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THE CURCUMIN CONTENT OF TEMULAWAK(*Curcuma xanthorriza* Roxb.) RHIZOME AS AFFECTED BY N, K AND MICRONUTRIENTS B, Fe, Zn

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ABSTRACT

A polybag experiment to study the influence of N, K, and micronutrients B, Fe, Zn and the curcumin content in temulawak was conducted in Malang from February – September 2012. Using RBD, 8 treatments (P1 Inseptisol, without fertilizer, P2 Inseptisol 300 urea kg.ha^{-1}, P3 Inseptisol 200 KCl kg.ha^{-1}, P4 Inseptisol 300 kg.ha^{-1} and 200 kg.ha^{-1} urea and KCl, P5 Alfisol, without fertilizer, P6 Alfisol 300 urea kg.ha^{-1}, P7 Alfisol 200 KCl kg.ha^{-1}, P8 Alfisol 300 kg.ha^{-1} and 200 kg.ha^{-1} urea and KCl in 3 replications. The micronutrients *in vitro* applied RCD by 4 treatments (MS medium, MS without B, Fe and Zn) in 10 replications. The results of experiment showed that dry weight of rhizome per plant in Inseptisol and Alfisol is 30.98 and 9.75 g, content of curcumin 6 month after planting was 3.60 and 4.72%. The highest rhizome weight of 8 months after planting was a combination of N and K of Inseptisol (48.28) and Alfisol (35.75 g per plant).The highest content of curcumin 6 months after planting was on Alfisol (7.99%) and Inseptisol (6.7%) by 200 KCl kg.ha^{-1}.The curcumin content in complete media was higher than that without B, Fe and Zn i.e. 6.26 compared with 1.86–2.39%.

Keywords : temulawak *(Curcuma xanthorriza* Roxb, synm. *Curcuma javanica),* N, K, B, Fe, Zn, curcumin content

INTRODUCTION

The use of temulawak *(Curcuma xanthorriza* Roxb. Synm.*Curcuma javanica*) as traditional medicine has kept increasing on the average of 5.4% per year for the last decade, Corinthian Infopharma Corpora (CIC, 2000). It has been triggered by the increasing public awareness of looking for alternative therapeutic efforts that have less side-effect by going back to nature and the rising price of chemical medication that has exceeded the affordability of societies.

The increased consumptionhas caused the traditional pharmaceutical industries to demand high quality, so that raw materials and *simplicia biopharmaca* could be processed into traditional medicine. The main active substance contained in temulawak rhizome is curcumin, serving as one of parameters that must befulfilled. As component of production, this natural, active substance is the resultant between genetic and environment, therefore, such active substance could not be guaranteed to be fixed (Raharjo and Rostiana, 2005). The amount of active substancesisvaried, which is possibly affected by environmental factors, such as climate, altitude site, and particularly the nutrients availability whichis related to the forming process of these active substances.

The availability of raw materials and temulawak extract from the farmer has faced the main obstacle, low production of rhizome and limited *simplicia* which contains curcumin that conforms to the standard at the marketplace. This is because the plant is mostly cultivated as intercropping in which the required nutrients for this plant have not been known yet. It runs the risk in relation to raw material quality and the active substance contained in low and varying rhizome.

The environmental effect, including the addition of N and K, as well as the availability of micronutrients of B, Fe, and Zn on curcumin, have been mostly observed for curcuma (*Curcuma longa*) in India, ginger in China, and *gingseng* in Korea (Gang and Ma, 2008; Akamine *et al*., 2007; Dexit *et al*., 2002;

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Chempakan and Parthasarathy, 2008). In temulawak, the effects of N and K as well as other environmental factors have, however,received less attention.

The application of fertilizer that contains suitable nutrients will improve the growth and yield of the plants. The application of P will increase the rhizome yield as high as in K, in which the application of K would increase the curcumin content. The application of N has significantly been able to increase the yield, but it has low curcumin content in comparison with K application (Behura, 2001). In addition to the macronutrients of N and K, the available micronutrients, such as B, Fe, and Zn, could increase yield and curcumin content in curcuma (*Curcuma domestica*) that belongs to the same family. It is presumed that low production and curcumin content are caused by improper harvest time.

The objectives of this research were to find out the effect of macronutrients, the combination of N and K, as well as the available micronutrients of B, Fe, and Zn on both yield and curcumin content of the temulawak.

MATERIALS AND METHODS

The research was conducted from February to September 2012 in two locations:the combination of N and K macronutrients was conducted in field, and micronutrients treatment was carried out in a laboratory.

The research was conducted in the screen house in Malangby applyingRandomized Block Design (RBD) with 8 treatments and three-time replication. Thematerials used were local variety of temulawakfrom Jember at 1 year of age (Kuswanto and Azizah, 2011). Twosoil types from Jember = Inseptisol and Sumenep = Alfisol (Repprot, 1989) were combined with 4 fertilizer applications: 1) control, 2) 300 kg urea ha^{-1}, 3) 200 kg KCl. ha^{-1}, 4) 300 kg urea and 200 kg KCl. ha^{-1}. Non-destructive observation, which was conducted once a month, started first until the eighth month with the following measures: number of leaf, length of plant, and leaf area. The destructive observation was conducted during the age of 6 and 8 months with the following measures: fresh weight and dry weight of the rhizome, as well as the curcumin content. Curcumin was measured three times for each treatment by TLC (Cammag Co. Ltd). The Mean and standard deviation of replication were determined by using analysis of variance with the difference test at 5 % level of significance which was used to compare treatment means.

The second experiment in micronutrients involved Randomized Complete Design RCD that consisted of 4 treatments: 1) MS medium (control), 2) MS medium without B, 3) without Fe, and 4) without Zn, each of which had 10 replications. Non-destructive observation was measured monthly for the number of leaf, plantlets, and roots. The destructive observation was conducted during the 8^{th} month for the fresh weight, dry weight of the plant and rhizome and the curcumin content in plantlets of temulawak. Curcumin was measured three times for each treatment by TLC (Cammag Co. Ltd).

Analysis of variance (ANOVA) was used in this research. The tests were carried out using the F testat the level of 5%. In the event of a significant differen the treatments occurring,further tests using LSD at 5% level followed.

RESULTS AND DISCUSSION

Response of N and K to the Growth of Temulawak

The growth parameters of temulawak (length of the plant, number of leaf, and leaf area) on the application of N and K alone and in combination are presented in Figure 1, 2, and 3. The length of temulawak plant grown in Inseptisol by adding 300 kg of urea ha^{-1} (P4) was higherthat that ofAlfisol soil by adding 300 kg ofurea and 200 kg of KCl ha^{-1} (P8) (Figure 1).

Adding N and K alone and combination of them would affect the growth, yield, and curcumin content. Adding N alone would increase the plant length and the leaf area, but not for K. However, the application of their combination had also increased both plant length and the leaf area. Yield of the temulawak rhizome was higher when the combination of N and K was added, and it was true for the application of N alone, but not for K applied alone. The highest content of curcumin in temulawak rhizome by the single application of K was different from the the single application of N, but single application of K was similar to the application of the combination. The result showed that Nitrogen would increase growth of the organ in correlate to photosynthesis, particularly the leaf area, in order to produce carbohydrate for vegetative growth (Taiz and Zeiger, 2010).

The combination of N and K triggered the photosynthetic activity that increased the leaf area and translocation of photosynthate to the plant organs. The number of leaf for temulawak grown in Inseptisol by 300 and 200 urea and KCl kg ha^{-1} (P4) and in Alfisol, by adding the same combination of fertilizers (P8) in which number of leaf for the plant grown in Inseptisol was similar to the plant grown in Alfisol (Figure 2). The number of leaf was more affected by genetic factor than environmental (Devy, 2009)

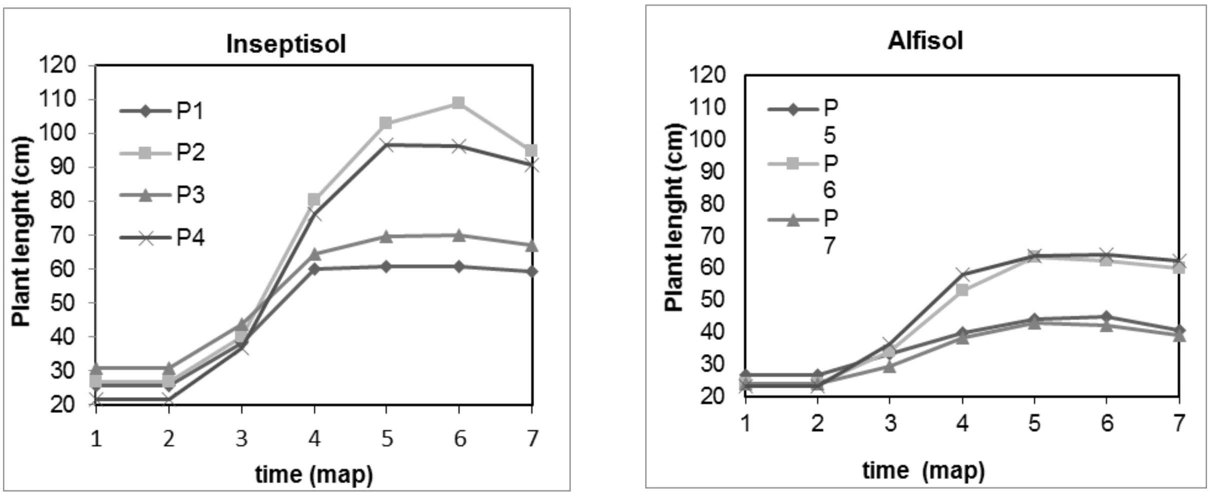

Figure1. Plant length of temulawak (*Curcuma xanthorriza*) grown in Inseptisol and Alfisol soilfollowing the fertilizer treatment (P1, P5 without fertilizer, P2, P6 300 kg Urea ha^{-1}, P3, P7 200 kg KCl ha^{-1}, P4, P8 300 and 200 urea and KCl kg ha^{-1}) during 1 – 6 month after planting (map)

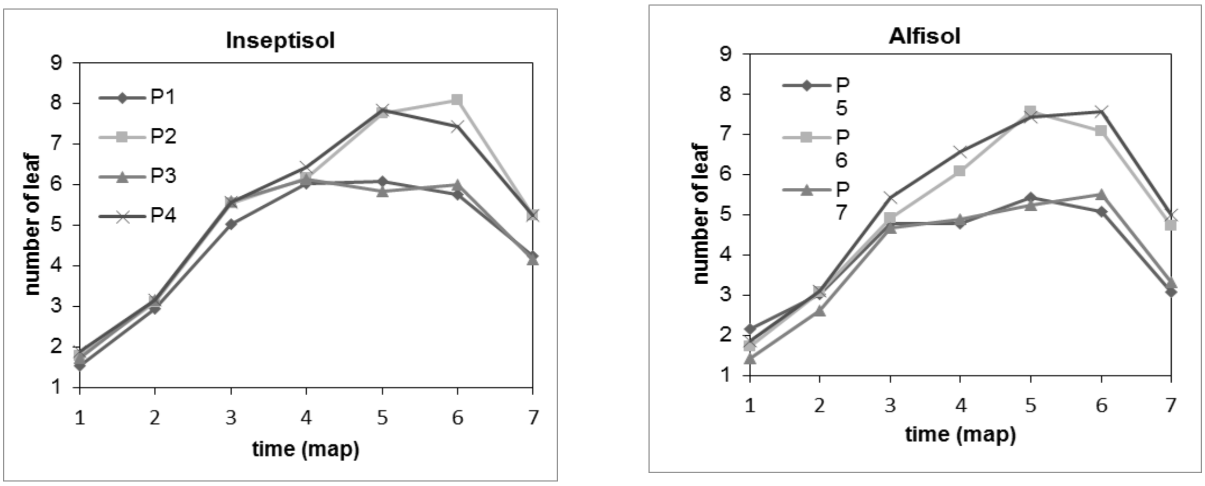

Figure 2.Number of leaf on temulawak (*Curcuma xanthorriza*) grown in Inseptisol and Alfisol with fertilizer treatment (P1, P5 Without fertilizer, P2, P6 300 kg Urea ha^{-1}, P3, P7 200 kg KCl ha^{-1}, P4, P8 300 and 200 urea and KCl kg ha^{-1}) during 1 – 7 month after planting (map)

In contrast, the maximum growth of the leaf area was attained at the age of 5 month after planting, and it was mostly affected by the soil characteristics. For the whole age of observation and treatments, the leaf area was wider for the plants grown in Inseptisol than those of Alfisol (Table 1), and this was due to the root growth that sustained the nutrients which supported the translocation toward the growth of leaf area for plants, where the plant grown in Inseptisol was higher than those grown in Alfisol.

Leaf area of the plant grown in Inseptisol with the application of 300 K and 200 urea and KCl kg KCl ha^{-1} (P4) in comparison with the plant in Alfisol with the same application of the combined fertilizers (P8) indicated thatthe leaf area of the plant grown in Inseptisol was greater than that of Alfisol (Table 1).

Table 1. Leaf area measurement of temulawak was grown in Inseptisol and Alfisol with fertilizer treatment ((P1, P5 Without fertilizer, P2, P6 300 kg Urea ha^{-1}, P3, P7 200 kg KCl ha^{-1}, P4, P8 300 and 200 urea and KCl kg ha^{-1}) during 2, 3, 4 and 5 month after planting (map)

Treatment	Leaf area (cm^2) during month after planting (map)							
	2		3		4		5	
P1	1368	b	1526	ab	1417	ab	13582	bc
P2	2802	c	4306	e	4158	c	41940	d
P3	1703	b	2106	bc	1613	ab	19420	c
P4	2984	c	4110	de	3305	c	39383	d
P5	625	a	897	a	667	a	2719	ab
P6	1452	b	2016	bc	1312	ab	10460	c
P7	719	a	811	a	643	a	1986	a
P8	1588	b	2234	c	1913	b	14513	c
BNT 5 %	477		688		688		11425	

Remarks: Numbers followed by the same letter in the same column show insignificant difference based on the Smallest Significant Difference Test in 5% content. P1 Inseptisol, Without Fertilizer, P2 Inseptisol + 300 kg Urea ha^{-1}, P3 = Inseptisol + 200 kg KCl ha^{-1}, P4 = Inseptisol + 300 kg Urea ha^{-1} + 200 kg KCl ha^{-1}, P5 = Alfisol, without fertilizer, P6 = Alfisol + 300 kg Urea ha^{-1}, P7 = Alfisol + 200 kg KCl ha^{-1}, P8 = Alfisol + 300 kg Urea ha^{-1} + 200 kg KCl ha$^{-1.}$
map : months after planting

Response of N and K to the Yield and Curcumin Content of the Temulawa

During observation, at the age of 6 months after planting, P2 and P4 produced greater dry weight of rhizome and had significant difference in comparison with the other treatments as a whole. During the observation, at the age of 8 months after planting, the rhizome weight of P2 was similar to P4. In contrast, during observation at the age of 6 months after planting, P7 , with P1, P5, and P6treatments, had higher content of curcumin, but it had insignificant difference inP3, P4,P8 treatments.During the observation at the age of 8 months after planting, P2, P3, and P8 had higher content of curcumin than that of the other treatments, but they had similar content in P2, P6, P7 treatments (Table 2 and 3).

Table 2. Dry weight of the rhizome of temulawak grown in Inseptisol and Alfisol with fertilizer treatments ((P1, P5 Without fertilizer, P2, P6 300 kg Urea ha^{-1}, P3, P7 200 kg KCl ha^{-1}, P4, P8 300 and 200 urea and KCl kg ha^{-1})in6 and 8 months after planting (map)

Treatment	**Dry weight of rhizome (g per plant)**			
	6 map		**8 map**	
P1	16.60	a	12.80	abc
P2	31.40	b	33.17	de
P3	12.90	a	24.43	cd
P4	41.33	b	44.15	e
P5	6.87	a	8.40	ab
P6	13.87	a	17.83	bc
P7	5.60	a	5.57	a
P8	14.87	a	19.42	bc
BNT 5 %	21.58		11.97	

Remarks: Numbers followed by the same letter in the same column show insignificant difference based on the Smallest Significant Difference Test in 5% content. P1 Inseptisol, Without Fertilizer, P2 Inseptisol + 300 kg urea ha^{-1}, P3 = Inseptisol + 200 kg KCl ha^{-1}, P4 = Inseptisol + 300 kg urea ha^{-1} + 200 kg KCl ha^{-1}, P5 = Alfisol, without fertilizer, P6 = Alfisol + 300 kg urea ha^{-1}, P7 = Alfisol + 200 kg KCl ha^{-1}, P8 = Alfisol + 300 kg urea ha^{-1} + 200 kg KCl ha$^{-1.}$
map : month after planting

The rhizome yield and curcumin content of both temulawak and turmeric were accomplish empirically, bringing the result of the experiment which showed that the nutrients condition in the field affected both yield and curcumin content in *Curcuma.* Identification for yield and curcumin on *Curcuma xanthorriza* and *Curcuma domestica* in Java and Madura, which was connected with Nand Mg, as well as P and K in the soil, have correlation with the curcumin content and rhizome weight. High curcumincontent (1.26%) and low weight of the rhizome (0.54 kg per plant) were obtained from Alfisol with low content of N and Mg (0.5 and 1.53) and medium content of P and K (7.8 and 2.86). Low curcumin content (0.59%) and heavy weight of rhizome (1.7 kg per plant) were obtained from Inseptisol with low content of N and Mg (0.10% and 1.89%, respectively) and high content of P and K (28.48% and 3.49%, respectively) (Wardiyati *at al.*, 2010).

Table 3. The curcuminc ontent of temulawa krizhomegrown in Inseptisol and Alfisol with fertilizer treatments (P1, P5 Without fertilizer, P2, P6 300 kg Urea ha^{-1}, P3, P7 200 kg KCl ha^{-1}, P4, P8 300 and 200 urea and KCl kg ha^{-1}) 6 and 8 months after planting (map)

Treatment	Curcumin content(%)			
	6 map			
P1	3.60	P1	3.60	P1
P2	6.37	P2	6.37	P2
P3	6.70	P3	6.70	P3
P4	6.65	P4	6.65	P4
P5	4.72	P5	4.72	P5
P6	5.53	P6	5.53	P6
P7	7.99	P7	7.99	P7
P8	6.88	P8	6.88	P8
BNT 5 %	1.54		BNT 5 %	

Remaks: Numbers followed by the same letter in the same column show insignificant difference based on the Smallest Significant Difference Test in 5% content. P1 Inseptisol, Without Fertilizer, P2 Inseptisol 300 kg Urea ha^{-1}, P3 Inseptisol 200 kg KCl ha^{-1}, P4 Inseptisol 300 and 200 ofurea and KCl kg ha^{-1}, P5 Alfisol, without fertilizer, P6 Alfisol 300 kg urea ha^{-1}, P7 Alfisol 200 kg KCl ha^{-1}, P8 Alfisol 300 and 200 of urea and KCl kg ha$^{-1.}$ map : months after planting

Akamine *et al*. (2007) claimed that the application of N (210 kg ha^{-1}), P (150 kg ha^{-1}), and K (150 kg ha^{-1}), both alone and in combination there were different weight and curcumin content of the curcuma (*Curcuma domestica*). The heaviest weight of rhizome was 165.9 g per plant affected the combination of N, P, and K which supported of the growth the upper part of the plant were the highest of length was 153.6 cm and 34 of leaves number. However the content of curcumin presented that the lowest was 0.16% and the highest was 21% were affected by K alone and it had the weight of rhizome was 18.2 g per plant which shown by the increasing growth of the upper part of the plant (length of the plant and number of leaf), each of which was 44.2 cm; 9.6, respectively.

The curcumin content was affected by the nutrient application and the harvest time. Regarding to harvest time during 6 months after planting, the highest content of curcumin resulted by temulak were grown in Alfisol with KCl 200 kg ha^{-1}.

The application of N could not increase the curcumin content of the rhizome, whereas the application of K in Alfisol soil could significantly increase the curcumincontent, eventhough it was comparable with the application of combined N and K in Alfisol and Inseptisol (Table 2). Nitrogen slightly increased P content to stimulate accumulation of curcumin, but not for K, and this study showed that K was the main element in forming curcumin in curcuma (*Curcuma domestica*) (Khartikeyan *et al*., 2006). Potassium is not only the important element in translocating photosynthate, but also in forming curcumin (Behura,2001). Kalium is used as cofactor for diverse enzymatic processes that relates to phosphorylation and hydrolysis of diverse compounds and as structural stabilizer for diverse nucleotides (Marschener, 2012). Imas *et al*. (2006) found that curcumin in curcuma had increased to 50% by the application of potassium 200 kg ha^{-1} in KCl form.

The Effect of Micronutrients B, Fe and Zn on Growth and Curcumin Content of*in vitro* Temulawak

The availability of B, Fe, and Zn in vitro media affected both growth and curcumin content in temulawak. Plantlets, which were cultured in MS medium, grew faster than those cultured in MS medium without B, Fe, and Zn. Plantlets, which were cultured in MS medium without Fe showed the lowest content in growth of plant length, numbers of plantlet and leaf in comparison with those in MS medium without other micronutrients. Therefore, it resulted in the lowest content of fresh weight and dry weight. It showed the lowest content of curcumin in MS medium without B in comparison with MS medium without any other micronutrients (Figures 4 and 5). The results showed that, beside the availability of these micronutrients for the growth and biosynthesis of the curcumin, each of these micronutrients had different functional criteria.

Response to the growth of *in vitro* temulawak, under the control treatment (MS medium and complete micronutrients) and unavailability of micronutrients B, Fe, and Zn, showed that MS medium without Fe showed the lowest yield of the plant growth (Figure 4). Also, the medium without Fe resulted in less fresh weight and dry weight of the total plants. Based on result of the analysis, it was found that there was insignificant influence between the control treatment and reduced micronutrients of B, Fe, and Zn on the parameter of plant length at the 8th month of age after planting, but on parameter for number of leaf, number of shoot and plantlets, micronutrient treatment hada significant effect. The control treatment, MS medium, and MS medium by reducing B, Fe, and Zn, had the same length of the plant, but for control treatment, it showedhigher content in comparison with reduced Fe for parameter on number of leaf and roots. The number of shoots showed the highest content under control treatment, whereas the number of plantlets was similar without the application of Boron. Iron had not only functioned as an activator of enzyme, but it also played an important role in chlorophyll synthesis. Fe was also involved in electron transport in the form of porphyrine, and it was related to the chloroplast protein synthesis (Marschner, 2012. Low photosynthate caused low yield of the plant organs, such as the number of leaves, roots, fresh and dry weight of total plants. Fe is an influential micronutrient in photosynthetic process, such as biosynthesis of chlorophyll, the transportation system of electron in photosynthesis, photosynthetic enzyme activities and carotenoid content, and the reducing of such micronutrient would cause reduced photosynthate as well and lead to inhibited growth and development of the plant (Marschner, 2012). Without Fe, the growth of the plant on each parameter of the number of leaves, roots, shoots, plantlets, fresh weight and dry as well as fresh weights would reduce.

Boron affected terpenoid metabolism line, such as structural component of the cell walls which were influential on membrane stability and other secondary metabolism (Marschner, 2012). Boron was involved in transporting sugar; therefore, deficiency of B would change the availability and content of the photosynthate, which would be translocated and used for curcumin accumulation and development of curcuma (*Curcuma domestica*) rhizome (Dixit *et al.*, 2002). Deficiency of B would change translocation of CO_2photosynthate to primary metabolite in relation to the accumulation of curcumin and essential oil of the curcuma (*Curcuma domestica*) (Dixit *et al.*, 2002). Deficiency of B would reduce the accumulation of sugar, amino acid, and organic acid in all position of leaves. It is ascertained that such reduced yield was due to reduced metabolite translocation to the rhizome. As a result, photosynthate assimilation serving as essential oil in leaf and curcumin in the rhizome also decreased.

As micronutrients, Zn did not only increase Zn in plant, but also K intake, in which K would improve the curcumin translocation from leaf to rhizome. The increasing Zn(ZnSO4) from 0 to 0.05 g m^{-3}could increase the curcumin content and the essential oil to 1.46 g m^{-3}.

Temulawak rhizome contains many chemical components in addition to curcumin and essential oil, as well as starch (amylum) as one of the greater components in temulawak(Hadipoentiyanti and Syahid, 2007). Besides, the starch is the yield of the primary metabolite which function as precursor in the next metabolic process, such as secondary metabolite (Taiz and Zeiger, 2010).

The curcumin synthesis as effective compounds is not only affected by characteristics of the soil, harvesting age, and availability of the nutrients, but it also relates to the starch (amylum) content in the rhizome.

The result of the analysis showed significant influence between micronutrient treatments in medium during observation for 8 months after planting on fresh weight and dry weight of plantlet, as well as the curcumin content (Figure 3 and 4). Control threatment had higher content of curcumin than other treatments, neither by applying B, Fe, nor Zn.

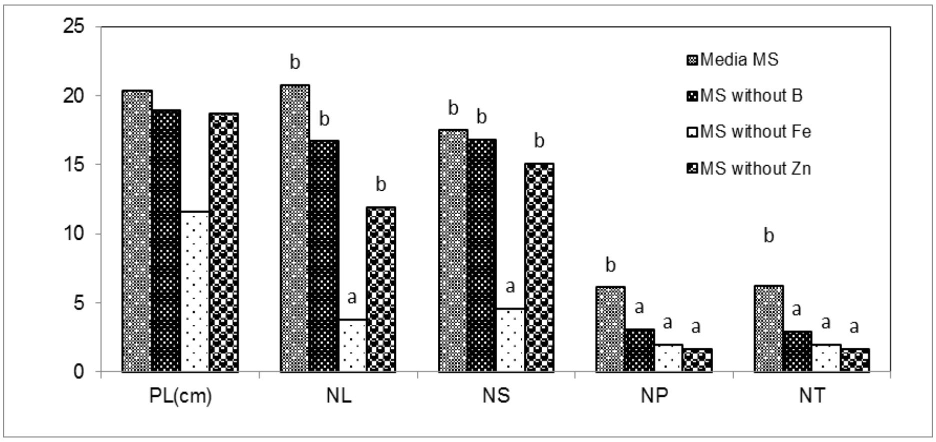

Figure 3. Hystogram PL = plant length, NL = number of leaf, NS = number of shoot andNP = number of plantletNT = Number of tiller *in-vitro*of temulawak (*Curcuma* xanthorriza) on 4 kinds of observation media within 8 months after planting

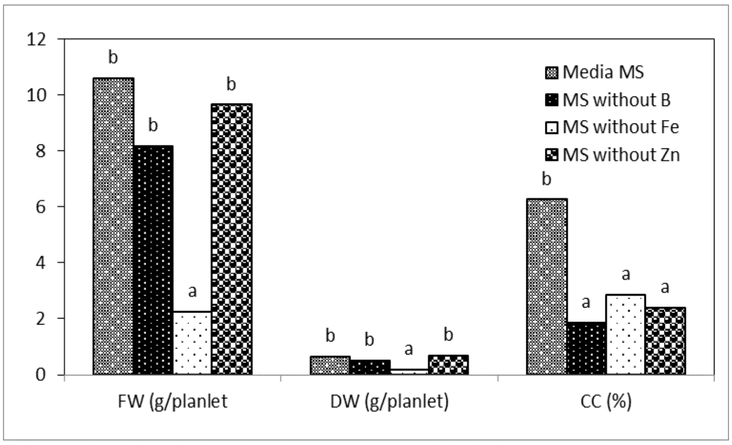

Figure 4. Hystogram for fresh weight of plantlet (FW), dry weight of plantlet (DW), and *in vitro* Curcumin content (plantlet) of the temulawak (*Curcuma xanthorriza*) on 4 kinds of observation media within 8 months

Control treatment, without B, and Zn had greater fresh weight and significant difference in comparison with treatment without Fe on fresh and dry weight of the plantlets. Control treatment had higher content of curcumin than other treatments, neither by applying B, Fe, nor Zn.

Iron is the important micronutrient in diverse photosynthetic process, such as biosynthesis of chlorophyll, transporting system of electron in photosynthesis and formation of chloroplast ultrastructure, as well as enzymatic activity in photosynthesis and carotenoid content (Morales *et al.*, 1998; Abadia, 1992). Structure and function of the whole photosynthetic tools are affected by iron deficiency (Marschener, 2012; Abadia, 1992). Low Fe caused low yield of photosynthate and less organs of the plant, such as the number of leaves, roots, fresh and dry weight of total plants (Taiz and Zeiger, 2010).

This is parallel to the result of the research by Rethinam and Sivaraman (1994); Dixit and Srivastava (2000), which states that the growth of curcuma is affected by deficient Fe and Zn. Greater proportion of accumulated photosynthate is required by plant for root growth and metabolism in rhizome plant, such as curcuma that reaches 30% of total accumulated photosynthate (Marschener, 2012). Therefore, both development of rhizome and curcumin accumulation have simultaneously depended on translocation of the formed metabolite in leaf.

The number of metabolites translocated to rhizome and produced by leaf and proportion, will highly affect size and yield, as well as biosynthesis and accumulation of curcumin. Finally, proportion of the photosynthate to rhizome becomes one of factors, which controls productivity regardless of biosynthetic capacity of the rhizome. Due to the active precursor produced in leaf, the photosynthetic capacity, which has become the characteristic, could become another regulating factor.

CONCLUSIONS

Weight oftemulawak rhizome in Inseptisol soil, with the application of N and K combination during the harvest time of 8 months was highest thanthat in Alfisol, each of which was 41.33 and 14.87 g per plant, respectively.

The curcumin content of temulawak rhizome in Alfisol soil, with the application of K alone during the harvest time of 6 map, was higher than that in Inseptisol, each of which was 7.99 and 6.7%, respectively.

Micronutrient availability in media was needed to produce curcumin for B, Fe and Zn. Media without B had the lowest curcumin(1.8%).

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