SHOOT, TOTAL PHENOLIC, AND ANTHOCYANIN PRODUCTION OF *Plectranthus amboinicus* WITH ORGANIC FERTILIZING

Produksi pucuk, total fenolik dan antosianin *Plectranthus amboinicus* dengan pemupukan organik

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(diterima 16 Desember 2013, direvisi 21 Desember 2013, disetujui 27 Desember 2013)

ABSTRACT

Bangun-bangun [*Plectranthus amboinicus*] is a functional vegetable that is used as lactagogue. This research was aimed to provide information of the effect of organic fertilizer on shoot, total phenolic, and anthocyanin production of bangun-bangun. This experiment was conducted at Bogor Agricultural University, Leuwikopo experimental station (Indonesia), from December 2012 to February 2013. The experiment was laid out in randomized block design with single factor with five combination of organic fertilizer treatments (combination of cow manure 12.3 t ha\(^{-1}\), rock phosphate 1.5 t ha\(^{-1}\), rice-hull ash 5.5 t ha\(^{-1}\) with three replications. The result showed that application of organic fertilizer increased of shoot production. Application of 12.3 t ha\(^{-1}\) cow manure + 1.5 t ha\(^{-1}\) rock phosphate + 5.5 t ha\(^{-1}\) rice-hull ash produced shoot dry weight ha\(^{-1}\) (57.33%) and metabolite production ha\(^{-1}\) (total phenolic 12.06%, anthocyanin 41.73%) higher than no fertilizing (P > 0.05). Application of cow manure + rock phosphate produced the lowest shoot dry weight ha\(^{-1}\) and metabolite production ha\(^{-1}\). The result of this research suggested that nitrogen, phosphorus, and potassium were needed on shoot production of bangun-bangun.

**Key words:** *Plectranthus amboinicus*, phenylalanine ammonia lyase, secondary metabolite

ABSTRAK

Bangun-bangun [*Plectranthus amboinicus*] merupakan salah satu jenis sayuran fungsional yang berfungsi sebagai Laktagogum. Percobaan ini dilakukan di Kebun Percobaan IPB Leuwikopo, Kecamatan Darmaga, Bogor, sejak Desember 2012 sampai Februari 2013 untuk mempelajari pengaruh pupuk organik terhadap produksi pucuk, total fenolik, dan antosianin bangun-bangun. Percobaan ini disusun menggunakan rancangan acak kelompok dengan tiga ulangan. Perlakuan yang diberikan yaitu lima kombinasi pemupukan organik (tanpa pemupukan, 12.3 t ha\(^{-1}\) pupuk kandang sapi + 1.5 t ha\(^{-1}\) batuan posfat, 12.3 t ha\(^{-1}\) pupuk kandang sapi + 5.5 t ha\(^{-1}\) abu sekam, 1.5 t ha\(^{-1}\) batuan posfat + 5.5 t ha\(^{-1}\) abu sekam, 12.3 t ha\(^{-1}\) pupuk kandang sapi + 1.5 t ha\(^{-1}\) batuan posfat + 5.5 t ha\(^{-1}\) abu sekam). Hasil percobaan menunjukkan bahwa pemberian pupuk organik meningkatkan produksi pucuk. Aplikasi 12.3 t ha\(^{-1}\) pupuk kandang sapi + 1.5 t ha\(^{-1}\) batuan posfat + 5.5 t ha\(^{-1}\) abu sekam memberikan bobot kering pucuk ha\(^{-1}\) (57.33%) dan produk metabolit ha\(^{-1}\) (total fenolik 12.06%, antosianin 41.73%) lebih tinggi dibandingkan tanpa pemupukan (P > 0.05). Aplikasi pupuk kandang sapi + batuan posfat memberikan produksi bobot kering pucuk ha\(^{-1}\) dan metabolit ha\(^{-1}\) terendah. Penelitian ini menunjukkan bahwa unsur nitrogen, fosfor, dan kalium diperlukan dalam produksi pucuk bangun-bangun.

**Kata kunci:** *Plectranthus amboinicus*, metabolit sekunder, phenylalanine ammonia lyase
INTRODUCTION

Bangun-bangun [Plectranthus amboinicus (Lour.) Spreng] is a potential wild tropical plant whose leaves have a distinctive aroma, so that it was known as aromatic plants and used as medicinal plant. Bangun-bangun has been used as breast milk stimulant (lactagogue) by Batak people (especially mothers after childbirth) in Indonesia for hundreds of years (Damanik et al., 2006). Coleus or Plectranthus is an important aromatic herb of the family Lamiaceae which is grown as a traditional medicinal herb in India that containing flavonoid and phenolic (Rasineni et al., 2008). Flavonoid is one group of phenolics compounds (Mualim, 2012). According to Ververidis et al. (2007) anthocyanin is part of flavonoid component that have antioxidant effect and function as cardioprotective.

One of the environmental factors that affect plant production is nutrient availability in the soil. Nutrient availability in the soil can be given by fertilizing, such as organic fertilizer. The use of organic fertilizer can increased nutrient availability in the soil, microorganism activity, and repaired soil structure (Dauda et al., 2008; Tu et al., 2006). Other research studied the effect of organic fertilizing on waterleaf (Susanti et al., 2008; Mualim, 2012). This research used three types of organic fertilizers i.e. cow manure (N source), rock phosphate (P source), and rice-hull ash (K source), but actually each organic fertilizer contained complete nutrient.

All phenolic compounds (included flavonoid) produced from the phenylpropanoid pathway. The first step for biosynthesis of the phenylpropanoid skeleton in higher plants is the deamination of L-phenylalanine to form trans-cinnamic acid. This reaction is catalyzed by phenylalanine ammonia–lyase (PAL, EC 4.3.1.24). PAL plays a crucial role at the interface between plant primary and secondary metabolism (Reichert et al., 2009).

PAL activity is increased by low nutrient level, light, and temperature (Tan, 1980). The biosynthesis of phenolic compounds has been associated with increased PAL activity. The high PAL activity and followed by increasing of phenolic compound in wet season, promoted synthesis of anthocyanin by shikimic acid pathway. In dry season, PAL activity was low, but total phenolic was high, the formation of phenolic compound was from malonic acid pathway (Mualim, 2012).

No information was found on the effect of organic fertilizing on shoot production and metabolite compounds of bangun-bangun. The purpose of this experiment was to study of the effect of organic fertilizing on shoot production and metabolite compounds of bangun-bangun.

MATERIAL AND METHODS

This experiment was conducted at IPB Leuwikopo Research Station, Darmaga, Bogor, under the shade of coffee tree with shade percentage ±35%. Location of the research station is at ±190 m above sea level. This experiment was conducted from December 2012 to February 2013. The experiment was laid out in randomized block design with single factor. Five combinations of organic fertilizing (no fertilizing, 12.3 t ha\textsuperscript{-1} cow manure + 1.5 t ha\textsuperscript{-1} rock phosphate, 12.3 t ha\textsuperscript{-1} cow manure + 5.5 t ha\textsuperscript{-1} rice-hull ash, 1.5 t ha\textsuperscript{-1} rock phosphate + 5.5 t ha\textsuperscript{-1} rice-hull ash, 12.3 t ha\textsuperscript{-1} cow manure + 1.5 t ha\textsuperscript{-1} rock phosphate + 5.5 t ha\textsuperscript{-1} rice-hull ash) were given as treatments (Table 1) and repeated three times, so there were 15 experimental units. Each experimental unit consisted of 10 plants so that there were 150 plants. Data were analyzed using ANOVA at 95% and Tukey’s HSD (Honestly Significant Difference) test with α 5% to compare means between each treatment.
Stem cuttings with the length of ±10-15 cm was planted in polybags and the composition of the media used for planting is soil : cow manure (2:1, v:v) for three weeks before planting in the nursery. Cow manure, rock phosphate, and rice-hull ash was given according to the treatment. These propagules with four pairs leaves planted on the plots with size of 5 m x 5 m with plant spacing 50 cm x 50 cm (population 40,000 plants ha⁻¹, 100 plants 25 m²).

The criteria of bangun-bangun shoot harvest is when the shoot with three pairs leaves were perfectly open and leaving one pair leave on the plant. First harvest at 8 WAP, and the second harvest at 12 WAP (week after planting in the nursery).

Observation was conducted on shoot production with shoot harvest criteria, and metabolite production of bangun-bangun, i.e., total phenolic and anthocyanin content and production. PAL activity was determined by using method from Dangcham et al. (2008). Anthocyanin content was determined by using method from Sims and Gamon (2002); and Mualim (2012) method was used for total phenolic. The material for total phenolic analyze using dry shoot from freeze dryer Flexy-DryTM MP (USA) that extracted with methanol and the aliquot used for analyze. Mixed solution extract was incubate used Eyela waterbath SB-24, whereas for measuring spectrophotometry used Shimadzu UV-1800 spectrophotometer (Japan) that connected with UV probe 2.34.

RESULTS AND DISCUSSION

Soil and plant nutrient content

Table 2 showed that nitrogen content in soil before organic fertilizer application were in medium status, however phosphorus and potassium content was low. Organic fertilizing decreased nitrogen content in soil after organic fertilizer application were in low status and increased nitrogen content in plant. This presumably caused by low N availability so that carbohydrates will be deposited in vegetative cells, causing them to thicken (Havlin et al., 2005) and presumably absorbed by plant for growth, especially for shoot production and by nutrient slow release from organic fertilizer with C/N ratio 9-10 were in low status (Table 3).

Application of organic fertilizer produced N nutrient content of plant higher than no fertilizing (Table 2). This presumably caused by the form of N absorb by plant is nitrate (NO₃⁻) and or ammonium (NH₄⁺). For plants, NH₄⁺ and NO₃⁻ are the most important form and both are produced from aerobic decomposition of soil organic matter. Soil N occurs as inorganic or organic N. Inorganic soil N includes NH₄⁺, NO₂⁻, NO₃⁻, N₂O, NO₃, dan N₂, whereas organic soil N occurs as proteins, amino acids, amino sugars, and other complex N compounds.

Table 1. Organic fertilizer treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Organic fertilizer (t ha⁻¹)</th>
<th>Nutrient contribution of organic fertilizer (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cow manure</td>
<td>Rock phosphate</td>
</tr>
<tr>
<td>P0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P1</td>
<td>12.3</td>
<td>1.5</td>
</tr>
<tr>
<td>P2</td>
<td>12.3</td>
<td>0</td>
</tr>
<tr>
<td>P3</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>P4</td>
<td>12.3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

¹ N content 1.29 % and K₂O content 1.10 %.
² P₂O₅ content 2.87 % (Land Resources and Soil Science Department Laboratory 2012).
The availability of \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) depends on the quantity of N mineralized from soil organic N. N mineralization is the conversion of organic N to \( \text{NH}_4^+ \) through two reactions, aminification and ammonification. Aminification converts proteins in residues to amino acids, amines, and urea, whereas ammonification converts organic N to inorganic \( \text{NH}_4^+ \) and then \( \text{NH}_4^+ \) converted to \( \text{NO}_3^- \) and \( \text{NO}_2^- \) by nitrification and absorbed directly by higher plants (N uptake) (Havlin et al., 2005).

The low content of P was caused by low soil pH (Table 3). The soil pH status before and after organic fertilizer application were in very low status (Table 2). This presumably caused by K\(^+\) uptake is influenced by the presence of other cations, particularly Ca\(^{2+}\) and Mg\(^{2+}\). Both Ca\(^{2+}\) and Mg\(^{2+}\) compete with K\(^+\) for uptake so that K\(^+\) uptake would be reduced as Ca\(^{2+}\) and Mg\(^{2+}\) are increased. In acid soils, Al\(^{3+}\) reduce K\(^+\) uptake, because toxic amounts of exchangeable Al\(^{3+}\) create an unfavorable root environment for nutrient uptake. When acid soils are limed, exchangeable Al\(^{3+}\) is converted to insoluble Al(OH)\(_3\). The reduction in exchangeable Al\(^{3+}\) reduces competition with K\(^+\), enabling K\(^+\) to compete with Ca\(^{2+}\) for vacant exchange sites. As a consequence, greater amounts of K\(^+\) can be adsorbed to CEC (cation exchange capacity) (Havlin et al., 2005).

**Shoot production of bangun-bangun**

Table 4 showed that fresh shoot weight plant\(^{-1}\), total fresh shoot weight plant\(^{-1}\), and shoot weight ha\(^{-1}\) of bangun-bangun was not affected by application of organic fertilizing (P >0.05). The
highest increase of shoot production in total fresh shoot weight plant\(^{-1}\) (71.04%) and shoot weight ha\(^{-1}\) (72.78%) was from cow manure + rock phosphate + rice-hull ash treatment compared with no fertilizing (P > 0.05). No fertilizing consistently produced the lowest fresh shoot weight plant\(^{-1}\), total fresh shoot weight plant\(^{-1}\), and fresh shoot weight ha\(^{-1}\). This showed that cow manure (N source), rock phosphate (P source), and rice-hull ash (K source) was required on shoot production of bangun-bangun.

Application of cow manure + rock phosphate + rice-hull ash produced the increasing in total fresh shoot weight plant\(^{-1}\) and shoot weight ha\(^{-1}\) higher than no fertilizing (Table 4), presumably caused by the nutrient availability, especially of macronutrients from organic fertilizing. Application of cow manure + rock phosphate + rice-hull ash produced soil and plant nutrient content higher than no fertilizing (Table 2). Nitrogen is one of macronutrients that plants require in greatest amounts. N has important role to the formation of proteins, N is an integral part of chlorophyll, which is the primary absorber of light energy needed for photosynthesis. The most essential function of P in plants is in energy storage and transfer. P is also an important structural component of nucleic acids, coenzymes, nucleotides, phosphoprotein, phospholipids, and sugar phosphates. K is important in osmotic pressure, charge balance, and involved in synthesis and transport of photosynthates to plant reproductive and storage organs (grains, fruits, and tubers) (Havlin et al., 2005). On other researches with different plant showed that N, P, and K nutrient increased plant height, fresh weight of leaf and number of leaf of Aloe indica (Hossain et al., 2007), onion (El-Bassiony, 2006; Ali et al., 2007), and waterleaf (Mualim et al., 2009).

### Shoot dry weight ha\(^{-1}\), total phenolic and anthocyanin production of Bangun-bangun

Table 5 showed that shoot dry weight ha\(^{-1}\) was affected by application of organic fertilizing (P <0.05). Cow manure + rock phosphate + rice-hull ash treatment produced shoot dry weight ha\(^{-1}\) higher than cow manure + rock phosphate treatment (P <0.05) but similar with no fertilizing and other organic fertilizer (P >0.05). Application of cow manure + rock phosphate produced the lowest shoot weight ha\(^{-1}\), total phenolic, and anthocyanin production. This presumably caused by nutrient slow release from organic fertilizer that related with acid soil in this experiment (Table 3). P immobile in acid soil and fixed by Fe/Al oxides so that presumably P was not available for plant.
Table 5. Shoot weight ha\(^{-1}\), total phenolic and anthocyanin production of bangun-bangun with organic fertilizing.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot weight (kg dry weight ha(^{-1}))</th>
<th>Total phenolic production (mg GAE kg(^{-1}) dry weight ha(^{-1}))</th>
<th>Anthocyanin production (µmol kg(^{-1}) dry weight ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fertilizing</td>
<td>50.17(^{ab}) ± 11.15</td>
<td>477.80(^{b}) ± 112.84</td>
<td>14.21(^{ab}) ± 3.81</td>
</tr>
<tr>
<td>Cow manure + rock phosphate</td>
<td>43.06(^{a}) ± 24.60</td>
<td>265.40(^{a}) ± 454.73</td>
<td>7.58(^{a}) ± 14.81</td>
</tr>
<tr>
<td>Cow manure + rice-hull ash</td>
<td>56.08(^{a}) ± 20.99</td>
<td>577.40(^{a}) ± 281.44</td>
<td>26.63(^{ab}) ± 5.71</td>
</tr>
<tr>
<td>Rock phosphate + rice-hull ash</td>
<td>63.02(^{a}) ± 28.86</td>
<td>514.70(^{a}) ± 260.08</td>
<td>32.15(^{a}) ± 5.97</td>
</tr>
<tr>
<td>Cow manure + rock phosphate +</td>
<td>78.93(^{a}) ± 25.03</td>
<td>535.40(^{a}) ± 331.54</td>
<td>20.14(^{a}) ± 6.84</td>
</tr>
<tr>
<td>rice-hull ash</td>
<td></td>
<td></td>
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</tbody>
</table>

Note: numbers in the same column followed by same letters were not significantly different at 5% Tukey’s HSD test (P > 0.05), ± SD of three replications, GAE = gallic acid equal.

Table 6. PAL activity, total phenolic, and anthocyanin content with organic fertilizing.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PAL activity (mg CAE g(^{-1}) protein)</th>
<th>Total phenolic (mg GAE g(^{-1}) dry weight)</th>
<th>Anthocyanin (µmol g(^{-1}) dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fertilizing</td>
<td>3.93(^{a}) ± 0.34</td>
<td>9.60(^{a}) ± 10.96</td>
<td>1.52(^{ab}) ± 0.77</td>
</tr>
<tr>
<td>Cow manure + rock phosphate</td>
<td>2.57(^{a}) ± 0.26</td>
<td>5.98(^{a}) ± 6.31</td>
<td>1.24(^{a}) ± 0.36</td>
</tr>
<tr>
<td>Cow manure + rice-hull ash</td>
<td>2.58(^{a}) ± 0.99</td>
<td>9.32(^{a}) ± 12.14</td>
<td>2.88(^{ab}) ± 0.87</td>
</tr>
<tr>
<td>Rock phosphate + rice-hull ash</td>
<td>3.03(^{a}) ± 0.73</td>
<td>7.86(^{a}) ± 9.77</td>
<td>4.32(^{a}) ± 1.60</td>
</tr>
<tr>
<td>Cow manure + rock phosphate + rice-hull</td>
<td>3.16(^{a}) ± 0.96</td>
<td>6.43(^{a}) ± 6.85</td>
<td>3.12(^{ab}) ± 0.77</td>
</tr>
<tr>
<td>ash</td>
<td></td>
<td></td>
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</tbody>
</table>

Note: numbers in the same column followed by same letters were not significantly different at 5% Tukey’s HSD test (P > 0.05), ± SD of three replications, CAE: cinnamic acid equal, GAE: gallic acid equal.

Total phenolic production of bangun-bangun was not affected by application of organic fertilizing (P >0.05) (Table 5). Application of cow manure + rock phosphate produced the lowest total phenolic production and similar with other treatments (P >0.05). Anthocyanin production was affected by application of organic fertilizing (P <0.05). Application of cow manure + rock phosphate had the lowest anthocyanin production, 46.66% lower than no fertilizing. Application of rock phosphate + rice-hull ash produced anthocyanin production higher than cow manure + rock phosphate (P <0.05) but similar with cow manure + rock phosphate + rice-hull ash (P >0.05).

**PAL activity, total phenolic, and anthocyanin content**

PAL activity and total phenolic was not affected by organic fertilizing (P >0.05). No fertilizing produced the highest PAL activity and total phenolic, but similar with application of cow manure + rock phosphate + rice-hull ash (Table 6). This presumably caused by low nutrient availability (N, P, and K). Anthocyanin content was affected by application of organic fertilizing (P <0.05). Application of cow manure + rock phosphate produced the lowest anthocyanin content, 18.42% lower than no fertilizing, and similar with application of cow manure + rock phosphate + rice-hull ash.

**Effect of rain intensity to Bangun-bangun metabolite compounds**

The differences of rain intensity in each time of harvesting caused the increasing of PAL activity, total phenolic, and anthocyanin content (Figure 1). The highest rain intensity occurred at the first harvest (8 WAP) that caused the increasing of humidity (Figure 1a) and then decreased at the second harvest (12 WAP). The decreased of rain intensity caused PAL activity (Figure 1b), total phenolic (Figure 1c), and anthocyanin content (Figure 1d) increased at 12
WAP, 15.90, 42.19, and 115.00%, respectively. High rain intensity at 8 WAP caused low light intensity so that it increased of PAL activity. The increasing of PAL activity followed by the increasing of total phenolic and anthocyanin content.

Mualim (2012) stated that phenolic compounds was produced by phenylpropanoid pathway with aromatic amino acid as precursor from shikimic acid pathway. Phenylalanine is one of aromatic amino acid and as precursor phenolic compound biosynthesis. Phenylalanine ammonia lyase is the first enzyme of phenylpropanoid biosynthesis that produced phenolic compounds (Reichart et al., 2009). The increasing of PAL activity showed that biosynthesis of phenolic compound also increased. Relation between the increasing of PAL activity with phenolic compound was showed in many research such as on Olea europaea (Garcia et al., 2009; Mualim et al., 2012), and corn (Gholizadeh, 2011).

CONCLUSION

Application of organic fertilizer increased of shoot production. Application of 12.3 t ha\(^{-1}\) cow manure + 1.5 t ha\(^{-1}\) rock phosphate + 5.5 t ha\(^{-1}\) rice-hull ash produced shoot dry weight ha\(^{-1}\) (57.33%) and metabolite production ha\(^{-1}\) (total phenolic 12.06%, anthocyanin 41.73%) higher than no fertilizing (P >0.05). Application of cow manure + rock phosphate produced the lowest shoot dry weight ha\(^{-1}\) and metabolite production ha\(^{-1}\). The result of this research suggested that nitrogen, phosphorus, and potassium were needed on shoot production of bangun-bangun.

Figure 1. (a) The histogram rain intensity with metabolite content of Bangun-bangun at the first (8 WAP) and second (12 WAP) harvesting, PAL activity (b), total phenolic (c), and anthocyanin content (d) of Bangun-bangun at 8 and 12 WAP; CAE: cinnamic acid equal, GAE: gallic acid equal, WAP: week after planting. Vertical line above histogram showed 95% confidence level.

Gambar 1. (a) Histogram intensitas curah hujan dengan kandungan metabolit pucuk bangun-bangun pada pemanenan 8 dan 12 MST, kandungan aktivitas PAL (b), total fenolik (c), dan antosianin (d) pucuk bangun-bangun pada pemanenan 8 dan 12 MST; MST: minggu setelah tanam; SAS: setara asam sinamat, SAG: setara asam galat, BK: bobot kering, PAL: phenylalanine ammonia-lyase, garis vertical di atas tiap balok data menunjukkan selang kepercayaan 95%).

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ACKNOWLEDGMENTS

This research was funded in part by Tropical Plant Curriculum Project SEAFAST Centre Bogor Agricultural University.

REFERENCES


