The Effects of NPK fertilizer, Manure and Vesicular Arbuscular Mycorrhiza (VAM) on the Growth, Biomass and Artemisinin Content of *Artemisia annua* L.
(Pengaruh pupuk NPK, Pupuk Kandang dan Mikoriza Vesikular Arbuskula (MVA) pada Pertumbuhan, Biomassa dan Kadar Artemisinin pada *Artemisia annua* L.)

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ABSTRACT

Most artemisinin is extracted from an annual herb *Artemisia annua* L. but the cultivation in Indonesia is limited by the low yield of the artemisin incontent that is not economically beneficial to industry. Improvement on cultivated varieties and cultivation techniques is therefore needed. This work aims to improve the cultivation techniques by evaluating the application effects of NPK, manure, and VAM on the artemisinin yield of *A. annua*. The experiment was set using a Split Split Plot Design involving three factors. First factor was the application of four dosages of NPK (0 kg ha^{-1} as a control; 40:40:40 kg ha^{-1}; 80:80:80 kg ha^{-1}; and 120:120:120 kg ha^{-1}). Second factor was manure addition (using 0 ton ha^{-1} and 150 tons ha^{-1}). Third factor wasthe application of mycorrhiza (0 g plant^{-1}and 15 g plant^{-1}). The experiment was divided into three different groups based on the seed sources (Bandung, Cibodas, and Tawangmangu plants) using similar treatments. The results showed that the plant growth (Relative Growth Rates/RGR of plant heights and number of branches), leaf yield, and total plant biomass were much affected by NPK. The addition of manure has less significant effect on those parameters. Only VAM seems to influence the artemisinin content. The highest artemisinin yield (5 kg ha^{-1}) was relatively low when compared to the production in USA, China, and Brazil. However the result shows that a low fertilizer input of 40:40:40 kg NPK ha^{-1} and an inoculation of VAM were recommended for cultivating *A. annua* resulting a significantly high yield of artemisinin.

Keywords: *Artemisia annua* L., Artemisinin content, NPK, Manure, VAM, Plant Growth, Biomass

ABSTRAK

Sebagian besar artemisinin diekstrak dari tanaman semusim *Artemisia annua* L. Budidaya tanaman tersebut di Indonesia is dibatasi oleh hasil artemisinin yang rendah dan tidak ekonomis bagi industri pengolahan. Perbaikan varietas tanaman dan teknik budidaya karenanya diperlukan. Tulisan ini bertujuan untuk meningkatkan teknik budidaya dengan mengevaluasi pengaruh pemberian NPK, pupuk kandang, dan MVA terhadap hasil artemisinin dari *A.annua*. Percobaan menggunakan rancangan petak petak terbagi dengan tiga faktor. Faktor pertama yaitu dosis pupuk NPK (kontrol, 40:40:40 kg ha^{-1}; 80:80:80 kg ha^{-1}, dan 120:120:120 kg ha^{-1}). Faktor kedua yaitu penambahan pupuk kandang (tanpa pupuk kandang dan 150 ton ha^{-1}). Faktor ketiga yaitu penambahan mikoriza (0 g tanaman^{-1} dan 15 g tanaman^{-1}). Penelitian ini dibagi menjadi tiga kelompok yang berbeda berdasarkan pada sumber benih (Bandung, Cibodas, dan Tawangmangu). Hasil penelitian menunjukkan bahwa pertumbuhan tanaman (Laju pertumbuhan relatif tinggi tanaman dan jumlah cabang), bobot daun, dan biomassa total tanaman lebih banyak dipengaruhi oleh NPK. Penambahan pupuk kandang tidak berpengaruh signifikan pada parameter tersebut. Aplikasi MVA cenderung mempengaruhi hasil artemisinin. Hasil artemisinin tertinggi (5 kg ha^{-1}) relatif rendah bila dibandingkan dengan produksi di Amerika Serikat, Cina, dan Brasil. Namun hasil pengamatan menunjukkan bahwa input pupuk rendah 40:40:40 kg NPK ha^{-1} dan inokulasi MVA dapat direkomendasikan untuk efisiensi budidaya *A. annua* yang menghasilkan artemisinin yang cukup tinggi.

Kata Kunci: *Artemisia annua* L., kadar Artemisinin, NPK, Pupuk Kandang, MVA, Pertumbuhan Tanaman, Biomassa

INTRODUCTION

Indonesia is one of the endemic areas for malaria. It is also known as one of the highest populated countries with high risk to be infected by *Plasmodium falciparum* (Andersen *et al*., 2011). This infection causes high mortality, particularly on infant (WHO, 2008). A figure of 1.5-2.7 million people died each year because of the infection (WHO 2000). Since 2006, WHO (2006) recommended an Artemisinin-based Combination Theraphy (ACT) as an effective strategy to combat malaria.

Artemisinin is one of chemical compounds occurring on *Artemisia annua* L. This plant is native to China and its adjacent areas and is known as a source of artemisinin and its derivats. This species contains approximately 0.1 to 0.8 % artemisinin (Ferreira *et al.* 2005). Although this plant originally occurs on subtropical climate, some cultivation experiments have shown that it also grows on tropical areas. Gusmaini & Nurhayati (2007) reviewed that *A. annua* possesses a high potency to be cultivated in Indonesia. However the cultivation constraints faced in this country are the availability of cultivars producing high artemisinin contents and the efficient cultivation techniques. It is because of artemisinin content is influenced by many factors such as climate, harvesting stage, genetic variation, and agronomic practice (Laughlin *et al.* 2002; Willcox *et al.* 2004; Ferreira *et al*. 2005).

One of the limiting factors on plant growth is mineral nutrient, especially Nitrogen (N) and Phosphorus (P) (Marschner 1995; Lambers *et al.* 2008). There are some reports on the role of the addition of NPK on artemisinin yield of *A. annua* (Laughlin *et al.* 2002; Ferreira *et al.* 2005; Özgüven*et al.* 2008; Davies *et al.* 2009; and Aftab *et al.* 2011). Those reports have shows the effect of nutrient addition on the enhancement of artemisinin yield via optimum biomass production. The nutrients addition are also could comes from organic sources such as farm yard manure or compost. The addition of organic fertilizer has been reported have positive effect on alleviation of volatiles organic compound (Malik *et al.* 2012), leaves protein content (Keshavarzi & Nik 2011), and artemisinin yield (Jha *et al.* 2011, Yeboah *et al.* 2012) compare to the control plant (without fertilizer) of *A. annua*. Hartatik & Widowati (2006), stated that manure only contain small amount of plant nutrient. Therefore, the aplication of manure aimed to improve biological and physical properties of soil than to supply plant nutrient. The application cost of organic fertilizer will be much higher than inorganic fertilizer. For example, to get similar effect on artemisinin yield, the plant need to add with 40 ton/ha of manure compare to 90 kg/ha of NPK (Yeboah *et al.* 2012). Combination application of organic and inorganic fertilizer would be more effective.

Plant nutritional status can also be improve by enhance nutrition uptake by associated soil microbes. One of the most widely known of soil microbes is Vesicular Arbuscular Mycorhiza (VAM). It is enhance nutrient uptake via enlarging root surface area (Koide 1991). Recently, Zeng *et al.* (2013), reviewed that arbuscular mycorrhiza affect secondary metabolites and production of active ingredient on medicinal plant. Some reports on the application of mycorrhiza on *A. annua* cultivation have shows that it has potencies to increase biomass and artemisinin yield (Kapoor *et al.* 2007; Chaudary *et al.* 2008). However, there are some evidence that different arbuscular mycorrizhal source have different effect on secondary metabolite of different plant species or cultivar and *vice versa* (Zeng *et al.* 2013). Chaudary *et al.* (2008) have showed that *Glomus macrocapum* was more effective than *G. fasciculatum* on artemisinin yield of two plant accession of *A. annua* but not on the other accession. This paper evaluates the effects of NPK, manure, and VAM aplication on the growth and artemisinin content of *A. annua*. There were some reports presenting the effects of N fertilizer, manure, and VAM applications on the artemisinin yield of *A. annua*. However these reports only showed the influences of single factors and none of those inform the interaction effects among these three factors. For example, Ferreira (2005), Özgüven *et al.* (2008), and Davies *et al.* (2009) only informed the effects of different levels of N fertiliser application on artemisinin yields. Jha *et al.* (2011) evaluated the effects of NPK fertiliser and manure on the *A. annua* biomass and artemisinin yields but not examined the interaction effects among the three factors. Chaudary *et al.* (2008) only compared the effects of P-added VAM to those of no P-added VAM on the growth and yield of *A. annua*. Later has been known that P fertilizer has no a significant effect on the artemisinin content of *A. annua* (Davies *et al.* 2011). This paper presents the interaction effects among NPK, manure, and FMA concentrations on some growth parameters, leaf and biomass yields, artemisinin content, and artemisinin yield.

MATERIAL AND METHODS

Seeds of *A. annua* were taken from three different sites: Cibodas Botanic Garden (Cibodas), Research and Development Center of Medicinal Plants and Traditional Medicine (Tawangmangu), and Research Station of Medicinal Plant of Kimia Farma (Bandung). Seeds were sowing in three different times. Seeds of Cibodas were sown on 14th March 2012, while seeds from Tawangmangu and Bandung were sown on 26thMarch 2012 and 10th April 2012, respectivelly.

All non sterilized seeds were sown on sterilized compost. One month after sowing, seedlings were transplanted into individual plastic pots. Seedlings were divided into two groups. The first group was added by mycorrhiza, while the other one was without it. The mycorrhiza inoculant (*Miza Plus* produced by IBRIEC) contain spore of *Acaulospora tuberculata* and some of non symbotic soil bacteria (N fixing bacteria, *Pseudomonas* sp., and *Serratia marcescens*). The inoculant has mycorrhizal spore density about 10^5 per gram. After two weeks within individual pots, seedlings were transplanted into 40 kg plastic bags of soil on the field. The growing medium was soil added by manure of 15 tons ha^{-1}. The manure is horse dung. Based on Hartatik & Widowati (2006), horse dung contain N, P, and K at 0.5; 0.25; and 0.3 %, respectivelly. Plant densities were 40,000 individuals ha^{-1}.

The experiments were set using a Split Split Plot Design with three factors. The first factor was the application of four different dosages of NPK (0 $kgha^{-1}$; 40:40:40 $kgha^{-1}$; 80:80:80 $kgha^{-1}$; and 120:120:120 $kgha^{-1}$). The second factor was manure addition (0 tons $ha^{-1}$and 150 tons ha^{-1}). The third factor was mycorrhiza applications (0 gram $plant^{-1}$and 15 gram $plant^{-1}$). The experiments were separated into three different groups based on their seed sources but with the same treatment.

Plant height (cm) and numbers of branches were measured monthly. Based on these data, Relative Growth Rates (RGR) *i.e.* plant heights and numbers of branches were counted. RGR were counted as (final plant size – initial plant size)/time interval. Time interval is the interval between planting time and flowering time. The time interval is three months for plant population from Bandung and Cibodas and two months for those from Tawangmangu. Plant biomass (wet and dry weights of leaves, twigs, and roots) were weighted after harvest. Harvesting time is before the plant start to flowering at around 3-4 months after planting.

Artemisinin content were analysed based on dried leaves from three month old plants. Leaves were dried on 40°C for 48 hours prior to extraction. Maseration technique of methanol for 24 hour/ sample was used for extraction. The extracts were filtrated and dried under vacum condition prior to weighing. Organic solvents; n-hexane, ethyl acetate, and methanol were used for partitioning and dried before being analyzed by HPLC.

Analysis of artemisinin content was conducted using Shimadzu HPLC coupled with UV-detector at 214 nm. Isocratic condition of 1 ml/ min of acetonitril 60% into reverse phase C-18 coloumn. Artemisinin content were quantified using calibration curve of artemisinin standard at a concentration of 125, 250, 500, and 1000 ppm, respectively.

RESULTS

Generally *A. annua* was responsive to fertilizer applications. Most of the growth parameters were more affected by the addition of NPK than manure or VAM addition (Table 1). The interaction effects of all treatments (NPK x Manure x VAM) were not significant for the most growth parameters. However, the interaction effects between two treatments significantly affected the RGR plant height and RGR plant branching, but not to plant biomass.

The mean of the relative growth rates (RGR) of *A. annua* plant heights ranged from 38 to 71 cm/month (Bandung), from 37 to 64 cm/month (Cibodas), and from 38 to 81 cm/month (Tawangmangu) (Figure 1). The highest plant height recorded was 320 cm found in the plant population from Bandung with the treatment of NPK of 120:120:120 $kgha^{-1}$, without manure and also without VAM addition. The lowest plant height was 12 cm that was found from Tawangmangu plants with a treatment of without NPK, manure, and VAM addition. Plant heights from Cibodas ranged from 43 to 240 cm at 3 months after planting. Plant population from Tawangmangu ranged from 12 to 213 cm in height at 2 months after planting, while plant population from Bandung ranged from 90 to 320 cm at 3 months after planting. Most of Tawangmangu's plants were harvested at two months after planting.

RGR of plant heights were signifanctly affected by the interaction between NPK and manure addition for all of plant accessions (Table 1). The interaction effects between NPK and VAM were only shown by plants from Tawangmangu. In contrast, the interaction effect between manure and VAM showed a weak influence to the RGR (i.e. plant height) of plants from Bandung and Tawangmangu.

The interaction effects between NPK and manure addition to RGR of plant height were shown on Table 2, showing that the highest RGR (height) was contributed by the NPK (120:120:120) and manure treatment, except on plants from Tawangmangu. The highest RGR (height) on Tawangmangu plants was from the NPK application (80:80:80) without manure addition. Below the NPK dosage of 80:80:80 $kgha^{-1}$, manure addition increased plant heights.

Mean of the relative growth rates of number of branches ranged from 10 to 18 branches/month (Bandung), 12-20 branches/month (Cibodas), and 2.5-21 branches/month (Tawangmangu) (Figure 2). The highest number of branches was 65 found in plant population from Bandung using the treatment of NPK of 120:120:120 kg ha^{-1}, without manure and without VAM addition. The lowest number of branches was found in plant from Tawangmangu using the treatment without NPK, manure, and VAM addition. The number of branches of plant from Cibodas ranged from 12 to 59 at 3 months after planting. Number of branches of plant population from Tawangmangu ranged from5 to 51 at 2 months after planting, while that of plant population from Bandung ranged from 10 to 65 at 3 months after planting.

The interaction effects between NPK and manure addition to RGR of number of branches were shown on Table 3, indicating that the RGR of number of branches was similar when the NPK fertilizer was added. The RGR of number of branches was different significantly between plants without NPK and manure and plants without NPK but with manure.

Biomass of *A. annua* was affected mostly by the application of NPK fertiliser. For some parameter, however, the interaction effect was significant although it can not be applied to all plant accessions. To simplify the result interpretation, the interaction effect was neglected. The effect of NPK application on plant biomass were shown on Table 4.

Table 1. Analysis of variance of the effect of NPK (N), Manure (M), and Vascular Arbuscular Mycorrhiza (VAM) on RGR of Plant Height (cm/month), RGR of Number of Branches (number of branch/month), and Biomass (gram/plant) of *Artemisia annua* L. ***= significant at $p<0.001$; **=significant at $p<0.01$; *=significant at $p<0.05$; and NS=Not significant.

Seeds Source	Growth and YieldParameter	Effect						
		NPK Dosage	Manure	VAM	Interaction			
		(N)	(M)	(V)	(NxM)	(NxV)	(MxV)	(NxMxV)
Bandung	RGR Plant Height	***	***	NS	**	NS	*	NS
	RGR Number of Branches	***	***	NS	**	NS	**	NS
	Wet Weight							
	Leaves	***	**	NS	NS	NS	NS	NS
	Flower	*	NS	NS	NS	NS	NS	NS
	Trunk	***	*	NS	NS	NS	NS	NS
	Roots	***	NS	NS	NS	NS	NS	NS
	Shoots	***	*	NS	NS	NS	NS	NS
	Total	***	*	NS	NS	NS	NS	NS
	Dry Weight							
	Leaves	***	*	NS	NS	NS	NS	NS
	Flower	*	NS	NS	NS	NS	NS	NS
	Trunk	***	NS	NS	NS	NS	NS	NS
	Roots	***	NS	NS	NS	NS	*	NS
	Shoots	***	NS	NS	NS	NS	NS	NS
	Total	***	NS	NS	NS	NS	NS	NS
Cibodas	RGR Plant Height	***	***	NS	***	NS	NS	NS
	RGR Number of Branches	***	***	NS	***	NS	NS	*
	Wet Weight							
	Leaves	NS	NS	NS	***	NS	NS	NS
	Flower	**	NS	NS	NS	NS	NS	NS
	Trunk	*	NS	NS	**	NS	*	NS
	Roots	NS	NS	NS	NS	NS	NS	NS
	Shoots	**	NS	NS	*	NS	NS	NS
	Total	**	NS	NS	*	NS	NS	NS
	Dry Weight							
	Leaves	NS	NS	NS	**	NS	*	NS
	Flower	**	NS	NS	NS	NS	NS	NS
	Trunk	*	NS	NS	*	NS	**	NS
	Roots	***	NS	NS	**	NS	NS	NS
	Shoots	**	NS	NS	**	NS	**	NS
	Total	**	NS	NS	**	NS	**	NS
Tawangmangu	RGR Plant Height	***	***	NS	***	***	*	NS
	RGR Number of Branches	***	***	*	***	*	***	NS
	Wet Weight							
	Leaves	*	*	NS	NS	NS	NS	NS
	Flower	**	NS	NS	NS	NS	NS	NS
	Trunk	***	*	NS	NS	NS	NS	NS
	Roots	***	NS	NS	NS	NS	NS	NS
	Shoots	***	*	NS	NS	NS	NS	NS
	Total	***	*	NS	NS	NS	NS	NS
	Dry Weight							
	Leaves	**	**	NS	NS	NS	NS	NS
	Flower	**	NS	NS	NS	NS	NS	NS
	Trunk	**	*	NS	NS	NS	NS	NS
	Roots	***	*	NS	NS	NS	*	*
	Shoots	***	*	NS	NS	NS	NS	NS
	Total	***	*	NS	NS	NS	NS	NS

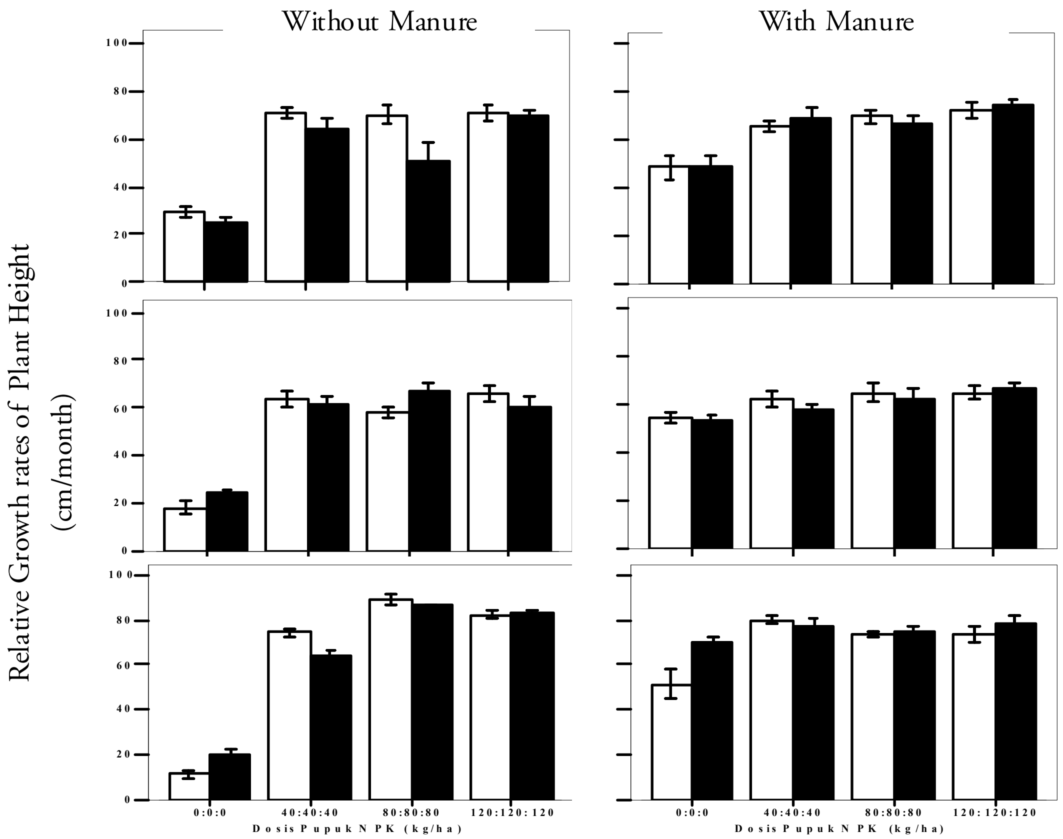

Figure 1. Relative Growth Rates of Plant Height (cm/month). White bars □ = withouth VAM black bars ■ = with VAM.

Table 2. Interaction Effects of NPK and Manure Treatment on RGR of Plant Height (cm/month) of *Artemisia annua* L..

NPK Treatment (Dosage Kg/ha)	RGR of Plant Height (cm/month) Without Manure VS With Manure					
	Bandung		Cibodas		Tawangmangu	
	T	*p*	*t*	*p*	*t*	*p*
NPK (0:0:0)	-5.96	<0.0001	-13.46	<0.0001	-10.36	<0.0001
NPK (40:40:40)	-0.07	0.94	0.77	0.44	-3.54	<0.01
NPK (80:80:80)	-1.46	0.15	-0.32	0.75	7.33	<0.0001
NPK (120:120:120)	-1.01	0.32	-0.76	0.45	2.44	0.02

In general, total wet and dry biomass increased with the NPK enhancement (Table 4). The figure shows that the biomass of *A. annua* from Tawangmangu is lower than Bandung and Cibodas. The highest total plant biomass (wet and dry) was found from plant with the NPK of 120:120:120 kgha^{-1} treatment, except plant from Cibodas. For plant from Cibodas, the biomass resulted from the application of different NPK dosages (40:40:40 kgha^{-1}, 80:80:80 kgha^{-1}, and 120:120:120 kgha^{-1}) was not significantly different.

Leaf biomass (wet and dry) also increased with NPK enhancement (Table 4). The figure shows that the leaf biomass of plant from

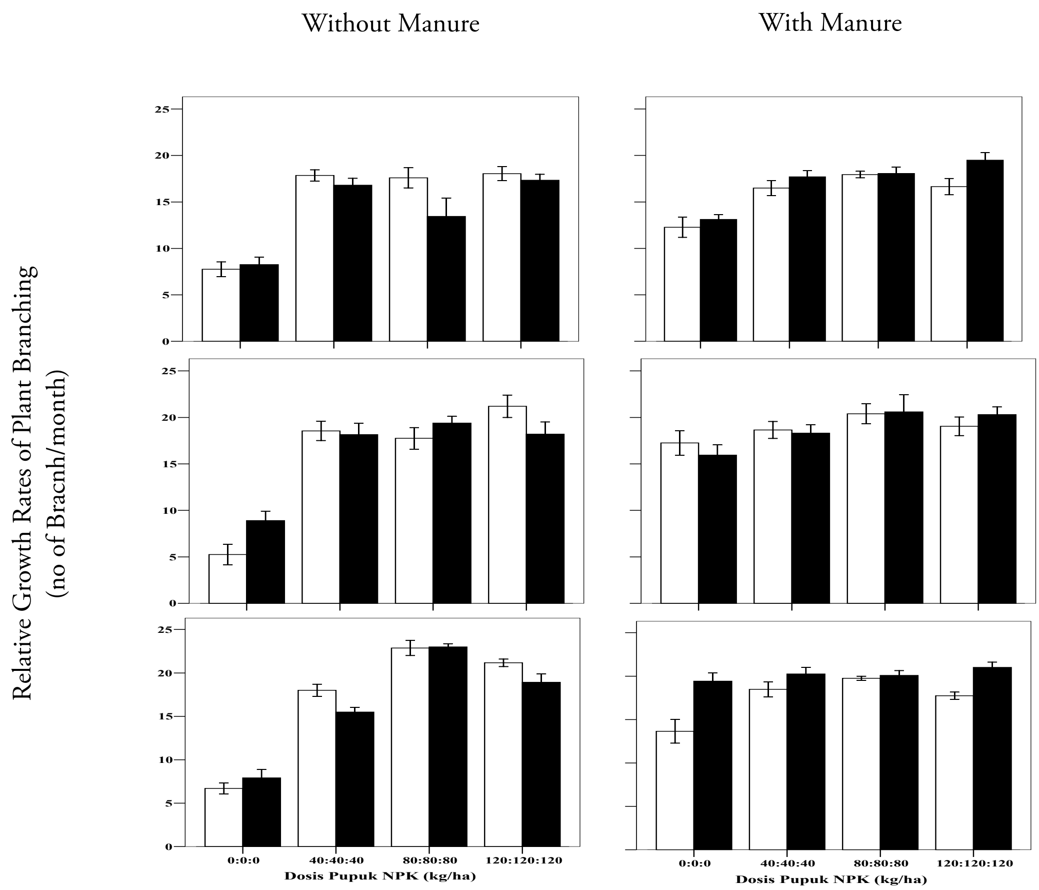

Figure 2. Relative Growth Rates of Plant Branching of *A. annua* L. under Various NPK Dosage, manure addition, and VAM addition. White bars □ = without VAM, black bars ■ = with VAM.

Table 3. Interaction Effects of NPK and Manure Treatment on Branches Number (Number of Braches/month) of *Artemisia annua* L..

NPK Treatment (Dosage Kg/ha)	RGR of Number of Branches (No.branch/month) Without Manure VS With Manure					
	Bandung		Cibodas		Tawangmangu	
	t	*p*	*t*	*p*	*t*	*p*
NPK (0:0:0)	-5.76	<0.0001	-7.96	<0.0001	-7.75	<0.0001
NPK (40:40:40)	0.31	0.75	-0,12	0.9	-3.31	<0.01
NPK (80:80:80)	-1.96	0.05	-1,53	0.13	5.54	<0.0001
NPK (120:120:120)	-0.46	0.64	0.02	0.98	0.86	0.39

Tawangmangu hada lower weight than that of Bandung and Cibodas. The highest leaf biomass was found from plant with the highest NPK dosage treatment, except plant from Cibodas where the biomass yielded from different NPK dosages application was not significantly different.

Artemisinin content from all accessions resulting from different treatments of *A. annua*ranged from 0.02 to 0.29 %. The highest content was found on plant without NPK and manure but with VAM addition treatment (Figure 3). The lowest content was found on plant without NPK,

Table 4. Effect of NPK Rates on Wet Weight and Dry Weight of Plant Biomass of *Artemisia annua* L from different location seed source. Number followed by similar word means not significantly different at α=0.05.

NPK Dosage	Wet Weight (g/plant)					Dry Weight (g/plant)				
	Leaf	Trunk	Root	Shoot	Total	Leaf	Trunk	Root	Shoot	Total
					Bandung					
Control	61.6 [a]	147.69 [a]	44.78 [a]	210.48 [a]	255.25 [a]	29.22 [a]	65.11 [a]	18.15 [a]	94.98 [a]	113.07 [a]
40:40:40	104.15 [b]	310.03 [b]	117.04 [b]	427.52 [b]	543.91 [b]	40.46 [ab]	145.3 [b]	45.84 [b]	191.83 [b]	241.78 [b]
80:80:80	139.91 [b]	365.98 [b]	149.25 [c]	527.1 [c]	676.35 [c]	49.3 [b]	166.09 [b]	51.38 [b]	225.42 [b]	276.8 [b]
120:120:120	186.13 [c]	528.47 [c]	171.04 [c]	739.09 [d]	910.13 [d]	72.37 [c]	262.63 [c]	76.29 [c]	346.98 [c]	423.24 [c]
Statistic	F=14.5;	F=43.34;	F=30.54;	F=46.26;	F=47.86;	F=11.99;	F=42.82;	F=32.11;	F=48.83;	F=49.22;
	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
					Cibodas					
Control	119.37 [a]	313.28 [a]	90.42 [a]	440.12 [a]	530.66 [a]	50.82 [a]	155.2 [a]	38.61 [a]	249.08 [a]	242.27 [a]
40:40:40	149.68 [ab]	482.62 [b]	145.83 [ab]	663.1 [b]	808.93 [ab]	65.18 [ab]	235.31 [b]	67.97 [b]	381.26 [b]	381.26 [b]
80:80:80	176.36 [b]	565.86 [b]	191.95 [ab]	797.83 [b]	989.78 [b]	77.11 [b]	256.92 [b]	94.23 [c]	448.89 [b]	448.89 [b]
120:120:120	171.32 [b]	529.21 [b]	300.21 [b]	760.01 [b]	1060.22 [b]	66.62 [ab]	253.23 [b]	86.7 [bc]	432.14 [b]	432.14 [b]
Statistic	F=2.37;	F=3.86;	F=2.06;	F=4.87;	F=4.33;	F=1.93;	F=2.86;	F=9.23;	F=5.01;	F=5.37;
	$p=0.07$	$p<0.05$	$p=0.11$	$p<0.01$	$p<0.01$	$p=0.13$	$p<0.05$	$p<0.001$	$p<0.01$	$p<0.01$
					Tawangmangu					
Control	56.52 [a]	110.7 [a]	44.68 [a]	181.58 [a]	224.66 [a]	26.48 [a]	56.93 [a]	21.48 [a]	90.01 [a]	111.49 [a]
40:40:40	84.27 [a]	201.31 [ab]	84.77 [ab]	323.05 [ab]	407.80 [ab]	32.19 [a]	97.21 [a]	36.31 [ab]	145.91 [a]	182.22 [ab]
80:80:80	93.53 [ab]	236.74 [b]	109.95 [bc]	387.82 [b]	497.78 [b]	39.52 [a]	113.99 [a]	52.26 [bc]	178.42 [a]	230.67 [b]
120:120:120	138.21 [b]	360.22 [c]	135.31 [c]	596.99 [c]	732.31 [c]	61.75 [b]	178.38 [b]	62.48 [c]	285.43 [b]	347.91 [c]
Statistic	F=3.81;	F=6.63;	F=6.81;	F=7.33;	F=7.62;	F=4.68;	F=6.28;	F=6.53;	F=7.3;	F=7.41;
	$p<0.05$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.01$	$p<0.01$	$p<0.001$	$p<0.001$	$p<0.001$

manure, and VAM from Cibodas and Tawangmangu. The results also show that artemisinin yield/plant (dry leaves weight x artemisinin content) has a similar trend to the artemisinin content. This confirms that high input (production) is not always correlated with high artemisinin yield.

DISCUSSION

Plant height and branch number of *A. annua* have been known influenced by seed source/ genetic factor (Firdaus 2008; Lestari *et al.* 2011; and Brisibe *et al.* 2012). However there have been controversies about the effects of fertilizer on plant height and branching. Özgüven *et al.*(2008) found that there was no effect of N fertilization levels to the plant height, while Davies *et al.* (2009) and Davies *et al.* (2011) said contrary. Our results showed that, genetically, plants from Bandung and Cibodas were higher and had more branches than those from Tawangmangu as they were shown on the control plants. The fertilization treatments only had a significant effect when all of the plants were compared to the control plants. There was only a minor difference among the fertilized plants. The plants were only given manure has a similar nutrient availability to plants that were given a dose of 40:40:40 NPK only. This is because of the NPK content of manure is not much different from the dose of NPK 40:40:40 kg/ha, except at P which implies only a third of it.

On the other side, the results showed that leaf yield and total biomass yield of *A. annua* were more affected by NPK fertilizing than by manure or VAM application. Jha *et al.* (2011) stated that manure addition enhanced the plant biomass compared to the control. However it was much

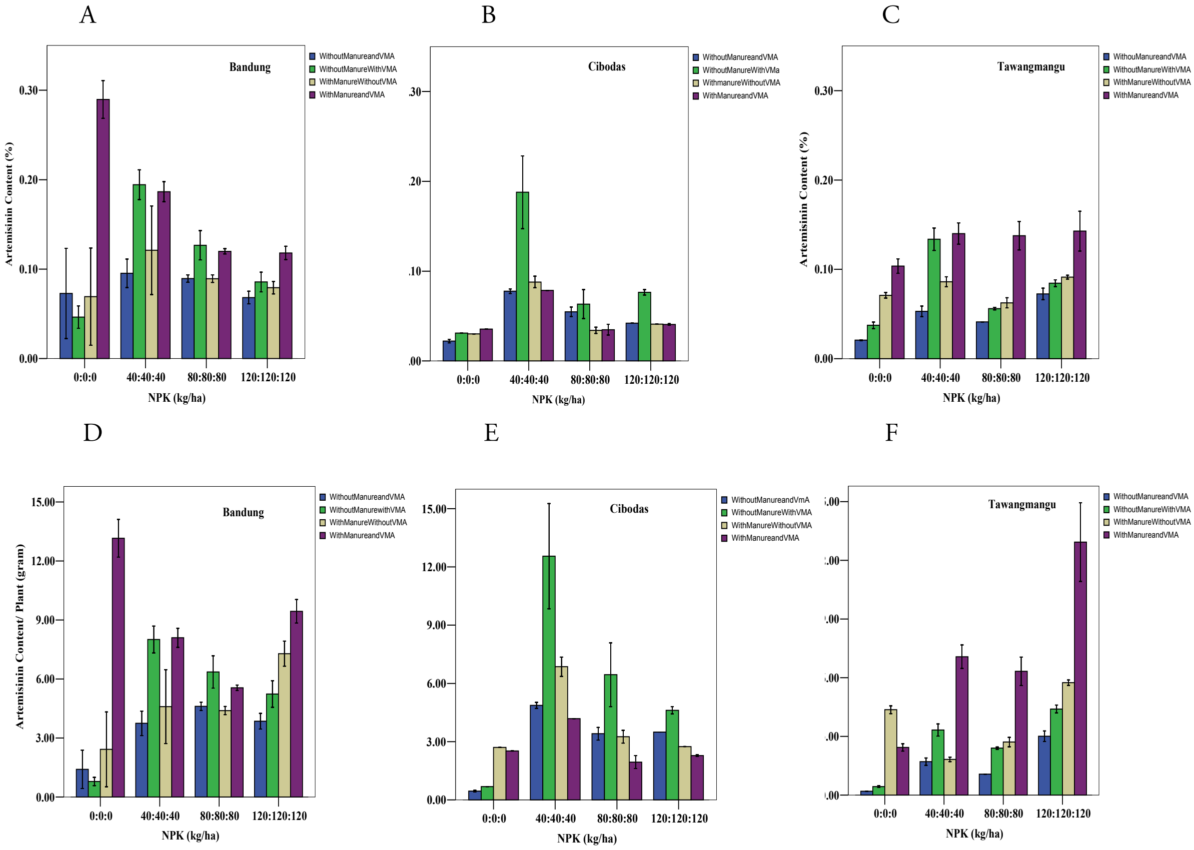

Figure 3. Artemisinin Content (A-C) and Artemisinin Yield per Plant (D-F) of *A.annua* L. from different Locations,Seed Sources and Treatments.

lower if compared to the plant treated with NPK only. Our results showed that the leaf yield and total biomass yield from the plants with and without manure on the same NPK rates were not significantly different.

The current results also showed that the addition of VAM did not affect the leaf yield and total biomass yield of *A. annua*. This result is similar to Rapparini *et al.* (2008) report. In contrast, Kapoor *et al.* (2007) stated that *A. annua* that was inoculated by VAM yielded more biomass than the control, and the similar experiment was also conducted by Chaudary *et al.* (2008). Their results suggest that the effect of VAM depends on the genetic of the plant.

Although the inoculation of VAM seems to not enhance the leaf yield and total biomass yield, but it increases the leaf artemisinin content. Most of the mycorrhizal plants have higher artemisinin contents than the non mycorhizal plants. This results were similar to what Kapoor *et al.* (2007) have found. They agreed that on mycorrhizal plants (either with or without P fertilizer addition), the artemisinin content is higher than that of plants treated only with P fertilizer or of control. This is probably caused by differences in the density of glandular trichome. Non- mychorrhiza plants, having glandular trichome density lower than that of plants with mycorrhiza plan (Kapoor *et al.* 2007). Artemisinin is a sesquiterpene lactone that synthesized in the glandular trichomes (Duke *et al.* 1994; Kjær *et al.* 2012). Graham *et al.* (2010) have showed that high artemisinin cultivar of *A. annua* have higher glandular trichome density than the low artemisin cultivar. Maes *et al.* (2011) showed that glandular trichome development of *A.annua* is influenced by a plant hormone that is Jasmonic Acid (JA). On the other hands, Hause *et al.* (2002) described that arbuscular mycorrhiza contribute to increased level of endogenous JA.

Artemisinin content is also affected by the addition of fertilizer, but there are also some discrepancies about the effect. Özgüven *et al.*(2008) argued that leaf artemisinin content increased concomitantly with N level addition. However Davies *et al.* (2009) stated that artemisinin content decreased with the raising of N fertilizer addition Our data indicated that there were some differences in artemisinin content between seed sources. Plants from Bandung and Cibodas follow Davies *et al.* (2009) report, but plants from Tawangmangu follow Özgüven *et al.*(2008) result.

Based on the results, the highest artemisinin yield per ha of *A. annua* wasfound on plants with no NPK fertilizer input and with manure and VAM addition. Chisaki & Horiguci (1997) reported that plant secondary metabolites are increase on the plant with nutrition deficiency. However, it is known that fertilization has effect on the artemisinin yield improvement through increased biomass artemisinin (Ferreira *et al.* 2005). This suggest that high doses of fertilization are inefficient in *A. annua* cultivation.

The optimum artemisinin yield in this experiment (5 kg artemisinin ha^{-1}) was still low if compared with the international yield rates that can reach 40 kg artemisinin ha^{-1} (Ferreira *et al.* 2005). This may be due to differences in genetic factor and the day length. This implies that one possible way to increase the production of artemisinin in Indonesia is by genetically manipulating plants with a high content of artemisin. The genetic engineering activities have been carried out by Widyatmoko *et al.* (2013). Currently these activities are just waiting for the results of the selection of plants with high artemisinin content.

In conclusion, to reach an optimum artemisinin yield, a low fertilizer input (NPK 40:40:40 kg ha^{-1}) on inoculated VAM plants is recommended for *A. annua* cultivation. There is no scientific recommendation on *A. annua* cultivation in Indonesia up to now. Thus this result can be one of the basic required recommendations for future use and cultivation of *A. annua* in Indonesia.

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