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Shoot Production and Metabolite Content of Waterleaf with Organic Fertilizer

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ABSTRACT

Waterleaf shoot (Talinum triangulare (Jacq.) Willd) is consumed as vegetable that contains some metabolites. The purpose of this research was to investigate the effect of organic fertilizer on shoot production and its metabolites i.e. protein, vitamin C, flavonoid, and peroxidase enzyme (POD) activity at 10, 12, and 14 weeks after planting. This experiment was conducted at Leuwikopo Experimental Station, Bogor, Indonesia from November 2012-February 2013. The organic fertilizers applied were cow manure (CM) (12.3 ton ha^{-1}), rock phosphate (RP) (1.5 ton ha^{-1}), and rice-hull ash (RH) (5.5 ton ha^{-1}). These organic fertilizers were combined into four treatments by using minus one test method and one control. Each treatment was repeated three times. The result showed that combination of organic fertilizer had the same effects on shoot production and metabolite content of waterleaf. It showed that the amount of organic fertilizers was not significantly sufficient to contribute nutrients to the plant.

Keywords: flavonoid, POD activity, repeated harvesting, Talinum triangulare, vitamin C

ABSTRAK

Kolesom (Talinum triangulare (Jacq.) Willd) dapat dikonsumsi pucuknya sebagai sayuran. Kolesom merupakan sayuran fungsional yang memiliki senyawa metabolit di daunnya. Tujuan penelitian ini adalah untuk mempelajari pengaruh pemupukan organik terhadap produksi pucuk kolesom (Talinum triangulare (Jacq.) Willd) dan senyawa metabolitnya seperti vitamin C, flavonoid, dan aktivitas enzim peroksidase (POD) dengan pemanenan berulang pada umur 10, 12, dan 14 minggu setelah tanam. Percobaan dilaksanakan di Kebun Percobaan Leuwikopo, Bogor, Indonesia pada bulan November 2012-Februari 2013. Pupuk organik yang digunakan adalah pupuk kandang sapi (PK) (12.3 ton ha^{-1}), rock phosphate (RP) (1.5 ton ha^{-1}), dan abu sekam (AS) (5.5 ton ha^{-1}). Pupuk organik kemudian dikombinasikan dalam lima perlakuan dengan metode minus one test dan satu perlakuan kontrol. Setiap perlakuan diulang tiga kali. Kombinasi pupuk organik tidak memberikan pengaruh nyata terhadap produksi dan kadar metabolit pucuk kolesom. Hal tersebut menunjukkan bahwa penambahan pupuk organik pada dosis tersebut belum dapat menyumbangkan hara yang cukup signifikan pada tanaman kolesom.

Kata kunci: aktivitas POD, flavonoid, pemanenan berulang, Talinum triangulare, vitamin C

INTRODUCTION

Waterleaf (*Talinum triangulare* (Jacq.) Willd) is a perennial plant that is known as medicinal plant. Waterleaf shoot can be consumed as vegetable. Waterleaf shoot can be harvested in 15 days interval (Susanti *et al*., 2011). The content of protein, fat, carbohydrate, fiber, and energy of waterleaf is 5.1, 1.33, 1.05, 8%, and 36.6 Kcal 100 g^{-1} DW, respectively (Kwenin *et al*., 2011). Based on dry weight, waterleaf contains some minerals such as Ca (2.44 mg 100 g^{-1}), K (6.10 mg 100 g^{-1}), Mg (2.22 mg 100 g^{-1}), Na (0.28 mg 100 g^{-1}), and Fe (0.43 mg 100 g^{-1}) (Mensah *et al*., 2008) and bioactive compound such as anthocyanin, alkaloid, flavonoid, saponin, and tannin (Mensah *et al*., 2008; Susanti *et al*., 2008; Mualim *et al*., 2009; Aja *et al*., 2010; Andarwulan *et al*., 2010). Furthermore waterleaf tuber has antioxidant activity (Estiasih and Kurniawan, 2007).

Waterleaf contains some primary and secondary metabolites. Vitamin C is the primary metabolite while flavonoid and lignin are secondary metabolites. Flavonoid is used by plants to protect them from UV rays (Taiz and Zeiger, 2002). In human, flavonoid functions as an antioxidant which is useful to prevent cancer (Ren *et al*., 2003). Lignin biosynthesis is affected by peroxidase (POD) activity (Boerjan *et al*., 2003). Lignin is relatively indigestible (Taiz and Zeiger, 2002) and therefore considered as dietary fiber (Muchtadi, 2001).

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Organic fertilizer improves soil structure and provides some macro and micronutrient for plants. Vegetable cultivation with organic fertilizer produces lower content of nitrates, higher content of phenolic compounds and vitamin C (Rembialkowska, 2007). Organic fertilizer also increases vitamin C content of waterleaf in wet season (Mualim, 2012). Cow manure and rice-hull ash are commonly used organic fertilizer, while rock phosphate is a natural sources of phosphorus. Cow manure is a good source of nitrogen (Havlin *et al*., 2005), and rice-hull ash is a source of potassium (Hadi, 2005).

Mualim (2012) analyzed the content of vitamin C, flavonoid, POD activity, and shoot production of waterleaf at 2, 4, and 6 weeks after planting (WAP), but no information regarding those metabolites content during the later stages. Therefore this research was aimed to investigate vitamin C, flavonoid, and POD activity content on shoot harvested at 10, 12, and 14 weeks after planting. The purpose of this research was to study the effect of various combination of organic fertilizer on shoot production content of vitamin C, flavonoid, and POD activity with repeated harvesting.

MATERIALS AND METHODS

This research was conducted at Leuwikopo research station, Department of Agronomy and Horticulture, Bogor, Indonesia from November 2012 to February 2013. The analysis of vitamin C, flavonoid and POD activity were conducted at Plant Analysis and Chromatography Laboratory, Department of Agronomy and Horticulture, IPB. Three kinds of organic fertilizers were used in this research, i.e. cow manure (12.3 ton ha^{-1}), rock phosphate (1.5 ton ha^{-1}), and rice-hull ash (5.5 ton ha^{-1}). The organic fertilizers were combined into four combinations and one control (without fertilizer application) (Table 1). The experiment was arranged in a randomized block design with three replications. Data were analyzed using ANOVA at 95% and *post-hoc* analysis using tukey test (α = 5%).

Five treatments of organic fertilizer applied two weeks before planting. Waterleaf stem cuttings were planted with spacing at 50 cm x 50 cm. Waterleaf was harvested at 10 weeks after planting (WAP) (first harvest), 12 WAP (second harvest), and 14 WAP (third harvest). Watering was not done during the experiment and only dependent on rainfall. Therefore data of rainfall intensity from Meteorology, Climatology, and Geophysics station (BMKG) Bogor was needed.

Fresh and dry weight of shoot per plant, fresh weight per shoot, and number of shoot were measured. Contents of vitamin C, flavonoid and POD activity were also analyzed in each harvesting time. Nitrogen, phosphorus, and potasium contents of the shoot were also observed in the third harvest.

Vitamin C content was determined using modified titrimetry method (Mualim *et al*., 2012). Five grams of shoot leaves were ground in 30 mL buffer (acetic acid (0.035 mol L^{-1}), sodium acetate (9.77x10-3 mol L^{-1}), EDTA 6.45x10-4 mol L^{-1}), and 5 g $CaCO_3$, and was centrifuged. Then, 10 mL supernatant was added with 1 mL amylum 1% and titrated with iodine.

POD activity was analysed followed Dangcham *et al*. (2008). Shoot leaves (0.5 g) were ground with 2.5 ml buffer extract (100 mmol L^{-1} Tris-HCl, pH 7.5, 1 mmol L^{-1} EDTA, 5 mmol L^{-1} $MgCl_2$, 0.05% Triton X-100, 2.5 mmol L^{-1} dithiothreitol). The mixture was centrifuged with 4,500 x g (10') and 25,000 x g (15'). Supernatant (0.1 mL) was added with 1.85 mL buffer extract, 0.85 mL aminoantipyrine, 0.5 mL phenol, and 0.02 mL H_2O_2. POD activity was determined using Shimadzu UV-1800 spectrophotometer in 512 nm for 4.5 minutes.

Flavonoid was determined using modified aluminium chloride colorimetric method (Mualim *et al*., 2012). Waterleaf shoot was freeze dried (Flexy-DryTM MP, USA) at -50 ºC. Dry leaves was ground and leaves powder (0.1 g) was extracted in 5 ml methanol, incubated in 60 ºC (60'), centrifuged at 4 500 x g (10') to get supernatant. Supernatant (0.1 mL) was added with 0.1 mL aluminium chloride (10%), potassium acetate (1 M) and 2.8 mL of water and incubated in 25 ºC (30'). The absorbance was measured by using Shimadzu UV-1800 spectrophotometer in 415 nm wavelength.

Nitrogen, phosphorus, potassium, C-organic, and soil pH were analyzed using method of Balittanah (2005). Content of N, P, K, C-organic in soil, and soil pH were analyzed before and after planting whereas N, P, K, and C-organic content of waterleaf shoot were analyzed at 14 week after planting. Soil and shoot sample were pooled from three replications. Therefore the data were not analyzed statistically.

Table 1. Organic fertilizer treatment combinations

Treatment	Rates (ton ha^{-1})			Nutrient addition (ton ha^{-1})		
	Cow manure[1]	Rock Phosphate[2]	Rice-hull ash[3]	N	P_2O_5	K_2O
Control	0	0	0	0	0	0
CM + RP	12.3	1.5	0.0	0.16	0.07	0.04
CM + RH	12.3	0.0	5.5	0.17	0.04	0.10
RP + RH	0.0	1.5	5.5	0.01	0.05	0.06
CM + RP +RH	12.3	1.5	5.5	0.17	0.08	0.10

Notes: [1]N content 1.29%, [2]P_2O_5 content 2.87%, and [3]K_2O content 1.10% (analysis from Soil Science and Land Resources Management, Bogor Agricultural University)

RESULT AND DISCUSSION

Nitrogen, Phosphorus, and Potasium Content in Soil and Waterleaf Shoot

Nitrogen and potassium content in soil before organic fertilizer application were in medium status, however content of phosporus was very low (Table 2). The low content of P was caused by low soil pH. Phosporus was precipitated into Fe/Al-P in low soil pH (Havlin *et al*., 2005).

Nitrogen was needed by plant for vegetative growth (Havlin *et al*., 2005). shoot harvesting maintained waterleaf plants at vegetative stage (Susanti, 2012). Nitrogen content in soil after planting was low. It showed that source of N may come from soil than organic fertilizer. Nutrient from organic fertilizer have to be mineralized in order to be available to plant, but all C/N ratio value from the treatment before and after fertilization was below 11 (Table 3). Low C/N ratio slows down the release of available nutrients.

The content of K in shoot by organic fertilizing was lower than control (Table 2). It was possible that K absorbtion by plant was inhibited by N absorbtion. The absorbtion of N presumably was in NH_4+ form from mineralization than NO_3-. Ammonium absorbtion by root inhibits K^+ absorbtion.

The largest source of P in this experiment probably was cow manure based on P content in waterleaf shoot. Although rock phosphate was a source of P but it was not available, so it was assumed that P came from cow manure. Research conducted by Garg and Bahl (2008) showed that application of poultry manure increased P availability to plant by increasing phosphatase activity.

Shoot Production and Metabolite Content of Waterleaf with Combination of Organic Fertilizer Application

Total dry weight of shoot, vitamin C content, flavonoid content, and POD activity was not affected by organic fertilizing (Table 4). It may caused by low nutrient availability from organic fertilizer and the slow released of nutrient. Waterleaf shoot production in this experiment was much lower than previous research conducted by Mualim (2012). Fresh waterleaf shoot production in dry season and wet season were about 250 g $plant^{-1}$ and 200 g $plant^{-1}$, respectively (Mualim, 2012).

Shoot fresh weight was not affected by organic fertilizer (Table 5). There was no significant fluctuation on shoot production from harvest to harvest, but the number of shoot increased (9.2, 11.4, and 16.7) and weight per shoot decreased (5.28, 4.03, and 2.90 g $shoot^{-1}$) (Figure 1) due to smaller shoot in the third harvest. Shoot harvesting induced growth of new smaller branches. Research by Sumpena (2008) in spinach showed that stem pruning increased plant height and lateral branches.

Table 2. N, P, K content in soil and waterleaf shoot

Treatment	N			P			K		
	Before	After	Plant	Before	After	Plant	Before	After	Plant
	...........%.............			.......ppm......		(%)	.........ppm......		(%)
Control	0.21	0.16	1.34	3.80	517.1	0.32	119.52	63.96	5.15
CM + RP	0.22	0.17	1.90	19.05	280.6	0.36	139.52	93.48	3.26
CM + RH	0.22	0.14	1.45	13.4	492.3	0.35	169.52	71.34	4.25
RP + RH	0.21	0.17	1.83	15.6	495.9	0.32	149.52	76.26	3.43
CM + RP +RH	0.22	0.17	1.39	22.15	513.5	0.38	169.52	95.94	3.53

Notes: CM = cow manure; RP = rock phosphate; RH = rice-hull ash; bef = soil analysis before planting; aft = soil analysis after planting; plant = analysis of shoot nutrient; P and K analysis before planting used Bray method to measure available P and K in soil. P and K analysis after planting analyzed by HCl method to measure total P and K in soil

Table 3. Soil pH, C-organic content, and C/N of soil

Treatment	Soil pH		C-org (%)			C/N	
	Before	After	Before	After	Shoot	Before	After
Control	5.6	5.5	2.2	1.5	49.6	10.2	9.4
CM + RP	5.6	5.5	2.2	1.6	51.2	10.2	9.4
CM + RH	5.6	5.9	2.2	1.4	49.7	10.2	9.6
RP + RH	5.6	5.4	2.2	1.6	50.5	10.2	9.4
CM + RP +RH	5.6	5.7	2.2	1.6	51.1	10.2	9.4

Notes: CM = cow manure; RP = rock phosphate; RH = rice-hull ash; before = organic fertilizer application; after = after planting; shoot = content in shoot

Vitamin C was positively correlated to flavonoid content (r = 0.88, P<0.05) but negatively correlated to K (r = -0.96, P<0.01), while flavonoid was negatively correlated to K (r = -0.96, P<0.05). Nitrogen is needed by plants to increase photosynthetic rate that caused increasing of photosynthate (Havlin *et al*., 2005). Photosynthate such as glucose and erythtrose was used to synthesize vitamin C and flavonoid (Valpuesta and Botella, 2004; Vogt, 2010).

Table 4. Shoot production and metabolite content of waterleaf

Treatment	Total shoot production (g dw plant^{-1})	Vitamin C (mg g^{-1} dw)	Flavonoid (mg QEg^{-1}dw)	POD activity (x10^{-3} U mg^{-1} protein^{-1})
Control	9.19±0.82	40.03±7.69	8.69±1.91	4.81±7.69
CM + RP	9.42±1.29	46.32±5.01	10.02±1.32	2.71±5.01
CM + RH	8.55±1.29	42.29±4.74	9.13±1.6	4.24±4.74
RP + RH	9.55±0.94	47.39±6.22	9.60±1.19	4.76±6.22
CM + RP +RH	9.28±0.29	46.69±4.07	9.50±1.25	3.65±4.07

Notes: Number followed by same letter was not significantly different based on tukey test at 5%, CM = cow manure; RP = rock phosphate; RH = rice-hull ash; dw = dry weight; QE = quercetin equivalent; U = unit; data were followed by ± standard error

Table 5. Shoot production of waterleaf with organic fertilizers and repeated harvesting

Treatment	Harvesting time (week after planting)			Total shoot production
	10	12	14	
	.... g fw plant^{-1}			
Control	43.92±4.36	35.61±5.58	51.12±7.16	130.64±11.04
CM + RP	41.88±1.62	47.11±7.76	48.27±17.58	137.26±20.41
CM + RH	39.97±4.60	58.78±6.58	44.65±2.69	143.41±7.66
RP + RH	57.13±0.67	48.73±13.17	57.02±3.13	162.88±11.60
CM + RP +RH	53.15±5.40	40.12±4.69	41.53±8.16	134.80±5.86

Notes: Number followed by same letter was not significantly different based on tukey test at 5%, CM = cow manure; RP = rock phosphate; RH = rice-hull ash; fw = fresh weight; data were followed by ± standard error

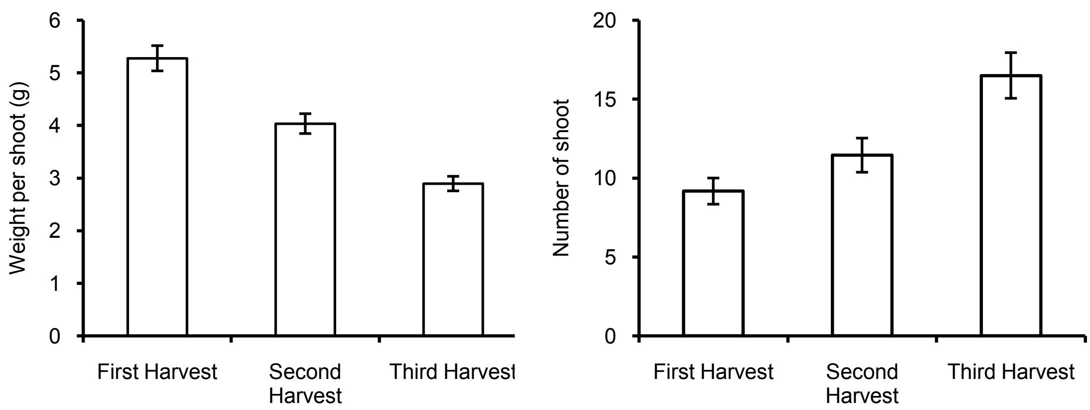

Figure 1. Weight per shoot and number of shoot at three times of harvesting

CONCLUSION

Shoot production of waterleaf in three times of harvesting were not affected by organic fertilizers. The same conditions also occurred on content of vitamin C, flavonoid, and POD activities. It may caused by the nutrient from organic fertilizer released slowly and it has not been able to contribute nutrient significantly to the plant.

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