Industrial Crops and Products 63 (2015) 269–275

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Effects of gamma-irradiated sodium alginate on lemongrass: field trials monitoring production of essential oil

Mohd. Idrees[a,*], Tariq A. Dar[a], M. Naeem[a], Tariq Aftab[a], M. Masroor A. Khan[a], Akbar Ali[a], Moin Uddin[b], Lalit Varshney[c]

[a] Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh 202002, India
[b] Women's College, Botany Section, Aligarh Muslim University, Aligarh 202002, India
[c] Radiation Technology Development Division, ISOMED, Bhabha Atomic Research Centre, Mumbai 400085, India

ARTICLE INFO

Article history:
Received 15 April 2014
Received in revised form
17 September 2014
Accepted 21 September 2014
Available online 18 October 2014

Keywords:
Lemongrass
Essential oil
Citral
Irradiated sodium alginate.

ABSTRACT

Essential oil of lemongrass (*Cymbopogon flexuosus* (Steud.) Wats.) is the major source of the natural citral used in pharmaceutical and cosmetic industries. The role of irradiated sodium alginate (ISA) as plant growth promoter was investigated for essential oil production of this commercially important crop. Two field experiments were conducted for two consecutive years on lemongrass to study the effect of five concentrations of ISA (0, 20, 40, 60, and 80 mg L^{-1}) in terms of plant growth and content and yield of essential oil and citral. Plant analysis was carried out at two growth stages, viz. 120 and 150 days after planting (DAP). There was a significant effect of ISA application on all the parameters including essential oil production and content and yield of citral at both growth stages. An increase of 35.85 and 23.08% was observed in ISA-60 mg L^{-1} over the control at 120 and 150 DAP, respectively. ISA-60 mg L^{-1} proved the best and enhanced essential oil yield by 103.41 and 94.86% over the control at 120 and 150 DAP, respectively. At 120 DAP, ISA-60 mg L^{-1} significantly improved citral yield by 181.03% over the control. Further, gel permeation chromatography (GPC) study revealed formation of lower-molecular-weight oligomers in irradiated samples, which might be responsible for plant growth promotion in the present study.

1. Introduction

The aroma-yielding plants or their distilled volatile oils are known to have been, and in many cases, still are in use in various human activities, from religious ceremonies and adornments, to remedies and personal use, even before the recorded history of mankind. The world trade regarding essential oils is expected to continue to expand tremendously in the foreseeable future, as a consequence of the growing number and preferences of consumers and a wide spectrum of the uses of these essential oil compounds (Sangwan et al., 2001). Among various plants, evaluated for essential oil, lemongrass (*Cymbopogon flexuosus* (Steud.) Wats.) has been recognized as a rich source of essential oil. At present, an ultra-recent radiation-mediated technique has emerged to increase the productivity of these plants. According to this technique, gamma-ray irradiations are employed to degrade and lower down the molecular weight of some natural polysaccharides like alginates, chitosan and carrageenan into small-sized oligomers. These oligomers, when applied to plants in the form of foliar sprays, elicit various kinds of biological and physiological activities, including promotion of plant growth, seed germination, shoot elongation, root growth, flower production, suppression of heavy metal stress, etc. Furthermore, application of these oligomers can shorten the harvesting period of various crops and helps in reducing the use of insecticides and chemical fertilizers (Hien et al., 2000; Ahni et al., 2001; Cabalfin, 2002; Hafiz et al., 2003; Luan et al., 2003). Out of several natural polysaccharides, sodium alginate (SA) is a polysaccharide with its large quantity available in nature. The radiolytically (using gamma rays) degraded oligomers are also reported to have been used as plant growth promoters in the form of foliar sprays (Kume, 2006; Mollah et al., 2009; Khan et al., 2011; Idrees et al., 2011; Aftab et al., 2011; Sarfaraz et al., 2011; Naeem et al., 2012a,b, 2014). Keeping the importance and increasing demand of the essential oil of lemongrass in mind, a hypothesis was designed to realize whether the application of gamma-irradiated sodium

* Corresponding author at: Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh 202002, India. Tel.: +91 9045434866.
E-mail address: idrees.amu@gmail.com (Mohd. Idrees).

http://dx.doi.org/10.1016/j.indcrop.2014.09.037
0926-6690/

alginate (ISA) could be useful as plant growth promoter to enhance the plant productivity as well as production of essential oil and other active constituents in lemongrass.

2. Materials and method

2.1. Plant materials and growth conditions

Two field experiments were conducted on lemongrass according to a simple randomized block design during the winter seasons of 2009 and 2010. The field was divided into the beds of 4 m^2. Slips of lemongrass were transplanted with spacing of 45 × 45 cm. Healthy slips of lemongrass were obtained from Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, UP, India. The soil samples were analyzed in the Soil-Testing Laboratory, Government Agriculture Farm, Quarsi, Aligarh. Physicochemical characteristics of the soil were: texture-sandy loam, pH (1:2) 7.5, E.C. (1:2) 0.48 m mhos $^{-1}$, available N, P and K 102.4, 7.8 and 145.9 mg kg^{-1} of soil, respectively. A uniform recommended basal dose of N, P and K (25.0, 11.0 and 21.0 mg kg^{-1} soil) was applied in the form of urea, single superphosphate and muriate of potash, respectively, at the time of planting. The treatments were consisted of different aqueous concentrations of ISA, viz. 0, 20, 40, 60 and 80 mg L^{-1}.

2.2. Gel permeation chromatography (GPC) analysis

Solid material of sodium alginate (Sigma Aldrich, USA) was sealed in a glass tube with atmospheric air. Sodium alginate samples were irradiated (Cobalt-60, GC-5000) in a Gamma Radiation Chamber (BRIT, Mumbai, India). The samples were irradiated by 520 kGy gamma radiation dose at a dose rate of 2.4 kGy/h. GPC analyses of sodium alginate samples were carried out using Hitachi EMerck HPLC/GPC system equipped with RI detector. The experimental conditions were as follows: mobile phase—water, flow rate—1.5 mL/min, column PL—Aquagel, mixed bed column, 300 mm × 10 mm, 20 μL injection loop. The average molecular weight of the un-irradiated sodium alginate samples was estimated to be about 695,131. Polyvinyl alcohol polymers of known molecular weight were used as standards. Different aqueous concentrations of ISA were finally prepared using double distilled water as spray treatment to the crop.

2.3. Determination of growth attributes

The crop performance was assessed in terms of growth and other physiological parameters, yield characteristics and quality attributes. At 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. The height and fresh weight of plants was measured thereafter.

2.4. Determination of physiological and biochemical attributes

2.4.1. Estimation of total chlorophyll and carotenoid contents

Total chlorophyll and carotenoid contents in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue from interveinal leaf area was grinded with 80% acetone using 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 carotenoid content, respectively, using the spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). Total chlorophyll content was assessed by totalling chlorophyll a and b contents. The photosynthetic pigments, thus measured, were expressed as mg g^{-1} FW.

2.4.2. Determination of carbonic anhydrase (CA) activity

The activity of carbonic anhydrase (EC 4.2.1.1) was measured in fresh leaves, using the method as described by Dwivedi and Randhawa (1974). Two hundred milligrams of fresh leaf pieces were weighed and transferred to Petri plates. The leaf pieces were dipped in 10 mL of 0.2 M cystein hydrochloride solution for 20 min at 4 °C. 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}.

2.4.3. Nitrate reductase (NR) activity

The activity of nitrate reductase (EC 1.6.6.1) was estimated by the intact tissue assay method developed by Jaworski (1971). The amount of nitrite (NO_2^-) formed was determined spectrophotometrically. Fresh pieces of chopped leaves, weighing 200 mg, were transferred to a plastic vial. The reaction mixture, contained in each vial, carried 2.5 mL of phosphate buffer (pH 7.5), 0.5 mL of potassium nitrate solution and 2.5 mL of 5% isopropanol. The vials were incubated for 2 h in dark at 30 °C for the manifestation of maximum enzyme activity. After incubation, 0.4 mL of the content was transferred from the vial to a test tube. To it, 0.3 mL of solution each of 1% sulphanilamide and 0.02% *N*-(1-naphthyl) ethylenediamine dihydrochloride (NED-HCl) was added. The test tubes were kept for 20 min at room temperature for colour development. The OD of the content was recorded at 540 nm using the spectrophotometer. The NR activity was expressed as nanomoles of nitrite produced per gram of fresh weight of leaf tissue per hour (nM NO_2^- g^{-1} FW h^{-1}).

2.4.4. Estimation of nitrogen (N), phosphorus (P) and potassium (K) contents in leaves

Leaf samples from each treatment were digested for the estimation of leaf-N, -P and -K contents. The leaves were dried in a hot air oven at 80 °C for 24 h. The dried leaves were powdered using a mortar and pestle and the powder was passed through a 72-mesh. The sieved leaf-powder was used for the estimation of N, P and K contents. One hundred mg of oven-dried leaf powder was carefully transferred into a digestion tube, to which 2 ml of AR (analytical reagent) grade concentrated sulphuric acid was added subsequently. This solution was heated on a temperature-controlled assembly at 80 °C for about two 2 h and then the content was cooled for about 15 min at room temperature. To the cooled content, 0.5 ml of 30% hydrogen peroxide (H_2O_2) was added. The addition of H_2O_2 was followed by gentle heating of the content as well as its cooling at room temperature. This step was repeated until the content of the tube turned colourless. The aliquot (peroxide-digested material), thus prepared, was used to estimate the per cent N, P and K contents in the leaves on dry weight basis.

2.4.4.1. Determination of N content. Leaf-nitrogen content was estimated according to the method of Lindner (1944) with slight modification by Novozamsky et al. (1983). The dried powder of leaves was digested in H_2SO_4 using a digestion tube. A 10 ml aliquot (peroxide digested material) was poured into a 50 ml volumetric flask. To it, 2 ml of 2.5 N sodium hydroxide and 1 ml of 10% sodium silicate solutions were added to neutralize the excess acid and prevent turbidity. A 5 ml aliquot of the peroxide digested material was poured into a 10 ml graduated test tube followed by addition of 0.5 ml Nessler's reagent. The OD (optical density) of the solution, thus obtained, was recorded at 525 nm using the spectrophotometer.