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Application of Smoke-saturated water boosts physiology and essential oil yield in *Mentha arvensis* L.

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Abstract

Some smoke-based compounds were also confirmed to influence a variety of physiological and developmental processes in plants. In this study, the influence of SSW (smoke-saturated water) using rhizome soaking and foliar spraying on growth and physiological, biochemical characteristics, glandular trichome, and final essential oil yield of *Mentha arvensis* L., a key economically important aromatic plant is investigated. Healthy rhizomes were immersed in SSW at 1:125 v/v, 1:250 v/v, 1:500 v/v and 1:1000 v/v for 24 h prior to planting, and at different concentrations of foliar treatments during crop growth. Both application strategies greatly improved plant height, biomass accumulation, leaf area, and leaf yield in plants treated with SSW at a dilution of 1:500 (v/v) compared with untreated controls. Increased chlorophyll fluorescence parameters, photosystem II efficiency, and levels of chlorophyll, carotenoids revealed enhanced photosynthetic performance. Moreover, SSW applied significant increases in the activities of the major metabolic enzymes namely nitrate reductase and carbonic anhydrase. SSW treatment induced accumulation of phenolics and flavonoids as well as marked enlargement of glandular trichomes. These physiological and biochemical benefits were characterized up to a remarkable increase in essential oil content and productivity, with higher concentrations of major molecules, i.e., menthol, menthone and menthyl acetate. In general, the results indicated that the optimal use of smoke-saturated water application of 1:500 (v/v) dilution of it (especially with optimum effectiveness) will be an eco-considerate treatment for enhancing growth performance in *Mentha arvensis* L. and essential oil yield and efficacy in *Mentha arvensis* L.

Keywords: *Smoke-saturated water;* Mentha arvensis *L.; physiological responses; essential oil yield; secondary metabolites*

1. Introduction

Medicinal and aromatic plants are an essential part of agriculture and pharmaceutical industries across a wide range of different industries, with diverse uses in medicine, cosmetics, food flavoring and traditional health systems (Rao, 2001; Verma *et al*., 2010). Of these, the genus Mentha L., a member of the family Lamiaceae worldwide is popularly known for its aromatic quality and great economic importance due to the existence of essential oils consisting of bioactive substances (Lawrence, 2007). In the context of the genus Mentha, over 30 species can be observed in both temperate and subtropical regions of the world, including many of them cultivated for commercial essential oil extraction (Kokkini, 1992). Japanese mint (*Mentha arvensis* L.) (Fig 1) is one of the widely cultivated mints and particularly in India, which is the top source of menthol-rich mint oil in the world (Singh *et al*., 2015). *M. arvensis* is extensively cultivated in northern Indian states like Uttar Pradesh, Punjab, and Himachal Pradesh, where climatic and soil conditions are conducive to high biomass and oil production (Chand *et al*., 2004). In addition to the previously recorded oil components of *M. arvensis* that have medicinal and commercial significance, they also incorporate menthone, menthyl acetate, limonene, cineole, pulegone, menthofuran, and carvone (Lawrence, 2007; Verma *et al*., 2010).

Fig. 1. Mentha arvensis L. plants at the vegetative growth stage.

Environmental cues and chemical signaling molecules play significant roles in plant growth and development that regulate physiological and metabolic activities (Taiz *et al*., 2015). In recent years, smoke originating from the plants has become a significant ecological factor to the germination, establishment of seedlings, and the growth of the plants in fire-prone ecosystems (Keeley and Fotheringham, 1998; Van Staden *et al*., 2000). Following observed improvements in post-fires regeneration, smoke appeared to be a source of biologically active extracts, which could induce plant development (Brown and Van Staden, 1997). Subsequent investigations shown that these growth promoting substances present in smoke are water soluble and can thus be trapped by aqueous solutions called smoke-saturated water (SSW) that retain the biological activity when applied as is in plants (Van Staden *et al*., 2004; Light *et al*., 2009). The use of SSW has been shown to improve seed germination, vegetative growth, photosynthetic efficiency and biomass accumulation in several types of plants used in agriculture and healthcare (Jäger *et al*., 1996; Kulkarni *et al*., 2011). Some of these compounds are particularly interesting analytically, as they seem to exert a strong influence on physiology and hormonal pathways in plants due to chemical and molecular signalling in the biochemical pathway systems (Flematti *et al*., 2004; Nelson *et al*., 2012). Based on primary growth responses on plants, smoke-derived treatments affect secondary metabolism and promote an elevated production of phenolics, flavonoids and other bioactive compounds themselves (Kulkarni *et al*., 2013). Essential oil biosynthesis and storage in aromatic plants is mainly carried out in glandular trichomes on leaf surfaces, and those factors that promote trichome development and metabolic activities directly contribute to the oil yield and quality (Gershenzon *et al*., 2000; Werker, 2000). The impact of smoke and smoke-derived solutions on perennial, rhizomatous aromatic crops including *Mentha arvensis* remains poorly understood despite increasing evidence of their positive impact. More specifically, the impact of SSW on physiological efficiency, metabolic enzyme function, glandular trichome growth, and production for essential oil in mint are less well characterized. Reflecting the high economic value of *Mentha arvensis*, and given the recent surge of interest in eco-friendly growth modulators, the current study investigated impacts of smoke-saturated water via rhizome soaking and foliar spraying. The objective of the study was to determine growth attributes, physiological and biochemical responses, glandular trichome characteristics, and essential oil yield and composition at various SSW concentrations and to develop a successful and sustainable approach to mint growth and productivity.

2. Material and Methods –

2.1 Experimental site and growth conditions

The experiments (1 and 2) were conducted during the summer season under natural environmental conditions in the net-house facility of the Department of Botany, AMU (Aligarh Muslim University), Aligarh, India. Plants were grown in earthen pots sized (25 cm diameter × 25 cm height) filled with a uniform mixture of garden soil and well-decomposed farmyard manure in a 5:1 ratio, following standard pot culture practices for mint cultivation (Chand *et al*., 2004). All pots were maintained under uniform irrigation and cultural conditions throughout the experimental period.

2.2 Preparation of smoke-saturated water (SSW)

Smoke-saturated water was prepared following the standard procedure described by Van Staden *et al*. (2004) with slight modifications. Dried wheat straw was combusted on a hot plate in a borosilicate petri dish and covered with an inverted glass funnel (Fig 2). The funnel outlet was joined via rubber tubing to a glass inlet tube inserted into a vacuum flask containing one litre of double-distilled water (DDW). A vacuum pump was used to facilitate continuous bubbling of smoke into the water.

Smoke generation was maintained by intermittently adding dried straw to ensure uniform combustion. The degree of smoke saturation was monitored by withdrawing aliquots of water at regular intervals and recording absorbance at 330 nm using a UV–visible spectrophotometer, as described earlier for smoke solutions (Kulkarni *et al*., 2011). After approximately 45 minutes, the water reached saturation and was designated as smoke-saturated water (SSW). The stock solution was filtered and stored at room temperature until further use.

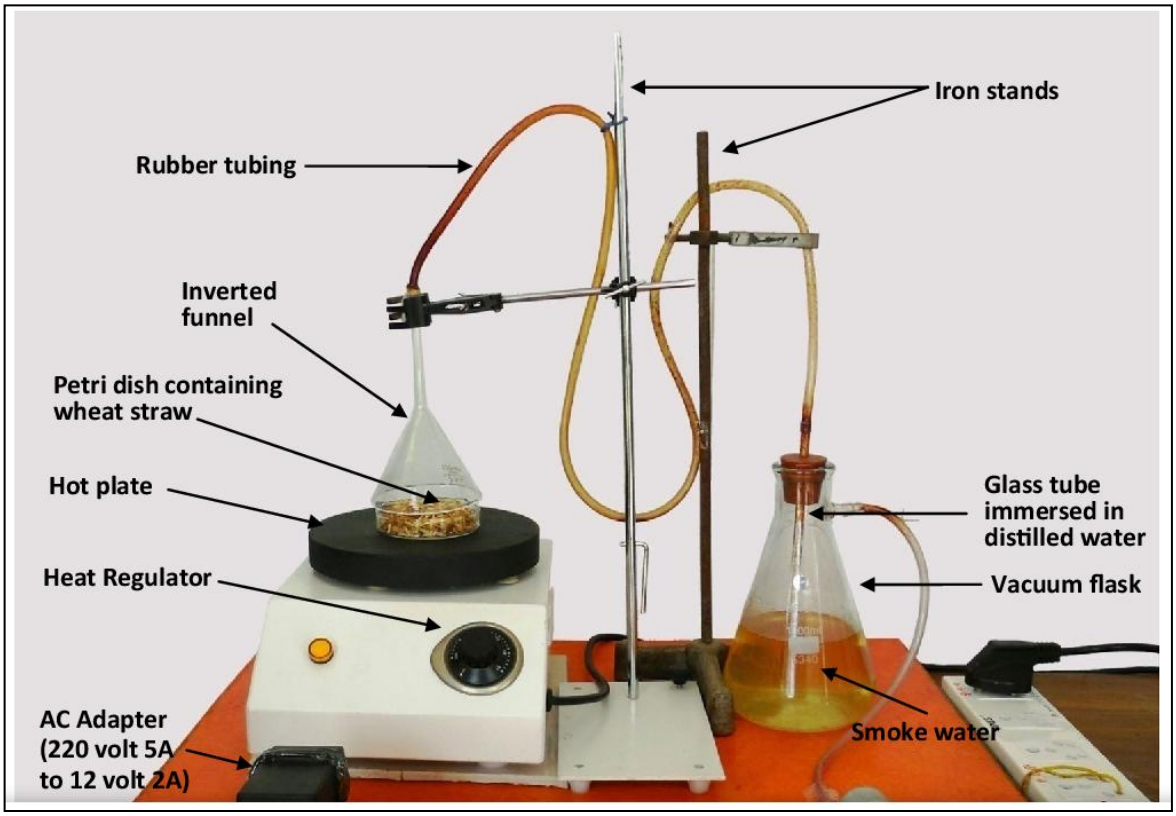


Fig. 2. Experimental setup specifically designed and assembled in the Advanced Plant Physiology Laboratory, Department of Botany, AMU, Aligarh, for producing smoke-saturated water.

Four aqueous dilutions of SSW (1:125 v/v, 1:250 v/v, 1:500 v/v, and 1:1000 v/v) were prepared using DDW. DDW alone served as the control treatment.

2.3 Plant material and treatment application

Healthy and uniform rhizomes of *Mentha arvensis* L. (var. Kosi) were selected for the experiment. Prior to planting, rhizomes were surface-sterilized using 1% sodium hypochlorite solution containing a few drops of Tween-20 for 10 minutes, followed by thorough rinsing with DDW to remove residual sterilant (Rady *et al*., 2013).

Five rhizomes were planted equidistantly in each pot. Two separate treatment methods were employed:
(i) **Rhizome soaking**, in which rhizomes were soaked for 24 h in different dilutions of SSW prior to planting, and

(ii) **Foliar application**, in which plants were sprayed with the respective SSW dilutions after establishment at appropriate growth stages.

Control plants received only DDW in both experiments.

2.4 Measurement of growth parameters

Growth parameters including plant height, fresh biomass (FW), dry biomass (DW), leaf area (LA), and leaf yield per plant (LYPP) were recorded at 100 days after planting (DAP), following standard procedures used for mint growth assessment (Singh *et al*., 2015). Plants were carefully uprooted, washed, and blotted dry. Plant height was measured using a measuring scale, while FW (fresh biomass) was recorded using an analytical balance. Samples were oven-dried at 80°C for 24 h to determine DW (dry biomass). LA (Leaf area) was measured using the graph paper method, and LYPP (leaf yield per plant) was calculated by weighing detached leaves.

2.5 Physiological parameters

2.5.1 Chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured on fully expanded leaves using a handy chlorophyll fluorometer (PAM-2000, Walz, Germany) following the protocol described by Maxwell and Johnson (2000). Leaves were dark-adapted for 30 minutes prior to measurement. Fv/Fm (Maximum quantum efficiency of photosystem II), [Y(II)] (effective quantum yield), (qP) (photochemical quenching), (qN) non-photochemical quenching, and ETR (electron transport rate) were recorded under actinic light conditions.

2.5.2 Estimation of photosynthetic pigments

Total chlorophyll and carotenoid contents were estimated following the method of Lichtenthaler and Wellburn (1983). Fresh leaf tissue was homogenized in 80% acetone, centrifuged, and absorbance of the supernatant was recorded at appropriate wavelengths using a UV–visible spectrophotometer. Pigment concentrations were calculated and expressed on a fresh weight basis.

2.6 Biochemical analyses

2.6.1 Nitrate reductase activity

Nitrate reductase activity was determined using the in vivo assay described by Jaworski (1971). Fresh leaf samples were incubated in phosphate buffer containing potassium nitrate and isopropanol, and nitrite formation was quantified spectrophotometrically. Enzyme activity was expressed as nmol NO_2^- g^{-1} fresh weight h^{-1}.

2.6.2 Carbonic anhydrase (CA) activity

Carbonic anhydrase (CA) activity was measured using the titrimetric method used by Dwivedi and Randhawa (1974). Fresh leaf tissue was incubated with sodium bicarbonate and indicator solution, and the amount of CO_2 released was determined by titration against standard acid.**2.6.3**

Estimation of TPC (total phenolic content)

Total phenolic content was measured using the Folin–Ciocalteu method as described by Singleton and Rossi (1965). Leaf extracts were reacted with Folin–Ciocalteu reagent and sodium bicarbonate, and absorbance was recorded at 650 nm. Results were expressed as gallic acid equivalents.

2.6.4 Determination of flavonoid content

Total flavonoid content was determined using the aluminium chloride colorimetric method following Chang *et al*. (2002). Absorbance was measured at 415 nm, and flavonoid concentration was expressed as catechin equivalents.

2.7 Scanning electron microscopy (SEM)

The leaf samples were prepared for scanning electron microscopy using standard fixation and dehydration methods (Werker, 2000). The samples were dehydrated using graded ethanol series and gold-coated and analyzed by SEM (scanning electron microscope) at USIF (University Sophisticated Instrumentation Facility), AMU, Aligarh. Image analysis software was used to determine the size of the glandular trichome.

2.8 GC–MS analysis of Essential oil

In the fresh leaves, essential oil was extracted in a Clevenger-type apparatus after the hydrodistillation process as recommended by Guenther (1972). Gas chromatography–mass spectrometry (GC–MS) was employed to analyze oil composition. Following Adams (2007), compounds were determined by using retention indices and mass spectral comparison with standard libraries.

2.9 Statistical analysis

The experiment was presented in a completely randomized design with six replicates per treatment. Statistical analyses were conducted with the software SPSS. Mean comparisons were conducted on standardized variables based on Duncan's multiple range test at $p \leq 0.05$, as found with Gomez and Gomez (1984). Results were reported as mean ± standard error.

3. Results

The effectiveness of the compounds on the growth performance, physiological efficiency, biochemical indices, the properties of the glandular trichome and the yield of essential oil by plants treated with SSW in different dilutions (1:500 – 1:500 diluted SSW) was compared between groups. The detailed information of the various parameters is provided based on them the outcomes are shown.

3.1 Growth attributes

Foliar application of SSW and rhizome soaking promoted vegetative growth of *Mentha arvensis* significantly bettered the vegetative growth of *M. arvensis*. SSW treatments resulted in significant improvements for plant height, fresh biomass (FW), dry biomass (DW), leaf area (LA), and leaf yield per plant (LYPP) values and in both the high-reaction indicated the highest level of response from SSW treatment and maximum response of the plants grew at the 1:500 (v/v) concentration (Table 1).

3.1.1 Plant height

On rhizome soaking procedure, it was shown that the treatment with SSW at 1:500 (v/v) significantly increased plant height with an increase about 23% over control. Such a response was statistically similar to the results of plants treated at 1:250 and 1:1000 (v/v). An increase of about 19.23% compared to untreated plants was observed with the foliar spray experiment at the same concentration (Table 1).

3.1.2 Fresh weight

In both experimental approaches, fresh biomass accumulation was significantly influenced by their application of SSW. Rhizome soaking with 1:500 (v/v) SSW yielded the sharp greatest increase (23.3%) to fresh weight versus the control, and was statistically at par with 1:250 and 1:1000 (v/v) treatments. Foliar application at the same concentration further showed a significant rise (19.30%) in fresh weight relative to control plants (Table 1).

3.1.3 Dry weight

Subsequent studies showed the large response of dry biomass produced under the SSW treatment. Regarding the rhizome soaking experiment, plants treated with SSW: 1:500 (v/v) increased dry weight at a maximum of 44.4% over the control. The same trend was also seen in foliar: Treatment with 1:500 (v/v), in which dry biomass was significantly improved by 35.36%. Responses were at best significant on average with measurements taking 1:250 and 1:1000 (v/v).

3.1.4 Leaf area

A significant effect was seen upon application of smoke saturated water through both rhizome soaking and foliar spray means on the Leaf area of *Mentha arvensis*. In the experiment to soaking the rhizomes, SSW was used in plants and the cells were watered with a dilution of 1:500 (v/v). This was the maximum expansion compared to the control plant. This increase was, statistically, the equivalent of plants treated at 1:250 and 1:1000 (v/v). Another positive response pattern was found under foliar application, with the 1:500 (v/v) SSW treatment greatly increasing leaf area in comparison to the untreated plants. Under optimal SSW treatment, however, leaf area increases, reflecting improved vegetative growth and higher photosynthetically active surface (Table 1).

3.1.5 Leaf yield per plant

In our experiments, there was a significant and concentration-dependent response in leaf yield per unit of plant to SSW application. The leaf yield per plant was highest in this case of 1:500 (v/v) SSW after the soaked rhizome and there was a significant improvement as compared to control. For example, this treatment was statistically matched with the 1:250 and 1:1000 (v/v) concentrations. Similarly, if SSW is added to the foliar spray experiment at 1:500 (v/v), leaf yield per plant increased significantly compared with untreated plants. Under optimal SSW concentration, improved biomass and leaf growth provide the basis of the improved leaf yield (Table 1).

Table 1. Effect of rhizome soaking and foliar application of smoke-saturated water (SSW) on growth attributes of Mentha arvensis L. recorded at 90 days after planting (DAP).

SSW Treatments (Rhizome Soaking)	Shoot length (SL) (cm)	Plant fresh weight (FW) (g)	Plant dry weight (DW) (g)	Leaf number plant^{-1} (LYPP)	Leaf area plant^{-1} (cm^{2}) (LA)
Control	67.4 ± 0.7^{d}	60.2 ± 0.5^{d}	9.2 ± 0.3^{c}	89.0 ± 0.5^{e}	2848.6 ± 24.5^{d}
T1	72.2 ± 0.9^{c}	65.3 ± 0.5^{c}	10.2 ± 0.2^{bc}	94.6 ± 0.6^{d}	3161 ± 9.1^{c}
T2	77.1 ± 0.4^{b}	70 ± 0.1^{b}	12.4 ± 0.7^{ab}	110 ± 0.5^{b}	3329.3 ± 23.4^{b}
T3	**83 ± 0.5^{a}**	**74.2 ± 0.3^{a}**	**13.3 ± 0.3^{a}**	**114.3 ± 1.2^{a}**	**3610 ± 20.4^{a}**
T4	76.13 ± 0.1^{b}	69 ± 0.1^{b}	10.2 ± 0.4^{bc}	100.6 ± 0.8^{c}	3176 ± 16.3^{c}
SSW Treatments (Foliar Application)	Shoot length (SL) (cm)	Plant fresh weight (FW) (g)	Plant dry weight (DW) (g)	Leaf number plant^{-1} (LYPP)	Leaf area plant^{-1} (cm^{2}) (LA)
Control	64.1 ± 0.3^{e}	59.2 ± 0.4^{e}	8.2 ± 0.2^{c}	85.6 ± 1.4^{d}	2639 ± 11.2^{e}
T1	68.2 ± 0.4^{d}	63.5 ± 0.3^{d}	8.7 ± 0.2^{c}	92.6 ± 1.4^{c}	2810 ± 6.5^{d}
T2	70 ± 0.5^{c}	65.1 ± 0.6^{c}	9.46 ± 0.2^{b}	100.3 ± 1.4^{b}	2990 ± 17.6^{c}
T3	**76.4 ± 0.5^{a}**	**70.6 ± 0.3^{a}**	**11.1 ± 0.2^{a}**	**110 ± 2.6^{a}**	**3120 ± 30.5^{a}**
T4	73.4 ± 0.3^{b}	68.3 ± 0.4^{b}	10.6 ± 0.2^{b}	105 ± 2.1^{b}	3010 ± 6.3^{b}

Each value represents the mean of six replicates ± SE. Means within a column followed by the same letter(s) do not differ significantly at $p \leq 0.05$. SSW = smoke-saturated water. Treatments: T1 = 1:125 v/v, T2 = 1:250 v/v, T3 = 1:500 v/v, and T4 = 1:1000 v/v.

3.2 Physiological parameters

3.2.1 Chlorophyll fluorescence

Application of smoke-saturated water (SSW) significantly improved chlorophyll fluorescence parameters in *Mentha arvensis* under both rhizome soaking and foliar spray treatments (Table 2). The **1:500 (v/v)** SSW treatment recorded the maximum enhancement in photosystem II efficiency.

In the rhizome soaking experiment, Fv/Fm, qP, qN, Y(II), and ETR increased by approximately **10%**, **15%**, **43%**, **23%**, and **26%**, respectively, compared to the control.

Similarly, foliar application of SSW at **1:500 (v/v)** resulted in the highest improvement, with increases of **12.10%** in Fv/Fm, **26.93%** in qP, **41.11%** in qN, **33.91%** in Y(II), and **22.12%** in ETR over untreated plants.

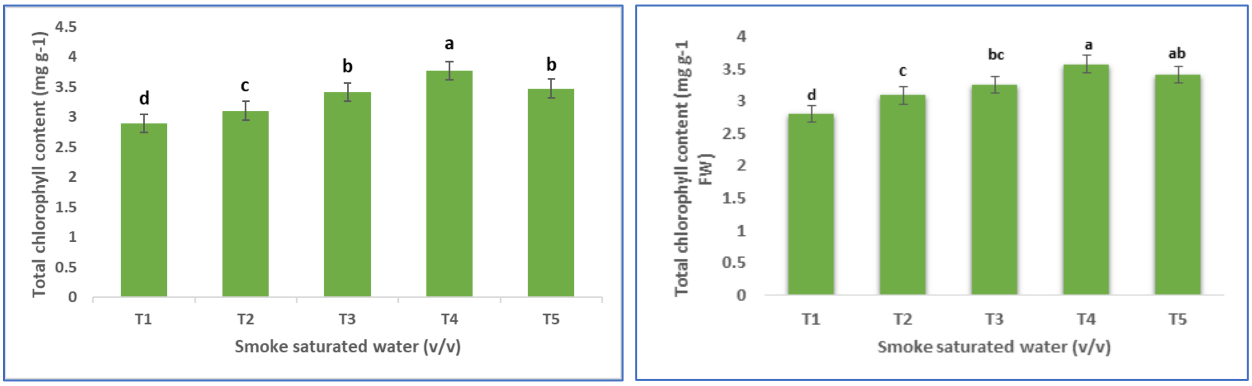
Table 2 *Effect of rhizome soaking and foliar application with SSW on chlorophyll fluorescence (Fv/Fm) and the related parameters regarding Mentha arvensis at 90 DAP (days after planting).*

SSW Treatments (Rhizome soaking)	Chlorophyll fluorescence (Fv/Fm)	qP	qN	Y(II)	ETR
Control	0.73±0.03^{d}	0.72±0.02^{e}	0.10±0.03^{e}	0.54±0.05^{d}	28.4±0.37^{e}
T1	0.75±0.05^{c}	0.74±0.05^{d}	0.12±0.04^{d}	0.56±0.04^{c}	30.1±0.23^{d}
T2	0.78±0.03^{b}	0.85±0.03^{c}	0.13±0.04^{c}	0.59±0.02^{b}	32.8±0.25^{c}
T3	**0.81±0.04^{a}**	**0.93±0.04^{a}**	**0.15±0.05^{a}**	**0.66±0.08^{a}**	**35.8±0.05^{a}**
T4	0.75±0.05^{c}	0.89±0.06^{b}	0.14±0.03^{b}	0.57±0.04^{c}	33.6±0.28^{b}
SSW Treatments (Foliar Application)	Chlorophyll fluorescence (Fv/Fm)	qP	qN	Y(II)	ETR
Control	0.72±0.001^{e}	0.67±0.003^{e}	0.09±0.005^{e}	0.51±0.004^{e}	25.9±0.23^{d}
T1	0.76±0.002^{d}	0.72±0.006^{d}	0.09±0.002^{d}	0.56±0.004^{d}	26.1±1.09^{c}
T2	0.79±0.002^{c}	0.81±0.004^{c}	0.10±0.005^{c}	0.61±0.003^{c}	27.6±0.61^{b}
T3	**0.81±0.004^{a}**	**0.85±0.002^{a}**	**0.12±0.004^{a}**	**0.69±0.003^{a}**	**31.6±0.24^{a}**
T4	0.80±0.002^{b}	0.84±0.005^{b}	0.11±0.002^{b}	0.66±0.004^{b}	29.2±0.41^{b}

Each value represents the mean of 6 replicates with ±SE. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). SSW –smoke-saturated water, qP – photochemical quenching, qN – non-photochemical quenching, Fv – variable fluorescence, Fm – maximum fluorescence, Y(II) – effective quantum yield of photosystem II, ETR – electron transport rate. (T1-1:125 v/v, T2 – 1:250 v/v, T3 – 1:500 v/v, T4 – 1:1000 v/v).

3.2.2 Total chlorophyll and carotenoid content

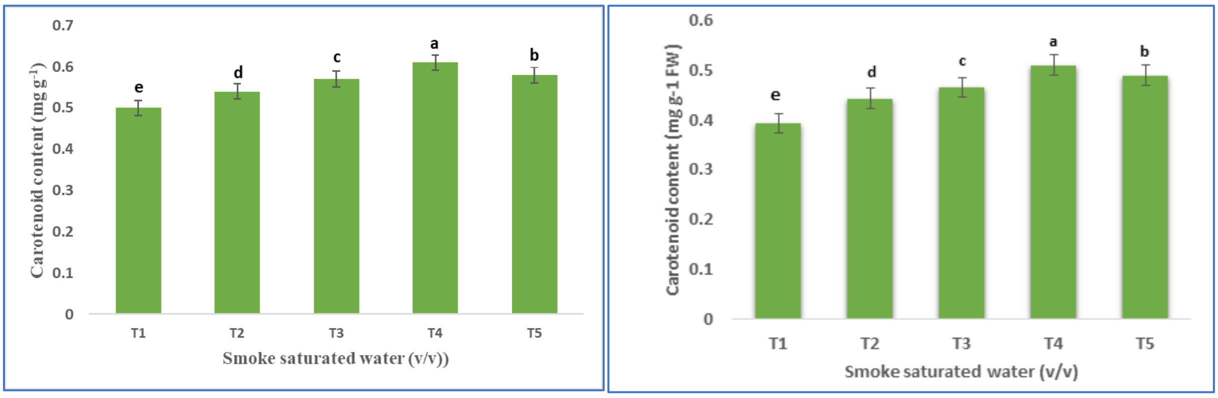
Total chlorophyll content increased significantly following SSW application in both experiments (Figure 3). Rhizome soaking with **1:500 (v/v)** SSW enhanced chlorophyll content by approximately **30%**, while foliar application resulted in a **25.70%** increase compared to the control.



A ***B***

Fig. 3. Effect of rhizome soaking and foliar application of smoke-saturated water (T5 = 1:1000, T4 = 1:500, T3 = 1:250, T2 = 1:125 v/v, and T1 = Control) on total chlorophyll content (mg g^{-1} FW) in Mentha arvensis L. (A and B).

Carotenoid content also showed a positive response to SSW treatment (Figure 4). In the rhizome soaking experiment, carotenoid levels increased by about **22%** at **1:500 (v/v)** SSW, whereas foliar application of the same concentration resulted in a higher increase of approximately **29.77%** over the control. Other concentrations produced moderate but statistically comparable responses.



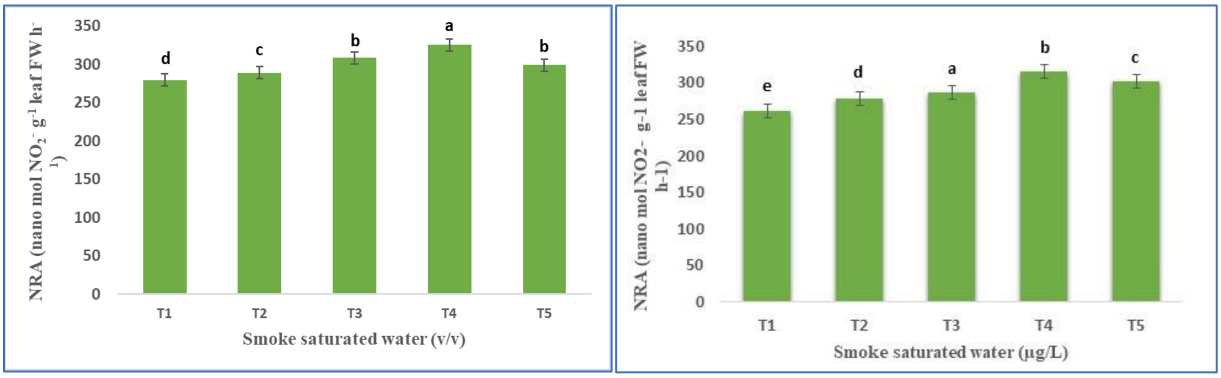
A *B*

Fig. 4. Effect of rhizome soaking (A) and foliar application (B) of smoke-saturated water (T5 = 1:1000, T4 = 1:500, T3 = 1:250, T2 = 1:125 v/v, and T1 = Control) on total carotenoid content (mg g^{-1} FW) in Mentha arvensis L.

3.3 Biochemical parameters

3.3.1 Nitrate reductase activity

The nitrate reductase activity increased remarkably after the application of smoke-saturated water (SSW), in both experimental designs (Figure 5). In rhizome soaking (RS) experiment, 1:500 (v/v) SSW treatment exhibited an increased rate of 15.96% compared with control. In a different vein, in the foliar spray (FS) experiment, treated plants at the same concentration showed a similar increase of approximately 15.63% compared to untreated plants. Other SSW concentrations exhibited moderate increases but showed statistical comparability to the ideal treatment.

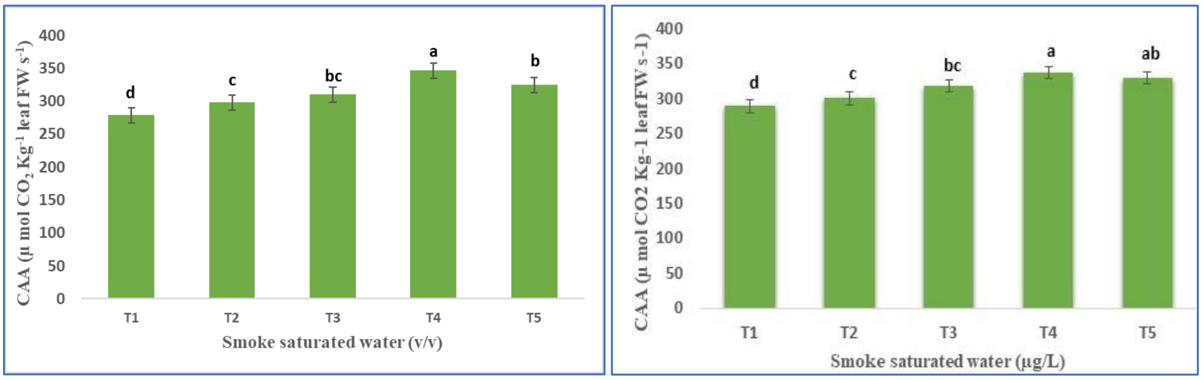


A ***B***

Fig. 5. Effect of rhizome soaking and foliar application of smoke-saturated water (T5 = 1:1000, T4 = 1:500, T3 = 1:250, T2 = 1:125 v/v, and T1 = Control) on nitrate reductase activity (nmol NO_2^- g^{-1} leaf FW h^{-1}) in Mentha arvensis L. (A and B).

3.3.2 Carbonic anhydrase activity

Carbonic anhydrase activity was shown to respond substantially to treatment with SSW in both experiments (Figure 6). In the RS experiment, the application of 1:500 (v/v) SSW increased enzyme activity by about 18.75% compared to the control. Likewise, in the FS experiment, a similar increase of ~19.23% was observed at the same concentration. The lower and higher concentrations resulted in smaller but significant increases.

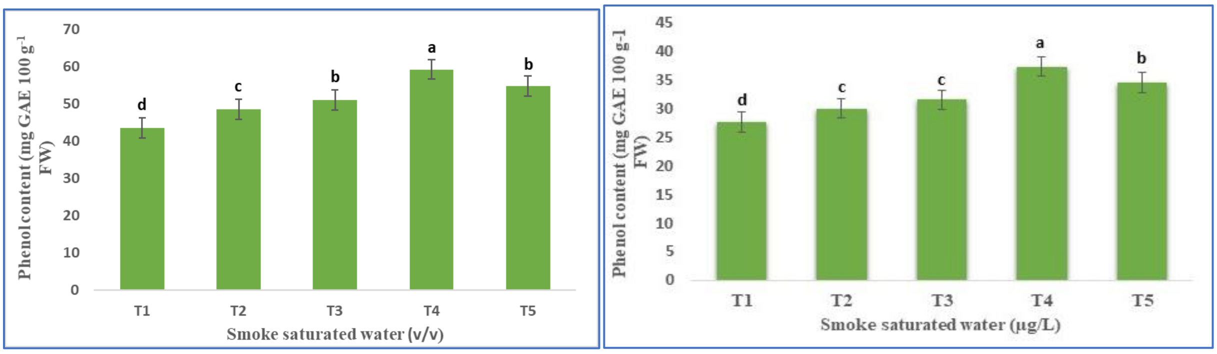


A ***B***

Fig. 6. Effect of rhizome soaking and foliar application of smoke-saturated water (T5 = 1:1000, T4 = 1:500, T3 = 1:250, T2 = 1:125 v/v, and T1 = Control) on carbonic anhydrase activity (µmol CO_2 kg^{-1} leaf FW s^{-1}) in Mentha arvensis L. (A and B).

3.3.3 Total phenolic content

The total phenolic contents of the sample increased markedly with SSW treatment for both experimental methods (Figure 7). In RS, the 1:500 (v/v) treatment produced a maximal increase of around 35.71% from the control. Likewise, phenolic content of FS improved by approximately 36.36% at the same concentration during the FS experiment. Other SSW treatments also exhibited moderate, statistically similar improvements. SSW application in both experiments showed a positive response to flavonoid accumulation (Figure 8).

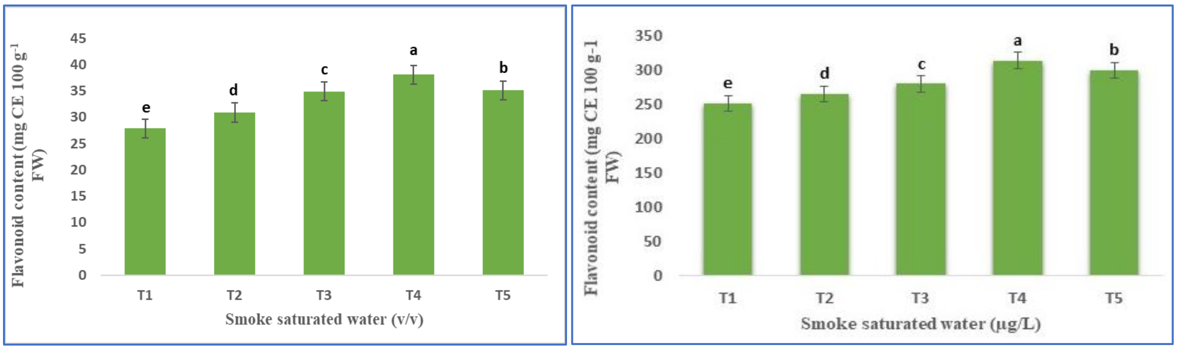


A ***B***

Fig. 7. Effect of rhizome soaking and foliar application of smoke-saturated water (T5 = 1:1000, T4 = 1:500, T3 = 1:250, T2 = 1:125 v/v, and T1 = Control) on total phenol content (mg GAE 100 g^{-1} FW) in Mentha arvensis L. (A and B).

3.3.4 Total flavonoid content

The RS experiment showed that plants, treated in SSW at 1:500 (v/v), increased total flavonoid level by around 36.00% versus controls. In the FS experiment, the percentage of increase was higher, reaching approximately 36.84% at the same concentration. Remaining treatments also exhibited significant but lower increases (Fig 8).



A ***B***

Fig. 8. Effect of rhizome soaking and foliar application of smoke-saturated water (T5 = 1:1000, T4 = 1:500, T3 = 1:250, T2 = 1:125 v/v, and T1 = Control) on flavonoid content (mg CE 100 g^{-1} FW) in Mentha arvensis L. (A and B).

3.4 Scanning electron microscopy (SEM) analysis

Changes in glandular trichome characteristics of *Mentha arvensis* leaves induced by smoke-saturated water (SSW) applied were observed on the SEM (Figure 9). Rhizome soaking (RS) showed an increase of approximately 24.75% compared to the control when the plant was soaked with 1:500 (v/v) SSW. Moderate increases were obtained in other concentrations, but they were statistically similar to the best treatment. The application of 1:500 (v/v) SSW in the foliar spray (FS) experiment had a significant increase in trichome size of about 14.00% relative to untreated plants. Smaller responses were observed with lower and higher concentrations. Overall, SSW supplementation improved glandular trichome growth, although a stronger effect was observed under rhizome soaking.

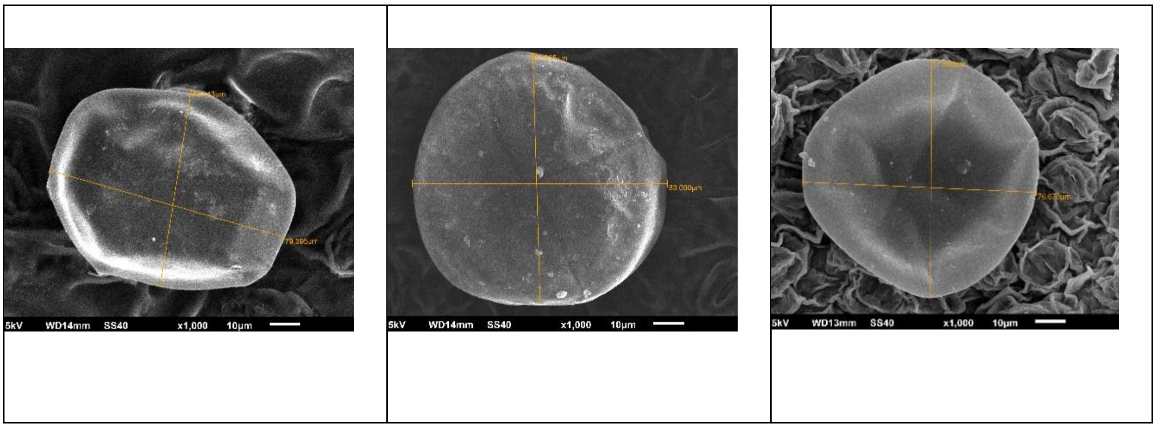
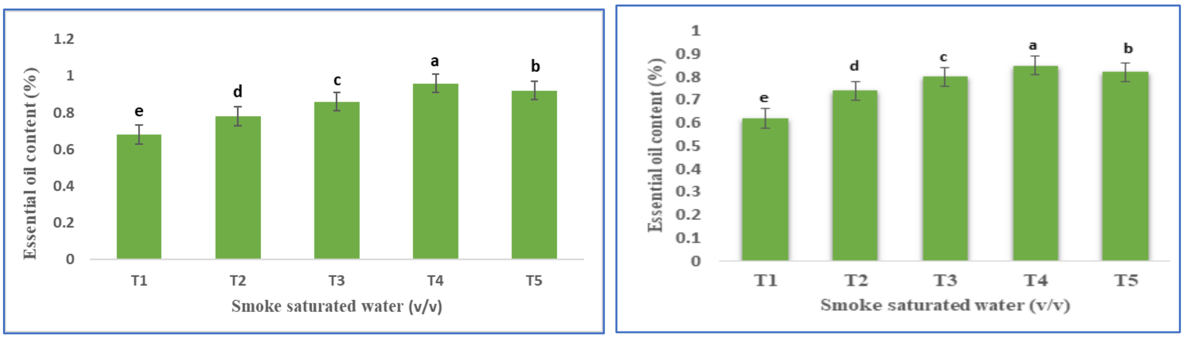


Fig. 9. Effect of smoke-saturated water (SSW) through rhizome soaking and foliar application on the size of leaf glandular trichomes in Mentha arvensis L., observed under a scanning electron microscope: (a) Control, (b) 1:500 (v/v) SSW — rhizome soaking, and (c) 1:500 (v/v) SSW — foliar application.

3.5 Essential oil content and yield

The amount and yield of essential oil were positively and significantly affected by SSW treatment in both experimental strategies (see Table 3, Fig 10). As noted above, the RS treatment with 1:500 (v/v) SSW led to a maximum level of improvement of 21.60% in the essential oil contents when compared with the control. This improvement was statistically equivalent to plants treated at 1:250 and 1:1000 v/v (Table 3). The essential oil content in the FS experiment increased by approximately 18.30% at 1:500 (v/v) in comparison to untreated plants. Other SSW treatments improved oil content similarly to the aforementioned, but only to a moderate degree.

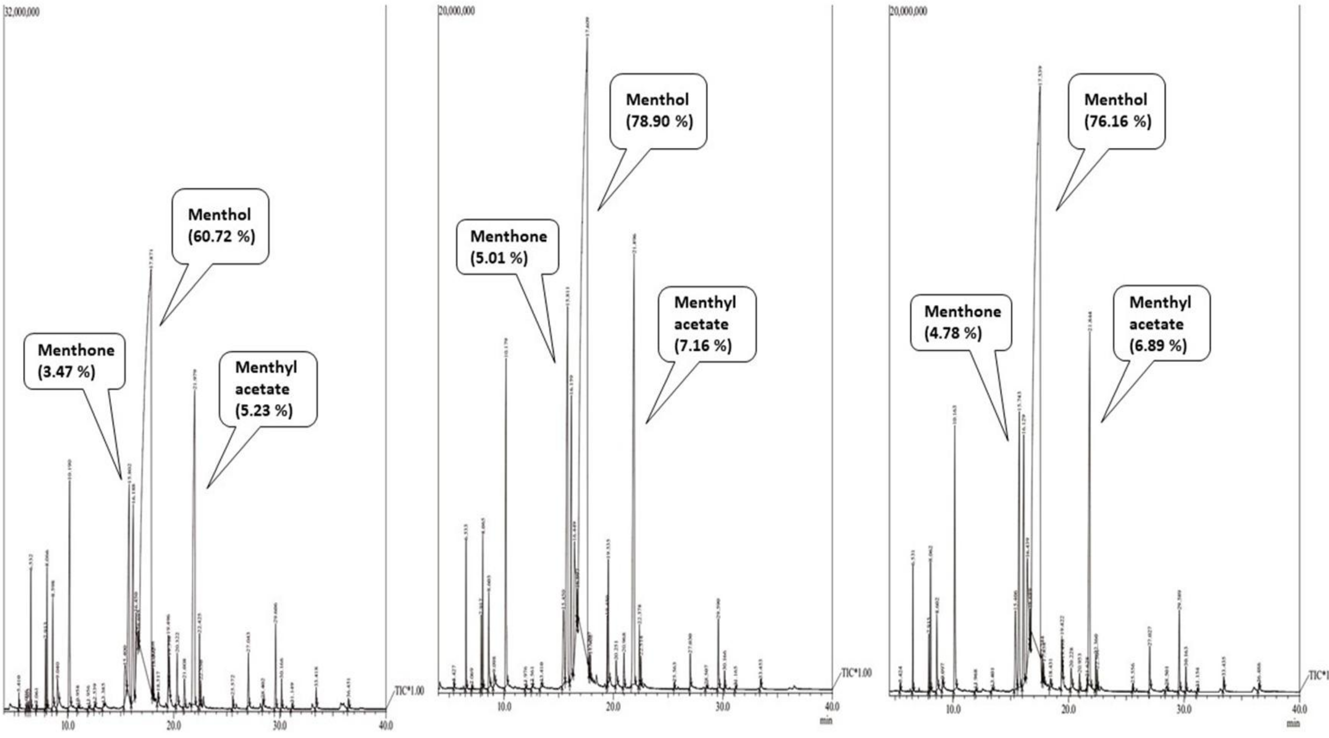


A *B*

Fig. 10. Effect of rhizome soaking and foliar application of smoke-saturated water (T5 = 1:1000, T4 = 1:500, T3 = 1:250, T2 = 1:125 v/v, and T1 = Control) on essential oil content (%) in Mentha arvensis L. (A and B).

3.6 Essential oil composition

Both qualitative and quantitative changes were observed in essential oil composition after SSW treatments (Table 4) by GC–MS analysis. In both RS and FS experiments, plants treated with SSW 1:500 (v/v) reported an increase in core constituents (especially menthol, menthone, and menthyl acetate) in comparison with control. For minor constituents, slight changes were also seen without any effects on quality of oil. In the rhizome soaking experiment, the improvement in essential oil composition was more significant than foliar application.



Control ***1:500 v/v SSW (Rhizome soaking)*** ***1:500 v/v SSW (Foliar application)***

Fig. 12. Optimal SSW concentration (1:500 v/v), applied through rhizome soaking and foliar spraying, enhanced menthol, menthyl acetate, and menthone contents compared with the control.

4. Discussion

A summary of the present study reveals that smoke-saturated water (SSW) exhibited a significant and favorable regulatory effect on growth, physiology, biochemical status as well as essential oil production of *Mentha arvensis* L, whereas the markedly improving of plant height, biomass accumulation, leaf appearance, leaf characteristics and overall growth performance of the plants, specially at dilution of 1:500 (v/v), all these data demonstrate that SSW is an extremely effective growth boosting plant. These tendencies are similar to previous studies which showed a potent vegetative, vigour and growth enhancer effect in smoke or smoke-bioactive-based formulations in vegetative growth, vigor and development in several plant species by virtue of bioactive signalling compounds such as smoke-based bioactive compounds (Keeley and Fotheringham, 1998; Van Staden *et al.*, 2000; Kulkarni *et al.*, 2011). The enhancement of photosynthetic efficiency achieved in the present study also enhances the support for the beneficial effect of SSW. The greatly improved chlorophyll fluorescence parameters, coupled with increased concentrations of chlorophyll and carotenoid indicate a photochemical improvement in effectuation and efficient light energetics. Higher Fv/Fm, Y(II), qP, and ETR values indicate better PSII activity, and electron transport in optimal SSW treatment may be effective. Comparable improvement of photosynthetic machinery in smoke solution has been also hinted on previous studies in which smoke-based compounds increased the capacity for light harvesting and photosynthetic capacity (Light *et al.*, 2009; Taiz *et al.*, 2015). Elevation in photosynthetic pigments may also signify enhanced chloroplast stability and metabolic competence (Lichtenthaler and Wellburn, 1983). These biochemical changes from in the current work corroborate the growth-promoting characteristics of SSW. Increased nitrate reductase and carbonic anhydrase activities indicate better nitrogen assimilation and carbon metabolism driving enhanced biomass composition. Similar physiological triggering under smoke exposure has been described previously, in which smoke compounds affected enzymes responsible for growth and metabolism (Rehman *et al.*, 2018). Furthermore, the substantial increase in phenolic and flavonoid content indicates augmented secondary metabolic activity, thus potentially resulting in better stress tolerance and antioxidative ability (Kulkarni *et al.*, 2013). An important result from this work is the stimulatory action of SSW on glandular trichomes development, as evidenced via SEM. Optimal concentration of enlarged peltate glandular trichomes would account for a structural increase in essential oil production, as these glandular trichomes are the major sites for essential oil biosynthesis and storage in mint species (Werker, 2000; Tiwari, 2016). Similarly, essential oil formation, production and the amount of main constituents (menthol, menthone, and menthyl acetate) were also more effective under SSW treatment. The enhanced profile is reminiscent of previous studies reporting that physiological level and metabolic regulation have a direct impact influencing the accumulation and quality of essential oil in Mentha species (Verma *et al.*, 2010; Tafrihi *et al.*, 2021). The study also shows that *Mentha arvensis* responds to SSW in a concentration-dependent way. Although all concentrations had positive effects, 1:500 (v/v) showed the greatest improvements in all growth, physiological, biochemical and oil parameters tested both in the case of rhizome soaking and as a matter of foliar spray. The study underlines the value of making the application strength more efficient, since the same effect of low or high quantities could not be obtained at low or large concentrations. Doses associated with comparable amount-dependent effects are already reported in a wider range of smoke-water studies by Van Staden and others (Van Staden *et al.*, 2004; Kulkarni *et al.*, 2011). In conclusion, SSW is recognized as an alternative biostimulant agent capable of facilitating primary growth processes and increase the secondary metabolite production in *Mentha arvensis*; It is also cost-effective and environmentally friendly and economic to manufacture. For *Mentha arvensis* production with the benefit of environmentally friendly benefits, SSW application as an initial strategy based on simplicity, as well as simple, inexpensive, low-cost, and sustainable, it is a probable good choice for increasing the quality of essential oil and yield of menthol mint. Field-scale trials and molecular studies must thus be performed to validate these effects and understand the exact pathways through which smoke regulates physiological function.

5. Conclusion

The findings in this study demonstrate that smoke-saturated water (SSW) is an effective biostimulant compound that promotes growth and physiological function including biochemical metabolism and essential oil development in *Mentha arvensis* L. The application of SSW, particularly at 1:500 (v/v), also led to significantly raised plant height, biomass, photosystem efficiency, pigment level, enzyme activities and secondary metabolites accumulation. Enlargement of glandular trichomes post-SSW treatment resulted in structural enhancement in essential oil yield and quality (including significant increases in major ingredients, menthol, menthone, menthyl acetate). SSW is overall a cost-effective, sustainable and accessible technology that has promise for enhancing productivity and essential oil profile in menthol mint cultivation. Field-scale investigation and molecular-level characterization of smoke-modulated regulation will add a further layer of value to its application to sustainable agricultural systems.

Declarations –

Competing Interest

There is no conflict of interest among the authors.

Author’s contributions –

SS drafted the original manuscript. ASC, SS and UHB prepared the draft of methodology of manuscript. MU and MMAK supervise and edited the final version of the manuscript figures and tables. All authors read and approved the final manuscript.

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