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Table: 1, Figs : 3

Sensitivity of *Brachiaria decumbens* and *Ipomoea cordifolia* to cyclic polysulfides from leaves of *Microlobius foetidus*

C.B. DA SILVA[1,*], E. SIMIONATTO[2], S. S. GEBARA[3], N. RÉ- POPPI[3], K.S.RECH[1], M. T. L. P. PERES[4], G.O. SANTOS[5], J. F. G. DIAS[1], S. M. W. ZANIN[1], O. G. MIGUEL[1] and M. D. MIGUEL[1].

Department of Pharmacy, Federal University of Paraná, Curitiba, CA 80210170, Brasil
E. Mail: cris.mpj@gmail.com.

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ABSTRACT

Volatile oils from leaves of *Microlobius foetidus* subsp. *paraguensis* (Benth.) M. Sousa & G. Andrade, a native and predominant species of cerrado regions of Pantanal and chaco regions of Porto Murtinho, were investigated by hydrodistillation (HD). In laboratory bioassays, the actions 6-concentrations of volatile oils (0%, 0.35%, 0.65%, 1.25%, 2.5% and 5.0%) were studied on the antioxidant defense system of *Ipomoea cordifolia*. The evaluation methods allowed the identification of 9-compounds (1,2,4-trithiolane, E-1,2,4-trithiolane-3,5-dimethyl, Z-1,2,4-trithiolane-3,5-dimethyl, 1,3,5-trithiane, 1,2,4,5-tetrathiane, 1,2,4,6-tetra-thiepane, 1,2,4,5,7-pentathiocane, 1,3,5,7,9-pentathiecane and 1,2,5,6-tetrathiocane) by the HD method and the substance 1,2,4-Trithiolane was the major constituent of volatile oils. The evaluated concentrations inhibited the growth of *I. cordifolia* seedlings and the effects were related to an increase in the concentration of the enzymes related to antioxidant defense system. It appeared that the actions of volatile oils were due to the oxidative stress.

Key words: Antioxidant defense, *Brachiaria decumbens,* Convolvulaceae, *Ipomoea cordifolia, Microlobius foetidus,* mimosoidae, volatile oils, volatilization.

INTRODUCTION

The *Microlobius* genus (Fabaceae family, Mimosoidae subfamily) is represented by *Microlobius foetida* species, with its two varieties: *foetidus* and *paraguensis*. It occurs from Mexico to Brazil, mostly in *Pantanal* regions, throughout the Bolivian and Paraguayan border (2,29). *Microlobius foetidus* subsp. *paraguensis* (Benth.) M. Sousa et G. Andrade, commonly known as *pau-alho* (garlic stick) reaches 18 m in height. It occurs in the Pantanal regions of Mato Grosso do Sul State, in pastures and roadsides and in Chaco regions of Porto Murtinho. It re-sprouts with great vigour after clear cutting and burning. Due to its strong garlic aroma, it has great potential as repellent against insects in agriculture and as pioneer specie for reforestations of degraded areas (29). Near *M. foetidus* trees other herbaceous species fail to develop, indicating interference in establishment of competing plants. This may be due to competition, release of volatile oils

[*]Correspondence author, [2]Department of Chemistry, State University of Mato Grosso do Sul, 79070-900, Naviraí, MS, Brazil, [3]Department of Chemistry, Federal University of Mato Grosso do Sul, 79070-900, Campo Grande Grande, MS, Brazil, [4]Department of Hydraulics and Transport, Federal University of Mato Grosso do Sul, 79070-970, Federal University of Mato Grosso do Sul, 79070-900, Campo Grande Grande, MS.

in to the environment or both. The Brazilian *cerrado* is the richest savanna for plant species in the world and is included in the list of global hot spots (25).

In *cerrado* the C_4 grass-plot species are predominant and present an adaptation in the photosynthesis process which grants them more productivity at high temperatures and low CO_2 contents (13). Therefore, they are well adapted and present an intimate relationship with the dynamics of fires in those surroundings. The absence of grass-plot species in such environment, where *M. foetidus a* is predominant, indicates the oxidative stress due to liberation of volatile oils which, once released in the environment, cause alterations in the antioxidant defence of competing species, eliminating the competition in surroundings.

Common weeds are difficult to control in field due to high seed production and their long viability. However, the great biodiversity of *cerrado* biome is severely threatened by numerous invasive exotic African grass species. The *Brachiaria decumbens* has spread in every cerrado fragment, outcompeting native herbs (27). *Ipomoea cordifolia* Carey ex Voight, is a tropical and subtropical weed, drought resistant and which has spread rapidly in floodplains of Pantanal of Mato Grosso South, competing with native plants in region (4)

The cyclic polysulfides are phytotoxic to the germination and initial growth of *Cucumis sativa, Lactuca sativa, Lycopersicum esculentum* and *Acacia farnesiana* (21); however, their exact pathways regarding oxidative stress in laboratory are not investigated. Most studies regarding the activity of antioxidant enzymes during the seed germination evaluated the alterations resulting from aging or stress (8,26), with few studies about the alterations in enzymatic systems, and even fewer dealing with these effects when mediated by volatile oils in the plant defence system during germination and initial laboratory growth.

This study aimed to identify the compounds in the volatile oils of *M. foetidus* and to assess in laboratory bioassays, the alterations in antioxidant defense systems during the germination, growth and development of *B. decumbens* and *I. cordifolia*.

MATERIALS AND METHODS

I. Extraction and identification of volatile oils

Fresh leaves of *M. foetidus* were hydrodistilled for 4.0 h in a Clevenger-type apparatus, followed by exhaustive extraction of distillate with hexane. The analysis of volatile oils was done by gaseous chromatography in a Varian CP-3800 instrument, equipped with a molten silica capillary column ZB-5 (5%-phenyl-95%-dimethylpolysiloxane) [30m x 0,25mm, film width 0,2 (μm)], obtained from Phenomenex (Torrance, CA, USA). The injecting conditions were: oxygen as carrier gas (1 mL/min); injector split/splitless at 200 °C; FID detector (flame ionization detector) at 280 °C; oven temperature of 50 to 250 °C with heating ramp of 4 °C/min.

CG-EM analyses were done in a Varian GC-MS-MS system equipped with Varian - 3900 gaseous chromatographer equipped with a ZB-5 capillary column, a 1077 injector, a CP-8410 automatic injector coupled with a Varian Saturn 2100 mass spectrometer operating with an electron impact of 70 e V, at the same analysis conditions of CG/FID.

Identification of oil components was based on comparisons of retention times, by determining and comparing the Kovats retention indexes and mass spectrums obtained from the NBS/NIST library with the indexes described by Adams (1). A n-alkane (C_8-C_{32}) homologous series was used to calculate the Kovats retention indexes.

II. Laboratory bioassays

For allelopathic activity tests, 250 mg of the essential oil was emulsified with Tween 80, in proportion of 1:1 (v/v) and dissolved in distilled water, obtaining the stock solution at concentration of 1.0%. The other concentrations (5.0; 2.5; 1.25 and 0.35 %) were prepared by dilution. Tween 80 solution at 1.0% v/v was used as control (2,33).

For the germination bioassays, Petri dishes (9.0 cm dia) containing a Whatman n°1 filter paper, received 5.0 mL of distilled water were used. Twenty five seeds were randomly sown on each disc of filter paper, with four replicates for each solution (11). After sowing, 3.0 mL of each solution concentration of volatile oils were distributed on filter papers and then Petri-dishes cover was closed, avoiding direct contact with seeds (2,33).

The Petri- dishes containing the diaspores were closed, wrapped with plastic film and placed in the germination chamber [with controlled light conditions (160 W), relative humidity (80%) and temperature, as per each target species (*Ipomoe*a at 25ºC and 12-h photoperiod and *Brachiaria* at 30°C and a 12-h photoperiod (11)]. The germination (%) was determined with criteria of radicle protrusion of > 2.0 mm in length (15). 4-5 days after incubation of *Ipomoe*a and *Brachiaria* incubation, respectively,

For growth bioassays, similar procedures as for germination were used without volatile oils. After germination (radicular protrusion - 2.0 mm), 80 seedlings were selected (4-replications, each with 20) for each treatment. Then, they were transferred to Petri-dishes containing the treatment solutions (20). The reading was taken 4-days after incubation; the elongation of primary root and hypocotyls/mesocotyl (10 seedlings per dish) were measured using plotting paper. Later, these seedlings were kept in over at 60 °C until a constant weight was achieved to determine the dry mass.

III. Total chlorophyll determination and formazan production

The aerial portions of seedlings of *I. cordifolia* and *B. decumbens* were crushed in a mortar and the chlorophyll was extracted with DMSO (12). The substances absorptions were measured by a spectrophotometer at wavelengths of 645 and 663 nm, and the Chlorophyll a, b, and total chlorophyll content were calculated (5).

Formazan productions in radicle cells was estimated through the reduction of triphenyl tetrazolium chloride (TTC). For this evaluation, the roots were clipped at 1 cm from the hood and their masses were recorded. They were then transferred to test-tubes, where 0.6% TTC (w/v) was added, along with 0.05 M phosphate buffer (pH 7.0). The test-tubes were maintained in desiccators for 2 h and then kept at 30 °C for 15 h. The solutions were then drained and the roots were washed once in distilled water. The test-tubes containing the roots were transferred again to bain-marie in boiling water (± 100 ºC); then ethanol 95% (v/v) was added. After cooling at room temperature, the roots were removed and ethanol 95% (v/v) was added to each solution. Absorbances were measured in a spectrophotometer at 530 nm of wavelength (35).

IV. Enzymes in scavenging of reactive oxygen species

(i) **Oxidative stress:** To evaluate the oxidative stress, seedlings were submitted to the volatile oils for 7 days and then crushed in a mortar with liquid nitrogen. The resultant mass was homogenized with 50 mM S-phosphate buffer, pH 7.0, containing 2 mM EDTA and PVP 1.0%. The supernatant was collected and used as crude extract for the dosages described below and the precipitate was discarded (22). The extracts were stored at -18 ° C until the analysis.

(ii) **Protein content:** Protein concentration of each enzyme extract was determined as per Bradford (20). Bovine serum albumin was used as a standard.

(iii) **L-Amylase:** To determine the α-amylase, the extract was placed in bain-marie at 70 °C for 20 min, transferred to test-tubes containing a 1.0% amide solution and then incubated at 30 °C for 5 min. Lugol's iodine was added and then the spectrophotometer readings at 620 nm were recorded (7). The results were expressed as µg of hydrolyzed amide min. μg^{-1}.

(iv) **Lipid peroxidaton (LP) activity:** It was measured in a medium containing TCA (Trichloroacetic acid) 0.1%, thiobarbituric acid 0.5%, and 0.1-0.4 mg protein of the enzyme extract (18). The results were measured at 534 nm and the activity was expressed as percentage of lipid peroxidation.

(v) **Polyphenol oxidase activity (PPO)**: It was determined as per Duangmal and Apenten (14) by measuring the conversion of catechol to quinone. The substrate was composed of 20 mM catechol, 100 mM S-phosphate buffer (pH 6.8) and 0.1–0.4 mg protein of enzyme extract. The reaction occurred at 30 °C and readings were taken every 10 s at 420 nm (ε, 2.47 mM^{-1} cm^{-1}).

(vi) **β-1,3-glucanase activity (GLU)** : It was measured in a medium containing the reaction mixture, which itself contained 50 mM acetate buffer (pH 5.0), laminarin 0.25%, and 0.1-0.4 mg of protein of the enzyme extract. The reducing sugar assay was determined as per method of Miller (23) and the absorbance was read at 540 nm.

(vii) **Phenylalanine ammonia-lyase activity (PAL):** It was measured in a medium; the reaction mixture containing 0.05 M tris-HCl (pH 8.0), 6.0 mM L-phenylalanine and 0.1-0.4 mg of protein of the enzyme extract (30). The absorbance was read at 290 nm and the specific enzymatic activity registered in mmoles cinnamic acid. mg $protein^{-1}$. min^{-1}.

(viii) **Peroxidase activity (POD)**: It was measured in a medium containing 25 mM K-phosphate (pH 6.8), 10 mM H_2O_2, 2.6 mM guaiacol, and 0.1–0.4 mg of protein of the enzyme extract. Tetraguaicol formation (ε, 25.5 mM^{-1} cm^{-1}) was measured at 470 nm (31).

(ix) **Activity of ascorbate peroxidase (APX)**: It was assessed as per Amako *et al.*, (3), with a reaction medium containing 50 mM K- phosphate (pH 7.0), 1 mM hydrogen peroxide, 0.5 mM ascorbic acid, 0.1 mM EDTA and 0.1–0.4 mg of protein of the enzyme extract. The ascorbate oxidation rate was estimated by monitoring the absorbance at 290 nm (ε, 36.0 mM^{-1} cm^{-1}).

(x) **Superoxide dismutase activity (SOD)***:* It was measured as per Giannopolitis and Ries (17). The medium contained 50 mM K-phosphate (pH 7.8), 6.5 mM methionine, 150 µM nitro blue tetrazolium NBT, 4 µM riboflavin, and 0.02-0.1 mg of protein of the enzyme extract. The reaction was started by switching on a light (20 W) and illuminating the medium for 20 min at 30ºC. One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the (NBT) photoreduction rate read at 560 nm, and the results were expressed as Units of SOD µg. protein^{-1}.

Statistical analysis

All data were submitted to analysis of variance, and when the treatments proved significantly effective ($P < 0.05$) than control, the averages were compared using Dunnett's test. When any of the presuppositions demanded by the parametric model failed to be met, non-parametric testes were used: Kruskal-wallis as an alternative to analysis of variance and Mann-Whitney as an alternative to Dunnett's test. All results were regarded considering the significance level p = 5%.

RESULTS AND DISCUSSION

Volatile oils

The volatile oils of the *M. foetidus* leaves were analysed. Nine compounds (1,2,4-trithiolane, E-1,2,4-trithiolane-3,5-dimethyl, Z-1,2,4-trithiolane-3,5-dimethyl, 1,3,5-trithiane, 1,2,4,5-tetrathiane, 1,2,4,6-tetrathiepane, 1,2,4,5,7-pentathiocane, 1,3,5,7,9-pentathiecane and 1,2,5,6-tetrathiocane), representing 77.2% of the oil composition. The identified compounds contained in the hydrodistillation-obtained oil were the sulfides: 1,2,4-trithiolane (29.6%), E-1,2,4-trithiolane-3,5-dimethyl (4.0%), Z-1,2,4-trithiolane-3,5-dimethyl (4.5%), 1,3,5-trithiane (9.4%), 1,2,4,5-tetrathiane (5.5%), 1,2,4,6-tetrathiepane (11.7%), 1,2,4,5,7-pentathiocane (0.8%), 1,3,5,7,9-pentathiecane (0.5%) and 1,2,5,6-tetrathiocane (11.2%) (Table 1). The hydrodistilled -obtained oil had yield of 0.84%, yellow colour and a characteristic odour.

Compounds were identified by mass spectrometry and comparison with data in literature (1). Based on this composition, these oils may be attributed with the pungent aroma of onions that is exhaled by the plant, and that is responsible for its popular name (*pau alho*; garlic stick).

Cyclic polysulfides have little occurrence in nature, with few reports in plants. The method employing hydrodistillation, in which direct contact between the vegetal material and water vapour occurs, may promote subsequent reactions within the molecules and these cyclic compounds. The presence of these substances are not very common in volatile oils of leaves. The presence of these sulfur-containing compounds turns the oil in a rather peculiar material regarding its chemical composition. The anticancer activity of sulfuric compounds has already been reported in literature (21), and the presence of cyclic polysulfides such as 1,2,4-trithiolane and 2,4,6-tetrathiepane has already been reported in brute extract of *Parkia* sp.

Table 1. Chemical composition of volatile sulfur compounds of *Microlobius foetidus* obtained by HD (Hydrodistillation)

Compounds [a,b]	M.F.	RI[c]	HD
1,2,4-trithiolane	$C_2H_4S_3$	1095	29.6 ± 2.2
E-1,2,4-trithiolane-3,5-dimethyl	$C_4H_8S_3$	1142	4.0 ± 0.4
Z-1,2,4-trithiolane-3,5-dimethyl	$C_4H_8S_3$	1150	4,5 ± 0.7
1,3,5-trithiane	$C_3H_6S_3$	1249	9.4 ± 0.6
1,2,5-trithiepane	$C_4H_8S_3$	1300	-
1,2,4,5-tetrathiane	$C_2H_4S_4$	1318	5.5 ± 0.0
1,2,3,4-tetrathiane	$C_3H_6S_4$	1354	-
1,2,4,6-tetrathiepane	$C_3H_6S_4$	1488	11.7 ± 0.5
1,2,4,5,7-pentathiocane	$C_3H_6S_5$	1530	0.8 ± 0.1
1,3,5,7,9-pentathiecane	$C_5H_{10}S_5$	1545	0.5 ± 0.0
1,2,5,6-tetrathiocane	$C_4H_8S_4$	1551	11.2 ± 1,1
Lenthionine	$C_2H_4S_5$	1597	-
Hexathiepane	CH_2S_6	1685	-
cyclic octaatomic sulfur	S_8	2006	-
Total			77.2

HD: Hydrolistillation, M.F: Molecular formula; [a]Compounds listed in order of elution from a ZB-5 column; [b]Identification, RI: Retention indices, GC-MS, gas chromatography-mass spectroscopy; [c]Programmed temperature retention indices determined on apolar ZB-5 column (50-250 °C; 3 °C min^{-1}).

Biological assays

The bioassays with *M. foetidus* volatile oils showed that these oils inhibited the germination and seedlings growth of *I. cordifolia* and *B. decumbens.* In the former, a higher concentration decreased the germination > 50%.

Among the two species assessed as bio-indicators, the volatile oils drastically inhibited the germination of *I. cordifolia* than *B. decumbens.* The inhibition in germination in *I. cordifolia* was ± 61% and 52% at concentrations of 5.0% and 2.5%, respectively (Fig. 1 A). A drastic reduction was observed in root and coleoptile growth of *B. Decumbens* (67%). At 5.0% concentration, the primary roots growth of *I. cordifolia* was decreased by 73%. While in *B. decumbens,* the 2.5% and 1.25% concentrations caused reduction as 60% and 53%, respectively (Fig. 1B). Similar inhibitory effects were observed in hypocotyl/ mesocotyl growth of *I. cordifolia* and *B. decumbens* (57% and 63%, respectively) at 5.0% concentration. The 2.5% concentration caused reduction of 54% in seedlings growth of *B. decumbens* (Fig. 1C). The highest concentrations (2.5% and 5.0%) decreased the dry weight of seedlings, and reduction was more drastic in *I. cordifolia* (48%) than in *B. decumbens* (26%) at 5.0% (Fig. 1D).

Chlorophyll rate and formazan production: Both these parameters were markedly decreased as a function of concentrations in both tested species. The decrease in chlorophyll rate in *I. cordifolia* and *B. decumbens* was of 6.2 µg/ml and 7.3 µg/ml, respectively, showing a greater sensitivity of former specie to volatile oils (Figs. 2A and B). Whitish spots were observed in both species, due to the chlorophyll decrease.

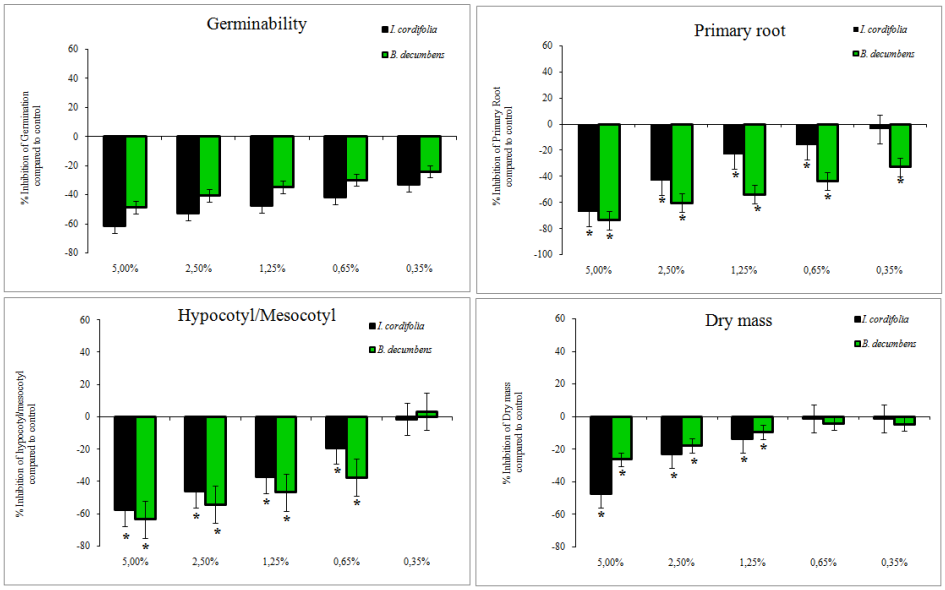

Figure 1. Effects of volatile oils concentrations of *M. foetidus* on the germination, growth of the primary root, hypocotyl/ mesocoptyl and dry mass of *I. cordifolia* and *B. decumbens*. *Treatment mean differs significantly ($p < 0.05$) as compared to control mean (Dunnett test).

I. cordifolia also showed increased sensitivity to the alellochemicals, when its chlorophyll amounts were evaluated. On the other hand, *B. decumbens* showed greater sensibility when evaluating the respiratory process with decrease in formazan synthesis (0.109 nm). These results are in concordance with a greater decrease in the underground portion, as the respiratory activity also affects root development (38).

Oxidative stress

Under normal conditions, chlorophyll reaches the singlet excitation state following the absorption of a photon. The excess energy from the chlorophyll in their triplet state is transferred to oxygen, causing formation of singlet oxygen. Singlet oxygen is highly reactive and causes bleaching of pigments and lipid peroxidation of membranes.

The oil concentrations increased the soluble proteins present in *I. cordifolia* and *B. decumbens*, with increases of 25.70 μg^{-1} and 23.44 μg^{-1}, respectively, with increased sensitivity of former species. The volatile oils increased the levels of antioxidant enzymes due to the oxidative stress in all evaluated concentrations (Fig. 2 C).

The decrease in the final germination (%) indicates that the volatile oils affected the energetic output of *I. cordifolia* and *B. decumbens* during their development. A low level of α-amylase was observed in the control group, indicating an energy expenditure that was necessary for the development of seedlings. For the volatile oil-treated species, an increase in these enzymes is observed, with highest α-amylase levels in *B. decumbens* (136 $\mu g.\ prot^{-1}$), which supports the effects observed on germination and growth (Fig. 2 D).

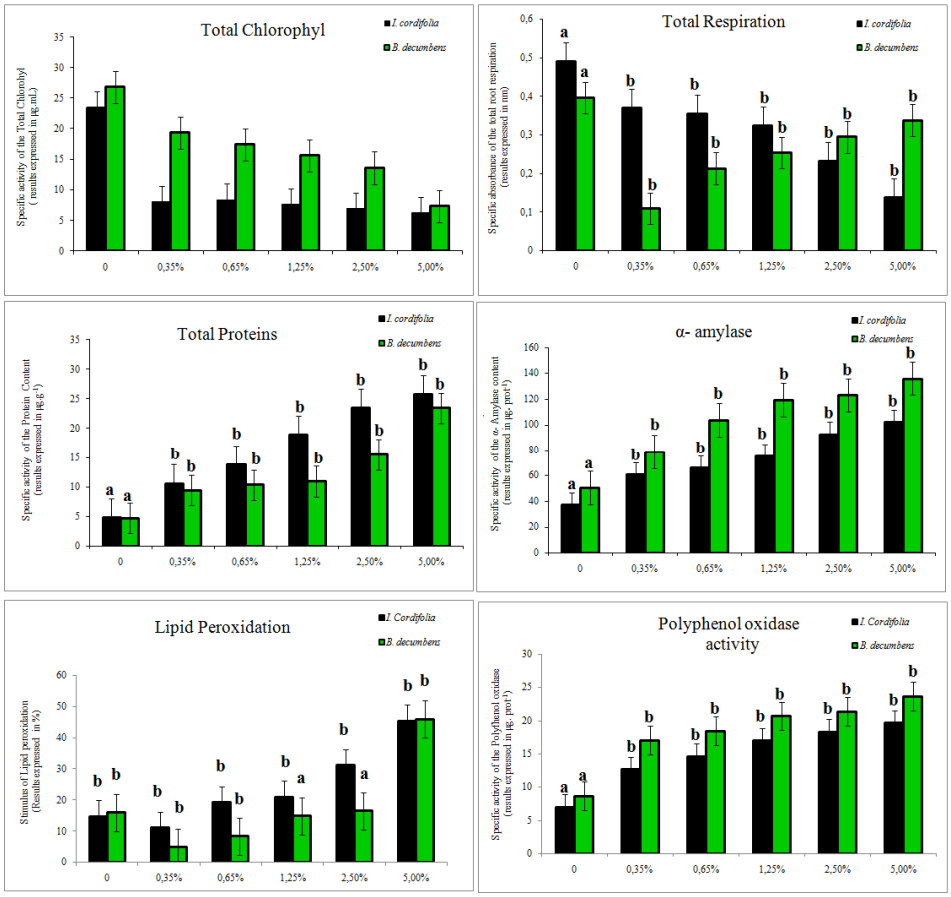

Figure 2. Effects of volatile oils concentrations of *M. foetidus* on the chlorophyll contents, respiration, Total proteins, α- amylase, Lipid peroxidation and Polyphenol oxidase in *I. cordifolia* and *B. decumbens* seedlings, in laboratory. Means followed by the same letter as control do not differ by the *Dunnett test* ($p < 0.05$).

Enzymes such as α-amylase possess a link between the germination speed reduction and the final germination (%), as this enzyme is produced by the aleurone layer in response to gibberellins activity. Released within the endosperm, this enzyme converts the amide to sugars, which are used for the growth of embryo (6).

Regarding the antioxidant defence, stimulus in the enzymatic responses were verified, with increase in MDA concentration caused by lipid peroxidation and the experimental conditions. An increase in MDA amount occurred in *I. cordifolia* in all used concentrations, with values over 3-folds the amount in control was 45% at 5.0% concentration. A similar effect was observed in *B. decumbens*, however, only 5.0% concentration caused significant MDA accumulation (46%), while the 0.65% and 0.35% concentrations decreased the MDA production (8.3% and 4%, respectively), indicating a low peroxidation rate than control (16%) (Fig. 2 E).

(i) **Lipid Peroxidation :** Other signs of stress, such as lipid peroxidation was also observed and the greatest effects were in *I. cordifolia*. Perhaps this has contributed to plant growth reduction Increases in the levels of lipid peroxidation was an indicator of lipid peroxidation and membrane damage. This occurs due to generation of ROS species, which and causes loss of membrane integrity. Exposure to volatile oils of *M. foetidus*, induced oxidative stress through the enhanced generation of ROS, which was accompanied by membrane damage, enhanced lipid peroxidation levels and by activation of antioxidant enzyme systems (9). Increased levels of scavenging enzymes indicate their induction as a secondary defence mechanism in response to *M. foetidus*.

(ii) **Polyphenoloxidase :** Increase in enzymatic levels of polyphenoloxidase were observed in both species. Both *I. cordifolia* and *B. decumbens* were sensitive to the volatile oils at the lowest concentration (0.35%) and stimulated this enzyme by 2-folds: 12.66 µg and 17.04 µg of protein, respectively. At higher conc (5.0%), the increase was 19.65 µg of protein to *I. cordifolia* and 23.63 µg of protein to *B. decumbens*, respectively (Fig. 2 F).

(iii) **β-1.3-glucanase :** An increase in its activity was observed in both *I. cordifolia* and *B. decumbens* at all tested concentrations. At 5.0% concentration, a great increase in the production rate occurred in *I. cordifolia* (176 µg of protein) than in *B. decumbens* (22 µg of protein) (Fig. 3 A).

(iv) **Phenylalanine ammonia-lyase** : The volatile oils at 5.0% concentration increased the production of this enzyme to 239 µg and 16.68 µg of protein for *I. cordifolia* and *B. decumbens*, respectively, (Fig. 3 B). An increase in antioxidant enzymes such as catalase was verified. At 5.0% concentration of volatile oil this enzyme showed 2-folds increases: 24 µg and 2.47 µg of protein for *I. cordifolia* and *B. decumbens*, respectively than control (Fig. 3 C).

(v) **Peroxidase :** The peroxidase production in *B. decumbens* seedlings increased to 14.5 µg of protein at 5.0%. *I. cordifolia* the increase was 10.9 µg of protein at 5.0% (Fig. 3 D). At 5.0% concentration of volatile oil the production of ascorbate peroxidase enzyme increased 3-folds in *I. cordifolia* and *B. decumbens* (30 µg of protein and 45.78 mg of protein, respectively). The volatile oil at 2.5% and 0.35% concentrations maintained this increase in *B. decumbens*: 42.63 µg 1 and 33.81 µg of protein, respectively (Fig. 6 E). The increase in the enzymatic activity of peroxidase and ascorbate peroxidase, is associated with the oxidative stress caused by volatile oils. These enzymes contribute to the response of plants to stress, leading to the production of reactive oxygen species.

(vi) **SOD :** The volatile oil in all concentrations stimulated the SOD activity in the seedlings: 197 µg and 203.25 µg of protein in *I. cordifolia* and *B. decumbens*, respectively, than control (Fig. 3 F). The results showed that the volatile oils increased the oxidative stress in evaluated species, as it interferes in the production of enzymes with antioxidant roles in vegetables. The effects were more pronounced in seedlings in the initial phases of development i.e. right after the imbibition of seed, the oils affected the beginning of respiratory process. The rupture of amide molecule was impaired, as demonstrated by the high α-amylase activity at the start of embryo development.

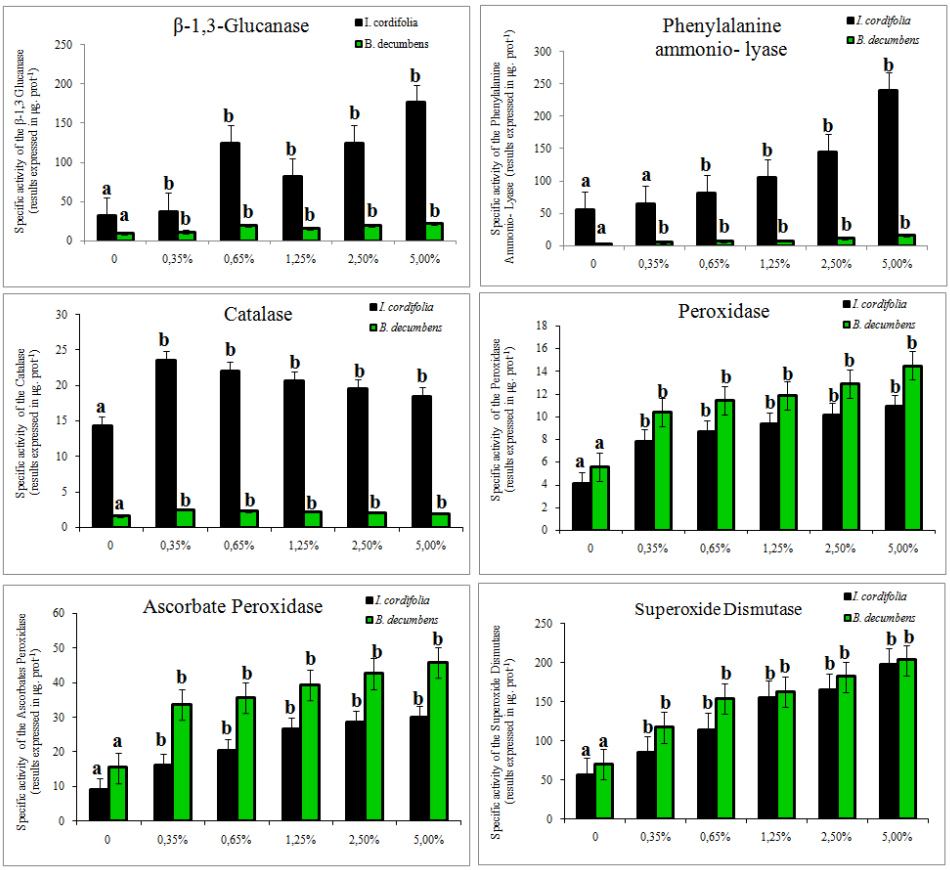

Figure 3. Effects of volatile oils concentrations of *M. foetidus* on the activity of the activity of β-1,3 glucanase, Phenylalanine ammonio- lyase, Catalase, Peroxidase, Ascorbate peroxidase and Superoxide dismutase in *I. cordifolia* and *B. decumbens* seedlings in laboratory. Means followed by the same letter as control do not differ by the *Dunnett test* ($p < 0.05$).

The sensitivity of evaluated species varied between the studied antioxidant enzymes as opposed to being specific to one species only. An increase in the glucanase and phenylalanine production occurred, which are related to apical necrosis and growth inhibition in the studied seedlings. The presence of injuries in the plants leads to deposition of β-1,3-glucane in the cellular wall in the form of callose, which may be form of protective mechanisms. Indeed, injury is prevented or reduced and the pathogens invasion is restricted (24). On the other hand, the phenylalanine ammonia-lyase catalyzes the series of metabolic reactions generating innumerous phenylpropanoid-based natural products (37), which are important for plant growth, but also provides the protection against ambient stress.

The occurrence of SOD, Cat, and POD activities in seedlings, along with significant alteration in respiration, suggests that production of reactive oxygen species is initiated as soon as mitochondrial respiration is resumed during the seed imbibition. Although the relative contribution of each process in respiration is not clear from our data, all these processes are stimulated in oxidative stress conditions (34).

Ascorbate, thus, could play a role in non-enzymatic antioxidant defence systems in primary roots of *I. cordifolia* and *B. decumbens*. The physiological roles of this family of enzymes in seed germination and seedling growth are not completely known. They function in tissue hydration (19), cell elongation (32), nitrogen storage (36), and breakdown of storage lipids (16). We cannot exclude, however, the possibility that lipid peroxidation activity was increased in response to cellular oxidative stress. Lipid peroxidation occurs during the seed germination with mitochondrial respiration (9), and the activation of lipoxygenase may be the immediate responses to structural changes in cell membrane induced by over-production of reactive oxygen species (6,28).

CONCLUSIONS

The occurrence of SOD, Cat, POD and APX in seedlings of *I. cordifolia* and *B. decumbens* is probably related to ROS production resulting from mitochondrial metabolism during the germination and initial growth. Regardless of the primary mechanisms of allelochemical induced oxidative stress, our data suggest that during seed germination and initial growth, a period when antioxidant enzyme activity increases and counteracts the harmful ROS effects produced during mitochondrial metabolism resumption, the presence of allelochemicals, which cause further oxidative stress, may leave the seeds/seedlings more vulnerable to cellular dysfunction and cell death.

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