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Research Article

Radiation Processed Carrageenan Improves Plant Growth, Physiological Activities, and Alkaloids Production in *Catharanthus roseus* L.

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Catharanthus roseus (L.) G. Don (Apocynaceae) is a medicinal plant that produces indole alkaloids used in cancer chemotherapy. Commercially important antineoplastic alkaloids, namely, vinblastine and vincristine, are mainly present in the leaves of *C. roseus*. Gamma-rays irradiated carrageenan (ICR) has been proven as plant growth promoting substance for a number of medicinal and agricultural plants. Considering the importance of ICR as a promoter of plant growth and alkaloids production in *C. roseus*, a pot experiment was carried out to explore the effect of ICR on the plant growth, physiological activities, and production of anticancer alkaloids in *C. roseus* at 120 and 150 days after planting (DAP). Foliar application of ICR (at 0, 20, 40, 60, 80, and 100 mg L^{-1}) significantly improved the performance of *C. roseus*. 80 mg L^{-1} of ICR enhanced the leaf yield by 29.2 and 35.4% and the herbage yield by 32.5 and 37.4% at 120 and 150 DAP, respectively, over the control. The spray of ICR at 80 mg L^{-1} increased the yield of vinblastine by 64.3 and 65.0% and of vincristine by 75.5 and 77.0% at 120 and 150 DAP, respectively, as compared to the control.

1. Introduction

Carrageenan ($C_{12}H_{17}O_{12}S$) is composed of D-galactose units linked alternately with α-1,4 and β-1,3 linkages. It is a mixture of water-soluble, linear, and sulfated galactans. Gamma-rays irradiation degrades the natural carrageenan into smaller oligomers with comparatively low molecular weight. Oligomers, obtained from radiolytically degraded carrageenan as well as those from other marine polysaccharides, have been used as plant growth promoters for several medicinal and agricultural crops [1–4]. In fact, foliar application of the degraded marine polysaccharides has been proved to stimulate various biological and physiological activities, including plant growth in general [1–3, 5–8], seed germination [1], shoot elongation [1, 9], root growth [10], flower production, antimicrobial activity, amelioration of heavy metal stress, and synthesis of phytoalexins [11–14]. Nonetheless, the use of irradiated carrageenan (and of other marine polysaccharides) in promoting plant growth and, thereby, augmenting the desired active constituents in medicinal and aromatic plants is inexpensive and safe [2].

There is immense need of enhancing the production of alkaloids of medicinally important plants in view of their massive demand worldwide. *Catharanthus roseus* (L.) G. Don (commonly known as "Sadabahar" or "Periwinkle," family Apocynaceae) is a medicinal plant that produces chemotherapeutically important alkaloids, namely, vinblastine and vincristine, which inhibit the growth of cancer cells, hindering the formation of mitotic apparatus during cell division [15]. Further, vinblastine has helped increase the chance of surviving childhood leukaemia, while vincristine is used to treat Hodgkins' disease [15]. However, the content

of these useful indole alkaloids is in traces in *C. roseus* leaves. In addition, L-tryptophan decarboxylase (TDC, EC 4.1.1.28) is the key enzyme, which is involved in the early stages of indole alkaloid biosynthesis [16], and catalyses the formation of tryptamine from tryptophan and therefore it plays a role in terpenoid indole alkaloids biosynthesis. The most research has focused on the role of TDC in the biosynthesis of pharmaceutically important terpenoid indole alkaloid in *C. roseus* plants [16–18].

Keeping in view the importance of ICR as a plant growth promoter and desired production of alkaloids of the crop, this study is aimed at investigating the effect of ICR on plant growth, leaf yield, activity of TDC enzyme, and content and yield of total and therapeutically specific alkaloids of *C. roseus* plants.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions. The pot experiment was conducted in naturally illuminated environmental 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) India. Healthy periwinkle seedlings of equal size were obtained from Woodbine Nursery, Civil Lines, Aligarh. The seedlings were then transplanted to earthen pots. Prior to transplantation, each pot (25 cm diameter × 25 cm height) was filled with 5 kg of homogenous mixture of soil and farmyard manure (4 : 1). Physical and chemical characteristics of the soil were texture sandy loam, pH (1 : 2) 7.5, E.C. (1 : 2) 0.46 dSm^{-1}, available N, P, and K 102.0, 7.8, and 145.6 mg kg^{-1} of soil, respectively. A uniform recommended basal dose of N, P, and K (15 : 25 : 25 kg ha^{-1}, resp.) was applied in the form of urea, single superphosphate, and muriate of potash, respectively, at the time of transplanting the seedlings. The experiment was conducted with simple randomized block design. Each treatment was replicated five times. Each pot contained a single healthy plant. The pots were watered as and when required.

2.2. Experimental Design and the Analysis of Growth, Yield, and Quality. Foliar spray of different concentrations of ICR was applied at 15 days interval when the plants were at 2-3 true leaf stage. Totally, five sprays of ICR were applied to the crop using a hand sprayer. The control plants were sprayed with distilled water only. Treatment of unirradiated carrageenan (20 mg L^{-1}) was not tested in the present study as it proved similar to that of water spray (control) in our previous experiment [3]. The applied ICR treatments were comprised of 0, 20, 40, 60, 80, and 100 mg L^{-1} of ICR. Plants were sampled for all physiological and biochemical parameters carried out at 120 and 150 DAP. All yield and quality attributes were also measured on these dates.

At sampling (120 and 150 DAP), five plants of each treatment were harvested and their roots were washed carefully with tap water to remove all adhering foreign particles. Water adhering to the roots was removed with blotting paper. Then, the plant fresh and dry weights were measured using an electronic balance.

2.3. Irradiation Procedure and GPC (Gel Permeation Chromatography) Analysis. Solid material of k-carrageenan (Sigma Aldrich, USA) was sealed in a glass tube with atmospheric air. The samples of carrageenan were irradiated (Cobalt-60, GC-5000) in a gamma radiation chamber (BRIT, Bhabha Atomic Research Centre, 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, and 20-microliter loop injection [3]. The molecular weight of the unirradiated commercial k-carrageenan sample was estimated to be about 100,000. Polyvinyl alcohol polymers of known molecular weight were used as standards. Different aqueous concentrations of irradiated carrageenan were finally prepared using double distilled water for the spray treatments.

2.4. Scanning Electron Microscopy (SEM) Analysis. The morphology structure of the carrageenan samples was examined using the scanning electron microscope (Philips XL 30 ESEM, Jeol, Japan). The samples were coated with gold. Scanning electron microscopy and elemental analysis were performed for the unirradiated as well as irradiated carrageenan samples (Figures 4(a) and 4(b)) at Ultra Sophisticated Instrumentation Facility (USIF), Aligarh Muslim University, Aligarh, India.

2.5. Determination of Growth Attributes. The growth attributes, namely, number of leaves per plant, average leaf area, and fresh and dry weight of plants were determined at 120 and 150 DAP. Plants from each treatment were uprooted carefully followed by recording the number of leaves and fresh weight per plant. Plants were washed and then dried in a hot-air oven at 80°C for 24 h prior for measuring the plant dry weight. Only 10% of the randomly selected leaves of each sample (consisting of five plants) were used to determine the leaf area using graph paper sheet [19]. The average area per leaf, thus determined, was multiplied with the total number of leaves to measure the total leaf area per plant.

2.6. Determination of Physiological Attributes

2.6.1. Net Photosynthetic Rate and Stomatal Conductance. Net photosynthetic rate and stomatal conductance were determined using intact leaves (the randomly selected youngest fully expanded leaves) from the five replicates. Measurements were made on sunny days at 1100 hours using an infrared gas analyzer (IRGA, Li-Cor 6400 Portable Photosynthesis System Lincoln, Nebraska, USA) at 120 and 150 DAP.

2.6.2. Total Contents of Chlorophyll and Carotenoids. Total content of chlorophyll and carotenoids in the leaves was estimated using the method of Lichtenthaler and Buschmann [20]. The fresh tissue from the interveinal area of leaf was grinded with 100% acetone using mortar pestle. The optical density (OD) of the pigment solution was recorded at 662,

645, and 470 nm to determine chlorophyll a, chlorophyll b, and total carotenoids content, respectively, using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). Total chlorophyll content was assessed by adding together the content of chlorophyll a and chlorophyll b. The content of each photosynthetic pigment was expressed as mg g^{-1} leaf FW.

2.6.3. Activity of Nitrate Reductase (NR). Activity of nitrate reductase (E.C. 1.6.6.1) was estimated in the youngest fully developed leaves by the intact tissue assay method developed by Jaworski [21]. Fresh chopped leaves, weighing 200 mg, were transferred to plastic vials. Each vial contained 2.5 mL phosphate buffer (pH 7.5), 0.5 mL potassium nitrate solution, and 2.5 mL of 5% isopropanol. The vials, containing the reaction mixture, were incubated for two hours at 30°C. After incubation, 1% sulphanilamide and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NED-HCL) were added. The test tubes were kept for 20 minutes at room temperature for maximum color development. The OD of the content was recorded at 540 nm. Activity of NR was expressed as nM NO_2^- g^{-1} FW h^{-1}.

2.6.4. Activity of Carbonic Anhydrase (CA). The activity of carbonic anhydrase (E.C. 4.2.1.1) was measured in the fresh leaves selected randomly, using the method described by Dwivedi and Randhawa [22]. Two hundred mg of the leaves (chopped leaf-pieces) was transferred to Petri plates. The leaf pieces were dipped in 10 mL of 0.2 M cysteine hydrochloride solution for 20 minutes at 4°C. The solution adhering to leaf pieces was removed with the help of a blotting paper, followed by immediately transferring them to a test tube containing 4 mL of phosphate buffer (pH 6.8). To it, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.022% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme activity was expressed as μM CO_2 kg^{-1} leaf FW s^{-1}.

2.6.5. Activity of Tryptophan Decarboxylase (TDC). The activity of tryptophan decarboxylase (E.C. 4.1.1.28) was carried out according to the method of Islas et al. [23]. Frozen transformed roots (1000 mg) were pulverized in a cold mortar to fine powder and homogenized with 1.25 mL of 0.1 M HEPES (pH 7.5), containing 3 mM, dithiothreitol, 5 mM EDTA, and 200 mg of polyvinylpolypyrrolidone. The extract was filtered through four layers of cheesecloth and then it was centrifuged at 18000 ×g for 30 min. The resulting supernatant was used as a source of enzyme for the TDC activity. The protein content of the enzyme extract was determined as described by Peterson [24], using albumin as standard. The activity of TDC enzyme was expressed as n mol $mint^{-1}$ mg^{-1} protein.

2.6.6. Yield and Quality Attributes. Leaf yield was recorded by weighing all plant leaves using an electronic balance. Herbage yield was measured weighing the total plant biomass excluding roots.

(1) Total Alkaloid Content in Leaves and Roots. Total alkaloid content was estimated in leaves and roots as described by Afaq et al. [25]. The leaves and roots were dried in a hot-air oven at 80°C for twenty-four hours. The samples were powdered and passed through a 72 mesh. Five hundred mg of the powdered sample was taken in a 100 mL round bottom reflux flask. To it, a known volume of ethyl alcohol was added. Then, the mixture was refluxed for 6 hours. Thereafter, it was filtered, followed by adding 50 mL of dilute HCl, and then the filtrate was transferred to a separating funnel, to which 50 mL of diethyl ether was added. The mixture was shaken for 15–20 minutes. The upper diethyl ether layer was discarded and the lower water layer was decanted to a beaker. The content, collected in beaker, was made slightly basic by adding ammonia solution. The decanted content was again transferred into a separating funnel with 50 mL of diethyl ether, followed by decanting the content again. To the final decant, anhydrous sodium carbonate was added. Then, the mixture was decanted in a preweighed dry porcelain dish, followed by evaporating the content till dryness; lastly, the dried content was weighed. Total alkaloid content in leaves/roots was calculated using the following formula:

$$\text{Total alkaloid content (\%)} = \frac{W_A - W_E}{W_R} \times 100, \quad (1)$$

where W_E = weight of empty porcelain dish (g), W_A = weight of porcelain dish after evaporation (g), and W_R = weight of the dried powder (g).

(2) Content of Vincristine, Vinblastine, and Vindoline Alkaloids

(a) Plant Material and Sample Extraction. Preparation of sample extraction and the chromatographic condition of high-performance liquid chromatography (HPLC) instrument were accomplished through the method of Uniyal et al. [26]. Freshly harvested leaves were oven dried at 80°C for 24 h and then grinded to fine powder. A volume (30 mL) of 90% ethanol was added to 5 g of leaf powder; it was left over night and then filtered. The residue was again extracted with 90% ethanol (3 × 30 mL) at room temperature (27°C), and the pooled alcoholic extract was filtered and concentrated *in vacuo* at 40°C. The dried residue was redissolved in ethanol (10 mL), diluted with water (10 mL), and then acidified with 3% hydrochloric acid (10 mL). This was then extracted with hexane (3 × 30 mL), the hexane extract was discarded, and the aqueous portion of the content was cooled to 10°C; it was basified with ammonium hydroxide to pH 8.5 and then was extracted with chloroform (3 × 30 mL). The combined chloroform extract was washed with water, evaporated to dryness, and redissolved in 1 mL of chloroform. After that, it was passed through a silica Sep-Pak cartridge (Waters), presaturated with chloroform, and it was washed successively with 5 mL each of chloroform and chloroform-methanol mixture (9 : 1, v/v) and dried over anhydrous sodium sulphate before being evaporated to dryness. The residue obtained

Table 1: Effect of six foliar concentrations of irradiated carrageenan (ICR) on a number of leaves and average leaf area per plant of *Catharanthus roseus* L. at 120 and 150 DAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$).

Treatments (mg L^{-1})	Number of leaves per plant		Average leaf area (cm^2)	
	120 DAP	150 DAP	120 DAP	150 DAP
ICR 0	200.0[f]	247.4[f]	10.27[f]	12.10[f]
ICR 20	214.3[e]	254.5[e]	10.46[e]	12.26[e]
ICR 40	220.0[d]	262.8[d]	11.03[d]	12.44[d]
ICR 60	226.9[c]	270.6[c]	11.30[c]	12.75[c]
ICR 80	233.2[a]	298.0[a]	11.66[a]	13.82[a]
ICR 100	229.0[b]	278.0[b]	11.47[b]	13.50[b]
LSD at 5%	4.0	5.2	0.13	0.11

Table 2: Effect of six foliar concentrations of irradiated carrageenan (ICR) on fresh and dry weights per plant of *Catharanthus roseus* L. at 120 and 150 DAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$).

Treatments (mg L^{-1})	Fresh weight per plant (g)		Dry weight per plant (g)	
	120 DAP	150 DAP	120 DAP	150 DAP
ICR 0	65.60[f]	71.63[f]	11.75[f]	12.70[f]
ICR 20	68.70[e]	74.90[e]	12.20[e]	13.43[e]
ICR 40	75.20[d]	86.84[d]	12.78[d]	15.70[d]
ICR 60	79.46[c]	90.36[c]	13.16[c]	16.24[c]
ICR 80	88.95[a]	101.35[a]	16.15[a]	18.16[a]
ICR 100	85.25[b]	98.10[b]	13.90[b]	16.90[b]
LSD at 5%	2.40	2.28	0.29	0.17

was dried to constant weight in order to determine the total alkaloid content. An aliquot (10 mg) of the crude alkaloid was dissolved in 1 mL of methanol, and 10 μL of it was subjected to HPLC analysis.

(b) HPLC Procedure. Chromatographic analysis was carried out using HPLC (Shimadzu, Japan, LC-10A). Solvents were filtered by using a Millipore system and analysis was performed on a Waters μ Bondapak C_{18} reversed-phase column, 10 mm (30 cm × 3.9 mm I.D.). A constant flow rate of 0.6 mL/min was used during analysis. The composition of mobile phase was optimized by using acetonitrile : 0.1 M phosphate buffer : glacial acetic acid (38 : 62 : 0.3); pH 4.14, flow rate 0.6 mL/min, column temperature 26°C, and detector wave length 254 nm. For standard, stock, solutions of vinblastine and vincristine sulphate were prepared, dissolving 1 mg of each in 1 mL of methanol. The solutions were subjected and the retention time (Rt) for vinblastine (Rt-8 min), vincristine (Rt-7 min), and vindoline (Rt-10 min) were noticed.

2.7. Statistical Analysis. The data were analyzed statistically using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA) according to simple randomized design. Means were compared using Duncan's multiple range test (DMRT) at $P < 0.05$. Least significant difference (LSD) was calculated and also employed in the tables and figures.

3. Results

3.1. Growth Attributes. The foliar application of ICR significantly improved growth attributes of *C. roseus* at 120 and 150 DAP. A progressive improvement in growth parameters with the increase in ICR concentration up to 80 mg L^{-1} was noted. However, at 100 mg L^{-1} of ICR, the values declined, but they were significantly better than the control (Tables 1 and 2). Treatment 80 mg L^{-1} of ICR gave the maximum values for growth characteristics both at 120 and 150 DAP. In comparison to the control, it increased the number of leaves by 16.6 and 20.5%, leaf area by 13.5 and 14.2%, plant fresh weight by 35.6 and 41.5%, and plant dry weight by 37.5 and 43.0% at 120 and 150 DAP, respectively (Tables 1 and 2).

3.2. Physiological and Biochemical Attributes. Application of ICR significantly enhanced the photosynthetic parameters (net photosynthetic rate and stomatal conductance) in the present study. 80 mg L^{-1} of ICR increased the net photosynthetic rate by 16.2 and 18.5% at 120 and 150 DAP, respectively (Figures 1(a) and 1(b)). It also increased the stomatal conductance by 9.80 and 13.8% at 120 and 150 DAP, respectively, as compared to the control (Figure 1(b)).

The depolymerised form of carrageenan (ICR) significantly increased the photosynthetic parameters in comparison to the control (Table 3). Of the ICR concentrations, 80 mg L^{-1} resulted in the greatest increase in the total

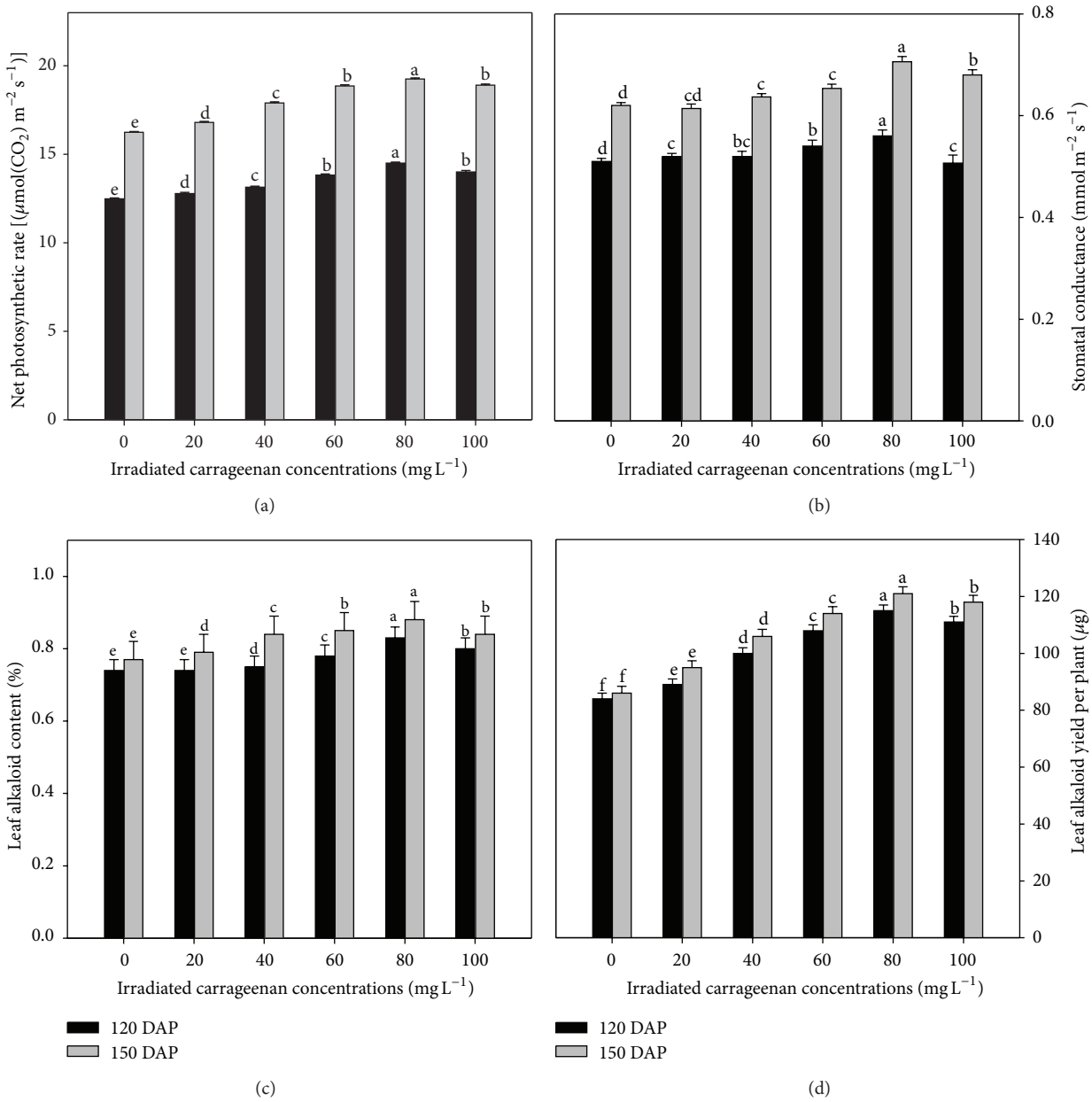

Figure 1: Effect of six concentrations of irradiated carrageenan (0, 20, 40, 60, 80, and 100 mg L^{-1}) on net photosynthetic rate (a), stomatal conductance (b), leaf alkaloid content (c), and leaf alkaloid yield (d) of *Catharanthus roseus* L. studied at 120 and 150 DAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$). Bars (⊤) represent the LSD at 5%.

Table 3: Effect of six foliar concentrations of irradiated carrageenan (ICR) on the total content of chlorophyll and carotenoids of *Catharanthus roseus* L. at 120 and 150 DAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$).

Treatments (mg L^{-1})	Total chlorophyll content (mg g^{-1} FW)		Total carotenoids content (mg g^{-1} FW)	
	120 DAP	150 DAP	120 DAP	150 DAP
ICR 0	1.032^{e}	1.214^{e}	0.185^{a}	0.195^{d}
ICR 20	1.065^{d}	1.278^{d}	0.188^{a}	0.199^{c}
ICR 40	1.098^{c}	1.328^{c}	0.184^{a}	0.199^{c}
ICR 60	1.135^{b}	1.374^{b}	0.185^{a}	0.200^{c}
ICR 80	1.180^{a}	1.415^{a}	0.186^{a}	0.209^{a}
ICR 100	1.148^{b}	1.380^{b}	0.185^{a}	0.204^{b}
LSD at 5%	0.030	0.034	NS	0.003

Table 4: Effect of six foliar concentrations of irradiated carrageenan (ICR) on activities of nitrate reductase, carbonic anhydrase, and tryptophan decarboxylase (TDC) of *Catharanthus roseus* L. at 120 and 150 DAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$).

Treatments (mg L^{-1})	Nitrate reductase activity [nM NO_2^- g^{-1} (FW) h^{-1}]		Carbonic anhydrase activity [mol (CO_2) kg^{-1} (FW) s^{-1}]		Tryptophan decarboxylase activity (nmol min^{-1} mg^{-1} protein)	
	120 DAP	150 DAP	120 DAP	150 DAP	120 DAP	150 DAP
ICR 0	214.2^{e}	224.9^{e}	5.34^{d}	5.45^{d}	20.0^{f}	22.0^{f}
ICR 20	222.0^{d}	232.4^{d}	5.65^{c}	5.67^{c}	20.5^{e}	22.5^{e}
ICR 40	230.0^{c}	248.0^{c}	5.73^{c}	5.76^{c}	21.0^{d}	23.0^{d}
ICR 60	236.5^{b}	260.8^{b}	5.85^{bc}	5.90^{bc}	21.6^{c}	24.0^{c}
ICR 80	244.6^{a}	270.7^{a}	6.13^{a}	6.30^{a}	22.5^{a}	25.7^{a}
ICR 100	238.4^{b}	264.0^{b}	5.93^{b}	5.98^{b}	22.0^{b}	24.7^{b}
LSD at 5%	5.42	6.20	0.18	0.20	0.02	0.02

Table 5: Effect of six foliar concentrations of irradiated carrageenan (ICR) on leaf yield and herbage yield per plant of *Catharanthus roseus* L. at 120 and 150 DAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$).

Treatments (mg L^{-1})	Leaf yield per plant (g)		Herbage yield per plant (g)	
	120 DAP	150 DAP	120 DAP	150 DAP
ICR 0	53.50^{f}	55.20^{f}	60.25^{f}	66.54^{f}
ICR 20	56.40^{e}	57.49^{e}	62.53^{e}	68.90^{e}
ICR 40	59.00^{d}	60.50^{d}	65.60^{d}	75.92^{d}
ICR 60	63.26^{c}	65.36^{c}	72.40^{c}	82.36^{c}
ICR 80	69.10^{a}	74.75^{a}	79.91^{a}	91.42^{a}
ICR 100	65.70^{b}	70.35^{b}	76.10^{b}	85.62^{b}
LSD at 5%	2.40	2.28	2.24	2.32

chlorophyll and carotenoids content. As compared to the control, application of ICR at 80 mg L^{-1} enhanced the total chlorophyll content by 14.3 and 16.6% at 120 and 150 DAP, respectively (Table 3). In addition, ICR increased the total carotenoids content by 7.18% at 150 DAP; however, ICR application did not affect the carotenoids content at 120 DAP (Table 3).

Application of ICR positively affected the activity of NR at both of the growth stages, with 80 mg L^{-1} of ICR increasing the NR activity by 15.2 and 20.4% at 120 and 150 DAP, respectively, over the control (Table 4). Application of ICR positively improved CA activity too at both growth stages. The activity of the enzyme increased to the maximum extent at 150 DAP (Table 4). Compared to the control, 80 mg L^{-1} of ICR resulted in 14.8 and 15.6% increase in CA activity at 120 and 150 DAP, respectively (Table 4).

The TDC activity was also positively affected by the application of ICR at both 120 and 150 DAP. The activity of the enzyme was maximum at 120 DAP. 80 mg L^{-1} ICR, improved the TDC activity by 12.5 and 16.8%, over the control, at 120 and 150 DAP, respectively (Table 4).

3.3. Yield and Quality Attributes. Concentration of ICR applied at 80 mg L^{-1} enhanced the leaf yield by 29.2 and 35.4% and the herbage yield by 32.5 and 37.4% at 120 and 150 DAP, respectively, over the control (Table 5). Application of ICR increased the content and yield of total alkaloids when compared to the control. The foliar spray of ICR at 80 mg L^{-1} resulted in the maximum content and yield of leaf alkaloids, increasing the value by 12.0 and 14.3% at 120 DAP and by 36.9 and 40.7% at 150 DAP over the control, respectively (Figures 1(a), 1(c), 1(b), and 1(d)). Similarly, 80 mg L^{-1} of ICR increased the content and yield of total root alkaloids by 12.5 and 14.6% at 120 DAP and by 45.8 and 62.7% at 150 DAP, respectively, over the control (Figures 2(a), 2(b), 2(c), and 2(d)).

As compared to the control, no increase in the content of anticancer alkaloids (vinblastine and vincristine) due to ICR applied on foliage (Figures 2(a), 2(b), 2(c), 2(d), 3(a), 3(b), 3(c), and 3(d)) was observed. However, the spray of ICR at 80 mg L^{-1} considerably increased the yield of both vinblastine and vincristine, exceeding the control by 64.3 and 65.0% at 120 DAP and by 75.5 and 77.0% at 150 DAP, respectively (Figures 2(a), 2(b), 2(c), 2(d), 3(a), 3(b), 3(c), and 3(d)).

Further, the application of ICR at 80 mg L^{-1} resulted in the maximum content and yield of vindoline alkaloid, augmenting the value over the control by 21.9 and 22.2% at 120 DAP and by 91.6 and 94.7%, at 150 DAP, respectively (Figures 3(a), 3(b), 3(c), and 3(d)).

4. Discussion

There is no report regarding the effect of ICR application on *C. roseus* so far. Hence, it might be considered as the

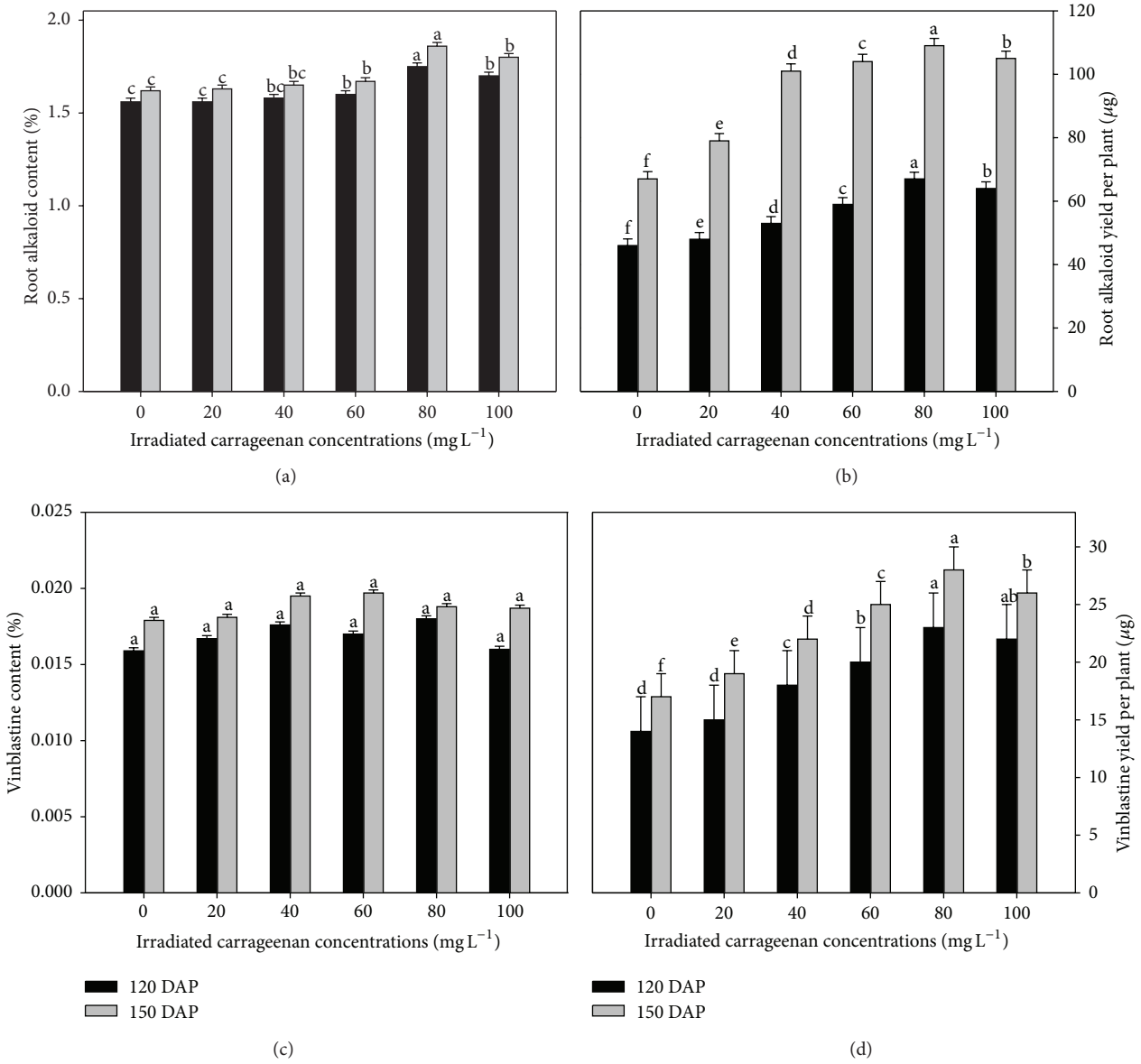

Figure 2: Effect of six concentrations of irradiated carrageenan (0, 20, 40, 60, 80, and 100 mg L^{-1}) on root alkaloid content (a) and root alkaloid yield (b), vinblastine content (c) and vinblastine yield (d) of *Catharanthus roseus* L. studied at 120 and 150 DAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$). Bars (⊤) represent the LSD at 5%.

first report of its kind, revealing the effect of ICR on plant growth, physiological activities, and the content and yield of alkaloids in this medicinally important plant. GPC of the unirradiated and irradiated carrageenan samples were carried out as reported previously [3]. GPC study revealed the formation of low molecular weight fractions in irradiated carrageenan samples, carrying less than 20,000 molecular weight oligomers, which might be responsible for plant growth promotion in this study. 80 mg L^{-1} was the most effective concentration of ICR that resulted in the highest values of growth attributes, herbage yield, and the content and yield of alkaloids of *C. roseus*. Like PGRs, which improve the plant defense by acting as signaling molecules, oligomers of carrageenan might act similar to those derived from alginate (alginate oligosaccharides) that behaved like an endogenous growth elicitor and functioned as a signal to trigger the synthesis of different enzymes and activate various responses of plants, exploiting the gene expression in Cd stress conditions [27]. In addition, the gamma-degraded marine polysaccharides, namely, sodium alginate, carrageenan, and chitosan, applied in the form of foliar sprays, have been reported to enhance plant growth in general [1–7, 28–32]. In the present investigation, the application of ICR 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). In the present study, application of radiation-derived oligosaccharides of carrageenan resulted

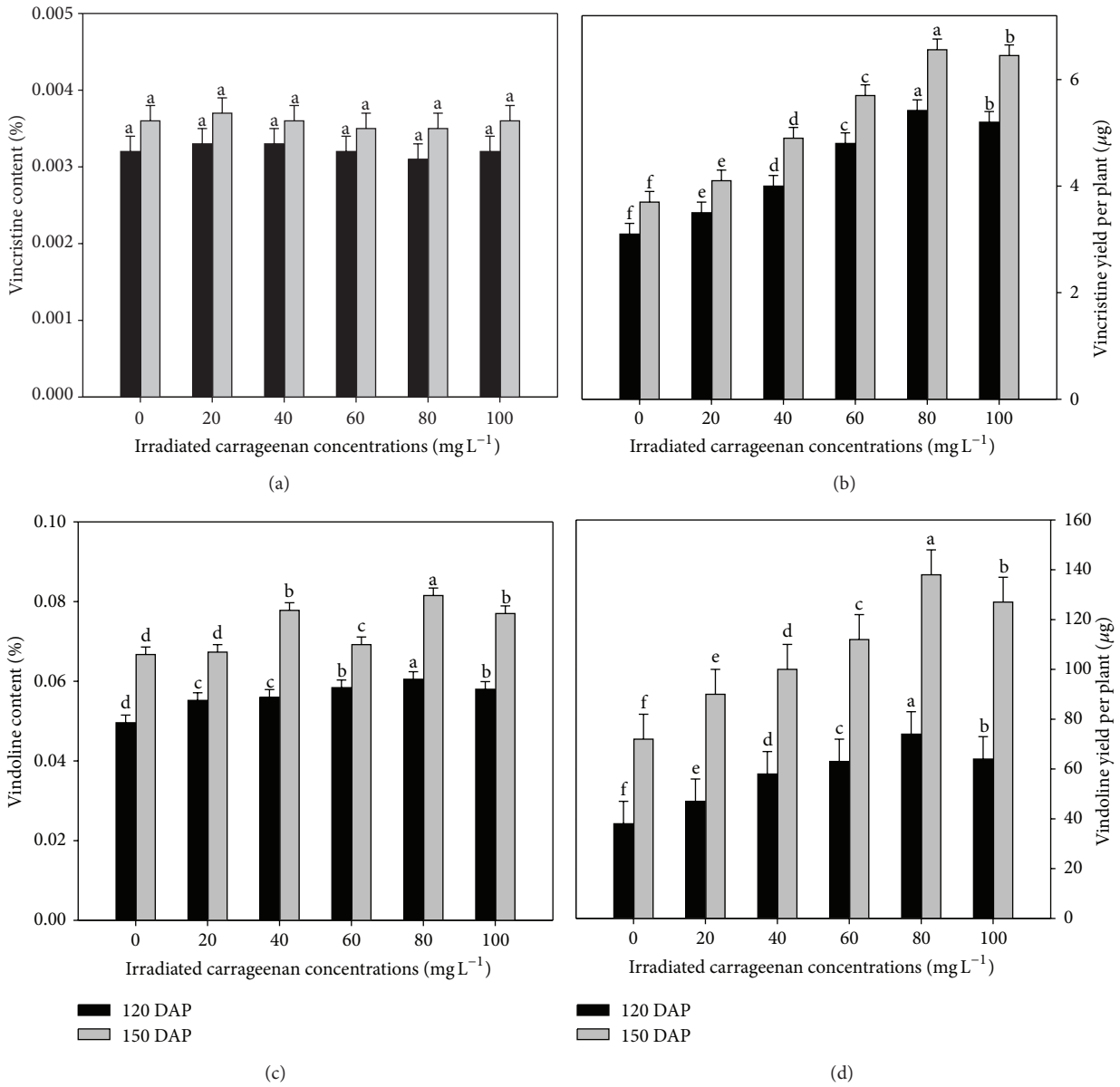

Figure 3: Effect of six concentrations of irradiated carrageenan (0, 20, 40, 60, 80, and 100 mg L^{-1}) on vincristine content (a) and vincristine yield (b) and vindoline content (c) and vindoline yield (d) of *Catharanthus roseus* L. studied at 120 and 150 DAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$). Bars (⊤) represent the LSD at 5%.

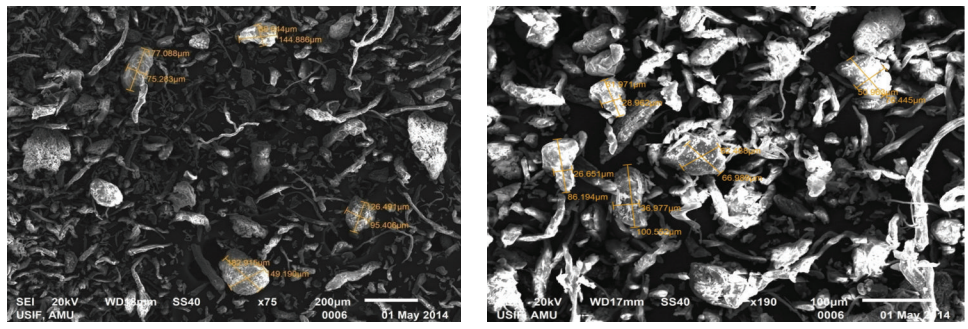

Figure 4: Scanning electron microscopy (SEM) of unirradiated carrageenan (a) and irradiated carrageenan (b) crystals.

in significant improvement in plant growth attributes. The present results are in conformity with the earlier findings that report the positive effect of degraded natural polysaccharides on the growth and development of various crops including barley [33], potato [34], red amaranth [30], *Artemisia* [5], opium [7], fennel [8], and mentha [3, 4]. Depolymerised carrageenan has been reported to promote major biological functions in plants [28]. It is also argued that the plants might have capacity to recognize the specific oligomers of natural polysaccharides, which trigger the growth, development, and defense responses of plants [11]. It is considered that there is specific structural and size requirement of the oligosaccharide for inducing the physiological processes in plants [11]. However, the phenomenon by which the radiolytically degraded polysaccharides stimulate the growth and development processes in plants still needs further investigations. Relleve et al. [28] suggested that a certain molecular weight of oligomers of degraded carrageenan was required to get optimum growth effects on rice plants. In fact, the biological activity of the irradiated k-carrageenan (at different gamma rays doses) has been studied in potato tissue culture [34].

Seemingly, the ICR-mediated increase in leaf area (Table 1) could trap more sunlight and conduct additional CO_2 to increase the rate of photosynthesis in comparison to control plants. In addition, the ICR-mediated enhancement in the leaf-chlorophyll content might have resulted in increased photosynthetic rate. The increase in photosynthetic rate due to application of irradiated sodium alginate (ISA) and k-carrageenan has been studied previously by several workers [3–5, 7, 8]. 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 [35]. In view of growth promoting effect of ISA, its application could result in an improvement in the growth of plant root and augmentation in shoot elongation, which brought about the increase in plant productivity and improvement in physiological parameters [2, 4, 36]. We presume that like ISA, the ICR might have enabled the plants to respond in somewhat similar mode regarding photosynthetic pigments and photosynthesis. This study also records a significant increase in the activities of NR and CA as a result of ICR application (Table 4). In this regard, our findings resemble to those that claim the synthesis of certain enzymes in the tissue culture of certain plants as a result of application of irradiated polysaccharides [37, 38]. The ICR-mediated improvement in physiological parameters might also justify the increase in plant fresh and dry matter contents in *C. roseus* plants in the present study (Tables 3 and 4; Figure 1). Application of ICR also enhanced the TDC activity positively at both stages (Table 3). It is documented that TDC enzyme played a role in enhancing the production of terpenoid indole alkaloids (TIA) biosynthesis [16, 39].

The ICR-stimulated increase in the growth and other physiological parameters studied might possibly culminate in the maximization of the leaf yield and herbage yield of the plants (Tables 1 and 2). Besides, the improved herbage yield and dry matter production in ICR-treated plants might result due to enhanced water and nutrient uptake from the soil, followed by smooth translocation of photosynthate and other metabolites to the sinks.

Depolymerised carrageenan was also effective in increasing the total alkaloid content as compared to water spray treatment (control). The increase in alkaloid content owing to application of ICR might be ascribed to the increase in the leaf nitrogen content that might have promoted amino acid synthesis leading to the improved alkaloid content in the leaves. To support our results, the promoting effect of the other degraded marine polysaccharides on alkaloids production in the case of opium poppy and *C. roseus* has earlier been reported by Khan et al. and Idrees et al., respectively [7, 31].

In the present study, no increase in the content of vinblastine and vincristine as compared to the control, when ICR concentrations were applied to the plant foliage (Figures 2(a), 2(b), 2(c), 2(d), 3(a), 3(b), 3(c), and 3(d)), was observed. However, ICR increased the overall production of vinblastine and vincristine due to increased production of plant biomass. Apart from this, the content and yield of vindoline was also increased in ICR-treated plants (Figures 3(a), 3(c), 3(b), and 3(d)). Moreover, an increase in the contents of these alkaloids is in accordance with the known fact that exogenous application of plant growth regulators might evoke the intrinsic genetic potential of the plant that may result in improved enzyme activities, uptake of nutrients, enhanced photosynthesis, and improved translocation of photosynthate and other metabolites to the reproductive parts [40]. This sustained increase in the above-mentioned parameters of the ICR-treated plants is expected to result in the maximization of total alkaloid content as well as yield of vincristine and vinblastine and also of vindoline in periwinkle plants in this investigation.

5. Conclusion

It is concluded that the optimized spray concentration ($80\ mg\ L^{-1}$) of irradiated carrageenan could be employed to improve the growth attributes, physiological activities, herbage yield, and content and yield of alkaloids of *C. roseus*. TDC enzyme might potentially be used for the production of terpenoid indole alkaloids biosynthesis. This treatment considerably increased the yield of vincristine, vinblastine, and vindoline in *C. roseus* plants. We hope that the application of radiation-processed carrageenan might be applied as plant growth promoter in future to achieve the desired quality of several medicinal and aromatic plants. Further, this technique is inexpensive and may be safely adopted by scientific community for boosting up the growth, yield, and quality of other medicinal and aromatic 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 the agricultural crops.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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