Improving copper stress tolerance in Mentha suaveolens L. by foliar application of salicylic acid

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Research Article

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Abstract

Background: Higher absorption and translocation of copper (Cu) in plant tissues can cause serious physiological and biochemical alterations. Salicylic acid (SA) is a signaling molecule responsible for inducing stress tolerance in plants. Therefore, SA spray could provide protection against several types of stress such as Cu toxicity. In this sense, a pot study was conducted to find out whether SA spraying (0.5 and 1 mM) could remedy the Cu (40 mM) toxicity in *Mentha suaveolens* plants

Results: Cooper stress caused a strong accumulation of Cu in the roots with a translocation of this metal to the leaves. Excessive accumulation of this metal decreased Ca, K and p contents. Copper stress also increased H_2O_2 accumulation and lipid peroxidation. However, SA spraying on these stressed plants decreased Cu concentrations in various parts of the plant. This decrease in Cu contents is accompanied by an increase in K, P and Ca concentrations. SA exerted also a remedial effect on the essential oils performance mainly at 0.5 mM. The histological study of the leaves of the *Mentha suaveolens* showed the presence of peltate and capitate glands which differ in size, stem and head. Copper decreased glands density but SA spraying increased glands number. Cu caused a variation in essential oils composition.

Conclusion: SA at 0.5 mM gives the best quality essential oils of all treatments. This concentration showed the greatest contents of the majority compounds (1,8-cineole, thujanone, camphor and Pulegone).

Background

The Lamiaceae (Labiatae) constitute a rich family in aromatic species characterized by the synthesis of essential oil of industrial and medicinal interest biosynthesized and accumulated in the glandular trichome distributed on the aerial vegetative and reproductive organs. The storage of essential oils in these structures can be used to limit toxicity to the plant itself, as many terpenes have been shown to be potentially toxic to plant tissues when released into surrounding cells [1]. The genus Mentha, comprising more than 25 species, produces around 2000 t of essential oil worldwide, making it the second most important genus producer of essential oils, after Citrus [2]. Like all Lamiaceae species, the genus Mentha has glandular trichomes responsible for the biosynthesis, secretion and accumulation of essential oil and non-glandular trichome (protective hairs) [3;4]. Glandular trichomes are subdivided into two types: peltate and capitate, which are distinguished by the size of the head, the length of the stem also by the material of secretion, the mode and time of secretion [5]. Cu is a natural trace element essential for plant growth. At low concentrations, Cu plays a crucial role in various processes such as protein and ion metabolism, photosynthesis, respiratory transport and nitrogen fixation [6]. Cu is characterized by its accumulation in the root cortex, which affects the uptake of other nutrients essential for plant growth [7;8]. Other work showed the negative effect of Cu stress on biomass and plant yield both in hydroponics and in soil [9]. In addition, several studies showed that exogenous SA contributes to the regulation of metabolic and physiological pathways in plants grown under Cu stress [10;11;12;13;14]. The curative effect of SA on plant growth under Cu stress conditions is related to its role in water content, nutrient uptake, chlorophyll pigments synthesis, growth, stomatal regulation, inhibition of ethylene biosynthesis, hormonal profile regulation and protein kinases synthesis [15;16;17;18]. In this study, we tested the remedial effect of SA on Cu toxicity on plant growth and mineral absorption, secretory gland density, essential oil content and its composition in *Mentha suaveolens*

Materials And Methods

Plant material and growth conditions

M. suaveolens cuttings were taken from the botanical garden of the Faculty of Science and Technology, Fez. Each cutting included at least two nodes. Culturing was carried out in plastic pots (3 kg capacity) containing 5 plants per pot and grown in a greenhouse.

Cu and SA treatments

After 30 days of plants transplantation (DAT), watering was performed with Cu. This Cu dose was chosen because a preliminary experiment showed that from 40 mM Cu induced growth inhibition of *M. suaveolens*. Cu was applied weekly over five-weeks (200 ml per pot). SA (0.5 and 1 mM) was applied by foliar spray at 60 DAT (These SA concentrations were chosen based on a preliminary screening experience). Three SA sprays were performed at a weekly interval using a hand sprayer (100 ml per pot).

This experiment included total six treatments: control, 0.5 mM salicylic acid (SA 0.5 mM), 1 mM salicylic acid (SA 1 mM), 40 mM Cu (Cu), 40mM Cu + 0.5 mM salicylic acid (Cu+ SA 0.5 mM) and 150 mM Cu + 1 mM salicylic acid (Cu+ SA 1 mM). Experiment was performed in five replicate and sampling was done at 100 DAT.

Cu and nutrients (K, P and Ca) content

Content of Cu and minerals (K, P and Ca) was determined according to Cottenie *et al.,* [19]. The root and shoot samples of *M. suaveolens* were dried at 100 °C for 48 hours. 100 mg of dried plant was calcined at 450 °C for 12 hours in a muffle furnace. The ash obtained was dissolved in 3 ml of nitric acid (0.1 N) and then filtered through Whatman filter paper 540 hardened ashes. The volume was adjusted to 20 ml with distilled water. Based on this solution, the assay was performed by inductively coupled plasma emission spectrometry (ICP-AES) to determine the content of Cu and minerals (K, P and Ca).

Hydrogen peroxide content

The extraction and determination of hydrogen peroxide are carried out according to the method described by Sergieve *et al.,* [20]. 1 g of leaves and roots are ground in 15 ml of trichloroacetic acid, the ground material is centrifuged for 15 min at 12,000 rpm. The assay is carried out by adding to 0.5 ml of supernatant, 0.5 of 10 mM phosphate buffer pH 7 and 1 ml of 1 mM potassium iodide then the optical density of the tubes is read at 390 nm.

Lipid peroxidation

Lipid peroxidation was determined as 2-thiobarbituric acid (TBA) reactive substances, i.e. malondialdehyde (MDA). Briefly, 0.25 g tissue sample was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA) followed by centrifugation at 10 000g for 5 min. To 1 ml aliquot of the supernatant, 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95^{o}C for 15 min and cooled immediately. The developed colour was extracted with 2 ml n-butanol, and the absorbance was measured at 532 nm followed by the subtraction of the value of nonspecific absorption at 600 nm. The level of lipid peroxidation was expressed as nmol of MDA g^{-1}fresh weight at an extinction coefficient of 155 mM cm^{-1} [21]. The results were presented as the average of three replicate estimations for each treatment.

Environmental scanning electron microscope

Microscopic observations of the fresh leaves were made using an environmental scanning electron microscope (Quanta 200, FEI Company). The microscope was equipped with a tungsten electron gun. Analyses were carried out under partial pressure of water vapour.

Count of peltate glandular trichome

Peltate glands counting was performed on the ventral side of the fresh leaves on a 1 mm^2 area, taking into consideration the basal (near the petiole), central and apical areas of the leaf. For each treatment, the glands number represents the average of glands of five plants and three leaves per plant [21].

Essential oil extraction

Essential oil extraction was carried out by hydrodistillation (Clevenger apparatus) of 100 g of parts aerial of *S. officinalis* dried in the free area. The extraction was carried out in 2 liters of distilled water for 180 min. The essential oil was collected, dehydrated with sodium sulfate and stored at 4°C. Essential oil yield was calculatedby the following formula [22]:

YEO (ml/100 g DM) = (V/DM x100)

YEO: essential oil yield of DM.

V: the volume of essential oil collected (ml).

DM: dry plant weight (g).

Essential oil analysis by GC–MS

Essential oil content was determined by gas chromatography (GC) (Agilent 7890A Series) coupled to mass spectrometry (MS) and equipped with a multimode injector, a BD-ASTMD 6584 column (15 m × 0.320 mm × 0.1 µm) and ionization by electronic impact. The protocol consists of: 1 µl of a solution of HE solubilized in chloroform was injected into the column by split mode 1: 2 using helium as carrier gas at 4 ml min^{-1}. The ion source and quadruple temperatures were 230 °C and 150 °C respectively. The oven temperature program was started at 30 °C and maintained 1 min then increased at 2 °C min^{-1} until 75 °C and maintained one minute then increased by 8 °C min−1 until 210 °C and kept constant for 1 min. The composition of the essential oil determined from the peak areas was calculated as a percentage of the total compounds existing in the sample detection using full scan mode between 30 and 1050 m z^{-1} with gain factor 5 and the identification was performed using NIST 2014 MS Library.

Statistical analysis

One-way analysis of variance was carried out for each parameter studied. Tukey's post hoc multiple mean comparison test was used to test for significant differences between treatments ($P \leq 0.05$). Univariate analysis was used to test significant differences in treatments, accessions, and their interaction for an individual parameter. All statistical analyses were performed with IBM. SPSS statistics, Version 22. The results of each experiment (biochemical assays) were repeated three times (20 times for morphological assays).

Results

Cu, Ca, K and P accumulation

M. suaveolens treatment with Cu (40 mM) increased the Cu concentration in various parts of the plant (Table 1). This accumulation takes place mainly in the roots (7.3mg/g MS) and its transfer to the leaves (4.3 mg/g MS). SA spraying decreased Cu absorption, which was found in trace amounts in the plant. K^+, P and Ca^{2+} absorption was also affected by Cu excess. There was a considerable drop in their content both in the leaves and in the roots. However, the application of SA to stressed plants results in an improvement in the levels of these elements in different parts of the plant mainly when SA was applied at 0.5 mM concentration. There is thus an improvement in root absorption of 48% for K^+, 739% for P and 44.50% for Ca^{2+}. In leaves, 60.60% for K^+ and 48.20% for Ca^{2+} and 455% for phosphorus.

Hydrogen Peroxide content

Cu stress greatly increased H_2O_2 concentration at leaf and root (fig. 1). These contents increased by 124.6% in the aerial part and 122% in the root parts. However, SA spraying on stressed plants greatly decreased H_2O_2 levels. This reduction can reach 50% mainly when SA was applied at 0.5 mM.

Malondialdéhyde content

Cu stress increased MDA levels (fig. 2); they were around 220% at the leaf and 880% at the root. On the other hand, SA spraying considerably reduced these levels mainly at 0.5 mM since this drop can reach 64% and 61% respectively in the leaves and roots.

Essential oil yield

In the absence of metallic stress, foliar SA spraying improved essential oil yield of *M. suaveolens* (Table 2). This improvement was all more important at higher SA concentration. The yield can reach (0.74%) with a concentration of 1 mM showing an improvement of 60.86%. In addition, the presence of Cu in the soil decreased essential oil yield of which can reach almost 54.34% compared to the control. On the other hand, under these Cu stress conditions, foliar SA spraying increased yield mainly at 0.5 mM when the yield can reach 1.43% showing an improvement of 581%.

Glandular trichomes abundance

In the absence of copper stress, SA at 0.5 mM improved glands density of the secreting essential oils in *M. suaveolens* (fig. 3). Copper stress decreased the peltate glands density (fig. 3 and 4b) in the three areas studied. These decreased reach almost 37.5% in the apical zone, 43.5% in the central zone and 27.6% in the basal zone (fig. 3). On the other hand, on these stressed plants, SA spraying mainly at 0.5 mM (fig. 4c) increased the glands number. Under these conditions, these increases reach 124%, 193% and 160% respectively in the basal, central and apical zones. Regarding the diameter, there is a slight decrease in diameter in stressed plants. On the other hand, we noted an increase in diameter in plants treated with SA at 0.5 mM (fig. 4) compared to plants stressed and not treated with SA. In these plants we notice the presence of some glands with a large diameter which goes up to 125.5 and 127.9 µm (fig. 5).

Essential oil composition

Table 2 shows the composition of the essential oils of *M. suaveolens*. Four categories of molecules were identified, the oxygenated monoterpenes, the hydrocarbon monoterpenes, the hydrocarbon sesquiterpenes and the oxygenated sesquiterpenes. The four main compounds of this oil are 1,8-cineole, thujanone, camphor and Pulegone. There is also a balance in the appearance and disappearance of certain molecules and the modification of the content of pre-existing molecules depending on the culture conditions.

Thus, copper stress increased oxygenated monoterpenes content by 24% and hydrocarbon sesquiterpenes content by 15%. It also leads to an increase in the content of major compounds, 1,8-cineole which ranges from 8.92 to 10.96%, thujanone from 7 to 10.73% and Pulegone from 10.32 to 11.32%. Furthermore, SA spraying on these stressed plants caused a very significant increase in oxygenated monoterpenes which can reach 67% compared to the control when SA is applied at 0.5 mM. Under these conditions, this phenomenon was due to the increase in the major compounds which showed the highest contents compared to all the treatments. Thus, 1,8-cineole goes from 8.92 to 14%, thujanone from 7 to 14.62 and Pulegone from 10.32 to 16.11%. This increase also concerns menthone, thujanol and menthole which respectively reach 96.41%, 77.82% and 88.79% compared to the control.

Note that under non-stress conditions, there was an improvement in the yield and chemical composition of essential oils under SA treatment at 0.5 Mm. SA caused an increase of 148.76% in the content of hydrocarbon sesquiterpenes, and of 62.56% in the levels of oxygenated monoterpenes. The latter is due to the increase in the majority compounds, 1,8-cineole from 8.92 to 11.87%, thujanone from 7 to 9.03, camphor from 10.06 to 16.44 and Pulegone from 10.32 to 15.11%.

Discussion

Copper stress had detrimental effects on plant growth and productivity. The inhibition of growth can be attributed to Cu toxicity on various processes such as respiration and photosynthesis [23]. This study showed that copper stress increased Cu concentrations in various parts of the plant. Copper accumulated much more in the roots than in the leaves. Collin 2011 [24] argued that the root apoplast is a major compartment of metal accumulation in plants. This increase in the Cu content was accompanied by a decrease in the K^+, P and Ca^{2+} contents. Indeed, Cu toxicity decreased the accumulation of mineral nutrients essential for plant growth [25; 26].

In addition, SA foliar spraying, mainly at 0.5 mM, improved plants growth. This improvement is linked to the decrease in Cu content in the leaves and roots and therefore to the improvement in the uptake of nutrients (Ca, k and p). These results are in agreement with those of Mei *et al.*, [13] on cotton, Es-sbihi *et al.*, [27; 28] on *Salvia officinalis*. Mostofa and Fujita (2013) suggested that the tolerance mechanism induced by SA is associated with exclusion of Cu or limited Cu uptake. In our study, SA-induced inhibition of Cu-upward process could be considered as one of the potential physiological effects of SA on *M. suaveolens* plants. According to Ahmad *et al.*, [30], the increased absorption of mineral nutrients may be due to the H^+ ATPase activity induced by SA. Es-sbihi *et al.*, [27; 28] have also showed that SA improved plant growth through the decrease in metal concentrations in leaves and roots of plants.

Excessive transport of Cu ions into plant tissues can accelerate ROS production [30], and subsequently damage cells and cellular organelles [31]. Cu, being one of the redox-active metals that catalyzes the Fenton reaction, can accelerate the generation of very damaging OH • radicals from O_2 and H_2O_2 [32]. Baryla *et al.*, [33] demonstrated that lipid peroxidation of membranes could be used as a biological test of toxicity for plants, because it is very sensitive to Cu.

In the present study, ROS (H_2O_2) production induced by Cu directly leads to damage to the lipid membranes of cells and is shown by the elevated MDA levels. This study also provides evidence that Cu-induced oxidative stress was effectively attenuated by SA spraying mainly at low doses (0.5 mM); low H_2O_2 and MDA contents were obtained. The observed low level of MDA content in roots and leaves indicates the protective role of SA in decreasing peroxidation of cell organelles. These results are in agreement with those of Kawano and Muto [34] and El tayeb *et al.*, [10] who also reported that the generation of OH • by Fenton-type reaction is lowered by SA. The latter can chelate transition metals, thus reducing the formation of OH • and / or can act directly as an OH scavenger. Mei *et al.*, [13] showed that treating cotton plants with SA results in an improvement in the activities of antioxidant enzymes such as SOD, POD and GR. In addition, we observed a decrease in the content of essential oils under the effect of copper stress, confirming the observations of Zheljazkov and Warman [35] in Mentha species. This decrease in performance can be attributed to the decrease in the number of essential oil secreting glands. On the other hand, SA spraying (mainly at 0.5 mM) in the presence of copper stress increased essential oils yield which was associated with an increase in the glands density. Idrees *et al.*, [36], Rowshan *et al.*, [37]; Khanam *et al.*, (2018) linked the positive effect of SA on essential oil yield to increased peltate gland count. Es-sbihi *et al.*, [28] showed a decrease in the essential oils contents of *S. officinalis* cultivated under zinc stress, whereas SA spraying of this plant leads to an increase in the gland density and the essential oil yield.

In addition, we observe many qualitative and quantitative variations regarding the chemical composition of essential oils. These changes result in the appearance or disappearance of certain compounds and changes in the content of pre-existing compounds. Copper stress positively influences the content of oxygenated monoterpenes and hydrocarbon sesquiterpenes and increases the content of the majority compounds, 1,8-cineole which varies from 8.92 to 10.96%, thujanone from 7 to 10.73%, camphor from 10.06 to 17.05% and the Pulegone from 10.32 to 11.32%. Idrees *et al.*, [36], Rowshan *et al.*, [38] and Hashimi *et al.*, [39] have shown the positive effect of SA on the enzymes responsible for the synthesis of terpenes and on the PAL activity (phenylalanine amonia lyase) key enzyme in the pathway of essential oil synthesis. On the other hand, we note that the SA (mainly at 0.5 mM) causes a very significant increase in oxygenated monoterpenes levels following the increase in the majority compounds. Note that the highest concentrations of these molecules were obtained with the combination SA / copper stress.

Pirbalouti *et al.*, [40] reported that SA foliar application reduced the effect of deficit water stress on the amount of the major essential oil compound of *Thymus daenensis*. It increased the carvacrol, α-thujone, α-pinene and p-cymene concentrations. These same authors added that SA can convert thymol to its isomer (carvacrol) and played an important signaling role in activating various plant defense responses, such as the biosynthesis of special secondary metabolites, which play the role phytoalexins in plants (Sirvent and Gibson, 2002). Es-sbihi *et al.*, [41] showed an improvement in the essential oils quality under SA at 0.5 mM in the presence of zinc stress.

Conclusion

Heavy metals in general, and Cu in particular, have caused a serious pollution problem in recent decades mainly due to industrial development. This pollution disrupts ecosystems by damaging soil, water surface, forests and crops. Cu is biopersistent, so it is difficult to eliminate and can accumulate in plant organs. This study shows the restorative effect of SA foliar spray, mainly at 0.5 mM, on essential oil synthesis and composition in *M. suaveolens*, grown under Cu stress. The positive effects of SA were linked to the decrease in excessive concentrations of Cu in different parts of the plant accompanied by better assimilation of other minerals. The

restorative effect of low-dose of SA on growth and essential oil synthesis of *M. suaveolens* grown under Cu stress can be attributed to inhibition of Cu root uptake.

Abbreviations

SA: Salicylic acid; DM: Dry matter; DAT: Days of plants transplantation.

Declarations

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Authors' contributions

All authors of this research paper have directly participated in planning, execution and analysis of the study.

All authors read and approved the final manuscript.

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Availability of data and materials

Additional data may be availed on request to the authors through the corresponding author.

We take legal responsibility for information, used procedures, data and results.

Ethics approval and consent to participate.

This research work meets all the ethical guidelines, adhering to the legal requirements of my country.

Consent for publication

The authors confirm that there is no conflict of interest and agree with submission of the manuscript to your journal.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table1: Content of Cuand mineral nutrients (P, K^+ et Ca^{2+}) in *M. suaveolens* exposed to different treatments of SA and copper stress in shoot and root.

		Cu (mglg DM)	*Ca (mglgDM)*	*K (mglg DM)*	*P (mglg DM)*
Roots	*Control*	Tr	13.86±0.02a	12.11±0.10A*	0.47±0.10A
	SA (0.5mM)	Tr	15.10±0.01b	15.07±0.01 B*	0.65±0.2B
	SA (1mM)	Tr	14.85±0.03c	10.52±0.06C*	0.97±0.1C
	Cu	7.3±0.03A	9.30±0.01d	9.48±0.05 D*	0.18±0.01D
	Cu+ SA(0.5mM)	Tr	13.44±0.03a	14.07±0.09E*	1.51±0.09E
	Cu + SA (1mM)	Tr	11.02±0.01a	11.07±0.4F*	0.83±0.07F
Leaves	*Control*	Tr	14.46±0.01a*	12.64±0.01A	1.25±0.01A*
	SA (0.5mM)	Tr	10.15±0.01b*	16.61±0.02B	1.49±0.03A*
	SA (1mM)	Tr	18.54±0.02c*	12.63±0.01A	1.01±0.01B*
	Cu	4.3±0.01a	9.57±0.01d*	8.83±0.02C	0.29±0.02A*
	Cu + SA (0.5mM)	Tr	14.18±0.11a*	14.18±0.03D	1.61±0.01A*
	Cu + SA (1mM)	0.12	13.18±0.03a*	13.22±0.02E	1.32±0.02A*

Table 2: Influence of SA and copper stress on essential oils yield and composition in *M. suaveolens*. The values followed by different letters are significantly different ($P \leq 0.05$).

	Control	SA 0.5 mM	SA 1mM	Cu	Cu +SA 0.5 mM	Cu+SA 1mM
Oils content (%)	0,46±0,01a	0,54±0,01b	0,74± 0,1c	0,21±0,01d	1,43± 0,08e	0,45±0,05 f
Components	***Peak area %***					
Hydrocarbon monoterpenes						
α-Pinene	0,88	0,5	0,47	0,39	0,47	0,26
β-Terpinene	nd	0,7	0,85	0,56	0,8	0,75
Limonene	2,28	2,54	1,38	1,59	1,45	1,49
α-Terpinene	1,2	1,25	1,5	0,2	2,2	1,2
Terpinolene	0,3	0,7	0,5	nd	0,9	0,6
γ-Terpinene	0,3	0,37	0,3	0,1	0,3	0,3
3-carene	1,34	1, 43	1,4	1,87	1, 98	1,94
Total (%)	**6,3**	**7,49**	**6,4**	**4,71**	**6,12**	**6,54**
Oxygenated monoterpenes						
1,8-Cineole	**8,92**	**11,87**	**10,06**	**10,96**	**14**	**11,4**
Thujanone	**7**	**9,03**	**8,89**	**10,73**	**14,62**	**12**
Thujanol	2,3	5,5	5,6	3,09	4,09	6,09
Terpineol	6,09	6,9	6,99	4,13	5,9	4,62
Thujone	0,6	3,42	2,4	2	2,4	2,57
Menthol	2,32	7,22	4,74	3,94	4,38	8,94
Menthone	2,23	5,22	5,74	3,94	4,38	1,94
Camphr	**10,06**	**16,44**	**15**	**10,58**	**17,05**	**15**
Bornyl acetate	0,43	0,57	0,65	0,46	0,87	0,83
Terpinen-4-ol	0,28	0,82	0,32	0,82	0,72	0,82
L-α-terpineol	0.32	0,4	0,8	0,32	0,47	0,85
Myrtenol	0.26	0,6	0,56	0,3	0,36	0.26
Estragol	0,82	0,42	0,4	0,9	0,56	0,94
Pulegone	**10,32**	**15,1**	**12,18**	**11,32**	**16,11**	**13,47**
Totale (%)	**51,37**	**83,51**	**76,14**	**63,49**	**85,91**	**79,47**
Hydrocarbon sesquiterpenes						

sabinene hydrate	0,6	0,66	0,29	0,13	0,5	1
Cariophyllene	0,34	0,59	0,84	0,87	0,7	0,28
Humulene	0,39	1,34	1,29	0,4	0,78	0,79
γ-Muurolene	0,34	0,73	0,25	0,98	0,69	0,79
Cadinene	0,4	0,82	0,4	0,2	0,62	0,94
β –Longipinene	0,2	0,25	0,50	0,32	0,24	0,2
Germacrene-D	0,56	2,65	3	0,36	0,23	0,6
Total (%)	**2,83**	**7,04**	**6,57**	**3,26**	**3,76**	**4,6**
Oxygenated sesquiterpenes						
Geranyl isovalerate	0,23	0,74	0, 94	0,18	0,94	0,22
Sabinol isovalerate	0,34	0, 93	0,21	0,11	0, 59	0,39
Caryophyllene oxide	0,34	0, 3	0,12	0,1	0,56	0,39
Humulene epoxide II	0,28	0,42	0,4	0,19	0,56	0,94
Viridiflorol	0,64	0,5	0,6	0,5	0,56	0,56
Cadinol	0,22	0,42	0,56	0,44	0,28	0,4
Caryophylladienol II	0,69	0,5	0,56	0,6	0,6	0,6
Total (%)	**2,74**	**2,58**	**2,45**	**2,12**	**3,5**	**3,1**
Total identified (%)	**63,24**	**98,81**	**91,56**	**73,58**	**99,29**	**93,71**

SA: Salicylic acid; nd: Not detected

Figures

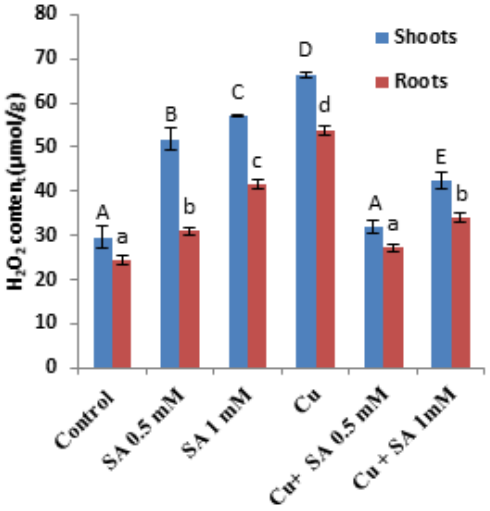

Figure 1

Changes of hydrogenperoxide contentsin *M. suaveolens*exposed to different treatment the SA and copper stress.

The values followed by different letters are significantly different (P ≤ 0.05)

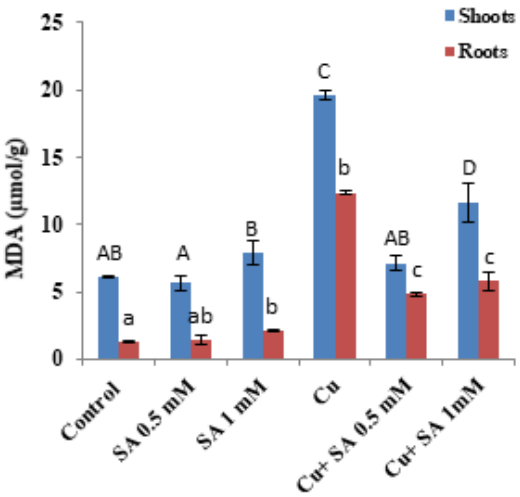

Figure 2

Changes of Malondialdéhyde contents in *M. suaveolens* exposed to different treatment the SA and copper stress.

The values followed by different letters are significantly different (P ≤ 0.05)

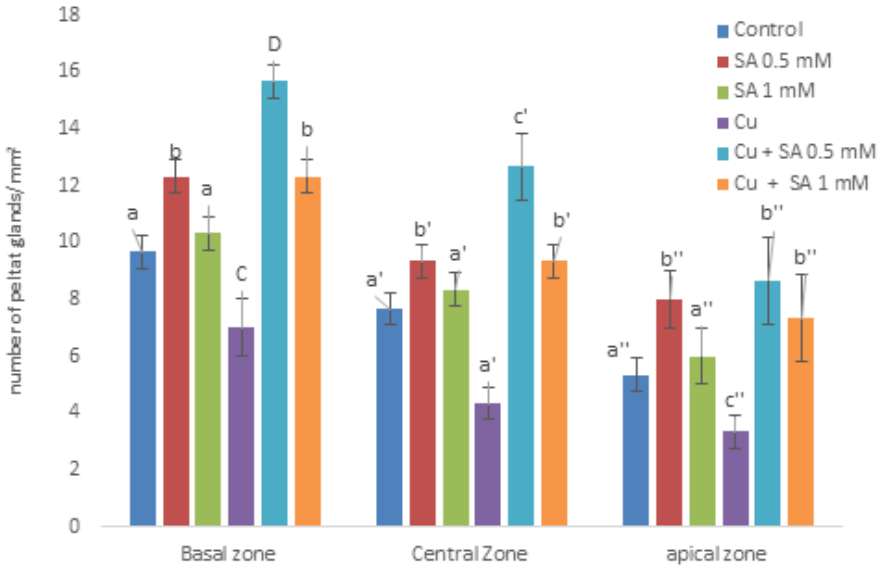

Figure 3

Abondance de glandes peltées sur la surface ventrale des feuilles de *M. suaveolens* exposées à différents traitements de stress au cuivre et de l'AS.

Les valeurs suivies de lettres différentes sont significativement différentes ($P \leq 0,05$)

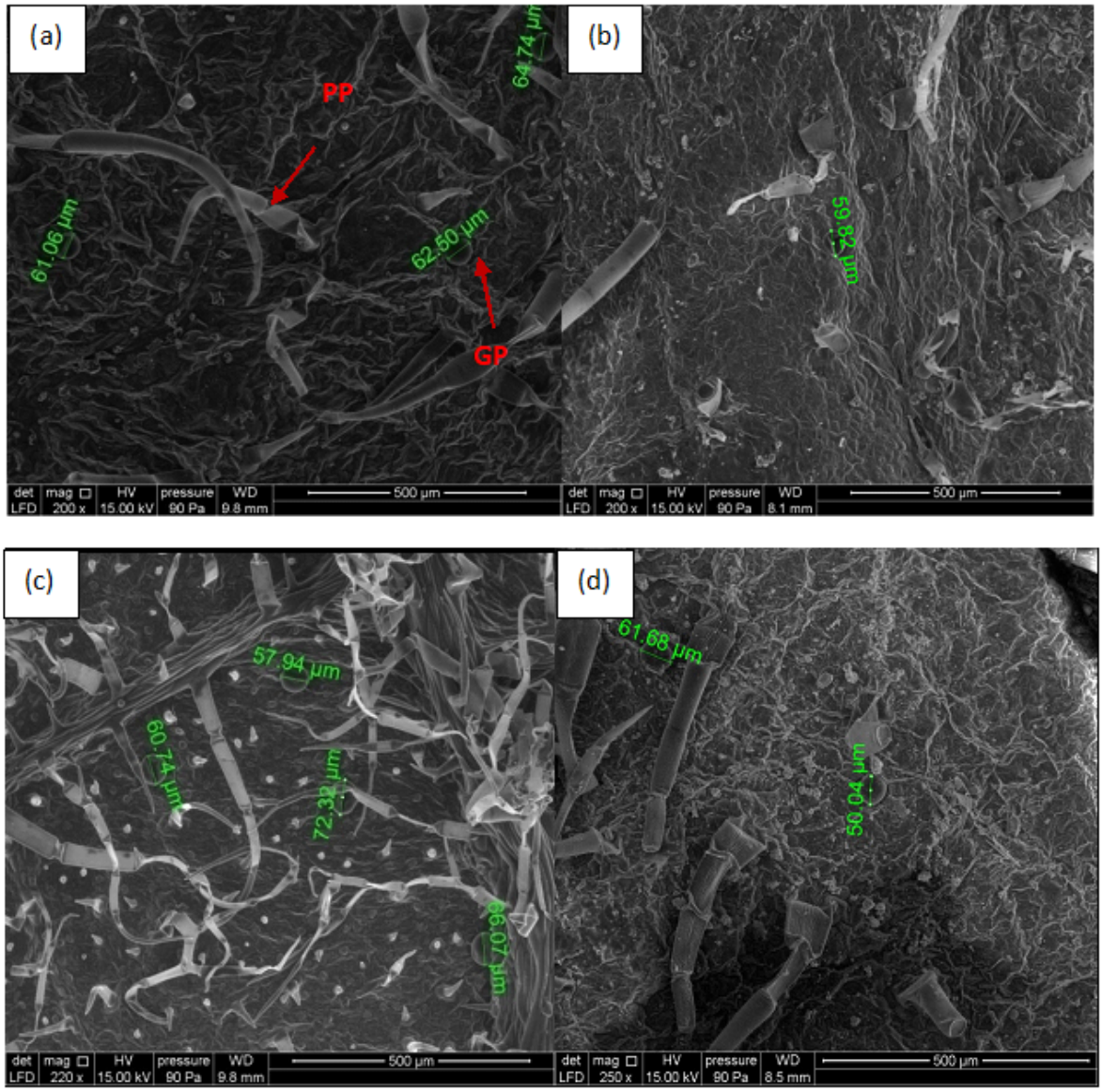

Figure 4

Observation par microscopie électronique à balayage environnementale chez les feuilles de *M.suaveolens.* Témoin(a), Cu (b), Cu+ AS 0.5mM (c), Cu+ AS 1mM (d). PG : Glandes peltées; PP: Poils de protection

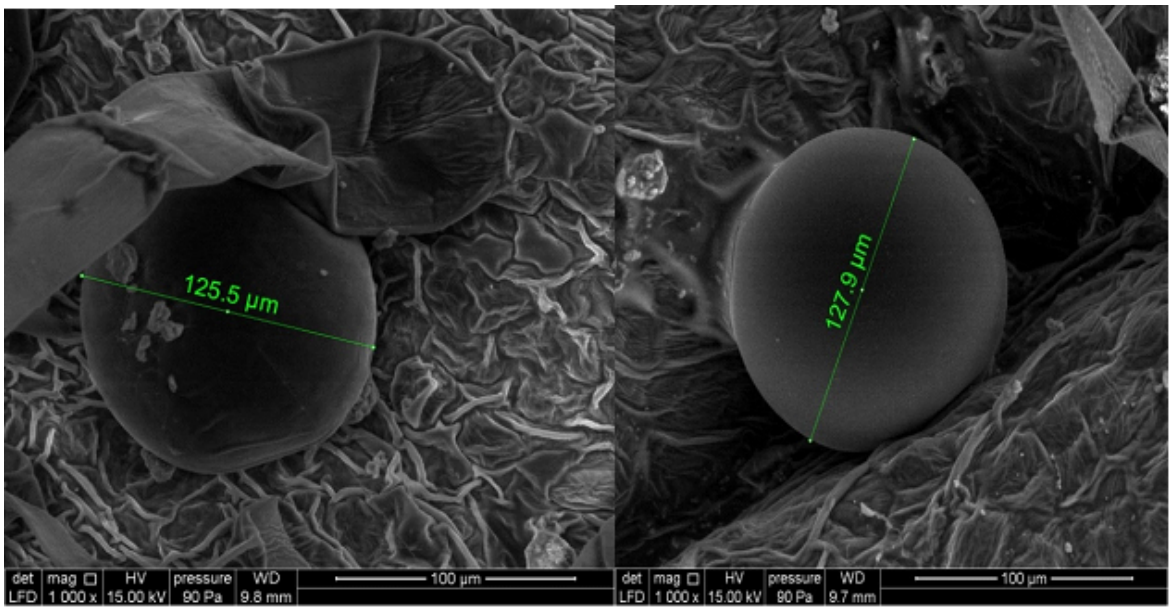

Figure 5

Observation en microscopie électronique à balayage environnementale de glandes peltées chez les feuilles de *M. suaveolens* traitée avec Cu et l'AS à 0.5Mm