

Preparation of Low Molecular Weight Natural Polymers by γ -Radiation and Their Growth Promoting Effect on Zea Maize Plants

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Abstract

Preparation of oligosaccharides from chitosan and Na-alginate were done using γ -rays and H_2O_2 as oxidizing agent. Structural and molecular weight changes of such oligosaccharides were determined by GPC, FT-IR and UV-Vis. spectroscopy. FT-IR and UV-Vis. studies revealed that during radiation degradation process, the main polysaccharide chain structure was almost remained. The effects of spraying 100 ppm of oligochitosan or oligoalginate or both of them with different ratios on the growth of zea maize plants showed an enhancement of plant growth performance and the productivity at the same time suggesting their possible use in agriculture purposes as growth promoters for plants.

Keywords: γ -rays, Degradation, Chitosan, Na-alginate, Growth Promoter, Zea Maize

1. Introduction

Recently, there is a tremendous potential for using polymers in agriculture. In the last decades functionalized polymers revolutionized the agricultural, horticultural and food industry with new tools for the molecular treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients etc.^[1] There is a worldwide trend to explore new natural products that act as growth promoters for plants and that control post-harvest pathogenic diseases, giving priority to that enhance the plant productivity, reduce disease incidence and avoid negative and side effects on human health as a result of the excessive application of synthetic agrochemicals. Among of them chitosan, alginate or carrageenan, a high molecular natural polymer, nontoxic, bioactive agent has become a useful appreciated compounds due to its bio-fertilizer, promotion of germination and shoot elongation,^[2] as a growth stimulator on growth and yield of rice, wheat, maize, black pepper, bean, cabbage, peanut, soybean, tomato, cotton, strawberry,^[3-5] in orchid tissue culture,^[6] fungicidal effects or elicitation of defense mechanisms in plant tissues,^[7-10] stimulation of growth of bifidobacteria to resist infection of diseases for plants particularly oligochitosan in agriculture as biotic elicitor to enhance defense responses against diseases^[11] and suppression of heavy metals stress.^[12]

Different processing technologies have been applied to transfer natural polymers into the marketable products. Radiation processing offers a clean and additive free method for preparation of value-added novel materials based on renewable natural polymers which can be used in various applications including health care, food, polymer processing industry and environment. To fulfill the demands of specific applications, the natural polymers need to possess different characteristics, for example, while for agricultural applications, radiation processing should lead to the formation of lower molecular products. For the environmental applications demand the formation of crosslinked network structures. To possess wound healing characteristics of natural polymers, incorporation of such as natural polymers with specific biocompatible and wound healing characteristics in synthetic polymers can produce biomaterials. Nowadays, radiation modification and degradation of natural polymers to obtain low molecular weight polysaccharides or oligosaccharides were used for development of new applications. Irradiation of alginate or chitosan led to the reduction of molecular weight by scission of glycosidic linkage.^[13-15]

Alginates are the major components of brown seaweed cell walls. Oligoalginates have been obtained by digestion of alginates with an alginate lyase, by treatment with γ -radiation, or by acid hydrolysis. In particular, it has been shown that alginates, depolymerized using γ -radiation, at concentrations of 0.5–1 mg/ml, enhance growth of rice and peanut plants cultivated hydroponically.^[16] In addition, a mixture of oligoalginates obtained by degradation of alginates with a bacterial alginate lyase, at a concentration of 0.5–3 mg/ml, stimulated growth of roots in lettuce,^[17] stimulated elongation of carrot and rice.^[18] Oligoalginates obtained by degradation of alginates with γ -radiation, at a concentration ranging from 0.02 to 0.1 mg/ml, increased shoot and root length, shoot dry weight, content of total chlorophylls and carotenoids, nitrate reductase activity involved in nitrogen assimilation, and alkaloid contents, mainly morphine and codeine, in opium poppy (*Papaver somniferum*) plants.^[9, 19]

Chitosan is one of the most important marine polysaccharide has many peculiar biological activities such as immunity, norcholesterol and antibacterial.^[20,21] Degradation of chitosan is usually used, turning chitosan into one with low molecular weight which exhibits good water solubility. The water-soluble chitosan with low molecular weight has some special biological, chemical and physical properties which are different from that of the ordinary chitosan such as antibacterial activity,^[22] antifungal activity^[23] and antitumor activity.^[24] Due to many unique properties such as biocompatibility, biodegradability, nontoxicity and nonantigenicity; chitosan has been widely applied in medicine, biotechnology, water treatment, agricultural and food science.^[25] Oligochitosans have received much attention as an alternative value-added product because of their bioactivity and other novel biological properties.^[26]

The main objective of the research was to elaborate the method of γ -irradiation of Na-alginate and chitosan to get different oligomers and studying the effect of such oligomers on the growth promotion and productivity of zea maize plants.

2. Materials and Methods

2.1. Materials

Chitosan (CS), degree of deacetylation 85% of high molecular weight, Aldrich. Na-alginate (Alg), of high molecular weight was supplied from Nice lab, India. Hydrogen peroxide (H₂O₂) 30%, Loba chemie. Zea maize seeds from Agrochemical Company for production of seeds and agricultural crops, Egypt.

2.2. Preparation of Oligoalginate and Oligochitosan by γ -irradiation

5% chitosan (in 1% lactic acid) or 5% Na-alginate were prepared then treated by 1% (v/w) H₂O₂ and mixed well to be irradiated by γ -rays at different doses of 25, 35 and 45 kGy.

2.3. Determination of the Molecular Weights

The number average molecular weights of the degraded polymers were determined by Gel permeation chromatography (GPC) 1100 Agilent instrument.

2.4. Ultra Violet Spectroscopy (UV-Vis)

UV absorbance was measured by a UV-Vis spectrophotometer JASCO V-560, Japan, in the range from 190 to 900 nm.

2.5. Infra-red Spectroscopy (FT-IR)

The transmittance was carried out in the form of KBr pellets in the range of 400-4000 cm⁻¹ using infra red spectrophotometer using JASCO FT-IR 6300, Japan.

2.6. Growth Promotion Test

The effects of spraying the oligoalginate, oligochitosan and the binary mixture of them on growth promotion were evaluated using zea maize plants as a model. The concentration of 100 ppm was used. The test field was divided into three groups of irradiation treatment of 25, 35 and 45 kGy. Each group divided into different separate lines. After plantation of 15, 30, 45 and 60 days, each three lines of plants were sprayed by 100 ppm solution of

- (a) Oligoalginate (Alg) prepared by γ -irradiation at 25 kGy
- (b) Oligochitosan (CS) prepared by γ -irradiation at 25 kGy
- (c) Oligoalginate/Oligochitosan (Alg/CS) (1/1) prepared by γ -irradiation at 25 kGy
- (d) Oligoalginate/Oligochitosan (Alg/CS) (1/2) prepared by γ -irradiation at 25 kGy
- (e) Oligoalginate/Oligochitosan (Alg/CS) (1/2) prepared by γ -irradiation at 25 kGy

The same method of spraying was repeated at for oligoalginate or oligochitosan prepared by γ -irradiation at 35 and 45 kGy. The growth rate of the plants is compared with control lines (untreated plant).

The increase in the grain yield of zea mzize plants after dryness for control and treated plants by oligoalginate, oligochitosan and binary mixture of them according to the following equation:

$$\text{The increase in grain yield (\%)} = ((G_t - G_c)/G_c) * 100$$

Where G_c and G_t are the grain yield of zea maize for control samples and the grain yield for zea maize plants treated by different types of prepared oligomers.

2.7. Statistical Analysis

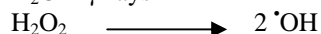
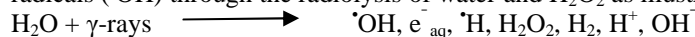
The experiment was carried out with three blocks with three replicates for each treatment. Data were statistically processed by analysis of variance (ANOVA).

3. Results and Discussion

3.1. Degradation of Chitosan and Na-alginate by γ -irradiation

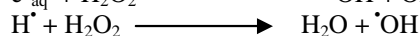
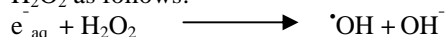
Fig. 1 shows the effect of γ -irradiation and oxidizing agents as H_2O_2 on the molecular weight of chitosan and Na-alginate solutions at the irradiation dose of 25-45 kGy. It was found that the molecular weight of chitosan or Na-alginate decreased with increasing the irradiation dose. Meanwhile, the addition of H_2O_2 during the irradiation process accelerates the degradation process, decreases chitosan and Na-alginate molecular weight and reduces the dose required for degradation of chitosan and Na-alginate. The molecular weight of unirradiated chitosan and Na-alginate was 1900 and 2400 kDa, respectively. Using 45 kGy, the molecular weight of chitosan and Na-alginate became 125 and 320 kDa, respectively. Meanwhile, in presence of H_2O_2 the molecular weight decreased to 10.5 and 15 kDa, respectively. It is obvious that there is a synergistic effect on the degradation rate of chitosan and Na-alginate when the radiation process was carried out in the presence of H_2O_2 which enhances and accelerates the rate of polymer scission. The results are agreed well with previous reports.^[15-16,27-29] After irradiation the resultant oligochitosan and oligoalginate solutions were directly used as a biotic elicitor for plants. In a previous report, the molecular weight obtained during γ -irradiation of chitosan (solid form as a paste) in presence of 10% H_2O_2 at 120 kGy was 8.5 kDa.^[15] While, in our experiment, 5% chitosan solution containing 1% H_2O_2 was degraded by γ -irradiation at 45 kGy to 10.5 kDa. The degradation rate of chitosan derivatives irradiated in solution is much higher than that irradiated in paste like form. The irradiation dose required for degradation of chitosan derivatives is low if compared with that in paste like form. The water radiolysis products like $\cdot OH$ radicals have a great effect on positive degradation of chitosan derivatives. The irradiation in solution form is better than that in solid form which reduces the required dose for degradation.

Polysaccharides are typical degradable materials under ionizing radiation through the β -(1-4) glycosidic bond cleavage resulting in the reduction of their molecular weights. The reason is due to the formation of hydroxyl radicals ($\cdot OH$) through the radiolysis of water and H_2O_2 as illustrated in the following equations:^[30]

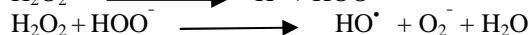
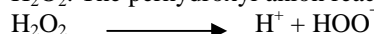


Furthermore, during the irradiation, e_{aq}^- and $\cdot H$ can react with

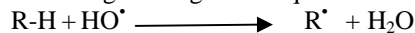
H_2O_2 as follows:



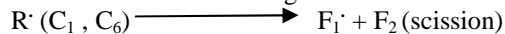
Hydrogen peroxide is substantially more acidic than water and formed perhydroxyl anion which is instable.^[31] The decrease in stability of H_2O_2 is caused by the instability of the HOO^- . γ -rays increase the decomposition of H_2O_2 . The perhydroxyl anion reacts with H_2O_2 to form the highly reactive hydroxyl radical ($\cdot OH$)



In general hydroxyl radical reacts with carbohydrates exceedingly rapidly, abstracting a C-bonded H atom according to the general equation:



These radicals then undergo further reactions before ending up as products as follows:



where R-H polysaccharide macromolecules, $R\cdot (C_n)$ and F_1, F_2 are fragments of the main chain after scission

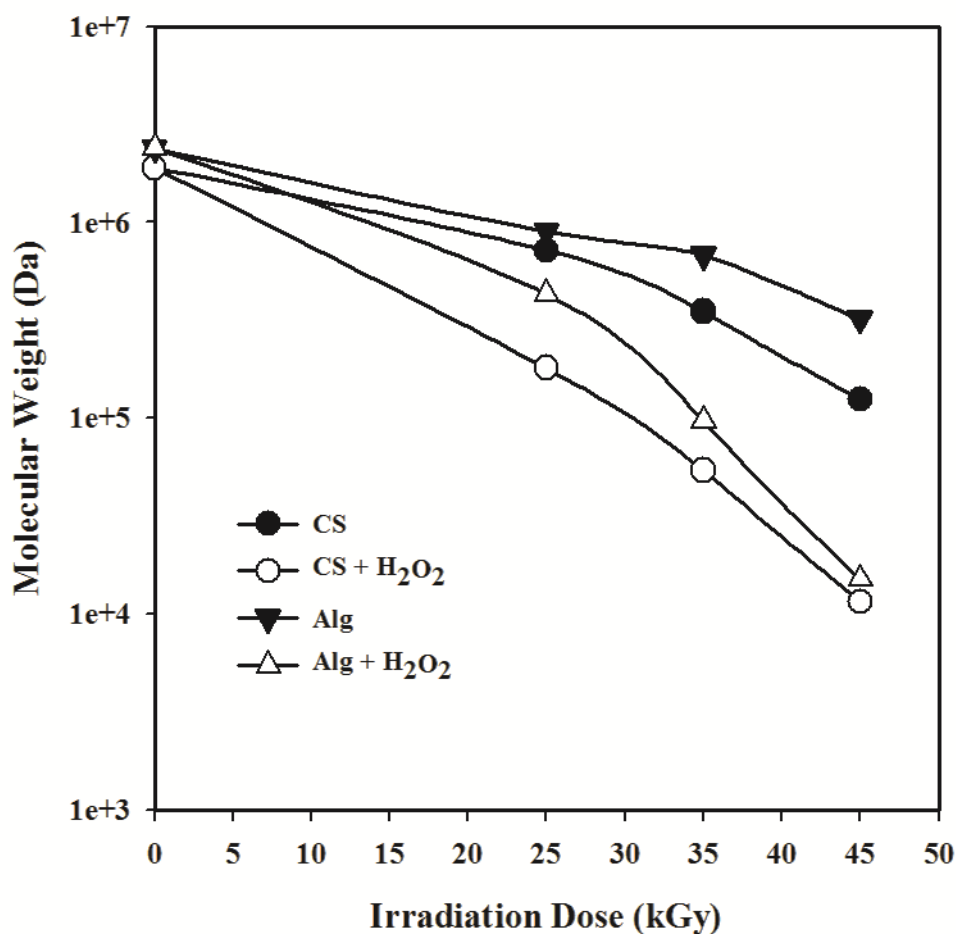


Fig. 1: The average molecular weights of chitosan and Na-alginate 5% solution using GPC after γ -irradiation at different doses; (●) chitosan + γ -rays, (○) chitosan + 1wt% H₂O₂ + γ -rays (▼) Na-alginate + γ -rays and (△) Na-alginate + 1wt% H₂O₂ + γ -rays.

3.2. Characterization of chitosan and alginate Oligomers

3.2.1. UV-Vis Spectroscopy

UV-Vis spectra of unirradiated and irradiated chitosan and Na-alginate were illustrated in Fig. 2. For the unirradiated and irradiated chitosan Fig. 2A, there are two absorption peaks at 275 and 315 nm were observed. The intensity of these peaks increased with increasing the irradiation dose. These two peaks may be due to the presence of unsaturated carbonyl and carboxyl groups. The obtained results are consistent with those reported previously.^[32]

For the unirradiated and irradiated Na-alginate Fig. 2B, there is a new absorption band around 275-280 nm and the band intensity increases with increasing the irradiation dose. This can be assigned to unsaturated bonds of Na-alginate formed after main chain scission and/or hydrogen abstraction reaction occurred during irradiation.^[33]

The aqueous solution Na-alginate pall yellowish color that changed to brown by radiation confirmed the formation of the unsaturated bonds. As the exposure dose increases, the brown color intensity changes to deep ones.

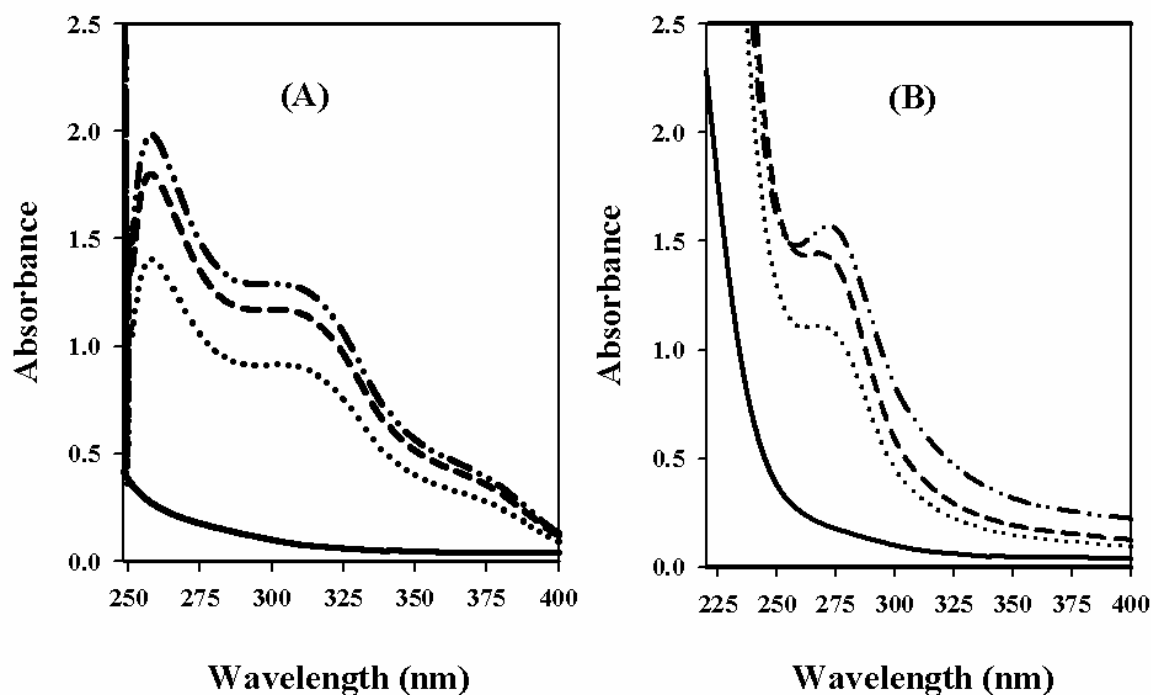


Fig. 2: UV-Vis spectra of (A) chitosan and (B) Na-alginate; unirradiated polymer (—) and the irradiated 5% polymer solution in presence of 1wt% H₂O₂ at (.....) 25, (---) 35 and (-·-) 45 kGy.

3.2.2. FT-IR spectroscopy

FT-IR spectra of unirradiated and irradiated chitosan and Na-alginate were illustrated in Fig. 3. FT-IR spectrum of chitosan Fig. (3 A, curve a) shows distinctive absorption bands appear at: 3440 cm⁻¹ (-OH and -NH₂), 2875-2920 cm⁻¹ (C-H stretching), 1638 cm⁻¹ (the stretching of amide C=O), 1598 (-NH bend), 1387 cm⁻¹ (amide -NH), 1156 cm⁻¹ and 1097 cm⁻¹ corresponds to the ether bond of saccharine ring. The spectrum of irradiated chitosan Fig. (3A, curves b, c and d) exhibited most of the characteristic absorption peaks of native chitosan but with some differences. For instance, the increase in the ratio of hydroxyl groups at 3445 cm⁻¹ due to the decrease in the inter- and intra-molecular hydrogen bonding between -OH and -NH₂ groups of chitosan resulting from the scission of glycosidic bonds leads to the formation of hydroxyl group, which is manifested as an increase in the ratio of hydroxyl group. The characteristic peaks at 1638 cm⁻¹ (-C=O) and 1385 cm⁻¹ (C-O stretch vibration) were shifted to higher wavenumber and its intensity increased with increasing the irradiation dose which manifested the formation of carbonyl or carboxyl after degradation. In general the changes in spectra confirmed that carbonyl or carboxyl groups were formed, partial amino groups were eliminated and/ or slightly some ring opening occurred during the radiation degradation process of chitosan.

In the spectrum of native Na-alginate in Fig. (3 B, curve a), the peaks at 3390 and 1095-1035 cm⁻¹ are attributed to hydroxyl and C-O-C groups, respectively. The asymmetric and symmetric stretching of carboxylate vibrations appeared at 1620 and 1420 cm⁻¹, respectively.^[34] The spectrum of irradiated Na-alginate Fig. (3 B curves b, c and d) exhibited most of the characteristic absorption peaks of native Na-alginate but with some differences. For instance, the bands at 1620 cm⁻¹ for carboxylate groups, at 3340 for OH groups and at 1035 cm⁻¹ and 1095 for (C-O stretching) which became broader and shift to another wavenumbers. The spectrum also showed the new bands appeared at 1725 cm⁻¹. The spectra indicated that the formation of carboxyl groups suggesting that ionizing radiation treatment under extreme conditions broke the glucosidic bonds with the change of the structure of reducing end residue and formation of -C=O groups. Also, the scission of glycosidic bonds leads to the formation of hydroxyl group, which is manifested as an increase in the ratio of hydroxyl group peak at 3425 cm⁻¹.

The FT-IR results of oligoalginate or oligochitosan exhibited most of the bands as the original one, indicating that the main polysaccharide structure of the resulting oligomers still remained, the proposed mechanism of degradation of chitosan and Na-alginate irradiated in liquid state could be illustrated in Scheme (1).

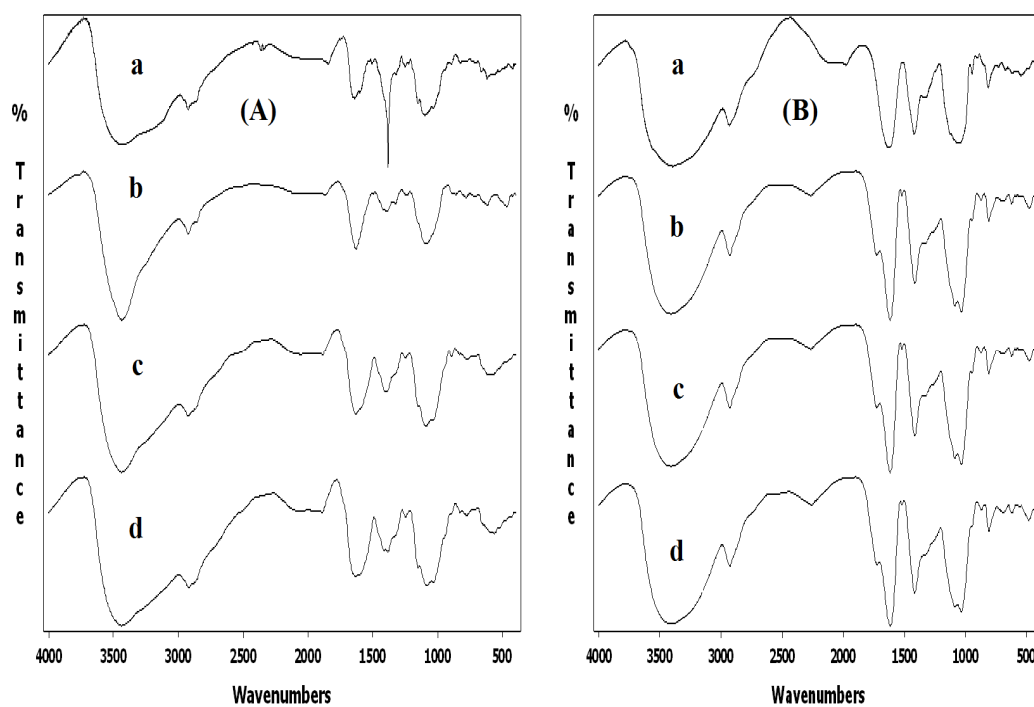
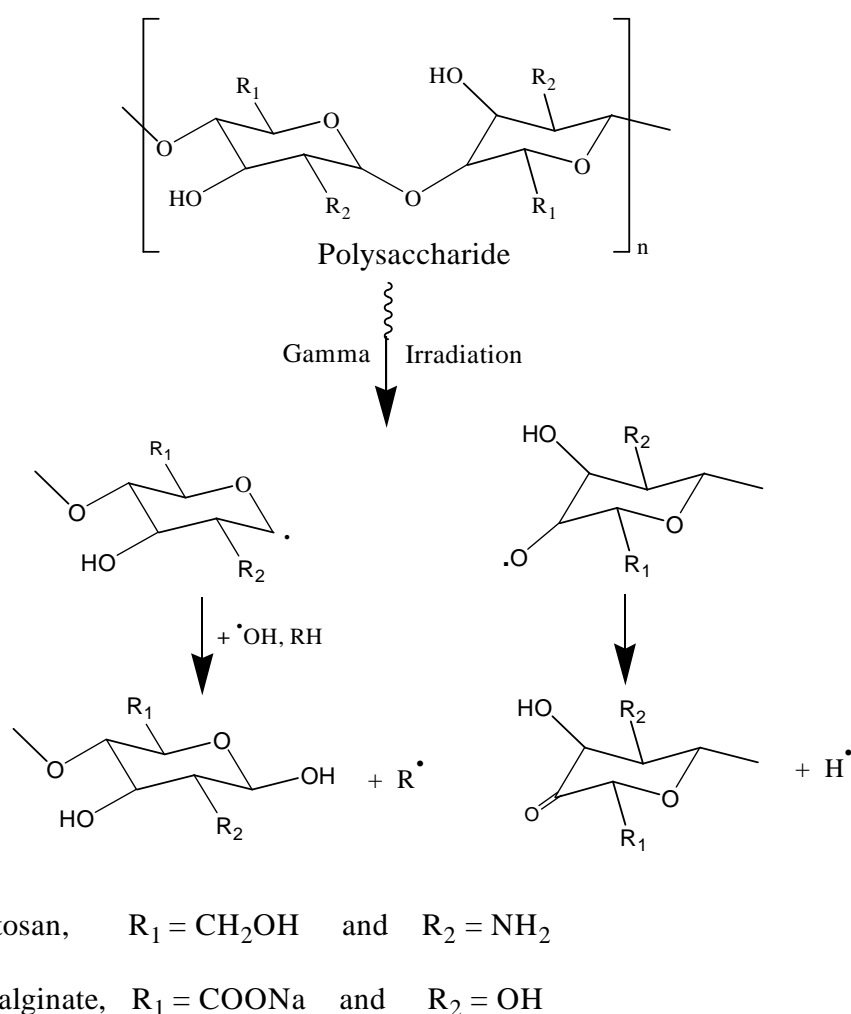


Fig. 3: FT-IR spectra of (A) chitosan and (B) Na-alginate; unirradiated polymer (a) and the irradiated 5% polymer solution in presence of 1wt% H₂O₂ at (b) 25, (c) 35 and (d) 45 kGy.



Scheme (1): Proposed mechanism of radiation induced degradation of chitosan and Na-alginate

3.3. Growth Promotion Effect of Chitosan and Na-alginate Oligomers on Zea Maize Plants

The aim of the agricultural tests was to examine the effect of spraying 100 ppm of oligoalginate, oligochitosan and binary mixture of them on growth promotion of the zea maize plants. Fig. 4. shows a picture of zea maize plants after 30 days of plantation and treated two times by spraying the prepared oligomers on the surface of plants. The treatment of plants was carried out three times of different ages at 15, 30 and 45 days of plantation. It was obvious that the treatment of zea maize plants with the different oligosaccharides obtained by γ -irradiation in presence of 1% H_2O_2 enhances zea maize plant growth promotion and performance.

Table (1) describes plant length (cm), paper width (cm), ear length (cm), 100 grain dry weight (g), dry ear weight (g) and grain yield/ acre (ardab) of total crop of zea maize obtained after the different treatment by spraying the plants 3times, completing the season of plantation (about 3 months and 20 days) and dryness. Fig. 5. shows the increase in grain yield (%) of crop zea maize after different treatment by prepared oligomers. It was observed that the highest growth was obtained for those plants treated by the oligomers prepared at 45 kGy of irradiation in presence of H_2O_2 . Compared to control one, the treatment of plants with different types of oligomers showed growth promotion effect followed the order of oligomers prepared at 45 kGy > oligomers prepared at 35 kGy > oligomers prepared at 25 kGy. The treatment of zea maize plants by oligoalginate was better than oligochitosan. The lower molecular weight of alginate obtained by γ -irradiation, the higher the growth promotion effect. The spraying of zea maize plants with binary mixture of oligoalginate/ oligochitosan especially the ratio of (2/1) has a promising effect on the growth. Compared to control plants, the increase in grain yield (%) of crop zea maize by treatment of oligoalginate prepared at 25, 35 and 45 kGy was 8.6, 31.7 and 47.3 %, respectively. While, the increase in grain yield (%) by the treatment of oligochitosan was 5.5, 26.2 and 40.6 %, respectively. The effect of mixing the oligoalginate and oligochitosan prepared at the same irradiation

has promising effect on increasing the grain yield crop. Also, with increasing the ratio of oligoalginate in the binary mixture increases the yield. The mixture of oligoalginate/ oligochitosan prepared at 45 kGy with the ratios of (1/1), (1/2) and (2/1) enhances the increase in yield crop as followed 39.8, 48.1, 53.9 %, respectively. The mixture of oligoalginate/ oligochitosan with ratio of (2/1) prepared at 45 kGy gives plant length (cm), paper width (cm), ear length (cm), 100 grain wt (g), ear wt (g) and grain yield/acre (ardab) 313, 12.2, 27, 40.54, 393 and 36.1, respectively if compared with that of control one (untreated) which gives 266, 9.8, 25, 22.35, 25 and 23.45, respectively.

In a previous report,^[15,35] the grain yield/acre (ardab) during the treatment of plants by γ -irradiation alginate or chitosan (solid form as a paste) in presence of 10% H₂O₂ at 120 kGy was 26.7 and 25.8 ardab, respectively. While, in our experiment, 5% alginate or chitosan solutions containing 1% H₂O₂ was degraded by γ -irradiation at 45 kGy enhanced to 35.5 and 32.8 ardab, respectively with an increase about 33 and 28 % in the yield, respectively. The irradiation in solution form is better than in solid form which reduces the required dose for degradation.

It was reported that the oligoalginate prepared by irradiation promoted the growth and development of crop plants (rice, peanuts, barley, and soybeans).^[16] Chitosan has a positive impact on plants growth. It was reported that irradiated chitosan solution is effective as plant growth enhancer. Chitosan enhances the vegetative growth in terms of the average values of stem length, number of growing leaves, including leaf width and length. The use of irradiated Na-alginate improved the quality of maize plant. The grain size, grain weight, total protein and total oil (%) increased if compared with the control one. The alginate oligomers generated by de-polymerization of Na-alginates have been reported to stimulate the plant growth, seed germination and shoot elongation in plants.^[16,36] They act as signal molecules that regulate plant growth and development as well as the defense reactions in plants by regulating gene expression. The results suggest that alginate derived oligosaccharide probably applied as leaf-sprays improved the growth attributes, enhanced the acceleration of the metabolic activities, photosynthetic capability and enzyme activities.^[8-10]

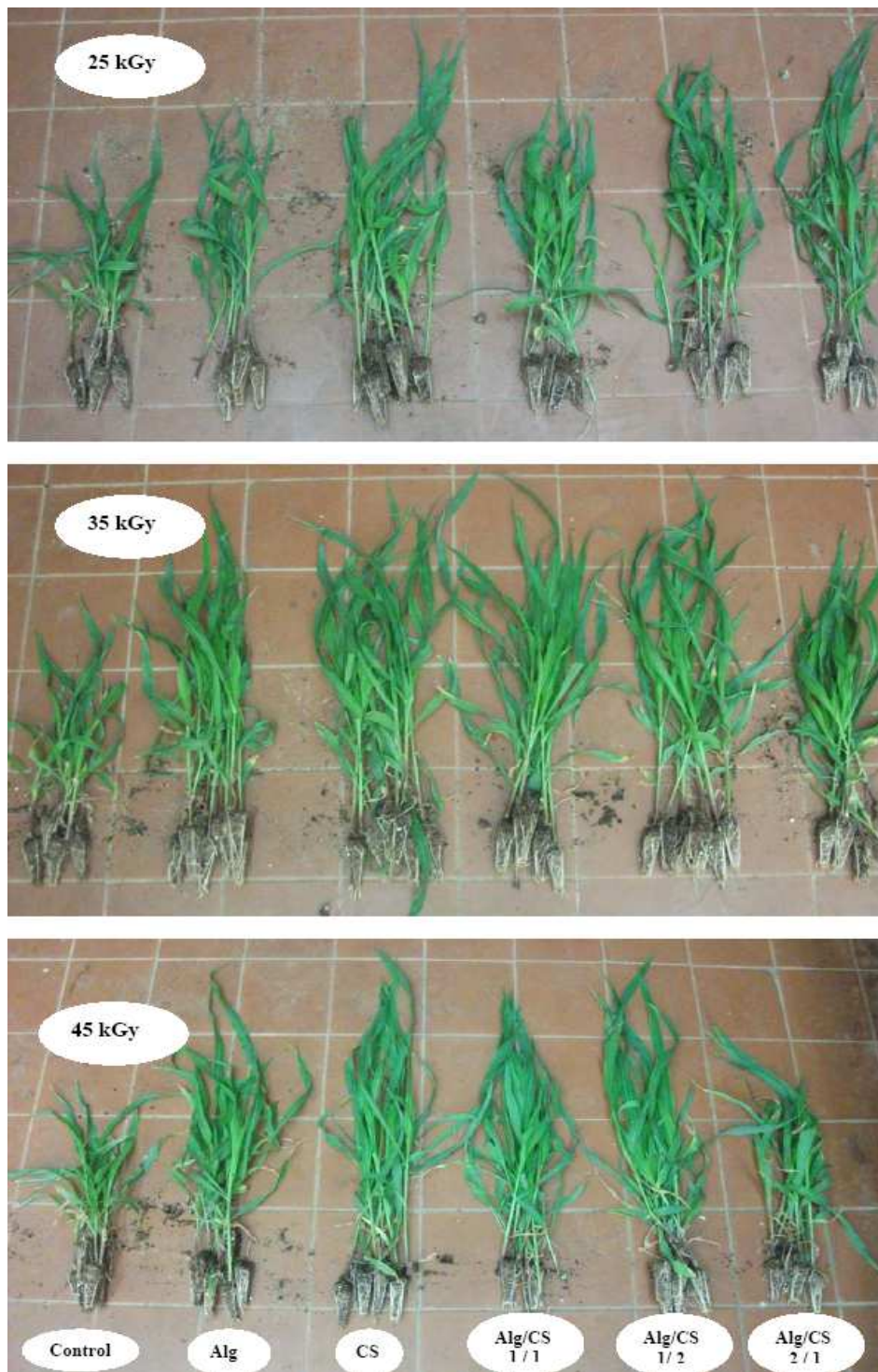


Fig. 4: Effect of spraying 100 ppm of oligoalginate or oligochitosan or binary mixture of them with different ratio and different irradiation doses on growth promotion of zeamaize plants after plantation of 30 days.

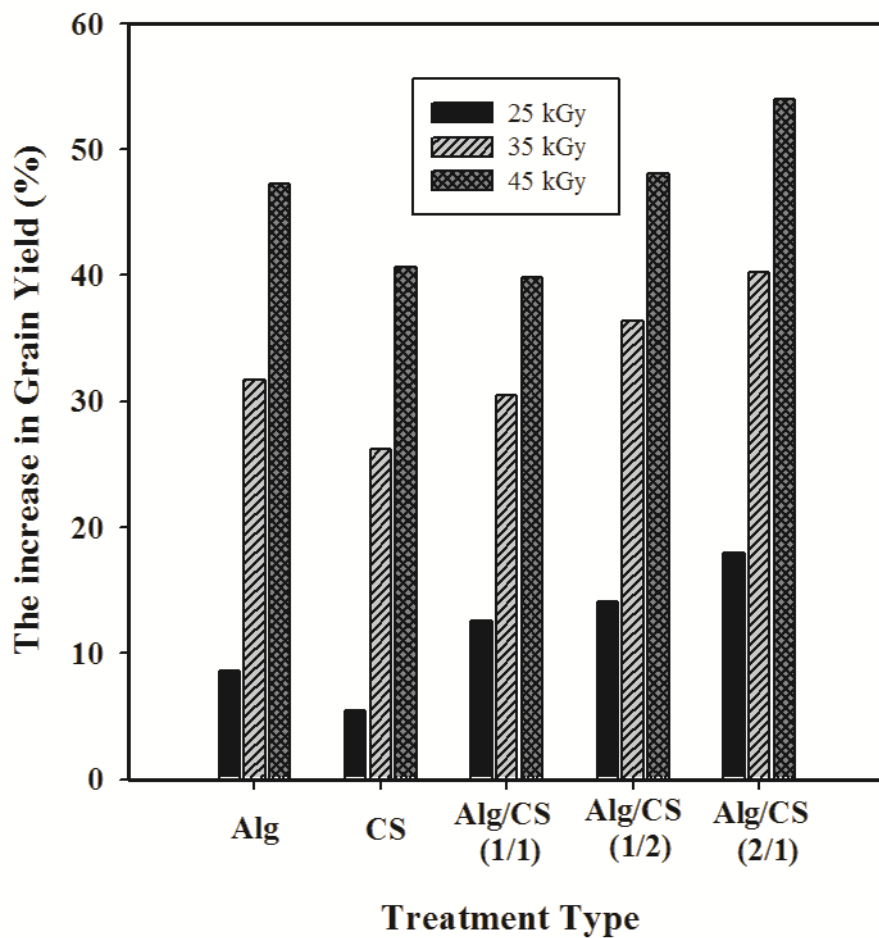


Fig. 5 : The increase in grain yield (%) of crop zea maize after treatment by different types of oligomers prepared by γ -irradiation in presence of H_2O_2 .

Table (1): Effect of spraying 100 ppm of oligoalginate, oligochitosan and binary mixture on crop yield of zea maize plants

Dose	Treatment	Plant length, (cm)	Paper width, (cm)	Ear length, (cm)	100 grain wt (g)	Ear wt (g)	Grain Yield/ acre (ardab)
0 kGy	Control	266	9.8	25	22.35	256	23.45
	Alg	263	10.3	26	24.73	278	25.47
	CS	268	10.5	25.8	23.25	270	24.74
25 kGy	Alg/CS(1/1)	274	10.7	26.4	25.19	288	26.39
	Alg/CS(2/1)	281	12.1	26.8	25.35	292	26.75
	Alg/CS(1/2)	283	12.2	27.5	26.15	302	27.67
	Alg	291	10.7	29	32.17	337	30.88
	CS	298	11.3	28	30.22	323	29.59
35 kGy	Alg/CS(1/1)	287	12.1	28	28.7	334	30.6
	Alg/CS(2/1)	291	12.3	28.5	32.95	349	31.98
	Alg/CS(1/2)	308	12.6	29.5	33.93	359	32.89
	Alg	307	10.6	29.2	38.61	377	34.54
	CS	314	11.8	28	36.27	360	32.98
45 kGy	Alg/CS(1/1)	309	11.3	27.6	34.47	358	32.8
	Alg/CS(2/1)	318	12.3	30.3	39.5	379	34.73
	Alg/CS(1/2)	313	12.2	27	40.54	393	36.1

4. Conclusion

γ -irradiation is a useful tool for degradation of alginate and chitosan. Spraying zea maize plants by 100 ppm of oligoalginate or oligochitosan or mixture of them prepared by γ -irradiation in presence of 1% H₂O₂ had a positive effect on plant growth and the productivity at the same time. The lower molecular weight of alginate and chitosan, the higher growth promotion effect obtained. The irradiation in solution form is better than that in solid form which reduces the required dose for degradation. Spraying zea maize plants by oligoalginate and/or oligochitosan prepared by γ -irradiation at 45kGy increase the grain yield crop about 47 and 40 %, respectively (Fig. 5). While, the combining of the two oligomers at the ratio of (2/1) increases the yield (%) to about 53 %, compared to the control plants (untreated). It can be concluded that the possible use of such oligosaccharides as growth promoters for plants in the agriculture applications.

6. Acknowledgment

This work is supported by The International Atomic Energy Agency (IAEA) under Research Contract No. 14425/RO Regular Budget Fund.

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