Effect of Gamma Irradiated Sodium Alginate on Malabar Spinach (*Basella alba*) and Spinach (*Spinacia oleracea*) as Plant Growth Promoter

Fahmida Parvin[1, 2], Farzana Yeasmin[1, 3], Jahid M M Islam[1], Elias Molla[3] and Mubarak A. Khan[1*]

[1]Institute of Radiation and Polymer Technology, Bangladesh Atomic Energy Commission, Dhaka, Bangladesh
makhan.inst@gmail.com, jahid.bmb@gmail.com
[2]Department of Environmental Sciences, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh
fahmidaprvn497@gmail.com
[3]Department of chemistry, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh

Abstract. Aqueous solution of sodium alginate (3% w/v) was irradiated by gamma radiation at various doses (5-50 kGy). These solutions were diluted to 1500 ppm and applied on malabar spinach (*Basella alba*) and spinach (*Spinacia oleracea*) to study the growth promotion behavior of sodium alginate. Viscosity and molecular weight of the irradiated sodium alginate was found to decrease with increasing radiation dose. Changes in morphological characteristics of those plants in different unit plots were found which is correlated with the use of sodium alginate. The dry matter (32.5%), plant height (33.55%), root length (27.36%), number of leaf (27.27%) were increased compared to the control vegetative plant production, when malabar spinach was nourished by sodium alginate irradiated at 12 kGy. In case of spinach; dry matter (320%), plant height (53.62%), root length (39.92%) and number of leaf (95.45%) were increased when nourished by sodium alginate irradiated at 20kGy.

Keywords: Sodium Alginate, Gamma Radiation, Growth Promoter, Malabar Spinach, Spinach.

Correspondence to: makhan.inst@gmail.com
Telephone +880 2 7788245, (office), Cell: +880 1819252292, Fax: +880 2 7789620

1 INTRODUCTION

Today's over populated world claims more production of crops to fulfill its demand, within its limited hand. For instance, the use of chemical fertilizer for the production of more crops is popular in the recent time. However, the foods are produced using commercial chemical plant growth promoters, might have some harmful effects (Mollah et al., 2009). Chemical fertilizers which are used in agricultural land create environmental pollution (air and water) and facilitate the growth of weeds. For that, scientists are now interested to use natural polymer or bio-fertilizer as plant growth promoter instead of chemical fertilizer.

Chitosan can be used as plant growth promoter but it is very expensive whereas alginates are widespread in nature; occurring in various organisms. Sargassum (brown algae) is the main source of sodium alginate (Na-alginate), which is abundantly found in Coral Island, St. Martin's Island in Bangladesh (Aziz et al., 2001). Na-alginate, the natural growth promoter is friendly for environment. Alginate is the major structural polysaccharide extracted from

brown seaweeds (Khan et al., 2010). Its empirical formula is $NaC_6H_7O_6$. It is composed of three types of block polymers namely polyglucuronate (poly-G), polymannuronate (poly-M) and copolymer of poly-G and poly-M in random sequences (Haug et al., 1967). Recently oligosaccharide derived from depolymerization of alginates by enzyme (referred to as oligoalginate) was reported to have novel features such as stimulation of growth of bifidiobacteria (Akiyama et al., 1992), promotion of germination and shoot elongation of plants (Yonemoto et al., 1993, Tomoda et al., 1994, Natsume et al., 1994). Oligoalginate is prepared by acid hydrolysis or enzymatic degradation of Na-alginate (Hien et al., 2000). On the other hand, radiation techniques can provide a useful tool for degradation of different polymers (Woods & Pikaev, 1994) including natural polysaccharide such as alginate (Kume & Takehisa, 1983) and Chitosan (Kume & Takehisa, 1982, Ulanski & Rosiak, 1992). Thus, degraded alginate and Chitosan can be produced by radiation depolymerization of corresponding polymers.

It was found that, the irradiated alginate of molecular weight less than 10 kD showed a strong effect on the growth promotion of rice and peanut (Hien et al., 2010). 3% solution of Na-alginate irradiated at 37.5kGy is effective for the growth promotion of red amaranth and the suitable concentration are about 150ppm (Mollah et al., 2009). But the effect of irradiated Na-alginate on malabar spinach (*Basella alba)* and spinach (*Spinacia oleracea)* has not been observed yet. Malabar spinach (Local name: Pooi shak; a kind of very common vegetable in Bangladesh) is high in vitamin A, vitamin C, iron, and calcium. It is low in calories by volume, but high in protein per calorie. On the other hand, Spinach is a good source of vitamin A, C, K, manganese, folate, magnesium, iron, calcium, potassium, dietary fiber, zinc etc. It helps to protect cardiovascular diseases, ovarian cancer and migraine. Cooked spinach is an excellent source of Iron, which is particularly important for anematic patients. Because of these high nutrition value, malabar spinach and spinach were chosen to evaluate the effect of irradiated Na-alginate.

In the present work, degraded alginate is prepared by exposing the Na-alginate to gamma rays and then physical parameters such as molecular weight, viscosity of irradiated Na-alginate were measured. After that, the effects of oligomers obtained from radiation-depolymerized alginates as a growth promoter of malabar spinach and spinach were investigated.

2 MATERIALS AND METHODS

2.1 Materials

Na-alginate powder used in this work was purchased from UNI-CHEM, China. Malabar spinach (Basella alba) and spinach (*Spinacia oleracea)* seeds were purchased from a local company named "Lalteer".

2.2 Irradiation of Na-alginate

Na-alginate powder was dissolved in water (3g Na-alginate in 100mL water) by stirring for 4h until completely dissolved. The Na-alginate solutions were irradiated by γ-rays generated from ^{60}Co source at various doses (5 to 50 kGy) at a dose rate of 7.50kGy/h.

2.3 Intrinsic viscosity and molecular weight measurement

For the determination of intrinsic viscosity and molecular weight, the Na-alginate powder was dissolved in a mixture of 0.1M NaCl, and intrinsic viscosity (η) was measured by Ostwald viscometer, which is obtained from the intercept of the Huggins *(*Billmeyer 1971*)*. The experiments were carried out at 25°C temperature. Molecular weight has been measured from intrinsic viscosity by using Mark-Houwink equation (Chuah et al., 2001, King 1994).

2.4 Fourier Transformed Infrared Spectroscopy (FTIR)

The FTIR spectra of the Na-alginate (both unirradiated and irradiated) films were recorded by FTIR Spectrophotometer (Perkin Elmer, Uk) in the wave number range 650–4000 cm^{-1} with resolution of 8 cm^{-1}. The FTIR spectrum was taken in a transmittance mode.

2.5 Application of degraded Na-alginate solution on plants

Malabar spinach and spinach seeds were sowed onto sixteen experimental blocks (8 blocks for malabar spinach and 8 for spinach) and each block was subdivided into three unit plots; thus the total number of unit plots were 48 (unit plot size: 0.5×0.5 m^2). Plots were prepared by adding natural fertilizer (cow dung) in soil. Irradiated 3% (w/v) Na-alginate solution was diluted to a concentration of 1500ppm. Then these irradiated (5-50 kGy radiation doses) solutions were sprayed to the plant; first time in 10 days after sown the seed then in every 7 days interval up to 50 DAS (Days after sown). The amount of solution sprayed was increased (50 to 250 mL of 1500-ppm concentration) with increasing the days after sown.

2.6 Measurement of morphological characteristics

The plant heights were measured from border of the container to the top of the main plant stem. The plant heights were not measured from the top of the soil; as the soil was condense with watering over time. In order to measure the number of leaves, every visible leaves of each plants were counted, including the tips of new leaves just beginning to emerge. In order to measure the leaf area, the leaves were traced on graph paper and the squares covered were counted to get an estimate of the surface area for each leaf. At 55 DAS the plants were harvested and lengths of roots were measured. The plants were dried in oven at 105°C for 12h (Mollah et al., 2009) and then the dried weights were taken.

2.7 Data analyses

The experiment was laid out in the randomized complete block design (RCBD) with three replications. Prior to statistical analysis, data were trimmed by removing the smallest and the largest plant from each harvest.

3 RESULT AND DISCUSSIONS

3.1 Effect of radiation on intrinsic viscosity, molecular weight and FTIR spectra of Na-alginate

The viscosity and molecular weight has decreased with the increase of radiation dose (**Fig. 1**). Molecular weight of Na-alginate is one of the most important characteristic in considering its applications in different fields. The figure shows the decrease of molecular weight of Na-alginate irradiated at various doses of γ-radiation, which indicates that the Na-alginate molecules have been broken down into smaller molecules. The molecular weight of unirradiated Na-alginate is about 9.87×10^4, but when Na-alginate solution is irradiated at 5 kGy, the molecular weight has reduced to 2.14×10^4 and at 50 kGy the molecular weight is 2.00×10^2. Mollah, et al. 2009 and Hien et al. 2000 also found almost similar types of results. This decrease of molecular weight was due to free radical depolymerization, which was induced by gamma radiation.

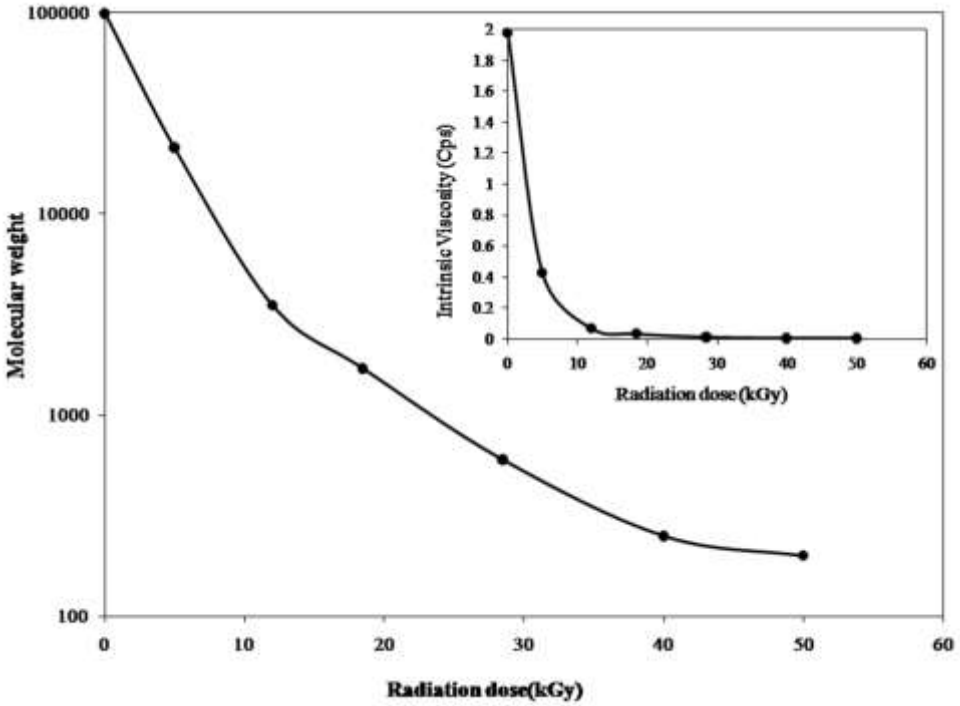

Fig. 1. Decrease of intrinsic viscosity and molecular weight of Na-alginate solution with various doses of gamma radiation.

Fig. 2 shows the FTIR spectra of film made from Na-alginate solution, both unirradiated and irradiated at 12 and 20 kGy. FTIR spectra of pure Na-alginate showed characteristic peaks at 3395 cm^{-1} for O-H stretching , 2933 cm^{-1} for C-H stretching, 1609 cm^{-1} for C=O stretching, 1416 cm^{-1} for C-H bending and at 1100 cm^{-1} for C=O stretching . For Na-alginate irradiated by 12 kGy and 20 kGy, peaks were obtained at almost similar regions. Therefore, there are similar types of value obtained for untreated and irradiated Na-alginate and no peaks for new functional groups are obtained for irradiated Na-alginate, which indicates that only polymer is broken down into smaller molecule but the main unit structure is not broken. From the molecular weight and FTIR spectra analysis, a mechanism can be proposed which is shown in **Fig. 3**. Similar type reaction mechanism of natural polymer has been reported by Mollah, et al., 2009, Charlesby, 1958 and Charlesby and Swallow, 1959.

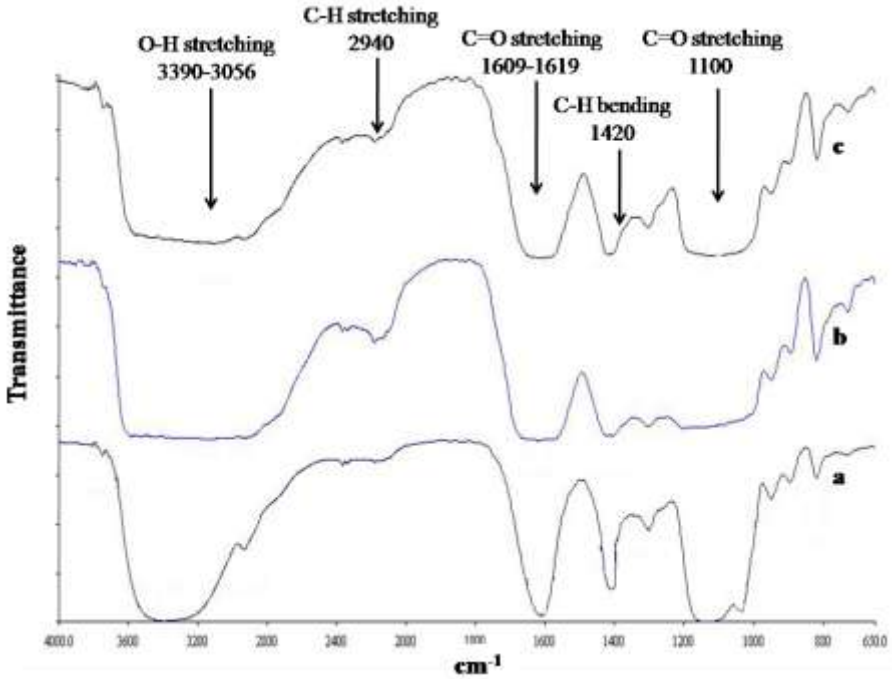

Fig. 2. FTIR spectra of Na-alginate solution a) unirradiated, b) irradiated at 12 kGy, c) irradiated at 20 kGy.

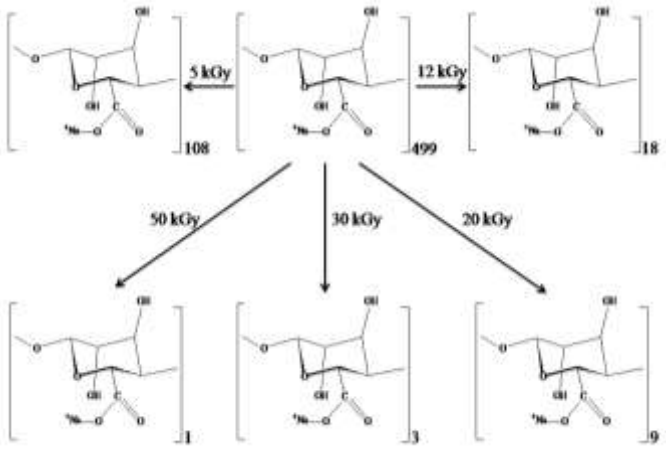

Fig 3. Gamma radiation induced depolymerization of Na-alginate.

3.2 Growth promotion effect of radiation degraded alginate on plants

Plant height, number of leaf, maximum leaf area

The plant height, number of leaf, maximum leaf area of malabar spinach and spinach against different radiation doses (including controlled sample) for upto 50 DAS are shown in **Table 1 and 2**. It was found that, application of irradiated Na-alginate showed a marked effect on the height of plants, number of leaf. The highest plant height and number of leaf average of malabar spinach was found with the treatment of Na-alginate irradiated at 12 kGy. The plant height and number of leaf average of malabar spinach nourished with only water (controlled

sample) and with the treatment of unirradiated Na-alginate solution is lower than the plant treated with irradiated (5-50 kGy) Na-alginate.

Table 1. Effects of irradiated Na-alginate solution of 1500 ppm on malabar spinach applied at different days after sown (DAS).

Treatment	Plant height average (cm)					No. of leaf average					Maximum leaf area (cm^2)				
	22 DAS	29 DAS	36 DAS	43 DAS	50 DAS	22 DAS	29 DAS	36 DAS	43 DAS	50 DAS	22 DAS	29 DAS	36 DAS	43 DAS	50 DAS
Control	12	20	29	39	49	4	9	15	19	22	10	29	47	131	156
Untreated	11	18	25	32.5	42	4	7	12	17	20	11	27	46	131	157
5 kGy	13	24	31	42.5	55	6	12	17	21	23	11	29	48	132	156
12 kGy	13.5	25.5	37	52	65	7	14	20	24	28	12	30	49	135	154
20 kGy	13	24	35	47	62	7	12	18	23	27	11	30	48	133	154
30 kGy	13	23	34	45	60	6	11	17	21	25	11	26	45	129	148
40 kGy	12	22	33	43	58	6	11	16	20	24	11	29	47	134	157
50 kGy	11	21	29	39	46	5	9	14	20	24	10	29	49	128	155

Table 2: Effects of irradiated Na-alginate solution of 1500 ppm on spinach applied at different days after sown (DAS).

Treatment	Plant height average (cm)					No. of leaf average					Maximum leaf area (cm^2)				
	22 DAS	29 DAS	36 DAS	43 DAS	50 DAS	22 DAS	29 DAS	36 DAS	43 DAS	50 DAS	22 DAS	29 DAS	36 DAS	43 DAS	50 DAS
Control	7.0	13	17	22	23	5	8	11	19	22	11	27	50	76	116
Untreated	8.5	14	21	27	29.5	6	11	21	28	31	13	58	65	109	150
5 kGy	8	15.5	21	27.5	31	7	12	18	28	32	19	67.5	78	131	186
12 kGy	10	16	22	28	32	7	15	23	35	40	23	69	80	133	198
20 kGy	11	17.5	25	32	35.4	6	16	25	38	43	28	77	85	159	265
30 kGy	10	15	21	25	26.5	7	11	19	25	28	26	67	75	92	237
40 kGy	7.5	10	13	16.5	20	7	8	12	20	22	15	33.5	45	50	100
50 kGy	7.2	9.4	11	15	17	6	8	9	17	20	14	29	35	48	91

For spinach, nourished with Na-alginate irradiated at 20 kGy, showed best results among the plants nourished by Na-alginate irradiated at six different radiation doses, unirradiated Na-alginate solution and control sample, in respect of these two-growth parameters (the highest plant height and number of leaf average). Irradiated Na-alginate solution has a very little effect on the leaf area of malabar spinach and spinach.

These results supported the result of Hien et al. 2000 and Mollah et al. 2009, where they explained that a certain molecular weight of degraded alginate may be suitable for plants growth. Recently oligosaccharide derived from depolymerization of alginates (referred to as oligoalginate) was reported to have novel features such as stimulation of growth of bifidiobacteria (Akiyama et al., 1992), promotion of germination and shoot elongation of plants (Yonemoto et al., 1993; Tomoda et al; 1994; Natsume et al, 1994).

Root length

Increase of root length indicates the growth of plant. **Fig. 4** shows the root length of malabar spinach and spinach as a function of different radiation dose on Na-alginate including controlled sample. Irradiated Na-alginate has increased the root length of malabar spinach and spinach compared to the controlled sample and sample nourished by unirradiated Na-alginate. In malabar spinach, highest root length was found by the treatment of Na-alginate irradiated at 12 kGy and amounts to 10.10 cm, whereas 7.93cm was found for controlled sample and 7.35 was found for plant treated with unirradiated Na-alginate solution. For spinach, highest root length at 50 DAS was found 18.05 cm with the treatment of Na-alginate irradiated at 20 kGy, whereas 12.90 cm found for controlled sample and 15.20cm for unirradiated Na-alginate solution. This indicates that irradiated Na-alginate solution has marked effect on root length.

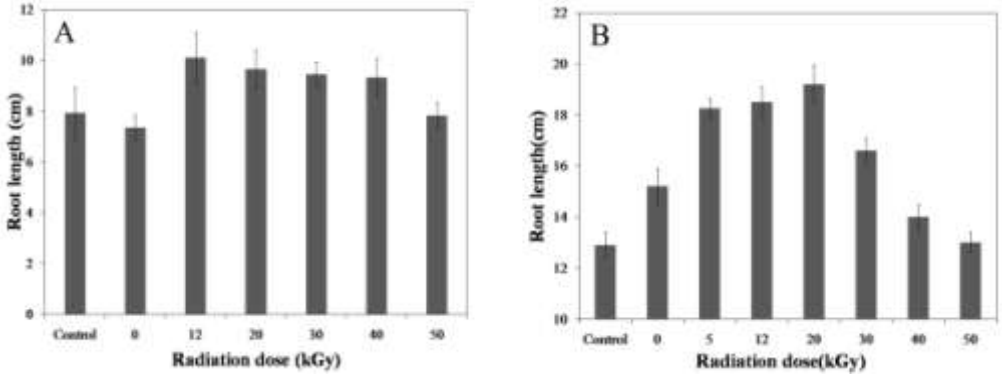

Fig. 4. Effect of irradiated Na-alginate on root length of the plants; A. Malabar spinach, B. Spinach.

Weight of plant

Fig. 5 shows the dry weight of malabar spinach and spinach respectively with various radiation doses of Na-alginate and controlled sample after uprooted the plant. Application of irradiated Na-alginate shows a marked effect on the dry weight of plants. Maximum weight was obtained 21.1 g for malabar spinach treated by 12 kGy irradiated Na-alginate. Dry weight for controlled sample was found 16.6 g and sample treated with unirradiated Na-alginate solution was 12.15g. For spinach, at 50 DAS, highest weight was obtained 6.19 g with the treatment of 20 kGy irradiated Na-alginate, whereas the weight for controlled sample is 1.55 g. The results suggest that foliar spraying of degraded alginate at certain concentrations causes an increase in the biochemical and physiological functions of plants that lead to an increase in dry matter (Hien et al., 2000).

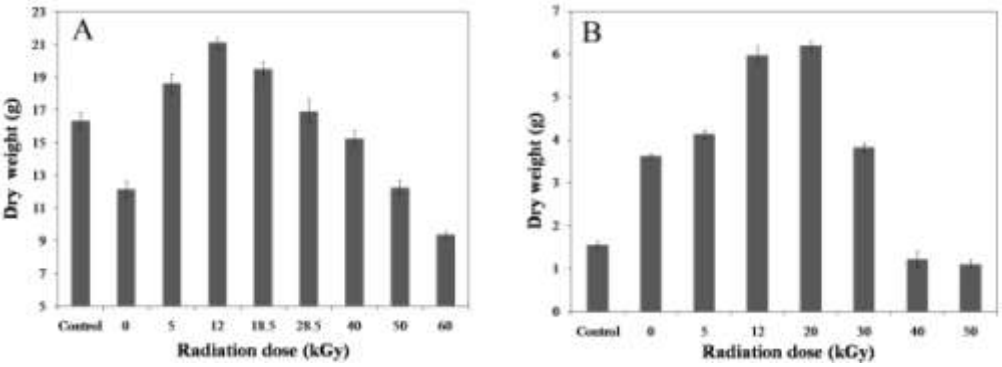

Fig. 5. Effect of irradiated Na-alginate on dry weight of the plants; A. Malabar spinach, B Spinach.

4. CONCLUSION

The experiments of this paper suggesting that gamma radiation degrades Na-alginate to form specific oligomers which can be used as plant growth promoter and eventually increases yield. It is also revealed that growth promotion activity of the oligomer is not only depends on the radiation dose but also crop variety which means dose optimization is required prior to apply as plant growth promoter (i.e. 12 kGy for Malabar spinach and 20 kGy for Spinach). So, there is a huge potentiality of irradiated Na-alginate solution to increase crop yield as it is natural, low cost and have no adverse effect on human or animal health and thus it can play a very important role to combat with challenge of emerging food demand.

Acknowledgment

We thank M.Z.I. Mollah, Radiation and Polymer Chemistry Laboratory, Institute of Nuclear Science and Technology, Bangladesh Atomic Energy Commission, for his valuable suggestion in this research.

References

Akiyama, H., Endo, T., Nakakita, R., Murata, K., Yonemoto, Y. and Okayama, K. (1992). Effect of depolymerized alginates on the growth of bifidobacteria. *Bioscience Biotechnology and Biochemistry*, *56*, 335–356.

Aziz, A., Nurul Islam, A.K.M., Pervin, R. (2001). Marine algae of St. Martin's Island, Bangladesh. *Bangladesh Journal of Botany*, *30 (2)*, 135–140.

Billmeyer, F. W., Jr. (1971). *Textbook of Polymer Science*, *2nd ed*. Wiley- Interscience, New York, p.129.

Charlesby, A. (1958). *Effects of Radiation on Materials*. Reinhold Publishing Corp., New York, p.261.

Charlesby, A.; Swallow, A.J. (1959). Radiation chemistry, *Annual Review of Physics and Chemistry*, *10*, 289.

Chuah , H. H., Lin, V. D. and Soni, U. (2001). Poly(trim ethylene terephthalate) molecular weight and Mark–Houwink equation, *Polymer*, *42(16)*, 7137-7139.

Haug, A., Myklestad, S., Larsen, B., Smidsrod, O. (1967). Correlation between chemical structure and physical properties of alginates. *Acta Chemical. Scandinavica*, *21*, 691-790.

Hien, N.Q., Naotsugu, N., Le, X.T., Fumio, Y., Vo, H.D., Hiroshi, M., Keizo, M., Tamikazu, K. (2000). Growth-promotion of plants with depolymerized alginates by irradiation. *Journal of Radiation Physics and Chemistry*, *59*, 97–101.

Khan, R. A., Khan, M. A., Das, A. K., Debnath, K. K., Dey, K., Kha, A., Saha, S., Huq, T., Noor, N., Sarker, B. and Saha, M. (2010). Thermo-Mechanical and Interfacial Properties of Calcium Alginate Fiber-Reinforced Linear Low-Density Polyethylene Composite. *Polymer Plastics Technological and Engineering*, *49*, 602–608.

King, K. (1994). Changes in the Functional Property and Molecular weight of sodium alginate following gamma irradiation. *Foods Hydrocolloids*, *8*.

Kume, T.; Takehisa, M. (1982). Effect of gamma-irradiation on Chitosan. In: *Proceedings of 2nd International Conference on Chitin and Chitosan*, Sapporo, Japan, pp. 66-70.

Kume, T.; Takehisa, M. (1983). Effect of gamma-irradiation on sodium alginate and carrageen an powder. *Argicultural and Biologica. Chemistry*, *47*, 889-890.

Mollah, M. Z. I., Khan, M. A., Khan, R. A. (2009). Effect of gamma irradiated sodium alginate on red amaranth (Amaranthus Cruentus L.) as growth promoter. *Journal of Radiation Physics and Chemistry*, *78*, 61-64.

Natsume, M., Kamo, Y., Hirayama, M., Adachi, J. (1994). Isolation and characterization of alginate- derived oligosaccharides with root growth-promoting activities. *Carbohydrate Research*, *258*, 187-197.

Tomoda, Y., Umemura, K., Adachi, T. (1994). Promotion of barley root elongation under hypoxic conditions by alginate lyase-lysate (A.L.L). *Bioscience Biotechnology Biochemistry*, *58*, 203-203.

Ulanski, P., Rosiak, J. (1992). Preliminary studies on radiation-induced changes in Chitosan. *Journal of Radiation Physics and Chemistry*, *39*, 53-57.

Woods, R.T., Pikaev, A.K. (1994). (Eds.), *Applied radiation chemistry: radiation processing*, Wiley, New York, pp. 341-391.

Yonemoto, Y., Tanaka, H., Yamashita, T., Kibatake, N., Ishida, Y., Kimura, A., Murata, K. (1993). Promotion of germination and shoot elongation of some plants by alginate oligomers prepared with bacterial alginate lyase. *Journal of Fermentation and Bioengineering*, *75*, 68-70.