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### Production Enhancement Technology of Pegagan (*Centella asiatica*)

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#### ABSTRACT

*Centella asiatica* has long been known as one of medicinal plants. Profound study is needed to be able to find out the responses of Pegagan to various treatments in order to increase centelloside content. It was also previously reported that the highest asiaticoside content was found at the lowland pegagan, Pantai Labu. Current study on planted lowland pegagan of Deli Serdang with 2.38% of asiaticoside content was aimed to know the response of the plants to phosphorus fertilizing, elicitor and harvesting time related to biomass production from leaves, petioles, roots and tendrils. Phosphorus fertilizer was applied in four levels: 0, 18, 36 and 54 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup>, which affected centelloside synthesis, and elicitor of methyl jasmonate was given at three levels: 0, 100, 200 µM, while harvesting time was set up at 56, 70 and 84 days after planting (DAP). Data showed that the best combination for biomass production was obtained when there was no elicitor and harvesting was done at 84 DAP. The best combination between methyl jasmonate hormone and harvesting period in both fresh weight of leaves and petioles as well as roots was obtained when there was no hormone and harvesting time was 84 days old.

#### INTRODUCTION

Up to the present, pegagan has been harvested in a natural way. In order to meet current demand increasing its production in a large scale and cultivating high quality pegagan are prerequisite. Many medicinal mixtures of *jamu* contain pegagan herbal. The demand for pegagan (*Centella asiatica*) has reached to 100 t, and PT. Sidomuncul, for example, needs 2 t of pegagan monthly while local factories need 25 t of pegagan in a year; unfortunately, local supply is only 4 t per year. Special virtue of pegagan with its chemical content is in its saponin compound, including its asiaticoside (Matsuda, Morikawa, Ueda, & Yoshikawa, 2001) and flavonol accumulation in leaf tissues (Bidel et al., 2015). Besides enhancing the process of wound healing and asiaticoside, a bioactive compound of pegagan can also be used in curing leprosy and tuberculosis (Mangas et al., 2006; Mangas et al., 2008; Mangas, Moyano, Hernandez-Vazquez, & Bonfill, 2009). Pegagan can also be used as blood

cleaning, blood circulation speeding, kidney stone crushing, antipiretic, haemostatica, anti-bacteria, anti-inflammation, hypotension, insecticide, anti-allergy. Saponin can also inhibit the production of excessive scar tissue (inhibit the incidence of keloid) (Mangas et al., 2008). Asiatic acid from *Centella asiatica* prevents memory deficits or memory increasing (Sirichoat et al., 2015) and protects against cognitive deficits (Chaisawang et al., 2017).

Some of the Indonesian herbal medicinal industries (agromedicine) state that the main obstacles of this industry are lacking of uniformity of raw material, production processes, problem solving, product expansion and marketing (Ghulamahdi, Aziz, Bermawie, & Hernani, 2007; Bermawie, Purwiyanti, & Mardiana, 2008).

Production of asiaticoside can be sped up by giving elicitor, a chemical substance produced by various sources, either biotic or abiotic, including physical factors that are able to trigger accumulation of secondary metabolite to animated organism. Methyl jasmonate is one of the elicitors which

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Noverita Sprinse Vinolina *et al.*: *Centella asiatica* Production.....

has been widely used in modulating physiological incidence in various high level plants leading to the accumulation of secondary metabolite (Lambert, Faizal, & Geelen, 2011; Zhao *et al.*, 2015). Pegagan plant grown in Deli Serdang is reported to be a potential plant which contains 2.38% of asiaticoside (Vinolina, Napitupulu, Siregar, Nainggolan, & Singh (2013). Current study was done in order to find out the effects of phosphorus fertilizer in combination with methyl jasmonate on pegagan production harvested at different harvesting time. Thus, it could be expected that this research would be able to answer the questions of (1) response of the plant to the dosetime of phosphorus combined with methyl jasmonate, and (2) correct harvesting time to obtain high quality of pegagan.

The research problems were high demand for simplicia, environment factors and uniformity in quality (standardization) which needed an effort to the cultivation and optimum production, and the quality of pegagan which could be obtained by conducting some agronomic practices. The objective of study was to obtain correct phosphorus dosetime, right concentration of methyl jasmonate, interaction among phosphorus dose time and the appropriate harvesting time to obtain optimal pegagan production.

## MATERIALS AND METHODS

The materials used were pegagan from Pantai Labu accession, Deli Serdang, TSP fertilizer, Urea and KCL, while the instruments needed in the research were digital scale, a digital camera, a GPS 12X12 channel Garmin, USA, some equipment for soil processing.

The research was conducted at Syahmad Village, Deli Serdang Regency, from January to October, 2013, using a Split Split Plot Design with three treatment factors:  $P_2O_5$  fertilizer at 4 levels:  $F_0 = 0 \text{ kg } P_2O_5 \cdot \text{ha}^{-1}$ ,  $F_1 = 18 \text{ kg } P_2O_5 \cdot \text{ha}^{-1}$ ,  $F_2 = 36 \text{ kg } P_2O_5 \cdot \text{ha}^{-1}$ , and  $F_3 = 54 \text{ kg } P_2O_5 \cdot \text{ha}^{-1}$ . The methyl jasmonate consisted of 3 phases:  $J_0 = 0 \text{ } \mu\text{M}$ ,  $J_1 = 100 \text{ } \mu\text{M}$ , and  $J_2 = 200 \text{ } \mu\text{M}$ . Harvesting time were  $H_1 =$  harvesting time of 56 DAP (day after planting),  $H_2 =$  harvesting time of 70 DAP, and  $H_3 =$  harvesting time of 84 DAP. In order to find out the influence of the treatment on the biomass production (the leaves and petioles, roots and tendrils with their fresh weight (g) and dry weight (g). The parameter measurement described below in observation, samples were performed in triplicates. The data was then analyzed

by using a univariate analysis (F-test) at the level of 5% and data were transformed  $(X+0.5)^{1/2}$ . Duncan's multiple range and equation correlation regression tests were conducted when there were found significancies between treatments.

## Research Activities

### Land Preparation

Some implementations were carried out, starting fromland preparation, calcification, plant preparation, planting, maintenance, application of methyl jasmonate, and harvesting. Land preparation was started by weeds and tillage clearance, then plotting of 108 plots at the size of 1.0 mx 1.0 m each. Interval between the blocks was 100 cm, while between the main plots was 100 cm and 50 cm as a distance between the plot. Calcification liming was done one week before planting, the dose for dolomite was 1.5 t ha<sup>-1</sup>.

### Plant Preparation and Planting

The plants of Deli Serdang accession was planted along this study. In order to allow the plants form their stolons, parent plants were grown two and a half months before stolons were formed and taken for further planting as uniform seedlings. For this purpose, the plants were grown at interval of 40x40 cm. Fertilization was done at planting with appropriate doses of P treatment, a third dose of Urea 300 kg ha<sup>-1</sup> and a dose of KCl 220 kg ha<sup>-1</sup>. Fertilizers were applied to the bolt around the planting hole.

### Maintenance

Maintenance was done in a series of activities, starting from fertilizing the plants at 20 and 40 DAP by applying Urea to each plant. Watering, weeding and replanting were also done. Watering was done in the afternoon and adapted to conditions on the ground. Stitching was done 2 weeks after planting seedlings which were provided separately. Methyl jasmonate was applied to the 50-day-old plants, either in plants' roots  $\pm 5 \text{ cc}$  or through the leaves around  $\pm 5 \text{ cc}$  per plant with a corresponding concentration of the treatment.

### Harvesting

Harvesting was done at the same time at 56, 70 and 84 DAP by dismantling all parts of the plant. Before harvesting, the soil was soaked with water to facilitate the dismantling of the plant and roots in the growing medium.

### Observations

Observation was done to the leaves and petioles, roots and tendrils with their fresh weight (g) and calculated as follows: harvested material was washed and drained separately into two parts, leaf and petiole; the root and tendril. The leaves and petioles, roots and tendrils of each study plot were measured initially for fresh weight. The leaf and petiole dry weight (g) were calculated by drying material in an oven set for 50°C for 3 days. The same activities were also done to measure roots and tendrils.

## RESULTS AND DISCUSSION

### Leaf and Petiole Fresh Weight

The highest leaf and petiole fresh weight was obtained from the treatment of 84 DAP harvesting time at the absence of methyl jasmonate ( $H_3J_0$ ) 13.041 ( $\pm$  188.511 g) and had insignificant disparity from harvesting time of 70 DAP with the giving of methyl jasmonate of 200  $\mu$ M ( $H_2J_2$ ), 11.676 ( $\pm$  166.418 g), followed by 70 DAP harvesting time but methyl jasmonate was present at 100  $\mu$ M ( $H_2J_1$ ) 11.435 ( $\pm$  152.057g) and 84 DAP harvesting time when methyl jasmonate was presented at 100  $\mu$ M ( $H_3J_1$ ) 10.408 (141.313 g). The lowest leaf and petiole fresh weight was found in the harvesting time of 56 DAP without giving methyl jasmonate ( $H_1J_0$ ) 7.454 ( $\pm$  61.670 g) (Table 1). A longer harvest time would produce more biomass, whereas a faster harvest biomass produced less, while the provision of methyl jasmonate would slightly depress growth as compared to administration of 200 and 100  $\mu$ M. The interaction of these two factors gave the highest yield fresh weight of leaves and petioles, and fresh weight of roots and tendrils found in the combination without giving methyl jasmonate with harvesting time 84 DAP. Jasmonate acid (JA) and methyl ester jasmonate were derived from linolenic acid catabolism and acted as a secondary metabolite which modulates several physiological processes of plants, including aging plants (Yendo,

de Costa, Gosmann, & Fett-Neto, 2010). Without giving methyl jasmonate, the aging process did not occur and the crop harvesting age was 84 DAP, causing the accumulation of photosynthate more than harvesting time 56 and 70 DAP so that biomass fresh weight of leaves and petioles, root fresh weight, and the highest tendrils reached in this treatment combination. The treatment of harvesting time and the giving of methyl jasmonate can be seen in Fig. 1.

In Fig.1, the harvesting time of 56 DAP produced leaf and petiole fresh weight in the maximum of 9.44 ( $\pm$  97 g) by giving methyl jasmonate of 99.75  $\mu$ M. Average disparity test of the treatment of methyl jasmonate and fertilizing phosphorous in leaf and petiole fresh weight is presented in Table 2.

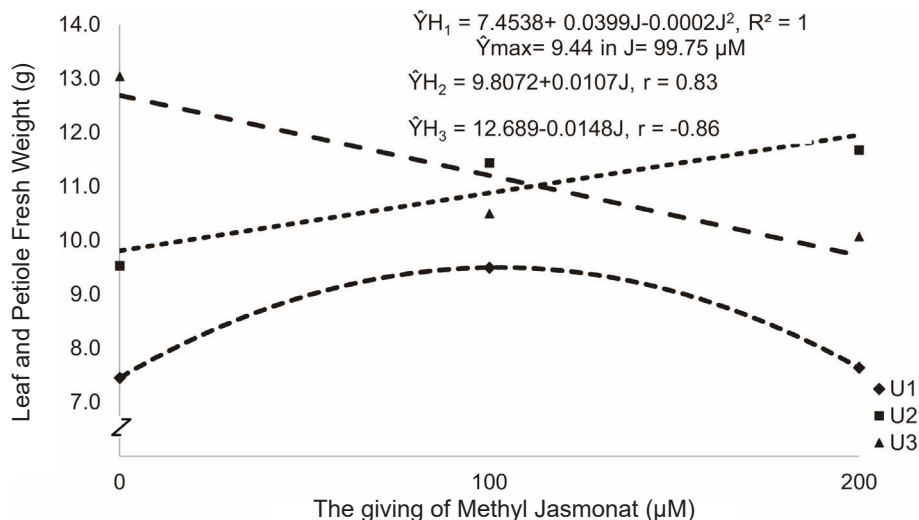
Table 2 indicates that the highest leaf and petiole fresh weight in the treatment of methyl jasmonate of 100  $\mu$ M and phosphorous fertilizing of 18 kg  $P_2O_5$ .ha<sup>-1</sup> ( $J_1F_1$ ) 12.698 ( $\pm$  197.054 g) had insignificant disparity from the treatment of 200  $\mu$ M methyl jasmonate and 54 kg  $P_2O_5$ .ha<sup>-1</sup> ( $J_2F_3$ ) 12.073 ( $\pm$  180.464 g) phosphorous fertilizer. The lowest leaf fresh weight was obtained when methyl jasmonate was given at 200  $\mu$ M but phosphorous fertilizing ( $J_2F_0$ ) was absent in 7.389 ( $\pm$  73.0 g). The interaction of these two factors significantly affected the weight of the fresh leaves and petioles per plot. On a fresh weight per largest plot in treatment provision contained methyl jasmonate 100  $\mu$ M and phosphorus fertilization 18 kg  $P_2O_5$ .ha<sup>-1</sup> ( $J_1F_1$ ) was obtained at the lowest fresh weight of 200  $\mu$ M and without giving phosphorus. This can occur because jasmonate acids are organic compounds that has function to inhibit the growth of certain plant parts and very strong encourage leaf senescence (Zeneli, Krokene, Christiansen, Krekling, & Gershenzon, 2006) stated that treatment with methyl jasmonate causing terpene concentration increased (The treatment of giving methyl jasmonate and phosphorous fertilizing can be seen in Fig.2).

**Table 1.** Average disparity test on pegagan leaf and petiole fresh weight (g) in the interaction between the treatment of harvesting time and methyl jasmonate

Clone codes	Methyl Jasmonate (J)			Average
	$J_0 = 0 \mu\text{M}$	$J_1 = 100 \mu\text{M}$	$J_2 = 200 \mu\text{M}$	
$H_1 = 56$	61.670 c	97.671 bc	73.527 c	77.623
$H_2 = 70$	115.242 b	152.057 ab	166.418 ab	144.572
$H_3 = 84$	188.511 a	141.313 ab	121.867 bc	150.564
<b>Average</b>	121.81	130.35	120.60	

DAP = days after planting

Noverita Sprinse Vinolina et al.: *Centella asiatica* Production.....



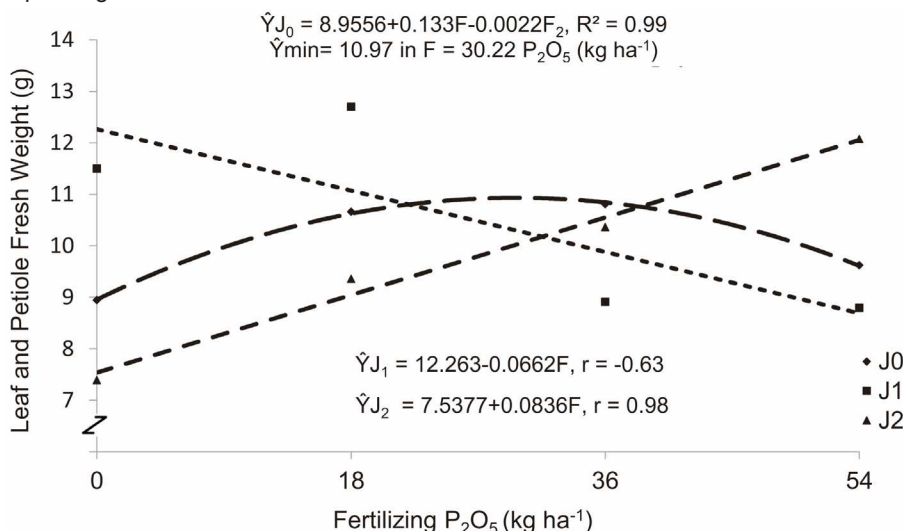
Remarks:  $H_1$  = harvesting time of 56 DAP (day after planting),  $H_2$  = harvesting time of 70 DAP, and  $H_3$  = harvesting time of 84 DAP

**Fig.1.** The influence of harvesting time and the giving of methyl jasmonate on leaf and petiole fresh weight (g)

**Table 2.** Average disparity test on leaves and petioles (g) in the interaction between the treatment of giving methyl jasmonate and fertilizing phosphorous

Clone codes	Methyl Jasmonate (J)				Average
	F <sub>0</sub> (0)	F <sub>1</sub> (18)	F <sub>2</sub> (36)	F <sub>3</sub> (54)	
J <sub>0</sub> = 0	109.324 bc	138.771 abc	126.897 abc	112.293 abc	121.808
J <sub>1</sub> = 100	148.830 ab	197.054 a	86.533 bc	88.970 bc	130.347
J <sub>2</sub> = 200	73.001 c	100.787 abc	128.165 abc	180.464 ab	120.604
<b>Average</b>	110.385	145.537	113.865	127.224	

DAP = days after planting



Remarks: Giving of methyl jasmonate J<sub>0</sub> = 0 µM, J<sub>1</sub> = 100 µM, and J<sub>2</sub> = 200 µM

**Fig.2.** The influence of phosphorous fertilizer in various concentrations of methyl jasmonate on leaf and petiole fresh weight (g)

In the absence of methyl jasmonate, the pegagan produced leaf and petiole fresh weight maximally at 10.97 ( $\pm$  128 g) when they were fertilized with phosphorous fertilizer of 30.22 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup>. In the presence of methyl jasmonate at 100  $\mu$ M, leaf and petiole fresh weight decreased when phosphorous fertilizer was given at 54 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup> (negative linear), but the presence of methyl jasmonate at 200  $\mu$ M and phosphorous fertilizer of 54 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup> increased leaf and petiole fresh weight. In this case, there was an interaction of phosphorus and methyl jasmonate, in influencing the fresh weight of leaves and petioles. As it was already mentioned above, the provision of methyl jasmonate would affect the concentration of terpenes and interacted with phosphorus in influencing the growth. In the previous study, the elicitor treatment in vitro culture did not only increase the production of saponins but could change the stoichiometric precursor final product. Elicitation not only affected the levels of saponins but also affected the saponin biosynthesis gene expression (Kim, O. T., Kim, M. Y., Hong, Ahn, & Hwang, 2004; Mangas *et al.*, 2006). The effect increased with increasing concentrations of elicitor (Bonfill, Mangas, Moyano, Cusido, & Palaz3n, 2011). In addition, it also served as a constituent of phosphorus metabolites and complex compounds, activators, cofactors or constituent of enzymes, as well as played its role in physiological processes (Salisbury & Ross, 1995).

#### Root and Tendril Fresh Weight

Table 3 shows that the highest root and tendril fresh weight are obtained in the treatment of harvesting time of 84 DAP and without methyl jasmonate (H<sub>3</sub>J<sub>0</sub>) 13.209 ( $\pm$  194.059 g). However, there was insignificant disparity between harvesting time of 70 DAP and when jasmonate was given at 100  $\mu$ M (H<sub>2</sub>J<sub>1</sub>) 12.565 ( $\pm$  190.542 g), followed by 70 DAP harvesting time with methyl jasmonate of 200  $\mu$ M (H<sub>2</sub>J<sub>2</sub>) 11.562 ( $\pm$  172.338 g), harvesting time of 84DAP and methyl jasmonate of 200  $\mu$ M (H<sub>3</sub>J<sub>2</sub>) 11.175 ( $\pm$  155.663 g) consecutively. The same

thing occurred in the growth of roots and tendrils, without giving methyl jasmonate it would produce the highest biomass weight. The lowest root and tendril fresh weight was noted from harvesting time of 56 DAP, without methyl jasmonate 0  $\mu$ M (H<sub>1</sub>F<sub>0</sub>) 6.801 ( $\pm$  50.383 g). Early harvesting would also affect the weight of the biomass, that the weight of fresh or accumulation of photosynthate produced would also be less. There was an interaction between the harvesting and delivery of methyl jasmonate where methyl jasmonate was given at age 50 DAP (The influence of harvesting time and the giving of methyl jasmonate treatment can be seen in Fig.3).

Fig.3 shows that the harvesting time of 56 DAP produces maximum root and tendril fresh weight of 8.70 ( $\pm$  85 g) when methyl jasmonate was given at 97.50  $\mu$ M. A longer harvesting time of 70 DAP increased root and tendril fresh weight to 9.59 ( $\pm$  119 g) when methyl jasmonate was given at 134.5  $\mu$ M (negative linear), by giving methyl jasmonate, suppression growth of leaves and petioles, but roots and tendrils tried to defend themselves by increasing the growth of roots and tendrils.

#### Leaf and Petiole Dry Weight (g)

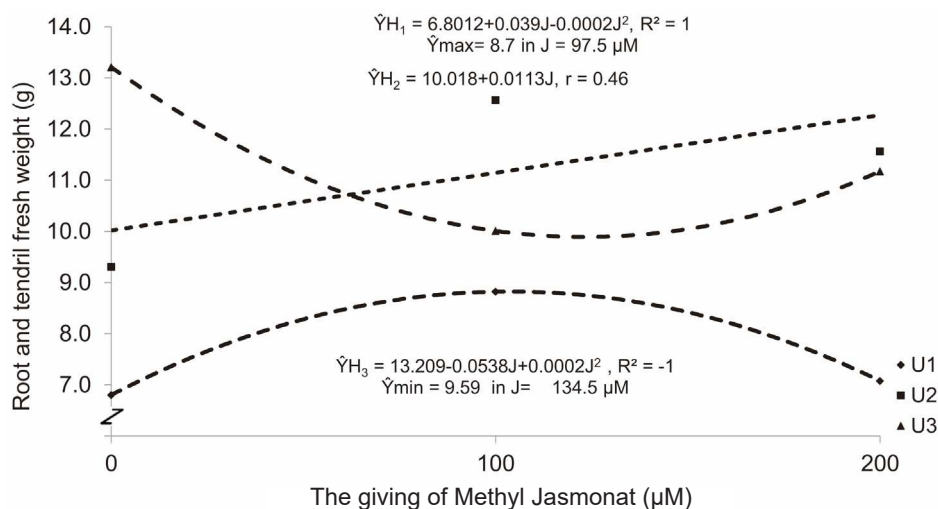
The three treatment factors and their interactions had insignificant influence on leaf and petiole dry weight (g). It was assumed that concentrations of methyl jasmonate given to the plants caused a lower ability in phosphate absorption, especially due to aging of leaves and roots following to the methyl jasmonate application. It was obviously clear that the higher treatment was given if the faster aging process existed. Reinbothe, C., Springer, Samol, & Reinbothe, S. (2009) stated that methyl jasmonate affecting the process of leaf aging led to cell's death and did not significantly affect the dry weight of leaves and petioles, since the methyl jasmonate increased plant's resistance to environmental factors (Lambert, Faizal, & Geelen, 2011). Current study noted that the pegagan plants were severely attacked by several diseases.

**Table 3.** Average disparity test of root and tendril fresh weight (g) of pegagan in the treatment of harvesting time and methyl jasmonate

Harvesting time (H) (DAP)	Methyl Jasmonate (J)			Average
	J <sub>0</sub> = 0 $\mu$ M	J <sub>1</sub> = 100 $\mu$ M	J <sub>2</sub> = 200 $\mu$ M	
H <sub>1</sub> = 56	50.383 e	85.824 cde	62.266 de	66.158
H <sub>2</sub> = 70	110.264 cde	190.542 ab	172.338 abc	157.714
H <sub>3</sub> = 84	194.059 a	128.400 bcd	155.663 abc	159.374
<b>Average</b>	118.24	134.92	130.09	

DAP = days after planting

Noverita Sprinse Vinolina et al.: *Centella asiatica* Production.....



Remarks:  $H_1$  = harvesting time of 56 DAP (day after planting),  $H_2$  = harvesting time of 70 DAP, and  $H_3$  = harvesting time of 84 DAP

**Fig.3.** The influence of giving of methyl jasmonate in various harvesting times on root and tendril fresh weight (g)

Plant growth highly depended on soil nutrients availability; Phosphorus (as phosphate,  $PO_4^{3-}$ ) is an integral component of important compounds of plant cell, especially along their life activities like carbohydrate metabolisms, photosynthesis, and energy cycles (Taiz & Zeiger, 2006). Phosphate also affected the asiaticoside content, a bioactive in pegagan leaves, as well as asiatic acids. Vinolina, Napitupulu, Siregar, Nainggolan, & Singh (2013) reported that the total amount of soil phosphate affected centelloside content of the pegagan. Phosphate stimulating the roots formation led to a better ability in absorbing nutrients and water, increasing total chlorophylls, increasing photosynthesis ability to produce photosynthates and presumably increasing the content of asiaticoside element.

Salisbury & Ross (1995) pointed out that the plant had never changed the form of phosphate, and thus it would be in its form as phosphate, either as phosphate itself or as binding to ester. Ester phosphate might come from sugar, alcohol, acids or polyphosphate that may function intermediate way of phosphate pentosa of primary metabolite and is derived as a precursor of secondary metabolite. Pegagan mostly contains triterpenoid, a derivative of primary metabolite along the mevanolate biosynthesis to produce geraniles pyrophosphate which leads to a formation of monoterpenoid and its derivatives. The pharnesyl pyrophosphate, however, increases production of sesquiterpenoid and conversion of

squalen to become triterpenoids. Vinolina, Napitupulu, Siregar, Nainggolan, & Singh (2013) noted that soil phosphate affected the asiaticoside, madecacoside, and asiatic acids of the dead plants.

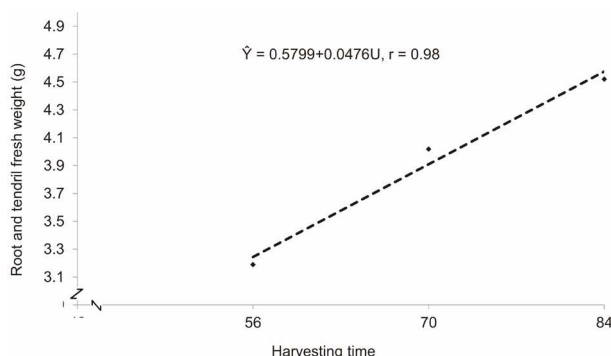
#### Root and Tendril Dry Weight (g)

Table 4 shows that root and tendril dry weight taken from the harvesting time of 84 DAP was 4.521 ( $\pm$  23.953 g) and had insignificant disparity to shorter of 70 DAP but had significant disparity when harvesting time was much shorter than 56 DAP (The disparity of harvesting time from root and tendril dry weight can be seen in Fig. 4). The influence of harvesting time on root and tendril dry weight was positive; the increase in harvesting time would cause root and tendril dry weight to increase. Dry weight of the roots and tendrils would increase longer harvest time. Growth continues at harvest age of 56, 70 and 84 DAP so that biomass will continue to increase with the accumulation of photosynthetic on increasing dry weight of roots and tendrils (Salisbury & Ross, 1995)

**Table 4.** Average disparity test on root and tendril dry weight (g) of pegagan in harvesting time treatment

Harvesting time (H) (DAP)	Average
$H_1 = 56$	12.291 b
$H_2 = 70$	19.037 a
$H_3 = 84$	23.953 a
<b>Average</b>	<b>18.427</b>

DAP = days after planting



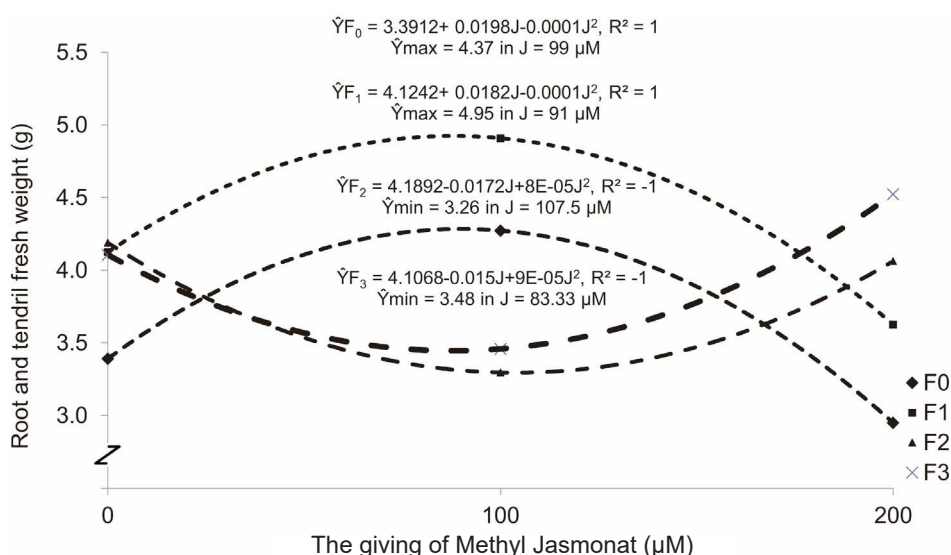
**Fig.4.** The influence of harvesting time on root and tendril dry weight (g)

Average disparity test of methyl jasmonate and phosphorous fertilizing on root and shoot dry weight is presented in Table 5. Table 5 shows that the highest root and tendril dry weight in the combination treatment of methyl jasmonate at 100

$\mu\text{M}$  and phosphorous fertilizing of  $18 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  ( $J_1F_1$ )  $4.909 (\pm 29.046 \text{ g})$ . These treatments had insignificant disparity from the combination of methyl jasmonate at  $200 \mu\text{M}$  and phosphorous fertilizing of  $54 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  ( $J_2F_3$ ), followed by methyl jasmonate and other phosphorous fertilizing:  $J_1F_0$ ,  $J_0F_2$ ,  $J_0F_1$ ,  $J_2F_2$ , and  $J_2F_1$  which had significant disparity from other treatments. The lowest root and tendril dry weight was noted from the combination treatment of methyl jasmonate  $200 \mu\text{M}$ , without phosphorous fertilizing ( $J_2F_0$ )  $2.950 (\pm 10.5 \text{ g})$  which had significant disparity from other treatments. Dry weight was the lowest for the treatment of awarding methyl jasmonate  $200 \mu\text{M}$  and without giving phosphorus, this was due to methyl jasmonate which was organic compounds formed by biosynthesis by the enzyme and served to inhibit the growth of some parts of certain plants and highly encouraged leaf senescence (Salisbury & Ross, 1995).

**Table 5.** Average disparity test of root and tendril dry weight (g) in the interaction of methyl jasmonate and phosphorous fertilizing

Methyl jasmonat(J) ( $\mu\text{M}$ )	Phosphorous fertilizing (F)				Average
	$F_0$ ( $0 \text{ kg P}_2\text{O}_5 \cdot \text{ha}^{-1}$ )	$F_1$ ( $18 \text{ kg P}_2\text{O}_5 \cdot \text{ha}^{-1}$ )	$F_2$ ( $36 \text{ kg P}_2\text{O}_5 \cdot \text{ha}^{-1}$ )	$F_3$ ( $54 \text{ kg P}_2\text{O}_5 \cdot \text{ha}^{-1}$ )	
$J_0 = 0$	15.067 bc	20.134 abc	18.306 abc	21.759 abc	18.817
$J_1 = 100$	20.228 abc	29.046 abc	11.237 abc	14.238 abc	18.687
$J_2 = 200$	10.524 e	14.870 abc	19.863 abc	25.853 abc	17.778
<b>Average</b>	15.273	21.350	16.469	20.619	



Remarks: Giving phosphorous:  $F_0 = 0 \text{ kg P}_2\text{O}_5 \cdot \text{ha}^{-1}$ ;  $F_1 = 18 \text{ kg P}_2\text{O}_5 \cdot \text{ha}^{-1}$ ;  $F_2 = 36 \text{ kg P}_2\text{O}_5 \cdot \text{ha}^{-1}$ ;  $F_3 = 54 \text{ kg P}_2\text{O}_5 \cdot \text{ha}^{-1}$

**Fig.5.** The influence of giving methyl jasmonate in various dose times of phosphorous on root and tendril dry weight (g)

The impact of methyl jasmonate on plant health and long-term anatomical and chemical changes caused by methyl jasmonate application on the growth and survival are unknown. Treatment of methyl jasmonate was proven to prevent the death of trees caused by bark beetles (Erbilgin, Krokene, Christiansen, Zeneli & Gershenzon, 2006). Phosphorus also greatly affects optimal growth, because its role is an essential part of the various sugar phosphates that play their role in the reactions to the dark phase of photosynthesis, respiration, and a variety of other metabolic processes. Phosphorus is also a part of nucleotides (RNA and DNA) and phospholipid membrane constituent (Salisbury & Ross, 1995). (The influence of giving methyl jasmonate and phosphorous fertilizing can be seen in Fig. 5).

At present, methyl jasmonate 200  $\mu\text{M}$ , root and tendril dry weight increased when phosphorous fertilizer was given at 54 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ , but root and tendril dry weight decreased when a lower concentration of methyl jasmonate (100  $\mu\text{M}$ ) was given

## CONCLUSION AND SUGGESTION

### Conclusion

The technology for optimum production of pegagan can use phosphorus fertilizers at 18 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ . The production of biomass (leaves, petioles, tendrils) is optimum when the methyl jasmonate hormone is absent. The technology to increase pegagan production with appropriate harvesting time and for optimum production is 84 DAP or over.

There was an interaction between concentration of methyl jasmonate hormone and harvesting time toward fresh weight of either leaves and petioles or roots and tendrils, and the best combination is without methyl jasmonate hormone and harvesting time of 84 DAP. There was no interaction effect among the three factor concentrations, methyl jasmonate and harvesting time toward all production parameters.

### Suggestion

Harvesting can be done on the parts of leaves and roots. In low land, the highest root and tendril dry weight was found in the treatment of methyl jasmonate (100  $\mu\text{M}$ ) and phosphorous fertilizing of 18 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ .

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Noverita Sprinse Vinolina *et al.*: *Centella asiatica* Production.....

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