Variation in Growth of *Centella asiatica* of Samosir – Indonesia Accession with Phosphorus Fertilizer Cultivated at Samosir Field

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**Abstract.** Indonesia has a variety of biological natural resources. One of the plants that thrive is pegagan (*Centella asiatica*). This study aims to investigate the response of pegagan to phosphorus fertilizer on growth components and phytochemical content. This plant is very useful because of the bioactive content contained in these plants. This research was conducted using a single factor with six levels of P$_2$O$_5$ fertilizer dosage with a non-factorial randomized block design repeated 3 times. The levels of fertilizer dosage are 0, 20, 40, 60, 80, 100 kg P$_2$O$_5$/ha. The application of phosphorus had no significant effects on growth component; length of petiole, total leaf area, number of stolons and biomass production of *Centella asiatica* in acid soil conditions. The doses of phosphorus given to *Centella asiatica* plants reaches 40 kg P$_2$O$_5$/ha (F2) in which the fertilized plants tend to offer higher results than those with no fertilizers. *Centella asiatica* can still grow well even though the land conditions are very acidic. The phytochemical screening of leaves and roots of *Centella asiatica* was studied and showed positive tests for saponin, tannin, phenolic, flavonoid, triterpenoid, steroid and glycoside and negative for alkaloid.

1. Introduction

*Centella asiatica* are widely used from nature. The medicinal plant that has been used from generation to generation and its bioactive can be felt by people who consume it. *Centella asiatica* is useful as a wound healer, rheumatism, asthma, inflammation, hemorrhoids, leprosy, dysentery, tuberculosis, fever, and an appetite enhancer. Japan uses a lot and imports medicinal and aromatic plants from China and India. China is the world’s biggest exporter for those plants [1].

*Centella asiatica* contains saponin compounds, including asiaticosides [2-3]. The bioactive of asiaticosides is able to speed up the wound convalescence and helpful for leprosy and tuberculosis treatment [4-5]. *Centella asiatica* in the large scale needs to be supported by good cultivation to produce quality product that required plant materials, guaranteed production levels and quality [6].

Pegagan (*Centella asiatica*) is recognized due to its bioactive compounds and has been employed as a treatment for numerous diseases [5, 8, 9]. The benefits offered by pegagan as a tropical plant leads to commercial cultivations in several countries such as China and India [10-11]. The increasing
demand of *Centella asiatica* both in national and international is mainly supplied by wild populations. This has resulted in the overexploitation of plants as has occurred worldwide [12]. Earlier studies on the centelloside amount in *Centella asiatica* such as madecasosside, asiaticoside, and asiatic acid have focused on the benefits of biochemical processes [13], concentration in the plant parts [14-16] and reports of their under controlled growth conditions [4]. Limited studies, especially in Indonesian region, deliver information on the influence of harvest time on the bioactive compounds concentration from *Centella asiatica* cultivated in the field. The production of pegagan field is various in quantity and quality over the year [2, 3, 6, 17, 18]. Field location, accession, and climatic conditions contributed to this inconsistency and deserves further study [13, 17]. Nowadays, an organic compound from natural materials is limited to compounds known as secondary metabolics. The use of plants as medicine is related to the chemical content contained in these plants, especially bioactive substances. Without the presence of a bioactive compound in plants in general, the plant cannot be used as medicine. Bioactive compounds found in plants are usually secondary metabolic compounds such as alkaloids, flavonoids, steroids, terpenoids, saponins, and others.

In this research, planting is carried out with agronomic action, giving phosphorus to see the consistency of the plant's response in biomass production and phytochemicals compounds. In previous studies, it was found that giving phosphorus without methyl jasmonate would result in higher biomass production and the asiaticoside content in leaves and roots would be affected so that the production of asiaticoside per g of dry matter would be higher [18-19]. The urgency of this research on *Centella asiatica* is due to the many benefits of *Centella asiatica* in various aspects. The benefits include diet in fish farming which has potential on the immune system mechanism in Nile tilapia [20], effective for inhibiting or preventing age-related retinal deterioration/ degeneration, useful for developing drugs or functional foods [21], protecting neuron cells [22], *Centella asiatica* herbs that are very useful [23]. So that various ways are done to increase the content of centelloside by utilizing methyl jasmonate [24-25]. The research objective was to obtain the response of pegagan to phosphorus fertilization on growth components and phytochemical content, potential plant material from North Sumatra.

2. Materials and methods

*Centella asiatica* of Samosir accession by giving phosphorus at a level that was increased from the previous study, analyzed the response of growth and photochemical content of *Centella asiatica*. The material used is *Centella asiatica* from Samosir accession. The tools required in this study were digital scales, digital cameras, soil processing equipment, meters, and others. This research was conducted using a single factor with 6 levels of P$_2$O$_5$ fertilizer dosage with a randomized block design repeated 3 times. The 6 levels of the fertilizer doses, named 0, 20, 40, 60, 80, 100 kg P$_2$O$_5$ / ha at the Samosir location.

2.1. Cultivation Field and Research Preparation

The cultivation field was located in the experimental field of Samosir at Salaon Toba Village, Ronggur Nihuta, Samosir, North Sumatra placed around 1100 m above sea level. This study started from March to Agustus 2020. The soil field at the cultivation was sampled and tested for pH, nitrogen, available phosphor, total organic carbon (C), and total K$_2$O based on potentiometric, Walkley-Black volumetric, Kjedhal volumetric, spectro-volumetric, and atomic absorption spectroscopic (AAS, HCl 25%) methods. Samples of soil were analyzed at the Socfindo laboratory, North Sumatra, Indonesia. Soil tillage was conducted to construct 18 units of 30 cm raised-soil beds or plots with the size of 1 m x 1 m. The gap between the plots was set at 0.5 m to allow easy access between the plots and maintain the separation of plants in different plots. The field was cleared from weeds.

2.2. Planting and Maintenance

The stolons separated from the mother plant were directly planted. Each plot contained four plants planted at 40 cm distance to each other. Urea was done for three times during the cultivation period at 0, 20, and 40 days after planting (DAP). In every fertilization period, the application dose of Urea was
30 g/plot (10 g/plot per each treatment) and KCl was 22 g/plot (after planting), distributed around all of the planting holes. Maintenance of cultivation consisted of regular watering, replanting crops and weeding. Weeding was done manually every day by removing weeds by hand from the soil. Control of diseases and pests was conducted every week to prevent the distribution of diseases and pests. Watering was done in the afternoon consistently by considering the conditions of weather in the field. Stitching was done in two weeks after planting to replace the dead plants. The plants were harvested at the same time at 12 weeks after planting (WAP) by collecting all parts of the plant.

2.3. Growth characteristics’ measurements
Length of petiole, total leaf area of mother plant, primary tendrils (tendrils that come out from the main plant) and secondary tendrils (tendrils that come out from the primary tendrils) were measured for their number and length every week for 12 WAP. The formed stolons were counted every week for 12 WAP from the formed stolons that came out from the tendrils.

2.4. Wet and Dry Weight Measurement
Harvested *Centella asiatica* were separated into two categories, the leaf part consisting of the leaves and petiols, and the root part consisting of the roots and tendrils and weighed for wet and dry weight.

3. Results and discussion

3.1. Length of Petiole (cm)
The length of petiole data at 4-12 WAP can be seen in Table 1. below. Analysis of variance of the 4-12 WAP showed that the phosphorus treatment did not significantly affect the length of the petiole, however, there was a tendency for F2 treatment to have the longest petiole length.

| Treatment | Length of Petiole (cm) (WAP) |
|-----------|-------------------------------|
| F0 = 0 kg P$_2$O$_5$/ha | 3.50 5.00 5.83 6.57 7.00 7.33 7.65 7.87 8.40 |
| F1 = 20 kg P$_2$O$_5$/ha | 4.00 4.90 5.33 5.87 6.37 6.97 7.20 7.80 7.87 |
| F2 = 40 kg P$_2$O$_5$/ha | 3.67 4.83 6.67 7.17 7.60 8.43 8.77 9.13 9.77 |
| F3 = 60 kg P$_2$O$_5$/ha | 3.33 4.00 5.17 6.00 6.83 7.07 7.37 7.60 7.80 |
| F4 = 80 kg P$_2$O$_5$/ha | 3.33 4.50 5.17 5.67 6.43 6.77 6.90 7.47 8.27 |
| F5 = 100 kg P$_2$O$_5$/ha | 3.67 5.00 5.67 6.50 7.07 7.53 7.90 8.40 8.67 |

3.2. Total Leaf Area (cm$^2$)

| Treatment | Total Leaf Area (cm$^2$) (WAP) |
|-----------|--------------------------------|
| F0 = 0 kg P$_2$O$_5$/ha | 4.86 14.23 36.87 96.77 185.38 340.88 479.40 595.15 708.89 1,085.88 1,409.52 |
| F1 = 20 kg P$_2$O$_5$/ha | 4.74 14.20 50.76 91.51 168.07 301.06 496.37 621.65 804.97 1,512.89 2,356.78 |
| F2 = 40 kg P$_2$O$_5$/ha | 5.25 12.78 33.18 90.44 204.72 376.04 514.80 599.86 808.34 1,903.33 2,339.03 |
| F3 = 60 kg P$_2$O$_5$/ha | 4.21 12.00 34.23 68.55 138.33 293.20 553.41 679.02 814.40 1,087.71 1,516.15 |
| F4 = 80 kg P$_2$O$_5$/ha | 3.69 11.37 31.61 72.58 176.38 336.80 468.57 613.57 742.40 1,055.24 1,944.57 |
Data on total leaf area (cm$^2$) at the age of 2-12 WAP observation is written in Table 2. The analysis of variance 2-12 MST showed that phosphorus treatment did not significantly affect the total leaf area, however, there was a tendency for F1 and F2 treatments to have the highest total leaf area compared to other treatments.

3.3. Number of Stolons (stolons)

Data on the number of stolons at 4-12 WAP observations can be seen in Table 3. The results of the 4-12 WAP analysis of variance showed that the phosphorus treatment did not significantly affect the number of stolons, however, there was a tendency for treatment F2 (40 kg P$_2$O$_5$/ha) to have the highest number of stolons compared to other treatments. Then followed by F4 treatment (80 kg P$_2$O$_5$/ha).

Based on Table 3, although the numbers are not significantly different, it was found that the highest average number of stolons in 9-12 WAP observation was in the treatment of phosphorus fertilizer dosage at 40 kg P$_2$O$_5$/ha.

### Table 3. Number of Stolons (stolons) at 4-12 WAP

| Treatment  | Number of Stolons (stolons) at 4-12 WAP |
|------------|----------------------------------------|
|            | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 |
| F0 = 0 kg P$_2$O$_5$/ha | 0.67 | 2.00 | 3.67 | 5.33 | 11.33 | 17.33 | 26.67 | 33.00 | 44.00 |
| F1 = 20 kg P$_2$O$_5$/ha | 0.67 | 2.00 | 4.00 | 6.33 | 13.00 | 19.33 | 26.33 | 36.00 | 45.00 |
| F2 = 40 kg P$_2$O$_5$/ha | 1.33 | 3.00 | 7.00 | 11.33 | 16.33 | 25.33 | 34.00 | 37.67 | 48.33 |
| F3 = 60 kg P$_2$O$_5$/ha | 1.00 | 3.00 | 5.33 | 8.33 | 14.33 | 21.00 | 26.00 | 31.67 | 40.00 |
| F4 = 80 kg P$_2$O$_5$/ha | 2.00 | 4.33 | 8.33 | 12.00 | 17.33 | 24.67 | 30.67 | 36.33 | 45.33 |
| F5 = 100 kg P$_2$O$_5$/ha | 1.67 | 3.67 | 7.00 | 12.00 | 14.67 | 21.67 | 29.00 | 34.33 | 42.33 |

Figure 1. Graph of Number of Stolons Development with Phosphorus Fertilization Dose Treatment (F0 = 0 kg P$_2$O$_5$/ha), (F1 = 20 kg P$_2$O$_5$/ha), (F2 = 40 kg P$_2$O$_5$/ha), (F3 = 60 kg P$_2$O$_5$/ha), (F4 = 80 kg P$_2$O$_5$/ha), (F5 = 100 kg P$_2$O$_5$/ha)
3.4. *Centella asiatica* Production

Table 4. Average Wet Weight Per Plot, Dry Weight of Leaves and Petiol, Dry Weight of Roots and Tendrils in Phosphorus Treatment

| Treatment   | WWP   | DWLP  | DWRP  |
|-------------|-------|-------|-------|
| F0 = 0 kg P₂O₅/ha | 723.33 | 61.30 | 64.93 |
| F1 = 20 kg P₂O₅/ha | 1,250.00 | 78.47 | 83.03 |
| F2 = 40 kg P₂O₅/ha | 1,320.00 | 104.70 | 119.20 |
| F3 = 60 kg P₂O₅/ha | 753.33 | 63.27 | 62.57 |
| F4 = 80 kg P₂O₅/ha | 1,060.00 | 82.70 | 94.13 |
| F5 = 100 kg P₂O₅/ha | 1,083.33 | 78.33 | 92.17 |

Note: Wet Weight Per Plot (WWP), Dry Weight of Leaves and Petiol (DWLP), Dry Weight of Roots Tendril (DWRP)

The average data of *Centella asiatica* plant production are listed in Table 4. The variance analysis showed that phosphorus treatment did not significantly affect wet weight per plot, dry weight per plot, wet weight of leaves and petiol, dry weight of leaves and petiol, wet weight of roots and tendrils, dry weight of roots and tendrils, however there was a tendency for treatment F2 (40 kg P₂O₅/ha) was the highest compared to other treatments.

3.4.1. *Wet Weight per Plot (g).* Data on average wet weight per plot with phosphorus fertilization can be seen in Table 4. Phosphorus fertilizer treatment did not significantly affect the wet weight per plot. The lowest wet weight per plot is acquired at 0 kg P₂O₅/ha without phosphorus fertilization (± 723.33 g) while the highest is at 40 kg P₂O₅/ha (± 1320.00 g) phosphorus fertilization treatment.

3.4.2. *Dry Weight of Leaves and Petioles (g).* Data on the average dry weight of leaves and crates are apparent in Table 4. The lowest dry weight of leaves and petiol per plot is obtained without phosphorus fertilizer at 0 kg P₂O₅/ha (± 61.30 g) while the highest was at 40 kg P₂O₅/ha (± 104.70 g) phosphorus treatment.

3.4.3. *Dry Weight of Roots and Tendrils (g).* Data on the average dry weight of roots and tendrils are presented in Table 4. The highest dry weight of roots and tendrils per plot was in the phosphorus fertilizer treatment at 40 kg P₂O₅/ha (± 104.70 g). The statistical analysis revealed that various phosphorus treatments did not significantly affect parameters of growth and production. Although the application of phosphorus, in general, does not have a significant effect, there is a trend of increasing yield with increasing doses of phosphorus given to *Centella asiatica* plants up to 40 kg P₂O₅/ha (F2) which the fertilized plants tend to produce higher yields than those of the unfertilized plants. The growth parameters such as length of petiole, total leaf area of parent plant, number of stolons, wet weight per plot, leaf dry weight, and root dry weight, were found in the treatment of phosphorus treatment at the level of 40 kg P₂O₅/ha. The existence of adequate nutrients will assist the plant growth. One of the macronutrients required by plants is phosphorus that has a major role in various plants life processes, such as photosynthesis, carbohydrate metabolism, and energy flow.
Table 5. Variation of soil chemical properties measured at before soil tillage

| Sample | Parameters       | Results     | Analytical Method                      |
|--------|------------------|-------------|----------------------------------------|
| Soil   | pH H\textsubscript{2}O | 2.76        | H\textsubscript{2}O (1:5)-Electrometry  |
|        | N-Kjehldahl      | 0.44 %      | Kjehldahl with Spectrophotometer       |
|        | Cation Exch.Cap  | 14.86 me/100 g | Amm. Acetate pH 7 with Spectrophotometer |
|        | P                | 0.21 %      | HNO\textsubscript{3} with Spectrophotometer |
|        | K-Total          | 0.08 %      | HNO\textsubscript{3} with AAS           |
|        | C/N              | 6.21        | -                                      |

Table 6. Phytochemical Screening of *Centella asiatica* Leaves and Roots of Samosir Accession [27]

| Phytochemical | Phytochemical Content |
|---------------|-----------------------|
|               | Leaves and petiols    |
|               | Roots and tendrils    |
| Alkaloid      | -                     |
| Saponin       | +                     |
| Tannin        | +                     |
| Phenolic      | +                     |
| Flavonoid     | +                     |
| Triterpenoid  | +                     |
| Steroid       | +                     |
| Glycoside     | +                     |

The prior study that soil P content influenced the centelloside content of *Centella asiatica* [17]. Phosphorus has a function to stimulate better root formation thereby the absorption of nutrients and water rises up, the content of leaf chlorophyll increases as increasing phosphorus application, plants can have well photosynthesis to yield photosynthe, and the amount of asiaticoside compounds upsurges [17]. Phosphorus will not be reducted in plants. The phosphorus stays as phosphate in its free form while it becomes esters when bound to organic compounds. Phosphate esters are made with sugars, alcohols, acids, or other phosphates (polyphosphates). This energy-rich compound is thought to be an intermediate for the pentose phosphate pathway of primary metabolites and is derived from precursors to secondary metabolites.

The phytochemical screening of *Centella asiatica* was examined and demonstrated positive tests for saponin, tannin, phenolic, flavonoid, triterpenoid, steroid, and glycoside and negative for alkaloid as shown in Table 6 [27]. Tannins acts as secondary metabolites that have responsibility for antimicrobial properties in various plants. Terpenoids and tannins could be associated with activities of analgesic and anti-inflammatory. Besides, tannins have contribution on astringency property that could heal wounds and inflamed mucous membrane rapidly [28, 31]. Saponins have been widely exploited as detergents, pesticides, and molluscicides; and have beneficial health effects. Phenolic compounds are one of the largest plant metabolites, which can be found everywhere [29]. Phenolic has biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, improvement of endothelial function, inhibition of angiogenesis and cell proliferation activities. *Centella asiatica* exhibited a positive result for flavonoids. Flavonoids are hydroxylated phenolic elements produced by plants as a result of microbial infection and have been discovered to be antimicrobial substances against various microorganisms. Glycosides function to decrease blood pressure.

*Centella asiatica* plants contain the most titerpenoid class compounds. Triterpenoids are derivative compounds of primary metabolite precursors that are bio-synthesized by the mevanolate pathway. Triterpenoids will produce geranyl pyrophosphates, which are primary metabolites forming
monoterpenoids and their derivatives, while farnesyl pyrophosphate escalates the forming of sesquiterpenoids and the alteration from squalene to triterpenoids. The elements of soil phosphorus affect the levels of asiaticoside, madecasoside and asiatic acid in several accessions were observed [2, 3, 17, 18, 30]. Centellosides include triterpenoid compounds biosynthesized in the cytoplasm through the mevalonic pathway. The centelloside biosynthetic pathway, where the last step is not yet clear, is required to conduct various studies in order to determine the response of *Centella asiatica* plants to the content and the production of very beneficial centellosides. Centellosides (asiaticosides, madecasosides, asiatic acids and madecacic acids) are a type of saponin ursane.

Farnesyl diphosphate synthase (FPS) has a crucial function in the development of plants organ. FPS has been found as a main regulatory enzyme in triterpene biosynthesis [32]. This biosynthesis may occur in leaves in which the asiaticoside amount magnifies with time. Phosphorus is also an essential element of a number of phosphate sugars, which holds important function in reactions in the dark phases of photosynthesis, respiration, and other metabolic processes. Phosphorus has major part in energy storage and transfer, also it is able advance the plants or dry matter production and increase the result quality [26].

4. Conclusion

The application of phosphorus had no effects on growth components; length of petiole, total leaves area of mother plant, number of stolons and biomass production of *Centella asiatica* in acid soil conditions. The screening of phytochemical in leaves part and roots part of *Centella asiatica* Samosir accession showed positive tests for saponin, tannin, phenolic, flavonoid, triterpenoid, steroid and glycoside and negative for alkaloid.

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