



Employing depolymerised sodium alginate, triacontanol and 28-homobrassinolide in enhancing physiological activities, production of essential oil and active components in *Mentha arvensis* L

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ABSTRACT

There is immense need of enhancing the content and yield of active constituents of medicinally important plants in view of their massive demand worldwide. Various phytohormones have proved effective in this regard. Gamma-rays irradiated sodium alginate (ISA), triacontanol (TRIA) and 28-homobrassinolide (HBR) have also proven as potent plant growth promoting substances for a number of agricultural and horticultural crops. Considering the medicinal importance, a pot experiment was conducted to explore the individual as well as combined effect of best foliar doses of ISA, TRIA and HBR on growth, yield and quality of mint (*Mentha arvensis* L.). The spray of ISA, TRIA and HBR, applied alone on plants, was positively effective. However, the effect of their combined application was much pronounced as compared to that of their individual application; it improved most of the plant growth attributes, physiological and biochemical parameters, herbage yield and the content and yield of active constituents of mint significantly studied at 100 and 120 DAP. Of the seven spray-treatments [(i) Control, (ii) 100 ppm ISA, (iii) 10^{-6} M TRIA, (iv) 10^{-7} M HBR, (v) 100 ppm ISA + 10^{-6} M TRIA, (vi) 100 ppm ISA + 10^{-7} M HBR, (vii) 100 ppm ISA + 10^{-6} M TRIA + 10^{-7} M HBR], 100 ppm ISA + 10^{-6} M TRIA + 10^{-7} M HBR proved to be the best. The combined application resulted in the highest content and yield of essential oil (EO) over the control by 42.1 and 43.9% and 114.0 and 121.7% at 100 and 120 DAP, respectively. This combined treatment of plant growth regulators (PGRs) also proved the best, increasing the content of menthol, L-menthone, isomenthone and menthyl acetate by 7.5 and 6.2%, 31.8 and 32.1%, 25.8 and 21.7%, and 40.1 and 37.2%, respectively, over the respective control at 100 and 120 DAP. As compared to the control, it increased the per plant yields of menthol, L-menthone, isomenthone and menthyl acetate by 129.9 and 135.5%, 190.0 and 194.4%, 162.5 and 164.3%, and by 225.0 and 187.5% at 100 and 120 DAP, respectively.

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1. Introduction

Gamma-rays irradiation with cobalt-60 degrades the natural polysaccharides, such as chitosan, carrageenan and sodium alginate, into smaller oligomers with comparatively low molecular weight. Oligomers, obtained from radiolytically degraded polysaccharides including those of irradiated sodium alginate (ISA), have valid applications in the field of agriculture, as plant growth promoters (Hien et al., 2000; Kume et al., 2002). Application of the degraded polysaccharides (oligomers) on plants promotes various

biological and physiological activities, including plant growth in general (Hien et al., 2000; Aftab et al., 2011; Khan et al., 2011; Sarfaraz et al., 2011; Naeem et al., 2012a,b), seed germination (Hien et al., 2000), shoot elongation (Natsume et al., 1994; Hien et al., 2000), root growth (Iwasaki and Matsubara, 2000), flower production, antimicrobial activity, amelioration of heavy metal stress, phytoalexin induction, etc. (Darvill et al., 1992; Kume et al., 2002; Luan et al., 2003; Hu et al., 2004).

Triacontanol (TRIA), a long chain primary alcohol ($C_{30}H_{61}OH$), has been realized as a potent plant growth promoting substance for various agricultural and horticultural crops. It increases the rate of several biochemical and physiological processes (Ries and Houtz, 1983; Ries, 1991; Naeem et al., 2009, 2012c) and, thereby, improves the plant growth as well as yield and quality characteristics of the crops (Ries, 1985).

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Recently, brassinosteroids (BRs) have emerged as a new group of growth promoting phytohormones. 28-Homobrassinolide (HBR) is one of the several BRs, the role of which in enhancing growth, productivity and quality of plants, via improving various physiological processes, has been established both under stress and normal conditions (Khrpach et al., 2000; Arora et al., 2011; Zhang et al., 2008; Swamy and Rao, 2011; Sharma et al., 2011; Naeem et al., 2012d).

Out of a large number of EO bearing plants, mint (*Mentha arvensis* L.) constitutes most important source of therapeutic agents used in the alternative systems of medicine (The Wealth of India, 1992). Further, mint oil has wide applications in pharmaceutical, agro-chemical and flavoring industries worldwide (Misra et al., 2000; Tassou et al., 2004). Keeping the importance and increasing demand of mint EO in mind, this study was conducted to find out the best concentration effect (Naeem et al., 2011, 2012a,d) of ISA, TRIA and HBR applied alone or in combination in order to enhance the productivity, physiological activities and production of EO of mint. There is no information regarding the combined effect of ISA, TRIA and HBR application on this medicinally important crop till date.

2. Materials and methods

2.1. Plant materials and growth conditions

The pot experiment was conducted in the natural conditions of the net house at Botany Department, Aligarh Muslim University, Aligarh, India. Prior to transplanting, each pot was filled with 5 kg homogenous mixture of soil and organic (cowdung) manure (4:1). Physico-chemical characteristics of the experimental soil mixture (4 parts soil:1 part cowdung manure) were: texture-sandy loam, pH (1:2) 7.5, E.C. (1:2) 0.48 dSm⁻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 (25:11:21 mg kg⁻¹ soil, respectively) was applied in the form of urea, single superphosphate and muriate of potash, respectively, at the time of planting. The experiment was conducted in randomized block design using earthen pots (25 cm diameter × 25 cm height). Each treatment was replicated five times. Each pot contained a single healthy plant. The pots were watered as and when required.

The best foliar dose of each of the PGRs used was selected on the basis of earlier findings of our study (Naeem et al., 2011, 2012a,d). The individual and combined application of optimized concentrations of ISA (100 ppm), TRIA (10⁻⁶ M) and HBR (10⁻⁷ M) was carried out at 10 days interval when the plants were at 2–3 true leaves stage to find out the most excellent response of mint crop. Total five foliar sprays of ISA, TRIA and HBR were applied to the crop using a hand sprayer. Un-irradiated sodium alginate was not tested in the present study as the chemical gave equal effect with that of control (Naeem et al., 2012a). Seven spray treatments [(i) Control, (ii) 100 ppm ISA, (iii) 10⁻⁶ M TRIA, (iv) 10⁻⁷ M HBR, (v) 100 ppm ISA + 10⁻⁶ M TRIA, (vi) 100 ppm ISA + 10⁻⁷ M HBR, (vii) 100 ppm ISA + 10⁻⁶ M TRIA + 10⁻⁷ M HBR] were applied, using distilled water as absolute control. The crop was harvested at 100 and 120 days after planting.

2.2. Irradiation and gel permeation chromatography (GPC) analysis

Solid form 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 also done accordingly (Naeem et al., 2012a). Different aqueous concentrations of irradiated sodium alginate

(ISA) were finally prepared using double distilled water as spray treatments.

2.3. Determination of growth attributes

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

2.4. Determination of physiological attributes

2.4.1. Estimation of total chlorophyll and carotenoids contents

Total content of leaf chlorophyll and carotenoids was estimated using the method of Lichtenthaler and Buschmann (2001). 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 the contents of chlorophyll a and b. The content of photosynthetic pigments was expressed as mg g⁻¹ leaf FW.

2.4.2. Determination of net photosynthetic rate and stomatal conductance

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

2.4.3. Determination of carbonic anhydrase (CA) activity

The activity of carbonic anhydrase (E.C. 4.2.1.1) was measured in the fresh leaves, using the method described by Dwivedi and Randhawa (1974). Two hundred mg of fresh leaf (chopped leaf-pieces) were transferred to Petri plates. The leaf pieces were dipped in 10 mL of 0.2 M cysteine hydrochloride solution for 20 min at 4 °C. The solution adhering at the cut surfaces of the leaf pieces was then removed with the help of a blotting paper followed by their transfer immediately to a test tube containing 4 mL of phosphate buffer of pH 6.8. To it, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.022% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme activity was expressed as μM CO₂ kg⁻¹ leaf FW s⁻¹.

2.4.4. Total phenol content

Total phenol content was estimated by the method described by Sadasivam and Manickam (2008). Five hundred mg of the leaves were grinded with 10 times volume of 80% ethanol, using mortar-pestle. The homogenate was centrifuged at 10,000 rpm (10,062 × g) for 10 min at 4 °C. The supernatant was evaporated to dryness, adding 5 mL of double distilled water (DDW) thereafter. Later, 0.5 mL of Folin-Ciocalteu Reagent and 2 mL of 20% Na₂CO₃ solution were added to each test tube. The OD of the solution, thus obtained, was measured at 650 nm against a reagent blank. Using the standard curve, the content of total phenols in the test samples was determined as mg phenol per 100 g of dry leaves.

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