ENHANCING PHOSPHATE AVAILABILITY AND GROWTH OF *C. asiatica* IN ANDISOLS THROUGH PHOSPHATE-SOLUBILIZING BACTERIA APPLICATION

PENINGKATAN KETERSEDIAAN FOSFAT DAN PERTUMBUHAN *C. asiatica* PADA ANDISOL MELALUI APLIKASI BAKTERI PELARUT FOSFAT

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ABSTRACT

Centella asiatica is a medicinal plant containing asiaticoside bioactive, this is considerably higher if grown in the highlands generally on Andisols. Conversely, phosphorus was deficient in Andisol soils. The experiment aimed to study effect of phosphate solubilizing bacteria (PSB) isolates from the rhizosphere of *C. asiatica* which can dissolve P, produce plant growth promoters, increase growth of *C. asiatica* and fertilization efficiency. The research was done at Soil Biology Laboratory, Agriculture Faculty, Universitas Padjadjaran and at the experimental field of Agricultural Technology Research and Assessment Installation Manoko, Lembang District, West Java, Indonesia. The research used a factorial randomized block design with three replications and two factors. The first factor were application techniques: control, root soaking, and soil watering. The second factor were dose of NPK fertilizer and soil conditioner: control, P fertilizer dose of 100%, soil conditioner, P fertilizer dose 75% + soil conditioner, and P fertilizer dose of 100% + soil conditioner. Three superior isolates were isolated from *C. asiatica rhizosphere* and have been identified as: *Paraburkholderia caribensis* strain MNL-133, *Paraburkholderia caribensis* strain DSM 13236, and *Pseudomonas aeruginosa* strain K19PSE24. The results showed that the application of PSB combined with a dose of P fertilizer and soil conditioner on *C. asiatica* affected the soil P available, the number of leaves, the number of stolons and plant dry weight.

Key words: Conditioner, Isolates, Soaking, Rhizosphere, Watering.

ABSTRAK

Pegagan (*Centella asiatica*) salah satu komoditas tanaman obat yang memiliki kandungan bioaktif asiatikosida. Asiatikosida lebih tinggi jika ditanam di dataran tinggi umumnya ordo Andisols. Defisiensi hara fosfor salah satu kendala budi daya pada tanah Andisol. Penelitian ini bertujuan mengkaji pengaruh bakteri pelarut fosfat (BPF) unggul yang diisolasi dari rizosfer

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tanaman pegagan yang mampu melarutkan P dan menghasilkan pemacu pertumbuhan tanaman pengaruhnya terhadap peningkatan pertumbuhan tanaman pegagan dan efisiensi pemupukan. Penelitian dilaksanakan di Laboratorium Biologi Tanah, Fakultas Pertanian UNPAD dan di kebun percobaan Instalasi Penelitian dan Pengkajian Teknologi Pertanian (IP2TP) Manoko, Lembang menggunakan Rancangan Acak Kelompok Faktorial dengan tiga ulangan. Faktor pertama adalah teknik aplikasi BPF: kontrol; perendaman akar; penyiraman pada tanah. Faktor kedua yaitu dosis pupuk NPK dan pembenah tanah, yaitu perlakuan kontrol, pupuk P dosis 100%, pembenah tanah, pupuk P dosis 75% + pembenah tanah, dan pupuk P dosis 100% + pembenah tanah. Isolasi BPF dari rhizosfer pegagan menghasilkan isolat unggul yaitu *Paraburkholderia caribensis* strain MNL-133, *Paraburkholderia caribensis* strain DSM 13236, and *Pseudomonas aeruginosa* strain K19PSE24. Hasil menunjukkan pemberian BPF dikombinasikan dengan dosis pupuk P dan pembenah tanah pada tanaman pegagan memberikan pengaruh terhadap P tersedia, jumlah daun, jumlah stolon, dan berat kering tanaman.

Kata kunci: Isolat, Pembenah, Penyiraman, Perendaman, Rhizosfer.

INTRODUCTION

The prospect of developing medicinal plant products is increasing, this is in line with the development of the modern medicine industry and traditional medicines which are also increasing. Asiatic Pennywort (*Centella asiatica*) is one of the leading medicinal plant commodities that has the potential to be developed. *C. asiatica* is a plant that has long been used as a traditional medicine, both in the form of fresh, dry and herbal ingredients (Nuraini & Rahayu, 2021). The efficacy and benefits of this plant include triterpenoid phytochemical components, saponins, alkaloids, flavonoids, tannins, steroids, and glycosides (Vinolina et al., 2012). The active substances contained in this plant include asiaticoside, medecacid and medecasoid (from the triterpenoid group), sitosterol and stigmasterol from the steroid group, vallerin, brahmoside, brahminoside from the saponin group (Padmiswari et al., 2023).

C. asiatica can grow from the lowlands to an altitude of 100-2500 m above sea level (Devkota & Jha, 2009). Cultivation of *C. asiatica* the highlands provide higher bioactive content (asiatikosida) than in the lowlands. Soils in the highlands are generally dominated by the Andisols order. Phosphorus (P) nutrient deficiency is one of the constraints in Andisols cultivation. Such soils are not only low in available phosphorus, but also fixate most of the supplied phosphorus, requiring large amounts of fertilizer to elicit a plant response. Phosphorus nutrient fixation is the main factor causing the low availability of this nutrient (Shen et al., 2011).

Other constraints of Andisols such as low acidity and availability of P. The availability of P is low due to the strong bonding of elemental P to soil colloids and the high retention of P > 80% (Ge et al., 2023). This high P retention causes inefficient use of P fertilizers (An et al., 2021). To overcome the problem of P in Andisols, continuous handling is carried out through the use of soil microbes which play a role in the transformation of P in the soil. These microbes are known as phosphate solubilizing bacteria (Timofeeva et al., 2022). Based on the results of the study, it was found that there was a connection between the element phosphorus (P) and the asiaticoside content of Asiatic Pennywort. This is associated with energy-rich phosphate compounds that mediate energy transfer phosphorylation in the process of plant organ growth and in producing secondary metabolites (Kim et al., 2010).

Phosphate solubilizing bacteria increase the availability of soil P through the activity of phosphatase enzymes which convert P-organic to P-inorganic so that it is available to plants through P mineralization and secretion of organic acids which convert insoluble P to soluble P in the soil (Fitriatin et al., 2020; Yu et al., 2022). The group of bacteria capable of releasing adsorbed P is phosphate solubilizing bacteria. Phosphate solubilizing bacteria produce organic acids, including citric, malic, oxalic, and acetate, which function as catalysts, chelating agents, and complex P-absorbing agents (Arcand & Schneider, 2006; da Silva et al., 2023). Phosphate solubilizing bacteria also secrete phosphatase and phytase enzymes which can mineralize organic P and produce phosphate (Mehrvarz & Chaichi, 2008; Kalayu, 2019). Another advantage of phosphate solubilizing bacteria is the ability to produce growth regulators or phytohormones (Fitriatin et al., 2020; Zhao et al., 2022).

Plants that were given a microbial consortium gave better crop yields (Hemasempagam & Selvaraj, 2011). Microbes in the rhizosphere of medicinal plants are largely unexplored. Therefore, experiments are needed to obtain superior microbes from the rhizosphere of medicinal plants that help in growth and nutrient uptake for plants and increase P dissolution and reduce the use of P fertilizers.

MATERIALS AND METHODS

Isolation and Selection of PSB

The phosphate solubilizing bacteria used were isolated from the rhizosphere of Asiatic Pennywort (*Centella asiatica*) using Pikovskaya medium consisting of $Ca_3(PO_4)_2$ 5 g; yeast extract 0.5 g; $(NH_4)_2SO_4$ 0.5 g; $MgSO_4$ $7H_2O$ 0.1g; KCl 0.2 g, $MnSO_4$ $2H_2O$ 0.1 mg; $FeSO_4$ 0.1 mg; glucose 10 g; agar 15 g; and 1 l distilled water (Atlas, 2010) with Dillution Plate Method. Sampling locations in the Agricultural Technology Research and Assessment Installation Manoko, Lembang, West Bandung, West Java. The choice of this location was based on where gotu kola grows at an altitude of 1,200 m above sea levels which is dominated by Andisols. Sampling for PSB isolation from the rhizosphere of Asiatic Pennywort was soil that attached to the roots (1-2 mm thick) (Zhu et al., 2017). After 48-72 hours of incubation, the presence of a clear zone around the colonies indicated the ability to solubilize phosphate qualitatively (Tamrela et al., 2021). After purification, isolates were selected based on their ability to dissolve phosphate, produce organic acids, phosphatase enzymes and growth regulators.

Selected PSB Application on Centella asiatica

The experiment was conducted at the Manoko Agricultural Technology Research and Assessment Installation, Lembang, West Java using a factorial randomized block design consisting of two factors with 3 replications. The first factor were application technique: control, root soaking, and soil watering. The second factor were dose of NPK fertilizer and soil conditioner: control, fertilizer P dose of 100%, soil conditioner, P fertilizer dose of 75% + soil conditioner, and P fertilizer dose of 100% + soil conditioner. The composition of soil conditioner: 50% compost + 20% biochar + 20% guano + 10% dolomite

The first stage in preparation for application was isolate refresh. Then, a gradual activation was carried out in the Pikovskaya liquid medium in an erlenmeyer tube. The inoculant was incubated on a shaker for 48 hours. Furthermore, the inoculant was transferred to a tube that already contains molasses looking media. Re-incubated for 96 hours. Ready to be used as a medium for soaking *C. asiatica* roots before planting. The planting medium 5 kg pot^{-1}.

C. asiatica seeds in the form of cuttings were first grown in polybags for 3 weeks to determine the uniformity of the seedlings before being transplanted. On average, the seeds of *C. asiatica* grown had 2 leaves. Seedlings that had been removed from the polybags and cleaned of the roots from the soil were then soaked with an isolate solution that had been activated and diluted to a population of 10^7 cfu ml^{-1}. Soaking was carried out for 30 minutes and only the roots (Kumar et al., 2020). The seeds were then planted in the planting holes provided.

Data were analyzed statistically using the SPSS program. The F test at a significant level of 5% was carried out to see the effect of the treatment factors. If the treatment factor had a significant effect on the response, then it was continued with Duncan's test at the 5% level of significance.

Observations were made on the number of leaves, stalk length and number of stolons, measurements were made 6 weeks after planting (WAP) and 12 WAP. Harvesting was done after the plants were 3 months old by taking the stems and leaves of the plants. Determination of Available P using the Bray Method was carried out at the Laboratory of Soil Chemistry and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran.

RESULTS AND DISCUSSION

The results of the isolation obtained 30 isolates of phosphate-dissolving bacteria and tested their ability to produce a clear zone (halozone). It was obtained that 25 isolates were able to produce clear zones and 5 isolates did not produce clear zones until the 7th observation. The ability to produce clear zones for each isolate was different.

Three superior isolates were obtained based on its ability to dissolve phosphate, produce organic acids, phosphatase enzymes and IAA. showed the best ability to dissolve phosphate in vitro, which was 5.04-7.00 $\mu g.g^{-1}.jam^{-1}$ and IAA phytohormone production in vitro was 6.611-7.385 ppm (Kartikawati et al., 2022).

The isolates were identified using DNA and Protein Sequence analysis based on the BLAST (Basic Local Alignment Search Tool) method were identified which were *Paraburkholderia caribensis* strain MNL-133, *Paraburkholderia caribensis* DSM 13236 strain, and *Pseudomonas aeruginosa* strain K19PSE24.

Soil P-available

Based on statistical tests on soil P available presented in Table 1. Treatment of phosphate solubilizing bacteria and combinations of fertilizer doses did not have a significantly different effect on all treatments. In the control treatment (without PSB) there was an increase in available P after application of P fertilizer with soil conditioner. However, the application of phosphate solubilizing bacteria gave effect on the watering treatment of phosphate solubilizing bacteria with a combination dose of 100% P fertilizer and fixer soil conditioner with an increase of 12,06%. The submersion treatment of phosphate solubilizing bacteria and the combination of doses of P fertilizer and soil conditioner and followed by the combination treatment of 100% fertilizer and soil conditioner also made a significant difference to plants without treatment.

Table 1. Effect of PSB and P fertilizer combination on soil P-available

Treatments	**P available (ppm)**				
	Control	P 100 %	Soil Conditioner (SC)	P 75% + SC	P 100% + SC
Control	118.85 A a	183.74 BC a	178.37 BC a	140.12 AB a	226.70 C a
Root soaking	90.94 A a	161.56 B a	162.25 B a	118.78 A a	240.27 C a
Soil watering	118.86 A a	192.63 B a	158.19 AB a	147 AB a	254.05 C a

Note: The mean number of treatments followed by the same capital letters (horizontal) and lowercase letters (vertical) is not significantly different according to Duncan's multiple range test at 5% level

Application of phosphate solubilizing bacteria with a combination of doses of P fertilizer and soil conditioner gives potential to increase soil P available. Phosphate solubilizing bacteria have a major influence on the availability of P in the soil because of their ability to secrete organic acids to break the bonds of P elements bound to other elements such as Ca and Mg in the soil (Kumar et al., 2022). This is consistent with research (Tian et al., 2021) which showed that P biofertilizers were able to increase the available P content of soil.

The addition of soil conditioner can improve soil P-available. Soil organic matter that can be utilized by phosphate solubilizing bacteria as a source of food and energy to grow and develop thereby increasing the PSB population (Arifin et al., 2021).

Number of Leaves

The response of *C. asiatica* to the application of phosphate solubilizing bacteria and a combination of fertilizers significantly increased the number of leaves. The number of leaves at 6 WAP treated with phosphate solubilizing bacteria and soil conditioner increased by 6.6% compared to the control (Table 2). The increasing in the number of leaves was 33,7% compared to the addition of soil conditioner. The number of leaves at 12 WAP treated with phosphate solubilizing bacteria and soil conditioner increased by 63.3% compared to the control. The increasing in the number of leaves at 12 WAP was 24.9% compared to the application of 100% dose of P fertilizer and soil conditioner.

The addition of microbial inoculants, the presence of nutrients in the soil can increase plant growth (Alori & Babaloba, 2018). Element P is useful for stimulating the number of leaves and increasing leaf area, flowering and fertilization, as well as stimulating seed formation (Kim & LI, 2018). The effectiveness of the fertilization can be achieved, so that it can support plant growth, including the number of leaves.

Table 2. The number of leaves of *C. asiatica* at the age of 6 and 12 weeks after planting

Treatments	Number of Leaves at 6 WAP				
	Control	P 100 %	Soil conditioner (SC)	P 75% + SC	P 100% + SC
Control	38.5 A b	41.75 AB ab	47.92 AB b	42.67 AB a	51.17 B b
Root soaking	21.42 A a	27.42 A a	23.08 A a	25.42 A a	30.83 A a
Soil watering	45.25 A c	44.0 A b	64.08 B a	39.58 A a	56.92 AB b
Treatments	**Number of Leaves at 12 WAP**				
	Control	P 100 %	Soil conditioner	P 75% + SC	P 100% + SC
Control	123.33 A a	152.83 A a	173.92 A a	168.5 A b	166.25 A ab
Root soaking	112.58 A a	123.17 A a	120.0 A a	103.83 A a	127.0 A a
Soil watering	123.08 A a	165.92 AB a	180.08 B a	160.83 AB ab	207.58 B b

Note: The mean number of treatments followed by the same capital letters (horizontal) and lowercase letters (vertical) is not significantly different according to Duncan's multiple range test at 5% level

The dosage of 100% P fertilizer and fixers was significantly different from other treatments because the nutrients contained in amelioran were relatively complete. The fertilizer contains all the macro and micro minerals needed by plants so that they can increase soil productivity and provide nutrients for plants. Dolomite application also increases soil pH which has an impact on increasing the availability of nutrients for plants (Krismawati et al., 2022). Element P is influential in the process of leaf formation. Element P is an important part in plant metabolism as a form of phosphate sugar which is needed by plants during photosynthesis. Photosynthesis that goes well will produce photosynthates that plants can use for plant growth and development (Muhamad et al., 2021).

Number of Stolons

The interaction between the application of PSB and P Fertilizer with soil conditioner increased the number of stolons of *C. asiatica* (Table 3). The number of stolons at 12 WAP treated with phosphate solubilizing bacteria increased by 6.6% compared to that without phosphate solubilizing bacteria and fertilizers. The addition of fixers also showed an increase in the number of stolons compared to 26.9% without the addition of bacteria and fertilizers.

The number of stolons in all treatments generally increased. Soil conditions including acid also affect the yield parameters. The application of phosphate solubilizing bacteria with doses of P fertilizer and soil conditioner had a significant effect on several treatment doses. Not all parameters have a real effect because *C. asiatica* is a plant whose stolons propagate in all directions, so fertilizer should be spread and instilled in all areas of the crop plot so that when the stolons begin to grow they get P intake.

From the results of observing the number of stolons, it can be concluded that giving PSB inoculants with soil watering increased the number of stolons. Phosphate solubilizing bacteria can stimulate plant growth or can be used as biostimulants (Hamid et al., 2021). This can increase the maximum plant height, maximum number of branches, maximum number of leaves, wet weight and dry weight. Phosphate solubilizing bacteria application can improve cell growth and cell elongation so that this increases the number of stolons Giving dolomite combined with organic fertilizers increased the number of tillers (Lestari & Maftu'ah, 2021).

Table 3. The number of stolons of *C. asiatica* at the age of 6 and 12 weeks after planting

Treatments	**Number of Stolons at 6 WAP**				
	Control	P 100 %	Soil Conditioner (SC)	P 75% + SC	P 100% + SC
Control	3.67 A ab	3.92 A a	4.08 A b	3.5 A a	4.08 A b
Root soaking	2.17 A a	2.5 AB a	2.17 A a	2.25 A a	4.08 B a
Soil watering	3.83 AB b	3.08 A a	4.08 AB b	3.42 AB a	4.42 B b
Treatments	**Number of Stolons at 12 WAP**				
	Control	P 100 %	Soil Conditioner (SC)	P 75% + SC	P 100% + SC
Control	6.17 A ab	6.0 A b	7.83 A b	6.33 A a	6.50 A a
Root soaking	4.42 A a	4.50 A a	5.50 AB a	5.83 B a	5.58 AB a
Soil watering	6.58 A b	6.08 A b	6.42 A ab	6.08 A a	6.5 A a

Note: The mean number of treatments followed by the same capital letters (horizontall) and lowercase letters (vertical) is not significantly different according to Duncan's multiple range test at 5% level

Fresh and Dry Weight

Plant fresh weight indicates the amount of water and organic matter contained in plant tissues or organs. The treatment of application of phosphate solubilizing bacteria and combination of fertilizer doses in some treatments was not significantly different and in others the results were significantly different. Phosphate solubilizing bacteria influence in plant fresh weight. The treatment of application of phosphate solubilizing bacteria and the combination of 100% P fertilizer and soil conditioner gave the highest value of 103.92 g followed by the watering treatment of phosphate solubilizing bacteria and soil conditioner of 81.28 g, the treatment of root soaking application of phosphate solubilizing bacteria and the combination of P fertilizer doses 100% and soil conditioner were 78.84 g. The PSB application by root soaking and soil conditioner were 62.97 g of fresh weight (Table 4).

Based on the results of the statistical analysis shown in Table 4, the application of PSB combined with P fertilizer and soil conditioner increased plant dry weight. The combination of doses of 100% P fertilizer and soil conditioner enhancer gave the highest value of 17.87 g followed by the watering treatment of phosphate solubilizing bacteria and the combination of doses of 100% P fertilizer and soil conditioner of 17.64 g and the fixer treatment of 16.70 g.

Table 4. The plant fresh weight of *C. asiatica*

Treatments	Plant Fresh Weight (g)				
	Control	P 100 %	Soil Conditioner (SC)	P 75% + SC	P 100% + SC
Control	64.17 A a	63.38 A a	92.92 A a	79.32 A a	95.88 A a
Root soaking	35.58 A a	48.10 AB a	62.97 BC a	54.69 ABC a	78.84 C a
Soil watering	46.38 A a	72.65 AB a	81.28 BC a	69.39 AB a	103.92 C a

Note: The mean number of treatments followed by the same capital letters (horizontal) and lowercase letters (vertical) is not significantly different according to Duncan's multiple range test at 5% level

Dry weight can be used as a measure of plant growth and development because it reflects the accumulation of organic compounds that plants have successfully synthesized. The effect of giving phosphate solubilizing bacteria and a combination of fertilizers on plant dry weight at 12 WAP (Table 5). Inoculation of phosphate solubilizing bacteria through root soaking was able to increase plant dry weight by 16.74% and watering PSB was able to increase by 39.51% higher than plants that were not given. This is due to PSB able to produce phytohormones thereby increasing plant growth. Changes or increases in dry weight values are an indicator of the effectiveness of phosphate solubilizing bacteria in relationship with plants. This is in line with Vieira et al. (2021) that phosphate solubilizing bacteria produce phytohormones such as IAA, gibberellic acid and cytokinins (zeatin), so that it can increase plant growth.

Table 5. The plant dry weight of *C. asiatica*

Treatments	Plant Dry Weight (g)				
	Control	P 100 %	Soil Conditioner (SC)	P 75% + SC	P 100% + SC
Control	7.29 A a	9.60 AB a	16.70 BC a	14.99 ABC a	17.87 C a
Root soaking	8.51 A a	10.54 A a	11.56 A a	12.41 A a	12.68 A a
Soil watering	10.17 A a	14.67 AB a	12.16 AB a	10.5 A a	17.64 B a

Note: The mean number of treatments followed by the same capital letters (horizontal) and lowercase letters (vertical) is not significantly different according to Duncan's multiple range test at 5% level

Increasing the growth and productivity of *C. asiatica* biomass through increasing nutrient uptake can occur due to the relationship mechanism of PSB that supports more optimal plant growth that is healthy and mutually beneficial. This is supported by Tariq et al. (2022) that phosphate solubilizing bacteria highly significant increased growth of Mint plant as indicated by an increase in plant fresh and dry weight.

CONCLUSIONS

1. The three superior isolates were isolated from *C. asiatica* rhizosphere and have been identified as follows: *Paraburkholderia caribensis* strain MNL-133, *Paraburkholderia caribensis* strain DSM 13236, and *Pseudomonas aeruginosa* strain K19PSE24.
2. Interaction of PSB and soil conditioner on *C. asiatica* had an effect on the number of leaves, number of stolons, and plant dry weight. The application of PSB and soil conditioner also affects the available P.

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