Application of chitosan for vegetative growth of kemiri sunan plant in marginal land

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Abstract. Kemiri sunan [*Reutealis trisperma* (Blanco) Airy Shaw] is a vegetable-oil producing plant with great potential to be developed as biodiesel feedstock. However, to develop Kemiri Sunan on marginal land, there is a necessity to innovate the cultivation including by applying fertilization or providing nutrients with plant supplements such as chitosan. This study used a Randomized Complete Block Design consisting of 2 factors. The first factor was the concentration of chitosan per plant: 10, 20, 30 and 40 ml/l. The second factor was the frequency of chitosan application: 3-, 4- and 5-time provision. The controlling variable was without chitosan application. The data were analyzed for the diversity and continued with Duncan Multiple Range Test at the level of 5%. The concentration of 20 and 30 ml/l chitosan and the frequency of 4-time chitosan resulted a better effect than other treatments. The vegetative growth of plants with chitosan administration was shown to have a better growth response than those without chitosan (control).

1. Introduction

Kemiri sunan [*Reutealis trisperma* (Blanco) Airy Shaw] is a vegetable oil producing plant of great potential for biodiesel feedstock, a newly developed alternative in Indonesia. It can also function as conservation plant because it has a rapid growth and deep root to withstand landslides and erosion so as to reclaim marginal lands. This plant is known to be highly adaptable to the environment, both in dry land and wet climate, strongly and deeply rooted, and survives on sloping land to withstand erosion. In addition, its lush canopy and its wide and dense leaves can absorb CO_2 and produce O_2 because the leaves will fall out in the dry season to form thick humus as a soil fertilizer.

Kemiri sunan is annual plant that can live and produce seeds until decades of age. The seeds production of this plant amounting 4-6 tons of dry seeds per hectare per year, equivalent to 2-3 tons of crude oil per hectare per year. Moreover, when extracted, the seeds will produce vegetable oil. Kemiri sunan's oil contain 50% of oleo stearic acid, a compound making kemiri sunan poisonous. This oil can be used for various purposes such as natural insecticides known to be very effective for killing pests [1], industrial raw materials for paints, varnishes, inks, wood preservatives, cosmetics, and pharmaceuticals. Given its various uses, kemiri sunan is definitely a potential plant for development.

On this basis, it is noteworthy to take heed on the vegetative growth of the plant, because its cultivation requires good management to assure the good yield. The cultivation phase taken place in a relatively long period of time will surely determine the production of crops per hectare, considering that kemiri sunan is classified as annual crops. In this research, kemiri sunan were planted in the Gunung Kelir Energy Garden as a garden model developed in collaboration between the Center for Research and

Development of Electricity Technology, New, Renewable Energy and Energy Conservation (P3TKEBTKE) Ministry of Energy and Mineral Resources of the Republic of Indonesia with The University of National Development "Veteran" Yogyakarta. This garden comprises of a dry hilly land and is categorized as marginal land with limited availability of water and nutrients and thin soil. To develop kemiri sunan on marginal land, it is necessary to invent the cultivation process by applying fertilization or providing nutrients with plant supplements using chitosan.

Chitosan is an organic nutrient derived from the processing of skin waste or shrimp shells, crabs, molds and others whose solutions contain macro and micro nutrients, and plant hormones to increase plant antibodies. Chitosan is environmentally friendly and easily degraded [2]. Based on test quality, chitosan contains 6.74% C-organic; 0.05% N; 0.01% P_2O_5, and 0.01% K_2O. It also constitutes of some degree of micro elements such as Fe, Cu, Zn and B of 8 ppm, 0.8 ppm, 7 ppm and 1 ppm respectively. The micro elements of Mn, Zn and Mo are not detected. Meanwhile, the heavy metal content of Cd was detected by only 0.02 ppm, while other heavy metals such as Pb, Co, As and Hg were not detected. Per solution of chitosan also contains growth and production hormone such as auxin (IAA) 319.11 ppm, cytokinin (zeatin) 18.46 ppm and gibberellin (GA3) 252.48 ppm [3].

Chitosan has been widely used in various plant commodities, among others to increase nitrogen fixation in soybean plants [4]; increase the germination and rooting of radish plants [5]; accelerate flowering time and increase the number of flowers in passion fruit plants [6]. In addition, it enhances plant and root growth in vines, accelerates flowering time, increases the number of flowers, fruit weight and yield of grapes [7]. The administration of chitosan is known to have a wide range of uses as it comes up with high affinity, contains zero toxic, is easily degraded, and is purely derived from nature. Chitosan regulates the plant's immune system and causes the excretion of an opposing enzyme. Moreover, chitosan not only activates cells, but also enhances defense capabilities against diseases and insects. Chitosan functions as a carbon source for microbes in the soil, accelerates the process of transforming organic compounds into inorganic compounds and helps root systems in plants to absorb more nutrients from the soil. This is so because chitosan is easily absorbed by the roots after being decomposed by bacteria in the soil [8].

In general, the administration of chitosan as an organic fertilizer plays an important role in supporting plant growth and productivity. To obtain the efficient and effective cultivation and growth of kemiri sunan, it is necessary to provide it with appropriate nutrient, both in terms of concentration and frequency. Appropriate concentration means the concentration measured based on the needs of the plant. On the other hand, inappropriate concentration leads to inefficient growth, and even damage to plants. The proper frequency of fertilizer provision means adjusting the provision based on the right time when the plant needs nutrient intake. This is intended so that plants can grow and develop optimally. The frequency of 3-time administration of chitosan is known to lead to a better effect on chitosan growth at 18 months [9].

Improved cultivation systems, especially in fertilization administrations, are expected to spur plant growth. Variations in chitosan treatment accordingly lead to varied results in each type of plant [10]. This pinpoints the fact that generally hormones act synergistically to promote responses, instead of working independently. This leads to the need for further research to determine the concentration and frequency of chitosan administration in young kemiri sunan. Therefore, this study investigated the application of chitosan for vegetative growth of kemiri sunan plant in marginal land.

2. Materials and methods

The study was conducted as a field research in an energy garden located in Gunung Kelir, Pleret Village, Pleret Subdistrict, Bantul Regency, Yogyakarta Special Province from January to May 2018. It involved some materials including Kemiri Sunan seedlings of high yielding varieties (KS2) ± 36 months old, chitosan, and insecticides.

The study used a Randomized Complete Block Design consisted of 2 factors. The first factor was the concentration of chitosan (C) per plant, consisted of 4 levels, namely: C1 (10 ml/l), C2 (20 m/l), C3 (30 ml/l), and C4 (40 ml/l). The second factor was the frequency of chitosan (F) application, consisted

of 3 levels, namely: F1: 3 times at 20, 40 and 60 days from the start of the observation, F2: 4 times at 15, 30, 45, and 60 the day from the start of the observation, and F3: 5 times at 20, 30, 40, 50 and 60 days from the start of the observation. These two factors resulted in 12 treatment combinations, which were repeated three times, with each of which consisted of 3 plants. Meanwhile, the researcher used kemiri sunan plant without any treatment of chitosan as the control. Thus, the total number involved amounted to 111 Sunan Kemiri plants.

The study was prepared with the selection and assortment of plants in the field to be included as the research object. As the initial phase of the observation, the selected plants were then observed and measured to see their agronomic character. The selected numbers of plants were then planted with a distance of 8 m × 8 m, within an area of ±7000 m^2. These plants were then treated with chitosan application by way of pouring the chitosan fertilizer to the roots of kemiri sunan.

The agronomic characters for vegetative growth of kemiri sunan were observed through the parameters of plant height (cm), number of leaves (strands), and the increase in stem diameter (cm), number of secondary branches, and width of leaf canopy (cm).

The resulted data were analyzed to see their diversity at the 5% level. To find out the differences between levels, the analysis was continued with Duncan Multiple Range Test at the level of 5%.

3. Results and discussions

3.1. Plants height

There were no interactions on plants treated with chitosan on the parameters of the initial plant height and on the height increase of plants aged 50, 70 and 90 days. The mean value of the initial plant height and height increase of plants aged 50, 70 and 90 days are presented in Table 1. Table 1 delineates that the treatment of concentration and frequency of chitosan application did not significantly affect plant height parameters at the age of 50, 70, and 90 days. Plants that were given chitosan had an average height increase of at the age of 70 days and 90 days which were significantly different from and higher than plants that were not given chitosan (control).

Table 1. Average height of plant at the initial observations and height increase of plants aged 50, 70 and 90 days (cm)

Treatments	Initial Observation (cm)	Height Increase of Plants (cm)		
		50 days	70 days	90 days
Chitosan concentration				
C1 (10 ml/l)	275.22^{a}	9.11^{a}	10.00^{a}	8.37^{a}
C2 (20 ml/l)	255.74^{ab}	7.93^{a}	14.48^{a}	10.89^{a}
C3 (30 ml/l)	225.41^{b}	4.78^{a}	11.56^{a}	15.93^{a}
C4 (40 ml/l)	254.33^{ab}	9.19^{a}	13.26^{a}	13.48^{a}
Chitosan frequency				
F1 (3 times)	251.36^{p}	7.72^{p}	12.06^{p}	10.31^{p}
F2 (4 times)	259.90^{p}	7.44^{p}	14.47^{p}	14.64^{p}
F5 (5 times)	247.22^{p}	8.08^{p}	10.44^{p}	11.56^{p}
Combined treatment	252.67^{x}	7.75^{x}	12.32^{x}	12.17^{x}
Without chitosan	251.89^{x}	6.33^{x}	4.33^{y}	4.11^{y}
Interaction	(-)	(-)	(-)	(-)

Average treatment between columns and rows followed by the same letter shows no significant difference in DMRT test at the 5% level. The sign (-) indicates no interaction.

3.2. Number of leaves

There were no interactions on the plants to be treated with chitosan in the parameters of the initial number of leaves observed and on the number of leaves at 30, 50, 70 and 90 days. The average number of leaves at the beginning of the observation and the increase in the number of leaves aged 30, 50, 70 and 90 days are presented in Table 2. It is prominent that the treatment of chitosan concentration

significantly affected the increasing number of leaves for the plants aged 30, 50 and 90 days. In addition, it is noteworthy that the concentration of chitosan which resulted in the greatest number of leaves was at a concentration of 20 ml/l and 30 ml/l. For the frequency of chitosan application, it is obvious that there was no significant effect on the number of leaf parameters at the ages of 30, 50, 70, and 90 days. Plants that were given chitosan resulted in more average number of leaves than plants with no chitosan (control).

Table 2. Average number of leaves at the observations and number of leaves aged 30, 50, 70 and 90 days (sheets)

Treatments	Initial observation (sheets)	Increasing Number of Leaves (Sheets)			
		30 days	50 days	70 days	90 days
Chitosan concentration					
C1 (10 ml/l)	166.15^{a}	10.33^{b}	23.26^{ab}	15.41^{a}	12.30^{b}
C2 (20 ml/l)	187.85^{a}	10.67^{b}	35.89^{a}	14.44^{a}	15.07^{a}
C3 (30 ml/l)	118.04^{a}	11.89^{b}	36.30^{a}	17.07^{a}	15.56^{a}
C4 (40 ml/l)	171.30^{a}	20.96^{a}	18.33^{b}	17.67^{a}	14.11^{ab}
Chitosan frequency					
F1 (3 times)	156.97^{p}	13.14^{p}	24.44^{p}	16.44^{p}	13.42^{p}
F2 (4 times)	149.72^{p}	11.94^{p}	33.64^{p}	15.31^{p}	13.64^{p}
F5 (5 times)	175.81^{p}	15.31^{p}	27.25^{p}	16.69^{p}	15.72^{p}
Combined treatment	160.83^{x}	13.36^{x}	28.44^{x}	16.15^{x}	14.26^{x}
Without chitosan	175.44^{x}	7.33^{y}	22.00^{y}	7.36^{y}	8.56^{y}
Interaction	(-)	(-)	(-)	(-)	(-)

Average treatment between columns and rows followed by the same letter shows no significant difference in DMRT test at the 5% level. The sign (-) indicates no interaction.

3.3. Stem diameter

The observation of various increase of stem diameter aged 30 days and 50 days indicated no interaction between the treatment of chitosan concentration and the frequency of chitosan application. The treatment of chitosan concentration and the frequency of each chitosan application had no significant effect on the increase of stem diameter aged 30 and 50 days. The mean value of the increase in stem diameter of 30 and 50 days is presented in Table 3.

Table 3. Average increase of stem diameter aged 30 and 50 days (cm)

Treatments	Increase of Stem Diameter (cm)	
	30 days	50 days
Chitosan concentration		
C1 (10 ml/l)	1.58^{a}	0.87^{a}
C2 (20 ml/l)	1.60^{a}	1.23^{a}
C3 (30 ml/l)	1.19^{a}	0.84^{a}
C4 (40 ml/l)	1.70^{a}	0.94^{a}
Chitosan frequency		
F1 (3 times)	1.81^{p}	0.80^{a}
F2 (4 times)	1.36^{p}	1.04^{a}
F5 (5 times)	1.38^{p}	1.07^{a}
Combined treatment	1.52^{x}	0.97^{x}
Without chitosan	1.49^{x}	1.55^{x}
Interaction	(-)	(-)

Average treatment between columns and rows followed by the same letter shows no significant difference in DMRT test at the 5% level. The sign (-) indicates no interaction.

The treatment of chitosan concentration and the frequency of chitosan application showed at 30 and 50 days, stem diameter increase response which did not differ between the levels. Plants given chitosan showed no different effect on the average increase in stem diameter compared to plants that were not given chitosan (control).

3.4. Width of leaf canopy

The observation of the width increases of canopy leaf growth aged 30, 50 and 70 days pinpointed that there was no interaction between the treatment of chitosan concentration and the frequency of chitosan application. The treatment of chitosan concentration and the frequency of chitosan application did not significantly influence the width of canopy leaves aged 30, 50 and 70 days. The mean value of the width of the canopy leaf growth aged 30, 50 and 70 days is presented in Table 4.

Table 4 shows that the increase in the width of the canopy of leaves aged 30, 50 and 70 days had no significant difference between each level of chitosan concentration and frequency of chitosan administration. Plants that were given chitosan showed a wider average width of the leaf canopy at the age of 50 and 70 days than the plants that were not given chitosan (control).

Table 4. The average width of the canopy leaf growth aged 30 days, 50 days and 70 days (cm)

Treatment	Width Increase of Canopy Leaves (cm)		
	30 days	50 days	70 days
Chitosan concentration			
C1 (10 ml/l)	13.07^{a}	14.63^{a}	15.15^{a}
C2 (20 ml/l)	14.30^{a}	22.92^{a}	13.19^{a}
C3 (30 ml/l)	18.30^{a}	8.11^{a}	11.11^{a}
C4 (40 ml/l)	24.48^{a}	14.07^{a}	13.26^{a}
Chitosan frequency			
F1 (3 times)	17.17^{p}	13.00^{p}	13.33^{p}
F2 (4 times)	19.36^{p}	10.44^{p}	14.64^{p}
F5 (5 times)	16.08^{p}	21.36^{p}	11.56^{p}
Combined treatment	17.53^{x}	14.94^{x}	13.18^{x}
Without chitosan	23.33^{x}	9.56^{y}	8.11^{y}
Interaction	(-)	(-)	(-)

Average treatment between columns and rows followed by the same letter shows no significant difference in DMRT test at the 5% level. The sign (-) indicates no interaction.

The results of the variability of stem diameter and width of leaf canopy at the beginning of observation and height increase at 30 days, increase in stem diameter at 70 and 90 days, number of secondary branches aged 90 days and width of leaf canopy at 90 days showed that there were interactions between the treatment of chitosan concentration and frequency giving chitosan. The mean value of stem diameter and width of leaf canopy at the beginning of observation and height increase of 30 days age plants, increase in stem diameter aged 70 and 90 days, increase in the number of secondary branches at 90 days and width increase of canopy leaf aged 90 days are presented in Table 5.

The combined treatment of 20 ml/l chitosan concentration and the frequency of chitosan administration of 4 times (C2F2) gave the highest increase in the 30-day aged plant of all other combined treatments. The combined treatment of 20 ml/l chitosan concentration and the frequency of chitosan application 5 times (C2F3) gave the largest increase in stem diameter of 70 and 90 days of all other combined treatments. The combined treatment of 20 ml/l chitosan concentration treatment and the frequency of chitosan application of 3 times (C2F1) gave the highest number of secondary branches aged 90 days compared to other combined treatments. The combined treatment of 30 ml/l chitosan concentration treatment and 4 times the frequency of chitosan application (C3F2) gave the widest increase in the width of leaf canopy aged 90 days compared to all other combined treatments.

In addition, it is also prominent that chitosan can be an alternative fertilizer to increase the vegetative growth of kemiri sunan. Of the observed parameters, it is revealed that the vegetative growth of the

kemiri sunan which was given chitosan showed a better growth rate than those not given chitosan (control). Increasing plant growth due to chitosan application is mainly triggered by chitosan's ability in improving plant metabolism. Chitosan is a form of polysaccharide that functions as a biological signal in cells and is able to regulate symbiotic defenses, as well as plant development processes [11]. Chitosan contains plant growth promotor in the form gibberellin, IAA, and Zeatin [12].

Table 5. Effect of chitosan concentration and frequency of chitosan administration on plant characteristics of kemiri sunan.

Combined Treatment	Initial observation			Increase			
	Stem Diameter	Width of Leaf Canopy	Plant Height 30 Days	Stem Diameter		Number of Secondary Branches 90 Days	Width of Leaf Canopy 90 Days
				70 days	90 days		
C1F1	17.79^{ab}	190.11^{b}	5.89^{c}	1.33^{bc}	1.32^{b}	2.78^{b}	14.11^{cd}
C1F2	19.32^{a}	233.00^{a}	13.22^{b}	1.17^{bc}	1.29^{b}	3.00^{b}	12.00^{d}
C1F3	16.62^{ab}	210.44^{ab}	14.11^{b}	0.91^{c}	1.22^{b}	3.44^{b}	13.11^{d}
C2F1	18.93^{b}	220.44^{ab}	5.69^{c}	1.57^{bc}	1.16^{b}	7.11^{a}	15.33^{cd}
C2F2	14.68^{b}	166.56^{bc}	22.44^{a}	0.78^{c}	1.43^{b}	3.11^{b}	20.33^{bc}
C2F3	17.34^{ab}	180.11^{b}	16.67^{ab}	2.83^{a}	2.23^{a}	3.56^{b}	23.33^{b}
C3F1	15.02^{b}	138.22^{c}	10.11^{b}	1.41^{bc}	1.34^{b}	3.44^{b}	15.00^{cd}
C3F2	10.91^{c}	145.78^{c}	16.78^{ab}	2.13^{b}	1.49^{b}	2.67^{b}	39.22^{a}
C3F3	13.17^{bc}	143.00^{c}	5.33^{c}	1.16^{bc}	1.33^{b}	3.22^{b}	16.22^{cd}
C4F1	12.47^{bc}	143.33^{c}	15.89^{b}	0.54^{c}	1.43^{b}	2.78^{b}	20.22^{bc}
C4F2	16.13^{ab}	167.78^{bc}	4.57^{c}	1.32^{bc}	1.78^{ab}	3.33^{b}	16.89^{cd}
C4F3	18.71^{b}	234.67^{a}	17.67^{ab}	0.99^{c}	1.38^{b}	2.89^{b}	14.67^{cd}
Average Combined Treatment	15.84^{x}	181.12^{x}	12.36^{x}	1.35^{x}	1.45^{x}	3.44^{x}	18.37^{x}
Without Chitosan	17.28^{x}	196.89^{x}	10.89^{x}	1.17^{x}	0.94^{y}	2.78^{x}	9.67^{y}

Average treatment between columns and rows followed by the same letter shows no significant difference in DMRT test at the 5% level

The stem is main source of plant growth, especially young plants [13]. Thus, the plant growth promoter of chitosan increases the rate of photosynthesis in producing photosynthate, thus helping in the enlargement of the stem. The increase in stem diameter is due to fairly good plant growth, because the nutrients are sufficiently available. Good growth is indicated by the higher rate photosynthesis. More carbohydrates are translocated via phloem and can be used to stimulate secondary growth, which is the expansion of stem cells and is indicated by a wider stem diameter.

To optimize vegetative growth, the application of chitosan should be done at the proper concentration and frequency. On the basis of this study, chitosan concentration of 20 ml/l and 30 ml/l are the most appropriate concentration. Appropriate concentration is intended to administer the plants with something exactly needed, not too much or too little because inadequate concentration of chitosan application will lead to stunted plant growth. On the other hand, inaccurate concentration means that the provision of nutrients is not in accordance with the needs of plants or nutrients because it is excessive and cause inefficiencies in plants and even harms. The frequency of chitosan application at 4 times is sufficient to support the vegetative growth of kemiri sunan. The proper chitosan application enriches the availability of nutrients in the soil needed by plants to support their growth.

4. Conclusion

There is an interaction between the treatment of chitosan concentration and the frequency of chitosan application to all vegetative growth of observed parameters of kemiri sunan except for the number of

leaves. The concentration of chitosan at 20 and 30 ml/l gives a better effect on most of the parameters observed. The frequency of 4-time chitosan application gives a better effect on most of the parameters observed. Plants that are treated with chitosan have better vegetative growth than those without chitosan (control).

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