Front. Agric. China 2011, 5(1): 122–127
DOI 10.1007/s11703-010-1056-0

RESEARCH ARTICLE

# Influence of alginate oligosaccharides on growth, yield and alkaloid production of opium poppy (*Papaver somniferum* L.)

Zeba H. KHAN, M. Masroor A. KHAN, Tariq AFTAB (✉), M. IDREES, M. NAEEM

*Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh 202 002, India*



**Abstract** **Opium poppy (*Papaver somniferum* L.), an important medicinal plant, produces several opiate alkaloids including morphine, thebaine, codeine, papaverine and noscapine. Polysaccharides, such as sodium alginate, have been used in depolymerized form as wonderful promoters of plant growth. The present study has revealed that application of alginate oligosaccharides (AO), obtained from sodium alginate irradiated by Co-60 gamma rays, significantly enhances certain physiological/biochemical parameters as well as the overall growth of opium poppy. The highest dose applied was the most effective in increasing the morphine and codeine contents as well as the overall yield of crude opium per plant.**

**Keywords** *Papaver somniferum* L., opium, alkaloids, morphine, alginate oligosaccharides

## Introduction

Opium poppy (*Papaver somniferum* L.) is one of the oldest cultivated medicinal plants that vary with different colors, shapes of flowers and capsules, alkaloid compositions and contents (Kapoor, 1995). Dry opium has long been considered as astringent, antispasmodic, aphrodisiac, diaphoretic, expectorant, hypnotic, narcotic and sedative. It contains the most effective analgesic alkaloid, morphine (Evans, 1989), together with other alkaloids including codeine, noscapine and thebaine, which are used in drugs (Bernath and Nemeth, 1999). The world requirement of morphine and codeine and their precursors and products is growing. The new uses of opium poppy alkaloids and analogs are being discovered.

It is now being realized that radiation processing can also be beneficially utilized either to improve the existing methodologies used for processing natural polymers or to impart value addition to agriculture by converting them into more useful forms (Sabharwal, 2004). Alginate is a natural polysaccharide, derived from brown algae, with a large available quantity in nature. Alginates have widespread application in food and drink, pharmaceutical and bioengineering industries (Gaseca, 1988; Hien et al., 2000). Depolymerised sodium alginate known as oligo-alginate is prepared by acid hydrolysis or enzymatic degradation of sodium alginate (Hien et al., 2000). Oligomers from depolymerisation of alginates were reported to have novel features such as stimulation of growth, promotion of germination and shoot elongation in plants (Yonemoto et al., 1993; Natsume et al., 1994; Tomoda et al., 1994; Jamsheer, 2010). Interestingly, biologically active oligosaccharides have been known to act as signal molecules that regulate plant growth and development as well as defense reactions by regulating gene expression (Albersheim and Darvill, 1985).

The application of ionizing radiation to degrade natural bioactive agents as growth-promoting substances is a novel emerging technology to exploit full genetic potential of crops in terms of growth, yield, and quality. Polysaccharides, such as sodium alginate, have been used as wonderful growth-promoting substances in their depolymerized form for various plants (Nagasawa et al., 2000). Radiation processing by Co-60 gamma rays (Nagasawa et al., 2000; Lee et al., 2003) offers a clean one-step method for the formation of low molecular weight oligomers. Hu et al. (2004) and Hegazy et al. (2009) investigated the responses of *Zea mays* plants treated with degraded sodium alginate by radiation and found that alginate oligosaccharides (AO) enhanced not only the plant growth but also the productivity. However, the promotive effects of AO on plants in terms of alkaloid

Received July 19, 2010; accepted September 9, 2010
Correspondence: Tariq AFTAB
E-mail: tarik.alig@gmail.com

production are yet to be studied extensively. The purpose of this work was to study the effectiveness of AO in enhancing the productivity and alkaloid content of opium poppy.

## Materials and methods

### Plant material and growth conditions

Seeds of opium poppy var. NBRI-5 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. Physico-chemical characteristics of the soil were: texture-sandy loam, pH (1:2) 8.0, E.C. (1:2) 0.48 m mhos·cm$^{-1}$, available N, P and K of 97.46, 10.21 and 147.0 mg·kg$^{-1}$, respectively, in soil. Then seeds were sown on November 15th, 2008 at a depth of 2 cm in earthen pots (25 cm diameter × 25 cm height) containing sandy-loam soil.

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

Sodium alginate was purchased from Sigma-Aldrich, USA. Sodium Alginate was sealed in a glass tube with atmospheric air. It was kept in water for swelling over night at room temperature to obtain 4% aqueous solution. Then the solution was stirred for several hours until completely dissolved. The samples were irradiated by gamma rays from Co-60 source at 520 kilo Gray (kGy). Different concentrations of AO viz. 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm and 120 ppm were finally prepared and used in the present study. Spray of distilled water was treated as control.

### Experimental design

A pot culture experiment was designed to analyze the changes in physiologic and biochemical attributes of opium poppy using the treatment with oligomers obtained from degraded sodium alginate in the net house at the Botany Department, AMU, Aligarh (27°52′N latitude, 78°51′E longitude, and 187.45 m altitude). Each treatment was replicated five times, and each replicate had three plants. One healthy plant was maintained per pot. The pots were watered thoroughly. Plants were grown under naturally illuminated environmental conditions.

### Growth and yield analyses

A simple randomized pot experiment was designed to assess various growth, physiological, biochemical, yield and quality attributes. The plants were sampled at 90 DAS; three plants of each treatment were harvested with the roots carefully washed with tap water to remove adhering foreign particles. Water adhering to the roots was removed with blotting paper, and fresh weights of plants were recorded. The plants were dried at 80°C for 24 h, and dry weights were then recorded.

### Estimation of total chlorophyll and carotenoid content

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

### Determination of nitrate reductase (NR) activity

Nitrate reductase (E.C. 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 mol·L$^{-1}$ phosphate buffer, 0.5 mL of 0.2 mol·L$^{-1}$ potassium nitrate, and 2.5 mL of 5% isopropanol. The nitrite formed subsequently was colorometrically determined at 540 nm after azocoupling with sulphanilamide and naphthylene diamine dihydrochloride. The NR activity was expressed as nmol·L$^{-1}$ $NO_2$·g$^{-1}$ FW·h$^{-1}$.

### Determination of carbonic anhydrase (CA) activity

Carbonic anhydrase (E.C. 4.2.1.1) activity was measured in fresh leaves using the method described by Dwivedi and Randhawa (1974). 200-mg fresh leaf pieces were weighed and transferred to Petri plates. The leaf pieces were dipped in 10 mL of 0.2 mol·L$^{-1}$ cystein hydrochloride solution for 20 min at 4°C. To each test tube, 4 mL of 0.2 mol·L$^{-1}$ sodium bicarbonate solution and 0.2 mL of 0.022% bromothymol blue were added. The reaction mixture was titrated against 0.05 mol·L$^{-1}$ HCl using methyl red as an indicator. The enzyme activity was expressed as mol·L$^{-1}$ $CO_2$·kg$^{-1}$ leaf FW·s$^{-1}$.

### Alkaloid estimation

The various alkaloids of opium were determined using HPLC (Perkin–Elmer; a series 200 liquid chromatograph, a series 600 link controller; LC-235 diode-array detector). From methanolic stock solution of morphine and noscapine (each 5 mg·mL$^{-1}$), codeine, thebaine, papaverine (each 1 mg·mL$^{-1}$), and brucine (internal standard 1 mg·mL$^{-1}$) standard solutions were prepared containing different amounts of the five alkaloids. Brucine internal standard solution was added to gum opium samples, and the samples were extracted twice with 2.5% (v/v) acetic acid. After adjustment of the pH to 9.0 with conc. ammonia solution was transferred to an Extrelut prepacked clean-up column. The alkaloids were eluted with 60 mL dichloromethane-isopropanol

(9∶1) and evaporated to dryness (Krenn et al., 2000). The extracts were dissolved in acetonitrile-water (60∶40) containing 0.8% heptafluorobutyric acid (15 mL) diluted to 30 mL with 0.1% aqueous heptafluorobutyric acid, and 3 µL was injected after filtration through a 0.45-µm millipore filter. The analyses on the porous stationary phase were performed as described elsewhere (Krenn et al., 2000). Separation and quantification on the non-porous stationary phase were performed on a 33 mm×4.6 mm inner diameter, 1.5-µm particle size, and Kovasil MS-C18 column. Multilinear gradient elution at room temperature was performed with mixtures of 0.1% aqueous heptafluorobutyric acid (A) and acetonitrile-water (60∶40) containing 0.8% heptaflurobutyric acid (B). The gradient profile was 0–0.03 min from 15 to 29% B; 0.3–1.9 min from 29 to 38% B and 1.9–2.4 min 38% B. The composition of the mobile phase during purging and regeneration of the column was 2.4–2.6 min from 38 to 100% B; 2.6–3.4 min from 100% B; 3.4–3.6 min from 100 to 15% B and 3.6–7.0 min 15% B. The flow rate was 1 mL·min$^{-1}$. The alkaloids were detected at 255 nm.

### Statistical analysis

Each pot was treated as one replicate, and all the treatments were repeated five times. The data were analyzed statistically using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Mean values were compared by the Duncan's Multiple Range Test (DMRT) at $P < 0.05$% level.

## Results and discussion

### Growth parameters

Application of 120 ppm was most effective in increasing the growth parameters (Fig. 1). The shoot and root lengths were 37.5% and 63.5% higher than those in the control, respectively (Table 1). The second best concentration in this regard was 100 ppm. The effect of 120 ppm was also significant with reference to the fresh and dry weights per plant, giving 59.8% and 47.6% higher values, respectively, compared with the control (Table 1). Plant growth regulators (PGRs) play a pivotal role in enhancing the growth and development of plants as well as the alkaloid content of herbs (Khan et al., 2007). Like PGRs, which are used to improve plant defense by acting as a signaling molecule, AO resembles an endogenous elicitor that functions as a signal to trigger the synthesis of enzymes and activate various responses through gene expression (Ma et al., 2010). It is known that polysaccharides such as alginates, carrageenan and chitosan in their depolymerised form have the novel properties of plant growth promotion (Darvill et al., 1992; Nutsume et al., 1994). Foliar spray of AO was significantly useful for most of the parameters studied, and the 120-ppm treatment was most effective. The increase in shoot length, root length, and the fresh and dry weights in treated plants conformed to the findings of Luan et al. (2003) and Qureshi (2010). In fact, gamma-irradiated sodium alginate appeared to facilitate absorption and utilization of mineral nutrients from the soil. The optimal supply of nutrients is important for optimum yield and quality of poppy seeds.


**Figure 1** Effect of Alginate Oligosaccharides (AO) on growth of opium poppy plants.
Note: (a): control; (b): 120 ppm treated plant.

### Physiological and biochemical parameters

The total chlorophyll content increased in the treated plants in a dose-dependent manner and was the maximum (43.1% higher in comparison to control) with 120-ppm treatment (Fig. 2(a)). Total carotenoid content also showed similar response, giving a 31.4% higher content over the control with 120 ppm (Fig. 2(b)). As to the significant enhancement in total chlorophyll and carotenoid content by application of irradiated sodium alginate, it is likely that the AO evoked the intrinsic genetic potential of the plant, causing increase in the chlorophyll and carotenoid contents. Since oligosaccharides (degraded alginate) have a role in inducing cell signaling in plants, leading to stimulation of various physiological processes (Farmer et al., 1991; Darvill et al., 1992; John et al., 1997), application of AO might seemingly improve pigment contents in our study.

**Table 1** Effect of different concentrations of Alginate Oligosaccharides (AO) on growth parameters of opium poppy plants

| treatments | shoot length/cm | root length/cm | shoot dry weight/g | root dry weight/g |
|---|---|---|---|---|
| control | 78.5±2.23$^{e}$ | 15.1±1.15$^{e}$ | 27.4±0.91$^{f}$ | 7.6±0.29$^{e}$ |
| 20 ppm | 82.1±2.45$^{d}$ | 15.7±1.45$^{e}$ | 28.5±0.92$^{ef}$ | 7.8±0.33$^{de}$ |
| 40 ppm | 83.9±2.21$^{d}$ | 16.5±1.79$^{de}$ | 30.6±1.26$^{e}$ | 8.3±0.53$^{d}$ |
| 60 ppm | 88.2±3.36$^{cd}$ | 17.8±2.08$^{d}$ | 33.2±1.53$^{d}$ | 8.9±0.67$^{cd}$ |
| 80 ppm | 91.5±3.14$^{c}$ | 19.7±1.77$^{c}$ | 36.4±1.71$^{c}$ | 9.5±0.82$^{c}$ |
| 100 ppm | 97.8±3.63$^{b}$ | 21.3±2.04$^{b}$ | 40.4±2.07$^{b}$ | 10.4±0.93$^{b}$ |
| 120 ppm | 108.5±4.04$^{a}$ | 24.7±2.38$^{a}$ | 45.4±2.41$^{a}$ | 11.2±1.16$^{a}$ |

Note: Means within a column followed by the same letter are not significantly different ($P \leqslant 0.05$). The data shown are means of five replicates±SE

Application of 120-ppm AO was most effective, followed by 100 ppm, giving 31.7% and 26.1% higher values, respectively, for NR activity (Fig. 2(c)). CA activity was the highest in the plants treated with 120-ppm AO and registered 28.2% higher values than that in the control (Fig. 2(d)). A significant increase in NR and CA activity was observed in the AO-treated plants. This facilitates the biomass accumulation in treated plants (Khan et al., 2007). According to Luan et al. (2003), the irradiated sodium alginate promoted enzyme activity and photosynthetic rate. Akimoto et al. (1999) found enzyme synthesis in a tissue culture supplied with alginate-derived oligomers.

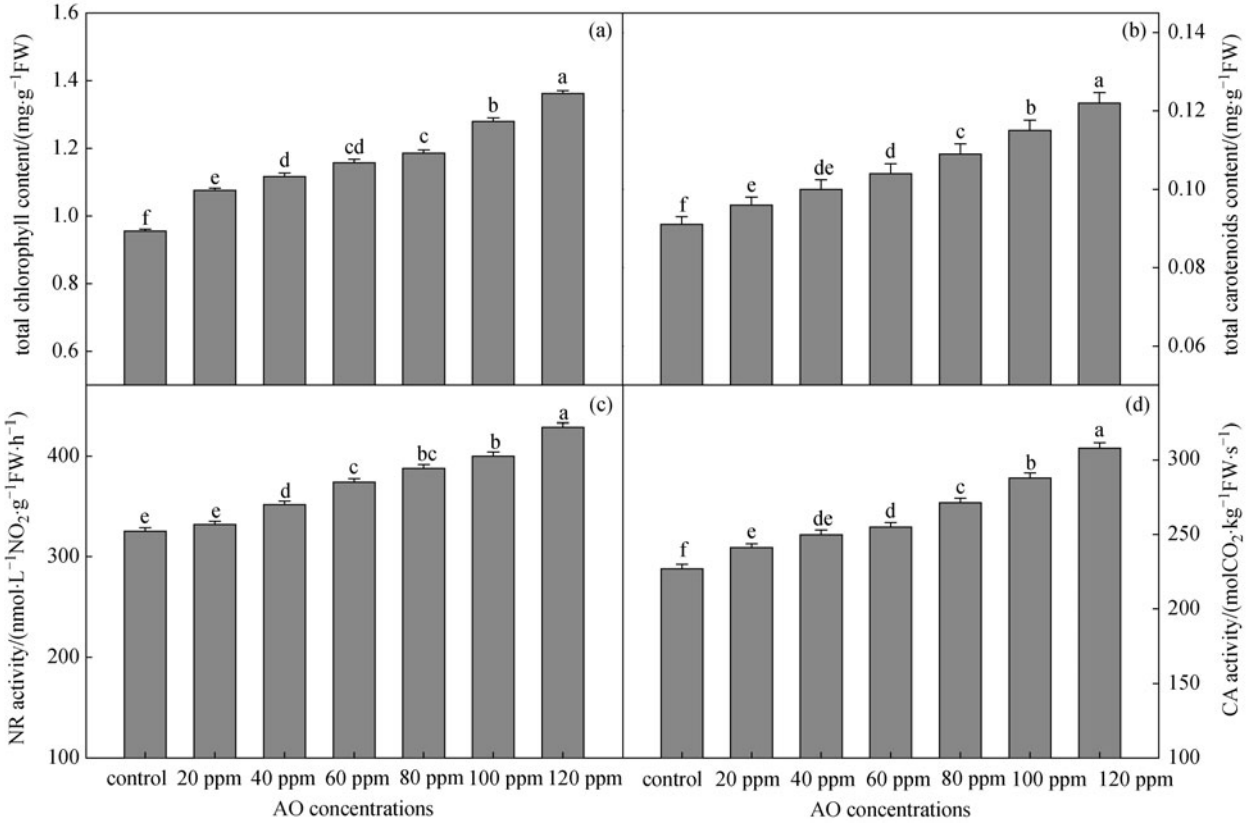


**Figure 2** Effect of different concentrations of alginate oligosaccharides (AO) on chlorophyll content (a), carotenoid content (b), NR activity (c), and CA activity (d) of opium poppy plants.
Note: Bars showing the same letter are not significantly different at $P \leqslant 0.05$ as determined by the Duncan's multiple range test. Error bars (⊤) show SE.

**Table 2** Effect of different concentrations of Alginate Oligosaccharides (AO) on yield parameters of opium poppy plants

| treatments | capsule dry weight/g | seed weight per capsule/g | crude opium per plant/mg |
|---|---|---|---|
| control | $2.61\pm0.02^{e}$ | $1.94\pm0.01^{f}$ | $103.4\pm2.31^{f}$ |
| 20 ppm | $2.73\pm0.02^{e}$ | $2.03\pm0.02^{f}$ | $105.5\pm2.52^{e}$ |
| 40 ppm | $2.92\pm0.03^{de}$ | $2.14\pm0.02^{e}$ | $109.8\pm2.26^{de}$ |
| 60 ppm | $3.21\pm0.03^{d}$ | $2.46\pm0.03^{d}$ | $117.2\pm2.59^{d}$ |
| 80 ppm | $3.67\pm0.03^{c}$ | $2.68\pm0.03^{c}$ | $133.4\pm2.91^{c}$ |
| 100 ppm | $4.16\pm0.04^{b}$ | $2.91\pm0.04^{b}$ | $149.8\pm3.46^{b}$ |
| 120 ppm | $4.78\pm0.05^{a}$ | $3.27\pm0.04^{a}$ | $167.3\pm3.74^{a}$ |

Note: Means within a column followed by the same letter are not significantly different ($P\leqslant0.05$). The data shown are means of five replicates±SE.

### Yield and quality parameters

Dry weight of capsules and seed yield were enhanced by the AO application, showing the maximum (91.8% and 83.1%, respectively) with 120 ppm (Table 2). Likewise, crude opium was the maximum (40.7% higher than that in the control) in the plants treated with 120 ppm AO (Table 2). An increase in growth and physiological parameters of treated plants was expected to culminate in maximization of capsule weight, seed yield and crude opium content.

The HPLC analysis of opium latex (crude opium) revealed the presence of four important alkaloids, viz., morphine, codeine, thebaine and noscapine, whereas papaverine was not detected in this variety of opium. Application of 120 ppm AO to the foliage increased the morphine content by 54.3%, while codeine was just doubled. On the other hand, thebaine content decreased, whereas noscapine content was insignificantly affected in response to 120-ppm AO treatment (Fig. 3). Secondary metabolites were influenced by PGRs (Aftab et al., 2010). Also, the alkaloid content in plant tissues was affected by the rate of biosynthesis and catabolism and varies with respect to plant development, diurnal variations, functionally different parts of plants and environmental factors (Jaleel et al., 2009). Additionally, the increase in nutrient content in treated plants would have a positive effect on the crude opium and morphine contents, as nitrogen is an integral part of all alkaloids. According to Kadar et al. (2001), the volume of capsule and their number per plant were affected by nitrogen supply. We found a correlation among plant weight, chlorophyll content and morphine concentration.

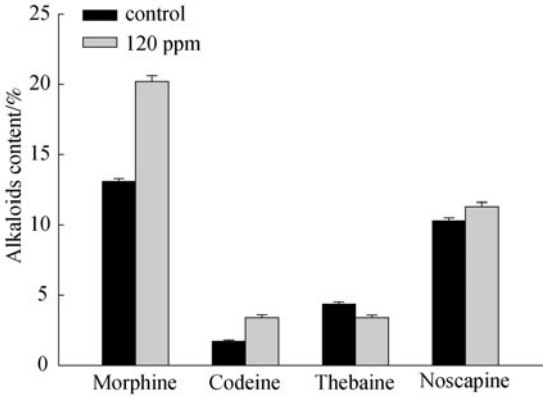


**Figure 3** Effect of 120 ppm alginate oligosaccharides (AO) on concentration of alkaloids in opium poppy plants.
Note: Bars showing the same letter are not significantly different at $P\leqslant0.05$ as determined by the Duncan's multiple range test. Error bars (T) show SE.

## Conclusion

The AO spray significantly affected plant growth, physiologic and biochemical variables, yield and alkaloids production in opium poppy. The 120-ppm concentration of AO proved the best in enhancing the overall plant performance. As to the specific alkaloids, morphine and codeine contents were increased considerably. The oligomer molecules produced by irradiated sodium alginate by Co-60 gamma rays seemed to have caused a triggering effect on the biosynthetic pathways of opiate alkaloids such that the precursors could synthesize morphine and codeine at a considerably high rate.

## Acknowledgements

The authors are indebted to Dr. Lalit Varshney, Head, ISOMED Group, Bhabha Atomic Research Centre (BARC), Mumbai, for providing the AO. Dr. Sudhir Shukla, Senior Scientist, National Botanical Research Institute, Lucknow, was kind enough to supply opium seeds.

## References

Aftab T, Khan M M A, Idrees M, Naeem M, Singh M, Ram M (2010). Stimulation of crop productivity, photosynthesis and artemisinin production in *Artemisia annua* L. by triacontanol and gibberellic acid application. J Plant Interact, 5(4): 273–281

Akimoto C, Aoyagi H, Tanaka H (1999). Endogenous elicitor-like effect

of alginate on physiological activities of plant cells. Appl Microbiol Biotechnol, 52(3): 429–436

Albersheim P, Darvill A G (1985). Oligosachharines. Sci Am, 253(3): 44–50

Bernath J, Nemeth E (1999). New trends in selection of poppy (*Papaver somniferum* L.). Int J Hortic Sci, 5: 69–75

Darvill A, Augur C, Bergmann C, Carlson R W, Cheong J J, Eberhard S, Hahn M G, Ló V M, Marfà V, Meyer B, Mohnen D, O'Neill M A, Spiro M D, van Halbeek H, York W S, Albersheim P (1992). Oligosaccharins—oligosaccharides that regulate growth, development and defence responses in plants. Glycobiology, 2(3): 181–198

Dwivedi R S, Randhawa N S (1974). Evaluation of rapid test for the hidden hunger of zinc in plants. Plant Soil, 40(2): 445–451

Evans W C (1989). Pharmacognosy. 13th ed. London: Baillère Tindall, 582–591

Farmer E E, Moloshok T D, Saxton M J, Ryan C A (1991). Oligosaccharide signaling in plants. Specificity of oligouronide-enhanced plasma membrane protein phosphorylation. J Biol Chem, 266(5): 3140–3145

Gaseca P (1988). Alginates. Carbohydr Polym, 8(3): 161–182

Hegazy E A, Abdel-Rehim H, Diaa D A, El-Barbary A (2009). Report: Controlling of degradation effects in radiation processing of polymers. In: Controlling of Degradation Effects in Radiation Processing of Polymers. Vienna, Austria: International Atomic Energy Agency, 64–84

Hien N Q, Nagasawa N, Tham L X, Yoshii F, Dang H V, Mitomo H, Makuuchi K, Kume T (2000). Growth promotion of plants with depolymerised alginates by irradiation. Radiat Phys Chem, 59(1): 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(1): 73–76

Husain A, Sharma J R (1983). Opium Poppy: CIMAP. Lucknow: Lucknow Publishing House

Jaleel C A, Riadh K, Gopi R, Manivannan P, Ines J, Al-Juburi H J, Chang-Xing Z, Hong-Bo S, Panneerselvam R (2009). Antioxidant defense responses: physiological plasticity in higher plants under abiotic constraints. Acta Physiol Plant, 31(3): 427–436

Jamsheer M K (2010) Response of beetroot (*Beta vulgaris* L.) to the application of phosphorus and gamma-irradiated sodium alginate. Dissertation for the Master Degree. Aligarh: Aligarh Muslim University

Jaworski E G (1971). Nitrate reductase assay in intact plant tissues. Biochem Biophys Res Commun, 43(6): 1274–1279

John M, Rohrig H, Schmidt J, Walden R, Schell J (1997). Cell signaling by oligosaccharides. Trends Plant Sci, 3(3): 111–115

Kadar I, Foldesi D, Voros J, Szilagyi J, Lukacs D (2001). Mineral fertilisation of poppy (*Papaver somniferum* L.) on calcareous loamy chernozem soil. II. Novenytermeles, 50: 467–478

Kapoor L D (1995). Opium Poppy: Botany, Chemistry, and Pharmacology. New York: Food Product Press, An Imprint of The Haworth Press, Inc.

Khan R, Khan M M A, Singh M, Nasir S, Naeem M, Siddiqui M H, Mohammad F (2007). Gibberellic acid and triacontanol can ameliorate the opium yield and morphine production in opium poppy (*Papaver somniferum* L.). Acta Agric Scand B Soil Plant Sci, 57: 307–312

Krenn L, Boros B, Ohmacht R, Jelinek L (2000). HPLC separation of opium alkaloids on porous and non-porous stationary phases. Chromatographia, 51(1): 175–178

Lee D W, Choi W S, Byun M W, Park H J, Yu Y M, Lee C M (2003). Effect of γ-irradiation on degradation of alginate. J Agric Food Chem, 51(16): 4819–4823

Lichtenthaler H K, Buschmann C (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. In: Curr Protoc Food Analyt Chem. New York: John Wiley and Sons, F4.3.1–F4.3.8

Luan L Q, Hien N Q, Nagasawa N, Kume T, Yoshii F, Nakanishi T M (2003) Biological effect of radiation-degraded alginate on flower plants in tissue culture. Biotechnol Appl Biochem, 38: 283–288

Ma L J, Li X M, Bu N, Li N (2010). An alginate-derived oligosaccharide enhanced wheat tolerance to cadmium stress. Plant Growth Regul, 62 (1): 71–76

Nagasawa N, Mitomo H, Yoshii F, Kume T (2000). Radiation induced degradation of sodium alginate. Polym Degrad Stabil, 69(3): 279–285

Natsume M, Kamo Y, Hirayama M, Adachi T (1994). Isolation and characterization of alginate-derived oligosaccharides with root growth-promoting activities. Carbohydr Res, 258: 187–197

Qureshi A H (2010) Effect of nitrogen and gamma-irradiated sodium alginate on the efficiency of beetroot (*Beta vulgaris* L.). Dissertation for the Master Degree. Aligarh: Aligarh Muslim University

Sabharwal S (2004). Report: Radiation processing in India: current status and future programme. In: Radiation Processing of Polysaccharides. Vienna, Austria: International Atomic Energy Agency, 9–16

Tomoda Y, Umemura K, Adachi T (1994). Promotion of barley root elongation under hypoxic conditions by alginate lyase-lysate (A.L.L.). Biosci Biotechnol Biochem, 58(1): 202–203

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 oilgomers prepared with bacterial alginate lyase. J Ferment Bioeng, 75(1): 68–70