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Cite as: AIP Conference Proceedings **1704**, 030003 (2016); https://doi.org/10.1063/1.4940072
Published Online: 22 January 2016

Mohd Hafez Mohd Isa, Muhamad Samudi Yasir, Abu Bakar Hasan, et al.

The effect of Gamma Irradiation on Chitosan and Its Application as a Plant Growth Promoter in Chinese Kale (*Brassica alboglabra*)

Mohd Hafez Mohd Isa[1,a)], Muhamad Samudi Yasir[2], Abu Bakar Hasan[3], Nur Izzah Md Fadilah[3], Abdul Rahman Hassan[3]

[1]*Faculty of Science and Technology, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia.*
[2]*School of Applied Physics, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.*
[3]*Faculty of Science and Technology, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia.*

[a)]Corresponding author: m.hafez@usim.edu.my

Abstract. This research project was conducted to study the effects of irradiation on chitosan and its potential application as a plant growth promoter. Chitosan in the form of flakes was irradiated with gamma rays at irradiation dosage of 50 kGy, 100 kGy, 200 kGy and 400 kGy. The effect of irradiation on chitosan in terms of intrinsic viscosity and average molecular weight was measured using Ubbelohde capillary viscometry technique and the results obtained showed irradiation at doses of up to 50 kGy had caused an extremely significant reduction of both parameters and this trend continued at higher irradiation doses, although the decrease were not significant. The effect of various concentrations of chitosan and irradiated chitosan on growth promotion of Chinese kale (*Brassica alboglabra*) was hydroponically grown and cultivated for 50 days. Statistical analysis showed addition of 10 ppm of irradiated chitosan of 200 kGy and 400 kGy, respectively, resulted in an extremely significant increase in the percentage weight gain of Chinese kale (*Brassica alboglabra*). Results obtained in this study showed the potential use of irradiated chitosan as a plant growth promoter for plants grown hydroponically.

Keywords: chitosan; gamma rays; Ubbelohde capillary viscometry; Chinese kale (*Brassica alboglabra*).

INTRODUCTION

Research and development activities to produce or promote modern farming techniques are being carried out around the world. Efficient farming methods should be more environmentally friendly, in addition to being able to provide a satisfactory return on agricultural products. The example of activities or techniques to develop modern agricultural methods is through the use of chitosan which is a natural substance. Chitosan, (1-4)-2-amino-2-deoxy-b-D-glucan, is deacetylated derivative of chitin and have been used commercially by the farmers for the purpose of soil enrichment and as a bio-active ingredient to increase the yield of livestock and plant production (Chandrakrachang 2002; Chmielewski et al. 2007). Chitosan is characterized by high molecular weight and low solubility in most solvents which limits its applications. The solubility of chitosan can be increased by lowering its molecular weight through chemical, radiation, or enzymatic reaction (Chmielewski et al. 2007). Chitosan oligomers or oligochitosan, as reported by Choi et. al. (2002) could be obtained through irradiation of chitosan dissolved in acetic acid. Previous research has established a connection between oligochitosan with the ability to induce plant defense mechanism and improving plant growth rate (Hai et al. 2003). In this research, the degradation of chitosan

Advancing Nuclear Science and Engineering for Sustainable Nuclear Energy Infrastructure
AIP Conf. Proc. 1704, 030003-1–030003-7; doi: 10.1063/1.4940072

was conducted through irradiation with gamma rays at specific irradiation doses. Irradiation of chitosan causes chain scission and, this can improve chitosan molecular mobility due to their shorter chain and size (Lim et. al. 1998).

Siri-Upathum (2002) reported oligochitosan in the range of 50 to 75 ppm, which was obtained through irradiation of chitosan using gamma rays, were able to increase the growth rate of orchids. Tham et. al. (2001) also have shown irradiation dosage of 70 to 150 kGy to be the most optimal irradiation dose of chitosan to increase the growth rate of the plant. Futhermore, Luan et. al. (2002) reported that 40 to 100 ppm of chitosan irradiated at 100 kGy could significantly stimulate plant growth in a tissue culture medium. Irradiation of macromolecules such as chitosan causes molecular chain to scission into smaller fragments. Molecular chain scission occurs randomly and the length of molecular fragment is not dependent on irradiation dosage. The probability of obtaining oligochitosan through irradiation will increase with increase in irradiation dosage. Therefore, in this study, it is expected that irradiated chitosan at a higher irradiation dosage will be able to increase plant growth rate. Moreover, addition of chitosan of various concentrations in plant hydroponics nutrient solution are expected to cause different effects on plant growth rates. In addition, the effect of irradiation on chitosan degree of deacetylation, intrinsic viscosity and average molecular weight shall also be determined in this study.

MATERIALS AND METHOD

Preparation of Irradiated Chitosan

Irradiation of chitosan was performed using gamma rays from Gammacell 220 (Atomic Energy of Canada Limited-Commercial Product) with radioactive cobalt-60 as the source of irradiation. Chitosan samples in the form of flakes were irradiated at 50 kGy, 100 kGy, 200 kGy and 400 kGy. Chitosan samples used in this research were obtained from Tokyo Kasei Kogyo Company (Japan). Chitosan samples were inserted into covered plastic containers before being put into the gamma cells for irradiation at room temperature.

Intrinsic Viscosity-Average Molecular Weight of Chitosan

Intrinsic viscosity and average molecular weight of chitosan was determined using Ubbelohde capillary type viscometer which allows the reading of flow times of the sample to be taken manually using a stop watch. Each measurement was taken at 30 ± 0.1 °C. The measurement firstly started with the determination of solvent flow times (t_0) and continued with flow times of samples (t_n) consisting of four different concentrations (C) of chitosan solution. The recorded flow times of chitosan solution were then used to measure viscosities which were determined using the equation as follows:

$$\text{Relative viscosity } (\eta_r) = \eta / \eta_0 \approx t/t_0 \quad (1)$$

$$\text{Specific viscosity } (\eta_{sp}) = \eta_r - 1 \quad (2)$$

$$\text{Reduced viscosity } (\eta_{red}) = \eta_{sp} / C \quad (3)$$

$$\text{Inherent viscosity } (\eta_{inh}) = (\ln \eta_r) / C \quad (4)$$

$$\text{Intrinsic viscosity } (\eta) = (\eta_{sp} / C)_{C=0} \quad (5)$$

The reduced viscosity and inherent viscosity were plotted against chitosan concentration. The value of intrinsic viscosity can be calculated by extrapolating graph of reduced viscosity and inherent viscosity to zero concentration. The Mark - Houwink equation was later used to obtain an estimate of the viscosity-average molecular weight of chitosan samples. *k* and *a* constant used in the Mark-Houwink equation was referred to values determined by Wang et. al. (1991) to the nearest degree of deacetylation of chitosan samples used in this study, which was 82 ± 2 %.

$$\text{Mark-Houwink, } [\eta] = kM_v^{a} \quad (6)$$

where M_v, a and k are average molecular weight and empirical constants of polymer-solvent system, respectively.

Growth Promotion Test

Chinese kale (*Brassica alboglabra*) obtained from Sakata Seed Corp. (Japan) were germinated by placing the seeds on a wet sponge for 10 to 15 days until the seeds produced 3 to 4 leaves. The seedlings were then transplanted into hydroponic reservoirs containing 20 L of Cooper's nutrient solution (Ismail, 1994) with the addition of 10 to 100 ppm of non-irradiated and irradiated chitosan. Average fresh weight of three to four plants in the 10^{th} and 50^{th} days of cultivation was weighted and recorded for measurement of percentage weight gain of plants for growth promotion test of chitosan.

Statistical Analysis

Data analysis consisted of calculating mean, standard deviation of the mean value and determination of the level of significance using the Tukey–Kramer multiple comparison test. The differences between measurements were considered significant at the level of $P < 0.05$.

RESULTS AND DISCUSSION

Intrinsic Viscosity-Average Molecular Weight of Chitosan

Based on the average of reduced and inherent viscosity, the intrinsic viscosity of chitosan samples was determined and are shown in Table 1. Irradiation of 50 kGy resulted to extremely significant decrease in the intrinsic viscosity of chitosan ($P < 0.001$). Increasing irradiation doses of up to 400 kGy led to a further decrease in intrinsic viscosity of irradiated chitosan, although the decrease was minimal and not significant ($P > 0.05$).

TABLE 1. Intrinsic Viscosity of Chitosan Samples

Chitosan Sample	Intrinsic Viscosity, [η] (dl/g)
Chitosan	33.10 ± 1.75
Chitosan 50 kGy	6.32 ± 0.43
Chitosan 100 kGy	3.70 ± 0.19
Chitosan 200 kGy	1.75 ± 0.03
Chitosan 400 kGy	0.98 ± 0.02

The Mark - Houwink equation was later used to determine the average molecular weight of chitosan based on the intrinsic viscosity of chitosan which was measured earlier. Figure 1 shows the effect of gamma irradiation to the decrease of average molecular weight of chitosan with increasing irradiation intensity. The average molecular weight of chitosan decreased significantly after 50 kGy of irradiation. Further increase in irradiation dosage of chitosan led to a further decrease in chitosan average molecular weight, although the decrease was minimal and insignificant. Results obtained in this experiment were in agreement with Ulanski and Rosiak (1992) and Tahtat et. al. (2012) with findings showing that gamma irradiation led to the decrease in intrinsic viscosity and average molecular weight of chitosan. The decrease of intrinsic viscosity and average molecular weight of chitosan through irradiation with gamma rays were due to a scission of polymer molecular chain (Lim et. al. 1998), rather than crosslinking.

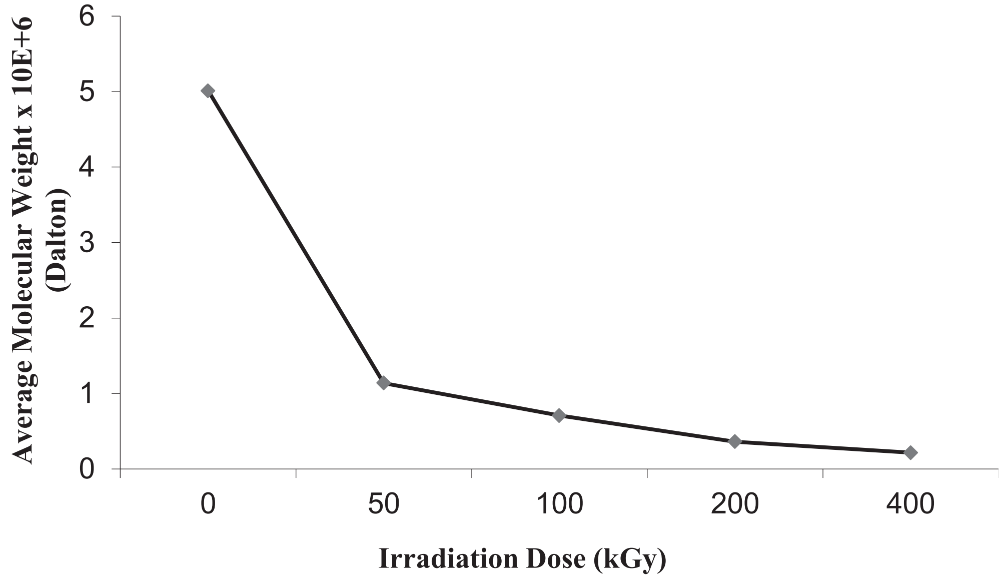

FIGURE 1. Average Molecular Weight of Chitosan.

Figure Growth-Promotion Effect of Irradiated Chitosan

Figure 2, Figure 3 and Figure 4, respectively, show a percentage weight gain of plant grown hydroponically with nutrient solution supplied with chitosan at a concentration of 10 ppm, 50 ppm and 100 ppm. In addition, Fig. 5 shows a comparison of plants at 50^{th} days of cultivation with the addition of various concentrations of 400 kGy of irradiated chitosan in the nutrient solution of hydroponic reservoir.

Figure 2 display plants supplied with 10 ppm of chitosan irradiated at 400 kGy that showed the highest percentage of weight gain in comparison to the other plants supplied with similar concentrations of chitosan of different irradiation dosage. Trends observed in Figure 2 were found to be similar to Figure 3 and Figure 4 in which 50 ppm and 100 ppm of 400 kGy of chitosan, respectively, showing the highest weight gain percentage.

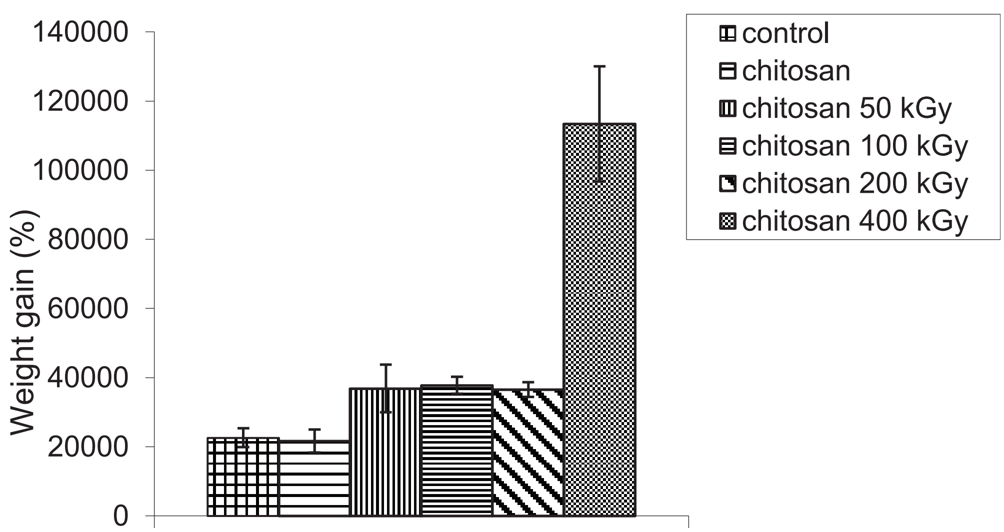

FIGURE 2. Weight gain of Chinese kale with addition of 10 ppm of chitosan in hydroponic nutrient solution.

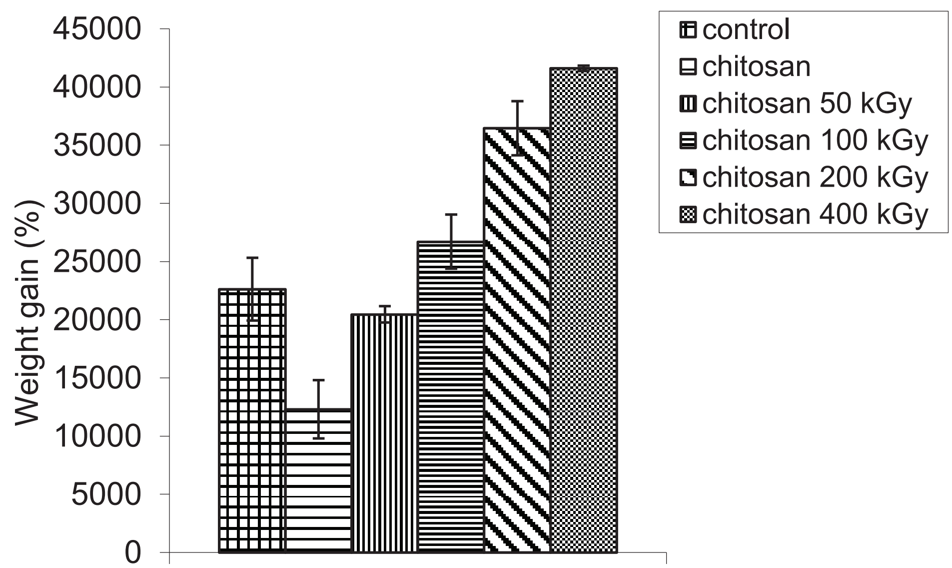

FIGURE 3. Weight gain of Chinese kale with addition of 50 ppm of chitosan in hydroponic nutrient solution.

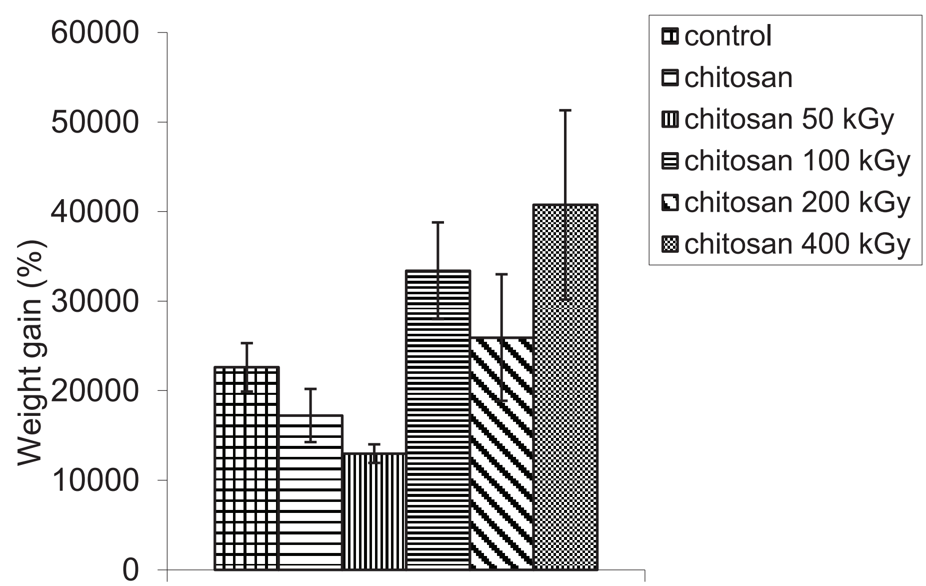

FIGURE 4. Weight gain of Chinese kale with addition of 100 ppm of chitosan in hydroponic nutrient solution.

FIGURE 5. Chinese kale at 50th days of cultivation with addition of 400 kGy of irradiated chitosan at various concentrations; (1) control; (2) 10 ppm of chitosan; (3) 50 ppm of chitosan; (4) 100 ppm of chitosan.

Tukey-Kramer multiple comparison test as in Table 2 shows addition of 10 ppm of 400 kGy of irradiated chitosan resulted in the extremely significant ($P<0.001$) increase in weight gain percentage of plants in comparison to the other irradiated chitosan. The addition of 50 ppm of 200 kGy and 400 kGy of irradiated chitosan as well would lead to extremely significant ($P<0.001$) increase in the weight gain percentage of plants in comparison to non-irradiated chitosan. Results obtained also show the addition of 50 ppm and 100 ppm of non-irradiated chitosan was less efficient and decreasing the growth rate of plants.

TABLE 2. statistical analysis (Tukey-Kramer multiple comparison test) on percentage weight gain of Chinese kale (*Brassica alboglabra*).

Chitosan	**Confidence level (P value)**		
	99.9 % (*P* < 0.001)	**99.0 % (*P* < 0.01)**	**95.0 % (*P* < 0.05)**
10 ppm	1. control – chitosan 400 kGy 2. chitosan – chitosan 400 kGy 3. chitosan 50 kGy – chitosan 400 kGy 4. chitosan 100 kGy – chitosan 400 kGy 5. chitosan 200 kGy – chitosan400 kGy	-	-
50 ppm	1. chitosan – chitosan 200 kGy 2. chitosan – chitosan 400 kGy	1. control – chitosan 200 kGy 2. control – chitosan 400 kGy 3. chitosan 50 kGy – chitosan 200 kGy 4. chitosan 50 kGy – chitosan 400 kGy	1. chitosan – chitosan 100 kGy 2. chitosan 100 kGy – chitosan 400 kGy
100 ppm	-	-	1. chitosan 50 kGy – chitosan 400 kGy

Previous studies have shown the use of low molecular weight of chitosan or oligochitosan with the ability to stimulate induction of phytoalexin in plants and thus increasing plant resistance against certain phytopathogens, as well as able to stimulate plant growth (Lehduwi et. al. 2002; Hai et. al. 2003). There is a link between irradiation of chitosan with random molecular chain scission into smaller fragments which as well increasing the probability of oligochitosan production. Rahman et. al. (2013) reported gamma irradiation causes scission of glycosidic linkage and thus decrease the molecular weight of chitosan. In addition, irradiated chitosan had a stimulatory effect on leaf area, length of roots and newly developed shoots, fresh and dry weights, and leaf area for which effectively stimulates the development of roots and shoots of plants (Rahman et. al. 2013). El-Sawy et. al. (2010) meanwhile, reported irradiated chitosan showed a strong effect on the growth of Faba bean plant and can be used in agriculture fields as a growth promoter.

CONCLUSIONS

The addition of 10 ppm of 200 kGy and 400 kGy irradiated chitosan (Mv = 2.16 to 3.62 x 10^5 Dalton) in the hydroponic nutrient solution resulted in the extremely significant ($P<0.001$) increase in the percentage weight gain of Chinese kale (*Brassica alboglabra*). This research has demonstrated degradation of chitosan at a specific irradiation dosage could further improve its characteristic as a plant growth promoter. Findings obtained in this research study indirectly broaden the scope of environmentally friendly modern farming methods with the potential of reducing the dependency on chemical fertilizers and pesticides, as well as increasing agricultural productivity through shorter duration taken for plant harvesting and maturity.

ACKNOWLEDGMENTS

The authors would like to acknowledge Nuclear Science Program, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM) for providing gamma irradiation facility for irradiation of chitosan.

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