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Depolymerised carrageenan enhances physiological activities and menthol production in *Mentha arvensis* L.

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ABSTRACT

Irradiated carrageenan (IC) could elicit plant growth promoting activities in plants. The effect of foliar spray of five concentrations of IC (20, 40, 60, 80 and 100 mg L^{-1}) was studied on *Mentha arvensis* L. in terms of plant growth, physiological attributes, herbage yield and the content and yield of essential oil and its components. Un-irradiated carrageenan and deionized water had no effect on the attributes studied. GPC study revealed formation of low molecular weight fractions in irradiated samples containing less than 20,000 molecular weight oligomers which are responsible for plant growth promotion in this study. 80 mg L^{-1} of IC was the most effective concentration which resulted in the highest values of growth attributes, herbage yield and the content and yield of essential oil and menthol content of the oil. It also improved the leaf-nutrient contents, photosynthetic rate and other physiological parameters. 100 mg L^{-1} of IC did not further improve the attributes studied, but it was always better than the control.

1. Introduction

Application of ionizing radiation to degrade natural bioactive agents and then using them as growth promoting substances is an emerging technology to exploit full genetic potential of crops in terms of growth, yield, and quality. Compared to the conventional techniques such as acid/base hydrolysis and enzymatic methods (Shimokawa, Toida, & Kawashima, 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 of sodium alginate and carrageenan (Abad et al., 2009; Lee et al., 2003; Nagasawa, Mitomo, Yoshii, & Kume, 2000; Relleve et al., 2000, 2005).

Gamma-rays irradiation degrades the natural polysaccharides, such as chitosan, carrageenan and sodium alginate, into smaller oligomers with comparatively low molecular weight. Oligomers, obtained from radiolytically degraded polysaccharides, have valid applications in the field of agriculture, as plant growth promoter (Hien et al., 2000; Kume, Nagasawa, & Yoshii, 2002). Application of these degraded polysaccharides (oligomers) on plants promotes various biological and physiological activities, including plant growth in general, seed germination, shoot elongation, root growth, flower production, antimicrobial activity, amelioration of heavy metal stress, phytoalexin induction, etc. (Aftab et al., 2011; Akiyama et al., 1992; De La Rosa, Abad, Relleve, & Aranilla, 2002; 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; Natsume, Kamao, Hirayan, & Adachi, 1994; Sarfaraz et al., 2011; Tomoda, Umemura, & Adachi, 1994; Yonemoto et al., 1993).

Out of a large number of essential oil bearing plants, mint (*Mentha arvensis* L.) constitutes most important source of therapeutic agents used in the alternative systems of medicine. It is a stimulant, tonic and vermifuge, having anti-spasmodic, diaphoretic, stomachic, carminative, antiviral, antifungal, antibacterial and choleretic properties (The Wealth of India, 1992). Mint oil has wide applications in pharmaceutical, agrochemical and flavoring industries worldwide (Misra, Hasan, & Kumar, 2000; Tassou, Nychas, & Skandamis, 2004).

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 a-1,4 and b-1,3 linkages. They are mixtures of water-soluble, linear, sulfated galactans. The use of carrageenan, to promote growth and the amount of essential oil along with the desired active constituents in medicinal plants, is inexpensive. Keeping the importance and increasing demand of the essential oil of mint, an assumption was made to understand whether the application of degraded oligomers of irradiated carrageenan (IC) could be useful to enhance the plant growth, physiological activities, and yield and quality attributes, including production of essential oil and menthol content in *M. arvensis* L.

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2. Materials and methods

2.1. Plant materials and growth conditions

The pot experiment was conducted in the natural conditions of the net house at Botany Department, Aligarh Muslim University, Aligarh, India. Prior to transplanting, each pot was filled with 5 kg homogenous mixture of soil and organic manure (4:1). Physico-chemical characteristics of the soil were: texture-sandy loam, pH (1:2) 7.5, E.C. (1:2) 0.48 m mhos cm^{-1}, available N, P and K 102.4, 7.8 and 145.9 mg per kg of soil, respectively. A uniform recommended basal dose of N, P and K (25:11:21 mg per kg soil, respectively) was applied in the form of urea, single superphosphate and muriate of potash, respectively, at the time of planting.

2.1.1. Irradiation and gel permeation chromatography (GPC) analysis

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. GPC of carrageenan samples were done on DIONEX ULTIMATE 3000 machine and the experimental conditions were as follows: mobile phase-water, flow rate-1.5 mL/min, column PL-Aquagel, mixed bed column, 300 mm × 10 mm, 20 μL loop injection.

The molecular weight of the un-irradiated commercial k-carrageenan sample was estimated to be about 100,000. Polyvinyl alcohol polymers of known molecular weight were used as standards. Radiation dose of 250 kGy was chosen as no significant change in molecular weight was reported beyond this dose in solid state irradiation of k-carrageenan (Relleve et al., 2005). Different aqueous concentrations of irradiated carrageenan (IC) were finally prepared using double distilled water as spray treatments.

2.1.2. Pot culture

The experiment was conducted in randomized block design in earthen pots (25 cm diameter × 25 cm height). Five concentrations of gamma-irradiated carrageenan solution (20, 40, 60, 80 and 100 mg L^{-1}) were applied, using distilled water as absolute-control and un-irradiated carrageenan (20 mg L^{-1}) as carrageenan-control. Total five foliar sprays of IC treatment were applied to the crop at 10 days interval using hand sprayer, when the plants were at the 2–3 true leaf stage. The crop was planted in February 2010 and harvested at 100 and 120 days after planting. Each treatment was replicated five times. Each pot contained a single healthy plant. The pots were watered as and when required.

2.2. Determination of growth attributes

The growth attributes *viz.* plant height, leaf-area, leaf-yield per plant and fresh and dry weights of plant were determined at 100 and 120 DAP. All the leaves of the plant were weighed to determine leaf-yield per plant. At 100 and 120 DAP, five plants of each treatment were uprooted, measuring the height and fresh weight of plant. The plants were dried in a hot-air oven at 80 °C for 24 h prior to record plant dry weight. Only 10% of the total leaves of each sample (consisting of five plants) were used to determine the leaf area using graph paper sheet (Watson, 1958). The mean area per leaf, thus determined, was multiplied with the total number of leaves to measure the total leaf area per plant.

2.3. Determination of physiological attributes

2.3.1. Estimation of total chlorophyll and carotenoids contents

Total chlorophyll and carotenoids contents in the leaves were estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue from the interveinal area of leaf was grinded with 100% acetone using a mortar and 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 spectrophotometer (Shimadzu UV-1700, Tokoyo, Japan). Total chlorophyll content was assessed by adding chlorophyll a and b contents. The photosynthetic pigments, thus measured, were expressed as mg g^{-1} leaf FW.

2.3.2. Determination of net photosynthetic rate and stomatal conductance

Net photosynthetic rate and stomatal conductance of the youngest fully expanded leaves were measured in three replicates on sunny days at 1100 h using an Infra Red Gas Analyzer (IRGA, Li-Cor 6400 Portable Photosynthesis System Lincoln, Nebraska, USA) at 100 and 120 DAP.

2.3.3. Determination of carbonic anhydrase (CA) activity

The activity of carbonic anhydrase (E.C. 4.2.1.1) was measured in the fresh leaves, using the method described by Dwivedi and Randhawa (1974). Two hundred mg of fresh leaf (chopped leaf-pieces) were 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 in to a test tube containing 4 mL 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 was expressed as μM CO_2 kg^{-1} leaf FW s^{-1}.

2.3.4. Leaf-digestion for N, P, and K estimations

Leaf samples from each treatment were digested for the estimation of leaf-N, -P and -K content. The leaves were dried in a hot-air oven at 100 °C for 24 h. The dried leaves were powdered using a mortar and pestle, passing the leaf-powder through a 72 mesh. Hundred mg of oven-dried leaf-powder was carefully transferred into a Kjeldahl digestion tube, followed by adding 2 mL of concentrated sulphuric acid. The mixture was heated on a temperature-controlled Kjeldahl assembly at 100 °C for about 2 h and then dissolved content was cooled for about 15 min at room temperature. 0.5 mL of 30% hydrogen peroxide (H_2O_2) was added in the content, followed by gentle heating and then cooling at room temperature. The aliquot (peroxide-digested leaf-material), thus prepared, was used to estimate the per cent N, P and K content in the leaves on dry weight basis.

2.3.4.1. Determination of leaf-N content. Leaf-N content was estimated according to the method of Lindner (1944) with slight modification by Novozamsky, Houba, van Eck, and van Vark (1983). A 10 mL aliquot was poured into a 50 mL volumetric flask. To it, 2 mL of 2.5 N sodium hydroxide and 1 mL of 10% sodium silicate solutions were added in it to neutralize the excessive acid and prevent turbidity, respectively. A 5 mL aliquot of the peroxide-digested leaf-material was poured into a 10 mL graduated test tube, followed by addition of 0.5 mL of Nessler's reagent. The OD (optical density) of the solution was recorded at 525 nm using the spectrophotometer.

2.3.4.2. Determination of leaf-P content. The method of Fiske and Subba Row (1925), with slight modification by Rorison, Spencer, and Gupta (1993), was used to estimate the leaf-P content. A 5 mL

aliquot was poured into a 10 mL graduated test tube. 1 mL of molybdic acid (2.5%) was added in it, followed by addition of 0.4 mL of 1-amino-2-naphthol-4-sulphonic acid. When the colour of the content turned blue, its volume was made up to 10 mL using double distilled water. The OD of the solution was recorded at 620 nm using spectrophotometer.

2.3.4.3. Determination of leaf-K content. Leaf-K was determined in the peroxide-digested leaf-material by a flame-photometer (Model, C150, AIMIL, India) with the help of emission spectra using specific filter. In the flame-photometer, the test solution was discharged through an atomizer in the form of a fine mist into a chamber, where it was drawn into a flame. Combustion of the elements produced light of a particular wavelength [λ max for K = 767 nm (violet)]. The light produced was passed through an appropriate filter to impinge upon a photoelectric cell that subsequently activated a galvanometer which displayed the K content on a digital screen.

2.4. Total phenol content

Total phenol content was estimated by the method described by Sadasivam and Manickam (2008). Five hundred mg of the leaves were grinded with 10 times volume of 80% ethanol, using mortar and pestle. The homogenate was centrifuged at 10,000 rpm (10,062 × g) for 10 min at 4 °C. The supernatant was evaporated to dryness, adding 5 mL of DDW (double distilled water) thereafter. Later, 0.5 mL of Folin-Ciocalteau reagent and 2 mL of 20% Na_2CO_3 solution were added to each test tube. The OD of the solution, thus obtained, was measured at 650 nm against a reagent blank. Using the standard curve, the concentrations of phenols in the test samples were determined as mg phenol 100 g^{-1} of the dry leaves.

2.5. Yield and quality parameters

Herbage yield of the crop was measured by weighing the total biomass per plant excluding the roots. The essential oil of mint was extracted and determined gravimetrically according to Guenther (1972). To estimate the essential oil, two fresh leaves were collected from each treatment per pot, followed by chopping and mixing them together. Thereafter, 1000 mg of the chopped leaf-pieces were used for the estimation the essential oil. The essential oil content in the leaves was extracted by distillation method for 3 h, using a Clevenger's apparatus.

The active constituents of the essential oil, namely, menthol, L-methone, isomenthone and menthyl acetate, were analyzed using the gas liquid chromatography (GLC, Nucon 5700, New Delhi, India) equipped with an AT-1000 stainless-steel column, a flame ionization detector and an integrator. Nitrogen was used as the carrier gas. The flow rates of nitrogen, hydrogen and oxygen were maintained at 0.5, 0.5 and 5 mL s^{-1}, respectively. The temperature schedule of GLC was as follows: detector temperature, 250 °C; oven temperature, 160 °C; injector temperature, 250 °C. The sample size was 2 μL for all the measurements. The identification of the active constituents was based on retention time of the particular constituent in the GLC column. The active constituents were quantified in per cent content, comparing their peaks with the peaks obtained from the reference standards reported in the literature.

2.5.1. Determination of specific gravity of essential oil

The specific gravity of the essential oil was determined at 25 °C with a 'specific gravity bottle' according to Afaq, Tajuddin, and Siddiqui (1994), using the following formula:

$$\text{Specific gravity} = \frac{\text{Weight of essential oil}}{\text{Weight of an equal volume of distilled water}}$$

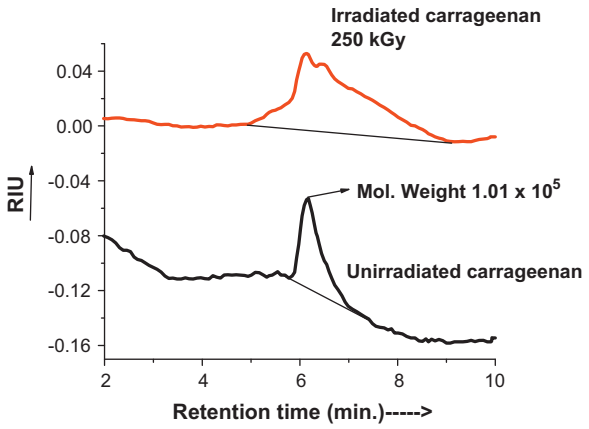

Fig. 1. The molecular weight distribution of un-irradiated and irradiated carrageenan. The profile has fraction eluting at higher retention time in comparison to un-irradiated sample indicating formation of lower molecular weight fragments in the 250 kGy irradiated sample. This fraction also contained less than 20,000 molecular weight oligomers of carrageenan which are responsible for plant growth promotion in this study. The molecular weight of the un-irradiated commercial k-carrageenan was estimated to be about 100,000.

2.5.2. Determination of refractive index of essential oil

The refractive index of the essential oil was determined according to Jenkins, Knevel, and Digangi (1967) employing an Abbe's Refractometer (Sipcon, New Delhi, India). Two to three drops of oil were placed on the double prism, clamping the prisms together firmly. The instrument was adjusted until the border line between light and dark halves of the view-field exactly coincided with the cross hairs of the telescope. The refractive index of oil was noted directly from the graduated scale. The mean of three readings was designated as refractive index of the oil. The refractive index of the oil was expressed as $N_D^{24^\circ}$, where $N_D^{24^\circ}$ denotes the index of the light refraction for the 'D' line (sodium light) measured at 24°.

2.6. 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 $p < 0.05$. Standard error was also employed in this regard.

3. Results and discussion

There is no report regarding the effect of IC application on this essential oil bearing medicinally important plant till date. Hence, it could be considered as the first report of its kind, revealing the effect of IC on plant growth, physiological attributes and the yield and content of essential oil in mint. Fig. 1 shows the molecular weight distribution of un-irradiated and irradiated carrageenan. The profile has fraction eluting at higher retention time in comparison to un-irradiated sample indicating formation of lower molecular weight fragments in the 250 kGy irradiated sample. This fraction also contained less than 20,000 molecular weight oligomers of carrageenan which are responsible for plant growth promotion in this study.

3.1. Growth attributes

The foliar application of IC with concentration up to 80 mg L^{-1} progressively and significantly improved the growth attributes of mint both at 100 and 120 DAP. Thereafter, the values significantly declined at 100 mg L^{-1}, but not to the extent of those obtained due to control (Table 1). The highest values of growth parameters

Table 1
Effect of seven concentrations of foliar sprays of irradiated carrageenan [0 (control), UN (un-irradiated), 20, 40, 60, 80 and 100 mg L^{-1}] on growth attributes of mint (*Mentha arvensis* L.) at 100 and 120 days after planting (DAP). Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Means of five replicates ± SE.

Growth attributes	Irradiated carrageenan concentrations (mg L^{-1})							
	DAP	Control	UN	20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}
Plant height (cm)	100	67.20 ± 1.15^{f}	68.24 ± 1.14^{f}	74.16 ± 1.14^{e}	78.36 ± 1.19^{d}	80.16 ± 1.20^{c}	86.42 ± 1.14^{a}	81.50 ± 1.12^{b}
	120	80.42 ± 1.21^{e}	81.20 ± 1.16^{e}	85.90 ± 1.70^{d}	88.48 ± 1.40^{d}	94.38 ± 1.84^{c}	106.64 ± 1.73^{a}	101.48 ± 1.70^{b}
Leaf-area per plant (cm^2)	100	2990.4 ± 18.20^{f}	3029.8 ± 19.16^{f}	30.96.4 ± 16.62^{e}	3240.6 ± 20.24^{d}	3346.4 ± 21.10^{c}	3601.9 ± 21.62^{a}	3486.2 ± 18.40^{b}
	120	4682.0 ± 18.36^{f}	4720.4 ± 16.90^{f}	4962.3 ± 14.621^{e}	5092.6 ± 16.48^{d}	5468.2 ± 14.56^{c}	5920.3 ± 15.70^{a}	5820.4 ± 14.82^{b}
Leaf-yield per plant (g)	100	14.40 ± 0.164^{f}	14.50 ± 0.140^{f}	16.32 ± 0.150^{e}	16.92 ± 0.164^{d}	18.20 ± 0.154^{c}	19.56 ± 0.160^{a}	18.70 ± 0.164^{b}
	120	27.28 ± 0.210^{f}	27.40 ± 0.222^{f}	28.48 ± 0.238^{e}	30.82 ± 0.218^{d}	34.16 ± 0.246^{c}	37.77 ± 0.249^{a}	36.82 ± 0.186^{b}
Fresh weight per plant (g)	100	54.62 ± 1.20^{e}	55.12 ± 1.16^{e}	58.26 ± 1.06^{d}	62.96 ± 1.12^{c}	67.49 ± 1.18^{b}	73.95 ± 1.24^{a}	72.18 ± 1.16^{b}
	120	66.48 ± 1.52^{f}	66.74 ± 1.50^{f}	70.42 ± 1.48^{e}	76.19 ± 1.52^{d}	84.42 ± 1.56^{c}	93.48 ± 1.60^{a}	90.86 ± 1.60^{b}
Dry weight per plant (g)	100	12.40 ± 0.215^{f}	12.48 ± 0.310^{f}	12.96 ± 0.247^{e}	13.76 ± 0.290^{d}	14.48 ± 0.254^{c}	16.10 ± 0.310^{a}	15.52 ± .0264^{b}
	120	15.14 ± 0.230^{e}	15.24 ± 0.240^{e}	15.96 ± 0.240^{d}	16.78 ± 0.282^{c}	18.36 ± 0.242^{b}	20.80 ± 0.312^{a}	20.19 ± 0.160^{c}

were attained at 120 DAP at 80 mg L^{-1} of IC. In comparison to the control, IC applied at 80 mg L^{-1}, increased the per plant values of shoot length by 28.6 and 32.6%, leaf-area by 20.5 and 26.5%, leaf-yield by 35.4 and 38.8%, fresh weight by 35.4 and 40.6%, and dry weight by 29.8 and 37.4%, at 100 and 120 DAP, respectively (Table 1). The growth and development of plants is governed by several exogenous and endogenous factors, including growth regulators (Taiz & Zeiger, 2006). Among exogenous factors, there are various plant growth promoters, which have direct or indirect influence on growth and development of the plant. Like PGRs, which improve the plant defense by acting as signaling molecules, AO (alginate oligosaccharides) resembles with an endogenous growth elicitor that functions as a signal to trigger the synthesis of different enzymes and activate various responses exploiting the gene expression (Ma, Li, Bu, & Li, 2010). It has already been reported that polysaccharides such as sodium alginate, carrageenan and chitosan, in their depolymerised form, are effective in promotion of germination and shoot elongation (Abad et al., 2009; Aftab et al., 2011; Hegazy et al., 2009; Hien et al., 2000; Hu et al., 2004; Khan et al., 2011; Kume et al., 2002; Luan et al., 2003; Mollah, Khan, & Khan, 2009; Natsume et al., 1994; Relleve et al., 2000; Sarfaraz et al., 2011; Tomoda et al., 1994). In the present investigation, the application of IC enhanced the leaf-area, which might obviously provide increased opportunity for light harvesting leading to the accumulation of enhanced plant dry matter, compared to the control (Table 1). The results obtained in present study showed significant improvement in plant growth attributes by the application of radiation-derived oligosaccharides of carrageenan. Similar results have also earlier been reported (Aftab et al., 2011; Khan et al., 2011; Sarfaraz et al., 2011). Depolymerised oligomers of carrageenan have been found to promote valuable biological functions (Relleve et al., 2000). It is known that the plants have capacity to recognize the oligomers or oligosaccharides which regulate growth, development and defense responses of plants (Darvill et al., 1992). In addition, there is a specific structural and size requirement of the oligosaccharide for inducing a range of effects in plants (Darvill et al., 1992; Shibuya & Minami, 2001). However, the phenomenon by which the radiolytically degraded polysaccharides stimulate the processes related to promotion of plant growth still needs further investigations.

Relleve et al. (2005) investigated the biological activity of oligosaccharides derived from irradiated k-carrageenan at different doses in potato tissue culture bioassay. 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 is required to get optimum growth effects on plants and that the degraded carrageenan might be easily produced via irradiation by gamma rays. The present results are in conformity with the findings that report the significant effects of degraded natural polysaccharides on various crops (Abad et al., 2009; Aftab et al., 2011; Jamsheer, 2010; Khan et al., 2011; Mollah et al., 2009; Natsume et al., 1994; Qureshi, 2010; Relleve et al., 2005; Sarfaraz et al., 2011; Tomoda et al., 1994).

3.2. Physiological attributes

A significant increase in net photosynthetic rate and stomatal conductance was recorded both at 100 and 120 DAP, at 80 mg L^{-1} of IC application (Fig. 2A and B). Compared to the control, application of IC at 80 mg L^{-1} accelerated the net photosynthetic rate by 12.3 and 15.6% at 100 and 120 DAP, respectively (Fig. 2A). Similarly, the application of IC at 80 mg L^{-1} increased the stomatal conductance by 7.8 and 11.4% at 100 and 120 DAP, respectively (Fig. 2B).

Depolymerised form of carrageenan (IC) also increased the chlorophyll content in the treated plants significantly (Fig. 2C). From all the IC concentrations, 80 mg L^{-1} resulted in the greatest increase in total chlorophyll content. Application of IC at 80 mg L^{-1} enhanced the total chlorophyll content by 12.8 and 13.8% at 100 and 120 DAP, respectively (Fig. 2C) in comparison to control. However, IC application did not affect significantly the total carotenoids content at any of the growth stages (Fig. 2D).

Presumably, as a result of IC-mediated increase in leaf area (Table 1), the IC treated plants could trap more sunlight and conduct additional CO_2 to increase the rate of photosynthesis in comparison to control plants. In addition, the enhancement in the chlorophyll content might have also resulted in increased photosynthetic rate. The increase in photosynthetic rate due to application of irradiated sodium alginate (ISA), a similar natural polysaccharide, has been studied previously by several workers (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 improved content of photosynthetic pigments and enhanced net photosynthetic rate (Farmer, Thomas, Michael, & Clarence, 1991). In view of growth promoting effect of ISA, its application could result in improvement in the growth of plant root and augmented shoot elongation and, thereby, might bring increase in plant productivity and improvement in physiological parameters (El-Rehim, 2006). Like ISA, the IC may, presumably, be expected to enable the plants to respond in somewhat similar mode regarding photosynthetic pigments and photosynthesis.

Application of IC positively improved CA activity at both the growth stages. The activity of the enzyme increased to the maximum extent at 120 DAP (Fig. 3A). Compared to the control, 80 mg L^{-1} of IC resulted in 17.8 and 21.7% increase in CA activity at 100 and 120 DAP, respectively (Fig. 3A). The spray of IC also increased the leaf phenolic content at both the sampling stages. The 80 mg L^{-1} of IC excelled the control by 8.0 and 9.7% at 100 and 120 DAP, respectively (Fig. 3B). Our findings are similar to those

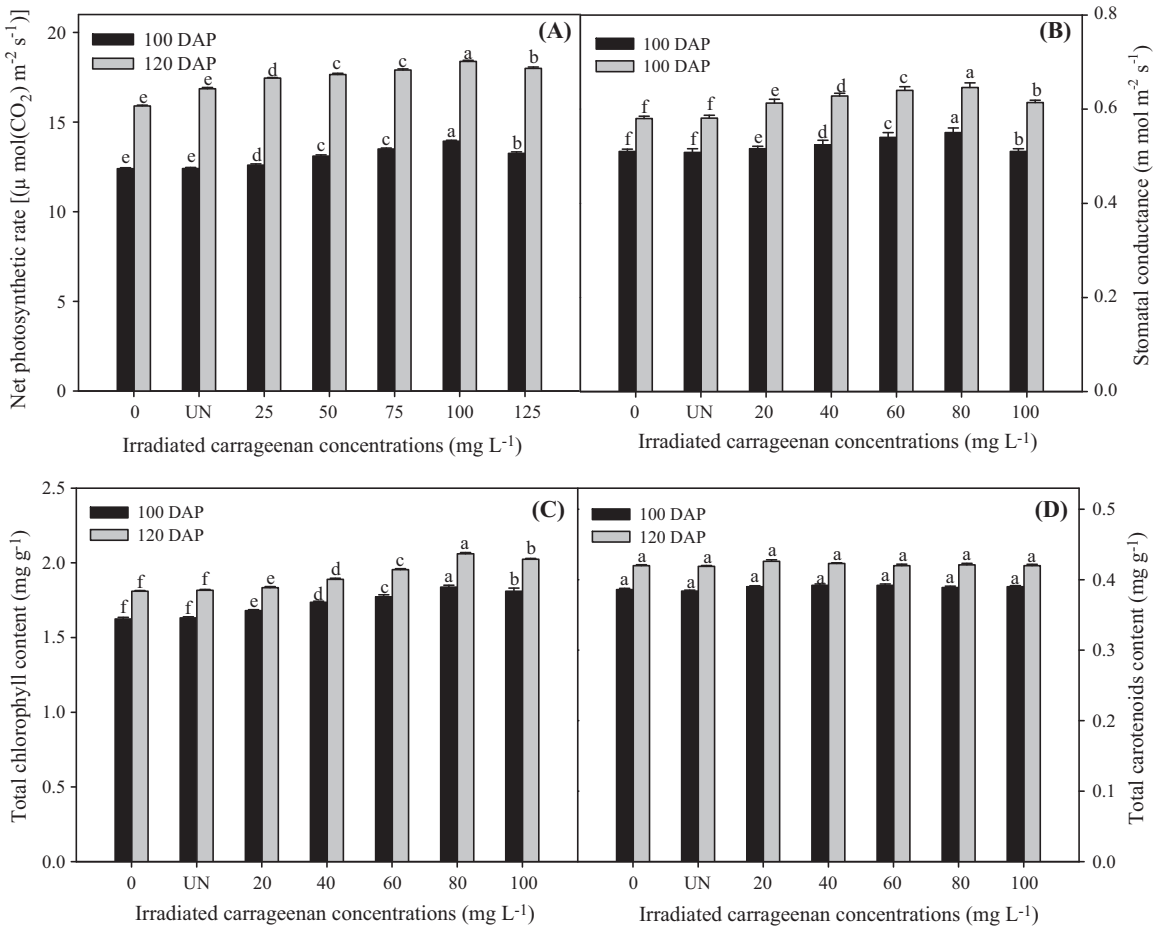

Fig. 2. Effect of seven concentrations of foliar sprays of irradiated carrageenan [0 (control), UN (un-irradiated), 20, 40, 60, 80 and 100 mg L^{-1}] on net photosynthetic rate (A), stomatal conductance (B) and total chlorophyll (C) and carotenoids (D) contents of mint (*Mentha arvensis* L.) studied at 100 and 120 days after planting (DAP). Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Error bars (⊤) show SE.

that claim the synthesis of certain enzymes in the tissue culture as a result of application of irradiated polysaccharides (Akimoto, Aoyagi, & Tanaka, 1999; Patier et al., 1995).

The leaf -N, -P and -K contents were also significantly enhanced by IC application, with 80 mg L^{-1} proving the best IC concentration (Fig. 3C–E). The application of IC at 80 mg L^{-1} significantly increased leaf-N by 14.5 and 15.3% at 100 and 120 DAP, respectively (Fig. 3C). Similarly, the aqueous spray of IC was also effective in increasing leaf-P and -K contents compared to the water-sprayed control plants at both the growth stages. However, leaf-P content was not affected by the IC spray at 120 DAP (Fig. 3D). The IC applied at the 80 mg L^{-1} concentration, increased the leaf-P content by 8.0% (at 100 DAP) and the leaf-K content by 10.5 and 7.46% at 100 and 120 DAP, respectively (Fig. 3D and E).

3.3. Yield and quality attributes

The application of 80 mg L^{-1} of IC concentration enhanced the herbage yield significantly, exceeding the control by 34.1 and 36.8% at 100 and 120 DAP, respectively (Table 2). In addition, the spray

Table 2
Effect of seven concentrations of foliar sprays of irradiated carrageenan [0 (control), UN (un-irradiated), 20, 40, 60, 80 and 100 mg L^{-1}] on yield and quality attributes of mint (*Mentha arvensis* L.) at 100 and 120 days after planting (DAP). Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Means of five replicates ± SE.

Yield & quality attributes	Irradiated carrageenan concentrations (mg L^{-1})							
	DAP	Control	UN	20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}
Herbage yield per plant (g)	100	36.28 ± 0.12^{f}	36.48 ± 0.14^{f}	37.90 ± 0.18^{e}	40.16 ± 0.12^{d}	42.34 ± 0.20^{c}	48.65 ± 0.15^{a}	46.28 ± 0.16^{b}
	120	52.36 ± 0.22^{f}	53.24 ± 0.21^{f}	56.19 ± 0.25^{e}	59.82 ± 0.24^{d}	63.18 ± 0.20^{c}	71.65 ± 0.26^{a}	68.10 ± 0.24^{b}
Essential oil-content (%)	100	0.646 ± 0.02^{e}	0.640 ± 0.01^{e}	0.689 ± 0.02^{d}	0.728 ± 0.02^{c}	0.778 ± 0.03^{b}	0.835 ± 0.02^{a}	0.782 ± 0.02^{b}
	120	0.952 ± 0.01^{f}	0.946 ± 0.01^{f}	0.989 ± 0.02^{e}	1.092 ± 0.02^{d}	1.196 ± 0.01^{c}	1.298 ± 0.02^{a}	1.216 ± 0.02^{b}
Essential oil-yield per plant (mL)	100	0.258 ± 0.01^{e}	0.254 ± 0.01^{e}	0.296 ± 0.02^{c}	0.362 ± 0.01^{d}	0.389 ± 0.02^{b}	0.446 ± 0.01^{a}	0.381 ± 0.02^{b}
	120	0.470 ± 0.01^{f}	0.465 ± 0.01^{f}	0.518 ± 0.01^{e}	0.624 ± 0.01^{d}	0.756 ± 0.01^{c}	0.881 ± 0.02^{a}	0.724 ± 0.01^{b}
Specific gravity of essential oil (g/cm^3)	100	0.893 ± 0.001^{a}	0.890 ± 0.001^{a}	0.890 ± 0.001^{a}	0.892 ± 0.001^{a}	0.891 ± 0.001^{a}	0.892 ± 0.001^{a}	0.890 ± 0.001^{a}
	120	0.892 ± 0.001^{a}	0.890 ± 0.001^{a}	0.890 ± 0.001^{a}	0.891 ± 0.001^{a}	0.890 ± 0.001^{a}	0.892 ± 0.001^{a}	0.890 ± 0.001^{a}
Refractive index of essential oil	100	1.462 ± 0.001^{a}	1.460 ± 0.001^{a}	1.460 ± 0.001^{a}	1.462 ± 0.001^{a}	1.462 ± 0.001^{a}	1.463 ± 0.001^{a}	1.461 ± 0.001^{a}
	120	1.462 ± 0.001^{a}	1.460 ± 0.001^{a}	1.460 ± 0.001^{a}	1.461 ± 0.001^{a}	1.462 ± 0.001^{a}	1.462 ± 0.001^{a}	1.462 ± 0.001^{a}

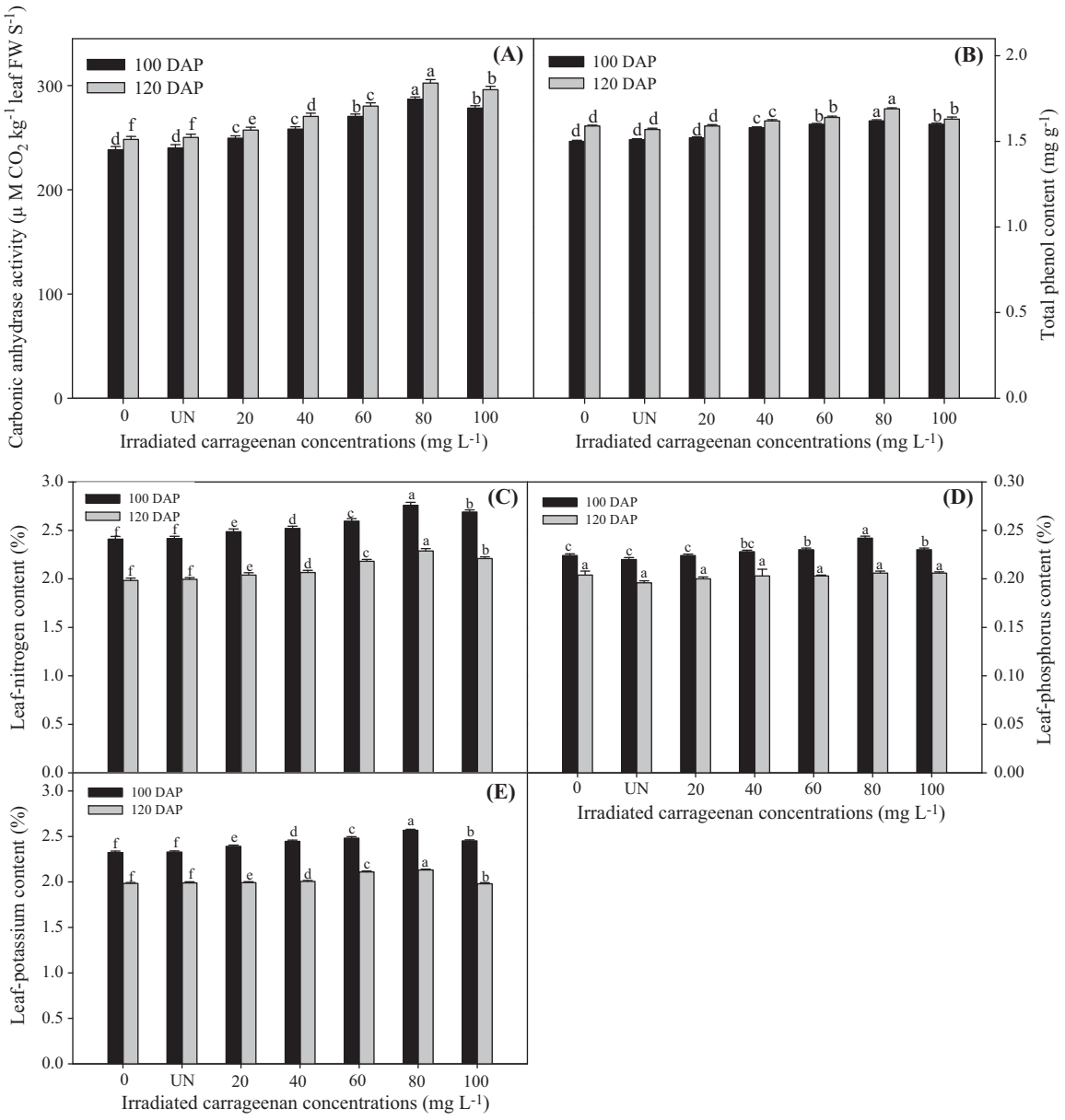

Fig. 3. Effect of seven concentrations of foliar sprays of irradiated carrageenan [0 (control), UN (un-irradiated), 20, 40, 60, 80 and 100 mg L^{-1}] on carbonic anhydrase activity (A) and total phenolic content (B) and leaf-nitrogen (C), -phosphorus (D) and -potassium (E) contents of mint (*Mentha arvensis* L.) studied at 100 and 120 days after planting (DAP). Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Error bars (T) show SE.

of IC at 80 mg L^{-1} also resulted in the maximum content and yield of essential oil (29.3 and 36.3%, 72.9 and 87.4% increase over the control) at both the stages. Specific gravity and refractive index of the essential oil were not improved by IC treatments at any growth stage (Table 2). Specific gravity is an important decisive factor as far as the quality and purity of essential oil is concerned. Refractive index plays an important role in the elucidation of structure of several constituents of essential oils after their separation and purification. It is a physical constant that can be used against the adulteration of drugs as it is helpful to check the identity and purity of a compound.

The significant increase in the above mentioned parameters of the IC treated plants might possibly culminate in maximization of the leaf-yield and herbage-yield of the mint plant (Tables 1 and 2). Moreover, the improved herbage yield and dry matter production might have resulted due to enhanced water and nutrient uptake from soil, followed by smooth translocation of photosynthates and other metabolites to the sinks in IC-treated plants. There was observed no increase in active components when the IC concentrations were applied to the foliage except that in menthol content (Table 3). The IC spray at 80 mg L^{-1} increased the menthol content by 5.34 and 5.73% compared to the control at 100 and 120 DAP, respectively (Table 3). As compared to control, 80 mg L^{-1} of IC considerably increased the yield of menthol (81.7 and 98.7%), L-menthone content (80.0 and 94.4%), isomenthone content (75.0 and 92.9%) and menthyl acetate content (75.0 and 75.0%) at 100 and 120 DAS (Table 3). In the present study, IC-mediated increase in the uptake of nutrients (leaf-N, -P and -K) might have enhanced the rate of photosynthesis and subsequently it could have improved the translocation of photosynthates and other metabolites to the sinks, leading to the improved content and yield of essential oil and its menthol content in IC sprayed plants (Table 2).

Table 3
Effect of seven concentrations of foliar sprays of irradiated carrageenan [0 (control), UN (un-irradiated), 20, 40, 60, 80 and 100 mg L^{-1}] on active constituents and its yield of mint (*Mentha arvensis* L.) at 100 and 120 days after planting (DAP). Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Means of five replicates ± SE.

Yield & quality attributes	Irradiated carrageenan concentrations (mg L^{-1})							
	DAP	Control	UN	20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}
Menthol content (%)	100	80.45 ± 0.02^{f}	80.35 ± 0.01^{f}	80.64 ± 0.02^{e}	81.55 ± 0.02^{d}	82.74 ± 0.02^{c}	84.75 ± 0.02^{a}	84.32 ± 0.01^{b}
	120	80.62 ± 0.02^{f}	80.46 ± 0.02^{f}	80.94 ± 0.02^{e}	81.64 ± 0.02^{d}	83.42 ± 0.02^{c}	85.24 ± 0.02^{a}	84.76 ± 0.02^{b}
Menthol yield per plant (mL)	100	0.208 ± 0.004^{e}	0.204 ± 0.004^{e}	0.239 ± 0.003^{d}	0.295 ± 0.004^{c}	0.322 ± 0.005^{b}	0.378 ± 0.003^{a}	0.321 ± 0.003^{b}
	120	0.378 ± 0.003^{e}	0.371 ± 0.004^{e}	0.419 ± 0.004^{d}	0.509 ± 0.004^{c}	0.631 ± 0.002^{b}	0.751 ± 0.004^{a}	0.629 ± 0.005^{b}
L-Menthone content (%)	100	3.94 ± 0.02^{a}	3.92 ± 0.03^{a}	3.94 ± 0.02^{a}	3.95 ± 0.01^{a}	3.94 ± 0.02^{a}	3.96 ± 0.01^{a}	3.94 ± 0.02^{a}
	120	3.93 ± 0.01^{a}	3.92 ± 0.01^{a}	3.90 ± 0.02^{a}	3.94 ± 0.03^{a}	3.92 ± 0.02^{a}	3.95 ± 0.02	3.93 ± 0.02^{a}
L-Menthone yield per plant (mL)	100	0.010 ± 0.001^{d}	0.009 ± 0.001^{d}	0.012 ± 0.001^{c}	0.014 ± 0.002^{bc}	0.015 ± 0.002^{b}	0.018 ± 0.002^{a}	0.015 ± 0.002^{b}
	120	0.018 ± 0.001^{d}	0.018 ± 0.001^{d}	0.020 ± 0.002^{c}	0.024 ± 0.002^{c}	0.029 ± 0.002^{b}	0.035 ± 0.002^{a}	0.028 ± 0.002^{b}
Isomenthone content (%)	100	2.94 ± 0.01^{a}	2.90 ± 0.01^{a}	2.92 ± 0.02^{a}	2.94 ± 0.01^{a}	2.93 ± 0.01^{a}	2.94 ± 0.01^{a}	2.92 ± 0.02^{a}
	120	3.04 ± 0.02^{a}	3.00 ± 0.01^{a}	3.02 ± 0.01^{a}	3.03 ± 0.02^{a}	3.04 ± 0.02^{a}	3.04 ± 0.01^{a}	3.00 ± 0.02^{a}
Isomenthone yield per plant (mL)	100	0.008 ± 0.001^{d}	0.007 ± 0.001^{d}	0.009 ± 0.002^{c}	0.010 ± 0.004^{b}	0.011 ± 0.002^{b}	0.014 ± 0.002^{a}	0.011 ± 0.001^{b}
	120	0.014 ± 0.002^{e}	0.014 ± 0.002^{e}	0.016 ± 0.001^{d}	0.019 ± 0.002^{c}	0.023 ± 0.002^{b}	0.027 ± 0.002^{a}	0.021 ± 0.001^{c}
Menthyl acetate content (%)	100	1.60 ± 0.01^{a}	1.60 ± 0.01^{a}	1.60 ± 0.01^{a}	1.61 ± 0.01^{a}	1.62 ± 0.02^{a}	1.63 ± 0.02^{a}	1.60 ± 0.01^{a}
	120	1.62 ± 0.01^{a}	1.64 ± 0.02^{a}	1.60 ± 0.01^{a}	1.61 ± 0.02^{a}	1.62 ± 0.01^{a}	1.62 ± 0.02^{a}	1.60 ± 0.02^{a}
Menthyl acetate yield per plant (mL)	100	0.004 ± 0.001^{c}	0.004 ± 0.001^{c}	0.004 ± 0.002^{c}	0.006 ± 0.002^{b}	0.006 ± 0.002^{b}	0.007 ± 0.002^{a}	0.006 ± 0.001^{b}
	120	0.008 ± 0.001^{d}	0.007 ± 0.001^{d}	0.008 ± 0.001^{d}	0.010 ± 0.002^{c}	0.012 ± 0.001^{b}	0.014 ± 0.002^{a}	0.011 ± 0.002^{b}

4. Conclusion

The application of gamma-irradiated carrageenan (IC) at 80 mg L^{-1} could significantly improve the growth attributes, physiological parameters, herbage yield and content and yield of the essential oil and its menthol content. This treatment also considerably increased the yield of L-menthone, isomenthone and menthyl acetate contents. Hence, the optimum concentration of IC may be utilized in future to enhance the productivity, quality and production of essential oil and other active constituents of medicinal and aromatic plants. Further, this technique may be safely adopted for boosting up the growth, yield and quality of medicinal and other crop plants. However, further investigations are required to comprehend the mechanism and mode of action of carrageenan-derived oligomers with regard to productivity and quality of medicinal and other crop plants.

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