Polygala extraaxillaris: OXIDATIVE STRESS IN *Brachiaria decumbens* MEDIATED BY VOLATILE OILS[1]

Polygala extraaxillaris: Estresse em ***Brachiaria decumbens*** *Mediado por Óleos Voláteis*

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ABSTRACT - The volatile oils extracted from the roots of *Polygala extraaxillaris* were analyzed to assess whether they increase oxidative stress in *Brachiaria decumbens* var. Piatã, as well as to assess their effect on cellular division and cytotoxicity in laboratory. Six concentrations were used (0%, 0.35%, 0.65%, 1.25%, 0.65%, and 5.0%) with four repetitions of 25 seeds. The substance 1-(2-hydroxyphenyl) - ethanone was identified as the major constituent of the volatile oils. The results showed that the highest concentrations of the oils resulted in an increase in the oxidative stress in *B. decumbens*, as well as alteration in germination and growth, with a consequent reduction in the process of cellular division, causing changes in the growth standard and antioxidant defense.

Keywords: reactive oxygen species, antioxidant enzymes, plant defense, signal grass.

RESUMO - Os óleos voláteis extraídos das raízes de ***Polygala extraaxillaris*** *foram analisados para avaliar se aumentam o estresse oxidativo em* ***Brachiaria decumbens*** *var. Piatã e, posteriormente, estimar o seu efeito na divisão celular e citotoxicidade em laboratório. Foram utilizadas seis concentrações (0%, 0,35%, 0,65%, 1,25%, 0,65% e 5,0%), com quatro repetições de 25 sementes. A substância 1 - (2-hidroxifenil) - etanona foi identificado como o principal constituinte dos óleos voláteis. Os resultados mostraram que as concentrações mais elevadas dos óleos causaram aumento no estresse oxidativo em* ***B. decumbens*** *e também alteraram a germinação e o crescimento, com consequente redução no processo de divisão celular, levando alterações no padrão de crescimento e defesa antioxidante.*

Palavras-chave: espécies reativas de oxigênio, enzimas antioxidantes, defesa de plantas, capim-braquiária.

INTRODUCTION

Essential oils are natural plant products that contain natural flavors and fragrances which provide characteristic odors (Mukhopadhyay, 2000). They are likely to break down quickly in the environment and could be used for weed control by organic farmers. Essential oils are classified as "generally regarded as safe" (GRAS) and can inhibit the growth of microorganisms in food (Beuchat, 2001). They also contain allelochemicals that inhibit seed germination and may be used to inhibit weed seed germination (Dudai et al., 1999).

The toxicity of ROS (reactive oxygen species), which causes oxidative damage to

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cell macromolecules, is well documented (De Wit, 2007; Chen, 2008). There is evidence that ROS also play normal physiological roles, acting as signaling molecules in many processes, including seed germination and growth (Ryan, 2000; Chen, 2008). The shift from a normal role in cell signaling to a toxic role with deleterious effects is probably associated with changes in ROS homeostasis that result from a shift in the balance between ROS-producing and ROS-scavenging processes (Pergo & Ishii- Iwamoto, 2011).

Cell growth in plants depends on a normal mitotic process. The growth-limiting effects of various kinds of stress were reported as factors in the control of cell division (Ding et al., 2010). The uniform division of all cell components allows a balanced growth for the organism (Reigosa et al., 2006).

The Brazilian *cerrado* is recognized as the richest savanna in the world as regards plant species, and it is on the list of global hot spots (Myers et al., 2000). However, the great biodiversity of the *cerrado* biome is being severely threatened by a number of invasive exotic species, namely the African grasses *Brachiaria* spp., *Melinis minutiflora*, *Andropogon gayanus*, *Panicum maximum*, *Hyparrhenia rufa*, once brought to the country as cattle forage. These grasses have spread in such magnitude that they occur in virtually every *cerrado* fragment, outcompeting native herbs (Pivello et al., 1999).

The *Polygala extraaxillaris* species is a sub-shrub with 7-60 cm height; as a taxonomic characteristic it has rudimentary, very narrow lateral petals (0.2 mm width), with more than half of its length stuck to the staminal sheath (Aguiar et al., 2008; Lüdke & Aguiar, 2008). The species is found in Paraguay, Argentina (Marques, 1979) and in the Brazilian states of Santa Catarina, Rio Grande do Sul, and Mato Grosso do Sul. It often occurs on the edges of roads, in dry or wet soils, in clean and shrubby fields (Marques, 1979). The high dominance of species of the genus *Polygala* in fields where there is competition with pasture species for livestock makes this genus interesting for the study of allelopathic activity. In *cerrados*, *P. extraaxillaris* has advanced massively throughout the native vegetation and formed monospecific patches with no other species growing below or close to it. In the dry season, the vegetative buds that are close to the soil surface act as a strategy to escape from grazing, and get closer to wetter areas, ensuring their survival (Caporal et al., 2010).

This study aimed to identify the compounds present in the volatile oils from *P. extraaxillaris* roots and verify that cause changes in oxidative stress, and cytotoxicity in *Brachiaria decumbens*.

MATERIALS AND METHODS

Plant Collection

Fully grown plants of *P. extraaxillaris* (about 70 individuals) were collected in the Pantanal region of Mato Grosso do Sul, and conditioned in a Styrofoam box with ice until extraction. A voucher specimen of the plant is found in the Botanical Museum of Curitiba (nº 357010).

Extraction of volatile oils

At the time of extraction, the roots were separated from the aerial parts and submitted to several extractions by hydrodistillation for 4 hours with a modified Clevenger device, followed by exhaustive extraction of the distillate with diethyl ether. After the solvent had been removed, the oil was dried with Na_2SO_4 and the yield of its crude form was calculated in relation to the fresh material, resulting in 0.97% (Farmacopéia Brasileira, 1988). The analyses in gas chromatography were performed on a Varian CP-3800 device, equipped with a capillary column of melted silica ZB-5 (5%-phenyl-95%-dimethylpolysiloxane; 30 m x 0.25 mm, 0.2 mm film thickness), obtained from Phenomenex (Torrance, CA, USA). The conditions of injection were: hydrogen carrier gas (1 mL/min); split/splitless injection at 220 °C; FID Detector at 280 °C; oven temperature between 50° and 250 °C, and heating ramp temperature at 3 °C min^{-1}. The analyses in GC-MS were performed using a Varian GC-MS-MS system, equipped with a Varian-3900 gas chromatographer with a ZB-5 capillary column, a 1077 injector, and a CP-8410 automatic injector, attached to a mass spectrometer (Varian Saturn 2100) operating with electron impact at 70 eV, under the same conditions of analysis in GC/FID.

The temperatures of the manifold, GC-MS interface and ion trap were 70, 240 and 200 °C, respectively. Helium (99.999 %) was used as carrier gas at a constant flow of 1.0 mL min^{-1}, employing an injection volume of 1 µL (split ratio of 1:20).

A mixture of C_9-C_{21} *n*-alkane diluted in *n*-hexane was prepared for temperature determination with programmed retention indices. Samples diluted in *n*-hexane were analyzed. Then, internal patterns (*n*-alkanes) were added to each sample to aid in the standardization of retention times; the identification of the components of the oils was based on comparison of the retention times and Kovats Indexes ($Ir_i = 100.n + 100.Dn.\ tr_i - tr_n / tr_m - tr_n$).

The MS scan parameters included electron impact ionization voltage of 70 eV, mass range of 41-380 *m/z*, and scan interval of 0.5 s. The identification of the oil components was based on comparison to the retention time, by determination and comparison with Kovats Retention Indexes and mass spectra of the NBS/NIST library, with the indices described by Adams (2001). A homologous series of *n*-alkanes (C_8-C_{32}) was used to calculate Kovats Retention Indexes. The identification of the substance 1-(2-hydroxyphenyl)-ethanone, present in the volatile oils, was confirmed by NMR spectra (^1H and ^{13}C), obtained in a Bruker DPX-300 spectrometer. The samples were dissolved in $CDCl_3$, taking the internal standard TMS as a reference.

Bioassays of phytotoxic activity

For the bioassays of phytotoxic activity, 1.250 mg of the essential oil was emulsified with Tween 80 (1.0%), and dissolved in distilled water, obtaining a stock solution at a concentration of 5.0%. The other concentrations (2.5; 1.25; 0.65 and 0.35%) were prepared by dilution. As control, a solution of Tween 80 at 1.0% v/v was used (Alves et al., 2004; Silva et al., 2009).

Brachiaria decumbens was obtained from commercial dealers. For the germination bioassays, Petri dishes (9.0 cm of diameter) containing Whatman nº 1 filter paper received 5.0 mL of distilled water. After this, 25 seeds were randomly sowed on each disc of filter paper, with four repetitions for each solution (Brasil, 2009). After sowing, 3.0 mL of each solution concentration were distributed in two filter papers, and then fixed on the cover of the Petri dishes, avoiding direct contact with the seeds.

The Petri dishes containing the diaspores were closed, wrapped with plastic film and taken to the germination chamber, with controlled light conditions (160 W), relative humidity (± 80%) and temperature of 30 °C and a 12-hour photoperiod (Brasil, 2009). After five days of incubation, the percentage of germination was determined, having as criterion a radicular protrusion of no less than 2.0 mm in length (Ferreira & Áquila, 2000).

For growth bioassays, similar procedures as those of the germination were used, although in the absence of volatile oils. After germination (radicular protrusion – 2.0 mm), 80 seedlings were selected (four repetitions of 20 apiece) for each treatment. Then, they were transferred to Petri dishes containing the treatment solutions (Laboriau, 1983). The reading was performed after four days of incubation; the elongation of the primary root and mesocotyl (10 seedlings per each dish) was measured using plotting paper. Later, these seedlings were taken into an incubator at 60 °C until constant weight was achieved, in order to determine dry mass.

Enzymatic assays

For the oxidative stress assays, a similar procedure to the one for germination was used, where 25 seeds germinated in distilled water were submitted to oil treatments, in triplicate. After seven days, the seeds were grounded in liquid nitrogen. The frozen powder was homogenized in 50 mM S-phosphate buffer (pH 7.0), 2 mM EDTA and PVP 1.0%. The supernatant was decanted and used as the enzyme source (Marques & Xavier Filho, 1991).

The protein concentration of each enzyme extract was determined according to Bradford (1976). Bovine serum albumin was used as a standard.

β-1,3-glucanase activity (GLU),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 by the method described by Miller (1959) and the absorbance was read at 540 nm.

Phenylalanine ammonia-lyase activity (PAL) was measured in a medium of reaction containing 0.05 M tris-HCl (pH 8.0), 6.0 mM L^{-1} phenylalanine and 0.1-0.4 mg of protein of the enzyme extract (Prusky et al., 1996). The absorbance was read at 290 nm and the specific enzymatic activity was registered in μmol^{-1} mg^{-1}.

Catalase activity (CAT) was measured in a medium containing 1M H_2O_2, 50 mM S-phosphate buffer (pH 6.0) and 0.1–0.4 mg protein of enzyme extract. The consumption of H_2O_2 was monitored at 240 nm (ε, 0.036 mM^{-1} cm^{-1}) (Aebi, 1984).

Peroxidase activity (POD) 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 (Pütter, 1974).

Superoxide dismutase activity (SOD) was measured according to Giannopolitis & Ries (1977). 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 30 °C. 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 mg per protein.

Lipid peroxidation (LP) was measured in a medium containing 0.1% trichloroacetic acid, 0.5% thiobarbituric acid, and 0.1–0.4 mg of protein of the enzyme extract (Gomes-Júnior et al., 2006). The absorbance was read at 534 nm and the enzymatic activity was expressed as a percentage of the stimulus to lipid peroxidation.

To evaluate cytotoxic activity, seeds of *B. decumbens* were initially germinated in water, and then submitted to the same concentrations that had been used in the germination bioassays during 24 hours. After this period, approximately half of the samples were collected and fixed in 70% alcohol. The techniques used to visualize the cells were those proposed by Fernandes et al. (2007, 2009), Fiskesjo (1985), and Leme et al. (2008), using Schiff's reactive, followed by acetic carmine (1.0%).

About 15000 cells were counted per tested concentration (1000 for each assay) at the concentrations of 0%, 0.35%, 0.65%, 1.25%, 2.5%, and 5.0%. The same number of cells was analyzed in the control test. By means of cytological analyses, abnormal cells were observed, and the mitotic index and rate of chromosomal aberrations were calculated.

All data were submitted to analysis of variance, and when the treatments proved significantly different ($p<0.05$) when compared to control, the means were compared using Dunnett's test. When any of the assumptions 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 $\alpha = 5\%$.

RESULTS AND DISCUSSION

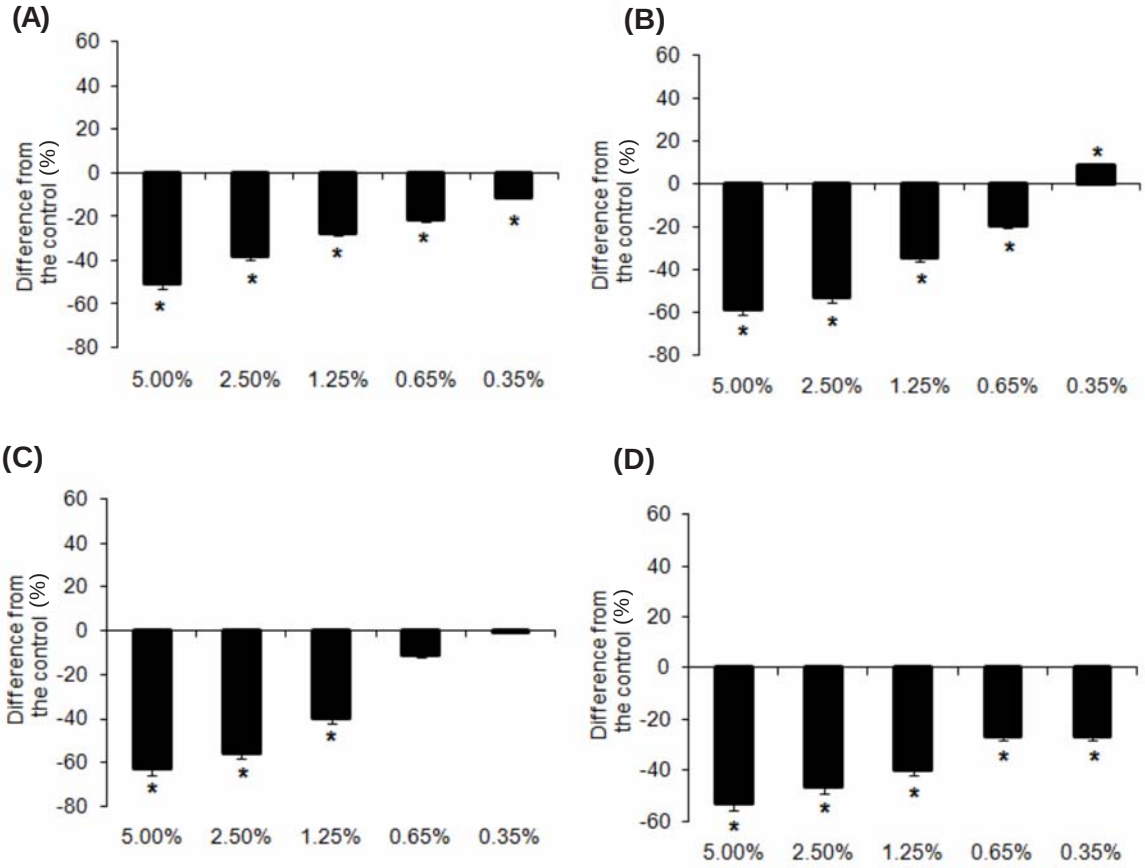
The analysis of the chemical constituents of essential oils by GC-MS and RMN (Table 1) revealed the presence of 1-(2-hydroxyphenyl)-ethanone, which represents 78% of the oil composition with a retention time of 17.354 minutes, and a Kovats Retention Index (KI) of 1161 (in the literature: 1160, by Adams, 2001). The compound appeared as a white solid, with a melting point of 4-6 ºC. The structure of 1-(2-hydroxyphenyl)-ethanone was mainly deduced from $^{1}H/^{13}C$ RMN, GC-MS and GC experiments. The 1-(2-hydroxyphenyl)-ethanone was identified based on its distinctive molecular ion at *m/z*=136, [m-H]-, and subsequent fragmentation to product ions at *m/z*=121, [M-H-15]-, m/z=93 [M-H-43]-, and m/z=65 [M-H-71]-. The ^{1}H RMN spectrum

($CDCl_3$) showed δ: 2.6 (3H, s), 6.9 (1H, 2d), 7.2 (1H, d,); 7.4 (1H, 2d); and 7.7 ppm (1H, d). The ^{13}C RMN spectrum ($CDCl^3$) showed δ: 26.2 (q); 118.4 (d); 118.9 (d); 119.7 (s); 130.6 (d); 136.4 (d) and 204.5 ppm (q).

Table 1 - ^1H and ^{13}C-NMR Spectral Data for 1-(2-hydroxyphenil)-ethanone

Position	^1H NMR (ppm)	*J* (Hz)	^{13}C NMR (ppm)
1	-	-	119.7 (s)
2	-	-	162.3 (s)
3	7.73 (d)	8.4	118.4 (d)
4	6.89 (dd)	7.4, 8.0	136.4 (d)
5	7.43 (dd)	7.8, 7.4	118.9 (d)
6	6.97(d)	7.2	130.6(d)
7	-		204.5 (s)
8	2.62 (s)	-	26.6 (q)

The germination results have demonstrated that all the concentrations affected the germinative process of *B. decumbens* and that only the highest concentration caused inhibition above 50% (Figure 1A). The oils from *P. extraaxillaris* roots interfered in the germination and growth of *B. decumbens*. The global process of germination consists of three partial processes: imbibition, activation and intraseminal growth. The difficulty in establishing the beginning and the end of each process accounts for the need of a macroscopic criterion to determine germination, i.e., the protrusion of the primary root (Ranal & Santana, 2006). The results are complemented by biometric tests (measurement of root and mesocotyls), which are important for determining seedling alterations (Piña-Rodriguez et al., 2004).



* Treatment means differ significantly; means followed by the same letter as control do not differ ($p < 0.05$) as compared to control means (Dunnett's test).

Figure 1 - Effect volatile oils of *Polygala extraaxillaris*, at different concentrations, upon the germinability (A), primary root growth (B), mesocotyl (C) and dry mass (D) of *B. decumbens*.

In the evaluation of the growth results, there was a marked inhibition on primary root and mesocotyl, verifying values of inhibition of the primary root at ± 58% and ±52% at the concentrations of 5.0% and 2.5%, respectively (Figures 1B). Regarding mesocotyl growth, inhibitions of ±63% were verified at the highest assayed concentration. The concentrations of 0.12% and 0.05% did not reduce mesocotyl growth (Figure 1C). Also, all concentrations have influenced dry mass. The final weight of ±53% for the concentration of 5.0% demonstrates that the oils affected the development of *B. decumbens* (Figure 1D).

The inhibition of primary root and mesocotyl was associated with an increase of the concentrations, the most intense effects being observed at 5.0%. The changes in the germination patterns could result from several effects at the primary level (Gusman et al., 2008). Among them, changes in membrane permeability, DNA transcription and translation, activity of secondary messengers, respiration by the scanvenger, expression of enzymes and receptors, or even the combination of all these aspects (Ferreira & Áquila, 2000).

Only the lowest concentration does not promote stretching of the primary root. Root elongation occurs by the action of substances that may affect membrane permeability and inhibit the absorption of water and nutrients, whereas these may be facilitated at low concentrations (Einhellig, 2006).

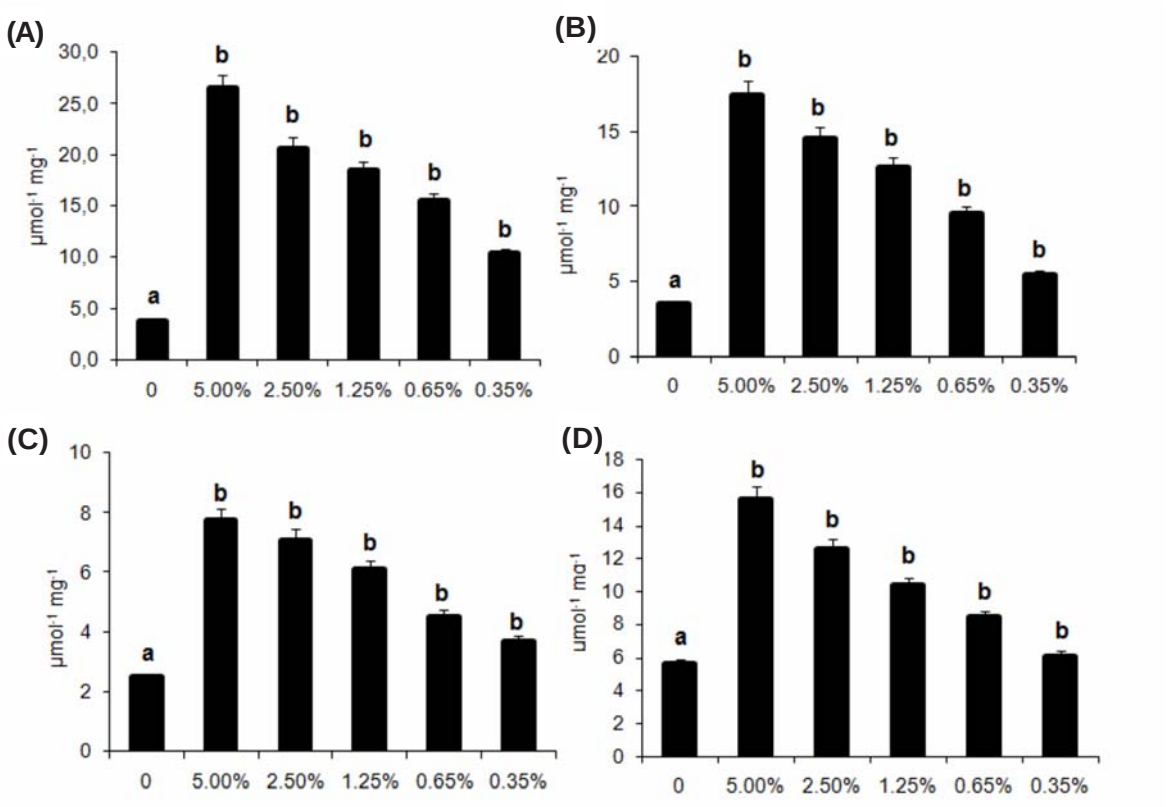
The pasture presence of the *P. extraaxillaris* species without being affected by competition with cultivated African grasses may be associated with the presence of volatile oils in the roots. Even in small quantities, these oils may be operating in the establishment of *P. extraaxillaris*, avoiding extinction in these locations. Some studies show that because of the intense productivity of grasses, which generate large amounts of biomass fuel – especially in the dry season, when its parts become desiccated epigean – the fire regime of the invaded areas may be altered, facilitating the occurrence of large fires (Hughes et al., 1991; Asner & Beatty, 1996; D'Antonio & Vitousek, 2002). Pott & Pott (1994) reported that the roots of the genus *Polygala* survived the fire, resprouting after rain.

The obtained results showed that volatile oils of *Polygala extraaxillaris* caused oxidative stress, which was verified by the increased activity of antioxidant enzymes as a result of higher oxidative stress levels presumably caused by volatile oils.

All concentrations have increased the activity of GLU and PAL in *B. decumbens*. The higher production of GLU (±26.57 μmol min^{-1} mg^{-1}) and PAL (±17.5 μmol min^{-1} mg^{-1}) occur in the concentration of 5,0% (Figure 2A and B). The levels of CAT and POD were also increased by ±7.75 and ±15.5 for CAT and POD, respectively, at the concentration of 5.0%; these effects were similar to GLU and PAL activities. Only the highest concentrations promoted an increase in POD and CAT activity, with the lower concentrations being insufficient to promote oxidative stress (Figure 2C and D).

Alterations in the antioxidant defense system of *B. decumbens* have been observed, indicating that the oils have increased oxidative stress. These results confirm the investigations regarding inhibition of ATP formation, proton capture and the rate of electron transport by the activity of p-hydroxyacetophenone (Céspedes et al., 2002), determining that the presence of oxygenated functions (hydroxyl) significantly increases the inhibitory potential of these compounds. Consequently, these radicals may directly affect the permeability of cell membranes, causing damage to DNA and proteins and leading to changes in germination and growth of the investigated species. The investigated enzymes were produced excessively by the examined species, with the purpose of protecting cells from the toxic effects of these radicals (Del Rio et al., 2002)

Enzymatic changes were also observed for β-1.3-glucanase and phenylalanine ammonia-lyase, which are responsible for the plant defense against reactive forms of oxygen (Morohashi & Matsushima, 2000). The increased production of glucanase and phenylalanine is probably associated with apical necrosis and growth inhibition in the evaluated seedlings. Studies showed that phenylalanine ammonia-lyase catalyzes the first of a series of metabolic reactions producing many natural products based on



* Treatment means differ significantly; means followed by the same letter as control do not differ ($p < 0.05$) as compared to control means (Dunnett's test).

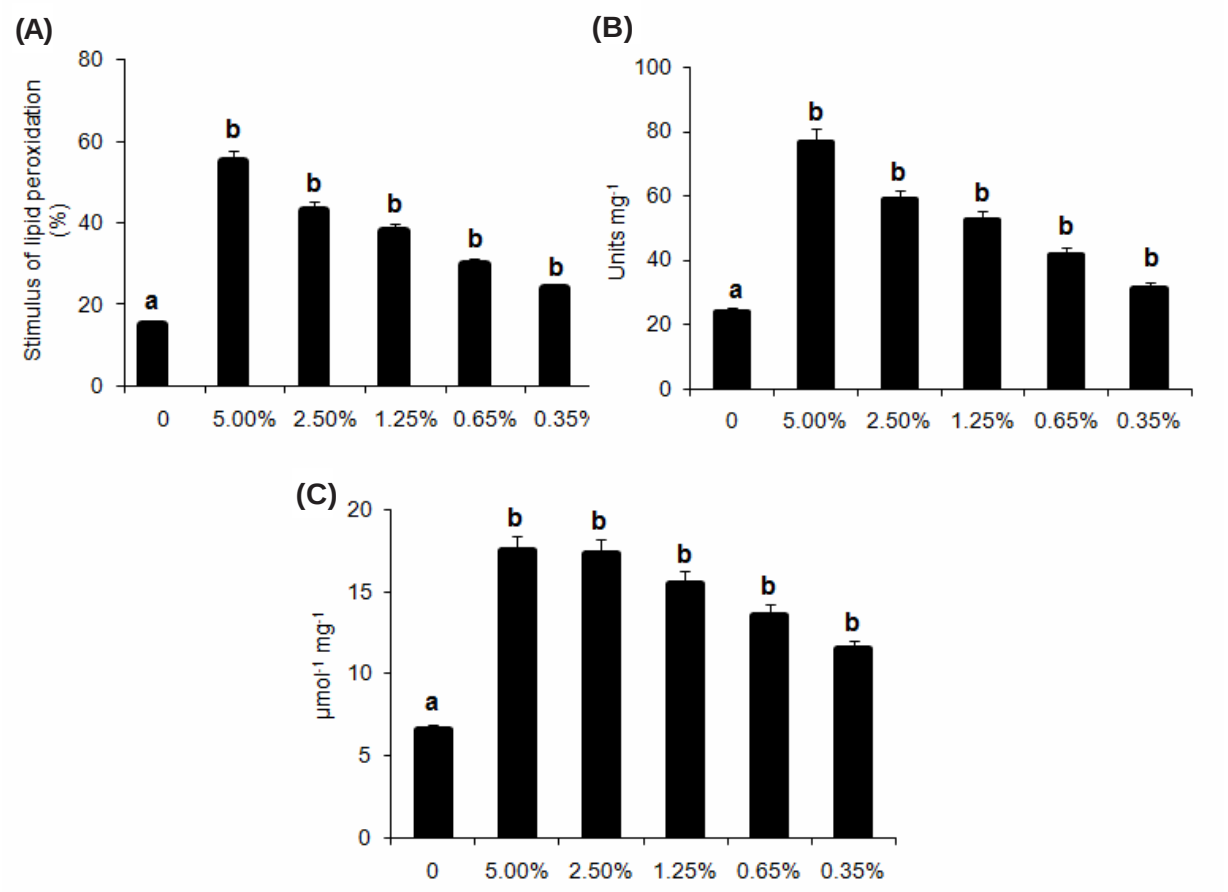
Figure 2 - Effect of different concentrations of the volatile oils of *Polygala extraaxillaris* upon *B. decumb*ens enzymes: Glucanase (A) Phenylalanine ammonia lyase (B), Catalase (C) and Peroxidase (D).

phenylpropanoids (Gayoso et al., 2004; Wen et al., 2005), which are important in plant growth and also act in the protection against environmental stresses (Dixon & Paiva, 1995; Cheng et al., 2001).

The oil at the assayed concentrations have increased membrane peroxidation (±55%) and SOD activity (±76.8%) in *B. decumbens* (Figure 3A and B). The increased activity of the involved enzymes in the defense system caused changes in the total content of proteins on the evaluated species (±17.52 at a concentration of 5.0%) (Figure 3C).

The increase in the enzymatic activity of catalase, peroxidase and superoxide dismutase is associated with the oxidative stress caused by the volatile oils. These are the main enzymes involved in EROS elimination during the germination process, and they are also associated with a wide range of physiological processes (Passardi et al., 2005; Almagro et al., 2009). Catalase, peroxidase and superoxide dismutase contribute to the response of plants to stress, leading to the production of reactive oxygen species in the resumption of mitochondrial processes during seed imbibition (Blokhin et al., 2003; Porta & Rocha-Sosa, 2006).

The increase in the oil concentrations caused marked reductions in the mitotic index (Table 2); the biggest depressive effect was verified for the concentration of 5.0%, with values of ±45%. Comparing the frequencies of the different phases of the mitosis for each treatment, a lower frequency on prophase and on other subsequent phases was observed as compared to control, where a decrease in cell division can be observed from the concentration of 0.65%.



*Treatment means differ significantly; means followed by the same letter as control do not differ ($p < 0.05$) as compared to control means (Dunnett's test).

Figure 3 - Effect of different concentrations of the volatile oils of *Polygala extraaxillaris* upon *B. decumb*ens enzymes: Lipid peroxidation (A), Superoxide dimutase (B) and Total proteins (C).

The growth interference of *B. decumbens* in the presence of volatile oils is associated with interferences in the process of cell division, by means of partial blocking of subsequent phases of division at higher concentrations, which may be associated with changes in the structure of chromosomes in prophase and metaphase, as well as disturbances in prophase and anaphase. Similar changes were observed in *A. cepa* cell chromatids submitted to a *Taxus baccata* aqueous extract (Majewska et al., 2000).

Only the 5.0, 2.5, and 1.25% concentrations caused cytotoxic changes, mainly in the presence of polynuclear cells, nuclear sprouts, cells with misshapen nucleus, polyploid cells, anaphase with chromosome loss and others (Table 2). The other concentrations did not provoke these effects. This volatile oil-caused interference, with its accented effects on the morphology of the radicular system, probably represents one of the mechanisms of action of the oils on the development of the test plant.

Cytotoxic changes, such as the presence of cells with polyploid nucleus, were also observed. These changes may have been due to the prevention of the cytokinesis process as a consequence of the difficulty in the formation of the phragmoplast. The obtained results suggest that the increase in the chromosome contraction can also occur as a result of the depolymerization and inactivation of the spindles, where the contraction of the chromosomes can promote chromosomic and chromatid adherences (Fernandes et al., 2009).

Table 2 - Mitotic index and different phases of cell division, as well as cytotocic activity in meristematic cells of *B. decumbens*, exposed to different concentrations of the oils of *P. extraaxillaris*

n=3	Control	5,00%	2,50%	1,25%	0,65%	0,35%
Mitotic index[1/]	87.44±1.38	45.78±0.19*	47.00±0.33*	59.67±0.33*	64.78±1.67*	70.67±0.67*
Prophase[1/]	165.00±1.05	72.00±1.00*	98.33±0.57*	115.33±1.76*	134.00±2.08*	166.33±1.20[ns]
Metaphase[1/]	62.67±2.08	42.67±1.52*	55.67±2.08[ns]	61.67±2.05ns	62.33±2.30ns	62.00±1.46[ns]
Anaphase[1/]	37.00±1.46	12.00±0.00*	14.33±1.15*	20.66±1.15*	25.33±2.03*	30.66±1.05*
Telophase[1/]	30.66±1.50	10.66±1.15*	12.66±2.03*	19.33±1.15*	20.66±1.55*	28.66±1.55[ns]
Interphase[1/]	21.00±1.00	9.66±1.52[ns]	15.33±2.51[ns]	15.00±0.00[ns]	16.33±1.52[ns]	16.66±1.85[ns]
n=3	Cytotoxic changes					
Type of change	Control	5,00%	2,50%	1,25%	0,65%	0,35%
PNC[1/]	0.00±0.00	0.61±0.41*	0.21±0.46*	0.12±0.28*	0.00±0.00 [ns]	0.00±0.00 [ns]
NB[1/]	0.00±0.00	0.35±0.29*	0.26±0.72*	0.18±0.04*	0.00±0.00 [ns]	0.00±0.00 [ns]
CMN[1/]	0.00±0.00	1.56±0.75*	1.22±0.29*	0.88±0.34*	0.00±0.00 [ns]	0.00±0.00 [ns]
PC[1/]	0.00±0.00	1.28±0.23*	2.33±0.08*	1.12±0.07*	0.00±0.00 [ns]	0.00±0.00 [ns]
ACL[1/]	1.33±1.52	2.89±0.33*	1.29±0.37[ns]	0.78±0.05[ns]	0.00±0.00 [ns]	0.00±0.00 [ns]
TPC[1/]	0.00±0.00	2.76±0.13*	1.56±1.23*	0.99±0.04*	0.00±0.00 [ns]	0.00±0.00 [ns]
AN[1/]	0.00±0.00	3.33±0.04*	1.42±0.22*	0.67±0.01*	0.00±0.00 [ns]	0.00±0.00 [ns]
ANI[1/]	0.00±0.00	3.52±0.01*	2.32±0.14*	0.78±0.12*	0.00±0.00 [ns]	0.00±0.00 [ns]
PCM[1/]	0.00±0.00	2.23±1.34*	1.98±0.71*	0.87±0.18*	0.00±0.00 [ns]	0.00±0.00 [ns]
MA[1/]	0.00±0.00	4.44±0.21*	2.35±0.09*	1.27±0.17*	0.00±0.00 [ns]	0.00±0.00 [ns]
ACN[1/]	0.00±0.00	1.23±0.35*	0.89±0.42*	0.37±0.05*	0.00±0.00 [ns]	0.00±0.00 [ns]
TCA[1/]	1.33±0.40	8.03±0.36*	15.83±0.77*	8.03±0.37*	0.00±0.00 [ns]	0.00±0.00 [ns]

[1/] Mean ± sd. The asterisk indicates a significant result (Dunnet's test $p<0.05$). [ns] Non significantly different. PCN: polynuclear cells; NB: nuclear buds; CMN: cells with misshapen nuclei; PC: polyploid cell; ACL: anaphase with chromosomal loss; TCB: telophase with chromosomal bridge; AN: amoeboid nuclei; ANI: amoeboid nuclei in interphase; LCM: loss of chromosomes in metaphase; MA: multipolar anaphase; ACN: amoeboid cell with a nuclei; TCA: cellular aberrations.

The chromosomic adherence is a common sign of toxic activity upon the genetic material and may probably have irreversible consequences to the cell (Fiskejo, 1985; Marcano et al., 1998). Many of the multipolar anaphases observed in this study also showed chromosome losses, and telophase with chromosomic bridges. This chromosomic disharmony, installed during the nuclear division, does not seem to prevent the nuclear membrane from restructuring itself. However, the membrane follows the irregular distribution of genetic material in the cell and causes misshapen nuclei (Gisselsson et al., 2004). The chromosomes that had been lost and left without the guidance of the spindle tend to migrate randomly to one of the daughter cells and derive cells with 2n ±1 chromosomes (Shamina et al., 2006).

The substance 1-2(hidroxyphenyl)-ethanone is the major constituent of the volatile oils of *P. extraaxillares* roots. The higher concentrations of these volatile oils are responsible for the oxidative stress in *B. decumbens*, causing interference in the germination and growth of the studied species, as well as changes in the process of cell division and cytotoxicity activity. Regarding this latter effect, the disorganization in the microtubules of mitotic spindles is mainly due to the lack of proper segregation of chromosomes, as observed in our study during cell division; it may be the main cause of growth inhibition. Likewise, the blocking of cytokinesis may be the main cause of the formation of polyploid cells.

The effects observed for mitotic activity can also contribute to understanding the effects of the essential oils on the growth of *B. decumbens*. Studies aimed at determining the synergistic activity exerted by the compounds present in the essential oils can

also contribute to understanding ecological relationships among species.

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