RESPONSE OF *Andrographis paniculata* ON PHOSPHATE AND ENDOPHYTIC BACTERIA CONSORTIA IN NUTRIENT SOLUTION MEDIA

Response Tanaman Sambiloto terhadap Fosfat dan Konsorsium Bakteri Endofit pada Media Larutan Hara

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ABSTRACT

Phosphate and endophytic bacteria are two necessary components in primary and secondary metabolic processes of king bitter (*Andrographis paniculata*). This research objectives to know the effect of P and endophytic bacteria consortia (EBC) to improve growth, yield and andrographolide content of *A. paniculata*. This experiment consisted of two stages: a) the response of *A. paniculata* to P concentrations in growth medium. The experiment was arranged in randomized block design with six treatments and four replications. The P concentrations were 0, 0.01, 0.05, 0.1, 1.0, and 2.0 mM KH_2PO_4. b) The response of *A. paniculata* to P concentration and EBC in nutrient solution media. The trial used a factorial randomized block design, 4 treatments, factorial, with six replications. The first factors were P consentration P; deficient P (0,1 mM KH_2PO_4), and P sufficient (1,0 mM KH_2PO_4). The second factors were EBC; without and with EBC (20CD). The results of the first stage indicated that P was deficient at 0.1 mM KH_2PO_4 and P sufficient at 1.0 mM KH_2PO_4. The results of the second stage showed that the P sufficient concentration significantly increased growth, and dry weight of herb. However, P deficient improved andrographolide content. The EBC significantly improved growth, increased dry weight of herb, and andrographolide yield. Although had no significant effect on andrographolide content. There were no interaction between P concentration and EBC in nutrient solution media.

Key words: *Andrographis paniculata,* andrographolide, endophytic bacteria, phosphate.

ABSTRAK

Fosfat (P) dan bakteri endofit merupakan dua komponen yang penting di dalam proses metabolit primer dan sekunder pada tanaman sambiloto. Tujuan penelitian ini untuk mengetahui pengaruh P dan konsorsium bakteri endofit (KBE) terhadap peningkatan pertumbuhan, produksi dan kadar andrografolid tanaman sambiloto. Penelitian ini terdiri dari 2 tahap: a) Respon tanaman sambiloto terhadap konsentrasi P pada media larutan hara, menggunakan rancangan acak kelompok, 6 perlakuan, dan 4 ulangan. Perlakuan P terdiri dari 0; 0,01; 0,05; 0,1; 1,0; dan 2,0 mM KH_2PO_4. b) Response tanaman sambiloto terhadap P dan KBE pada media larutan hara, Percobaan menggunakan rancangan acak kelompok, faktorial, 4 perlakuan dan 6 ulangan. Faktor pertama adalah konsentrasi P terdiri dari P kurang (0,1 mM KH_2PO_4) dan P cukup (1,0 mM KH_2PO_4). Faktor kedua adalah bakteri yaitu tanpa KBE dan dengan KBE (20CD). Hasil penelitian pada tahap pertama menunjukkan bahwa diperoleh dosis P kurang untuk tanaman sambiloto (0,1 mM KH_2PO_4) dan dosis P cukup (1,0 mM KH_2PO_4). Hasil penelitian pada tahap kedua menunjukkan

bahwa Pemberian P cukup mampu meningkatkan pertumbuhan, dan bobot kering herba. Sebaliknya, P kurang meningkatkan kadar andrografolid. KBE mampu meningkatkan pertumbuhan, bobot kering herba, dan produksi andrografolid, tetapi tidak mempengaruhi kandungan andrografolid. Tidak ada interaksi antara P dan KBE dalam media larutan hara.

Kata kunci : *Andrographis paniculata*, *andrografolid, bakteri endofit, fosfat.*

INTRODUCTION

Phosphate (P) is one of the essential elements for plant growth and development, as well as for secondary metabolic process especially terpenoids group. In primary metabolic processes, P is able to supply energy required for photosynthesis process, sugar production, and nucleic acids synthesis (Saber *et al*., 2005). Plant growth and production were often limited by low P availability in the soil (Hernandez *et al*., 2007). Plants adapt low P availability by using P efficiently. There are many factors affecting nutrient absorption by plants.

Various attempts were made to increase P availability in the soil, such as the application of inorganic and organic P fertilizers, and also microorganisms. Inorganic P fertilizer can immediately supply P available and be used by plants. However, excessive dosage will be inefficient and even inhibit plant growth. The high rate of P absorption by soil causes more than 90% of P fertilizer is quickly converted into unavailable forms. Plants will grow slowly at soil with low P. The efforts have to perform to increase available P for optimum plant growth, such as applying phosphate fertilizer (Watson and Mullen, 2007).

Phosphate has significant role both in primary and secondary metabolic processes. It had significantly enhanced bioactive compounds on medicinal plant *Salvia miltiorrhiza* (Lu *et al*. 2013), and *Lens culinaris* Medic (Sarker and Karmoker, 2011). The dosage of P fertilizer up to 150 kg ha^{-1}, increased essential oils content of *Mentha piperita* (Sulandjari *et al*., 2007). Bahl *et al*. (2000) reported P fertilization at 30 kg ha^{-1} or more, significantly increased oil content of sunflower. Phosphate played a key role in andrographolide biosynthesis, particularly in the formation of isopentenyl pyrophsophate (Vickery and Vickery, 1986; Dubay *et al*., 2003).

The use of microorganisms, such as bacteria, as plant growth promoter has significant effect on plant growth and yield. Commonly, endophytic bacteria were widely used on food crops including rice, sugarcane and beans. Conversely, the information of the application on medicinal plants is very limited and not yet known. Several studies have mentioned the use of endophytic bacteria as growth promoter to produce phytohormones and supply nutrients. It also gave positive response to the host plant. *Pseudomonas fluorescence* could stimulate root growth of maize (Benizri *et al*., 1998), while *Bacillus* sp and other microbes could promote the growth of green bean sprouts (Aryantha *et al*., 2004).

Endophytic and soil bacteria have the same roles in enhancing plant growth. These bacteria have one or several mechanisms that can be beneficial to plants, such as biological control of pest through competition, to produce antibiotics (Lugtenberg and Kamilova, 2009), phytohormones (Shi *et al*., 2009), induced resistance (Sturz and Nowak, 2000), increased nutrient availability through N fixation (Rolfe and Wienman, 2001), and increased solubility of organic and inorganic P (Hussain *et al*., 2013).

Application of P and endophytic bacteria were expected to increase primary and secondary metabolites of *A. paniculata*.

This study provided overview of P requirement and endophytic bacteria role in supporting plant growth and andrographolide content of *A. paniculata*. The purposes of this study were to: 1) obtain the concentration of P deficient and P sufficient in nutrient solution media for *A. paniculata*, and 2) determine the effect of P and endophytic bacteria on *A. paniculata* growth in nutrient solution media.

MATERIALS AND METHOD

The research was conducted at Cimanggu Garden, Indonesian Spice and Medicinal Crops Research Institute, Bogor West Java from June until November 2013.

Plant Materials

The materials used were *A. paniculata (*Sambina 1 variety) from Cimanggu Bogor, West Java, Indonesia.

Endophytic Culture

The endophytic bacterial culture (20CD) were isolated from *A. paniculata* leaf tissues. The 20CD endophytic bacteria consortia consisted of four isolates of *Bacillus* sp.

Nutrient Solution Media

Nutrient solution composition were 1.5 mM $Ca(NO)_3.4H_2O$, 1.0 mM NH_4NO_3, 0.4 mM $MgSO_4.7H_2O$, 0.5 ppm $MnSO_4.4H_2O$, 0.02 ppm $CuSO_4.5H_2O$, 0.05 ppm $ZnSO_4.7H_2O$, 0.5 ppm H_3BO_3, 0.01 ppm $NH_2Mo_7O_{24}$, 68 µM Fe EDTA, 1 mM KCl.

Methods

The experiments were conducted in 2 stages:

A. Andrographis paniculata response to P in the nutrient solution media

The nutrient solution was combined with several concentrations of P. This experiment used a randomized block design, six treatments and four replications. The treatments consisted of 0, 0.01, 0.05, 0.1, 1.0 and 2.0 mM KH_2PO_4. The seeds were germinated for 2 months, and then transplanted into 1 l pot^{-1}containing treatment media after the seedlings had 4-6 leaves. Water lost by transpiration was replaced by deionized water every day. pH was maintained at approximately 5.5. Plants maintained in this condition for 4 weeks. Plant height, number of leaves, lenght of root, length of leaves, shoot and root dry weight, leaf area (LA), and leaf area ratio (LAR) were measured at 4 weeks after planting (WAP).

B. Andrographis paniculata response to P and endophytic bacteria consortia in nutrient solution media

This study used a randomized block design, factorial, 4 treatments and 6 replications. The first factors were without endophytic bacteria consortia; and with endophytic bacteria consortia. The second factors were P deficient and P sufficient Lugtenvernt. P deficient was 0.1 mMKH_2PO4 and P sufficient was 1.0mM KH_2PO4. The treatments combination consisted of: 1) P deficient (0.1 mM KH_2PO_4), 2) P sufficient (1.0 mM KH_2PO_4), 3) P deficient+endophytic bacteria consortia (P deficient+EB), and 4) P sufficient+endophytic bacteria consortia (P sufficient+EB).

The same nutrient solution media, as in stage A was used in this experiment. Endophytic bacteria consortia were applied 10 ml pot^{-1} at concentration 10^{10} cfu ml^{-1}, 3-4 days after planting. Plants were grown for 4 WAP. The parameters observed were plant height, leaf number, root length, herb and root dry weight, andrographolide content and yield. The content of andrographolide was de termined and the results were obtained by HPLC. Andrographolide yield was ditermined from multiplication of andrographolide content and herb dry matter.

Statistical Analysis

The data were analyzed using Analysis of Variance (ANOVA) and further using Duncan's Multiple Range Test (DMRT) 5% if there was any significant effect of treatments.

RESULTS AND DISCUSSIONS

A. Andrographis paniculata response to P in the nutrient solution media Plant growth components

Phosphate significantly increased plant height, leaf number, and root length compared to controls. Plant growth was optimum at 1.0 mM KH_2PO_4, and then decreased at 2.0 mM KH_2PO_4. Performance of *A. paniculata* growth showed at Figure 1.

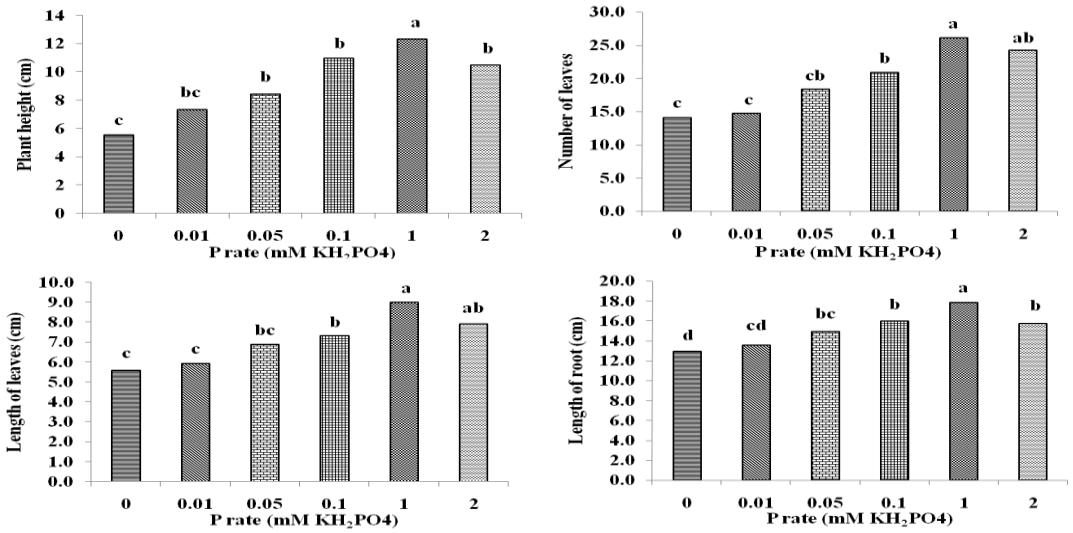

Figure 1. Effect P on *A. paniculata* growth in nutrient solution media *Leaf area and Ratio leaf area*

The P application significantly increased leaf area (LA), and leaf area ratio (LAR). LAR was biomass dry weight per unit of leaf area, indicating photosynthesis results. The 1.0 mM KH_2PO_4 gave higher LAR than 0.1 and 2.0 mM KH_2PO_4 (Table 2). It utilized more efficient assimilates for leaf formation. On the contrary, 0-0.05 mM KH_2PO_4 resulted in low LAR causing low dry weight of yield and leaf area (Table 1 and 2).

Table 1. Effect of P on leaf area and leaf area ratio in nutrient solution

Treatments	LA (cm^2)	LAR (cm^2g^{-1})
0 (without P)	24.12 d	111.52 c
0.01 mM KH_2PO_4	37.48 c	137.37 bc
0.05 mM KH_2PO_4	62.58 b	174.52 ab
0.1 mM KH_2PO_4	85.38 a	197.43 a
1.0 mM KH_2PO_4	97.04 a	156.68 b
2.0 mM KH_2PO_4	65.24 b	199.61 a

Note: numbers followed by the same letter in the same column were not significantly different at 5% DMRT.

Dry matter of yield

Phosphate application at 0.05; 0.1 and 1.0 mM KH_2PO_4 significantly increased dry weight of root and shoot at 4 WAP. The 1.0 mM KH_2PO_4 produced the highest root and shoot dry weight (Table 2). Lu *et al*. (2013) mentioned that P application increased root dry weight of root, shoot and biomass of *S. miltiorrhiza*. Similar result on tomato was reported by Basirat *et al*. (2011), indicateing that dry weight of yield increased following the increase of P concentration.

Table 2. Dry matter yield in nutrient solution at 4 WAP

Treatment	Root dry weight ($gplant^{-1}$)	Shoot dry weight ($gplant^{-1}$)	Ratio of root-shoot dry weight
0 (without P)	0.07 d	0.15 d	0.47
0.01 mM KH_2PO_4	0.09 bd	0.19 cd	0.47
0.05 mM KH_2PO_4	0.10 bc	0.26 c	0.38
0.1 mM KH_2PO_4	0.12 ab	0.33 b	0.36
1.0 mM KH_2PO_4	0.13 a	0.49 a	0.26
2.0 mM KH_2PO_4	0.08 cd	0.25 c	0.32

Note: numbers followed by the same letter in the same column were not significantly different at 5% DMRT.

P concentration less than 1.0 mM KH_2PO_4 was defined as P deficient. Both growth and dry matter production was lower than 1.0 KH_2PO_4, although there was no symptoms of P deficiency. This suggested plant adaptation to P deficiency. Plant adaptation to P deficiency could be through root modification and carbon translocation from shoot to root (Wang *et al*., 2008). This was indicated by higher ratio of root : shoot dry weight at P deficient. On the P deficient condition, higher root dry weight than shoot, the contrary P sufficient condition, higher shoot dry weight than the root (Table 2). These results were in accordance to Jebara *et al*. (2005). Changes in carbon partitioning caused by P supply shortage, produced higher root dry weight than shoot at low P levels (Boutraa, 2009).

Furthermore, 0.1 mM KH_2PO_4 considered as P deficient because plant growth was not optimal, thus P did not fulfill the requirement. The 1.0 mM KH_2PO4 was considered as sufficient because it gave optimal growth. Moreover, plant growth declined at 2.0 mM KH_2PO4, though there was no sign of damage. Plant growth at 0.1 mM KH_2PO_4 treatment was able to follow growth at 1.0 mM KH_2PO_4, because plant could use P more efficient at low P environment.

B. *Andrographis paniculata* response to P and endophytic bacteria consortia in nutrient solution media

Plant growth components

Phosphate and endophytic bacteria applications significantly affected plant growth. The P sufficient and endophytic bacteria increased plant height and leaf number. There was no interaction between P and endophytic bacteria consortia to all parameters (Table 3, 4, 5).

Table 3. Effect of P and endophytic bacteria consortia on growth of *A. paniculata* at 4 WAP

Treatments	Plant height (cm)	Number of leaves	Length of root (cm)
P factor			
P deficient	9.08 b	21.75 b	13.53 a
P sufficient	12.52 a	26.14 a	14.45 a
Endophytic bacteria factor			
Without endophytic bacteria consortia	9.13 b	19.47 b	15.31 a
With endophytic bacteria consortia	12.47 a	28.41 a	12.67 b

Note: numbers followed by the same letter in the same column were not significantly different at 5% DMRT.

Dry matter yield

P sufficient produced higher shoot dry weight than P deficient, but root:shoot ratio increased at deficient P. Endophytic bacteria consortia gave higher dry weight of roots and shoots than nonendophytic bacteria consortia. However, it declined ratio of root:shoot (Table 4).

Plant experienced nutrient stress at P deficient condition, thus enhancing root : shoot ratio. The growth of shoot was greatly reduced, causing the decrease of photosynthesis rate and assimilates. Plants adapted to stress through several mechanism including transporting photosynthates from shoot to roots, to fulfill P requirement (Cakmak, 1994). Consequently, carbohydrates accumulated in the roots and net photosynthesis decline (Hernandez *et al*., 2007). This was indicated by higher root:shoot ratio than P sufficient (Table 4). The similar result was indicated in wheat (Jian *et al*., 2003).

Table 4. Effect of P and endophytic bacteria on *A. paniculata* dry weight of yield at 4 WAP

Treatments	Shoot dry weight	Root dry weight	Root:shoot ratio
	(g plant^{-1})		
P factor			
P deficient	0.51 b	0.11 a	20.73 a
P sufficient	0.67 a	0.12 a	18.69 b
Endophytic bacteria factor			
Without endophytic bacteria consortia	0.46 b	0.08 b	19.85 a
With endophytic bacteria consortia	0.72 a	0.14 a	17.32 b

Note: numbers followed by the same letter in the same column were not significantly different at 5% DMRT.

The increase of plant height (37.9%), leaf number (20.2%), and dry weight of shoots (31.4%) showed a positive response of *A. paniculata* on sufficient P application. This study indicated the role of P significantly increased growth and dry matter. Phosphate plays important role in many processes of energy formation including nucleic acid synthesis, photosynthesis, glycolysis, respiration, synthesis and stability of membrane, enzyme activation/inactivation, redox reactions, carbohydrate metabolic, and nitrogen fixation (Vance *et al.,* 2003). At P sufficient conditions, optimal growth and plant metabolism processes would be undisturbed.

Phosphate also had role in cell membranes formation. The availability of P can increase rate of phospholipid synthesis.

It has positive effect on plant metabolic processes such as respiration, ion uptake and energy formation. The increasing of energy formation would facilitate chloroplast photophosphorylation to improve the rate of photosynthesis (Blair and Edwards, 2000). The plant will be able to produce more carbohydrates, and dry matter yield. These results were consistent with Lu *et al*. (2013), that P fertilization increased the yield of medicinal plants (*Thevetia periviana*).

The application of endophytic bacteria consortia promoted growth and increased plant dry matter yield significantly. The 20CD endophytic bacteria consortia produced IAA, and GA3. Phytohormone can stimulate plant growth through plant cell division. Endophytic bacteria promote plant growth by producing plant growth promoting substances and N fixing from atmosphere (Sturz *et al*., 2000). Thus, the plant can grow optimally. This is consistent with research Puente *et al*., (2004); Fitri and Gofar (2010), that endophytic bacteria can increase plant growth and produce phytohormones; IAA, GA3 and abscisic acid (ABA) (Baca and Elmerich, 2003; Feng *et al*., 2006; Boiero *et al*., 2007; and Lins *et al*., 2014).

Andrographolide Content and Yield

Andrographolide content of P deficient was higher than P sufficient. However, P had no significant effect on andrographolide yield. Environmental factor affected crop yield and bioactive compounds. Accumulation of bioactive compound can be induced by environmental stress. Phosphate availability is one of environmental factors affecting primary and secondary metabolism. Environmental stress, such as nutrients deficiency or excess, affects levels of several secondary metabolites (Kirakosyan, 2004).

On P deficient condition, plants will undergo stress, thus producing more secondary metabolic. John *et al*. (2009) revealed that secondary metabolic increased in the plants following P deficient. Moreover, Sarker and Karmoker (2011) reported P deficiency increased active compounds, such as proline and phenolics, in roots and stems of *Lens culinaris* Medic, as well as enhanced anthocyanin levels in the leaves. The increasing of secondary metabolic was one of indicator of plant suffered stress due to P deficiency (Sarker and Karmoker, 2011).

Table 5. Effect of P and endophytic bacteria consortia on andrographolide content and yield of *A. paniculata*

Treatments	Andrographolide content (%)	Andrographolide yield (g plant^{-1})
P factor		
P deficient	2.76 a	0.015 a
P sufficient	2.34 b	0.016 a
Endophytic bacteria factor		
Without endophytic bacteria consortia	2.52 a	0.012 a
With endophytic bacteria consortia	2.58 a	0.018 b

Note: numbers followed by the same letter in the same column were not significantly different at 5% DMRT.

CONCLUSIONS

Phosphate signicantly increased growth and dry matter yield of *A. paniculata*. It was defined 0.1 mM KH_2PO4 as P deficient and 1.0 mM KH_2PO4 as P sufficient. Andrographolide content at P deficient was higher than sufficient P, although P indicated no significant effect on andrographolide yield. Endophytic bacteria consortia significantly increased growth and dry matter yield of *A. paniculata*. Endophytic bacteria consortia produced higher andrographolide yield than without endophytic bacteria, whereas andrographolide content showed no significant different on both treatments. No interaction between P and endophytic bacteria consortia.

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