

*Full Length Research Paper*

# **An evaluation of the effects of irradiated sodium alginate on the growth, physiological activities and essential oil production of fennel (*Foeniculum vulgare Mill.*)**

**Adeeba Sarfaraz<sup>1</sup>, M. Naeem<sup>1\*</sup>, Shafia Nasir<sup>1</sup>, Mohd Idrees<sup>1</sup>, Tariq Aftab<sup>1</sup>, <sup>1</sup>Nadeem Hashmi, M. Masroor A. Khan<sup>1</sup>, Moinuddin<sup>1</sup> and Lalit Varshney<sup>2</sup>**

<sup>1</sup>Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh 202 002, India.

<sup>2</sup>Head Advanced Materials Section, ISOMED, Bhabha Atomic Research Centre, Mumbai -400085, India.

Accepted 1 September, 2010

**In the present study, sodium alginate, degraded by Co-60 gamma rays, was used to evaluate the efficacy of irradiated sodium alginate (ISA) on *Foeniculum vulgare Mill.* The aim of this study was to find out the effects of various concentrations of ISA viz. deionised water (control) and UN (un-irradiated), 20, 40, 60, 80, 100 and 120 ppm ISA in order to get the best response of fennel in terms of various attributes. The growth attributes (shoot and root lengths, number of leaves, fresh and dry weights per plant), yield and quality attributes (number of umbels per plant, number of umbellets per umbels, 100-seed weight, seed yield per plant, content and yield of essential oil) and biochemical parameters (total chlorophyll and carotenoids contents, carbonic anhydrase activity, nitrate reductase activity and proline content) were determined at 70 days after sowing. The results obtained by treatment with un-irradiated sodium alginate showed poorest effect and gave equal value to the control for all the studied attributes and did not significant to each other in its effect. Of the eight ISA concentrations, 80 ppm proved to be the best concentration compared to the other foliar concentrations of ISA. The present work revealed that ISA, applied as leaf-sprays at concentrations of 20 to 120 ppm, improved growth, yield and quality attributes and biochemical parameters of fennel significantly. However, further investigations are required to comprehend the mechanism and mode of action of alginate-derived oligomers for plant productivity and quality.**

**Key words:** *Foeniculum vulgare Mill.*, irradiated sodium alginate (ISA).

## **INTRODUCTION**

It is now being realized that irradiation products of natural bioactive agents, such as various polysaccharides, can also be beneficially utilized to improve the existing methodologies used to impart value addition in agriculture by converting these bioactive agents into more useful form (Sabharwal, 2004). Sodium alginate is a natural polysaccharide, derived from brown algae, available in large quantities in nature. As compared to the conventional techniques, like acid/base hydrolysis, and

enzymatic methods (Shimokawa et al., 1996), radiation processing of bioactive agents by Co-60 gamma rays offers a clean one-step method for the formation of low molecular weight oligomers (Nagasawa et al., 2000; Lee, 2003).

Polysaccharides such as sodium alginate, in their depolymerised form have a novel property to act like plant growth regulators have shown spectacular effects on plant productivity. Sodium alginate was irradiated at 520 kilo Gray (kGy) by Co-60 gamma rays and the degraded alginate products (small size oligomers) was used to confirm its effectiveness as a plant growth promoter for fennel crop. However, these degraded

\*Corresponding author. E-mail: [naeem\\_phd@yahoo.co.in](mailto:naeem_phd@yahoo.co.in).

oligomers when applied to different plants, elicited various kind of biological and physiological activities, including promotion of plant growth in general, seed germination, shoot elongation, root growth, flower production, antimicrobial activity, suppression of heavy metal stress, phytoalexin induction Yonemoto et al., 1993; Hadwiger, 1994; Zakaria et al., 1995; Ohta et al., 1999; Hien et al., 2000; Ahni et al., 2001; Tham et al., 2001; Cabalfin, 2002; Kume et al., 2002; Mohd Hafez et al., 2003; Hu et al., 2004; Natsume et al., 1994; Tomoda et al., 1994; Luan et al., 2005; Hegazy et al., 2009).

Fennel (*Foeniculum vulgare* Mill.) has been long considered as a medicinal and spice herb. It is medicinally used as laxative, stomachic, carminative, stimulants and for prevention of colic in infants. Both oil and fruits are recommended for eye diseases, burning sensations, fever, thirst, wounds, dysentery and leprosy (The Wealth of India, 1992).

Keeping the immense medicinal value of fennel in mind, an assumption has been made to find out whether these gamma-rays degraded oligomers of alginates as foliar application could ameliorate growth, physiological activity and yield attributes as well as production of essential oil in fennel.

## MATERIALS AND METHODS

### Plant material and soil

Healthy seeds of *F. vulgare* Mill. were initially surface sterilized with 95% ethyl alcohol for 5 min and then washed thoroughly with double distilled water before sowing. Prior to seed sowing, 5.0 kg homogenous mixture of soil and farmyard manure (4:1) was filled in each pot. The soil samples were tested at the Government Soil Testing Laboratory, Quarsi Farm, Aligarh. The experimental soil was sandy loam with pH (1:2) 7.5, E.C (1:2) 0.57 mmhos/cm, and available N, P and K 98.45, 7.15 and 141.8 mgkg<sup>-1</sup> soil, respectively.

### Irradiation of sodium alginate by Co-60 gamma rays

Sodium alginate (SA) was purchased from Sigma-Aldrich, USA. SA samples were irradiated by gamma rays from Co-60 source at 520 Kilo Gray (kGy). It was sealed in a glass vial with atmospheric air. Different concentrations of irradiated sodium alginate (ISA), viz. 0 (control), 20, 40, 80 and 120 ppm were finally prepared using double distilled water.

### Experimental design and pot culture

A pot culture experiment was conducted to statistically analyze the ISA-affected changes in physiological and biochemical parameters and growth as well as quality attributes of *F. vulgare* Mill. at the Botany Department, A.M.U., Aligarh (27° 52' N latitude, 78° 51' E longitude, and 187.45 m altitude). The experiment was conducted in the natural conditions of net house using earthen pots (25 cm diameter × 25 cm height) according to randomized complete block design. Two controls were taken for this purpose. In the first control, the plants were sprayed with deionized water (DDW) and in the second control the plants were sprayed with 20 ppm unirradiated

alginate (UN). Five levels [viz. 0 (control), 20, 40, 80, and 120 ppm] of oligomers obtained from degraded sodium alginate (ISA) were employed as treatments. 20 ppm was prepared by dissolving 2 mg of the SA in 100 ml DDW.

Similarly, all the above treatments were prepared. Therefore, there were six different concentrations of ISA and two controls, thus, consists of 8 treatments. Each treatment was replicated five times. About 90% of the seeds germinated within 5-6 days of sowing. After a month of germination, the seedlings were thinned at the rate of one plant per pot. Lastly one healthy plant was maintained per pot. Seeds were sown at a depth of 2 cm. The first foliar spray of alginates was given to fennel at 35 days after sowing (DAS) and the spraying was continued at an interval of 10 days using a hand sprayer. Thus, the total number of sprays was 10. The pots were watered as and when needed.

### Growth and yield characteristics

Growth attributes of the *F. vulgare* L. were determined at 70 days after sowing (DAS). At this stage, five plants of each treatment were carefully harvested with the roots and washed thereafter with tap water to remove adhering foreign particles. Water adhering to the roots was removed with blotting paper. Fresh weights of plants were recorded using an electronic balance. The clean and blot-dried plants were oven-dried at 80°C for 24 h. Dry weights of plants were recorded thereafter. The shoot length and root length of the plants was measured with the help of a meter scale. Number of umbels per plants and number of umbellets per umbel were counted. Hundred seeds were counted and seed yield per plant was calculated accordingly.

### Biochemical parameters

The youngest fully developed fresh leaves were used for the analysis of various biochemical parameters.

### Total chlorophyll and carotenoids content

Total chlorophyll and carotenoids content in fresh leaves were estimated by the method of Lichtenthaler and Buschmann (2001). The fresh tissue from the interveinal leaf-area was ground using a mortar and pestle using 80% acetone. The absorbance of the extract-solution was recorded at 662 and 645 nm for chlorophyll estimation and at 470 nm for carotenoids estimation using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan).

### Nitrate reductase (NR) activity

Nitrate reductase (EC 1.6.6.1) activity in the fresh leaves was determined by the intact tissue assay method of Jaworski (1971). Chopped leaf pieces (200 mg) were incubated for 2 h at 30°C in a 5.5 ml reaction mixture, which contained 2.5 ml of 0.1 M phosphate buffer, 0.5 ml of 0.2 M potassium nitrate, and 2.5 ml of 5% isopropanol. Subsequently, the nitrite formed was colorometrically determined at 540 nm after azocoupling with sulphanilamide and maphthyl ethylemediamime. The NRA was expressed as n M NO<sub>2</sub> g<sup>-1</sup> FW h<sup>-1</sup>.

### Carbonic anhydrase (CA) activity

Carbonic anhydrase (E.C. 4.2.1.1) activity was measured in fresh leaves using the method of Dwivedi and Randhawa (1974). 200 mg of fresh leaf pieces were transferred into Petri plates. The leaf

pieces were dipped in 10 ml of 0.2 M cystein hydrochloride solution for 20 min at 4°C. To each test tube, 4 ml of 0.2 M sodium bicarbonate solution and 0.2 ml of 0.022% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme activity was expressed as  $\mu\text{M CO}_2\text{ kg}^{-1}\text{ leaf FW s}^{-1}$ .

#### Estimation of proline (PRO) content

The PRO content was estimated by the method of Bates et al. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. The supernatant was used for the estimation of PRO content. The reaction mixture consisted of 2 ml acid ninhydrin and 2 ml of glacial acetic acid, was boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene and absorbance was read at 520 nm.

#### Estimation of essential oil content

Dried fruits were powdered using a mortar and pestle. The essential oil content of crop was extracted by using Clevenger method. Plants material was hydro-distilled using a Clevenger's apparatus for 3 h. The essential oil was extracted and determined gravimetrically (Guenther, 1955). The essential oil was dried over anhydrous sodium sulphate and stored in sealed glass vials at 4°C for analysis. The content of essential oil was calculated on the dry weight basis.

#### Statistical analysis

Each pot was treated as one replicate and all the treatments were replicated five times. The data was analyzed statistically using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). The least significant difference was calculated for the significant data at  $P < 0.05$ .

## RESULTS

The data indicates that all the ISA treatments showed promotive effects on all the growth, yield and quality attributes and physiological and biochemical parameters. However, spray of treatment un-irradiated sodium alginate resulted in the lowest effect. It was statistically equal to the control for most of the parameters studied. Of the eight ISA concentrations, 80 ppm proved to be the best compared to other ISA concentrations.

#### Growth parameters

There was a progressive increase in values with the increase in ISA concentration up to 100 ppm. Thereafter, the values declined significantly. However, the treatment 100 ppm showed parity with treatment 80 ppm ISA in its effects (Table 1). The maximum values of growth characteristics were observed at 80 ppm ISA. Application of ISA at 80 ppm significantly increased shoot length per plant by 47.8 and 46.3% as compared to control and 20

UN ppm at 70 DAS, respectively. Treatment ISA at 80 ppm also increased plant root length by 49.3 and 41.1% exceeding the control and 20 UN ppm (Table 1). An exceed in number of leaves per plant was noted at 80 ppm ISA by 58.8% over the control and 54.2% as compared to that of 20 UN at 70 DAS, respectively. As compared with that of control and 20 UN treatment, maximum fresh weight per plant was registered in 80 ppm ISA by 68.8 and 54.0%, respectively (Table 1). Whereas, dry weight was also recorded maximum at 80 ppm by 69.1 and 54.4% as compared to the control and 20 UN, respectively (Table 2).

#### Physiological and biochemical parameters

Application of ISA (80 ppm) improved maximum total chlorophyll content by 22.2% over the control and 10.8% as compared with that of 20 UN, respectively. The effect of this treatment was statistically at par with that of 100 ppm (Figure 1). Maximum carotenoid content was found in 80 ppm by 27.5% as compared with that of control and 23.7% 20 UN at 70 DAS, respectively (Figure 1). Carbonic anhydrase activity was positively affected by the application of ISA. The spray of 100 ppm ISA exhibited the highest values of CA activity. Compared to the control and 20 UN (water spray treatment), 80 ppm of ISA resulted in 25.74 and 24.78% increase in CA activity, respectively. Similarly, activity of NR was also enhanced in ISA treated leaves. ISA at 80 ppm increased the NR activity by 26.96 and 25.48% as compared to control and UN, respectively (Figure 2). ISA treated leaves produced maximum proline content and it was noted with 120 ppm by 49.4% as compared to the control. However, the value decreased upto 80 ppm as compared to control and 20 UN, respectively, in this regard (Figure 2).

#### Yield characteristics

Maximum number of umbels was registered in 80 ppm by 68.0% over the control and 56.5% as compared with that of 20 UN at harvest, respectively (Table 2). A spray of ISA at 80 ppm produced maximum number of umbellets per umbel by 70.4% over the control and 65.3% as compared with that of 20 UN at harvest, respectively was recorded (Table 2). Hundred seed weight was found significant and maximum weight was recorded in 80 ppm treatment which was 33.1% more as compared with that of control and exceeds 30.4% over UN 20 ppm (Table 2). The seed yield was maximally registered in 80 ppm by 62.3% over the control and 27.2% as compared to that of 20 UN, respectively (Table 2). The application of ISA at 80 ppm enhanced the fruit yield significantly by 62.3% over the control and 27.2% as compared to that of 20 UN, respectively (Table 2). Application of ISA increased content and yield of essential oil when compared to the

**Table 1.** Effect of various concentrations of ISA [0 (control), UN (un-irradiated), 20, 40, 60, 80, 100 and 120 ppm]. on growth parameters of fennel (*F. vulgare* Mill.). Each value is mean of five replicates. LSD ( $p \leq 0.05$ ) was employed to separate the means in the table.

Growth parameters	ISA concentrations (ppm)								LSD at 5%
	Control	20 UN	20 ppm	40 ppm	60 ppm	80 ppm	100 ppm	120 ppm	
Shoot length per plant (cm)	29.7	30.0	35.2	37.2	40.4	43.9	37.2	36.8	2.1
Root length per plant (cm)	14.0	14.5	16.0	17.4	19.1	20.9	19.7	18.1	1.1
Number of leaves per plant	6.8	7.0	8.8	9.2	10.4	10.8	10.4	10.2	0.9
Fresh weight per plant (g)	23.0	25.2	30.7	33.1	35.8	38.8	32.5	31.7	2.2
Dry weight per plant (g)	6.2	6.8	8.5	9.3	10.3	10.5	8.8	7.7	0.7

**Table 2.** Effect of various concentrations of ISA [0 (control), UN (un-irradiated), 20, 40, 60, 80, 100 and 120 ppm]. on yield characteristics of fennel (*F. vulgare* Mill.). Each value is mean of five replicates. LSD ( $p \leq 0.05$ ) was employed to separate the means in the Table.

Yield attributes	ISA concentrations (ppm)								LSD at 5%
	Control	20 UN	20 ppm	40 ppm	60 ppm	80 ppm	100 ppm	120 ppm	
Number of umbels per plant	12.2	13.1	15.1	17.3	18.7	20.5	16.3	14.8	1.2
Number of umbellets per umbels	9.8	10.1	14.6	12.2	16.7	11.3	10.9	10.4	1.1
100- seed weight (g)	0.329	0.336	0.355	0.377	0.427	0.438	0.367	0.362	0.016
Fruit yield per plant (g)	0.929	0.971	1.05	1.06	1.20	1.24	1.19	1.17	0.041
Essential oil content (%)	2.84	2.85	2.87	2.90	2.95	3.12	2.97	2.87	0.04
Essential oil yield per plant (ml)	0.030	0.030	0.030	0.031	0.035	0.038	0.035	0.033	0.003

un-treated plants. As presented in Table 2, the spray of ISA at the rate of 80 ppm resulted in the maximum content and yield of essential oil (9.86 and 26.7% increase over the control).

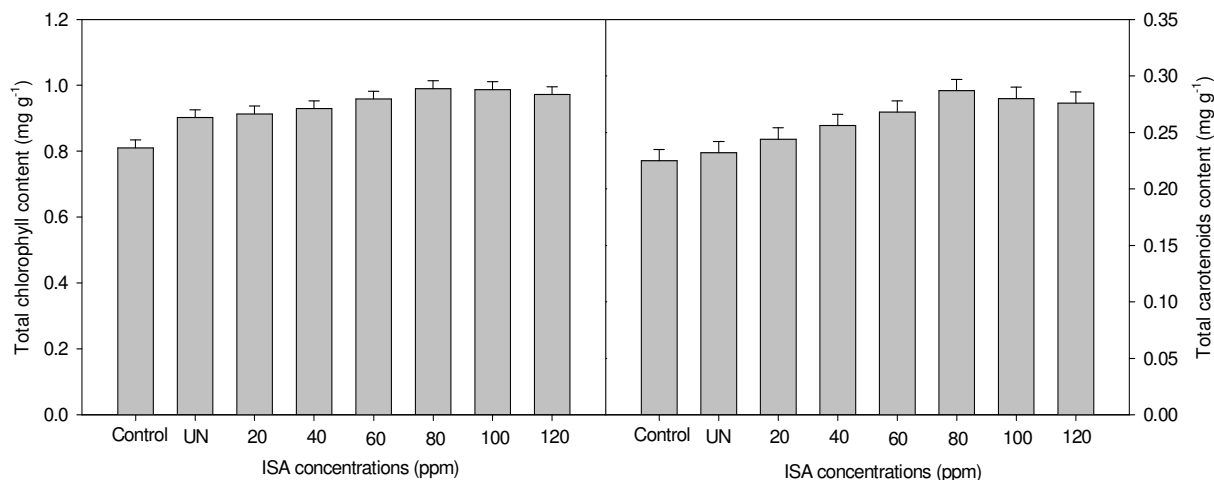
## DISCUSSION

Several exogenous and endogenous factors regulate the growth, development and yield of a plant (Srivastava and Srivastava, 2007). Among exogenous factors, various plant growth promoters are known which have direct or indirect influence on growth of the plant. It has already

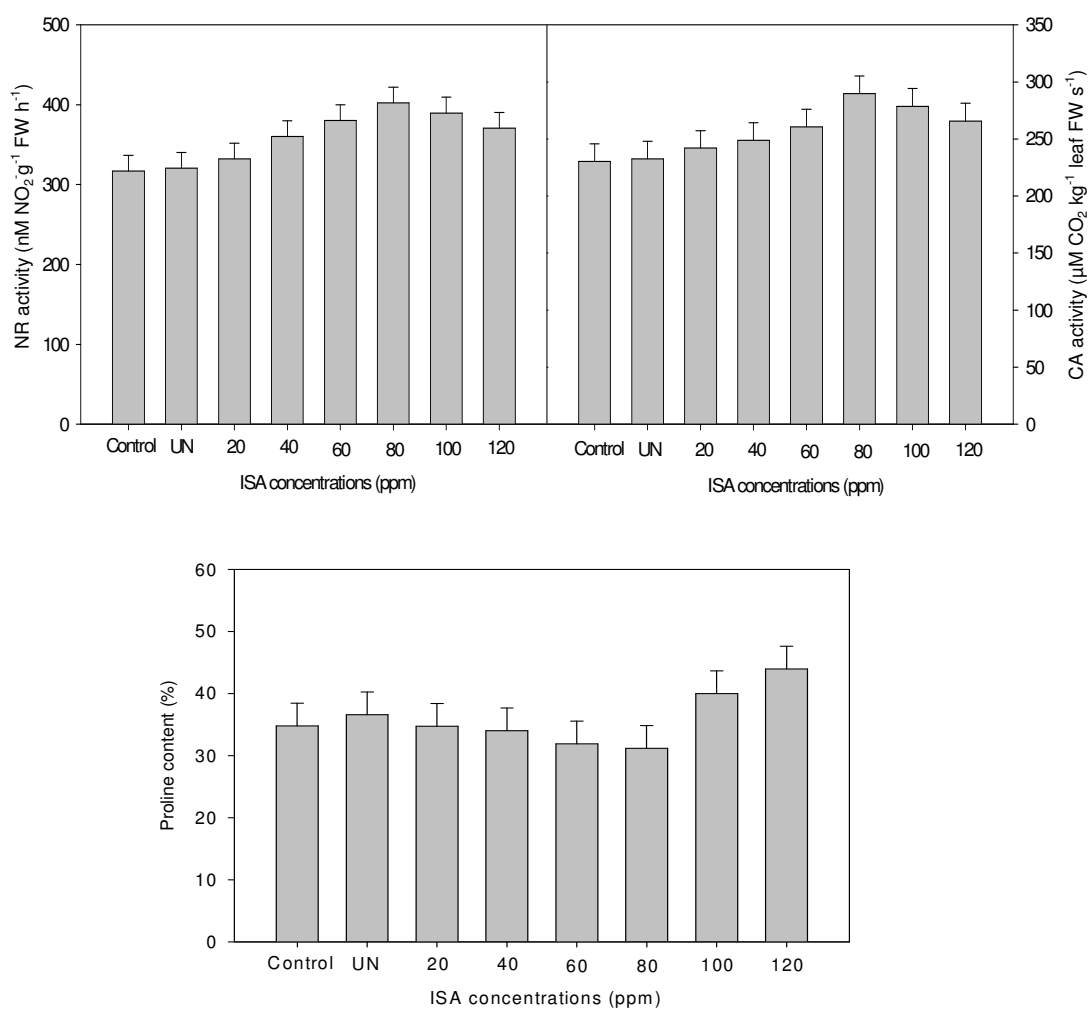
been reported that polysaccharides such as alginate, carrageenan and chitosan in their depolymerised form have the novel properties of promotion of germination and shoot elongation (Darvill et al., 1992; Natsume et al., 1994; Tomoda et al., 1994; Nagasawa et al., 2000; Hien et al., 2000; Kume et al., 2002; Luan et al., 2003). In fact, biologically active oligosaccharides have been known to act as signal molecules that regulate plant growth and development and defense reactions in plants by regulating gene expression (Albersheim and Darvill, 1985). The results (Tables 1 to 2 and Figures 1 to 2) suggest that foliar sprays of radiation degraded sodium

alginate improve the growth attributes (shoot and root lengths, number of leaves, fresh and dry weights), biochemical parameters (total chlorophyll content, carotenoids content, activities of NR and CA and proline content) and yield and quality attributes (number of umbels per plant, number of umbellets per umbel, 100-seed weight and fruit yield, content and yield of essential oil) of fennel.

Previous studies have shown that a range of concentrations of radiation degraded sodium alginate depend upon the source and unit (kGy) of irradiation for a particular plant (Tomoda et al., 1994). Oligomers, produced by depolymerisation



**Figure 1.** Effect of various concentrations of foliar sprays of ISA [0 (control), UN (un-irradiated), 20, 40, 60, 80, 100 and 120 ppm] on total chlorophyll and carotenoids contents fennel studied at 70 DAS (means of five replicates). Bars (┐) shown LSD ( $p \leq 0.05$ ).



**Figure 2.** Effect of various concentrations of foliar sprays of ISA [0 (control), UN (un-irradiated), 20, 40, 60, 80, 100 and 120 ppm] on activities of CA and NR, and proline content studied at 70 DAS (means of five replicates). Bars (┐) shown LSD ( $p \leq 0.05$ ).

of alginates, have been reported to have triggered the stimulation of growth, promotion of germination and shoot elongation in plants. Among the different concentrations of radiation degraded oligomers of sodium alginate (irradiated at 520 kGy), 80 ppm concentration exposed promotive effects on all the growth parameters studied (Table 1). In lines with the present results, it is earlier reported that significant improvement in plant growth attributes by the application of radiation-derived oligosaccharides of alginate. Also, Hien et al. (2000) reported that when the range of 10 to 500 kGy Co-60 gamma rays exposed chitosan, proved very effective for considerable plant growth promotion. Furthermore, the reports also show that chitosan when irradiated at suitable radiation dose, and applied on plants through hydroponics system or through foliar application, has become a successful method in modern commercial farming. The results are in conformity with the findings of Hien et al. (2000), Thama et al. (2001), Kume et al. (2002), Luan et al. (2003, 2005), Natsume et al. (1994), Tomoda et al. (1994), Chmielewska et al. (2007), Khan (2009), Qureshi (2010) and Jamsheer (2010) in case of various crops.

Foliar spray of degraded products of SA at a certain concentration causes an increase in the biochemical and physiological function of plant that leads to an increase in dry matter and yield. Mollah et al. (2009) also confirmed the growth promoting effect of irradiated sodium alginate in *Amaranthus cruentus*. Since the important role of oligosaccharides (degraded alginate) in inducing cell signaling in various plants leading to stimulation of various physiological processes had been revealed (Hein et al., 2000), the application of ISA in the present study improved the photosynthetic pigment content.

The de-polymerization of alginate was first initiated during the radiation process of SA. Furthermore, the absorption of oligomers acted as a growth promoter, which resulted in plant root and shoot elongation and, thereby led to promotion and increase in plant productivity and improvement in physiological parameters compared with the unsprayed plant (El-Rehim, 2006). Furthermore, depolymerized sodium alginate play a role of cell signaling in plant for the induction of phytoalexins (Farmer et al., 1991; Darvill et al., 1992). Due to this property, the plants treated with radiation degraded alginate show an increase not only in growth but also in disease resistant capacity. In the present study, we noticed that activities of NR and CA were positively regulated by various concentrations of ISA. In this regard, our findings are similar to those that report the synthesis of certain enzymes in the tissue culture after addition of alginate derived oligomers (Akimoto et al., 1999).

Proline is an important component of the defense system of the plants to counter any stress generated in plants. Proline occurs widely in higher plants and accumulates in larger amounts than other amino acids (Abraham et al., 2003), regulates the accumulation of useable nitrogen. It might be possible that any kind of

stress generated by applied higher concentration (100 and 120 ppm) causes a physiological disturbance that lead to an activation of the enzymes of proline biosynthesis and suppression of those of its degradation and consequently the accumulation of higher level of proline (Figure 2). However, lower concentrations of ISA decreased the proline level as compared to control and 20 ppm UN. The results brought out from this study, if we sprayed 100 or 120 concentrations which is higher than 80 ppm, it might be possible that a stress will be generated in the plants itself. On the other hand, optimised concentration control and reduced the level of proline content as well.

It could be concluded that radiation degraded sodium alginate might enhance the growth, biochemical parameters, yield and quality attributes including essential oil production, with 80 ppm of ISA resulting in the highest values of fennel (*F. vulgare*). The results also show that best concentration of radiation degraded alginate (radiated at 520 kGy) to enhance the growth, physiological activities and yield and quality attributes of the crop is 80 ppm. Furthermore, the present work also suggests that alginate (depolymerised form) when irradiated at suitable radiation dose, and applied on plants through foliar application, has become a successful method in modern commercial farming. Not only this but such application can shorten the harvesting period of certain medicinal crops and help in reducing the dependency to insecticide and chemical fertilizers. However, the phenomenon which stimulates the processes related to promotion of plant growth still needs further investigations.

## REFERENCES

- Abraham E, Rigo G, Szekeley G, Nagy R, Koncz C, Szabados L (2003). Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in arabidopsis. *Plant Mol Biol* 51:363-372
- Ahni KJ, Park L, HJ, Byun, MW (2001). The production of chitosan and alginate monomer and oligomer by irradiation. Session 59A, carbohydrate, 2001, IFT Annual Meeting-New Orleans, Louisiana, USA.
- Akimoto C, Aoyagi H, Tanaka H (1999). Endogenous elicitor-like effect of alginate on physiological activities of plant cells. *Appl. Microbiol. Biotechnol.*, 52: 429-436.
- Albersheim P, Darvill AG (1985). Ologosachharines. *Sci. Am.*, 253: 44-50.
- Bates LS, Waldeen RP, Teare ID (1973). Rapid determination of free water stress studies. *Plant Soil*, 39: 205
- Cabalfin EG (2002). Status of radiation treatment of liquid samples in the Philippines. FNCA 2002 Workshop on Application of Electron Accelerator, Takasaki, Japan, Dec. pp. 16-20.
- Chmielewska AG, Migdala BW, Swietoslawskic J, Swietoslawskid J, Jakubaszeka U, Tarnowskib T (2007). Chemical-radiation degradation of natural oligoamino-polysaccharides for agricultural application. *Rad. Phys. Chem.*, 76: 1840-1842.
- Darvill A, Auger C, Bergmann C, Carlson RW, Cheong JJ, Eberhard S, Hahn MG, Lo VM, Marfa V, Meyer B, Mohnen D, O'Neill MA, Spiro MD, Halbeek H, York WS, Albersheim P (1992). Oligosaccharin-oligosaccharides that regulate growth, development and defense response in plants. *Glycobiology*, 2: 181-198.
- Dwivedi RS, Randhawa NS (1974). Evaluation of rapid test for the

- hidden hunger of zinc in plants. *Plant Soil*, 40: 445-451.
- El-Rehim AHA (2006). Characterization and possible agricultural application of polyacrylamide/sodium alginate crosslinked hydrogels prepared by ionizing radiation. *J. App. Polym. Sci.*, 101: 3572-3580.
- Farmer EE, Thomas DM, Michael JS, Clarence AR (1991). Oligosaccharide signaling in plants. *J. Biol. Chem.*, 266: 3140-3145.
- Guenther E (1955). *The essential oils 1*. Van Nostrand, New York, pp. 316-319.
- Hadwiger LA (1994). Chitosan as crop growth regulator. Asia-Pacific Chitin and Chitosan Symposium (APCCS), Universiti Kebangsaan Malaysia, 24-27 May. p. 25.
- Hafez M, Muhamad O, Muhammad SY, Ahmad MS (2003) Irradiated chitosan as plant promoter. *Prosiding Simposium Biologic Gunaan Ke-7*, Mines Beach Resort and Spa, Seri Kembangan, Selangor, 3-4 June.
- Hegazy EA, Abdel-Rehim H A, Diao DA, El-Barbary A (2009). Report: Controlling of degradation effects in radiation processing of polymers. In: *Controlling of Degradation Effects in Radiation Processing of Polymers*. International Atomic Energy Agency. Vienna, Austria. pp. 64-84.
- Hien NQ, Nagasawa N, Tham LX, Yoshii F, Dang HV, Mitomo H, Makuuchi K, Kume T (2000). Growth promotion of plants with depolymerised alginates by irradiation. *Radiat. Phys. Chem.*, 59: 97-101.
- Hu X, Jiang X, Hwang H, Liu S, Guan H (2004). Promotive effects of alginate-derived oligosaccharide on maize seed germination. *J. Appl. Phycol.*, 16: 73-76.
- Jaworski EJ (1971). Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Commun.*, 43: 1247-1279.
- Jamsheer MK (2010). Response of beetroot (*Beta vulgaris* L.) to the application of phosphorus and gamma-irradiated sodium alginate. M.Sc. Thesis. Aligarh Muslim University, Aligarh.
- Khan ZH (2009). Influence of Co-60 gamma irradiated oligomers of sodium alginate on the growth, yield and alkaloids production of opium poppy (*Papaver somniferum* L.). M.Sc. Thesis. Aligarh Muslim University, Aligarh.
- Kume T, Nagasawa N, Yoshii F (2002). Utilization of carbohydrates by radiation processing. *Radiat. Phys. Chem.*, 63: 625-627.
- Lee DW, Choi WS, Byun MW, Park HJ, Yu YM, Lee CM (2003). Effect of  $\gamma$ - irradiation on degradation of alginate. *J. Agric. Food Chem.*, 51: 4819-4823.
- Lichtenthaler HK, Buschmann C (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. In: R.E. Wrolstad, Editor, *Current Protocols in Food Analytical Chemistry*. John Wiley and Sons (New York), pp. F4.3.1-F4.3.8.
- Luan LQ, Hien NQ, Nagasawa N, Kume T, Yoshii F, Nakanishi TM (2003). Biological effect of radiation-degraded alginate on flower plants in tissue culture. *Biotechnol. Appl. Biochem.*, 38: 283-288.
- Luan LQ, Ha VT, Nagasawa N, Kume T, Yoshii F, Nakanishi TM (2005). Biological effect of irradiated chitosan on plants *in vitro*. *Biotechnol. Appl. Biochem.*, 41: 49-57.
- Mollah MZI, Khan MA, Khan RA (2009). Effect of gamma irradiated sodium alginate on red amaranth (*Amaranthus cruentus* L.) as growth promoter. *Radiat. Phys. Chem.*, 78: 61-64.
- Nagasawa N, Mitomo H, Yoshii F, Kume T (2000). Radiation induced degradation of sodium alginate. *Poly. Degrad. Stabi.*, 69: 279-285.
- Natsume M, Kamao Y, Hirayan M, Adachi J (1994). Isolation and characterization of alginate derived oligosaccharides with root growth promoting activities. *Carbohydr. Res.*, 258: 187-197.
- Ohta K, Zanigui A, Konishi N, Idosoki T (1999). Chitosan treatment affects plants growth and flower quality in *Eustoma grandiflorum*. *Hort Sci.*, 34: 233-234.
- Qureshi AH (2010). Effect of nitrogen and gamma-irradiated sodium alginate on the efficiency of beetroot (*Beta vulgaris* L.)
- Sabharwal S (2004). Report: Radiation processing in India: current status and future programme. In: *Radiation Processing of Polysaccharides*. International Atomic Energy Agency. Vienna.
- Shimokawa YT, Toida T, Kawashima T (1996). Isolation and structural analysis of polysaccharide containing galactofuranose from the cell walls of *Bifidobacterium infantis*. *J. Bacteriol.*, 178: 317-320.
- Srivastava NK, Srivastava AK (2007). Influence of gibberellic acid on  $^{14}\text{CO}_2$  metabolism, growth, and production of alkaloids in *Catharanthus roseus*. *Photosynthetica*, 45: 156-160.
- Tomoda Y, Umemura K, Adachi T (1994). Promotion of barley root elongation under hypoxic conditions by alginate lyase-lysate (A.L.L.). *Bios. Biotechnol. Biochem.*, 58: 203-203.
- Yonemoto Y, Tanaka H, Yamashita T, Kitabatake 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. *J. Ferment. Bioengin.*, 75: 68-70.
- Thama LX, Nagasawa N, Matsuhashib S, Ishikab NS, Itob T, Kume T (2001). Effect of radiation-degraded chitosan on plants stressed with vanadium. *Radiat. Phys. Chem.*, 61: 171-175.
- The Wealth of India (1992). *Raw Materials (Revised Edition)*, Vol. 3, C.S. Ambusta (Editor in Chief), Publication and Information Directorate, CSIR, New Delhi.
- Zakaria MB, Wan Muda WM, Abdullah MP (1995). Chitin and chitosan-the versatile environment friendly modern material. Penerbit Universiti Kebangsaan Malaysia, Bangi.