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Depolymerized carrageenan ameliorates growth, physiological attributes, essential oil yield and active constituents of *Foeniculum vulgare* Mill

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ABSTRACT

Irradiated carrageenan (IC) elicits an array of plant defense responses and biological activities in plants. An experiment was carried out in the naturally illuminated conditions of net house in order to assess the effects of foliar spray of IC on agricultural performance of fennel (*Foeniculum vulgare* Mill.), which is a high-value essential oil bearing medicinal crop used in pharmaceutical, food and cosmetic industries. There were applied four IC concentrations (40, 60, 80 and 100 mg L^{-1}) as foliar sprays. Application of IC significantly improved the growth attributes, physiological and biochemical parameters, essential oil yield and the contents of main components of essential oil of fennel. IC applied at 80 mg L^{-1} enhanced these parameters maximally. Unirradiated carrageenan and deionized water had no effect on the attributes studied. Moreover, GLC analysis revealed a significant increase in the components of essential oil, viz. fenchone (4.48–7.82%) and anethole (78.38–86.08%) compared to the control.

1. Introduction

Natural polysaccharides such as sodium alginate, carrageenan and chitosan are considered to be the potent elicitors of plant defence responses (John, Rohrig, Schmidt, Walden, & Schell, 1997; Mercier et al., 2001). It is documented that the polysaccharides purified from seaweeds as well as the oligosaccharides derived from polysaccharides have the ability to trigger plant defence responses (Kessmann, Daniel, & Barz, 1988; Kloareg & Quatrano, 1988; Potin, Bouarab, Kupper, & Kloareg, 1999). These oligomers have valid applications in the field of agriculture as plant growth promoters (Hien et al., 2000; Kume, Nagasawa, & Yoshii, 2002). In fact, they have been reported to promote various biological and physiological activities in plants, including plant growth in general, seed germination, shoot elongation, root growth, flower production, antimicrobial activity, amelioration of heavy metal stress, etc. (Abad et al., 2009; Aftab, Khan, Idrees, Naeem, & Moinuddin Hashmi, 2011; Bi, Iqbal, Arman, Ali, & Hassan, 2010; Kume et al., 2002; Naeem, Idrees, Aftab, Moinuddin, & Varhney, 2011; Relleve et al., 2000; Sarfaraz et al., 2011).

Carrageenans are sulfated anionic polymers that comprise the main structural polysaccharides in red seaweed (Rhodophyceae). They are composed of D-galactose units linked alternately with α-1,4 and β-1,3 linkages. They are mixtures of water-soluble, linear, sulfated galactans. Carrageenans are classified as kappa (j-), iota (i-) and lambda (k-) according to the number and position of sulfate groups. Mercier et al. (2001) reported that k-carrageenan elicited an array of plant defence responses in tobacco possibly through the effect of its high sulfate content; it induced signaling and defence gene expression in plants. Application of IC has exerted spectacular effects on plant growth and other physiological activities, leading to improved productivity of plants (Abad et al., 2009; Bi et al., 2010; Kume et al., 2002; Naeem et al., 2011; Relleve et al., 2000, 2005).

Fennel *(Foeniculum vulgare* Mill.) has long been considered as a medicinal and spice herb. Further, it is a high-value medicinal crop used as stimulant, diuretic, digestive, carminative, sedative, galactagogic, emmenagogic, expectorant and antispasmodic agent (Charles, Morales, & Simon, 1993; Chiej, 1984). Additionally, fennel seeds are widely used in the preparation of various dishes like soups, sauces, pastries, confectioneries, pickles, meat curries, etc. (Tanira, Shah, Mohsin, Ageel, & Quereshi, 1996). The essential oil of fennel plays an important role in pharmaceutical and other industries as well (Abdallah, El-Gengaihi, & Sedrak, 1978). Keeping the immense medicinal value of fennel in mind, the present study was carried out to find out whether the foliar application of gamma-rays degraded carrageenan (carrageenan oligomers) could ameliorate growth, physiological activities, yield attributes and production of essential oil of fennel. In this regard, a pot experiment was

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conducted in the naturally illuminated net house, using randomized block design.

2. Materials and methods

2.1. Irradiation and preparation of carrageenan

Solid material of k-carrageenan (Sigma–Aldrich, USA) was sealed in a glass tube with atmospheric air. The samples of carrageenan were irradiated in Co-60 Gamma Chamber, GC-5000 supplied by BRIT, Mumbai, India, at a dose rate of 2.4 kGy/h. The samples were irradiated to a total dose of 250 kGy. Different aqueous concentrations of IC were finally prepared as spray treatments using double distilled water.

2.2. Experimental set up and growth analyses

Authentic seeds of fennel were procured from Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. The experiment was conducted in randomized block design in earthen pots (25 cm diameter × 25 cm height) in the naturally illuminated environmental conditions in the net house of the Department of Botany, Aligarh Muslim University, Aligarh (27°53′N latitude, 78°51′E longitude, and 187.45 m altitude). Different concentrations of gamma-irradiated carrageenan solution (40, 60, 80 and 100 mg L^{-1}) were applied. Each treatment was replicated four times and each replicate-pot contained two plants. Plants were sprayed three times with IC. The first spray was carried out at 40 days after sowing (DAS), while the second and third sprays were applied after one and two weeks, respectively. Control plants were sprayed with deionized water. Performance of the crop was assessed in terms of growth attributes, physiological and biochemical parameters, essential oil yield and main components of the oil. Growth, physiological and biochemical attributes were determined at flowering stage (70 DAS). Four plants from each treatment were harvested and washed carefully with tap water to remove all adhering foreign particles, surface drying the plants thereafter using blotting paper. Later, the growth attributes, viz. plant height, number of umbels per plant and plant fresh weight were recorded. Plant samples were dried at 80 °C for 24 h using a hot-air oven to obtain plant dry weights. Yield attributes were recorded at the time of harvest (150 DAS). Umbels were threshed and cleaned to get seeds. Weight of 1000 seeds, number of seed per umbel and seed-yield per plant were determined subsequently.

2.3. Physiological and biochemical analyses

2.3.1. Estimation of total chlorophyll and carotenoid contents

Total contents of chlorophyll and carotenoids in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue from interveinal leaf area was grinded using mortar-pestle containing 80% acetone. The optical density (OD) of the pigment-extract solution was recorded at 662 and 645 nm (for the content of chlorophyll a and b, respectively) and at 470 nm (for carotenoids content) using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The photosynthetic pigments were expressed as mg g^{-1} FW.

2.3.2. Determination of nitrate reductase (NR) activity

The NR activity was estimated by the intact tissue method given by Jaworski (1971). The method is based on the reduction of nitrate to nitrite as per the following biochemical reaction

$$NO_3 + NADH + H^+ \xrightarrow{NR} NO_2 + NAD^+ + H_2O$$

The nitrite formed was determined spectrophotometrically. 200 mg of fresh chopped leaves was transferred to each of the plastic vials employed. The reaction mixture, containing 2.5 mL of phosphate buffer (pH 7.5), 0.5 mL of 0.2 M potassium nitrate solution and 2.5 mL of 5% isopropanol, was incubated for 2 h in dark at 30 °C. To 0.4 mL of the incubated mixture, 0.3 mL each of 1% sulfanilamide and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NED-HCl) was added subsequently. The test tubes were kept for 20 min at room temperature for maximum color development. The OD of the colored solution was recorded at 540 nm using the spectrophotometer. The NR activity was expressed as nM NO_2^- g^{-1} FW h^{-1}.

2.3.3. Determination of carbonic anhydrase (CA) activity

The activity of carbonic anhydrase was determined in the fresh leaves using the method of Dwivedi and Randhawa (1974). 200 mg of fresh leaf tissue was transferred to Petri plate, followed by incubation of the leaf tissue in 10 mL of 0.2 M cysteine hydrochloride solution for 20 min at 4 °C. Thereafter, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.02% bromothymol blue solution was added to the homogenate. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. CA activity was expressed as μM CO_2 kg^{-1} leaf FW s^{-1}.

2.3.4. Estimation of leaf-N content

Leaf-N content was estimated in the aliquot according to the method of Lindner (1944) with a slight modification made by (Novozamsky, Houba, van Eck, & van Vark, 1983). A 10 mL of the aliquot was poured into a 50 mL volumetric flask, followed by additions of 2 mL of 2.5 N sodium hydroxide and 1 mL of 10% sodium silicate solution in order to neutralize the excessive acid and prevent turbidity, respectively. 5 mL aliquot of this solution was poured into a 10 mL graduated test tube and then 0.5 mL of Nessler's reagent was added. Regarding estimation of leaf-N content, the optical density of the solution was recorded at 525 nm, using the spectrophotometer.

2.3.5. Estimation of leaf-P content

The method of Fiske and Subba Row (1925), with a slight modification, introduced by Rorison, Spencer, and Gupta (1993), was used to estimate the leaf-P content in the aliquot. 5 mL of the aliquot was poured into a 10 mL graduated test tube, followed by additions of 1 mL of molybdic acid (2.5%) and 0.4 mL of 1-amino-2-naphthol-4-sulfonic acid. The content was kept at room temperature for color development, followed by making the volume up to 10 mL with double distilled water. For leaf-P estimation, the OD of the solution was recorded at 620 nm, using the spectrophotometer.

2.3.6. Estimation of essential oil content

The crop was harvested when the fennel fruits were at the half-yellow stage. 500 g of fresh fruit tissue was taken from each treatment and crushed with distilled water using an electric grinder. The oil extraction was determined gravimetrically according to Guenther (1972) by water distillation method using a Clevenger's apparatus (Borosil, India). The volume of the extracted essential oil was determined thereafter. The oil samples were dehydrated over anhydrous sodium sulfate and stored in the sealed glass vials at 4 °C for further analyses by gas liquid chromatography.

2.3.7. Gas liquid chromatography (GLC) analysis

The components of essential oil were analyzed by GLC (Nucon 5700, New Delhi, India) equipped with AT-1000 stainless steel column, a flame ionization detector and an integrator. Nitrogen was used as a carrier gas. The flow rates of nitrogen, hydrogen and oxygen were 0.5, 0.5 and 5 mL s^{-1}, respectively. The GLC apparatus was

Table 1
Effect of foliar application of different concentrations of irradiated carrageenan (40, 60, 80 and 100 mg L^{-1}) on growth attributes of *Foeniculum vulgare* Mill. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of four replicates ± SE.

Growth parameters	Irradiated carrageenan concentrations				
	Control	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}
Plant height (cm)	129 ± 1.26	134 ± 1.46^{c}	140 ± 1.47^{b}	158 ± 1.74^{a}	136 ± 1.53^{c}
Fresh weight per plant (g)	320 ± 1.54^{d}	326 ± 0.85^{d}	383 ± 1.43^{b}	468 ± 2.41^{a}	345 ± 1.62^{c}
Dry weight per plant (g)	72.88 ± 0.37^{d}	77.18 ± 0.60^{d}	84.58 ± 0.49^{c}	120.12 ± 0.68^{a}	96.79 ± 0.63^{b}

run with the following specifications: detector temperature 250 °C; oven temperature 160 °C; injector temperature 250 °C; sample size 2 μL. The identification of the active constituents was made on the basis of retention time and their quantification was carried out comparing the experimental peaks with those obtained from the reference standards reported in the literature.

2.4. Statistical analysis

Each experimental pot was treated as one replicate and all the treatments were replicated four times. The data were analyzed statistically according to randomized block design using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Mean values of the results were statistically compared using Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

3. Results and discussion

3.1. Vegetative growth

Foliar application of various concentration of IC proved better than control in terms of growth attributes (Table 1). Application of 80 mg L^{-1} of IC proved better than other IC concentrations. It significantly enhanced plant height by 22.4% and fresh and dry weight of plant by 46.2 and 65.0%, respectively (Table 1). Exogenous factors include various plant growth promoters, which have direct or indirect influence on growth and development of the plant. Like PGRs, which improve the plant defence by acting as signaling molecules, CO (carrageenan oligosaccharides) resemble with growth elicitors that trigger the synthesis of different enzymes and activate various plant responses exploiting the gene expression (Ma, Li, Bu, & Li, 2010). The effect of natural polysaccharides, such as sodium alginate, carrageenan, and chitosan has been positive for plant growth parameters (Abad et al., 2009; Aftab et al., 2011; Hegazy, Abdel-Rehim, Diaa, & El-Barbary, 2009; Hien et al., 2000; Hu, Jiang, Hwang, Liu, & Guan, 2004; Khan, Khan, Aftab, Idrees, & Naeem, 2011; Kume et al., 2002; Luan et al., 2003; Mollah, Khan, & Khan, 2009; Natsume, Kamao, Hirayan, & Adachi, 1994; Relleve et al., 2000; Sarfaraz et al., 2011; Tomoda, Umemura, & Adachi, 1994). As per the results obtained in the present study, there was significant improvement in plant growth attributes by the application of radiation-derived oligosaccharides of carrageenan. Similarly, Sarfaraz et al. (2011) reported significant enhancement in the shoot and root length, number of branches, leaves and flowers, and plant fresh and dry weight in fennel as affected by different concentrations of sodium alginate under normal conditions. It is argued that the plants have capacity to recognize the oligomers or oligosaccharides of natural polysaccharides that regulate growth, development and defence responses of plants (Darvill et al., 1992). The growth-promoting effects of degraded natural polysaccharides on growth and yield characteristics of various crops have, in fact, been reported by several workers (Abad et al., 2009; Aftab et al., 2011; Jamsheer, 2010; Khan et al., 2011; Natsume et al., 1994; Qureshi, 2010; Relleve et al., 2005; Sarfaraz et al., 2011; Tomoda et al., 1994).

3.2. Physiological and biochemical characteristics

3.2.1. Photosynthetic pigments

Application of depolymerized form of carrageenan (IC) at 80 mg L^{-1} significantly increased the content of chlorophyll 'a' (by 16.5%), chlorophyll 'b' (by 31.0%) and the total chlorophyll content (by 34.1%) at 70 DAS as compared to the control (Table 2). Increment in the chlorophyll content due to application of IC might be ascribed to a favorable effect of IC application on photosynthesis as well as on the overall growth of the plant (Table 1). In fact, various workers have reported positive effect of irradiated sodium alginate (ISA) regarding photosynthetic pigments and rate of net photosynthesis (Aftab et al., 2011; Khan et al., 2011; Sarfaraz 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, Thomas, Michael, & Clarence, 1991).

3.2.2. Nitrate reductase (NR) activity

IC, applied at 80 mg L^{-1}, increased the NR activity maximally, the value being 47.4% higher as compared to the control (Table 2). N and P mediated increase in the uptake of various nutrients and the resultant increase in the NR activity has earlier been established under normal conditions (Samiullah, Moinuddin, Ansari, & Afridi, 1998). Thus, one of the reasons of IC-enhanced NR activity in this study might be the IC-enhanced leaf-N, and -P contents (Table 2). The positive effect of IC application on NR activity has also been reported by Naeem et al. (2011) in the case of *Mentha arvensis* L.

Table 2
Effect of foliar application of different concentrations of irradiated carrageenan (40, 60, 80 and 100 mg L^{-1}) on physiological biochemical and parameters of *Foeniculum vulgare* Mill. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of four replicates ± SE.

Physiological and biochemical parameters	Irradiated carrageenan concentrations				
	Control	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}
Chlorophyll 'a' content (mg g^{-1})	1.70 ± .015^{c}	1.73 ± .062^{c}	1.75 ± .005^{bc}	1.98 ± .015^{a}	1.79 ± .008^{b}
Chlorophyll 'b' content (mg g^{-1})	0.71 ± 0.17^{d}	0.74 ± .011^{dc}	0.76 ± .011^{bc}	0.93 ± .011^{a}	79 ± .020^{b}
Total chlorophyll content (mg g^{-1})	1.55 ± 0.023^{c}	1.56 ± 0.023^{c}	1.65 ± 0.023^{b}	2.08 ± 0.017^{a}	1.71 ± 0.005^{c}
Leaf-N content (%)	2.18 ± 0.041^{c}	2.24 ± 0.041^{c}	2.54 ± 0.070^{b}	2.98 ± 0.165^{a}	2.62 ± 0.071^{b}
Leaf-P content (%)	0.31 ± 0.026^{c}	0.32 ± 0.026^{c}	0.35 ± 0.018c	0.47 ± 0.041^{a}	0.38 ± 0.020^{c}
NR activity (nM NO_2^- g^{-1} FW h^{-1}).	290.79 ± 1.35^{d}	392.35 ± 2.37^{c}	402.58 ± 0.90^{b}	428.78 ± 0.80^{a}	409.16 ± 2.00^{bc}
CA activity (μM CO_2 kg^{-1} FW s^{-1})	1.80 ± .014^{d}	1.83 ± .010^{d}	1.92 ± .010^{c}	2.46 ± .011^{a}	1.85 ± .013^{d}

Table 3
Effect of foliar application of different concentrations of irradiated carrageenan (40, 60, 80 and 100 mg L^{-1}) on yield attributes of *Foeniculum vulgare* Mill. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of four replicates ± SE.

Yield attributes	Irradiated carrageenan concentrations				
	Control	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}
Umbel number per plant	8.38 ± 0.20^{c}	8.48 ± 0.21^{c}	10.16 ± 0.20^{c}	15.02 ± 0.32^{a}	10.24 ± 0.20^{c}
Seed number per plant	92.00 ± 0.88^{d}	102.30 ± 1.85^{c}	115.27 ± 1.95^{b}	132.85 ± 1.90^{a}	116.36 ± 1.87^{b}
1000 seed weight (g)	12.51 ± 0.56^{d}	12.60 ± 0.20^{d}	15.65 ± 0.56^{b}	21.06 ± 0.88^{a}	15.85 ± 0.33^{b}
Seed yield (g)	13.75 ± .015^{d}	13.79 ± .015^{d}	18.86 ± .015^{b}	23.26 ± .015^{a}	13.96 ± .015^{d}

Additionally, the increase in NR activity of plant with increasing carrageenan concentration in the present investigation is in agreement with the findings reported by Aftab et al. (2011) regarding ISA mediated increase in overall plant growth and other yield and quality parameters in *Artemisia annua* L.

3.2.3. 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 CO_2 to carbonic acid, thereby increasing the availability of CO_2 to RuBisCO in photosynthesis (Badger & Price, 1994). The application of IC at 80 mg L^{-1} proved optimum for the CA activity at vegetative stage. It registered 36.6% higher activity of the enzyme compared to the control (Table 2). Such a plant response to IC application is expected because the deploymerized natural polysaccharides have been reported to increase the stomatal conductance significantly (Naeem et al., 2011), which might facilitate the diffusion of additional amounts of CO_2 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 IC-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 IC-treated plants might be responsible for the enhanced rate of CO_2 fixation (not measured in this study) that could have resulted in significant increase in fresh and dry weights of the plants (Table 1). Regarding IC-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, Aoyagi, & Tanaka, 1999; Patier et al., 1995).

3.2.4. Leaf-N and -P contents

Leaf-N and -P contents were also significantly enhanced by IC application, with 80 mg L^{-1} proving the best. It increased the leaf-N and -P content by 36.7 and 51.6%, respectively, at 70 DAS (Table 2). In conformity with these results, Naeem et al. (2011) reported significant increase in the uptake of these elements (N and P) at an IC concentration of 80 mg L^{-1} in *M. arvensis* L. Such an impact of IC application could be ascribed to the IC-mediated increase in overall growth of plants (Table 1), which accordingly demanded for higher uptake of these nutrients from the soil, leading to their significant accumulation in the leaves.

3.3. Yield attributes

The foliar application of radiation-derived oligosaccharides of carrageenan significantly improved the seed yield and yield attributes, with 80 mg L^{-1} of IC proving the best. In comparison to the control, IC applied at 80 mg L^{-1} increased the number of umbel per plant, number of seeds per umbel, 1000 seed-weight and seed yield per plant by 79.2, 44.4, 68.3, and 69.1%, respectively (Table 3). Radiation-derived oligomers of carrageenan have been found to promote valuable biological functions (Relleve et al., 2000). It is considered that the plants have capacity to recognize the oligomers or oligosaccharides which regulate growth, development and defence responses of plants (Darvill et al., 1992). Relleve et al. (2005) investigated the biological activity of the oligosaccharides derived from irradiated k-carrageenan at different doses in potato tissue culture bioassay. Earlier, Relleve et al. (2000) reported an IC-induced weight gain in treated rice seedlings. They suggested that a certain molecular weight of oligomers of degraded carrageenan was required to get the optimum effects on plant growth and that the degraded carrageenan might be easily produced via irradiation by gamma rays. Expectedly, the increase in seed yield and yield attributes might result due to the enhanced water and nutrient uptake from soil, followed by smooth translocation of photosynthates and other metabolites to the sinks in IC-treated plants. In this context, our results are in line with regard to various medicinal plants (Aftab et al., 2011; Khan et al., 2011; Sarfaraz et al., 2011).

3.4. Yield of essential oil and its main components

Among various concentrations, 80 mg L^{-1} of IC gave the best results regarding yield and main components of essential oil of fennel. As compared to the control, it maximally augmented the per-plant yield of essential oil (66.3%) and the contents of main constituents of essential oil, viz. anethole (86.0%) and fenchone (7.82%) at 150 DAS (Table 4). According to Anand et al. (2008), anethole and fenchone are the main components of fennel essential oil. Anethole bears anti-cancer activity, fenchone is responsible for the fragrance of the fennel oil and trans-anethole is included as a flavoring agent in various food products (Lawrence, 1997). In the case of medicinal and aromatic plants, the exogenous application of IC has been found positively effective in improving growth, yield and essential oil content of *M. arvensis* (Naeem et al., 2011). Increment in the yield of essential oil and that in the contents of its active constituents might be due to the IC-stimulated vegetative growth, population

Table 4
Effect of foliar application of different concentrations of irradiated carrageenan (40, 60, 80 and 100 mg L^{-1}) on essential oil yield and active constituents of *Foeniculum vulgare* Mill. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of four replicates ± SE.

Oil yield and active constituents	Irradiated carrageenan concentrations				
	Control	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}
Essential oil yield (mL/plant)	0.98 ± .023^{d}	1.23 ± .023^{d}	1.36 ± .023^{c}	1.63 ± .023^{a}	1.48 ± .023^{b}
Fenchone (%)	4.48 ± 0.056^{c}	4.97 ± 0.090^{c}	5.64 ± 0.104^{b}	7.82 ± 0.060^{a}	7.58 ± 0.056^{a}
Trans-anethole (%)	78.38 ± 1.19^{c}	80.46 ± 1.19^{c}	81.52 ± 1.00^{b}	86.08 ± 1.63^{a}	81.65 ± 1.19^{b}

of leaf oil glands, nutrient accumulation (N and P) and also due to the beneficial effect of IC on plant metabolism and enzymes activities responsible for mono or sesqueterpene-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 Naeem et al. (2011) on *M. arvensis* and Sarfaraz et al. (2011) on fennel. However, a positive effect of foliar spray of IC on fennel oil yield and the contents of its main constituents fenchone (4.48–7.82%) and anethole (78.38–86.08%) have been reported for the first time in this study.

4. Conclusion

The application of irradiated carrageenan resulted in significant improvement in growth attributes, physiological and biochemical parameters, essential oil yield and the contents of its main constituents in fennel, with 80 mg L^{-1} proving the best IC concentration. These findings confirm the earlier findings carried out regarding the effect of other irradiated natural polysaccharides on growth, yield and/or quality of other crops. However, such an IC-dependent enhancement of growth, yield and quality of fennel has been worked for the first time in this investigation. Further, this research may also help to find out the optimum concentration of IC and/or other irradiated natural polysaccharides for different medicinal and aromatic plants to enhance the productivity, quality and production of essential oil and other active constituents.

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