Plant Growth Regul (2011) 65:195–206
DOI 10.1007/s10725-011-9588-8

ORIGINAL PAPER

Triacontanol-mediated regulation of growth and other physiological attributes, active constituents and yield of *Mentha arvensis* L.

M. Naeem · M. Masroor A. Khan · Moinuddin · Mohd. Idrees · Tariq Aftab

Received: 20 August 2010 / Accepted: 15 March 2011 / Published online: 26 March 2011

Abstract Triacontanol (TRIA) has been realized as a potent plant growth promoting substance for a number of agricultural and horticultural crops. 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. The mint plant has marvelous medicinal properties. In view of enhancing growth, yield and quality of this medicinally important plant, a pot experiment was conducted according to simple randomized block design. The experiment was aimed at studying the effect of four concentrations of TRIA (10^{-0}, 10^{-7}, 10^{-6} and 10^{-5} M) on the performance of mint with regard to growth and other physiological attributes, crop yield and quality attributes and the yield and contents of active constituents of the plant. The growth and other physiological parameters as well as yield and quality attributes were studied at 100 and 120 DAP. The foliar application of TRIA at 10^{-6} M concentration significantly enhanced most of the growth and other physiological attributes, crop herbage yield and the yield and content of active constituents (menthol, L-methone, isomenthone and menthyl acetate) of mint at both the stages. However, the next higher concentration of TRIA (10^{-5} M) exhibited slightly negative effect and did not further increase the values of the attributes studied, but it proved significantly better than the control. Application of TRIA significantly enhanced the yield and content of all the active constituents determined by GLC technique.

M. Naeem (✉) · M. M. A. Khan · Moinuddin · M. Idrees · T. Aftab
Department of Botany, Plant Physiology Section, Aligarh Muslim University, Aligarh 202 002, India
e-mail: naeem_phd@yahoo.co.in; naeemgaur@gmail.com

Keywords Photosynthesis · CA · Active constituents · TRIA · *Mentha arvensis*

Introduction

Triacontanol (TRIA), a long chain primary alcohol ($C_{30}H_{61}OH$), has been realized as a potent plant growth promoting substance for a number of agricultural and horticultural crops. TRIA, being a plant growth promoter, improves the plant growth as well as yield and quality characteristics of various crops (Ries 1985) and increases the rate of several biochemical and physiological processes (Ries and Houtz 1983; Ries 1991; Naeem et al. 2009, 2010).

The demand for essential oil-bearing medicinal plants have increased exponentially in recent years in both developing and developed countries and are expected to expand tremendously in the foreseeable future. Since the supply of essential oils is severely lagging behind its demand, it is the need of the hour to maximize the essential oil yield of medicinal plants. India is the largest mint oil producer, with an annual production of essential oil of 15,000–20,000 tonns (Chand et al. 2004). India is the world's dominant producer of crude oil of *Mentha arvensis* and is also an exporter of its processed derivative, the natural menthol (Misra et al. 2000).

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, vermifuge, anti-spasmodic, diaphoretic, stomachic, carminative, antiviral, antifungal, antibacterial and choleretic agent (The Wealth of India 1992). Mint oil has a wide application in pharmaceutical, agrochemical and flavouring industries

worldwide (Misra et al. 2000; Tassou et al. 2004). Keeping the importance and increasing demand of the essential oil of mint in mind, a hypothesis was designed to realize whether the application of TRIA could be useful to enhance the productivity, production of essential oil and other active constituents in *Mentha arvensis* L.

The positive role of TRIA in increasing growth, yield and quality as well as physiological processes of various medicinal plants including *Artemisia annua*, *Coriandrum sativum, Cymbopogon flexuosus, Lavandula dentate, Mentha arvensis, Ocimum carnosum, Papaver somniferum* and *Pelargonium* species has earlier been reported by various workers (Shukla et al. 1992; Aftab et al. 2010; Idrees et al. 2010; Misra and Srivastava 1991; Balyan et al. 1994; Srivastava and Sharma 1990, 1991; Gupta et al. 1995; Bhattacharya and Rao 1996). However, there is meager information regarding the effect of TRIA on this medicinally important crop till date except that of Srivastava and Sharma (1991), who reported that TRIA positively increased fresh and dry matter production, photosynthetic characteristics as well as essential oil yield of Japanese mint.

Materials and methods

Plant materials and growth conditions

The experiment was conducted in earthen pots in the natural conditions of the net house at the Botany Department, A.M.U., Aligarh (27°52′N latitude, 78°51′E longitude, and 187.45 m altitude). Healthy rhizomes of *Mentha arvensis* L. were procured from Sambhal, Distt. Moradabad, UP (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, 5 kg homogenous mixture of soil was filled in each pot (25 cm diameter × 25 cm height). Before sowing, 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) was applied in the form of urea, single superphosphate and murate of potash, respectively at the time of planting.

Pot culture

The experiment was conducted arranging the pots according to simple randomized block design. The effect of foliar application of TRIA was determined on the basis of growth, yield and quality attributes and the content of essential oil and active constituents. Totally, five foliar sprays of different concentrations of TRIA (10^{-0}, 10^{-7}, 10^{-6} and 10^{-5} M) were applied at 10 days interval to the crop when the plants were at 2–3 true leaf stage using a hand sprayer. All the attributes studied were determined at 100 and 120 days after planting (DAP). The crop was sown on 10th Feb, 2009 and harvested in the month of June, 2009. Forty five pots were maintained during the experiment. Each treatment was replicated five times. Each pot contained a single healthy plant. Plants were grown under naturally illuminated environmental conditions. The pots were watered as and when required.

Determination of growth attributes

The crop performance was assessed in terms of growth and other physiological attributes, yield and quality attributes, and the levels of active constituents. At 100 and 120 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 height and fresh weight of plant was measured. Each treatment was replicated five times.

Leaf-yield was recorded by weighing all plant leaves using an electronic balance. After taking plant fresh weight, the plants were dried at 80°C for 24 h using a hot air oven and the dry weight of plants was recorded thereafter. The leaf-area was obtained with the help of a graph paper sheet. The 10% of total leaves of each plant sample (consisting of five plants) was determined with the help of graph paper sheet and then mean area per leaf, thus determined, was multiplied with the total number of leaves to measure the leaf area per plant.

Determination of physiological attributes

Estimation of total chlorophyll and carotenoids content

Total chlorophyll and carotenoids content in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue from interveinal leaf area was ground using a mortar and pestle containing 100% acetone. 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 were measured on sunny days at 1100 hours using youngest fully expanded leaves with the help of Infra Red Gas Analyzer (IRGA, Li-Cor 6400 Portable Photosynthesis System Lincoln, Nebraska, USA). Before recording the measurements, the IRGA was calibrated and zero was adjusted approximately every 30 min during the measurement period. Net photosynthetic rate as well as stomatal conductance was recorded three times for each treatment. Photosynthesis was measured at 100 and 120 DAP.

Determination of carbonic anhydrase (CA) activity

Carbonic anhydrase (E.C. 4.2.1.1) activity was measured in fresh leaves, using the method as described by Dwivedi and Randhawa (1974). Two hundred mg 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}.

Estimation of N, P and 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 100°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 100°C for about two 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 colorless. 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.

Determination of N content

Leaf-nitrogen content was estimated according to 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.

Determination of P content

The method of Fiske and Subba Row (1925), with slight modification by Rorison et al. (1993), was used to estimate the leaf-phosphorus content in the peroxide-digested material. A 5 ml aliquot was poured into a 10 ml graduated test tube. To it, 1 ml of molybdic acid (2.5%) was added, followed by addition of 0.4 ml of 1-amino-2-naphthol-4-sulphonic acid. When the colour of the content turned blue, its volume was made up to 10 ml using double distilled water. The OD of the solution, thus obtained, was recorded at 620 nm using the spectrophotometer.

Determination of K content

Leaf-potassium content was determined in the peroxide-digested material by a flame-photometer (Model, C150, AIMIL, India) with the help of emission spectra using specific filter. In the flame-photometer, the solution (peroxide-digested material) was discharged through an atomizer in the form of a fine mist into a chamber, where it was drawn into a flame. Combustion of the elements produced the light of a particular wavelength [λ max for K = 767 nm (violet)]. The light, thus produced, was passed through an appropriate filter to impinge upon a photoelectric cell that subsequently activated a galvanometer.

Total phenol content

Total phenol content was estimated by the method as described by Sadasivam and Manickam (2008). Exactly 1.0 g of the leaf sample was ground using a mortar and pestle with 10 times volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm, saving the supernatant. The supernatant was evaporated to dryness, adding 5 ml of DDW (double distilled water) thereafter. Later, 0.5 ml of Folin–Ciocalteu 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.

Determination of yield and quality attributes

Herbage yield of the crop was measured weighing the total biomass per plant excluding the roots. The quality parameters, including the active constituents, were determined accordingly, estimating the essential oil-yield per plant, menthol yield per plant, L-menthone yield per plant, isomenthone yield per plant and menthyl acetate yield per plant.

Determination of specific gravity

The specific gravity of the essential oil was determined using a 'specific gravity bottle'. The weight of distilled water and the essential oil was determined using a specific gravity bottle at room temperature (25°C). The same bottle was emptied and dried. It was filled with the oil up to the mark and weighed, maintaining the room temperature at 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

The refractive index of the essential oil was determined by the method described by Jenkins et al. (1967) using an Abb'e Refractometer (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 field of view exactly coincided the cross hairs of the telescope. Rotating the compensator prisms, to obtain a sharp uncoloured border line. Then there was noted the refractive index of the oil 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 $N_D^{24^\circ}$. Where $N_D^{24^\circ}$ denotes the index of refraction for the 'D' line (sodium light) measured at 24°.

Isolation and compositional analysis of essential oil

The essential oil content was extracted and determined gravimetrically according to Guenther (1972). The fresh leaves were chopped into small pieces. The essential oil content in the leaves was extracted by distillation for 3 h, using a Clevenger's apparatus and the essential oil was dried over anhydrous sodium sulphate and preserved in sealed glass vials at 4°C for the GLC analysis of the oil.

The active constituents (menthol, L-methone, isomenthone and menthyl acetate) of the essential oil 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. GLC temperature schedule was as follows: detector temperature, 250°C; oven temperature, 160°C; injector temperature, 250°C. The sample size was 2 µl invariably. The identification of active constituents was based on the retention time. They were quantified as per cent content comparing their peaks with the peaks obtained from the reference standards reported in the literature.

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 $P < 0.05\%$ level. Standard error was also employed to separate the means in the tables and figures.

Results and discussion

Growth attributes

The influence of the TRIA spray was significant ($P < 0.05$) on plant height, leaf-area, leaf-yield per plant and fresh and dry weight per plant at 100 and 120 DAP. Of the four TRIA concentrations, the spray of 10^{-6} M TRIA proved the best (Table 1). It increased the values of all the growth attributes over the respective controls both at 100 and 120 DAP. The application of TRIA at 10^{-6} M enhanced the plant height, leaf-area, leaf-yield, plant fresh weight and plant dry weight by 53.8 and 56.6%, 39.6 and 46.5%, 62.3 and 65.7%, 50.3 and 53.6% and 56.7 and 58.6% at 100 and 120 DAP, respectively, compared with the control (Table 1). In contrast, 10^{-5} M TRIA caused an adverse effect on all the growth attributes at both the growth stages and gave significantly lower values in comparison to 10^{-6} M TRIA, but it proved significantly better than the control (Table 1). As observed in this study, a significant enhancement in the values of plant height in the TRIA treated plants could presumably be referred to the well

Table 1 Effect of four concentrations of foliar spray of TRIA (10^{-0}, 10^{-7}, 10^{-6} and 10^{-5} M) on growth attributes of mint (*Mentha arvensis* L.) at 100 and 120 DAP

Growth attributes	TRIA concentrations (M)				
	DAP	10^{-0}	10^{-7}	10^{-6}	10^{-5}
Plant height (cm)	100	72.35 ± 1.31[d]	90.45 ± 1.27[c]	111.25 ± 1.17[a]	108.78 ± 1.14[b]
	120	85.69 ± 1.25[d]	102.60 ± 1.04[c]	134.15 ± 1.05[a]	126.94 ± 0.981[b]
Leaf-area per plant (cm^2)	100	2,950.6 ± 17.4[d]	3,698.3 ± 14.3[c]	4119.0 ± 13.4[a]	3,890.2 ± 17.6[b]
	120	4,684.5 ± 12.8[d]	5,419.0 ± 14.1[c]	6,862.8 ± 15.0[a]	6,574.6 ± 16.6[b]
Leaf-yield per plant (g)	100	14.26 ± 0.125[d]	18.25 ± 0.130[c]	23.15 ± 0.132[a]	22.62 ± 0.115[b]
	120	27.24 ± 0.217[d]	35.14 ± 0.232[c]	45.14 ± 0.231[a]	43.78 ± 0.230[b]
Fresh weight per plant (g)	100	50.93 ± 1.39[d]	65.70 ± 1.45[c]	76.56 ± 1.02[a]	73.25 ± 1.26[b]
	120	62.84 ± 1.18[d]	70.83 ± 1.40[c]	96.52 ± 1.09[a]	90.54 ± 1.06[b]
Dry weight per plant (g)	100	11.76 ± 0.306[c]	14.75 ± 0.589[b]	18.43 ± 0.407[a]	15.74 ± 0.647[b]
	120	15.12 ± 0.381[c]	17.93 ± 0.681[b]	23.98 ± 0.338[a]	19.67 ± 0.618[b]

Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$). Means of five replicates ±SE

known effect of exogenous application of TRIA on elongation of internodes through cell division and expansion (Taiz and Zeiger 2004). With regard to *Mentha arvensis* L., Srivastava and Sharma (1991) found a positive correlation between plant height and fresh and dry weights per plant, with the TRIA affecting the three growth parameters positively. Such a positive effect of TRIA on *Mentha arvensis* L. is in line with our results in this regard. Since these parameters reflect the overall growth of the plant, TRIA might help to boost up the overall growth, yield and quality of the plant. Additionally, the growth-promoting effect of TRIA on various attributes, including height and fresh and dry weights of plant, has earlier been reported by various workers regarding different medicinal plants (Misra and Srivastava 1991; Srivastava and Sharma 1991; Bhattacharya and Rao 1996; Kumaravelu et al. 2000; Muthuchelian et al. 2003; Giridhar et al. 2005; Naeem and Khan 2005; Chaudhary et al. 2006; Khan et al. 2006, 2007, 2009; Sharma et al. 2006; Naeem et al. 2009, 2010; Aftab et al. 2010; Idrees et al. 2010).

Physiological attributes

All the physiological attributes were significantly affected by the application of TRIA at both the growth stages (Fig. 1; Table 2). However, TRIA application did not affect P content at 120 DAP (Fig. 2d). Of the four TRIA concentrations applied, foliar application of TRIA at 10^{-6} M maximally accelerated the rate of photosynthesis and stomatal conductance at 120 DAP, exceeding the control by 20.6 and 23.1% and by 12.0 and 18.0%, respectively. (Fig. 1a, b). Since the stomatal conductance showed considerable improvement in the TRIA treated plants, a significant increase in the rate of photosynthesis could obviously be expected (Fig. 1). Such an increase in photosynthesis has previously been reported as an important plant response to TRIA, which, in turn, might be associated with the increase in leaf chlorophyll content (Ivanov and Angelov 1997; Naeem et al. 2009, 2010). Earlier studies have also revealed a TRIA-mediated increase in the rate of both CO_2 fixation and photosynthesis in *Mentha arvensis* L. (Srivastava and Sharma 1991) as well as in other plants (Srivastava and Sharma 1990; Misra and Srivastava 1991; Ivanov and Angelov 1997; Kumaravelu et al. 2000; Chen et al. 2003; Muthuchelian et al. 2003; Naeem et al. 2009; 2010). However, TRIA-attributed increase in stomatal conductance and carbonic anhydrase activity (that might persuade the significant increase in P_N in this study) is the first report of its kind in the case of *Mentha arvensis* L.

In this study, the exogenous application of TRIA improved the chlorophyll and carotenoids contents significantly. The TRIA, applied at 10^{-6} M concentration, caused a significant increase in the total chlorophyll and carotenoids contents, exceeding the control by 25.2 and 26.7% at 100 DAP and by 14.13 and 15.6% at 120 DAP, respectively (Fig. 1c, d). Compared to that at 10^{-6} M TRIA, the values of the chloroplast pigments decreased at 10^{-5} M TRIA; however, this TRIA concentration gave significantly higher values of the photosynthetic pigments in comparison to the control (Fig. 1a–d). Srivastava and Sharma (1991) also found a significant positive effect of TRIA spray on chlorophyll content of *Mentha arvensis* L. The increased content of chloroplast pigments in TRIA-sprayed leaves could presumably be attributed to the TRIA-attributed increase in the number and size of chloroplasts as revealed by Ivanov and Angelov (1997) and Muthuchelian et al. (2003) in different plants.

Foliar application of TRIA increased carbonic anhydrase (CA) activity, with 10^{-6} M proving the best concentration.

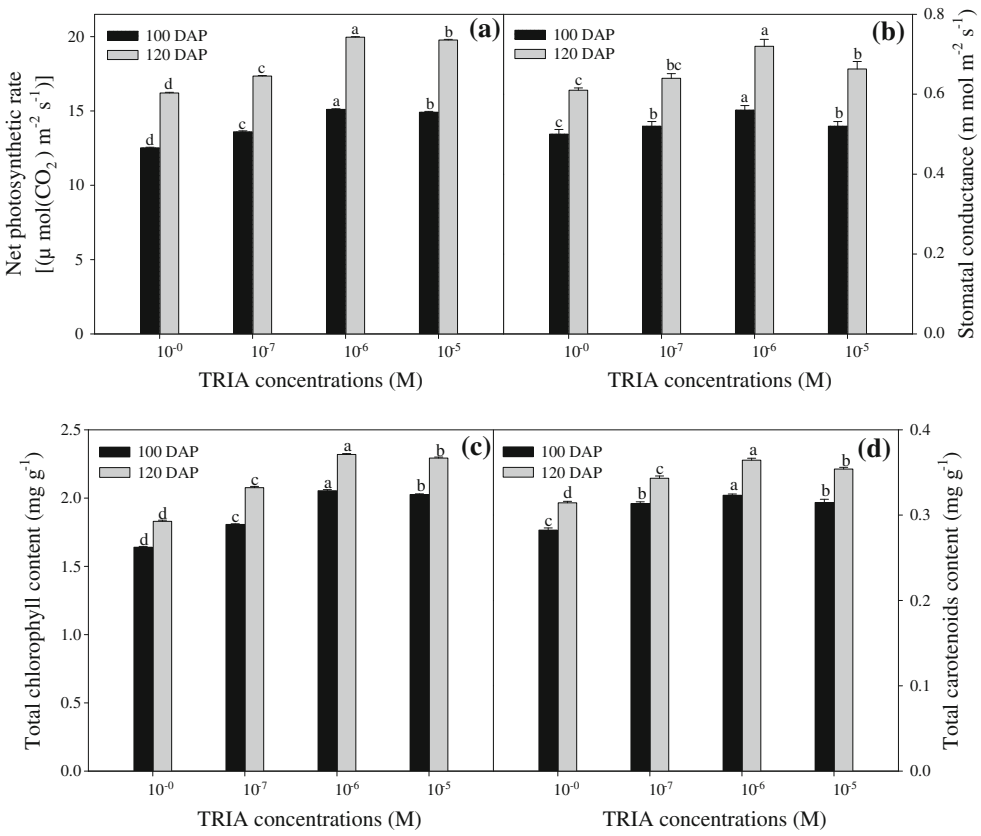

Fig. 1 Effect of four concentrations of foliar sprays of TRIA (10^{-0}, 10^{-7}, 10^{-6} and 10^{-5} M) on net photosynthetic rate (**a**) and stomatal conductance (**b**), total chlorophyll content (**c**) and total carotenoids content (**d**) of mint (*Mentha arvensis* L.) studied at 100 and 120 DAP. Means within a column followed by the *same letter(s)* are not significantly different ($P \leq 0.05$). *Error bars* (⊤) show SE

Table 2 Effect of four concentrations of foliar spray of TRIA (10^{-0}, 10^{-7}, 10^{-6} and 10^{-5} M) on yield and quality attributes of mint (*Mentha arvensis* L.) at 100 and 120 DAP

Yield and quality attributes	TRIA concentrations (M)				
	DAP	10^{-0}	10^{-7}	10^{-6}	10^{-5}
Herbage yield per plant (g)	100	36.40 ± 0.234^{d}	44.62 ± 0.289^{c}	56.48 ± 0.225^{a}	53.30 ± 0.229^{b}
	120	50.72 ± 0.173^{d}	61.39 ± 0.243^{c}	85.59 ± 0.202^{a}	80.38 ± 0.223^{b}
Essential oil-content (%)	100	0.643 ± 0.002^{d}	0.689 ± 0.001^{c}	0.902 ± 0.001^{a}	0.870 ± 0.002^{b}
	120	0.850 ± 0.002^{d}	0.924 ± 0.002^{c}	1.219 ± 0.002^{a}	1.164 ± 0.002^{b}
Essential oil-yield per plant (ml)	100	0.254 ± 0.003^{d}	0.364 ± 0.002^{c}	0.540 ± 0.003^{a}	0.482 ± 0.002^{b}
	120	0.462 ± 0.002^{d}	0.548 ± 0.002^{c}	0.998 ± 0.004^{a}	0.946 ± 0.002^{b}
Specific gravity of essential oil (g/cm^3)	100	0.894 ± 0.002^{a}	0.894 ± 0.001^{a}	0.896 ± 0.001^{a}	0.893 ± 0.001^{a}
	120	0.895 ± 0.001^{a}	0.895 ± 0.001^{a}	0.896 ± 0.001^{a}	0.894 ± 0.001^{a}
Refractive index of essential oil	100	1.461 ± 0.001^{a}	1.460 ± 0.001^{a}	1.462 ± 0.001^{a}	1.461 ± 0.001^{a}
	120	1.463 ± 0.002^{a}	1.462 ± 0.001^{a}	1.465 ± 0.001^{a}	1.464 ± 0.00^{a}

Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$). Means of five replicates ±SE

In the present study, TRIA treated leaves showed improved CA activity, exceeding the control (unsprayed leaves) by 27.5 and 29.4% at 100 and 120 DAP, respectively (Fig. 2a). Such a plant response to TRIA application is expected because TRIA increased the stomatal conductance significantly (Fig. 1b) that might have facilitated a comparatively higher diffusion of carbon dioxide (the substrate for CA) into the stomata. In fact, CA catalyzes the reversible hydration of CO_2, thereby, making available the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) in the chloroplast stroma. The enhancement of CA activity due to TRIA application might also be ascribed to the de novo synthesis of CA, which might involve the genes associated with its transcription and translation in the cells (Okabe et al. 1980).

The enhancement of CA activity in the TRIA treated plants might have, presumably, been responsible for the enhanced rate of CO_2 fixation that, accordingly, could have

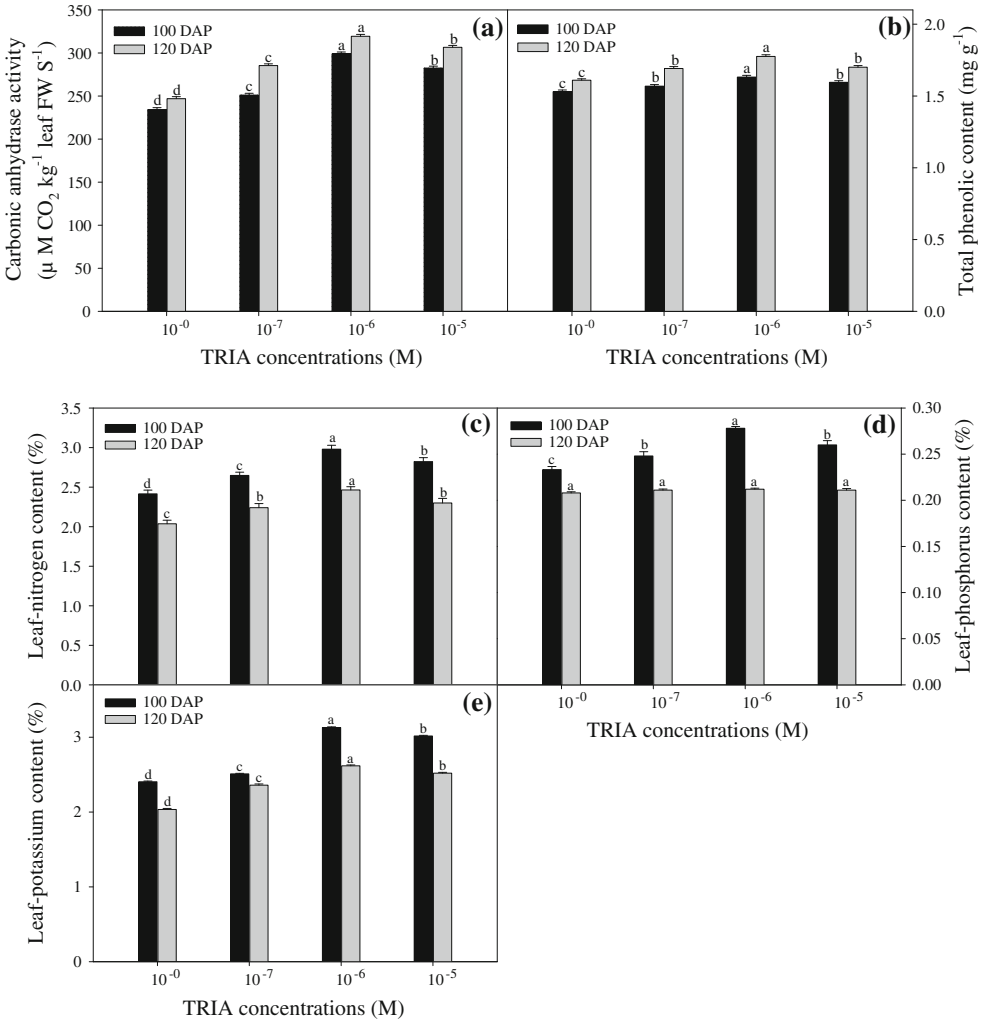

Fig. 2 Effect of four concentrations of foliar sprays of TRIA (10^{-0}, 10^{-7}, 10^{-6} and 10^{-5} M) on carbonic anhydrase activity (**a**) and total phenolic content (**b**) and leaf-nitrogen (**c**), -phosphorus (**d**) and -potassium (**e**) contents of mint (*Mentha arvensis* L.) studied at 100 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

resulted in significant increase in the fresh and dry weights of TRIA-treated plants (Table 1). Such a response of plants to TRIA application has also been reported by Ries and Houtz (1983), Srivastava and Sharma (1990), Misra and Srivastava (1991), Kumaravelu et al. (2000), Muthuchelian et al. (2003), Naeem et al. (2009, 2010) and Idrees et al. (2010).

The TRIA application also improved the leaf-phenolic content at both the sampling stages. In this study, TRIA, applied at 10^{-6} M, increased the total phenolic content maximally. The TRIA application resulted in an increase in phenolic content by 6.7 and 10.3% over the control at 100 and 120 DAP, respectively (Fig. 2b). Thereafter, at 10^{-5} M TRIA, the content of phenols decreased significantly (Fig. 2b). The leaf phenolic contents reflect the free radical scavenging capability of the plant that may help the plant to maintain the normal growth at later growth stages, at which frequent production of free radicals takes place, inducing bad effects of aging (Dimitrios 2006). The significant effect of TRIA on phenol content has also been reported by Kumaravelu et al. (2000) in green gram.

The application of TRIA improved the leaf-N, -P and -K contents at both the growth stages compared to the control. The TRIA, applied at 10^{-6} M, resulted in the highest values; however, in comparison to 10^{-6} M TRIA, 10^{-5} M TRIA gave significantly lower values (Fig. 2c, e). A foliar spray of TRIA at 10^{-6} M increased the leaf-N content by 23.4 and 21.0% over the un-sprayed plants at 100 and 120 DAP, respectively (Fig. 2c). The TRIA applied at 10^{-6} M increased the leaf-P content at 100 DAP by 19.31% (Fig. 2d); while there was no effect of TRIA at 120 DAP in this regard. The control gave lowest leaf-P content at both the stages. As compared to the control, the maximum leaf-K content, recorded at 10^{-6} M TRIA, was 30.1 and 28.7% higher at 100 and 120 DAP, respectively (Fig. 2e). Enhancement in leaf-nutrient contents, particularly the nitrogen, due to TRIA application could be attributed to the compositional or chemical change in plants leading to alterations in nitrogen concentration (Knowles and Ries 1981). The positive results obtained in this study in response to TRIA application might be ascribed to such a

specific role of TRIA in plants. The present findings are in accordance with the results obtained by several other workers in this regard (Knowles and Ries 1981; Ries and Houtz 1983; Kumaravelu et al. 2000; Sharma et al. 2002; Chaudhary et al. 2006; Khan et al. 2006, 2007, 2009; Naeem et al. 2009, 2010; Idrees et al. 2010).

Yield and quality attributes

Of the four TRIA concentrations, 10^{-6} M enhanced the herbage yield maximally, outyielding the control by 55.2 and 68.8% at 100 and 120 DAP, respectively (Table 2). The effect of TRIA was also prominent on content and yield of essential oil in comparison to the non-treated plants. TRIA applied at 10^{-6} M brought about the highest content and yield of essential oil, surpassing the control by 40.3 and 43.4% and 112.6 and 116.0% at 100 and 120 DAP, respectively (Table 2). On the other hand, TRIA application did not improve specific gravity and refractive index of the essential oil significantly at any growth stage (Table 2).

Specific gravity is an important decisive factor as far as the quality and purity of an essential oil is concerned. The specific gravity of mint oil (0.894) in this study lies within the given range of values for essential oils. This study revealed that the refractive index of mint essential oil (1.461) was more than the refractive index of pure water (1.333). Refractive index plays an important role in the elucidation of structure of several constituents of essential oils after their separation and purification. In fact, the refractive index is a physical constant that can be used against the adulteration of drugs as it is helpful to check the identity and purity of a compound.

The growth and essential oil production of aromatic plants may be altered using several plant growth regulators (Singh and Misra 2001; Sangwan et al. 2001). Exogenous application of TRIA has earlier been found beneficial in improving the herbage yield as well as leaf-artemisinin content in *Artemisia annua* (Shukla et al. 1992; Aftab et al. 2010). Srivastava and Sharma (1991) investigated the effect of various levels of TRIA on *Mentha arvenis* L. under control conditions in the glasshouse. They reported that TRIA application at 0.1 g m^{-3} increased the herb yield, essential oil yield as well as fresh and dry matter production of *Mentha arvensis*. In addition, application of TRIA significantly increased the plant height, number of leaves and leaf length of lemongrass (Balyan et al. 1994). Similarly, it increased the values of yield attributes in rose-scented geranium (Bhattacharya and Rao 1996). Being a potent PGR, TRIA is expected to augment the growth, yield and quality of *Mentha arvensis* L. like other PGRs. In this regard, Kewalanand et al. (1998) reported that the values for growth parameters viz., plant height, leaf area, leaf to stem ratio, oil content, fresh herbage, oil yield and menthol content were significantly enhanced due to application of 40 mg l^{-1} GA_3 in Japanese mint. Ethrel and gibberellic acid also influenced the partitioning of primary photosynthetic metabolites and, thus, modified the plant growth and essential oil accumulation in spearmint (Singh et al. 1999). Moreover, there was reported a significant increase in essential oil accumulation in Japanese mint (*Mentha arvensis*) due to the application of chlormequat chloride by Farooqi and Sharma (1988). Likewise, the spirostane analogues of brassinosteroids were found to increase the production of leaves as well as to elevate the levels of essential oils in hydroponically grown mint (Maia et al. 2004).

In the present study, the improved content and yield of essential oil in TRIA treated plants could perhaps be ascribed to the enhanced rates of photosynthesis (Fig. 1b) as pointed out by Srivastava and Sharma (1991) in the case of *Mentha arvensis* L. In fact, a photosynthetic model in relation to essential oil production in *Mentha piperita* was proposed by Burbott and Loomis (1967), which was later modified by Clark and Menary (1980). The model showed that the balance between production and utilization of photosynthates was an important determinant of oil accumulation and major components of the oil. As per the model, the rates of photosynthesis as well as the factors affecting photosynthesis were suggested to be the determining factors regarding oil accumulation (Srivastava and Sharma 1991). Since photosynthesis leads to the increase in total biomass of the plant, Srivastava and Sharma (1991) revealed a positive correlation between P_N and the fresh and dry weights of the plant and argued that significant increase in the oil yield was due to the increase of biomass in TRIA-treated plants of *Mentha arvensis* L. They further claimed that the oil quality (as judged by the menthol/menthone ratio) was practically not affected by TRIA application except at 0.1 and 4 g m^{-3} TRIA, where the change was due to increase and decrease in menthol content, respectively. They maintained that increase in P_N in the mint resulted in the increase in oil percentage and menthol content, whereas the decrease in P_N was accompanied by lower menthol and higher menthone content that led to the positive correlation of P_N with oil content and yield. In the present study, we did not work out these correlations. However, in TRIA-treated plants, we found a progressive increase in content and yield of active components of *Mentha arvensis* as compared to the control, (Fig. 3a–d; Table 3) that might be ascribed to the TRIA-mediated improvement in overall growth of the plants as revealed by TRIA-enhanced leaf-N, -P and -K contents, photosynthesis and growth and other physiological attributes. However, a positive effect of TRIA on mint oil components such as content and yield of isomenthone and

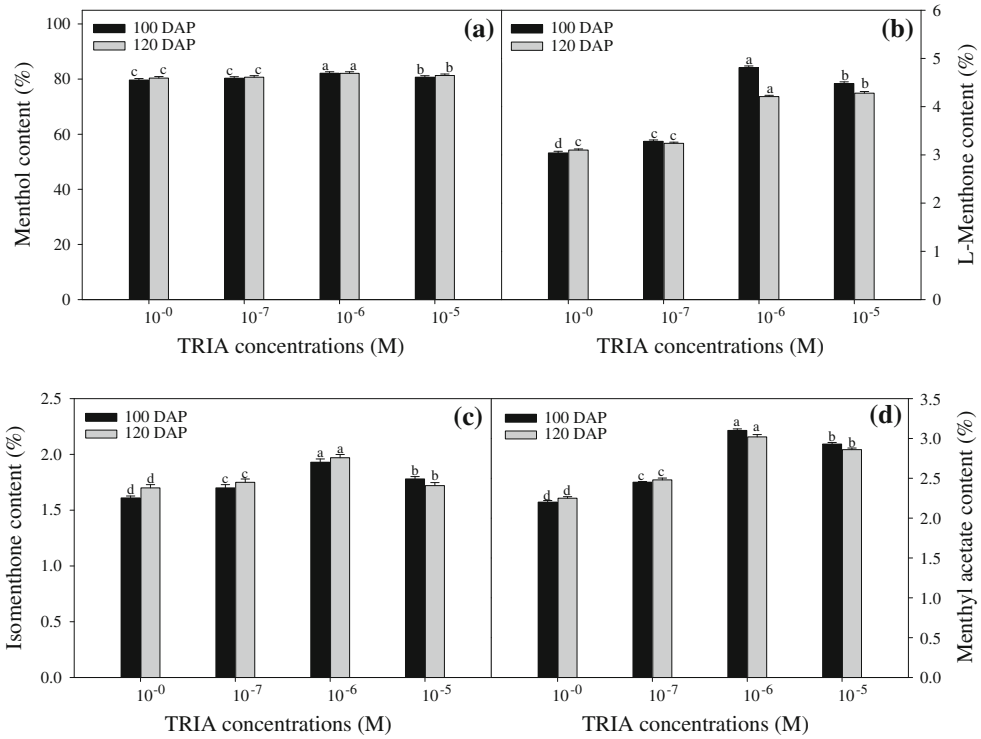

Fig. 3 Effect of four concentrations of foliar sprays of TRIA (10^{-0}, 10^{-7}, 10^{-6} and 10^{-5} M) on menthol content (**a**), L-menthone content (**b**), isomenthone content (**c**), and menthyl acetate content (**d**) of mint (*Mentha arvensis* L.) studied at 100 and 120 DAP. Means within a column followed by the *same letter(s)* are not significantly different ($P \leq 0.05$). *Error bars* (⊤) show SE

Table 3 Effect of four concentrations of foliar spray of TRIA (10^{-0}, 10^{-7}, 10^{-6} and 10^{-5} M) on yield of active constituents of mint (*Mentha arvensis* L.) at 100 and 120 DAP

Quality attributes	TRIA concentrations (M)				
	DAP	10^{-0}	10^{-7}	10^{-6}	10^{-5}
Menthol yield per plant (ml)	100	0.202 ± 0.004^{d}	0.292 ± 0.005^{c}	0.443 ± 0.003^{a}	0.389 ± 0.004^{b}
	120	0.371 ± 0.004^{d}	0.442 ± 0.004^{c}	0.819 ± 0.005^{a}	0.769 ± 0.003^{b}
L-Menthone yield per plant (ml)	100	0.008 ± 0.002^{d}	0.012 ± 0.004^{c}	0.026 ± 0.004^{a}	0.022 ± 0.004^{b}
	120	0.014 ± 0.003^{d}	0.018 ± 0.004^{c}	0.042 ± 0.004^{a}	0.041 ± 0.003^{b}
Isomenthone yield per plant (ml)	100	0.004 ± 0.002^{d}	0.006 ± 0.002^{c}	0.010 ± 0.003^{a}	0.009 ± 0.001^{b}
	120	0.008 ± 0.002^{d}	0.010 ± 0.003^{c}	0.020 ± 0.002^{a}	0.016 ± 0.003^{b}
Menthyl acetate yield per plant (ml)	100	0.006 ± 0.001^{d}	0.009 ± 0.001^{c}	0.017 ± 0.002^{a}	0.014 ± 0.002^{b}
	120	0.010 ± 0.002^{d}	0.014 ± 0.002^{c}	0.030 ± 0.002^{a}	0.027 ± 0.003^{b}

Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$). Means of five replicates ±SE

menthyl acetate have been reported for the first time in this study.

Of the four concentrations, 10^{-6} M TRIA proved the best, increasing significantly the contents of menthol, L-menthone, isomenthone and menthyl acetate by 3.11 and 2.18, 58.22 and 35.81, 19.87 and 15.88 and 40.91 and 34.22% over the corresponding control at 100 and 120 DAP, respectively (Table 3). As compared to the control, 10^{-6} M TRIA increased the per plant yields of menthol, L-menthone, isomenthone and menthyl acetate by many folds, enhancing the values of these components by 119.3 and 120.8%, 225.0 and 200.0%, 150.0 and 150.0% and 183.3 and 200.0% at 100 and 120 DAP, respectively (Table 3). That TRIA application significantly increased the menthol and menthone contents of the mint has also been reported by Srivastava and Sharma (1991). In fact, it has earlier been reported that the composition of the essential oil of *Mentha arvensis* L. might be altered using plant growth regulators (Srivastava and Sharma 1991; Singh et al. 1999; Sangwan et al. 2001; Swamy and Rao 2009). Like that mediated by TRIA, the increase in the essential oil content in lavender, spearmint, Japanese mint, and coriander as a result of application of EBL, ethrel and gibberellic acid has also been reported by several workers (Srivastava and Sharma 1991; Youssef and

Talaat 1998; Singh et al. 1999; Swamy and Rao 2009; Idrees et al. 2010).

The positive effect of TRIA on essential oil yield might be attributed to the TRIA-improved plant growth and metabolism as revealed by study. It seems that TRIA might enhance the intrinsic genetic potential of the mint plants to produce additional quantity of essential oil. The enhanced essential oil yield in TRIA-treated leaves might also be ascribed to the increased uptake of leaf-nutrients (N, P and K) that could have been subsequently used to enhance the formation of photosynthates and other metabolites with regard to oil formation. In fact, TRIA induces the activation of a number of membrane bound enzymes (Ries and Houtz 1983; Savithiry et al. 1992). The stimulation of these enzymes leads to dephosphorylation of L(+) forms of AMP, ADP and ATP, resulting in the formation of 9-β-L(+) adenosine, which triggers the cascade of events leading to rapid physiological responses (Ries et al. 1990; Ries 1991). Ries and Houtz (1983) suggested that TRIA, like other plant hormones, might activate enzymes or alter the function of cell membranes, which could trigger cascading effects resulting in increased metabolism and enhanced accumulation of various critical intermediate compounds. Thus, TRIA-enhanced plant growth, photosynthesis (and the related parameters such as leaf nutrients, chloroplast pigments and carbonic anhydrase activity) and the overall plant metabolism might have accounted significantly for oil accumulation in the present study. These results corroborate the findings of Shukla and Farooqi (1990), Srivastava and Sharma (1991), Ries and Houtz (1983), Khan et al. (2006, 2007, 2009) and Idrees et al. (2010) regarding various medicinal plants.

Srivastava and Sharma (1991) maintained that inhibitory effect of high dose of TRIA (4 g m^{-3}) on the oil yield was largely due to its negative effect on biomass production and that there was no direct effect of TRIA on biosynthesis of oil or its quality. Further, they added that inhibitory effect of high TRIA dose on photosynthetic rate (and ultimately on fresh and dry matter yield and the content of oil and its yield) was largely due to the toxic effect of Cu and Zn that, unlike Fe and Mn, continued to increase in the mint leaves even at higher doses of TRIA. Such a negative effect of higher dose of TRIA (10^{-5} M), in comparison to that of 10^{-6} M TRIA, might have also possibly lowered the values of various parameters in our study. However, in our study, this TRIA dose was significantly better than the control.

Conclusion

The foliar spray of TRIA enhanced the values of all the attributes studied, improving the overall performance of the crop. It improved significantly the crop growth and yield, photosynthesis and other physiological attributes as well as the content and yield of active constituents. The optimum concentration of TRIA (10^{-6} M), brought out by the present study, might presumably be considered applicable for maximizing the productivity and quality of mint that is used as an important drug in the modern as well as alternative systems of medicine. Thus, application of TRIA as spray could be used to enhance the crop productivity as well as the production of essential oil and other active constituents in *Mentha arvensis* L.

Acknowledgments Financial support by the Science and Engineering Research Council, Department of Science & Technology, Government of India, New Delhi, for the award of Young Scientist to Dr. Mu. Naeem (Project No. SR/FT/LS-003/2008) is also gratefully acknowledged.

References

Afaq SH, Tajuddin, Siddiqui MMH (1994) Standardization of herbal drugs. A.M.U. Press, Aligarh

Aftab T, Khan MMA, Idrees M, Naeem M, Singh M, Ram M (2010) Stimulation of crop productivity, photosynthesis and artemisinin production in *Artemisia annua* L. by triacontanol and gibberellic acid application. J Plant Interact 4:273–281

Balyan SS, Pal S, Prabhu D (1994) Triacontanol effect on growth and yield parameters on CKP-25 variety lemongrass. Indian Perfum 38:60–64

Bhattacharya AK, Rao BR (1996) Effect of triacontanol and mixatalol on rose scented geranium (*Pelargonium* sp.). J Essent Oil Res 8:383–388

Burbott AJ, Loomis WD (1967) Effects of light and temperature on the monoterpenes of pepper mint. Plant Physiol 42:20–28

Chand S, Patra NK, Anwar M, Patra DD (2004) Agronomy and uses of menthol mint (*Mentha arvensis*)—Indian perspective. Proc Indian Natural Sci Acad B70(3):269–297

Chaudhary BR, Sharma MD, Shakya SM, Gautam DM (2006) Effect of plant growth regulators on growth, yield and quality of chilli (*Capsicum annum* L.) at Rampur, Chitwan. J Inst Agric Anim Sci 27:65–68

Chen X, Yuan H, Chen R, Zhu L, He G (2003) Biochemical and photochemical changes in response to triacontanol in rice (*Oryza sativa* L.). Plant Growth Regul 40:249–256

Clark RJ, Menary RC (1980) Environmental effects on peppermint (*Mentha piperita* L.). II. Effects of temperature on photosynthesis, photorespiration and dark respiration in peppermint with reference to oil composition. Aust J Plant Physiol 7:693–697

Dimitrios B (2006) Sources of natural phenolic antioxidants. Trends Food Sci Technol 17:505–512

Dwivedi RS, Randhawa NS (1974) Evaluation of rapid test for the hidden hunger of zinc in plants. Plant Soil 40:445–451

Farooqi AHA, Sharma S (1988) Effect of growth retardants on growth and essential oil content in Japanese mint. Plant Growth Regul 7:39–45

Fiske CH, Subba Row Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66:375–400

Giridhar P, Rajasekara T, Ravishanka GA (2005) Improvement of growth and root specific flavour compound 2-hydroxy-4-methoxy benzaldehyde of micropropagated plants of *Decalepis*

hamiltonii Wight & Arn., under triacontanol treatment. Scientia Hort 106:228–236

Guenther E (1972) The essential oils: history-origin in plants production-analysis, vol 1. Robert E. Krieger Publishing Company, Huntington

Gupta S, Kumar A, Khosla MK (1995) Physiological analysis of growth in *Ocimum carnosum* L.K. et. Otto. with triacontanol treatment. Indian Perfum 39:107–111

Idrees M, Khan MMA, Aftab T, Naeem M (2010) Synergistic effects of gibberellic acid and triacontanol on growth, physiology, enzyme activities and essential oil content of *Coriandrum sativum* L. The Asian Aust J Plant Sci Biotechnol 4:24–29

Ivanov AG, Angelov MN (1997) Photosynthesis response to triacontanol correlates with increased dynamics of mesophyll protoplast and chloroplast membranes. Plant Growth Regul 21:145–152

Jenkins GL, Knevel AM, Digangi FE (1967) Quantitative pharmaceutical chemistry, 6th edn. McGraw Hill Book Company, London

Kewalanand, Singh JN, Pandey CS (1998) Effect of plant growth regulators on the growth, herbage and oil yield of Japanese mint (*Mentha arvensis*) and its economics there from. J Med Arom Plant Sci 20:725–730

Khan MMA, Mujibur-Rahman M, Naeem M, Mohammad F, Siddiqui MH, Khan MN (2006) Triacontanol-induced changes in the growth yield and quality of tomato (*Lycopersicon esculentum* Mill.). Electron J Environ Agric Chem 5:1492–1499

Khan R, Khan MMA, Singh M, Nasir S, Naeem M, Siddiqui MH, Mohammad F (2007) Gibberellic acid and triacontanol can ameliorate the optimum yield and morphine production in opium poppy (*Papaver somniferum* L.). Acta Agric Scand Section B Soil Plant Sci 57:307–312

Khan MMA, Bhardwaj G, Naeem M, Moinuddin, Mohammad F, Singh M, Nasir S, Idrees M (2009) Response of tomato (*Lycopersicon esculentum* Mill.) to application of potassium and triacontanol. Acta Hort (ISHS) 823:199–207

Knowles NR, Ries SK (1981) Rapid growth and apparent total nitrogen increases in rice and corn plants following applications of triacontanol. Plant Physiol 68:1279–1284

Kumaravelu G, David LV, Ramanujam MP (2000) Triacontanol-induced changes in the growth, photosynthetic pigments, cell metabolites, flowering and yield of green gram. Biol Planta 43:287–290

Lichtenthaler HK, Buschmann C (2001) Extraction of photosynthetic tissues: chlorophylls and carotenoids. Curr Protoc Food Anal Chem F4.2.1–F4.2.6

Lindner RC (1944) Rapid analytical methods for some of the more common inorganic constituents of the plant tissues. Plant Physiol 19:76–89

Maia NB, Bovi OA, Zullo MAT, Perecin MB, Granja NP, Carmello QAC, Robaina C, Coll F (2004) Hydroponic cultivation of mint and vetiver with spirostane analogues of brassinosteroids. Acta Hortic (ISHS) 644:55–59

Misra A, Srivastava NK (1991) Effects of the triacontanol formulations "Miraculan" on photosynthesis, growth, nutrient uptake, and essential oil yield of lemongrass (*Cymbopogon flexuosus*) Steud, Watts. Plant Growth Regul 10:57–63

Misra PN, Hasan SA, Kumar S (2000) Cultivation of aromatic plants in India, 1st edn. Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow

Muthuchelian K, Velayutham M, Nedunchezhian N (2003) Ameliorating effect of triacontanol on acidic mist-treated *Erythrina variegata* seedlings changes in growth and photosynthetic activities. Plant Sci 165:1253–1257

Naeem M, Khan MN (2005) Effect of foliar spray of triacontanol on growth performance of hyacinth bean. Bionotes 7:62

Naeem M, Khan MMA, Moinuddin, Siddiqui MH (2009) Triacontanol stimulates nitrogen-fixation, enzyme activities, photosynthesis, crop productivity and quality of hyacinth bean (*Lablab purpureus* L.). Sci Hortic 121:389–396

Naeem M, Idrees M, Aftab T, Khan MMA, Moinuddin (2010) Changes in photosynthesis enzyme activities and production of anthraquinone and sennoside content of coffee senna (*Senna occidentalis* L.) by triacontanol Internat. J Plant Develop Biol 4:53–59

Novozamsky I, Houba VJG, van Eck R, van Vark W (1983) A noval digestion technique for multi-element plant analysis. Commun Soil Sci Plant Anal 14:239–248

Okabe K, Lindlar A, Tsuzuki M, Miyachi S (1980) Effects of carbonic anhydrase on ribulose 1,5-biphosphate carboxylase and oxygenenase. FEBS Lett 114:142–144

Ries SK (1985) Regulation of plant growth with triacontanol. Critical Rev Plant Sci 2:239–285

Ries S (1991) Triacontanol and its second messenger 9-β-L(+)-adenosine as plant growth substances. Plant Physiol 95:986–989

Ries S, Houtz R (1983) Triacontanol as a plant growth regulator. HortScience 18:654–662

Ries S, Wert V, O'Leary NFD, Nair M (1990) 9-β-L(+)-adenosine: a new naturally occurring plant growth substance elicited by triacontanol in rice. Plant Growth Regul 9:263–273

Rorison IH, Spencer RE, Gupta PL (1993) Chemical analysis. In: Hendry GAE, Grime JP (eds) Methods in comparative plant ecology. Chapman and Hall, New York, pp 156–161

Sadasivam S, Manickam A (2008) Biochemical methods, 3rd edn. New Age International (P) Ltd Publishers, New Delhi

Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS (2001) Regulation of essential oil production in plants. Plant Growth Regul 43:3–21

Savithiry S, Wert V, Ries S (1992) Influence of 9-β-L(+)-adenosine on malate dehydrogenase activity in rice. Physiol Plant 84:460–466

Sharma MK, Joolka NK, Sharma N (2002) Effect of triacontanol and paclobutrazol on photosynthetic efficiency, carbohydrate metabolism and leaf nutrient status of nonpareil almond. Progress Hortic 34:117–118

Sharma K, Kaur H, Thind SK (2006) Kinetin and triacontanol effects on leaf characteristics, nitrate reductase activity nodulation and yield in soybean *Glycine max* (L.) Merrill under reduced light intensity. Environ Ecol 24:426–429

Shukla A, Farooqi AHA (1990) Utilization of plant growth regulators in aromatic plant production. Curr Res Med Arom Plants 12:152–157

Shukla A, Farooqi AHA, Shukla YN, Sharma S (1992) Effect of triacontanol and chlormequat on growth, plant hormones and artemisinin yield in *Artemisia annua* L. Plant Growth Regul 11:165–171

Singh P, Misra A (2001) Influence of gibberellin and ethrel on growth chlorophyll content and enzyme activities and essential monoterpene oil in an efficient genotype of *Mentha spicata* var. MSS-5. J Med Arom Plant Sci 22 & 23:283–286

Singh P, Srivastava NK, Mishra A, Sharma S (1999) Influence of ethrel and gibberellic acid on carbon metabolism, growth, essential oil accumulation in spearmint (*Mentha spicata*). Phtosynthetica 36:509–517

Srivastava NK, Sharma S (1990) Effect of triacontanol on photosynthesis, alkaloid content and growth in opium poppy (*Papaver somniferum* L.). Plant Growth Regul 9:65–71

Srivastava NK, Sharma S (1991) Effect of triacontanol on photosynthetic characters and essential oil accumulation in Japanese mint (*Mentha arvensis* L.). Photosynthetica 25:55–60

Swamy KN, Rao SSR (2009) Effect of 24-epibrassinolide on growth, photosynthesis and essential oil content of *Pelargonium graveolens* (L.) Herit. Russ J Plant Physiol 56:616–620

Taiz L, Zeiger E (2004) Plant physiology, 4th edn. Sinauer Associates, Inc., Publishers, Sunderland

Tassou CC, Nychas GJE, Skandamis PN (2004) Herbs and spices and antimicrobials. In: Peter KV (ed) Handbook of herbs and spices. Woodhead Publishing Limited, Abington, Cambridge, UK, pp 35–53

The Wealth of India (1992) Raw materials. In: Ambusta CS (ed) Publication and information directorate, vol 2. CSIR, New Delhi, pp 349–352

Youssef AA, Talaat IM (1998) Physiological effect of brassinosteroids and kinetin on the growth and chemical constituents of lavender plant. Ann Agric Sci (Cairo) 43:261–272