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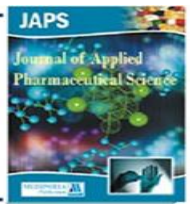

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Irradiated sodium alginate improves plant growth, physiological activities and active constituents in *Mentha arvensis* L.

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ABSTRACT

Sodium alginate, irradiated by Co-60 gamma rays in solid state, elicits plant growth promoting responses in various plants. Irradiated sodium alginate (ISA) was applied as a foliar spray on mint (*Mentha arvensis* L.) to investigate its effect on plant growth, physiological attributes and herbage yield as well as on content and yield of essential oil and its components (menthol, L-menthone, isomenthone and methyl acetate). A simple pot experiment was conducted applying five concentrations of ISA, *viz.* 25, 50, 75, 100 and 125 mg L^{-1}, as foliar sprays. GPC study revealed formation of lower molecular weight oligomer fractions in irradiated samples which could be responsible for plant growth promotion in the present work. Of the five ISA concentrations, 100 mg L^{-1} proved the best. As compared to the control, the ISA applied at 100 mg L^{-1} resulted in the highest values of all physiological parameters at 100 and 120 days after planting.

Keywords: Active constituents, carbonic anhydrase, irradiated sodium alginate, *Mentha arvensis*, photosynthesis.

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 (Naeem *et al.*, 2012). Compared to the conventional techniques such as acid/base hydrolysis and enzymatic methods (Shimokawa *et al.*, 1996), radiation processing of bioactive agents by Co-60 gamma rays offers a clean one-step method for the formation of low molecular weight oligomers of sodium alginate (Nagasawa *et al.*, 2000; Lee, 2003). Sodium alginate is a natural polysaccharide. It is derived from brown algae and is available in large quantities. Polysaccharides such as sodium alginate have been successfully used as plant growth promoting substances in their depolymerized form. Gamma-rays irradiation degrades the sodium alginate into smaller oligomers with comparatively low molecular weight. Application of these oligomers on plants results in various biological and physiological activities, including promotion of plant growth in general, seed germination, shoot elongation, root growth, flower production, antimicrobial activity, amelioration of heavy metal stress, phytoalexin induction, etc. (Hien *et al.*, 2000; Kume *et al.*, 2002; Hu *et al.*, 2004; Hegazy *et al.*, 2009; Aftab *et al.*, 2011; Idrees *et al.*, 2011). The medicinal plants, bearing essential oil, have increased exponentially in recent years in both developing and developed countries and are expected to expand tremendously in the foreseeable future (Weiss, 1997). Since the supply of essential oils is severely lagging behind their demand, it is the need of the hour to maximize the oil production of medicinal plants.

Out of a large number of essential oil bearing plants, mint (*Mentha arvensis* L.) constitutes the 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 *et al.*, 2000; Tassou *et al*., 2004). The aim of the present study was to investigate whether the application of irradiated sodium alginate (ISA) could be used to enhance the growth, physiological activities, yield attributes and the production of essential oil and other active constituents in *Mentha arvensis* L.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The experiment was conducted in earthen pots (25 cm diameter × 25 cm height) in the natural conditions of the net house at the Botany Department, Aligarh Muslim University, Aligarh (27° 52' N latitude, 78°51' E longitude, and 187.45 m altitude). Healthy rhizomes of *Mentha arvensis* L. were procured from Sambhal, Moradabad, Uttar Pradesh (India). They were surface sterilized with 0.02% $HgCl_2$ solution for 5 min with frequent shaking and then thoroughly washed with de-ionized water. Prior to transplanting, each pot was filled with 5 kg homogenous mixture of soil. Before transplantation, the soil samples were collected randomly from different pots and analyzed subsequently for the soil characteristics. The samples were analyzed in the Soil-Testing Laboratory, Government Agriculture Farm, Quarsi, Aligarh. 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 (25.0, 11.0 and 21.0 mg per kg soil, respectively) was applied in the form of urea, single superphosphate and muriate of potash, respectively, at the time of planting.

Irradiation and Gel Permeation Chromatography (GPC) Analysis

Solid material of sodium alginate (Sigma Aldrich, USA) was sealed in a glass tube with atmospheric air. The samples of sodium alginate were irradiated in a Gamma Chamber (Cobalt-60, GC-5000) made by BRIT, Mumbai, India. The samples were irradiated to 520 kGy gamma radiation dose at a dose rate of 2.4 kGy/h. GPC of sodium alginate samples were done on Hitachi EMerck HPLC/GPC system using RI detector. The experimental conditions were as follows: Mobile phase-water, flow rate-1.5mL/min, column PL-Aquagel, mixed bed column, 300 mm × 10 mm, 20 micro liter injection loop.

The average molecular weight of the un-irradiated sodium alginate samples were estimated to be about 6,95,131. Polyvinyl alcohol polymers of known molecular weight were used as standards. Different aqueous concentrations of irradiated sodium alginate (ISA) were finally prepared using double distilled water as spray treatments.

Pot Culture

The pot experiment was conducted according to simple randomized block design. There were applied five concentrations of ISA (25, 50, 75, 100 and 125 mg L^{-1}), using distilled water and un-irradiated sodium alginate (25 mg L^{-1}) as absolute-control and sodium alginate-control, respectively. The ISA treatments were applied as foliar sprays to the crop at 10 days interval using a hand sprayer, when the plants were at the 2-3 true leaf stage. The crop was planted in February 2009 and harvested at 100 and 120 days later. Plants were grown under naturally illuminated environmental conditions. Each treatment was replicated five times. Each pot contained a single healthy plant. The pots were watered as and when required.

The crop performance was assessed in terms of growth attributes, physiological activities, herbage yield and content as well as yield of active constituents of *Mentha arvensis* L.

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 leaves of the plant were weighed to determine leaf-yield per plant. At 100 and 120 DAP, five plants of each treatment pot were uprooted and their roots were washed carefully with tap water to remove all adhering foreign particles. The water, adhering to the roots, was removed with blotting paper, measuring height and fresh weight of plant. After taking plant fresh weight, the plants were dried at 80°C for 24 h using a hot air oven, recording the plant dry weight thereafter. The leaf-area was obtained with the help of a graph paper sheet. Merely 10% of total leaves of each plant 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 leaf area per plant.

Determination of Physiological Activities

Estimation of Total Chlorophyll and Carotenoids Contents

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

Determination of Net Photosynthetic Rate and Stomatal Conductance

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

and 120 DAP. Before recording the measurements, the IRGA was calibrated and zero was adjusted approximately every 30 minutes during the measurement period. Net photosynthetic rate (P_N) as well as stomatal conductance was recorded three times for each treatment.

Determination of Carbonic Anhydrase (CA) Activity

The activity of carbonic anhydrase (E.C. 4.2.1.1) was measured in fresh leaves, using the method as 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 minutes at 4°C. To each test tube, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.022% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme was expressed as μM CO_2 kg^{-1} leaf FW s^{-1}.

Total Phenol Content

Total phenol content was estimated by the method as described by Sadasivam and Manickam (2008). Leaf sample (500 mg) was ground 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, saving the supernatant. 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 were added to each 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 leaves.

Yield and Quality Parameters

Herbage yield of the crop was measured weighing the total biomass per plant excluding the roots. The quality parameters, *viz.* content and yield of essential oil, menthol, L-menthone, isomenthone and menthyl acetate were determined as described below:

Isolation and Compositional Analysis of Essential Oil

We collected fresh leaves from each treatment pot. Later, we chopped and mix them together. Thereafter, we took sufficient quantity of chopped leaf-pieces for the estimation the essential oil. The essential oil of mint was extracted and determined gravimetrically according to Guenther (1972).The essential oil content in the leaves was extracted by distillation method for 3 h, using a Clevenger's apparatus. The essential oil was dried over anhydrous sodium sulphate and preserved in sealed glass vials at 4 °C for the GLC analysis.

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 a 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 the retention time of the particular constituent in the GLC column. The active constituents were quantified as per cent content, comparing their peaks with the peaks obtained from the reference standards reported in the literature.

Determination of Specific Gravity of Essential Oil

The specific gravity of the essential oil was determined at 25°C using a 'specific gravity bottle'. The specific gravity bottle was filled with the oil up to the mark and weighed at room temperature (25°C). The exact weight of the oil was determined by subtracting the weight of the empty bottle from the total weight of the bottle filled with the oil. The specific gravity was determined according to Afaq et al. (1994), using the following formula:

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

Determination of Refractive Index of Essential Oil

The refractive index of the essential oil was determined by the method described by Jenkins *et al.*, (1967) using the Abb'eRefractometer (Sipcon, New Delhi, India). Two or three drops of the oil were placed on the double prism, clamping the prisms together firmly. The light source was fixed, so that light could be reflected through the prisms and the instrument was adjusted until the border line between the light and dark halves of the view-field exactly coincided the cross hairs of the telescope. The compensator prisms were rotated in order to obtain a sharp uncolored border line. The refractive index of the oil was noted directly from the graduated scale. The instrument was rotated and again the refractive index was determined until three similar readings were obtained. The mean of the recorded readings was designated as the refractive index of the oil. The refractive index of the oil was expressed as $N_D^{24°}$. Where $N_D^{24°}$ denotes the index of the refraction for the 'D' line (sodium light) measured at 24°.

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). Means were compared using Duncan's Multiple Range Test (DMRT) at $P<0.05$. Standard error was also employed to separate the means in the tables and figures.

RESULTS AND DISCUSSION

The ISA, applied as leaf-sprays at 25 to 100 mg L^{-1} concentration, showed promotive effects on all the growth attributes, physiological activities, herbage yield, content and yield of essential oil and oil-quality parameters. Fig. 1 shows the molecular weight distribution of un-irradiated and irradiated

Table 1. Effect of seven concentrations of foliar sprays of ISA [0 (control), UN (un-irradiated), 25, 50, 75, 100 and 125 mg L^{-1}] on growth attributes of mint (*Mentha arvensis* L.) at 100 and 120 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		ISA concentrations (mg L^{-1})						
	DAP	Control	UN	25	50	75	100	125
Plant height (cm)	100	70.76±1.10^{f}	72.16±1.17^{f}	78.44±1.04^{e}	82.60±1.17^{d}	92.76±1.21^{c}	115.14±1.10^{a}	106.70±1.10^{b}
	120	84.24±1.11^{e}	86.80±1.06^{e}	93.32±1.77^{d}	97.70±1.42^{d}	119.60±1.80^{c}	137.64±1.69^{a}	126.18±1.78^{b}
Leaf-area per plant (cm^2)	100	2975.1±20.17^{f}	3038.6±19.20^{f}	3266.4±23.10^{e}	3473.5±23..20^{d}	3669.3±24.28^{c}	4170.9±23.28^{a}	3780.1±24.24^{b}
	120	4732.5±17.90^{f}	4769.6±13.94^{f}	5271.2±13.61^{e}	5647.8±21.50^{d}	6065.9±13.09^{c}	6845.8±15.72^{a}	6390.6±14.87^{b}
Leaf-yield per plant (g)	100	14.10±0.162^{f}	14.20±0.132^{f}	14.50±0.129^{e}	15.26±0.152^{d}	16.82±0.173^{c}	20.64±0.162^{a}	19.30±0.162^{b}
	120	26.96±0.230^{f}	27.28±0.225^{f}	28.56±0.236^{e}	31.18±0.208^{d}	33.70±0.305^{c}	40.34±0.216^{a}	38.92±0.179^{b}
Fresh weight per plant (g)	100	53.28±1.10^{e}	54.40±1.11^{e}	62.18±1.04^{d}	73.08±1.04^{c}	76.16±1.17^{b}	84.82±1.21^{a}	75.10±1.20^{b}
	120	65.14±1.63^{f}	65.82±1.58^{f}	73.50±1.64^{e}	82.70±1.61^{d}	93.10±1.61^{c}	106.43±1.60^{a}	90.10±1.61^{b}
Dry weight per plant (g)	100	11.62±0.205^{e}	12.34±0.300^{e}	13.95±0.317^{d}	15.99±0.297^{c}	17.76±0.234^{b}	19.20±0.318^{a}	17.92±.0277^{b}
	120	14.29±0.262^{e}	14.58±0.231^{e}	15.41±0.239^{d}	18.84±0.289^{c}	21.44±0.232^{b}	23.94±0.332^{a}	18.65±0.167^{c}

Control: deionized water (control 1). UN: Unirradiated sodium alginate applied at 25 mg L^{-1} concentration (control 2).

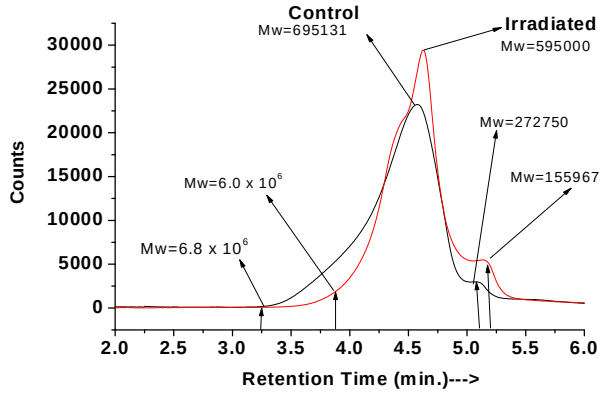

Fig. 1. This shows the molecular weight distribution of un-irradiated and irradiated sodium alginate. The average molecular weight of the un-irradiated sodium alginate samples were estimated to be about 6,95,131. The distribution curve in the GPC profile shows shifting of whole graph to higher retention time indicating radiation degradation of sodium alginate on irradiation and forming lower molecular weight oligomers. This average molecular weight of 6,95,131 was observed in the control and 5,95,000 for the irradiated samples. However, considering the molecular weight values in the Fig. 1, it may be said that these value may fall in natural variation of SA. The lower molecular weight fraction (less than 100,000) which is coming at the end of the profile is very small.

sodium alginate. The distribution curve in the GPC profile shows the elution of different molecular weight fractions w.r.t to time. The profile shows shifting of whole graph to higher retention time indicating radiation degradation of sodium alginate on irradiation and forming lower molecular weight oligomers. This average molecular weight of 6,95,131 was observed in the control and 5,95,000 for the irradiated samples. However, considering the molecular weight values in the Fig. 1, it may be said that these value may fall in natural variation of SA. The lower molecular weight fraction (less than 100,000) which is coming at the end of the profile is very small. Therefore, it is difficult to say which molecular weight fraction of SA acts as a stimulant and investigations on this aspect is in progress. Compared to other ISA concentrations, 100 mg L^{-1} proved to be the best, while 125 mg L^{-1} ISA did not further improve the growth attributes; however, it enhanced the above mentioned parameters significantly in comparison to the water spray control. Spray treatment using unirradiated sodium alginate (25 mg L^{-1}) resulted in the lowest effect. It was statistically equal to the control (water spray) for most of the parameters studied (Tables 1-3, Figs. 2-3).

Growth Attributes

Application of different concentrations of ISA improved the growth attributes of *Mentha arvensis* L. significantly ($P \leq 0.05$) both at 100 and 120 DAP. There was a progressive increase in values with the increase in ISA concentration up to 100 mg L^{-1}. Thereafter, the values declined significantly (Table 1). The maximum values of growth characteristics were attained at 120 DAP with 100 mg L^{-1} ISA. As compared to the control, application of 100 mg L^{-1} ISA significantly increased the shoot length, leaf area, leaf yield and fresh and dry weights of the plant (Table 1).

Several exogenous and endogenous factors regulate the growth, development and yield of a plant (Srivastava and Srivastava, 2007). Among exogenous factors, various plant growth promoters are known, which have direct or indirect influence on growth and development of the plant. 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. The ISA treatment enhanced the leaf-area, which could observably provide increased opportunity for light harvesting that ultimately could manifest itself in significantly higher dry matter in the present investigation, compared to the control (Table 1). Oligomers, produced by depolymerisation of alginates, have been reported to have triggered the stimulation of growth, promotion of germination and shoot elongation in plants (Darvill *et al.*, 1992; Nutsume *et al.*, 1994; Tomoda *et al.*, 1994; Nagasawa *et al.*, 2000, Aftab *et al.*, 2011, Khan *et al.*, 2011, Sarfaraz *et al.*, 2011). In line with the present results, significant improvement in plant growth attributes by the application of radiation-derived oligosaccharides of sodium alginate has earlier been reported. The irradiation of SA by gamma rays affects the overall polymer cross-linking process; consequently, its application influences the biological properties of the plant cells (El-Rehim 2006). Previous studies have claimed that a range of concentrations of radiation degraded sodium alginate depend upon the source and unit (kGy) of irradiation for a particular plant (Tomoda *et al.*, 1994). However, the phenomenon

Table 2. Effect of seven concentrations of foliar sprays of ISA [0 (control), UN (un-irradiated), 25, 50, 75, 100 and 125 mg L^{-1}] on yield and quality attributes of mint (*Mentha arvensis* L.) at 100 and 120 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 and quality attributes	ISA concentrations (mg L^{-1})							
	DAP	Control	UN	25	50	75	100	125
Herbage yield per plant (g)	100	35.96±0.16^{f}	36.40±0.13^{f}	38.88±0.14^{e}	42.00±0.14^{d}	45.46±0.14^{c}	55.82±0.14^{a}	52.90±0.15^{b}
	120	50.45±0.26^{f}	51.60±0.19^{f}	54.81±0.29^{e}	58.96±0.25^{d}	66.02±0.13^{c}	80.12±0.26^{a}	77.24±0.26^{b}
Essential oil-content (%)	100	0.648±0.01^{e}	0.640±0.01^{e}	0.692±0.02^{d}	0.741±0.04^{c}	0.810±0.01^{b}	0.884±0.02^{a}	0.774±0.01^{bc}
	120	0.950±0.009^{f}	0.948±0.02^{f}	1.032±0.02^{e}	1.130±0.02^{d}	1.218±0.01^{c}	1.348±0.02^{a}	1.278±0.02^{b}
Essential oil-yield per plant (mL)	100	0.254±0.01^{e}	0.248±0.01^{e}	0.290±0.01^{c}	0.371±0.02^{d}	0.438±0.02^{b}	0.508±0.01^{a}	0.404±0.008^{bc}
	120	0.454±0.01^{f}	0.446±0.01^{f}	0.532±0.01^{e}	0.680±0.01^{d}	0.821±0.01^{c}	0.954±0.01^{a}	0.734±0.01^{b}
Specific gravity of essential oil (g/cm^3)	100	0.892±0.001^{a}	0.890±0.001^{a}	0.891±0.001^{a}	0.892±0.001^{a}	0.894±0.001^{a}	0.894±0.001^{a}	0.892±0.001^{a}
	120	0.894±0.001^{a}	0.892±0.001^{a}	0.892±0.001^{a}	0.893±0.001^{a}	0.894±0.001^{a}	0.895±0.001^{a}	0.894±0.001^{a}
Refractive index of essential oil	100	1.463±0.001^{a}	1.462±0.001^{a}	1.462±0.001^{a}	1.462±0.001^{a}	1.464±0.001^{a}	1.464±0.001^{a}	1.463±0.001^{a}
	120	1.464±0.001^{a}	1.462±0.001^{a}	1.462±0.001^{a}	1.463±0.001^{a}	1.464±0.001^{a}	1.465±0.001^{a}	1.463±0.001^{a}

Control: deionized water (control 1). UN: Unirradiated sodium alginate applied at 25 mg L^{-1} concentration (control 2).

Table 3. Effect of seven concentrations of foliar sprays of ISA [0 (control), UN (un-irradiated), 25, 50, 75, 100 and 125 mg L^{-1}] on content and yield of active constituents of mint (*Mentha arvensis* L.) at 100 and 120 DAP. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Means of five replicates ± SE.

Content and yield of active constituents	ISA concentrations (mg L^{-1})							
	DAP	Control	UN	25	50	75	100	125
Menthol content (%)	100	81.49±0.02^{a}	81.32±0.02^{a}	80.31±0.01^{e}	81.35±0.02^{a}	81.34±0.02^{a}	80.40±0.02^{a}	79.30±0.01^{a}
	120	81.79±0.04^{a}	81.35 ±0.02^{a}	81.49±0.02^{a}	81.35±0.02^{a}	81.39±0.01^{a}	81.84±0.03^{a}	81.33±0.02^{a}
Menthol yield per plant (mL)	100	0.207±0.003^{f}	0.202±0.003^{f}	0.233±0.003^{e}	0.302±0.004^{d}	0.356±0.005^{b}	0.408±0.004^{a}	0.320±0.004^{c}
	120	0.371±0.003^{f}	0.362±0.004^{f}	0.434±0.005^{e}	0.553±0.004^{d}	0.668±0.004^{b}	0.781±0.004^{a}	0.597±0.004^{c}
L-Menthone content (%)	100	3.91±0.02^{e}	3.95±0.02^{e}	4.20±0.01^{d}	4.65±0.02^{c}	4.80±0.02^{b}	5.07±0.03^{a}	4.65±0.03^{c}
	120	3.90±0.02^{f}	3.96±0.01^{f}	4.36±0.02^{e}	4.52±0.02^{d}	4.68±0.01^{c}	4.91±0.03^{a}	4.75±0.02^{b}
L-Menthone yield per plant (mL)	100	0.009±0.001^{f}	0.009±0.001^{f}	0.012±0.002^{e}	0.017 ±0.003^{d}	0.021±0.002^{c}	0.026±0.004^{a}	0.019±0.002^{b}
	120	0.018±0.001^{f}	0.018±0.001^{f}	0.023±0.002^{e}	0.031±0.002^{d}	0.038±0.003^{b}	0.047±0.004^{a}	0.035±0.002^{c}
Isomenthone content (%)	100	2.96±0.01^{e}	2.94±0.01^{e}	2.98±0.01^{d}	3.10±0.01^{c}	3.15±0.02^{b}	3.28±0.02^{a}	3.10±0.01^{c}
	120	3.04±0.01^{c}	3.06±0.02^{c}	3.10±0.01^{c}	3.12±0.02^{b}	3.15±0.01^{b}	3.29±0.02^{a}	3.00±0.02^{c}
Isomenthone yield per plant (mL)	100	0.008±0.001^{d}	0.007±0.001^{d}	0.009±0.002^{c}	0.012±0.004^{bc}	0.014±0.003^{b}	0.017±0.004^{a}	0.013±0.001^{b}
	120	0.014±0.002^{e}	0.014±0.002^{e}	0.016±0.003^{d}	0.021±0.003^{c}	0.026±0.003^{b}	0.031±0.003^{a}	0.022±0.002^{c}
Menthyl acetate content (%)	100	1.60±0.01^{f}	1.60±0.01^{f}	1.62±0.01^{e}	1.70±0.01^{d}	1.75±0.02^{c}	1.83±0.02^{a}	1.65±0.01^{b}
	120	1.65±0.01^{e}	1.64±0.02^{e}	1.68±0.01^{e}	1.70±0.02^{d}	1.72±0.01^{c}	1.79±0.02^{a}	1.68±0.02^{b}
Menthyl acetate yield per plant (mL)	100	0.004±0.001^{d}	0.004±0.001^{d}	0.005±0.002^{c}	0.006±0.002^{c}	0.008±0.002^{a}	0.009±0.002^{a}	0.007±0.001^{ab}
	120	0.008±0.001^{e}	0.007±0.001^{e}	0.009±0.001^{d}	0.011±0.002^{c}	0.014±0.001^{b}	0.017±0.002^{a}	0.012±0.002^{b}

Control: deionized water (control 1). UN: Unirradiated sodium alginate applied at 25 mg L^{-1} concentration (control 2).

which stimulates the processes related to promotion of plant growth still needs further investigations. The present results are in conformity with the findings of Nutsume *et al*., (1994), Tomoda *et al*., (1994), Mollah *et al*., (2009), Jamsheer (2010), Qureshi (2010), Aftab *et al.*, (2011), Khan *et al*., (2011), Naeem *et al.*, (2012) and Sarfaraz *et al.*, (2011) regarding various crops.

Physiological Activities

The application of de-polymerized form of sodium alginate significantly enhanced the photosynthetic parameters (net photosynthetic rate and stomatal conductance) in the present study. A significant increase in net photosynthetic rate and stomatal conductance was recorded at 100 and 120 DAP with the application of ISA at 100 mg L^{-1} concentration. Compared to the control, application of the 100 mg L^{-1} ISA concentration significantly accelerated the net photosynthetic rate at 100 and 120 DAP, respectively (Fig. 2 A). The 100 mg L^{-1} ISA also increased the stomatal conductance significantly compared to the control at 100 and 120 DAP, respectively (Fig. 2 B). Depolymerised sodium alginate significantly increased the photosynthetic parameters in the treated plants as depicted in Fig. 2 C and D. Of the seven concentrations of ISA, 100 mg L^{-1} resulted in the greatest increase in the photosynthetic parameters. As regards photosynthetic pigments (total chlorophyll and carotenoids content), application of ISA at 100 mg L^{-1} proved most beneficial. There was the highest increase in pigments at 120 DAP. As compared to the control,

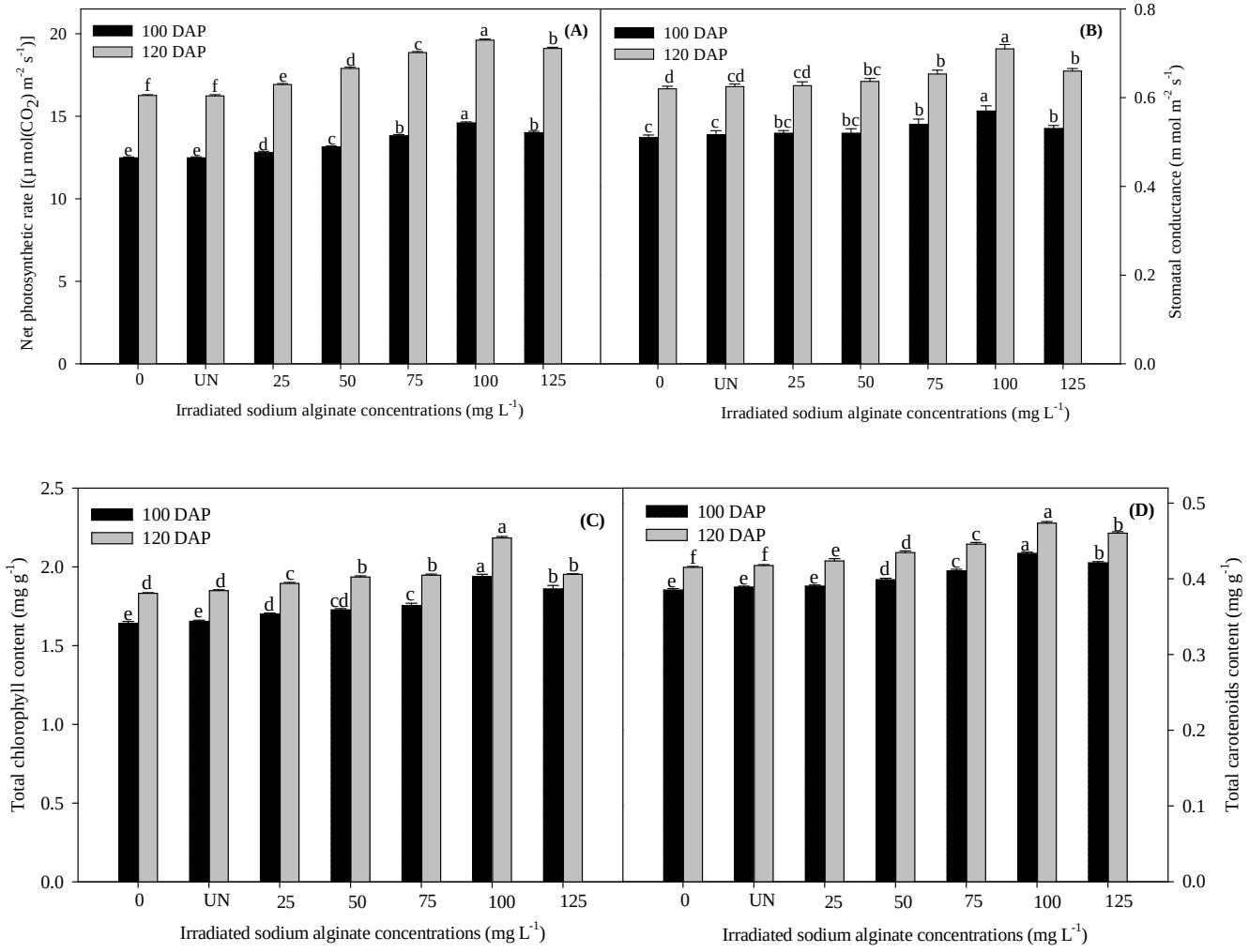

Fig. 2. Effect of seven concentrations of foliar sprays of ISA [0 (control), UN (un-irradiated), 25, 50, 75, 100 and 125 mg L^{-1}] on net photosynthetic rate (A), stomatal conductance (B) and total chlorophyll (C) and carotenoids (D) contents of mint (*Mentha arvensis* L.) studied at 100 and 120 DAP. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Error bars (┬) show SE.

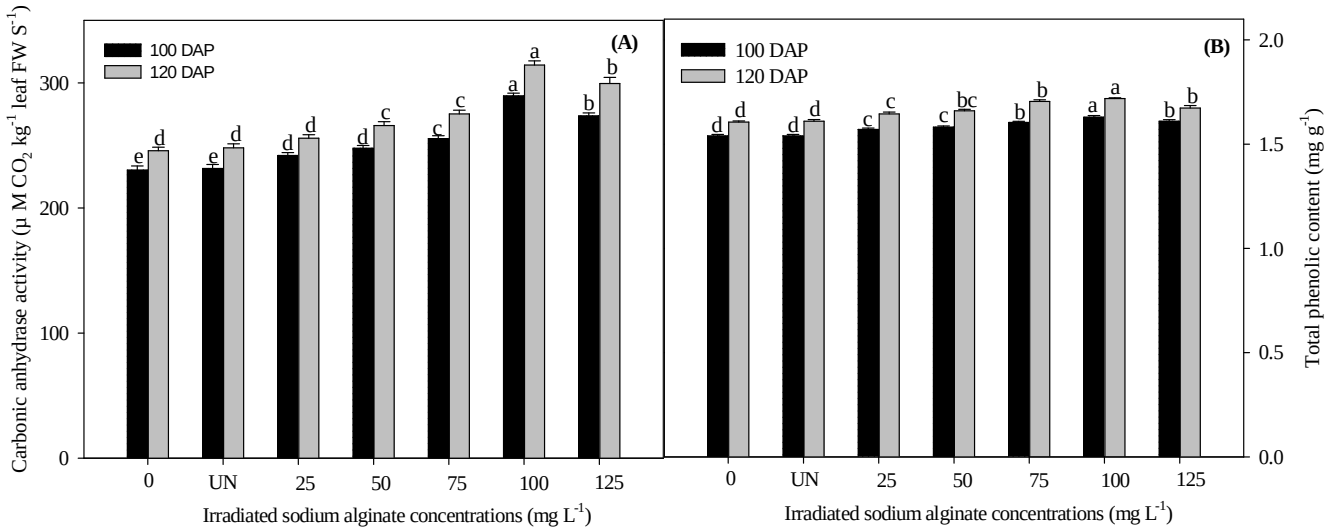

Fig. 3. Effect of seven concentrations of foliar sprays of ISA [0 (control), UN (un-irradiated), 25, 50, 75, 100 and 125 mg L^{-1}] on carbonic anhydrase activity (A) and total phenolic content (B) of mint (*Mentha arvensis* L.) studied at 100 and 120 DAP. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Error bars (┬) show SE.

application of ISA at 100 mg L^{-1} ISA significantly enhanced the total chlorophyll content at 100 and 120 DAP, respectively (Fig. 2 C). Both the pigments were also significantly enhanced by the application of ISA at 120 DAP. The ISA application at 100 mg L^{-1} proved the best. Total carotenoids content significantly increased with the increasing levels of ISA both at 100 and 120 DAP (Fig. 2 D). Presumably, ISA treated plants could trap more sunlight to increase the rate of photosynthesis as compared to the control plants. The improved content of photosynthetic pigments might have resulted in the increased photosynthetic rate due to foliar application of ISA in our previous lab studies also (Aftab *et al.*, 2011; Khan *et al.*, 2011; Sarfaraz *et al.*, 2011). Since there has been revealed a role of oligosaccharides (degraded alginate) in inducing cell signaling leading to stimulation of various physiological processes in various plants (Farmer *et al.*, 1991), the application of ISA in the present study has induced photosynthetic capacity and improvement in pigment content.

Furthermore, since the of oligomers of sodium alginate might act as a growth promoters, they could result in improved plant root and shoot elongation and, thereby led to promotion and increase in plant productivity and improvement in physiological parameters compared with the unsprayed plants (El-Rehim, 2006).

Carbonic anhydrase activity was also positively affected by the ISA application. The spray of 100 mg L^{-1} ISA exhibited the highest values of CA activity at both the growth stages. The activity of the enzyme increased to the maximum extent at 120 DAP (Fig. 3 A). In this regard, our findings are similar to those that claim the synthesis of certain enzymes in the tissue culture following addition of alginate derived oligomers (Akimoto *et al.*, 1999). The ISA application at 100 mg L^{-1} also increased the level of leaf phenolic content at both the sampling stages (Fig. 3 B). The ISA application at 120 mg L^{-1} gave the significantly reduced phenolic content (Fig. 3 B).The positive results obtained in this regard in response to ISA application might be ascribed to such a specific role of oligomers degraded by Co-60 gamma rays technique in plants.

Yield and Quality Attributes

Application of ISA increased essential oil content and its yield significantly when compared to the un-treated plants (control). Compared to the control, the application of ISA at 100 mg L^{-1} concentration significantly enhanced the herbage yield as well as content and yield of essential oil at 100 and 120 DAP, respectively (Table 2). Specific gravity and refractive index of the essential oil were not improved by ISA treatments at any sampling stage (Table 2). As compared to the control, there was no progressive increase in the active components of the essential oil (particularly menthol content), when various ISA concentrations were applied to the foliage (Table 3). However, application of ISA significantly enhanced other active constituents of the essential oil. The spray of ISA at 100 mg L^{-1} increased the contents of L-menthone, isomenthone and methyl acetate at 100 and 120 DAP (Table 3). Furthermore, 100 mg L^{-1} ISA considerably increased the yields of menthol and the content of L-menthone, isomenthone and menthyl acetate at both the stages (Table 3). In the present study, enhanced photosynthesis and improved translocation of photosynthates and other metabolites to the sinks that, in turn, could contribute to the improved content and yield of essential oil in ISA sprayed plants (Table 2). Similarly, the significant increase in the above mentioned parameters of the ISA treated plants might possibly culminate in maximization of the leaf-yield and herbage-yield of the mint plant in the present study (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 ISA-treated plants.

CONCLUSION

In conclusion, application of ISA at 100 mg L^{-1} might significantly improve the growth attributes, physiological activities, herbage yield and content and yield of essential oil and its components.

Thus, there is possibility to improve growth, yield and quality of different medicinal and other crops using the irradiated sodium alginate. However, further investigations are required to comprehend the mechanism and mode of action of alginate-derived oligomers for productivity and quality of the medicinal and other crop plants.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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