○ **Chemical and Biological Technologies in Agriculture**

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Effects of boron toxicity on growth, oxidative damage, antioxidant enzymes and essential oil fingerprinting in *Mentha arvensis* and *Cymbopogon flexuosus*

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Abstract

Background: Boron (B) is an essential micronutrient and necessary to plants for their proper growth. The concentration of B in soil and plant is greatly critical for the growth and productivity. Even at the low amount available in soils, B can be extremely toxic to plants especially in semi-arid and arid environments. In the present study, the effects of high B concentrations (2.5, 5, 10, 20 and 30 mg/kg) were elucidated on two important essential oil-bearing plants; *Mentha arvensis* and *Cymbopogon flexuosus* which are aromatic and antimicrobial herbs having well-known medicinal values.

Results: Application of different concentrations of B showed growth inhibitory effects on plant as evident by shoot and root lengths, fresh and dry weights of shoot in the studied plants. Treatments of B also reduced the total chlorophyll and carotenoid content, chlorophyll fluorescence and reduced the activities of carbonic anhydrase and nitrate reductase enzymes. Moreover, B stress considerably increased the proline content and lipid peroxide content as compared to control. The activities of antioxidant enzymes like catalase, peroxidase and superoxide dismutase were also significantly increased under B stress. The content of essential oil of *M. arvensis* and *C. flexuosus* increased at 2.5 mg/kg of B and decreased with further increase in concentrations of B.

Conclusion: The findings of present work suggest that increasing concentrations of B inhibited growth and photosynthetic pigments, increased oxidative damage and activities of antioxidant enzymes; however, a mild stress of B increased essential oil production in *M. arvensis* and *C. flexuosus* plants.

Keywords: Aromatic plants, Boron, Essential oil, Oxidative damage

Background

Boron (B) is an important micronutrient and plays a crucial role in the growth and development of plants [1]. Boron plays significant role in several processes that are necessary for the growth of higher plants such as cell wall synthesis, lignification process, auxin metabolism, sugar and carbohydrate metabolism, photosynthesis, nucleic acid metabolism and several enzyme activities

[2, 3]. Depending on the type of soil, amount of organic manure and precipitation, B concentration varies ranging from 10 to 300 kg^{-1} [4]. Application of B-rich fertilizers can overcome the deficiency of B, but to alleviate morbidity is a major issue [5]. High B levels in the soil, particularly in arid and semi-arid regions inhibit plant growth and decrease crop yield and quality [6]. B toxicity causes chlorosis and necrosis in the marginal regions of mature leaves [7], structural damage to thylakoids thus inhibits photosynthesis and limits CO_2 uptake [8]. Mechanisms of B toxicity found in literature are: (1) changes in the cell wall structure, (2) alteration in the function of

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energy molecules, such as ATP, and (3) binding to RNA in order to interrupt the mechanism of cell division and expansion [9, 10]. Also, B toxicity disturbs electron transport, provides molecular oxygen as an alternative source for non-utilized electrons and light energy, leading to the overproduction of reactive oxygen species (ROS) such as superoxide (O_2^-), hydroxyl (OH^-) radicals, that causes membrane damage, that eventually leads to the cell death [11, 12]. An increase in concentration of ROS under high B concentration was found in tomato [13] and *Artemisia annua* [14–16]. Increase in the concentration of ROS in response to B toxicity, has been reported in many crops such as apple root stock [17], tomato [18], wheat [19], and barley [20]. Moreover, accumulation of hydrogen peroxide (H_2O_2) and an increase in lipid peroxidation in the cells and tissues caused by B toxicity has been reported in many plants [17–19, 21]. Ochiai et al. [22] compared B tolerance and intolerance in barley cultivars, and concluded that metabolite was insufficient to explain the tolerance mechanism in tolerant cultivar. Moreover, it was found that roots actively participate in the efflux of B, and accumulate low B in both root and shoots, which can be the reason of tolerance in barley [22]. However, our knowledge of B toxicity effects on essential oil-bearing plants is very limited.

Mentha arvensis L. (Lamiaceae), usually known as corn mint, field mint, wild mint or menthol mint and *Cymbopogon flexuosus* (Steud.) Wats., commonly known as lemongrass, are two major essential oil-bearing plants mainly cultivated in tropical and subtropical regions. In the foreseeable future, the global market of essential oils is expected to increase enormously, due to wide spectrum of the uses of these essential oil compounds, results in the growing number and preferences of consumers [23]. Among all the species of genus *Mentha*, essential oil of *M. arvensis* has the maximum share in global mint market, due to highest content of menthol (by product), used in pharmaceutical, cosmetics, perfumery and food industry [24]. The main constituent of *C. flexuosus* essential oil is citral, which provides lemon or rose-like smell to the plant, used in flavor, cosmetics, perfumery and pharmaceuticals industries [25]. Under B deficit conditions, growth of *M. arvensis* was severely affected [26], grain set failure [27] and reduced reproductivity was noted in wheat [28]. Micronutrients generally found to play significant role in growth, yield and oil content of essential oil-bearing plants and can affect oil quality and quality as they are involved in photosynthesis and utilize photosynthates, which are possibly involved in storage, synthesis and catabolism of monoterpenes [26]. Scarcity of boron, copper, manganese and molybdenum had positive effects on menthol content while menthone content showed negative effects in *M. arvensis* [26]. Pande et al.

[29] narrated positive effects of iron on growth, chlorophyll content and oil yield in menthol mint. Differential effects of magnesium, manganese, iron, zinc and boron on growth and oil content was observed in *Cymbopogon martini* [30]. In three species of *Cymbopogon*, viz. *C. flexuosus*, *C. martini* and *C. winterianus*, cramped supplementation of manganese, iron, molybdenum, copper and zinc showed stimulated growth and oil yield in lemongrass [31]. Essential oil of both the plants have immense pharmaceutical value and the main objective of the current study is to document the morphological and biochemical changes including essential oil, in the plants under various concentrations of B, as no such studies have been performed earlier.

Materials and methods

Plant material and stress treatment

Mentha arvensis plants have been initiated from rhizome segments while *C. flexuosus* plants were commenced from slips of the plants obtained from CIMAP, Lucknow. The plants were grown in a naturally illuminated greenhouse using pots with a diameter of 25 cm, containing 5.0 kg consistent mixture of farmyard manure and soil (1:4). Physiochemical characteristics of soil; texture: sandy loam, pH: 8.0, EC (1:2): 0.48 m mhos/cm, available N, P and K: 97.46, 10.21 and 147.0 mg/kg soil, respectively.

Available B in the experimental soil was 0.5 mM, which is considered as safe limit for the agricultural crops. Boron was applied to the soil at the concentration of 0 (control) 2.5, 5, 10, 20 and 30 mg/kg soil, in the form of boric acid (H_3BO_3), 1 month after transplantation. Every treatment was replicated five times having five plants in each replicate. The experiment was conducted following simple randomized block design, and the plants were sampled 45 days after treatment for the different parameters.

Measurement of the growth characteristics

Plants were uprooted from the pots, washed to remove soil and all adhered foreign particles. Then, the plants were surface dried using blotting paper and then root and shoot height was recorded. Roots of the plants were removed carefully and the fresh weight of the shoot was recorded using an electronic balance. Dry weight of the plants was recorded after the samples were dried in hot air oven for 48 h at 80 °C.

Estimation of boron in plant tissues

B contents in the roots and leaves were determined by azomethine-H method [32]. The plant samples were reduced to dry ash in a muffle furnace at 500 °C for 6 h, and the carbon-free residue was then dissolved in 0.1 M

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HCl. Root and leaf B contents were determined in terms of milligrams per kilogram.

Determination of photosynthetic parameters

Total chlorophyll and carotenoid content were measured in the fully expanded young leaves by the method of [33]. Briefly, leaf tissue was ground in 80% acetone and then the absorbance of the extracted pigments was recorded at 662 nm and 645 nm for chlorophyll and at 480 nm for carotenoid content using a spectrophotometer (Shimadzu UV 1700, Tokyo, Japan). The photosynthetic pigments measured thus were expressed as mg g^{-1} FW.

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

Estimation of activities of carbonic anhydrase and nitrate reductase

The activity of carbonic anhydrase (CA) was measured by the method of Dwivedi and Randhawa [34] while the activity of nitrate reductase (NR) was measured by the method developed by Jaworski [35] as described in our previous paper [14].

Determination of proline

The proline content was estimated using the method of Bates et al. [36]. The leaf material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000×g rpm. The supernatant was used to estimate the proline content. The reaction mixture, consisting of 2 mL of acid ninhydrin and 2 mL of glacial acetic acid, was boiled at 100 °C for 1 h. After terminating the reaction in an ice bath, the proline was extracted with 4 mL of toluene and its absorbance was recorded at 520 nm.

Estimation of lipid peroxidation rate (TBARS content)

Lipid peroxidation in leaves was determined by the content of total 2 thiobarbituric acid reactive substances as described by the method of Cakmak and Horst [37]. TBARS were extracted from 500 mg freshly chopped leaves, ground with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The mixture is then centrifuged at 12,000×g for 5 min, an aliquot of 1 mL of the supernatant was added to 4 mL of 0.5% (w/v) TBA in 20% (w/v) TCA. Samples were incubated at 90 °C for 30 min. Thereafter, the reaction was stopped using an ice bath. The mixture was centrifuged at 10,000×g for 5 min, and the absorbance of the supernatant was recorded at 532 nm with the help of a spectrophotometer and the values were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. TBARS content was expressed as nanomoles per gram FW.

Antioxidant enzymes assay

Fresh leaf tissue was homogenized with an extraction buffer containing 0.5% (v/v), Triton-X-100 and 1% (w/v) polyvinyl pyrrolidone in potassium-phosphate buffer (100 mM, pH 7.8) using chilled mortar and pestle. The homogenate was centrifuged at 15,000×g for 5 min at 4 °C. The supernatant obtained after centrifugation was used for the assay of catalase, peroxidase and superoxide dismutase.

Catalase (CAT) activity was measured according to the method given by Chandlee and Scandalios [38] with a minor modification. The enzyme activity was expressed in units per milligram protein ($U = 1$ mM H_2O_2 reduced per minute per milligram protein).

Peroxidase activity (POX) was assayed by the method of Kumar and Khan [39]. The activity was expressed in units per milligram protein. One unit of the enzyme activity corresponded to an amount of the enzyme that caused an increased in the absorbance by 0.1 min^{-1} mg^{-1} protein.

Superoxide dismutase (SOD) activity was assayed by the method of Beauchamp and Fridovich [40]. One unit is defined as the amount of change in the absorbance by 0.1 h^{-1} mg^{-1} protein.

Determination of essential oil (EO) attributes

The content and quality attributes were determined in terms of content of EO per treatment and menthol and methyl acetate content in *M. arvensis* and citral and geraniol content in *C. flexuosus.*

The EO was extracted through hydro-distillation method using Clevenger's apparatus (Borosil, India) and then quantified gravimetrically. The fresh leaves (200 g) were chopped into small pieces. Essential oil was extracted by distillation of leaves for 4 h. The extracted oil was dried over anhydrous sodium sulphate and subsequently preserved in sealed glass vials at 4 °C for gas chromatography and mass spectrometry analysis of the EO. The amount of essential oil obtained from the plant material (leaves) was calculated as:

$$\text{EO content (\%v/w)} = [\text{Observed volume of oil (mL)} / \text{Weight of sample (g)} \times 100].$$

GC–MS analysis

Essential oil active-constituents were determined using GC–MS at Jawaharlal Nehru University, Delhi (India).

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For GC–MS an TG-WAX MS fused silica capillary column (30 m × 0.32 mm, i.e., film thickness 0.25 μm) was used in a Perkin Elmer Clarus® 680 GC interfaced with a Clarus® SQ 8 C Quadrupole mass spectrometer. Programmed split/splitness (PSS) injector temperature was 250 °C with a split ratio of 1:40. Column oven was programmed from 40 to 120 °C at the rate of 3 °C/min and hold of 9, then at 120 °C to 140 °C at the rate of 2 °C/min and hold on 2 min and finally at 140 °C to 250 °C at the rate of 5 °C/min and hold of 2 min. Helium was used as carrier at constant flow of 1 mL/min. Source temperature was 220 °C; ionization energy 70 eV; and mass scan range 40–450 amu. Characterization was achieved on the basis of retention time, elution order, TurboMass NIST 2011 libraries version 2.3.0, Wiley registry of mass spectral data 9th edition.

Statistical analysis

Descriptive statistics were presented as mean ± standard error of mean. One-way ANOVA was used to test differences among the treatments. Duncan's multiple range test (DMRT ≤ 0.05%) was also used to identify different treatment followed by ANOVA. Statistical significance level was considered as 5% and SPSS (ver: 22) statistical program was used for all statistical computations.

Results

To evaluate the effect of different concentrations of B on the growth, physiological, biochemical and essential oil parameters of *M. arvensis* and *C. flexuosus* were studied. In all tested concentrations of B, growth was maximally inhibited at 30 mg/kg B and minimally at 2.5 mg/

kg B in both the plants as compared to control (Table 1). At 30 mg/kg B, shoot length was decreased by 46.9% in *M. arvensis* and 84.5% in *C. flexuosus*, root length was decreased by 59.8% and 82.5% in *M. arvensis* and *C. flexuosus*, respectively, as compared to control (Table 1). The inhibition of shoot fresh weight by 67.3% in *M. arvensis* and 50.7% in *C. flexuosus* was noticed while shoot dry weight was significantly decreased by 71.6% and 56.8% in case of *M. arvensis* and *C. flexuosus*, respectively, as compared to control (Table 1). B concentration in plant tissues increased with increasing B concentration in the soil in concentration-dependent manner, with B concentration of root being higher compared to that of leaf (Table 1). B accumulation was found to be more in *M. arvensis* compared to *C. flexuosus*. The photosynthetic pigments were decreased as the applied B concentration was increased. Maximum reduction of these pigments was noted when 30 mg/kg B was supplied to plants, while application of 2.5 mg/kg B showed minimum inhibition (Table 2). The total chlorophyll content decreased significantly by 36.9% in *M. arvensis* and 33.4% in *C. flexuosus* and total carotenoid content inhibited by 22.4% in *M. arvensis* and 23.9% in *C. flexuosus* in leaves of the plants as compared to control at 30 mg/kg B application (Table 2). Further, treatment of 30 mg/kg B maximally decreased chlorophyll fluorescence by 16.7% in *M. arvensis* and 16.5% in *C. flexuosus* (Table 2).

Activities of assimilation of key enzymes (NR and CA) were also decreased with the increase in concentration of B. Maximum reduction in the activity of these enzymes was observed when 30 mg/kg B was applied (Table 2). NR activity was inhibited by 41.7% in *M. arvensis* and

Table 1 Effect of different concentrations of B on growth attributes and B content of *M. arvensis* and *C. flexuosus*

Plants/ treatments	Shoot length (cm)	Root length (cm)	Shoot FW (g)	Shoot DW (g)	Shoot B (mg/kg)	Root B (mg/kg)
M. arvensis						
Control	54.75 ± 1.94[a]	24.57 ± 1.02[a]	26.05 ± 1.58[a]	9.02 ± 0.95[a]	Not detected	0.19 ± 0.02[f]
2.5 B	52.84 ± 1.23[ab]	23.61 ± 0.93[a]	24.25 ± 1.31[b]	7.87 ± 0.81[b]	0.14 + 0.01[e]	0.42 + 0.02[e]
5 B	50.72 ± 1.58[b]	21.72 ± 0.86[b]	22.62 ± 0.20[c]	6.45 ± 0.28[c]	0.39 + 0.02[d]	0.93 + 0.07[d]
10 B	45.07 ± 1.33[c]	17.20 ± 0.82[c]	17.45 ± 0.08[d]	4.60 ± 0.19[d]	0.87 + 0.04[c]	1.71 + 0.12[c]
20 B	38.27 ± 1.17[d]	13.82 ± 0.79[d]	12.42 ± 0.17[e]	3.21 ± 0.12[e]	1.75 + 0.08[b]	3.41 + 0.21[b]
30 B	29.12 ± 1.06[e]	09.87 ± 0.46[e]	08.52 ± 0.21[f]	2.57 ± 0.07[f]	3.34 + 0.16[a]	7.23 + 0.39[a]
C. flexuosus						
Control	187.84 ± 11.08[a]	56.10 ± 3.52[a]	124.56 ± 6.86[a]	28.28 ± 2.84[a]	Not detected	0.09 ± 0.01[f]
2.5 B	181.43 ± 09.94[ab]	52.63 ± 3.46[b]	116.45 ± 6.09[b]	24.12 ± 2.46[b]	0.06 + 0.01[e]	0.17 + 0.02[e]
5 B	165.51 ± 10.81[b]	47.23 ± 2.98[c]	109.75 ± 5.45[c]	22.23 ± 2.42[b]	0.13 + 0.01[d]	0.38 + 0.05[d]
10 B	141.46 ± 08.65[c]	39.78 ± 2.27[d]	89.65 ± 4.94[d]	18.64 ± 2.14[c]	0.29 + 0.02[c]	0.64 + 0.07[c]
20 B	127.55 ± 07.80[d]	31.64 ± 1.54[e]	73.56 ± 3.54[e]	15.31 ± 1.88[d]	0.49 + 0.03[b]	1.51 + 0.09[b]
30 B	113.38 ± 07.43[e]	21.82 ± 1.18[f]	61.43 ± 3.19[f]	12.23 ± 1.49[e]	1.01 + 0.05[a]	3.42 + 0.14[a]

Data represent the mean value ± standard error from five replicates (*n* = 5). The different lower case letters represent statistically significant differences between treatments (*p* < 0.05)

Table 2 Effect of different concentrations of boron on photosynthetic and biochemical attributes of *M. arvensis* and *C. flexuosus*

Plants/ treatments	Total chlorophyll content (mg g^{-1} FW)	Total carotenoid content (mg g^{-1} FW)	Chlorophyll fluorescence (F_V/F_M)	Nitrate reductase activity (NO$_2$ g^{-1} FWh^{-1})	Carbonic anhydrase activity (CO$_2$ kg^{-1} FWs^{-1})
M. arvensis					
Control	1.44 ± 0.08[a]	0.554 ± 0.008[a]	0.789 ± 0.008[a]	382.67 ± 3.79[a]	345.23 ± 3.68[a]
2.5 B	1.36 ± 0.07[b]	0.532 ± 0.006[c]	0.762 ± 0.006[b]	354.45 ± 2.86[b]	305.45 ± 2.81[b]
5 B	1.37 ± 0.07[b]	0.541 ± 0.005[b]	0.758 ± 0.006[b]	352.34 ± 2.94[b]	297.67 ± 2.73[b]
10 B	1.31 ± 0.06[c]	0.518 ± 0.003[d]	0.725 ± 0.005[c]	322.78 ± 2.76[c]	265.45 ± 2.49[c]
20 B	1.20 ± 0.06[d]	0.487 ± 0.002[e]	0.698 ± 0.004[d]	286.12 ± 2.59[d]	233.12 ± 2.22[d]
30 B	0.91 ± 0.04[e]	0.432 ± 0.002[f]	0.654 ± 0.004[e]	223.43 ± 2.25[e]	202.34 ± 2.04[e]
C. flexuosus					
Control	1.23 ± 0.04[a]	0.678 ± 0.008[a]	0.796 ± 0.009[a]	412.56 ± 4.52[a]	294.45 ± 3.32[a]
2.5 B	1.12 ± 0.04[b]	0.643 ± 0.007[b]	0.773 ± 0.006[b]	389.76 ± 3.77[b]	256.34 ± 2.86[b]
5 B	1.10 ± 0.03[b]	0.624 ± 0.007[c]	0.768 ± 0.005[b]	370.56 ± 3.67[c]	245.31 ± 2.27[c]
10 B	0.97 ± 0.03[c]	0.621 ± 0.007[c]	0.729 ± 0.003[c]	354.70 ± 3.38[d]	212.34 ± 2.10[d]
20 B	0.92 ± 0.03[d]	0.576 ± 0.006[d]	0.702 ± 0.002[d]	315.76 ± 2.90[e]	193.23 ± 2.00[e]
30 B	0.82 ± 0.03[e]	0.516 ± 0.006[e]	0.663 ± 0.002[e]	278.80 ± 2.26[f]	165.45 ± 1.86[f]

Data represent the mean value ± standard error from five replicates ($n = 5$). The different lower case letters represent statistically significant differences between treatments ($p < 0.05$)

32.5% in *C. flexuosus* at 30 mg/kg B, while the activity of CA showed similar response and decreased by 41.3% in *M. arvensis* and 43.9% in *C. flexuosus* at 30 mg/kg B (Table 2).

Lipid peroxidation rate after application of different concentrations of B was enhanced rapidly and maximum values were noted at 30 mg/kg B as compared to control in case of both crops. The most toxic effects were observed at 30 mg/kg B, showing the highest membrane damage (Table 3). Proline content in leaves was significantly increased at all the concentrations of B as compared to control, however, the highest values were observed at 30 mg/kg B (Table 3). High concentrations of B increased the activity of CAT, POX, and SOD as

Table 3 Effect of different concentrations of boron on proline, TBARS and antioxidant enzymes of *M. arvensis* and *C. flexuosus*

Plants/ treatments	Proline content (mg plant^{-1})	TBARS content (nmol g^{-1} FW)	CAT activity (units mg^{-1} protein)	POX activity (units mg^{-1} protein)	SOD activity (units mg^{-1} protein)
M. arvensis					
Control	5.34 ± 0.21[f]	3.21 ± 0.28[e]	17.75 ± 0.87[e]	22.10 ± 0.66[f]	4.55 ± 0.23[e]
2.5 B	5.98 ± 0.22[e]	3.34 ± 0.34[e]	19.42 ± 1.30[d]	24.34 ± 0.92[e]	4.68 ± 0.27[e]
5 B	6.49 ± 0.35[d]	4.45 ± 0.46[d]	21.23 ± 1.90[d]	28.61 ± 1.10[d]	5.56 ± 0.30[d]
10 B	7.34 ± 0.48[c]	5.58 ± 0.51[c]	25.34 ± 2.28[c]	32.14 ± 1.43[c]	6.78 ± 0.32[c]
20 B	9.98 ± 0.64[b]	7.12 ± 0.58[b]	31.11 ± 2.72[b]	38.45 ± 1.61[b]	7.22 ± 0.35[b]
30 B	11.21 ± 0.72[a]	9.01 ± 0.72[a]	39.23 ± 3.20[a]	48.12 ± 2.10[a]	8.67 ± 0.36[a]
C. flexuosus					
Control	4.21 ± 0.24[f]	2.04 ± 0.17[f]	23.34 ± 0.94[e]	19.12 ± 0.50[f]	3.12 ± 0.16[e]
2.5 B	4.82 ± 0.30[e]	2.62 ± 0.32[e]	24.23 ± 1.19[e]	21.23 ± 0.83[e]	3.24 ± 0.18[e]
5 B	5.35 ± 0.41[d]	3.19 ± 0.56[d]	27.42 ± 1.44[d]	23.56 ± 1.55[d]	4.17 ± 0.22[d]
10 B	6.37 ± 0.63[c]	4.31 ± 0.67[c]	31.71 ± 1.07[c]	28.99 ± 1.65[c]	5.51 ± 0.22[c]
20 B	7.87 ± 0.81[b]	5.74 ± 0.71[b]	38.39 ± 1.14[b]	33.87 ± 1.69[b]	6.45 ± 0.23[b]
30 B	10.12 ± 0.96[a]	7.01 ± 0.83[a]	44.12 ± 1.33[a]	42.12 ± 1.87[a]	8.04 ± 0.32[a]

Data represent the mean value ± standard error from five replicates ($n = 5$). The different lower case letters represent statistically significant differences between treatments ($p < 0.05$)

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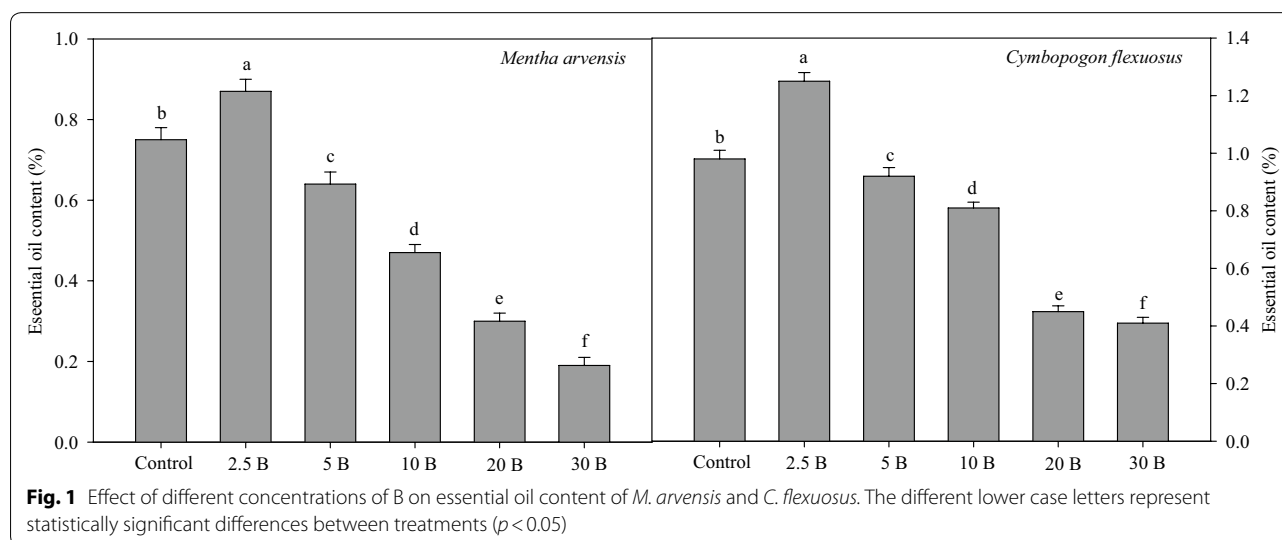
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compared with the control in both plants. Activities of CAT and POX were maximally increased when 30 mg/kg B was applied to *M. arvensis* and *C. flexuosus* plants. Also, the activity of SOD almost doubled as compared to control in *M. arvensis* and *C. flexuosus* at 30 mg/kg B (Table 3).
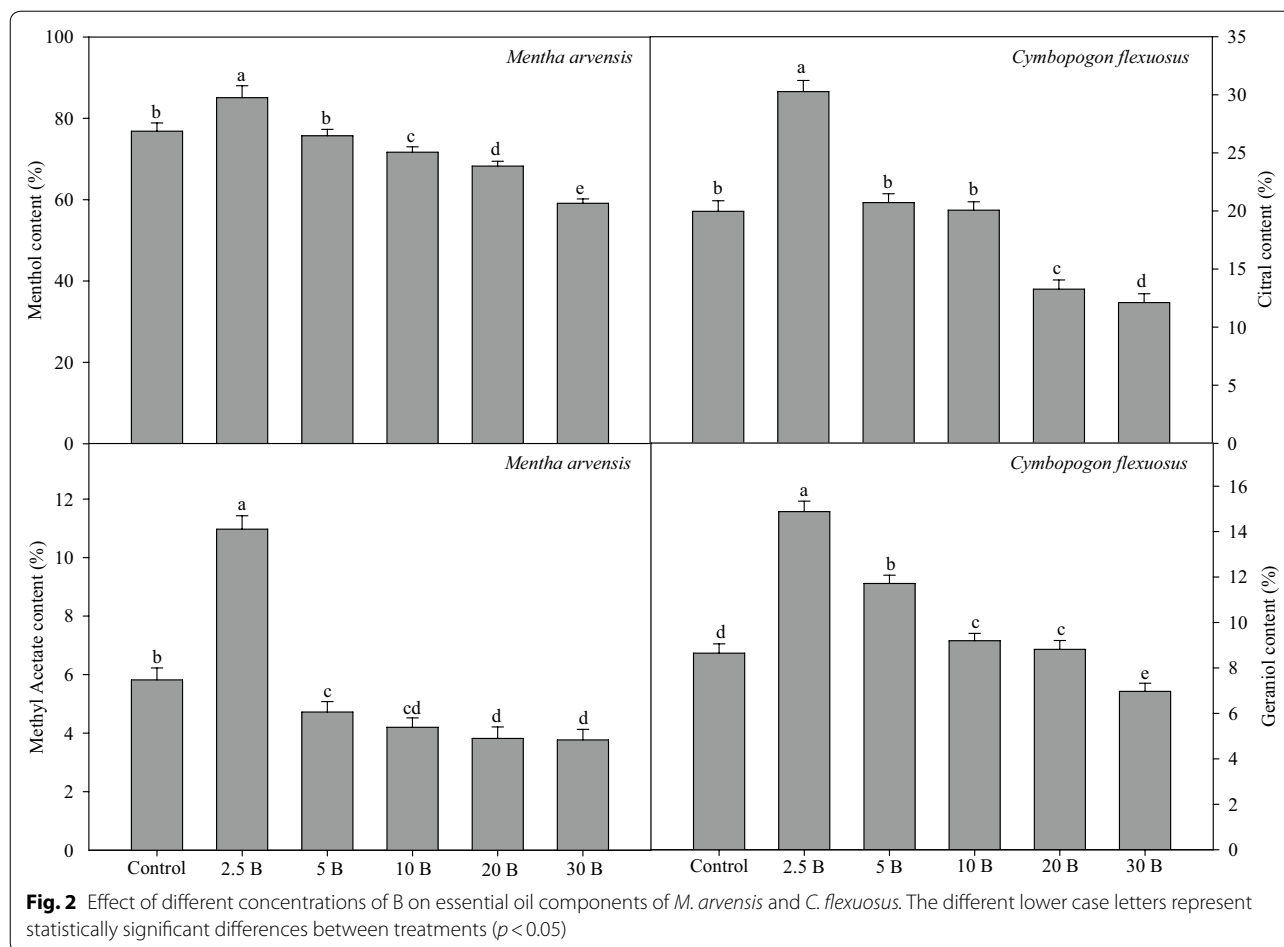
Interestingly, EO content was significantly increased at 2.5 mg/kg B in *M. arvensis* and *C. flexuosus* plants. However, further increase in the applied B concentrations decreased the EO content in both the plants (Fig. 1). GC–MS data revealed the percentage of components present in essential oil of studied plants. In case of *M. arvensis*, menthol and menthyl acetate content was significantly increased at 2.5 mg/kg B as compared to control (Figs. 2, 3). The content of citral and geraniol was increased at 2.5 mg/kg B in *C. flexuosus* as compared to the control. Surprisingly, a significant increase in geraniol content was also noticed at 5 mg/kg B in *C. flexuosus* (Figs. 2, 3).

Discussion

Boron is involved in many physiological and biochemical processes during plant growth, but the exposure of plants to high levels of B results in a reduction in growth parameters. Boron nutrition requirement varies considerably among different crops. *M. arvensis* and *C. flexuosus* grown under high concentrations of B exhibited remarkable decrease in growth parameters. The shoot and root lengths of both the plants were reduced as the concentration of applied B was increased. Inhibition of root and shoot growth was reported earlier under excess B concentrations in grapevine [19], barley [41], tomato [18], *Artemisia* [16] and in wheat [42] as B stress causes antimitotic and mito-depressive effect that eventually leads to the decrease in cell division.

Various processes of dissimilar susceptibility lead to the response of high B and intercellular B concentration is found to be associated with B toxicity [43]. In *Arabidopsis*, findings on molecular basis of reduction in root growth under B stress revealed that expression of genes involved in cell wall modification, ABA response and ABA signaling was enhanced under high B concentration while the expression of genes involved in water transporters was repressed, suggesting that inhibition of root growth under B stress is associated with water stress response [28]. High B concentration significantly decreased fresh and dry weights of *M. arvensis* and *C. flexuosus* and similar results were found in case of agricultural crops such as wheat, tomato, and pepper [44–46]. Reduction in photosynthetic enzymes activities under high B concentration alter the electron transport system, that ultimately results in low CO_2 assimilation and a direct correlation has been found in reduced shoot biomass and decreased photosynthetic CO_2 assimilation and decline of sucrose paralleled by increments of glucose and fructose concentration in leaves [47, 48]. Roots play numerous roles during plant growth and development and are typically the first part of the plant to encounter several types of environmental stresses [49]. Results of present study revealed that there was a higher accumulation of B in roots compared to that in leaves with increasing B concentrations (Table 1). In this context, our results corroborate the findings of Kaya et al. [50], who reported comparatively a higher accumulation of B in roots of tomato than in other plant parts. In fact, the ability of the plants to tolerate high B could be due to the capacity of the plants to accumulate more B in roots and a lesser amount of the B being transported to shoots. However, B uptake

Fig. 1 Effect of different concentrations of B on essential oil content of *M. arvensis* and *C. flexuosus*. The different lower case letters represent statistically significant differences between treatments ($p < 0.05$)

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Fig. 2 Effect of different concentrations of B on essential oil components of *M. arvensis* and *C. flexuosus.* The different lower case letters represent statistically significant differences between treatments ($p < 0.05$)
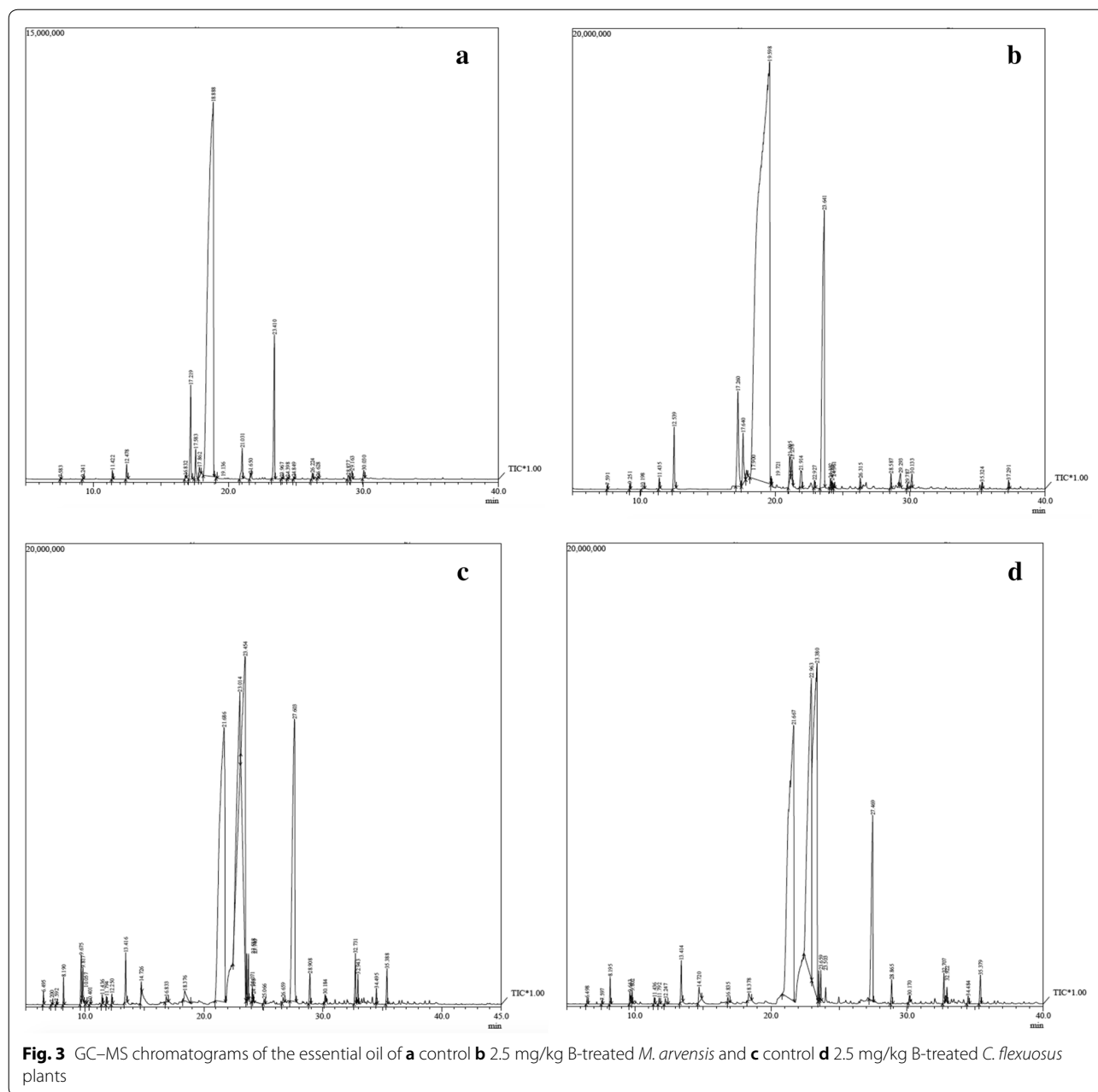
responses of the crops might differ significantly with regard to the susceptibility of crops to various stresses.

In the present study, chlorophyll fluorescence 'F_V/F_M' (maximum quantum yield of photosystem II) was measured to check the effect of high concentration of B on PS II. The high doses of B decreased the quantum yield dramatically in both the plants showing severe damage to PS II. Decrease in F_V/F_M has earlier been reported in the leaves of Navelina orange plants under high B concentration and same results were found in apple rootstocks [51, 52]. *M. arvensis* and *C. flexuosus* leaves showed chlorotic patches on the margins of leaves and necrosis on the leaf tip under excess B, might be the reason of low chlorophyll and carotenoid concentration in both plants. It was reported that ribose moieties of ATP, NADH, or NADPH, usually binds to B under high internal B concentration, causing disruption in metabolism and inhibits normal activities of biosynthetic pathways [53]. Stress conditions usually affect the chlorophyll and carotenoid content in leaves [54]. Reduction in chlorophyll and carotenoid contents was found in Arabidopsis [47], wheat [1, 42], and pepper [53] under excess B. Moreover, reduced F_V/F_M

in leaves under excess B was reported in many species, showed photoinhibition that ultimately leads to the formation of ROS [55]. A significant decrease was observed in the activities of NR and CA in both plants under excess B. Formation of borate ester with ribose by the inhibition of protein synthesis under B toxicity is found to disrupt the normal function of several enzymes, thus the plant metabolism [56]. Excess B affected the NR activity in wheat, barley and tomato [18, 20, 21, 44]. Cervilla et al. [18] reported that excess B enhanced the activity of glutamine synthase and glutamine synthetase while lowering the activity of nitrate reductase and nitrite reductase that eventually caused assimilation of ammonium in tomato leaves.

Proline content increased with the increasing concentrations of B in both the plants. Proline plays a crucial role in osmoregulation, protecting enzyme from denaturation and scavenges ROS as hydroxyl radical scavenger. Accumulation of proline in leaves under excess B is directly correlated with stress tolerance in plants [1]. High proline content under excess B was noticed in several crops [57]. TBARS content of both the plants was

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Fig. 3 GC–MS chromatograms of the essential oil of **a** control **b** 2.5 mg/kg B-treated *M. arvensis* and **c** control **d** 2.5 mg/kg B-treated *C. flexuosus* plants

measured to determine lipid peroxidation under B stress. Boron toxicity increased MDA content in a dose-dependent manner. Similar results were found in many crops [13, 19]. It is well documented that high level of H_2O_2 in plant cells causes the formation of oxidative stress [58]. Moreover, excess H_2O_2 can damage membrane integrity resulting in the formation of hydroxyl radical that causes lipid peroxidation [14]. It is found that singlet oxygen, product of triplet state chlorophyll highly damages the cellular components and increases lipid peroxidation that further initiates the production of highly reactive radicals

and lipid hydroperoxides, known to be the indicator of stress conditions [59].

The activity of antioxidant enzymes such as CAT, POX and SOD were measured in shoots of both plants as these are the lipid peroxidation scavenging enzymes. Increased activities of antioxidant were observed in plants to combat and minimize the toxic effects of ROS such as, superoxide radicals, hydroxyl alkoxy radicals and singlet oxygen [47]. In the present study, B toxicity increased antioxidant enzyme activities 2–3 folds with increasing B concentrations. Previous researches

support our results as antioxidant enzyme activities increased under B toxicity [11, 13]. Excess of B causes inhibition of α tocopherol synthesis, main component of antioxidant defense mechanism that results in the membrane damage by free radicals [60]. SOD plays crucial role in plant stress tolerance mechanism and increased level of SOD under stress conditions is an indication of oxidative stress tolerance, as it breaks O_2^- into H_2O_2 in various compartments of plant cells [11, 61]. Excess B increased SOD activities in the leaves of various plants [14, 62]. High B concentration in leaves affects CAT activity differently depending on the plant species and CAT scavenges H_2O_2 produced in peroxisome by breaking it into water and oxygen through glycolate cycle and β-oxidation of fatty acids, showing protective role under stress conditions [11, 63].

Interestingly, the content of EO and its active constituents (menthol and menthyl acetate in case of *M. arvensis* and citral and geraniol in case of *C. flexuous*) were enhanced under the boron treatment at 2.5 mg/kg (Figs. 2, 3). The impact of B stress on both crops has not earlier been studied. It is worth emphasizing that the content of essential oil was more responsive to low concentration of B than the vegetative growth, which is especially crucial and is the main objective for cosmetic and pharmacy industry. In fact, it has been reported that if the concentration of B is relatively low in plants, it plays a prominent role in metabolic processes [64, 65]. It was reported by various researchers that B changes the EO content as well as metabolite profiles in crops [66, 67]. Primary and secondary metabolisms in essential oil-bearing plants such as mints and citronella are dependent on nutritional status of plants [68]. The present results exhibited differences in the content of EO of *M. arvensis* and *C. flexuosus*, which is in conformity with the results reported by Sugier et al. [69]. However, there are a few studies in context of ecological effects of abiotic stress on secondary metabolites rather than genetically influenced [70].

Conclusions
All the concentrations of applied B, reduced the growth of *M. arvensis* and *C. flexuosus*, however, the highest concentration, i.e., 30 mg/kg B was found to be most deleterious and maximally decreased the growth attributes. TBARS content and activities of antioxidant enzymes were increased under B stress, as these parameters are indicative of oxidative stress. Interestingly, oil content was significantly increased in both the plants under mild B stress (2.5 mg/kg B). An increase in menthol and menthyl acetate content was noticed at 2.5 mg/kg B in *M. arvensis* and in the content of citral and geraniol in *C. flexuosus* as compared to the control.

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