THE INFLUENCE OF CONCENTRATION AND FREQUENCY OF CHITOSAN DISTRIBUTION ON THE VEGETATIVE GROWTH OF KEMIRI SUNAN PLANT

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ABSTRACT

Kemiri sunan (*Reutealis trisperma* (Blanco) Airy Shaw) is one of the plants that produce vegetable oil which can be processed into biodiesel and can be planted on marginal land. In order to have a better vegetative growth of plants, fertilization technology can be carried out on immature plants by giving chitosan. This research was carried out in Kelir Mountain energy garden from January to May 2018 using a complete randomized block design of two factors, namely concentration and frequency of chitosan distribution. The results showed that there was an interaction between the treatment of chitosan concentration and the frequency of chitosan distribution in the addition of stem diameter in 70 and 90 days after the initial observation and the increase in the number of the secondary branches on 90 days after the initial observation. The concentration of 20 and 30 ml / l chitosan and the frequency of 4 times chitosan distribution gave a better effect than other treatments. Plants that were treated with chitosan had a better vegetative growth than those without chitosan (control).

Keywords: vegetative growth, kemiri sunan, chitosan

INTRODUCTION

Kemiri sunan (*Reutealis trisperma* (Blanco) Airy Shaw) is one type of plant that contains vegetable oil which is potential as a raw material for biodiesel (Herman et al., 2009). Biodiesel from kemiri sunan has advantages over other vegetable sources, including high yields and has a long production life. Kemiri sunan plants can be used as conservation plants because their growth is fast and their roots are deep so they are able to withstand the landslides and erosion, reclaim marginal lands so it increases the economic value of the land. This plant has a high adaptability to the environment, is able to grow in dry land of wet climate, has strong and deep roots, is able to survive on sulfur land so it can withstand the erosion, its lush and its wide and dense leaves can absorb CO^2 and produce a lot of O_2 and the leaves will fall out in the dry season so that it can form a thick humus as a soil fertilizer (Pranowo and Rusli, 2012).

Kemiri sunan plants are able to produce seeds as much as 4-6 tons of dry beans per hectare per year which is equivalent to 2-3 tons of crude oil per hectare per year. Kemiri sunan seeds

when being extracted will produce vegetable oil. Vossen and Umali (2002) *cit*., Syafruddin and Wahyudi (2012) states that kemiri sunan oil containing 50% ∝-eleostearate acid is a compound that causes poisonous kemiri sunan oil so it cannot be consumed. This oil can be used for various purposes such as natural insecticides which are very effective for killing pests (Burkill, 1966 *cit*., Syafruddin and Wahyudi, 2012), industrial raw materials for paint, varnish, ink, wood preservatives, cosmetics, and pharmaceuticals. Paying attention on the various uses of kemiri sunan, this plant has the potential to be developed.

Chitosan is an organic compound derived from chitin, derived from chitin biomaterial which is currently widely used for various purposes such as plant growth promoters, natural biopesticides to protect plant crops from bacterial and fungal attacks, and as a coating material for various plant seeds (Uthairatanakij *et al*., 2007 *cit*., Anisa, 2014). The provision of chitosan in agriculture can reduce environmental stress due to the dryness or nutrient deficiency, and improve seed viability, vigor and production. The distribution of chitosan is also able to increase the chlorophyll content so it increases the effectiveness of photosynthesis (Anonim, 2016). In addition, chitosan acts as a fertilizer to strengthen the growth (Anisa, 2014). The distribution of chitosan must be done with the right concentration and frequency in order to be able to increase the vegetative growth of kemiri sunan plants.

Chitosan has a wide range of uses with high not toxic affinity, is easily degraded, and has raw materials derived from the 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 the defense capabilities against the diseases and insects. Chitosan has an effect on agriculture, for example it acts as a carbon source for microbes in the soil, accelerates the process of transforming organic compounds into inorganic compounds and helps the root system in plants to absorb more nutrients from the soil. Chitosan is absorbed by the roots after being broken down by bacteria in the soil. The usage of chitosan in agriculture, even without chemical fertilizers, increases the population of microbes in large numbers and the process of transforming nutrients from organic to inorganic which is more easily absorbed by plant roots (Boonlertnirun *et al*., 2008 *cit*., Ianca, 2010). Chitosan also contains growth regulating substances such as giberelin: GA3, GA5, GA7, Auxin (Indole Acetic Acid) and Cytokines (Kinetin and Zeatin) (Anonim, 2013).

Vegetative growth is characterized by various plant growth and development activities related to the leaf formation and enlargement, the formation of apical or lateral meristems and growth into branches, and the expansion of plant root systems. The vegetative growth of kemiri sunan plants is an important thing to know which will be expected to support better generative growth of plants so that the plants can provide an optimum productivity. Therefore, the technology innovation of kemiri sunan cultivation is done, one of which is by the chitosan distribution (Sasmita and Haryanto, 2016) by conducting research entitled "The Effect of Concentration and Frequency of Chitosan Distribution on the Vegetative Growth of Kemiri Sunan Plants".

MATERIALS AND RESEARCH METHODS

The research was conducted at the Energy Garden located in Gunung Kelir, Pleret Subdistrict, Pleret District, Bantul Regency, Special Province of Yogyakarta, which was a garden developed under the collaboration between the Center for Research and Development of Electricity Technology, New, Renewable Energy and Energy Conservation (P3TKEEBTKE) Ministry of Energy and Resources of Mineral Power of the Republic of Indonesia and Universitas Pembangunan Nasional “Veteran” Yogyakarta. This Energy Garden is a pilot plant for vegetable energy sources, one of which is based on kemiri sunan. The research took place from January to May 2018.

The materials used in this research were seedlings of ± 36 months old high yielding varieties (KS2), Chitosan, and Insecticides. The tools used were hoes, buckets, stirrers, calipers, rulers, and stationery.

The research used a Randomized Complete Block Design consisting of 2 factors. The first factor was the concentration of chitosan (C) consisting of 4 levels, namely: C1 (10 ml / l), C2 (20 ml / l), C3 (30 ml / l), and C4 (40 ml / l). The second factor was the frequency of chitosan (F) distribution, consisting of 3 levels, namely: F1: 3 times distributin at 20, 40 and 60 days from the start of observation, F2: 4 times distribution at 15, 30, 45 and 60 days from the start of observation, and F3: 5 times distribution at 20, 30, 40, 50 and 60 days from the start of the observation. Of these two factors, 12 treatment combinations were obtained, repeated three times, and each treatment combination consisted of 3 plants. Being a control i\wass without chitosan treatment, so the total number = 111 kemiri sunan plants.

Plant preparation began with the plants selection and determination in the field which would be the object of research. In the field, the planting distance of kemiri sunan was 8 m x 8 m, so that the number of plants that had been determined were in the land of of ± 7000 m^2 area.

Chitosan was distributed according to the concentration and time, for the distribution of 15 days in F2 treatment, 20 days in F1 and F3 treatments, 30 days in F2 and F3 treatment, 40 days in F1 and F3 treatments, 45 days in F2 treatment, 50 days in F3 treatment, and 60 days in F1, F2, and F3 treatment.

The observation of agronomic characters for the vegetative growth of kemiri sunan plants was carried out at the age of 70 and 90 days after the initial observation on the parameters of plant height (cm), number of leaves (strands), and increase in stem diameter (cm), number of secondary branches, and width of leaves (cm).

The observation data were analyzed for the diversity at the 5% level. To find out the differences between levels, the analysis continued with DMRT (Duncan Multiple Range Test) at the level of 5%.

RESULTS AND DISCUSSION

Plant height (cm), number of leaves (strands) and stem diameter (cm) at the beginning of the observation

The results of variance showed that there were interactions between plants that would be treated with chitosan concentration and the frequency of chitosan distribution on the stem diameter parameters at the beginning of the observation. The average plant height, number of leaves and stem diameter are presented in Table 1.

Table 1. Average plant height (cm), number of leaves (strands) and plant stem diameter (cm) of kemiri sunan at the beginning of observation

Observed Parameter	Consentration of Chitosan (C)	Frequency of Chitosan Distribution (F)			
		F1	F2	F3	Average
Plant Height	C1	272.56	295.56	257.56	275.22 a
	C2	289.78	247.00	230.44	255.74 ab
	C3	211.44	241.11	223.67	225.41 b
	C4	231.67	254.11	277.22	254.33 ab
	Average	251.36 p	259.90 p	247.22 p	252.67 (-) x
	Control				251.89 x
Number of Leaves	C1	158.56	202.00	137.89	166.15 a
	C2	199.78	148.56	215.22	187.85 a
	C3	151.33	79.67	123.11	118.04 a
	C4	118.22	168.67	227.00	171.30 a
	Average	156.97 p	149.72 p	175.81 p	160.83 (-) x
	Control				175.44 x
Plant Steam	C1	17.79 ab	19.32 a	16.62 abc	17.91
	C2	18.93 ab	14.68 abcd	17.34 abcd	16.65
	C3	15.02 abcd	10.91 d	13.17 bcd	13.03
	C4	12.47 cd	16.13 abc	18.71 ab	15.77
	Average	15.80	15.26	16.46	15.84 (+) x
	Control				17.28 x

Description: The mean (average) treatment between columns and rows followed by the same letter showed no significant difference in the DMRT test at the 5% level. The sign (-) showed no interaction.

Table 1 showed that the initial observations for plants with the C1F2 code had the largest stem diameter compared to the diameter of the other plants. The average plant height, number of leaves and stem diameter between plants that would be treated with chitosan and control were not different.

Number of Secondary Branches (fruit), Leaf Width (cm) at the beginning of observation

The results of variance showed that there were interactions between plants that would be treated with chitosan concentration and the frequency of chitosan distribution to the width of the leaf at the beginning of the observation. The average number of secondary branches and the width of the leaf are presented in Table 2.

Table 2. Average number of secondary branches (fruit) and width of leaf (cm) kemiri sunan at the beginning of observation

Observed Parameter	Consentration of Chitosan (C)	Frequency of Chitosan Distribution (F)			
		F1	F2	F3	Average
Number of Secondary Branches	C1	12.89	15.44	11.11	13.15 a
	C2	13.33	7.67	10.33	10.44 a
	C3	7.78	3.33	5.11	5.41 b
	C4	4.67	8.11	15.22	9.33 ab
	Average	9.67 p	8.64 p	10.44 p	9.58 (-) x
	Control				13.22 x
Width of Leaf	C1	190.11 abcd	233.00 ab	210.44 abc	211.19
	C2	220.44 ab	166.56 bcd	180.11 abcd	189.04
	C3	138.22 d	145.78 cd	143 cd	142.33.
	C4	143.33 cd	167.78 bcd	234.67 a	181.93
	Average	173.03	178.28	192.06	15.84 (+) x
	Control				17.28 x

Description: The mean (average) treatment between columns and rows followed by the same letter showed no significant difference in the DMRT test at the 5% level. The sign (-) showed no interaction.

Table 2 showed that plants that would be treated with chitosan with the C4F3 code had the widest leaf. The average of plants that would be given chitosan and would not be given chitosan (control) had a number of secondary branches and the width of the leaf that were no different.

Plant Height Increase (cm)

The results of variance showed that there was no interaction between chitosan concentration and the frequency of chitosan distribution on the plant height on 70 days and 90 days after initial observation. The average height increase of plants is presented in Table 3.

Table 3 below showed that in observing the high growth of 70-day-old plants, the real difference occured in each of the single factors. The concentrations of 20 ml / l (C2) and 40 ml / l (C4) gave higher plant growth than other concentrations. The frequency of 5 times chitosan distribution (F3) gave higher plant height. The 90 days increase of the plant height after the initial observation between the treatment concentration and frequency of chitosan of each level did not show a different effect. Plants that were given chitosan showed the effect of higher and higher plant growth than plants that were not given chitosan (control).

Table 3. Average height increase of kemiri sunan plants on 70 and 90 days observations after the initial observation

Plant Height Increase	Concentration of Chitosan (C)	Frequency of Chitosan Distribution (F)			
		F1	F2	F3	Average
70 days after the initial observation	C1	26,11	35,44	29,00	30,18 ab
	C2	23,02	41,33	47,67	37,34 a
	C3	34,45	29,56	17,22	27,08 b
	C4	33,11	38,35	34,00	35,15 a
	Average	29,17 r	36,16 q	55,96 p	40,43 (-) x
	Control				29,55 y
90 days after the initial observation	C1	6.22	8.89	10.00	8.37 a
	C2	10.67	10.78	11.22	10.89 a
	C3	10.22	26.22	11.33	15.93 a
	C4	14.11	12.67	13.67	13.48 a
	Average	10.31 p	14.64 p	11.56 p	12.17 (-) x
	Control				4.11 y

Description: The mean (average) treatment between columns and rows followed by the same letter showed no significant difference in the DMRT test at the 5% level. The sign (-) showed no interaction.

Amount of Leaves (strands)

The results of variance showed that there was no interaction between chitosan concentration and the frequency of chitosan distribution on the number of leaves at 70 days and 90 days after the initial observation. The average number increase is presented in Table 4.

Table 4 below showed that the observation of the increasing number of leaves aged 70 days after the initial observation at 30 ml / l (C3) concentration treatment showed a different effect with a smaller average number of leaves than the other concentrations. The chitosan frequencies on each level did not show a different effect. In observing the increasing number of leaves aged 90 days after the initial observation, the real difference only occured in each single factor. The concentrations of 30 ml / l (C2) and 40 ml / l (C3) gave more leaves than other concentrations. The frequency of 5 times chitosan distribution (F3) gave more number of leaves. Plants that were given chitosan showed the influence on the different and more number of leaves than plants that were not given chitosan (control).

Table 4. Average increase in the number of kemiri sunan leaves on the 70 and 90 days observations after the initial observation

Number of Leaves Increase	Concentration of Chitosan (C)	Frequency of Chitosan Distribution (F)			
		F1	F2	F3	Average
70 days after the initial observation	C1	45,45	71,44	69,23	62,04 a
	C2	60,78	68,67	53,56	61,00 a
	C3	57,45	49,67	49,55	52,22 b
	C4	52,45	53,78	64,66	56,96 a
	Average	54,02 p	60,89 p	59,25 p	58,06 (-) x
	Control				36,89 y
90 days after the initial observation	C1	11.22	13.11	12.56	12.30 b
	C2	14.22	13.67	14.44	14.11 ab
	C3	13.56	12.78	20.33	15.56 a
	C4	14.67	15.00	15.56	15.07 a
	Average	13.42 b	13.64 ab	15.72 a	14.26 (-) x
	Control				8.56 x

Description: The mean (average) treatment between columns and rows followed by the same letter showed no significant difference in the DMRT test at the 5% level. The sign (-) showed no interaction.

Stem Diameter Increase (cm)

The results of variance showed that there was an interaction between chitosan concentration and the frequency of chitosan distribution on the increase of the stem diameter at 70 days and 90 days after the initial observation. The average increase in stem diameter is presented in Table 5.

Table 5 below showed that the 70 days observations since the initial observation of C2F3 treatment combination (concentration of 20 ml / l and frequency of 5 times distribution) gave a different effect on the increase of stem diameter than the other treatment combinations. At the 90 days observation since the initial observation, the combination of C3F2 treatment (concentration of 30 ml / l and frequency of 4 times distribution) gave a greater increase in the stem diameter than other treatment combinations. The average increase in stem diameter on the 90 days observations after the initial observation for plants given chitosan showed a different and greater effect than plants that were not given chitosan (control).

Table 5. Average increase of the diameter of kemiri sunan stems on 70 and 90 days observations after the initial observation

Increase of Diameter Stem	Concentration of Chitosan (C)	Frequency of Chitosan Distribution (F)			
		F1	F2	F3	Average
70 days after the initial observation	C1	3,98 bc	4,06 b	2,74 c	3,59
	C2	4,37 b	3,60 bc	5,68 a	4,55
	C3	3,96 b	3,53 bc	3,30 bc	3,59
	C4	2,97 c	3,83 b	3,95 b	3,58
	Average	3,83	3,75	3,92	3,83 (+) x
	Control				4,21 x
90 days after the initial observation	C1	1.32 b	1.29 b	1.22 b	1.28
	C2	1.16 b	1.43 b	1.49 b	1.36
	C3	1.34 b	2.23 a	1.33 b	1.64
	C4	1.43 b	1.78 ab	1.38 b	1.53
	Average	1.31	1.68	1.36	1.45 (+) x
	Control				0.94 y

Description: The mean (average) treatment between columns and rows followed by the same letter showed no significant difference in the DMRT test at the 5% level. The sign (-) showed no interaction.

Number of Secondary Branches

The results of the variance showed that there was an interaction between chitosan concentration and the frequency of chitosan distribution on the average number of secondary branches increased on the 90 days after the initial observation. The average increase in stem diameter is presented in Table 6.

Table 6 below showed that the observation on the number of secondary branches on the 70 days after the initial observation at 30 ml / l (C3) concentration treatment showed a different effect with a smaller number of secondary branches compared to the other concentrations. Each level did not show a different effect for the chitosan frequencies. The average number of secondary branches increased on the 90 days observations after the initial observation, the number of secondary branches was more than the other treatments for C2F1 treatment combination (concentration of 20 ml / l and frequency of 3 times distribution). The number of secondary branches was different and more numerous between plants that were given chitosan with plants that were not given chitosan (control).

Table 6. Average increase in the number of secondary branches of kemiri sunan plants on the 70 and 90 days observations after the initial observation

Number of Secondary Branches	Concentration of Chitosan (C)	Frequency of Chitosan Distribution (F)			
		F1	F2	F3	Average
70 days after the initial observation	C1	25,22	28,55	23,67	25,81 a
	C2	29,23	13,67	30,00	24,30 a
	C3	16,89	11,23	10,78	12,96 b
	C4	10,44	39,67	31,33	27,15 a
	Average	20,45 p	19,47 p	23,95 p	22,55 (-) x
	Control				21,55 x
90 days after the initial observation	C1	2.78 b	3.00 b	3.44 b	3.07
	C2	7.11 a	3.11 b	3.56 b	4.59
	C3	3.44 b	2.67 b	3.22 b	3.11
	C4	2.78 b	3.33 b	2.89 b	3.00
	Average	4.03	3.03	3.28	3.44 (+) x
	Control				2.78 y

Description: The mean (average) treatment between columns and rows followed by the same letter showed no significant difference in the DMRT test at the 5% level. The sign (-) showed no interaction.

Leaf Width Increase (cm)

The results of variance showed that there was an interaction between chitosan concentration and the frequency of chitosan distribution on the average width of the leaf growth on the 90 days from the initial observation. The average width of the leaf is presented in Table 7.

Table 7 showed that there was a significant difference occurred in the chitosan concentration factor after observing the increase of the the 70-day-old leaf width, where the concentrations of 20 ml / l (C2) and 40 ml / l (C4) gave wider width of the leaf than the other concentration. The frequency factor of chitosan distribution in each level showed the same effect. On the 90 days observation after the initial observation, the average increase of the width in the C3F2 treatment combination (30 ml / l concentration and 4 times the frequency of distribution) gave the largest increase in leaf compared to the other treatment combinations. There was a wide and different width of leaf between the plants given chitosan and not given chitosan (control).

Table 7. The average leaf width increase of the kemiri sunan plant canopy on the 70 and 90 days observations after the initial observation

Leaf Width Increase	Concentration of Chitosan (C)	Frequency of Chitosan Distribution (F)			
		F1	F2	F3	Average
70 days after the initial observation	C1	42,22	49,78	36,55	42,85 b
	C2	41,44	38,89	70,88	50,40 a
	C3	43,00	41,33	28,22	37,52 b
	C4	47,34	47,78	60,33	51,81 a
	Average	43,50p	44,45 p	48,99 p	46,65 (-) x
	Control				41,00 y
90 days after the initial observation	C1	14.11 cd	12.00 d	13.11 d	13.07
	C2	15.33 cd	20.33 bc	23.33 b	19.67
	C3	15.00 cd	39.22 a	16.22 cd	23.48
	C4	20.22 bc	16.89 cd	14.67 cd	17.26
	Average	16.17	22.11	16.83	18.37 (+) x
	Control				9,67 y

Description: The mean (average) treatment between columns and rows followed by the same letter showed no significant difference in the DMRT test at the 5% level. The sign (-) showed no interaction.

The use of chitosan can be an alternative in an effort to increase the vegetative growth of kemiri sunan plants. The distribution of chitosan with a concentration of 20 ml / l, 30 ml / l and 40 ml / l generally gave a better effect than the concentration of 10 ml / l. The frequency of 4 times and 5 times chitosan distribution in general gave a better effect than the frequency of 3 times chitosan distribution and could increase the vegetative growth of kemiri sunan plants. The results showed that from the observed parameters, the vegetative growth of the kemiri sunan plant which was given chitosan showed a better growth rate compared to plants that were not given chitosan (control). The role of chitosan in improving plant metabolism caused the increase plant growth. 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 (Dzung, 2010). Chitosan contains Plant Growth Promotor in the form of giberelin, IAA, and Zeatin (Rekso, 2005). According to Mawgoud et al. (2016), chitosan is known to increase the number of leaves, chlorophyll, and the availability of amino acids for plants.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

1. There was an interaction between the treatment of chitosan concentration and the frequency of chitosan distribution on the parameters of the increase in stem diameter (C2F3), the increase of the number of secondary branches (C2F1) and the width of the leaf (C3F2).
2. The concentration of chitosan 20 ml / l, 30 ml / l, and 40 ml / l gave a better effect than the concentration of 10 ml / l in all parameters observed.
3. Frequency of 4 and 5 times chitosan distribution gave a better effect on most of the parameters observed compared to the frequency of 3 times distribution.
4. Plants that were treated with chitosan had a better vegerative growth than those without chitosan (control).

Recommendation

The frequency of 4 times chitosan distribution is recommended to be applied to kemiri sunan plants because it gives the same good effect as the 5 times chitosan distribution. Besides, seen from the aspect of efficiency in using chitosan, the 4 times distribution was more economical than 5 times, be able avoide the waste.

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THANKING

A great gratitude was given to Kemenristek Dikti for the fund to conduct this research.