Regulation of functional activities and essential oil production in *Vetiveria zizanioides* L. Nash after γ-irradiated sodium alginate elicitation

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Abstract: Upon irradiation, marine polysaccharides undergo depolymerization, leading to formation of oligosaccharides that elicit various biological activities in plants. Taking a step further on the previously established growth-promoting activity of irradiated sodium alginate (ISA), structural rearrangements in ISA were analyzed using complementary techniques to develop an understanding of the structure–property relationship. The essential oil (EO) of vetiver (*Vetiveria zizanioides* L. Nash) immensely benefits the perfumery industry, proving itself as an economically important crop. A pot experiment was designed to test the effect of water-soluble ISA fractions on the growth, physiology, and EO production of vetiver. The structural characterization of radiation-induced sodium alginate was carried out using SEM, FT-IR, and UV-Vis spectroscopy. Of the various treatments employed, ISA-120 (ISA applied at 120 mg L⁻¹) proved the best for most of the parameters studied, including fresh and dry weight of plants and photosynthetic attributes. As compared to the control (water-spray treatment), foliar feeding of ISA-120 resulted in an increase of 21.2% in chlorophyll content, while this treatment enhanced the chlorophyll fluorescence by 21.8% at 300 days after transplanting. Application of ISA-120 also increased the EO content by 21.1% and EO yield by 47.6%. Gas chromatography revealed an increase of 30.1% and 92.6% in the values of content and yield of khusimol, respectively, over the control.

Key words: Irradiated sodium alginate, *Vetiveria zizanioides*, FT-IR, scanning electron microscopy, essential oil, gas chromatography

1. Introduction

Sodium alginate (NaC₆H₇O₆) is the sodium salt of alginic acid. Commercially available alginates mainly find their source from brown algae (family Phaeophyceae) (Craige et al., 1984; Avella et al., 2007). It is a linear copolymer with homopolymeric blocks of (1→4)-linked β-D-mannuronate (M) and its C-5 epimer, α-L-guluronate (G) residues, covalently linked together in different blocks. The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks), or alternating M and G-residues (MG-blocks) (Hecht and Srebnik, 2016). Sodium alginate has wide use across a variety of industries including food, textile printing, pharmaceutical, and bioengineering industries (Gacesa, 1988; Hien et al., 2000). Structural modifications like grafting, cross-linking, or degradation in sodium alginate are most likely to expand its application. To bring about these modifications, radiation technology is a convenient tool. It is a clean, one-step process to degrade the polysaccharides unlike the other two methods of chemical and enzymatic hydrolysis. The radiolytic degradation of the polysaccharides results not only in the reduction of molecular weight of the polysaccharide concerned but also facilitates certain changes in its structure, like the introduction of double bonds and the addition of polar end groups. Such changes in the degraded polysaccharides are considered to stimulate some critical physiological activities in plants such as plant growth promotion, shoot elongation, root growth, flower production, antimicrobial activity, alleviation of heavy metal stress, or phytoalexins induction (Natsume et al., 1994; Hien et al., 2000; Kume et al., 2002; Hu et al., 2004; Aftab et al., 2011; Idrees et al., 2011). As per the investigation carried out by Iwasaki and Matsubara (2000), the alginate oligomers with tetramer to hexamer degrees of polymerization exhibited a potent growth-promoting and elicitor effect on plants. Additionally, these oligomers (oligosaccharides) are claimed to reduce the harvest period of different crops, thus curbing the exploitation of chemical fertilizers and insecticides (Darvill et al., 1992; Nagasawa et al., 2000; Ahni et al., 2001; Cabalfín, 2002;
Vetiver is a perennial bunchgrass of the family Poaceae, native to India. In western and northern India, it is popularly known as khus. The roots as well as aboveground plant parts are known to benefit mankind. The roots serve the dual purpose of sustaining the environment as they form an intertwined network in the soil, making the soil environment suitable for land rehabilitation. Vetiver roots are also known to regulate soil and water conservation and remediate polluted sites (Brandt et al., 2006; Antiochia et al., 2007). Moreover, the roots of vetiver also synthesize a specific essential oil (EO), which is highly valued for its aromatic and pharmaceutical properties. The major odor-influencing constituent of the oil is khusimol, which is considered to be the ‘fingerprint’ of vetiver oil (Demole et al., 1995). Apart from its direct applications in the perfumery industry, vetiver oil in its dilute form is extensively used commercially in food and cosmetic industries and in aromatherapy and pharmacology (Chen et al., 2003; Lavania, 2003; Danh, 2007; Bhushan et al., 2013). Moreover, the byproducts left after the extraction of vetiver oil are extensively used as a refrigerant for household and coolant purposes. They are also employed to make handicrafts, paper and straw board, and soft as well as durable fabric.

The need of the hour is to amplify the production of vetiver by the application of plant growth promoters (PGPs) so as to bring infertile lands, particularly in the northern parts of India, into instant use and augment the production of vetiver EO in order to support the country’s economy. The present study was conducted with the following objectives: to characterize the structural and functional changes in sodium alginate after gamma-irradiation and to assess the effects of foliar application of ISA on the productivity (EO yield) and quality (khusimol content) of vetiver EO.

2. Materials and methods

2.1. Experimental plants and growth conditions

The pot experiment conducted on vetiver (Vetiveria zizanioides L. Nash) was designed according to a simple randomized design in the natural conditions of the net-house at the Department of Botany, Aligarh Muslim University, Aligarh, India (27°08′N, 78°08′E and 187.45 m altitude). The slips of vetiver were obtained from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. Prior to transplantation, each pot was filled with 5 kg of a homogeneous mixture of soil and organic manure (4:1). Samples of the pot soil were analyzed in the central laboratory for soil and plant analysis (Indian Agricultural Research Institute, New Delhi). Physical and chemical characteristics of the experimental soil were: texture-sandy loam, pH: 8.07, EC: 0.36 dS m⁻¹, available N, P, and K: 167.4, 74.6, and 186.0 mg per kg of soil, respectively. As per fertilizer recommendations for vetiver, a uniform basal dose of N (as urea), P (as diammonium phosphate), and K (as muriate of potash) was applied before transplantation at 85.5, 102.3, and 45.0 mg kg⁻¹ soil, respectively.

2.2. Irradiation of sodium alginate by Co-60 gamma rays

Samples of sodium alginate (Sigma Aldrich, USA) were irradiated by gamma rays at 520 kGy using Co-60 as source at the Bhabha Atomic Research Centre (BARC), Mumbai, India. Different aqueous concentrations of ISA, 0 (control), 40, 80, 120, and 160 mg L⁻¹, were utilized beforehand for the use of foliar application in the present study.

2.3. FT-IR spectroscopy

Characterization of sodium alginate in the solid phase was carried out using FT-IR (PerkinElmer FT-IR Spectrometer, USA) in the region of 400–4000 cm⁻¹. Samples were taken in KBr pellets and values are given in cm⁻¹. The spectrometer was continuously purged with dry air during the measurements. Baseline correction and normalization were performed for all the spectra.

2.4. UV-Vis spectroscopy

UV-Vis spectroscopy of sodium alginate was carried out at room temperature using a Lambda 950 UV-Vis-NIR Spectrophotometer (PerkinElmer Lambda Spectrometer) in the region of 200–800 nm. The polymer concentration of the aqueous solution used for the spectroscopy was 0.025% (w/v).

2.5. Scanning electron microscopy (SEM) analysis

The morphological structures of the unirradiated and ISA samples were examined by SEM (JEOL, JSM-6510 LV, Japan) at the Ultra Sophisticated Instrumentation Facility Centre, Aligarh Muslim University, Aligarh, India. Prior to scanning, the samples were coated with gold. The surface characteristics of the samples were evaluated at an accelerating voltage of 20 kV. A secondary imaging detector was used for imaging at the 500 µm scale.

2.6. Pot culture

There were six treatments, including a control (deionized water). The radiation-processed sodium alginate was applied to the plants in different concentrations [0 (control), 40, 80, 120, and 160 mg L⁻¹] through foliar application. Five spray treatments were applied at an interval of 7 days with the help of a hand sprayer. Each
treatment was replicated three times. Each pot contained a single healthy plant. The sampling (plant analysis) was carried out at 300 days after transplanting (DAT). The crop performance was assessed in terms of growth attributes, physiological and biochemical attributes, and content as well as yield of EO and active constituents of vetiver.

2.7. Determination of growth attributes
Growth attributes, namely fresh and dry weights of plants, were measured at 300 DAT. Three plants of each treatment were uprooted and washed carefully with tap water to remove all adhered foreign particles. Thereafter, the plants were surface-dried using blotting paper. The fresh weight of the shoot and root of the blot-dried plants was recorded using an electronic balance. Subsequently, plant samples were dried at 80 °C for 48 h using a hot-air oven in order to measure the dry weight shoot and root separately.

2.8. Determination of physiological and biochemical attributes
Physiological and biochemical parameters, namely photosynthetic parameters and enzyme activities, were determined at 300 DAT using standard techniques as described below.

2.8.1. Estimation of total chlorophyll and carotenoid contents
The content (concentration) of chlorophyll and carotenoids was estimated in the fresh leaves according to the method of MacKinney (1941) and MacLachlan and Zalik (1963), respectively. Fresh tissue from the interveinal leaf area was ground with 80% acetone using a mortar and pestle. The optical density (OD) of the pigment extract was recorded at 645 and 663 nm for chlorophyll content and at 480 and 510 nm for total carotenoids content, using a spectrophotometer (Shimazu UV-1700, Tokyo, Japan). The photosynthetic pigments thus measured were expressed as mg g⁻¹ FW.

2.8.2. Estimation of photosynthetic parameters
Photosynthetic parameters of net photosynthetic rate (Pₙ), internal CO₂ concentration (Cᵢ), and stomatal conductance (gₛ) were measured with the help of an infrared gas analyzer. Chlorophyll fluorescence (Fᵥ/Fₘ value) was measured in fresh leaves using the method of Dwivedi and Randhawa (1974). Chopped leaf pieces (0.2 g) were transferred to a test tube. Leaf pieces were then dipped in 10 mL of 0.2 M cysteine hydrochloride solution for 20 min at 4 °C. To each test tube, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.002% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as an indicator. The enzyme activity was expressed as mol CO₂ kg⁻¹ leaf FW s⁻¹.

2.8.3. Estimation of chlorophyll fluorescence
Chlorophyll fluorescence (Fᵥ/Fₘ value) was measured in diurnal time using a saturation-pulse fluorometer PAM-2000 (Walz, Effeltrich, Germany). All measurements were carried out on the first pair of unifoliolate fully expanded leaves. The upper surface of the leaf was clipped to measure the chlorophyll fluorescence.

2.8.4. Estimation of carbonic anhydrase activity
The activity of carbonic anhydrase (EC 4.2.1.1) was measured in fresh leaves using the method of Dwivedi and Randhawa (1974). Chopped leaf pieces (0.2 g) were transferred to a test tube. Leaf pieces were then dipped in 10 mL of 0.2 M cysteine hydrochloride solution for 20 min at 4 °C. To each test tube, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.002% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as an indicator. The enzyme activity was expressed as mol CO₂ kg⁻¹ leaf FW s⁻¹.

2.8.5. Estimation of nitrate reductase activity
The activity of nitrate reductase (EC 1.7.1.1) was estimated by the intact tissue assay method developed by Jaworski (1971). Fresh chopped leaves, weighing 0.2 g, were transferred to a plastic vial. Each vial contained 2.5 mL of phosphate buffer (pH 7.5), 0.5 mL of potassium nitrate solution, and 2.5 mL of 5% isopropanol. After incubation for 2 h at 30 °C, 0.4 mL of the vial's content was transferred to a test tube. To it, 0.3 mL each of 1% sulfanilamide and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NED-HCL) solution was added. Lastly, the vials were incubated at room temperature for 20 min for maximum color development; the vial content was diluted to a volume of 5 mL with distilled water. The OD of the content was recorded at 540 nm using a spectrophotometer. Nitrate reductase activity was expressed as nanomoles of nitrite produced per gram of fresh weight of leaf tissue per hour (nmol NO₂⁻ g⁻¹ FW h⁻¹).

2.9. Determination of yield and quality attributes
The yield and quality attributes were determined, estimating the EO yield per plant and khusimol yield per plant at 300 DAT.

2.9.1. Isolation and compositional analysis of essential oil
The vetiver EO was extracted through hydrodistillation method using Clevenger's apparatus (Borosil, India) and then it was quantified gravimetrically according to Guenther (1972).

2.9.2. Estimation of EO content
The fresh roots (200 g) were chopped into small pieces. EO was extracted by distillation of roots for 10 h. The extracted oil was dried over anhydrous sodium sulfate and subsequently preserved in sealed glass vials at 4 °C for the gas chromatography analysis of the EO. The amount of EO obtained from the plant material (roots) was calculated as: EO content (% v/w) = [observed volume of oil (mL)/weight of sample (g)] × 100.

2.9.3. GC analysis
The content of the EO active constituent (khusimol) was determined using a gas chromatography apparatus [7890B (Agilent) USA] equipped with a capillary column HP5 (coated with polyimide and fused silica) of the size 30 m × 0.320 mm, a flame ionization detector, and an injector. Nitrogen was used as the carrier gas. The GC temperature...
schedule was as follows: detector temperature, 300 °C; oven temperature, 250 °C; injector temperature, 250 °C. The sample size was perpetually 0.2 µL. The initial temperature was 100 °C with a hold time of 20 min; it was increased to 270 °C at the rate of 4 °C per minute. Identification of the active constituent (khusimol) was based on retention time. Khusimol was quantified as the percent content comparing its peak with the peak obtained from the reference standard reported in the literature (Adams, 2007).

2.10. Statistical analysis
Each pot was treated as one replicate and each treatment contained three replicates. The data were analyzed statistically using SPSS 22 (IBM Corp., Armonk, NY, USA) according to a simple randomized design. Means were compared using the Duncan multiple range test (DMRT) at $P \leq 0.05$. Standard error (±SE) was also employed to distinguish the mean values.

3. Results
The following section is a brief appraisal of the effects of ISA on vetiver. Among the various ISA concentrations employed, 120 mg L$^{-1}$ maximally increased the values of most of the parameters in comparison to the control.

3.1. Structural and functional changes in irradiated sodium alginate
FT-IR spectroscopy and UV spectra of ISA elucidate the structural and functional changes in ISA. The FT-IR spectroscopy reports illustrated some convincing results (Figure 1). The broad absorption bands obtained in the range of 1095 to 1037 cm$^{-1}$ could be attributed to the stretching of C=O and the hydroxyl group, respectively (Sartori et al., 1997). The peaks at 1628 cm$^{-1}$ correspond to the O-H bond bending vibrations, and the broad absorption peaks appearing near 1619 and 1420 cm$^{-1}$ could be assigned to the asymmetric and symmetric stretching of carboxylate group vibrations. The spectrum of ISA exhibited most of the characteristic adsorption peaks of unirradiated sodium alginate (UISA), but with some differences. As can be inferred from Figure 1, the peak intensity of ISA was increased as compared to that of UISA, indicating the formation of new C=O and -OH groups due to the scission of glycosidic bonds of sodium alginate, reducing end residue; it indicates the change in the sodium alginate structure after irradiation by gamma rays. This may be manifested in an increase in the ratio of -OH group peaks and broad C=O peaks (El-Mohdy, 2012; Rao et al., 2013; Sellimi et al., 2015).

Figure 2 depicts the UV spectra of the irradiated and unirradiated forms of sodium alginate; it reveals the formation of a new absorption band at approximately 265 nm in ISA, which was absent in UISA. The formation of the new peak could be attributed to the formation of carbonyl (C=O) groups after the main chain scission of alginate and hydrogen abstraction, followed by the ring opening in the

![Figure 1. FT-IR spectra of unirradiated sodium alginate and irradiated sodium alginate.](image-url)
radiation-induced degradation process (Luan et al., 2009; El-Mohdy, 2012).

3.2. Gamma-irradiation mediated size reduction in sodium alginate through SEM analysis
SEM analysis showcased a clear reduction in particle size of sodium alginate after gamma irradiation. As per SEM analysis, UISA particle size ranged between 174.4 and 399.4 µm, whereas ISA particle size ranged between 34.8 and 89.3 µm (Figures 3a and 3b).

3.3. Foliar feeding by ISA improved growth as well as fresh and dry weight of plants
ISA-120 (ISA applied at 120 mg L–1) significantly augmented the fresh and dry weight of shoots and roots in comparison to that of the control (water-spray treatment). This treatment surpassed the control by 21.2% and 20.3% regarding shoot fresh weight and shoot dry weight per plant, respectively. Regarding root fresh weights and root dry weights per plant, it exceeded the control by 21.9% and 22.4%, respectively (Table 1).

3.4. Amplification in photosynthetic parameters in ISA-applied plants
As per Table 2, foliar application of ISA increased the content of total chlorophyll and total carotenoids, NPR, internal CO₂ concentration, stomatal conductance, and Fv/Fm value in vetiver plants. As a result of application of ISA-120, the photosynthetic parameters also attained the maximal values. The application of ISA 120 mg L–1 enhanced the total content of chlorophyll (21.19%) and carotenoids (11.6%) compared to the control (measurements made at 300 DAT). ISA-120 also exceeded the control with regard to net photosynthetic rate, internal CO₂ concentration, and stomatal conductance by 21.2%, 21.7%, and 21.6%, respectively. Application of ISA-120 also enhanced the chlorophyll fluorescence value, exceeding the control by 21.8% (Table 2).

3.5. Enhancement in the activities of enzymes by foliar application of ISA
Enzymatic activities were measured in the water-sprayed control as well as in ISA-treated plants. Foliage-applied ISA stimulated the activities of assimilation of key enzymes NO₃⁻ and CO₂ (NR and CA, respectively). ISA-120 also resulted in a maximal increase in NR activity, excelling the control by 20.2% (Table 2). However, increasing the ISA concentration above 120 mg L–1 resulted in a progressive decrease in the values (Table 2). Maximal value of CA activity was also recorded as a result of application of ISA-120. This treatment showed a 21.3% increase in the CA activity compared to the control (Table 2).

3.6. Enhancement in the content and yield of EO and its active constituent by ISA
Foliar feeding of ISA also enriched the content and yield of EO as well as the EO active constituent (khusimol). Among different concentrations of ISA, ISA-120 proved the best regarding the content and yield of vetiver EO. As compared to the control, ISA-120 maximally increased the EO content (21.1%) as well as the EO yield (47.6%) in vetiver plant (Figures 4a and 4b). Khusimol is one of the main components of vetiver EO. The GC chromatograms
Figure 3. Scanning electron microscopy (SEM): unirradiated sodium alginate (a), irradiated sodium alginate (b).

Table 1. Effects of various concentrations of ISA (0, 40, 80, 120, and 160 mg L⁻¹) on growth attributes of vetiver recorded at 300 days after transplanting (DAT). Values of means within a row followed by the same letter(s) are not significantly different according to DMRT (P ≤ 0.05).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ISA concentrations (mg L⁻¹)</th>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Shoot fresh weight (g)</td>
<td>389.12 ± 4.97&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shoot dry weight (g)</td>
<td>103.8 ± 1.16&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Root fresh weight (g)</td>
<td>196.2 ± 3.45&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Root dry weight (g)</td>
<td>57.4 ± 0.90&lt;sup&gt;e&lt;/sup&gt;</td>
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clearly revealed that ISA significantly improved the active constituents of the vetiver EO. Of the different ISA concentrations, 120 mg L⁻¹ proved the best as it increased the content value of khusimol by 30.1% compared to the control (Figure 4c). As compared to the control, khusimol yield was significantly enhanced (92.6%) when ISA-120 was sprayed on the foliage of vetiver plants (Figures 4d, 5a, and 5b).

### 4. Discussion

PGPs critically determine the extent of growth by establishing a strong source–sink relationship, which leads to better supply of nutrients, thereby ensuring efficient cell metabolism (Patel and Golakia, 1988). Low-molecular-weight oligomers obtained by irradiation-mediated depolymerization of the natural polysaccharides (such as sodium alginate, chitosan, and carrageenan) have been reported to act as plant growth promoting substances and are believed to act in the same way as PGP (El-Rehim, 2006; Mollah et al., 2009; Aftab et al., 2016; Dar et al., 2016; Idrees et al., 2016; Ahmad et al., 2017; Naeem et al., 2017).

Depolymerization of sodium alginate (powder form) requires high irradiation doses (El-Mohdy, 2012) and the change in the molecular weight of alginate (if any) as a result of radiation-based degradation is estimated by GPC (Hien et al., 2000). The GPC profile helps in unraveling the gamma radiation-mediated depolymerization of polysaccharides as a result of chain scission of the complex natural polysaccharide. Followed by gamma irradiation of sodium alginate, the distribution curve showed elution of varying molecular-weight fractions against time (Idrees et al., 2012). GPC analysis revealed a shift in the graph towards higher retention time, which depicted the decrease in the molecular weight of sodium alginate brought about by its degradation upon gamma irradiation. The resultant low-molecular-weight oligosaccharides in the GPC graph illustrated an average molecular weight of 595,000 Da compared to the control (unirradiated sodium alginate), weighing 695,131 Da. The GPC distribution curve further revealed lower-molecular-weight oligomers at the end of the profile, possessing molecular weights of less than 100,000 Da. Since these fractions are small enough to penetrate easily through the stomatal aperture upon foliar feeding, they are expected to trigger growth-promoting activities in plants. It is speculated that after gamma irradiation these oligosaccharides bind to certain receptor proteins inducing different signaling pathways, thereby eliciting the improved growth and yield in plants (El-Mohdy, 2012). The present experiment was conducted considering the growth-triggering effect of these oligosaccharides in various other MAPs (Khan et al., 2011; Idrees et al., 2014; Naeem et al., 2015; Aftab et al., 2016; Dar et al., 2016).

### Table 2.

Effect of various concentrations of ISA (0, 40, 80, 120, and 160 mg L⁻¹) on the physiological and biochemical attributes of vetiver recorded at 300 days after transplanting (DAT).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ISA concentrations (mg L⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Total chlorophyll content (mg g⁻¹ FW)</td>
<td>1.675 ± 0.009δ</td>
</tr>
<tr>
<td>Total carotenoids content (mg g⁻¹ FW)</td>
<td>0.542 ± 0.007β</td>
</tr>
<tr>
<td>N R activity (nM NO₂ g⁻¹ FW h⁻¹)</td>
<td>331.4 ± 4.19 δ</td>
</tr>
<tr>
<td>C A activity (µM CO₂ kg⁻¹ leaf FW s⁻¹)</td>
<td>233.76 ± 2.82ε</td>
</tr>
<tr>
<td>Internal CO₂ concentration (µmol CO₂ kg⁻¹ FW s⁻¹)</td>
<td>269.6 ± 2.84ε</td>
</tr>
<tr>
<td>Chlorophyll fluorescence (Fv/Fm)</td>
<td>0.708 ± 0.006ε</td>
</tr>
<tr>
<td>Net photosynthetic rate (µmol CO₂ m⁻² s⁻¹)</td>
<td>13.14 ± 0.18δ</td>
</tr>
<tr>
<td>Stomatal conductance (µmol CO₂ m⁻² s⁻¹)</td>
<td>0.217 ± 0.003ε</td>
</tr>
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</table>

Values of means within a row followed by the same letter(s) are not significantly different according to DMRT (P ≤ 0.05).
Foliar application of ISA showed remarkable effects on growth characteristics, physiological attributes, and EO content and yield of vetiver (Table 1). ISA-120 proved the best for most of the parameters studied; however, the values regarding all the parameters portrayed the unfavorable effect of higher ISA concentrations beyond 120 mg L$^{-1}$. Progressive improvement in the growth parameters by foliar feeding of ISA has been established with regard to *Foeniculum vulgare* (Sarfaraz et al., 2011), *Mentha arvensis* (Naeem et al., 2012), *Vicia faba* (El-Mohdy, 2012), *Eucalyptus citriodora* (Ali et al., 2014), *Catharanthus roseus* (Naeem et al., 2015), *Artemisia annua* (Aftab et al., 2016), and *Trigonella foenum-graecum* (Dar et al., 2016). It was also found that alginate oligomers have a functional resemblance to endogenous growth elicitors and may act as signaling molecules, which apparently activate the plant’s responses by stimulating gene expression and synthesizing various enzymes.

A progressive improvement was observed in physiological and biochemical parameters as a result of the foliar application of ISA (Table 2). Here too, 120 mg L$^{-1}$ ISA proved optimum for photosynthetic pigments (total contents of chlorophyll and carotenoids), chlorophyll fluorescence, photosynthetic rate, internal CO$_2$ concentration, and stomatal conductance (Table 2). The beneficial effect of ISA on the enzyme activities was also registered in the present study. Improvement in physiological attributes might be attributed to the ISA-mediated stimulation of gene expression, leading to an improvement in enzymatic activities, photosynthetic pigments, and net photosynthetic rate (Farmer et al., 1991; John et al., 1997).

Nitrogen assimilation in plants, catalyzed by the nitrate reductase (NR) enzyme, is a highly regulated reaction at the transcriptional, posttranscriptional, and postranslational levels, where the inputs are supplied by light, photosynthesis, CO$_2$, oxygen availability, and nutrient status (von Wiren et al. 2000). The progressive increase in NR activity in this investigation might be ascribed to the ISA-mediated enhancement in the leaf
nutrient contents (Table 2). The findings of the present study can be corroborated by those conducted on opium poppy (Khan et al., 2011), lemongrass (Idrees et al., 2014), and *Artemisia* (Aftab et al., 2016). The positive effect of ISA application on NR activity was also reported by Naeem et al. (2015) and Dar et al. (2016) in periwinkle and fenugreek, respectively.

Carbonic anhydrase is a zinc metalloprotein that is known to catalyze reversible interconversion of HCO$_3^-$ and CO$_2$ (Xin Bin et al., 2001; Khan et al., 2004). It represents 1%–2% of the total soluble protein present in the leaf (Oakabe et al., 1984), as evident by its presence in all photosynthesizing tissues. As carbonic anhydrase is directly related to photosynthesis in higher plants, it is considered to maintain the growth and development in plants by controlling the synthesis of carbon compounds (Khan, 1994; Henry, 1996). It catalyzes the reversible hydration of CO$_2$ to carbonic acid, enhancing the accessibility of CO$_2$ to RuBisCo in plants. In this investigation, the augmentation in the CA activity in ISA-treated plants over the control might be related to the enhanced photosynthesis in the treated plants, as indicated by Siddiqui et al. (2008). The observed effect is consistent with the previous findings reported by Aftab et al. (2013) with regard to *Artemisia* and by Idrees et al. (2016) in connection with coriander.

Synthesis of essential oil (EO) in plants is directly or indirectly linked to the process of photosynthesis since carbohydrate precursors are invariably required for EO synthesis. The irradiated polysaccharides are believed to have a positive effect on gene expression related to
synthesis of some important enzymes involved in the terpene biosynthetic pathway (Ahmad et al., 2017). As presented in Figures 4a–4d, the content and yield of EO was significantly increased by the application of various concentrations of ISA. Enhancement in growth, enzyme activities, nutritional status, and photosynthesis, as evident from the present study (Tables 1 and 2), might have led to proficient assimilation, efficient translocation, and partitioning of photosynthates that could have resulted in increased biosynthesis of carbohydrates, thereby improving the growth of the plants. In turn, increased carbohydrate levels and their potential diversion to secondary metabolism might have contributed to the elevated levels of EO in plants (Swamy and Rao, 2009). As discussed earlier, vetiver oil is a complex sesquiterpene that is an outcome of the cytosolic mevalonate-dependent pathway triggered by association of two acetyl coenzyme A units. The precursors in this pathway are isopentenyl diphosphate (IPP) and its allylic isomer, dimethylallyl diphosphate (DMAPP). A concomitant secondary metabolic pathway takes place in the plastids, commonly known as the MEP pathway. Although this subcellular compartmentalization allows both of the pathways to operate independently in plants, there is evidence that they cooperate in the biosynthesis of certain metabolites (Laule et al., 2003; Kloer et al., 2006). As per the GC reports of this investigation, significant increase in the active constituent of vetiver oil (khusimol) over the water-sprayed control was recorded as a result of ISA application. The peaks in the chromatograms were compared with the standard chromatograms of khusimol (Adams, 2007) in order to quantify the khusimol content in the leaves. Khusimol content and khusimol yield per plant were maximally increased by ISA-120 over the control in the present study (Figures 4c, 4d, 5a, and 5b). Increase in the content of khusimol in the present study is perhaps because of increased de novo synthesis of the enzymes involved in the biosynthesis of this sesquiterpene. According to Schalk and Deguerry (2013), the khusimol results from oxidation of zizaene, which is apparently synthesized from FPP by the activity of zizaene synthase. As per the results of yield attributes shown in the GC chromatograms (Figures 5a and 5b), it could be conjectured that ISA may have some stimulatory role in the biosynthetic pathway mainly at two steps, one at the conversion of zizaene from FPP by zizaene synthase and the other at the oxidation of zizaene into khusimol by cytochrome P450 reductase.

In conclusion, gamma irradiation exposure results in the depolymerization of sodium alginate into low-molecular-weight oligosaccharides. Depolymerization also leads to certain structural changes, which cause the oligosaccharides to elicit various beneficial responses in plants with regard to growth, yield, and quality. The study revealed that foliar feeding by ISA was influential in enhancing the growth, physiological, and biochemical attributes; it proved remarkably proficient in increasing the content and yield of the EO. The highest values with regard to overall performance of the plant were obtained with ISA applied at 120 mg L⁻¹. The quantity as well as the quality of the EO was also improved as the percentage of key active constituents like khusimol was significantly enhanced. This technique is safe, as it aims at the exposure of natural polysaccharides to radiations and not the plant or plant parts as such. It is a convenient agrotechnique as it involves a clean one-step degradation format. Additionally, this technique is an economical and ecofriendly way to improve and utilize the growth potential of medicinal herbs like vetiver.

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