EFFECT OF DIFFERENT DOSES OF GAMMA-IRRADIATED SODIUM ALGINATE AND NITROGEN FERTILIZER ON GROWTH, PHYSIOLOGY AND YIELD ATTRIBUTES OF MENTHA PIPERITA L. IN NORTHERN HIMALAYAS

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ABSTRACT

A pot experiment was conducted in a year, in order to observe the combined effect of a unique dose of irradiated sodium alginate (ISA), along with the different concentration doses of nitrogen fertilizer on the overall growth, physiology and yield attributes of *Menthapiperita L.* for 120 days after plantation (DAP) in Srinagar (J&K), India. A unique concentration of irradiated sodium alginate ISA_{80} (80 mgL^{-1}) was prepared along with the doses of nitrogen (N_{80}) in the form of 20 kg ha^{-1} , 40 kg ha^{-1} , 60 kg ha^{-1} , 80 kg ha^{-1} and 100 kg ha^{-1} and 120 kg ha^{-1} . The total of seven treatments were prepared viz, T1 ($ISA_{80} + N_{20} \text{ kg ha}^{-1}$), T2 ($ISA_{80} + N_{40} \text{ kg ha}^{-1}$), T3 ($ISA_{80} + N_{60} \text{ kg ha}^{-1}$), T4 ($ISA_{80} + N_{80} \text{ kg ha}^{-1}$), T5 ($ISA_{80} + N_{100} \text{ kg ha}^{-1}$), T6 ($ISA_{80} + N_{120} \text{ kg ha}^{-1}$) and T7 (Control as Double Distilled Water). Among various concentrations of irradiated sodium alginate and nitrogen fertilizer, T4 ($ISA_{80} + N_{80} \text{ kg ha}^{-1}$) proved to be the best over the control in *Menthapiperita L.* Among the growth attributes, the plant height showed increase by (41.04%), fresh weight by (54.08%), dry weight by (67.89%) were as the leaf area per plant showed the maximum increase of 21.35 % over the control in *Menthapiperita L.* Besides, the same trend was noticed while analyzing the physiological and biochemical parameters which showed the maximum increase of 31.48 % in Chlorophyll content, Carotenoid content by 35.71 %, Nitrate Reductase activity by 23.14 %, Carbonic Anhydrase activity by 27.38 % and Chlorophyll Fluorescence by 41.86 % by the foliar application of T4 ($ISA_{80} + N_{80} \text{ kg ha}^{-1}$) over the control in *Menthapiperita L.* However, at T4 ($ISA_{80} + N_{80} \text{ kg ha}^{-1}$), the yield attributes like essential oil content showed the increment by (43.33%), essential oil yield by (111.11%), herbage yield by (42.36%) over the control in *M. piperita L.*

KEYWORDS: Irradiated Sodium Alginate & *Menthapiperita L.*

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INTRODUCTION

Aromatic plants, since the time immortal have been proven beneficial in terms of essential oil derivatives and other plant extracts. Polysaccharidelike sodium alginate is obtained from naturally occurring brown algae Sargassum in huge quantities. Sodium alginate, along with chitosan is known to be the elicitors for the defense mechanisms in the plants (John *et al.*, 1997). Gamma irradiated sodium alginate has shown the tremendous growth promoting activities and behaves as a bio-fertilizer (Mollahet *al.*, 2009). Enhanced shoot elongation, seed germination, as well as root modifications were observed by the foliar spray of gamma irradiated sodium alginate

(Natsumeet *al.*, 1994; Hu *et al.*, 2004). *Menthapiperita* L. belongs to the family Lamiacea and is widely cultivated commercial crop in the northern semi-arid and sub-tropical regions of India. India is currently producing nearly 18000 tonnes of mint oil per year and has emerged as one of the largest supplier of mint oil and menthol (Patra *et al.*, 2008). Menthol mint is a potential source of natural menthol and other constituents like mint terpenes, menthone, isomenthone, menthofuran which are widely used in drug industry, pharmaceutical and variety of cosmetic products. Alginates have been used in varied ways for food, pharmaceutical and drinking purposes. Keeping in view the importance of *Menthapiperita* L., an experiment was conducted to work out the effect of ISA and N on the crop productivity, photosynthetic capacity, and essential oil production of the plant.

MATERIAL AND METHODS

Plant Materials, Location of Cultivation and Growth Conditions

A pot experiment was conducted in the botanical garden of Kashmir university, Srinagar during the year 2017. The plant materials were collected from IIIM Srinagar (J&K). Small plantlets of *Menthapiperita* L. were grown in the earthen pots of botanical garden (University of Kashmir). The pot experiment was conducted during 2016-2017. The experiment was conducted in randomised block design in earthen pots (25 cm height x 25 cm diameter). The plants were sown in the month of April. At least three replicates of each treatment were planted in the pots. Each earthen pot was filled with 5 Kg homogenous mixture of soil and organic manure in the ratio of 4:1. The soil analysis was done at agricultural farms soil testing unit at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K) (Table 1).

Table 1: Soil Profile (Srinagar, JK)

Parameters	Values
Texture	Sandy Loam with low amount of silt
pH	5.8 – 6.13
Conductivity dsm^{-1}	0.59
Available Nitrogen (mg/Kg Soil)	98.47
Available Phosphorous (mg/Kg Soil)	145.01
Available Potassium (mg/Kg Soil)	6.8
Soil Temperature ($^{\circ}\text{C}$)	12.31

Irradiation of Sodium Alginate

Solid form of (Sigma Aldrich, USA) was properly sealed in a long glass tube with atmospheric air. The samples of sodium alginate were irradiated in a Gamma Chamber (Cobalt-60, GC-5000) manufactured by Bhabha Atomic Research Centre (BARC, Zakura, Srinagar), India. The samples were irradiated to 520 kGy gamma radiation dose at a dose rate of 2.4 kGy/h.

Estimation of Growth Parameters

The plants from each treatment were uprooted carefully and then shoot and root lengths were recorded. Plants were washed with tap water to remove adhering foreign particles and the growth parameters were recorded carefully. The unique dose of irradiated sodium alginate (ISA) and multiple doses of Nitrogen (N) fertilizer were selected on the basis of earlier studies Aftabet *al.*, (2011 a, b). The plants were irrigated as and when required. The treatments consisted of Control (foliar spray of double distilled water) along with the irradiated sodium alginate concentration i.e. 80 mgL^{-1} (ISA₈₀), and Nitrogen doses in the form of 20, 40, 60, 80, 100 and 120 kg ha^{-1} were given prior to the plantation. The first foliar spray of

ISA was given to the plants after 40 days of plantation. Five foliar sprays were given to the plants under experimentation.

Estimation of Physiological and Biochemical Parameters

Determination of Chlorophyll Content and Carotenoid Content

Total content of leaf chlorophyll and carotenoids was estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue from the interveinal area of leaf was grinded with 100% acetone using mortar-pestle. The optical density (OD) of the pigment solution was recorded at 662, 645 and 470 nm to determine chlorophyll a, chlorophyll b and total carotenoids content, respectively, using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). Total chlorophyll content was assessed by adding the contents of chlorophyll a and b. The content of photosynthetic pigments was expressed as mg g^{-1} leaf FW.

Determination of Carbonic Anhydrase (CA) Activity

The activity of carbonic anhydrase was measured in the fresh leaves, using the method described by Dwivedi and Randhawa (1974). Two hundred mg of fresh leaves (chopped leaf pieces) was transferred to Petri plates. The leaf pieces were dipped in 10 mL of 0.2 M Cystein hydrochloride solution for 20 min at 4°C. The solution adhering at the cut surfaces of the leaf pieces was then removed with the help of a blotting paper followed by their transfer immediately to a test tube containing 4 mL of phosphate buffer of pH 6.8. To it, 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 $\text{M CO}_2 \text{ kg}^{-1} \text{ leaf FW s}^{-1}$.

Determination of Nitrate Reductase Activity (NR) Activity

Nitrate reductase activity in the 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. The nitrite that formed subsequently was calorimetrically determined at 540 nm after azocoupling with sulphanilamide and naphthylene diamine dihydrochloride. NR activity was expressed as $\text{nM NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$.

Estimation of Yield Attributes

Estimation of Essential Oil Content and Yield

After attaining full maturity at 120 DAP the crops were subjected to harvesting process. From each treatment 500g of fresh plant parts including leaves were taken and crushed. Oil extraction process was estimated according to the (Guenther 1972). Considerable amount of chopped leaves were taken into account for EO estimation and EO content was extracted through Clevenger's apparatus used for three hours.

Statistical Analysis

The data were analyzed statistically using SPSS- 17 statistical software (SPSS Inc., Chicago, IL, USA). Means were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. Standard errors were taken into consideration.

RESULTS

The present study revealed that the different concentration of irradiated sodium alginate and nitrogen fertilizer applied to the plants improved the performance of *Menthapiperita* L. significantly. Further, this study revealed that the combined application of irradiated sodium alginate and Nitrogen fertilizers gave the better performance on the overall growth attributes, physiological and biochemical attributes as well as essential oil composition and essential oil yield in *Menthapiperita* L. as compared to the control. (Table 2, 3 &4). Out of the seven spray treatments, the combination of doses ISA₈₀ + N₈₀ (T4) proved to be the best as compared to the control. The details are as follows:

Growth Attributes

As compared to the control, the significant effect of foliar spray of different treatments was proved to be beneficial for overall growth of the plant i.e. plant height, fresh weight, dry weight and leaf area per plant. Among the various combined treatments of the plant growth regulators T4 i.e. (ISA₈₀ (80 mg L⁻¹) + N₈₀ (80 kg N ha⁻¹), the plant height was increased by 41.04%, fresh weight and dry weights of the plants showed increase by 54.08% and 67.25%, leaf area per plant by 21.35 % respectively over the control in *Menthapiperita* L. Like other plant growth regulators, Alginate Oligosaccharides (AO's) resemble the growth elicitors that initiate the biosynthesis of various enzymes including the activation of gene expressions (Ma *et al.* , 2010).

Physiological and Biochemical Attributes

Photosynthetic Pigments

As far as chlorophyll content is concerned, the application of depolymerized form of ISA at T4 (ISA₈₀ (80 mg L⁻¹) along with nitrogen, N₈₀ (80 kg N ha⁻¹) significantly increased the total content of chlorophyll by (31.48%) and that of carotenoids (35.71%) at 120 DAP as compared to the T7 control (Table 3) in *Menthapiperita* L.

Nitrate Reductase (NR) Activity

ISA applied along with Nitrogen doses, increased the NR activity maximally. The increase in NR activity by (23.14 %) was shown by the T4 (ISA₈₀ (80 mg L⁻¹) along with nitrogen, N₈₀ (80 kg N ha⁻¹) over the control T7 in *Menthapiperita* L. (Table 3).

Carbonic Anhydrase Activity

The application of T4 (ISA₈₀ (80 mg L⁻¹) along with nitrogen, N₈₀ (80 kg N ha⁻¹) proved optimum for the CA activity at 120 DAP. It registered 27.38% higher activity of the enzyme compared to the control T7 in *Menthapiperita* L. (Table 3).

Yield Attributes

Essential oil content was significantly increased by the combined effect of ISA and Nitrogen doses. The best response was seen in T4 (ISA₈₀ (80 mg L⁻¹) along with nitrogen, N₈₀ (80 kg N ha⁻¹) which showed the enhancement of EO by (43.01%), essential oil yield by (111.11%) and herbage yield by 42.36% respectively as compared to the control in *Menthapiperita* L. (Table 4).

Table 2: Effect of Gamma – Irradiated Sodium Alginate and Nitrogen Fertilizer on the Growth Attributes of *Menthapiperita*L. After 120 Days of Plantation

Growth Attributes	Treatment Concentrations						
	T1 ISA ₈₀ +N ₂₀	T2 ISA ₈₀ +N ₄₀	T3 ISA ₈₀ + N ₆₀	T4 ISA ₈₀ + N ₈₀	T5 ISA ₈₀ + N ₁₀₀	T6 ISA ₈₀ + N ₁₂₀	T 7 Control
Plant Height (cm)	99.10 ± 2.89 ^d	107.21 ± 1.18 ^c	110.65 ± 2.58 ^{bc}	124.40 ± 0.85 ^a	121.20 ± 1.85 ^b	94.41 ± 1.68 ^e	88.20 ± 1.41 ^f
Leaf-area per plant (cm ²)	1296.46 ± 23.09 ^d	1335.06 ± 19.70 ^{cd}	1441.81 ± 5.83 ^a	1409.17 ± 18.67 ^{ab}	1378.11 ± 6.80 ^{bc}	1208.13 ± 15.22 ^e	1161.22 ± 7.71 ^e
Fresh Weight per plant (g)	37.41 ± 1.11 ^{de}	39.50 ± 1.32 ^{cd}	43.71 ± 1.41 ^{bc}	52.85 ± 1.27 ^a	44.41 ± 0.99 ^b	47.21 ± 1.27 ^b	34.30 ± 1.29 ^e
Dry Weight per plant (g)	10.72 ± 0.19 ^e	11.32 ± 0.21 ^d	12.67 ± 0.50 ^c	15.99 ± 0.14 ^a	13.07 ± 0.14 ^b	10.35 ± 0.25 ^f	9.56 ± 0.30 ^g

Table 3: Effect of Gamma – Irradiated Sodium Alginate and Nitrogen Fertilizer on the Physiological and Biochemical Attributes of *Menthapiperita*L. After 120 Days of Plantation

Physiological and Biochemical Attributes	Treatment Concentrations						
	T1 ISA ₈₀ +N ₂₀	T2 ISA ₈₀ +N ₄₀	T3 ISA ₈₀ + N ₆₀	T4 ISA ₈₀ + N ₈₀	T5 ISA ₈₀ + N ₁₀₀	T6 ISA ₈₀ + N ₁₂₀	T 7 Control
Chlorophyll Content	1.18 ± 0.02 ^{cd}	1.21 ± 0.03 ^c	1.31 ± 0.02 ^b	1.42 ± 0.01 ^a	1.31 ± 0.05 ^b	1.12 ± 0.01 ^{de}	1.08 ± 0.02 ^e
Carotenoid Content	0.32 ± 0.02 ^{bc}	0.33 ± 0.03 ^b	0.32 ± 0.01 ^b	0.38 ± 0.01 ^a	0.31 ± 0.01 ^{bc}	0.30 ± 0.01 ^{bc}	0.28 ± 0.02 ^c
Nitrate Reductase activity	344.30 ± 2.51 ^e	354.11 ± 3.04 ^d	361.80 ± 2.76 ^c	383.11 ± 2.65 ^a	371.20 ± 3.33 ^b	319.10 ± 2.63 ^f	311.11 ± 3.11 ^g
Carbonic Anhydrase activity	223.01 ± 2.15 ^e	247.11 ± 2.90 ^d	253.22 ± 1.97 ^c	265.60 ± 2.82 ^a	259.10 ± 2.74 ^b	216.05 ± 1.89 ^f	208.50 ± 3.35 ^g

Table 4: Effect of Gamma – Irradiated Sodium Alginate and Nitrogen Fertilizer on the Yield Attributes of *Menthapiperita* L. After 120 Days of Plantation

Yield Attributes	Treatment Concentrations						
	T1 ISA ₈₀ +N ₂₀	T2 ISA ₈₀ +N ₄₀	T3 ISA ₈₀ + N ₆₀	T4 ISA ₈₀ + N ₈₀	T5 ISA ₈₀ + N ₁₀₀	T6 ISA ₈₀ + N ₁₂₀	T 7 Control
Essential Oil Content (%)	0.40 ± 0.01 ^b	0.39 ± 0.01 ^{bc}	0.31 ± 0.01 ^c	0.43 ± 0.01 ^a	0.33 ± 0.01 ^c	0.39 ± 0.01 ^b	0.30 ± 0.02 ^{cd}
Essential Oil Yield (%)	0.14 ± 0.01 ^{bc}	0.15 ± 0.01 ^b	0.12 ± 0.01 ^{bcd}	0.19 ± 0.01 ^a	0.13 ± 0.02 ^{bcd}	0.12 ± 0.02 ^{cd}	0.09 ± 0.01 ^d
Herbage Yield (g)	37.21 ± 1.30 ^b	39.30 ± 0.97 ^b	41.49 ± 3.12 ^b	45.70 ± 2.58 ^a	40.20 ± 1.40 ^b	33.20 ± 2.42 ^c	32.10 ± 0.90 ^c

DISCUSSIONS

Growth Attributes

The data presented in (Table 2) indicated that combined effect of ISA and Nitrogen doses have promotive effects on all the growth parameters studied. The foliar application of T4 (ISA₈₀ + N₈₀) exhibited the significant response to enhance the growth attributes as compared to the control plants (T 7). These results are in significance with those of Hein *et al.*, 2000) who reported that gamma – irradiated SA, applied at concentration of 20-100 ppm, enhanced the productivity of carrot, cabbage, barley, rice, peanut, tea during the field investigations. Alginate derived oligosaccharides promoted the biomass or dry weight of tomato seedling (Ruizhiet *al.*, 2009). According to El-Rehim (2006) and Mollahet *al.* (2009), the oligomers derived from de-polymerization of alginate released from the copolymer were absorbed by plants. The influence of natural polysaccharides, such as carrageenan, sodium alginate and chitosan has proved to be significant for plant growth parameters (Abad *et al.*, 2009; Aftabet *al.*, 2011; Hegazyet *al.*, 2009; Hien *et al.*, 2000; Hu *et al.*, 2004; Khan *et al.*, 2011; Kumeet *al.*, 2002; Mollahet *al.*, 2009; Natsumeet *al.*, 1994; Relleveet *al.*, 2000; Sarfarazet *al.*, 2011; Tomodaet *al.*, 1994).

The absorption of oligomers acted as a growth promoter, which resulted in elongation of plant root and shoot of plants and, in turn, resulted in an increase in plant productivity and significant improvement in physiological parameters compared with the untreated plants. They maintained that ISA might have growth promoting activities like other plant growth promoters and could act as a bio-fertilizer. Exogenous factors include various plant growth promoters, which have direct or indirect influence on growth and development of the plants. The effect of irradiated natural polysaccharides, such as sodium alginate, carrageenan and chitosan has been reported positive for plant growth parameters in various earlier studies (Natsumeet *al.*, 1994; Tomodaet *al.*, 1994; Hien *et al.*, 2000; Relleveet *al.*, 2000; Kumeet *al.*, 2002; Hu *et al.*, 2004; Abad *et al.*, 2009; Mollahet *al.*, 2009; Jamsheer, 2010; Qureshi, 2010; Sarfarazet *al.*, 2011; Khan *et al.*, 2011; Aftabet *al.*, 2011, 2013; Naeemet *al.*, 2011, 2012; Idreeset *al.*, 2012).

Physiological and Biochemical Attributes

Photosynthetic Pigments

Increment in the chlorophyll and carotenoid content due to the combined application of ISA and Nitrogen doses (Table 3) might be ascribed to a favorable effect of ISA and Nitrogen doses on photosynthesis, as well as on the overall growth of the plant. In fact, various workers have reported positive effect of ISA and N on the total content of chlorophyll and carotenoids in the leaves of artemisia (Aftabet *al.*, 2013), lemongrass (Idreeset *al.*, 2012), mentha (Naeemet *al.*, 2011, 2012), fennel (Sarfarazet *al.*, 2011), red amaranth (Mollahet *al.*, 2009) opium poppy (Khan *et al.*, 2011). The ISA has also been reported to induce cell signaling, leading to stimulation of various physiological processes in various plants, including ISA-mediated improvement in the content of photosynthetic pigments and net photosynthetic rate (Farmer *et al.*, 1991).

NR Activity

A significant increase in leaf-NR activity by ISA and N in this study might be related to the increase in leaf-N and -P content that might have possibly increased the nitrate concentration in leaves. In fact, the substrate (nitrate) concentration essentially induces functional NR activity (Hewitt & Afridi, 1959) by producing nitrate sensing protein of unknown nature (Campbell, 2002). The results of the present study are concordant with those obtained regarding artemisia (Aftabet *al.*, 2013), lemongrass (Idreeset *al.*, 2012), and in the case of fennel (Sarfarazet *al.*, 2011), and those of regarding

opium poppy (Khan *et al.*, 2011). The positive effect of ISA and N concentration on NR activity has also been reported by Naeem *et al.*, 2011; in the case of *Mentha arvensis* L.

Determination of Carbonic Anhydrase (CA) Activity

CA is one of the most abundant zinc containing proteins in plants. It has an active role in photosynthesis, which is evident by its presence in all photosynthesizing tissues. It catalyzes the reversible hydration of CO₂ to carbonic acid, thereby increasing the availability of CO₂ to RuBisCO in photosynthesis (Badger & Price, 1994). The application of ISA₈₀ + N₈₀ mg L⁻¹ proved optimum for the CA activity. It registered 26.79% % higher activity of the enzyme compared to the control (Table 3). Such a plant response to ISA and doses of Nitrogen is expected because, the depolymerized natural Polysaccharides have been reported to increase the stomatal conductance significantly (Naeem *et al.*, 2011), which might facilitate the diffusion of additional amounts of CO₂ through the stomata to be acted upon by CA, resulting in the enhanced CA activity. Further, a probable reason for the enhancement of CA activity could be the ISA-mediated de novo synthesis of CA, which might involve transcription/translation of the genes associated as has been reported for other degraded natural polysaccharides (Knowles & Ries, 1981). Expectedly, the enhancement of CA activity in the combined application of ISA and N treated plants might be responsible for the enhanced rate of CO₂ fixation (not measured in this study) that could have resulted in significant increase in fresh and dry weights of the plants (Table 2). Regarding ISA-mediated CA activity, our findings are similar to those that claim the synthesis of certain enzymes in the tissue culture, as a result of application of irradiated natural polysaccharides (Akimoto *et al.*, 1999; Patier *et al.*, 1995).

Yield Attributes

As revealed by the data of leaf analysis, the absorption of nutrients was increased in treated plants, which might have led to better nutrient status, efficient assimilation and translocation of photosynthates, improved growth and yield of leaves, and greater yield of oil and active constituents. Hence, the enhanced values of yield attributes with ISA and Nitrogen fertilizers might be ascribed to the role of ISA in plant growth stimulation in general (Hien *et al.*, 2000; Thamet *et al.*, 2001; Kumeet *et al.*, 2002; Luan *et al.*, 2005; Mollahet *et al.*, 2009; Khan *et al.*, 2011; Aftabet *et al.*, 2011; Sarfarazet *et al.*, 2011; Naeemet *et al.*, 2011, 2012; Idreeset *et al.*, 2012). Increment in the yield of EO and that in the contents of its active constituents might be due to the ISA-stimulated vegetative growth, population of leaf oil glands, nutrient accumulation (N and P) and also due to the beneficial effect of ISA and Nitrogen on plant metabolism and enzymes activities responsible for mono or sesquiterpenes biosynthesis, which could have been subsequently used to enhance the formation of metabolites with regard to oil formation. This conclusion is in accordance with the findings of, Sarfarazet *et al.*, (2011), Hashmi *et al.*, (2012), Idreeset *et al.*, (2012) and Naeemet *et al.*, (2012).

CONCLUSIONS

The application of irradiated sodium alginate and Nitrogen fertilizer resulted in significant improvement in growth attributes, physiological and biochemical parameters in addition to enhancing the essential oil content and essential oil yield along with the herbage yield. The best ISA concentration proved to be the ISA₈₀ + N₈₀. However, ISA dependent enhancement of growth, yield and quality of *Mentha piperita* L. has been worked out for the first time in this investigation. Further, this research may also instigate the scientists to find out the optimum concentration of ISA or other irradiated natural polysaccharides for different medicinal and aromatic plants in order to enhance the productivity, quality and

production of EO and other active constituents. Furthermore, the technique is highly safe as we used the gamma radiations only to depolymerize the sodium alginate rather exposing the plants or seeds. In addition to that, this is one step processing method to obtain the oligosaccharides. Above that, the application of ISA and Nitrogen doses used in this study is very economical and beneficial.

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