ASM Sc. J., 13, Special Issue 6, 2020 for InCOMR2019, 30-35

Effect of Irradiated Chitosan on Kenaf (*Hibiscus cannabinus* L.) by Foliar Application

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Kenaf (*Hibiscus cannabinus* L.) has been selected as the most promising fibre producing plant in Malaysia. The production of kenaf is still low although it has been extensively used in various industrial application. The present study was designed to determine the effect of difference concentration of irradiated chitosan (50, 100, 150, 200 and 250ppm) and its potential application on growth attributes in kenaf seedlings. Irradiation of chitosan was performed using liquid gamma irradiator (Natural Rubber Latex at Malaysian Nuclear Agency, Malaysia, dose rate 0.88kGy/hr). Foliar spraying was used at 7 day interval to investigate four growth parameter including plant height, root length, dry and fresh weight. Statistical analysis showed the application of irradiated chitosan at 200Gy and 100ppm resulted in an extremely significant increase in kenaf seedlings with 37.55cm plant height, 2.01g of dry weight and 5.72g of fresh weight. However, at higher degree of exposure chitosan to gamma radiation, 400Gy at 250ppm, there is a significantly reduction of kenaf growth. No significant differences in root length was observed in kenaf growth when supplemented with irradiated chitosan at any concentration. These results suggest the possibilities utilize of gamma-irradiated chitosan in kenaf plant as growth stimulator. Thus, irradiated chitosan could be alternative tools in modern agriculture to ensure food security.

Keywords: chitosan; foliar application; gamma radiation; *Hibiscus cannabinus*; plant growth promoter.

I. INTRODUCTION

Kenaf, *Hibiscus cannabinus* L. is a short day, annual, herbaceous plant belong to the family of Malvaceae. It represents an interesting crop and mostly cultivated for the outer bark (bast) on the stem for high quality fibre production which notable for their economic, industrial and horticultural importance (Bourguignon, 2016). Kenaf plant has become the popular choice to fill the global demand for fibrous materials due to the shortages of trees in many areas and other environmental issues (Hossain *et al.*, 2011). Recent years have seen considerable research into the adaptability of kenaf with local climate, efficient agricultural practices for kenaf cultivation, harvesting and mechanization as well as fibres processing and downstream application development. In Malaysia, kenaf plantation is being implemented in Pahang, Kelantan, Terengganu, Perak, Johor and Melaka. However, kenaf production is still low compared to other countries with around 5 to 10 tonnes per hectare. The highest yield achieved was about 9.8 tonnes

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per hectare (Paridah *et al.*, 2017). In addition, photoperiod insensitive and late flowering have been identified as the main issue required for growing kenaf plants in a tropical country like Malaysia (Ayadi *et al.*, 2018). The low production of kenaf is the major drawback in exporting this commodity. To meet the population demand, plant growth promoters (PGP) such as chitosan has been used to increase the crop yields. As it is convenience, cost and labour efficiency, many countries such as Japan, South Korea, Poland and China have been practising the used of chitosan in many crops (Sultana *et al.*, 2015).

Chitosan (poly-P-(1-4)-N- acetyl-D-glucosamine) has been identified as a key factor that stimulate plant growth, provide barrier and protection of edible product, induce abiotic and biotic stress and tolerance in diverse horticultural commodities. It is a natural, impregnable and affordable product of deacetylation process, are used in a wide range of industries due to its valuable properties. The solely producer of chitosan mainly come from the shell of crustaceans, such as shrimp, lobster, crab, squid. Naturally, degradation of chitin by deacetylation process produce a high molecular weight (Mw) of chitosan with limitation of applications. Contradictory, low Mw of chitosan consists of special biological properties extensively applied as immunity stimulator for animal and plant, antimicrobial, antioxidant and antitumor property (Hien *et al.*, 2004).

Numerous degradation techniques can be used to produce low Mw chitosan including chemical, enzymatic hydrolysis and radiation process. However, radiation (ɤ-rays, electron beam) is an effective and preferable mechanism for breakdown of polymer from the perspective of eco-friendly processing technology (Haji-Saeid *et al.*, 2010). A number of study has been reported the efficiency application of irradiated chitosan in promoting growth in crops such as rice, wheat, coffee, faba bean and soybean (Chamnanmanoontham *et al.*, 2015; El-Sawy *et al.*, 2010; Dzung *et al.*, 2011; Phu *et al.*, 2017 & Zhang *et al.* 2016) and ornamental plants including orchid tissue and chrysanthemum (Hien, 2004 & Nge *et al.*, 2006). Due to our concern, this is the first study reported the application of irradiated chitosan on growth promotion on kenaf seedlings. In view of the above, this research work sought to determine the effect of irradiated chitosan with different concentration on growth of kenaf seedlings under Malaysian conditions. It is hope that, irradiated chitosan can reduce the demand application of hazardous chemical throughout cultivation, ensure the food security by increasing crops yield and reducing post-harvest lost.

II. MATERIALS AND METHOD

A. Plant Material and Growth Condition

Seeds of *Hibiscus cannabinus* L. were surface sterilized by soaking in 80% ethanol for 5 min followed by 3 to 4 washing with autoclaved distilled water. Seeds were sown in a small polythene bags containing top (loamy soils) and kept under ambient temperature. The fertilizer used were NPK green at 450 kg/ha after 1 day of planting and NPK blue at 450 kg/ha after 14 days of plants. Additionally, pesticide and herbicide were also applied to control insect and weeds.

B. Preparation of Irradiated Chitosan

Chitosan samples (Degree of deacetylation 90.6 %) used in this research were purchased from China. A chitosan stock solution (3% w/v) in lactic acid (2% w/v) was prepared. Irradiation of chitosan was performed using liquid gamma irradiator of an activity, 94,818 Ci. (Natural Rubber Latex at Malaysian Nuclear Agency, Malaysia, dose rate 0.88 kGy/hr). The irradiated 3% chitosan stock solution was used to prepare the irradiated series of the chitosan solutions (100 Gy, 200 Gy, and 400 Gy).

C. Experimental Design and Foliar Application Using Irradiated Chitosan

A pot experiment was carried out in a simple randomized design in the natural condition at net house at Faculty of Applied Science, Universiti Teknologi MARA Cawangan Negeri Sembilan. Effect of irradiated chitosan applied on growth of *Hibiscus cannabinus* L. was conducted in five different concentrations of irradiated chitosan that were 0, 50, 100, 150, 200 and 250 ppm. Four times foliar spray after germination (on day 7, 14, 21 and 30 at field stages) were carried out. In the control treatment, only water was sprayed. Plant height, root length, dry weight and fresh weight were recorded at harvesting time.

D. Determination of Plant Growth Attributes

Effect of irradiated chitosan on growth attributes of the *Hibiscus cannabinus* L. were verified after 30 days of sowing. Five plants from each treatment were meticulously uprooted and foreign particles were removed by washing at running tap water. Adhering water at roots were removed by using blotting paper. Plant were oven dried at 80 °C for 24 h and the dry mass of plants were recorded. Electronic balance was utilized to measure the fresh weight of the clean and blot-dried plants. Plant height and root length were measured by using the meter scale.

E. Statistical Analysis

Each treatment was conducted in triplicate and the results were expressed as a mean. Data were subjected to statistical analysis using analysis of variance (ANOVA, SPSS Version 23) to compare the means among the group. If there are statistically significant difference exist, least significant difference (LSD) test were used to compare the means at 0.05 probability level ($p < 0.05$).

III. RESULT AND DISCUSSION

Utilization of value product such as chitosan in agricultural field has been remarkably interest. Due to their beneficial properties as plant growth promoter, various low Mw of chitosan has been produced by irradiation (ɤ-rays, electron beam) process. However, there are only limited study reported the application of irradiated chitosan on agricultural performance on plant. However, until now, no research works have been considered the importance effect of irradiated chitosan on kenaf. As far as our concern, this study will be the first report on the effect of irradiated chitosan on growth of kenaf seedlings. Based on the result, application of irradiated chitosan demonstrated the improvement in plant growth attributes in kenaf seedlings.

Figure 1. Kenaf growth on different dosage of acute gamma radiation

The effect of irradiated chitosan on morphological traits such as plant height and root length were shown in Figure 1. Result revealed that the unradiated chitosan (control plant) exhibited the growth of kenaf seedlings of the above parameters. The control plant demonstrated the shortest plant height compared to irradiated plant with chitosan. In addition, root length also showed the lowest growth in control plant as shown in Figure 1. These results indicated that application of irradiated chitosan had tremendous effect on performance on kenaf plant. There are consistent with Pratana and Sililuk (2016), who reported the increasing number of growth and development in corn supplemented with irradiated chitosan. According to Dzung *et al.* (2011), the plants have the capability to identify oligosaccharides (chitosan) which in turn stimulate vital process of plants on every level of biological process. Furthermore, Dar *et al.* (2015), reported that chitosan increased enzyme activity of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease), and boost the nitrogen uptake in the functional leaves which improved plant growth and development in plant. Similar phenomenon may be seen in the recent study. Foliar application of chitosan and irradiated chitosan demonstrated a significant variation in kenaf growth. From this study, gamma radiation was found to be most effective and enhanced the plant growth by degrading the molecules of chitosan.

Table 1. Plant height and root length of kenaf seedlings after foliar spraying at different concentration of irradiated chitosan

Dosage (Gy)	Plant height (cm) Concentration of chitosan (ppm)					
	0	50	100	150	200	250
Control	28.50 [d]	29.52 [d]	31.51 [d]	30.72 [d]	30.32 [d]	30.21 [d]
100	32.51 [b]	32.71 [b]	35.82 [b]	35.80 [b]	33.51 [b]	35.40 [b]
200	34.13 [a]	34.45 [a]	41.44 [a]	37.08 [a]	36.75 [a]	36.44 [a]
400	29.68 [c]	29.73 [c]	31.65 [c]	30.77 [c]	30.60 [c]	30.56 [c]

Dosage (Gy)	Root length (cm) Concentration of chitosan (ppm)					
	0	50	100	150	200	250
Control	15.07 [a]	15.37 [a]	15.49 [a]	15.48 [a]	15.42 [a]	15.09 [a]
100	15.13 [a]	15.40 [a]	15.53 [a]	15.52 [a]	15.48 [a]	15.43 [a]
200	15.25 [a]	15.52 [a]	15.63 [a]	15.54 [a]	15.53 [a]	15.47 [a]
400	15.12 [a]	15.39 [a]	15.56 [a]	15.51 [a]	15.45 [a]	15.20 [a]

Note: Mean within a column followed by the same letter are not significantly different (p<0 .05)

Table 1 showed that, growth-promoting attributes significantly increased with increasing radiation dose of chitosan up to 200Gy and concentration of chitosan up to 100ppm. Result in this study was aligned with Isa *et al.* (2016) who reported increasing dosage of gamma radiation and concentration of chitosan showed a positive effect on the growth of Chinese kale. However, the mechanism by which these oligomers (acquired through gamma radiation) stimulate plant growth and development is remains unclear. According to Rahman *et al.* (2013), gamma irradiation cause scission of glyosidic linkage and thus decrease the molecular weight of chitosan as well as increasing the progressively impact on plant growth.

Due to high gamma radiation dose of chitosan, foliar application of irradiated chitosan at 400Gy in 250 ppm slightly decreased plant height, dry weight and fresh weight of kenaf seedling (Table 2). It can be shown that, by increasing in dose of gamma radiation until a certain dosage, it will be led to the negative effect in the stimulatory of chitosan and therefore reduce the plant growth. This former explained referred to the result of foliar-applied chitosan at 400Gy showed the lowest value of all growth attributes tested in kenaf among irradiated-chitosan plant.

Comparable among chitosan treatment, the highest growth promotion effect on kenaf seedling were recorded in the sample irradiated at 200Gy and 100ppm chitosan treatment with 41.4cm plant height, 6.26g fresh weight and 2.30g dry weight. In contrast, the lowest growth of kenaf seedling were observed in 400Gy and 250ppm with 30.56g plant height, 4.14g fresh weight and 1.63g dry weight.

Table 2. Fresh weight and dry weight of kenaf seedlings at various concentration of irradiated chitosan at various concentration of irradiated chitosan

Dosage (Gy)	Dry weight (g) Concentration of chitosan (ppm)					
	0	50	100	150	200	250
Control	1.25 [d]	1.28 [d]	1.30 [d]	1.28 [d]	1.26 [d]	1.12 [d]
100	1.70 [b]	1.76 [b]	1.94 [b]	1.92 [b]	1.77 [b]	1.79 [b]
200	1.94 [a]	2.08 [a]	2.30 [a]	2.17 [a]	2.15 [a]	2.11 [a]
400	1.62 [c]	1.63 [c]	1.68 [c]	1.65 [c]	1.64 [c]	1.63 [c]

Dosage (Gy)	Fresh weight (g) Concentration of chitosan (ppm)					
	0	50	100	150	200	250
Control	3.14 [d]	3.34 [d]	3.70 [d]	3.36 [d]	3.37 [d]	3.17 [d]
100	4.68 [b]	5.18 [b]	5.34 [b]	5.16 [b]	5.07 [b]	5.05 [b]
200	5.30 [a]	5.82 [a]	6.26 [a]	6.08 [a]	5.52 [a]	5.32 [a]
400	4.09 [c]	4.21 [c]	4.45 [c]	4.21 [c]	4.18 [c]	4.14 [c]

Note: Mean values having the same superscript within column are not significantly different (p<0 .05)

The stimulating effect of chitosan on plant growth may be attributed to an increased in the accessibility and transportation of water and major nutrients such as nitrogen, phosphorus and potassium through adjusting cell osmotic pressure and gradual accumulation of free-radical damage (ORS) by increased antioxidant and enzyme activities (Guan *et al.*, 2009). Root length did not show any statistically differences between different irradiation dose and concentration of chitosan on foliar-applied irradiated chitosan plant. This is attributed to inherent genotypic different and the amount of food reserved mobilized which alternatively contributed for longer root length in seedling (Islam *et al.*, 2015). Based on this result, it can be said that foliar application of irradiated chitosan can improve growth attributes of kenaf seedling. Thus, it is an alternative method to develop a new cultivar of kenaf with high fibre characteristics which will help to boost the production of fibres in Malaysia. However, more experiment should be carried out in various location and season to draw a reliable conclusion with respect to the irradiated chitosan foliar application for growth attributes improvement of kenaf.

IV. CONCLUSION

The addition of 100ppm of 200Gy-irradiated chitosan by foliar spraying resulted in the extremely significant ($p<0.05$) increased in plant growth attributes (plant height, fresh and dry weight) of kenaf seedlings. This research has demonstrated degradation of chitosan at a specific irradiation dosage could further improve its characteristic as promoting plant growth.

V. ACKNOWLEDGEMENTS

Author would like to thank UiTM and Agency Nuclear Malaysia for providing all the necessary facilities for the successful completion of the research work. The authors wish to thank the Agency Nuclear Malaysia for permission to publish this paper.

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