# Evaluation of the chemical composition of aquatic environments with leaf litter decomposition of *Eucalyptus urophylla* S.T. Blake (Myrtaceae) and their toxicity in *Allium cepa* L. (Amaryllidaceae)

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# Abstract

Allelochemicals from eucalyptus released into the environment, mainly by leaves, can have a toxic effect on local biota, including in aquatic environments. Therefore, the present study evaluates the toxic activity of the water containing leaves of *Eucalyptus urophylla* S.T. Blake (Myrtaceae) in decomposition using *Allium cepa* L. (Amaryllidaceae) as a test organism. The toxicity and the cytotoxicity evaluation were performed using onion bulbs (*A. cepa*). The toxicity was assessed by inhibiting root growth. The cytotoxicity was evaluated by using a comparison of the mitotic index (MI) and the negative control. The extraction of eucalyptus metabolites from water of the decomposition tests and creek water was performed by solid-phase microextraction (SPME). The chemical characterization was done by gas chromatography coupled to mass spectrometry (GC-MS). There was inhibition of the root growth of *A. cepa*, indicating toxicity of the compounds released in the water during the decomposition. The cytotoxicity tests did not indicate a toxic effect. However, there were identified some mutations, cell death, and morphological changes in the roots. 26 compounds were identified on samples of water acquired from decomposition tests.Fenchone, 2-ethyl-1-hexanol, *cis*-dihydrocarvone, and *trans*-dihydrocarvone were identified in all samples. The results highlight the importance of studies and monitoring of aquatic environments near eucalyptus.


# 1. Introduction

The expansion of eucalyptus culture has attracted attention because it is associated with different negative impacts on the environment. Among the environmental concerns is the planting of *Eucalyptus* species close to rivers, dams, etc. This concern is due to the presence of allelopathic compounds, mainly in the leaves, which impair the development of other plants and hinder the process of microbiological decomposition of the residues (Silva &Costa 2004).

Allelochemicals are bioactive secondary metabolites produced by plants resulting from chemical interactions between plants and other organisms that cause interference in plant growth (Latif et al. 2017). These compounds have very specific ecological and physiological functions involved in biotic and abiotic interactions in ecosystems. The main functions are related to protection against herbivory, the attraction of pollinators, and water and temperature control (Simões et al. 2010). Among the most common allelochemicals are terpenes, alkaloids, phenolic compounds, steroids, long-chain fatty acids, and unsaturated lactones (Rice 1984). Specifically, for the *Eucalyptus* genus, there are compounds such as 1,8-cineol (eucalyptol, which is the major compound in most species), piperitone, felandrene, and volatile aldehydes, including aliphatics and aromatics, which have toxic effects (Araújo et al. 2010) and are inhibitory microbial (Canhoto &Graça 1999).

The eucalyptus leaves dispersed in the burlap during the rainy season are carried to rivers and ponds through leaching, and, in contact with water, they release chemical constituents. The permanence of these in the aqueous environment represents a potential risk to aquatic organisms (Abelho &Graça 1996). These effects can be verified through toxicity tests that are used to evaluate the potentially toxic effect of chemicals compounds present in the contaminated water and aim to collect information to record the elements and chemical compounds involved, and, inherent in the contaminated water and thus used for comparison with the standard values allowed (Brota 2012).

Among the toxicity tests are those in the ecotoxicology area, which covers the study of the behavior and transformations of chemical agents in the environment, as well as their effects and responses on living organisms (Júnior et al. 2013). In this vein, studies have been conducted in aquatic ecosystems, through tests with organisms that allow the identification of problems and the monitoring of these ecosystems (Cesar et al. 1997). Thus, a toxicity test was performed with *Allium cepa* L. (onion), which is considered effective when used to assess the quality of waters. This test consists of studying macroscopic parameters, root growth inhibition values, and cytological parameters such as cell aberrations in metaphases or anaphases and mitotic cells (Fiskesjö 1988). The use of *A. cepa* becomes interesting due to its high sensitivity, low cost, speed, and ease of manipulation and allows it to determine the reduction of the mitotic index and the formation of chromosomal aberrations (Leme &Marin-Morales 2009). Furthermore, the test has been internationally validated as a bioindicator of environmental contamination (Evseeva et al. 2003) and is commonly used to assess the genotoxic and cytotoxic potential of substances (Barbério et al. 2009).

In this context, this research aimed to evaluate the toxic activity of decomposition water of leaves of *Eucalyptus urophylla* S.T. Blake using *A. cepa* as a test organism and to identify the compounds presents on aqueous extract by gas chromatography coupled to mass spectrometry (GC-MS).

## 2. Material And Methods

## 2.1. Leaves of *E. urophylla* and creek water

Leaves of *E. urophylla* and creek water were collected by a litter of eucalyptus at 18°42'09.5"S and 49°10'01.1"W contiguous to the riparian vegetation of the Córrego da Areia. This watercourse belongs to the hydrographic basin of the Paranaíba River and is located in the Canápolis, Minas Gerais.

The identification of *E. urophylla* was done with the aid of a botanical identification key, by comparison with images present in Flores et al. (2016) and by species confirmation through a photographic record by a specialist.

## 2.2. Simulated test of the leaf’s decomposition in water

The leaves of *E. eucaliptus* (6.0 g) and distilled water (1.0 L) were placed in glass containers, closed, and stored in an environment with low lighting. The experiments were organized into 4 groups based on the decomposition time: 5, 10, 15, and 30 days (adapted from Zoratto (2007)). After each decomposition period, the decomposition was filtered to perform biological and chemical analysis.

## 2.3. Toxicity and cytotoxicity analysis

The bioassay followed the protocol of Fiskesjo (1988). Fifteen bulbs of *Allium cepa* of similar sizes (about 3,5 cm in diameter), originating from organic culture and acquired in the Itumbiara, Goiás, were used. The primordial ring (where the roots emerge) was carefully cleaned and the golden bark of the bulbs was removed.

The decomposition water of leaves was used in concentrations of 25%, 50%, and 100% in distillate water. Three bulbs were used for each concentration and they were in contact with the samples for seven days. Three

bulbs were exposed to distilled water as a negative control, and three bulbs as a positive control were prepared using copper sulfate (0.06 g $L^{-1}$).

After seven days the toxicity was assessed by the root growth index obtained from the average growth of the three largest roots of each bulb. The influence of concentrations and days of decomposition on root growth was performed with ANOVA Factorial test using the R program (*R Core Team* 2018).

The cytotoxicity through bulb roots was evaluated in a microscope (Leica Zeiss Modelo DMC2900) with slides prepared with cut roots and placed in an ethanol/acetic (3:1) acid fixing solution, counting 5,000 cells for each treatment and controls. Changes in Morphological cell indicating cell death and the frequency of mitotic index (MI, according to the equation (Francisco 2011): MI = (number of cells in division / total number of cells observed) x 100) were analyzed. The values obtained were compared with those obtained in the negative control, using the chi-square test.

## 2.4. Chemical characterization by gas chromatography coupled to mass spectrometry (GC-MS)

The compounds presented in the decomposed water, extract from eucalyptus leaves, and in the water from the Córrego da Areia were extracted by the SPME method and analyzed in sequence by GC-MS. Polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber with $d_f$ 65 µm (Sigma-Aldrich) was used in SPME. For extraction, 5 mL of sample was placed in headspace flasks with 20 mL capacity and sealed. It was heated at 60º C under stirring for 10 min. Next, the fiber was added to the GC-MS of Shimadzu (QP2010 model) for analysis of the chemical composition. The conditions used were: capillary column type DB-5 (J&W, 30m×0.25mm×0.25m), helium as a carrier gas with a constant flow of 1.0 mL $min^{-1}$; injector temperature of 200º C; detector temperature of 230º C; interface temperature of 275º C, splitless injection mode; 10 min of desorption time of SPME; the temperature of the oven was 40º C for 5 min, after this the oven temperature increase to 125º C at 2.5º C $min^{-1}$, and increase to 245º C at 10º C $min^{-1}$, maintained for 15 min; the ionizing potential of 70 eV; the range of *m/z* 40-650. The identification of the compounds by this technique was based on the similarity index (SI) obtained by the software (LabSolution-GC-MS Solution) was also used by comparison with spectra present in the Nist27, Nist147, Wiley7, Wiley229, and Shim2205 libraries.

# 3. Results And Discussion

## 3.1. Toxicity test

The growth of the roots can occur by the production of new cells, which happens in the meristematic zone, and by the extension of the already formed cells, which occurs both in the meristemic zone and in the root elongation zone (Cutler et al. 2011). However, root growth can be compromised in the presence of toxic substances (Fiskesjö 1988). Thus, was evaluated the toxicity of decomposition water of leaves of *Eucalyptus urophylla* S.T. Blake using *A. cepa*. The results of the toxicity analysis through root growth inhibition are summarized in Table 1, which is observed as the mean root growth value of *A. cepa* exposed to different concentrations of the decomposition water of leaves in different periods of days.

Table 1
Mean root growth values in centimeters (cm), treatments, and controls for the three largest roots of each *Allium cepa* bulb.

| Sample concentration (%) | Mean root growth (cm) | | | |
|---|---|---|---|---|
| | 5 days | 10 days | 15 days | 30 days |
| 100 | 1.49 ±0.41 | 1.51 ±0.19 | 3.32 ±0.18 | 2.43 ±0.15 |
| 50 | 2.29 ±0.25 | 2.63 ±0.17 | 3.34 ±0.30 | 5.18 ±0.39 |
| 25 | 3.71 ±0.29 | 3.1 ±0.19 | 3.94 ±0.42 | 6.17 ±0.08 |
| Control (+) * | 0.12 ±0.06 | 0.12 ±0.06 | 0.12 ±0.06 | 0.12 ±0.06 |
| Control (-) ** | 5.79 ±0.13 | 5.79 ±0.13 | 5.79 ±0.13 | 5.79 ±0.13 |
| **Note**: *control (+): copper sulfate, **control (-): distilled water | | | | |

The influence of the sample concentrations and the decomposition time of the eucalyptus leaves in water are summarized in Figure 1. From this result, it was possible to observe that the growth of the root of the *A. cepa* bulbs, about the control group, was influenced by both the concentration and the decomposition time of the leaves in water (ANOVA Factorial; $F = 7.608$; $P < 0.001$). The samples with 100% leaf decomposition water concentration and periods of the 5 and 10 days of decomposition were the most inhibited the root growth.

The sample with 25% concentration and 30 days of decomposition inhibited root growth the least when compared to the control group.

The inhibition of root growth is indicative of the presence of compounds with toxic effects. Ribeiro (1997) showed the toxicity of industrial effluents through tests to inhibit *A. cepa* roots (Ribeiro 1997). Using the same test, Moreira *et al*. (2014) showed the toxic effect of the pesticide deltamethrin (Moreira et al. 2014). The inhibition growth of the *A. cepa* root in leaf decomposition water may be related to the release of chemical constituents from leaves in decomposition (Abelho &Graça 1996). Thus, the inhibition of *A. cepa* root growth may have been due to the presence of terpenes. Monoterpenes can inhibit germination and root growth in several species since these compounds can cause the reduction of mitotic activity and lipid globules formation in plants (Steven &Gayland 1993).

*In vitro* ecotoxicological tests using crustaceans of the species *Daphnia laevis* and *Daphnia similis* as bioindicators have already indicated the ecotoxicological potential of the volatile oil of the litter of *E. urograndis* (hybrid species), in addition to deleterious effects on aquatic biota (Araújo et al. 2010). Also, ecotoxicological evaluations showed the toxic effect of the natural compounds produced by *E. grandis* and *E. urophylla* using leaf decomposition water with essential oils and extracts of different polarities of these two species, even in small concentrations. Both species warn of possible harmful impacts on aquatic ecosystems located around areas with eucalyptus cultivation (Zoratto 2007).

# 3.2. Cytotoxicity test

The cytotoxicity test was assessed using the mitotic index (MI), which is determined through the rate of cell division (Francisco 2011). The MI values were compared with those obtained in the negative control (Table 2)

and the results of the chi-square test were not significant ($X^2$ = 1.348; DF = 9; p < 0.998). A low MI, compared to the control group, indicates that there were chemical changes capable of interfering with the growth and development of the organism, while a higher MI than that observed in the control group indicates an increased cell division, which is harmful because it causes disorderly multiplication and tumor formation (Leme &Marin-Morales 2009). Thus, it can be inferred that there was no effect of days and concentration on the MI. However, it is possible to observe changes in the cell cycle of the roots exposed to different concentrations on the slides, besides some aberrations and signs of cell death (Fig. 2, 3 and 4). The roots of the positive control did not show sufficient growth for making slides, so it was not possible to calculate the MI of this group.

Table 2
Miotic index (%) of *A. cepa* roots with different concentrations (%) of the decomposition water of eucalyptus leaves in different periods (days).

| Sample concentration (%) | Mitotic index - MI (%) | | | |
|---|---|---|---|---|
| | 5 days | 10 days | 15 days | 30 days |
| 100 | 20.34 | 21.98 | 21.04 | 21.06 |
| 50 | 25.10 | 23.38 | 23.96 | 23.42 |
| 25 | 23.12 | 21.90 | 28.98 | 21.04 |
| Control (-) * | 34.98 | 34.98 | 34.98 | 34.98 |
| **Note**: *Control (-): distilled water. | | | | |

Slides with histological sections from the negative control showed a better visualization of the cells, as well as their nuclei and cell division stages. The nucleus of the control cells is well condensed, which contributes to better staining. In the other slides, the roots exposed to the treatments with the water of decomposition of the leaves of *E. urophylla*, the nucleus is compartmentalized and little condensed, which prevents the fixation of the dye and, consequently, the visualization. In some cases, as in (Fig. 2c), the presence of many vacuoles means cell death, and it is not possible to visualize the cell's genetic material. Some studies using the same bioindicator, but subjected to tests with different substances, showed similar mutations during the division process (Leme &Marin-Morales 2009, Mendes 2008, Paula et al. 2015).

It is worth mentioning that the roots of the present study also showed different morphological characteristics from the control group, especially those exposed to 100% concentrations in the periods of 15 and 30 days. The roots of this treatment, even showing root growth, had a soft texture and thin thickness, which made it difficult to make good quality slides.

# 3.3. Chemical composition

The chemical composition of volatile compounds presents in the leaf decomposition water (in different periods) and the aqueous extract of the leaves of the litter of *E. urophylla* were extracted by the SPME method and identified by GC-MS. (Table 3 and Fig. 5) show all compounds identified and lists according to their retention time. A total of 26 compounds were identified in the aqueous extract and decomposition water of leaves, where was identified at least 87% of compounds present in the samples.

Table 3
Chemical composition present in the leaf decomposition water at different periods (days) and in the aqueous extract of the leaves of the litter of *E. urophylla*.

| | | **5 days** | **10 days** | **15 days** | **30 days** | **fresh leaves** |
|---|---|---|---|---|---|---|
| RT (min) | Compounds | TIC (%) | TIC (%) | TIC (%) | TIC (%) | TIC (%) |
| 12.53 | α-pinene (1) | - | - | 4.6±0.71[a] | - | 11.10±0.46[e] |
| 18.01 | cymene (*o* or *p*) (2) | - | - | - | - | 2.81±0.69[e] |
| 18.55 | eucalyptol (3) | - | - | - | - | 60.97±0.63[e] |
| 18.59 | 2-ethyl-1-hexanol (4) | 10.6±0.89[a] | 12.59±0.61[d] | 8.59±0.43[d] | 5.01±1.20[c] | - |
| 20.97 | *cis*-linalyl oxide (5) | 2.32±1.35[a] | 3.61±1.00[a] | - | 3.43±1.09[a] | - |
| 21.73 | fenchone (6) | 8.73±1.31[a] | 8.04±0.75[e] | 7.26±1.36[e] | 12.3±0.21[e] | - |
| 23.02 | 2,2,6-trimethyl-3-keto-6-vinyltetrahydropyran (7) | - | - | - | 4.43±0.21[c] | - |
| 23.33 | fenchol (8) | - | - | - | - | 1.01±0.20[c] |
| 24.06 | α-campholenal (9) | - | - | - | - | 0.89±0.06[c] |
| 24.76 | *trans*-pinocarveol (10) | - | - | - | - | 1.68±0.24[e] |
| 25.01 | camphor (11) | 9.48±0.50[a] | 7.89±0.10[a] | - | 20.67±0.32[a] | - |
| 25.99 | *trans*-pinocamphone (12) | 6.20±0.61[e] | 5.32±0.02[e] | 8.70±1.41[e] | - | - |
| 26.13 | pinocarvone (13) | 8.01±0.26[e] | 1.44±0.59[e] | 1.25±0.38[e] | - | 1.26±0.23[e] |
| 26.39 | isoborneol (14) | - | - | - | - | 1.37±0.20[c] |
| 26.77 | *cis*-pinocamphone (15) | 3.36±0.08[d] | 8.68±0.32[d] | 3.84±1.05[a] | - | - |
| 27.77 | *trans*-isocarveol (16) | 10.6±0.48[d] | 11.47±0.61[e] | 11.79±0.26[d] | - | - |
| 27.91 | α-terpineol (17) | - | - | - | - | 2.38±0.55[c] |
| 28.20 | *cis*-dihydrocarvone (18) | 8.67±0.24[e] | 10.02±0.09[e] | 16.56±0.11[e] | 24.26±0.12[e] | - |
| 28.59 | *trans*-dihydrocarvone (19) | 7.14±0.14[e] | 8.77±0.04[d] | 18.91±0.55[d] | 21.77±0.29[e] | - |

**Note**: RT: retention time; Identification method by similarity index with mass spectral database: a=Nist08s, b=Nist08, c= Wiley139, d=Wiley229, e=Shim225.

| | | 5 days | 10 days | 15 days | 30 days | fresh leaves |
|---|---|---|---|---|---|---|
| 29.29 | iso-dihydrocarveol (20) | 3.96±0.29$^{c}$ | 5.52±0.14$^{d}$ | 6.43±0.13$^{d}$ | - | - |
| 30.02 | nerol (21) | 5.94±0.16$^{d}$ | 3.71±0.18$^{d}$ | - | - | - |
| 36.71 | α-terpinyl acetate (22) | - | - | - | - | 10.55±0.27$^{d}$ |
| 39.95 | *trans*-caryophyllene (23) | - | - | - | - | 1.47±0.13$^{c}$ |
| 43.84 | aromadendrene (24) | - | 0.67±0.09$^{b}$ | 1.06±0.64$^{c}$ | 2.45±0.13$^{d}$ | 1.85±1.00$^{b}$ |
| 44.16 | caryophyllene oxide (25) | 4.90±4.90$^{e}$ | - | - | - | - |
| 44.34 | alloaromadendrene (26) | - | 0.65±0.10$^{a}$ | - | - | 1.11±0.10$^{d}$ |
| | Total identified (%) | 89.94 | 87.69 | 88.97 | 94.31 | 98.43 |

**Note**: RT: retention time; Identification method by similarity index with mass spectral database: a=Nist08s, b=Nist08, c= Wiley139, d=Wiley229, e=Shim225.

In the analysis of composition water from 10 days of decomposition had the largest number of compounds (14 compounds) while of the 30 days had the lowest (8 compounds). The chemical class (Table 4) of the volatile constituents shows that at least 55% of compounds are oxygenated monoterpenes, with this class representing approximately 80% of the constitution of the aqueous extract. Sesquiterpene hydrocarbons and oxygenated sesquiterpenes were found in small amounts, where the highest (21.77 %) was observed in the decomposition water of leaves of 5 days. These classes of compounds have different functions, such as insecticides and repellents (Reis et al. 2016), antifungal (Kh &Abdelgaleil 2017), among others. Sesquiterpenes and monoterpenes were also the main compounds released by *E. urophylla* by leaves leaching and decomposition in the litter in a study that evaluates the compounds present in soil water (He et al. 2014).

Table 4
Chemical class distribution of compounds presents in the leaf decomposition water at different periods (days) and in the aqueous extract of the leaves of the litter of *E. urophylla*.

| Functional groups | 5 days (%) | 10 days (%) | 15 days (%) | 30 days (%) | fresh leaves (%) |
|---|---|---|---|---|---|
| Monoterpenes | - | - | 4.6 (1) | - | 13.91 (2) |
| Oxygenated monoterpenes | 55.25 (10) | 70.17 (10) | 74.62 (8) | 78.99 (3) | 80.09 (8) |
| Sesquiterpenes | - | - | 1.06 (1) | - | 4.43 (3) |
| Oxygenated sesquiterpenes | 21.77 (1) | 1.32 (2) | - | 2.45 (1) | - |
| Oxirane | - | - | - | 4.43 (1) | - |
| Oxilane | 2.32 (1) | 3.61 (1) | - | 3.43 (1) | - |
| Aliphatic alcohol | 10.6 (1) | 12.59 (1) | 8.59 (1) | 5.01 (1) | - |

The major compounds identified in the aqueous extract were eucalyptol (60.97 %), α-pinene (11.10 %), and α-terpinyl acetate (10.55 %), all monoterpenes. These compounds are found in other *Eucalyptus* species in different proportions, as observed in other studies that evaluated the composition of essential oils (Baptista et al. 2015, Sebei et al. 2015). Hepp, Delanora, and Trevisan (2009) also identified secondary compounds during the decomposition of leaves of *E. grandis* in a stream for different periods, with some compounds similar to those found in this study, such as eucalyptol, fenchol, pinocarveol, pinocamfone, and terpineol.

The main compounds identified in the decomposition water of leaves of all days were all oxygenated monoterpenes. As observed in Table 3, α-pinene is presented only in the 15 days sample and the aqueous extract, the caryophyllene oxide is presented only in the 5 days sample and in aqueous extract and the 2,2,6-trimethyl-3-keto-6-vinyltetrahydropyran is presented only in the 30 days sample. There is also a significant increase in the percentage of the *cis*-dihydrocarvone and *trans*-dihydrocarvone compounds over the days of decomposition of leaves, reaching 24.26 and 21.77%, respectively, in the 30 days sample. The compounds aromadendrene and alloaromadendrene appeared only in samples of 10 and 15 days and 10 and 30 days, respectively.

According to the data presented, the 5 and 10 days periods showed similarities between the compounds, with nerol being a compound unique to these two periods. Nerol is a monoterpene found in many essential oils (Andrei &Comune 2005, Peterson et al. 2006), that has biological potentials potentialities in several in vitro and in vivo test systems (Marques et al. 2013). This monoterpene is found in several medicinal plants, which are reported in the literature with antioxidant, antimicrobial and neuroprotective activities (Allahverdiyev et al. 2004, Escobar et al. 2010, Kennedy et al. 2003). Moreover, nerol is used as a fragrance in cosmetics and cleaning products (Santos et al. 1996).

The action is reported in the central nervous system (CNS), as an antidepressant, anxiolytic, antinociceptive and anticonvulsant, in addition to having antioxidant, anti-inflammatory, antimicrobial and anthelmintic activities (Almeida et al. 2014, Coelho 2017, Escobar et al. 2010, Lapczynski et al. 2008, Silva et al. 2014, Silva et al. 2012, Topal et al. 2008). Nerol can provide attractive, repellent and even toxic activity to insects and microorganisms, and provides an antispasmodic effect (Coelho 2017, Escobar et al. 2010, Silva et al. 2014). This chemical component has been shown to be toxic to Artemia salina and in the evaluation of hemolysis in mice at a concentration of 500 µg / mL, nerol showed hemolytic and cytotoxic effect at the cellular level, causing membrane rupture (Coelho 2017).

# 4. Conclusion

This study shows that the compounds of *E. urophylla* released during the decomposition of the leaves in the water have an effect by the inhibition of *Allium cepa* root growth, mainly in the first 10 days.

The results of the cytotoxicity analysis did not indicate a significant toxic effect. However, it was possible to observe morphological changes, some mutations, and cell death in the histological sections of the *A. cepa* root.

The analysis by GC-MS identified 26 compounds released by the leaves of *E. urophylla*, most of which belong to the class of oxygenated monoterpenes. The compounds 2-ethyl-1-hexanol, fenchone, *cis*-dihydrocarvone,

and *trans*-dihydrocarvone are important in the water decomposition of leaves and eucalyptol, α-pinene, and α-terpinyl acetate in the aqueous extract.

Because of the results presented here, this research corroborates the risk of foreign materials for aquatic biota, especially in small environments with less water flow, such as in temporary puddles present in the litter, which are used, for example, for the reproduction of amphibians. Thus, it is important to monitor the aquatic environments close to the eucalyptus, as well as respect a minimum distance established by law and use natural barriers that prevent the eucalyptus leaves from being leached to the watercourse.

# Abbreviations

GC-MS - gas chromatography coupled to mass spectrometry, DF - degrees of freedom, DVB – divinylbenzene, MI - mitotic index, PDMS - polydimethylsiloxane, RT - retention time, SPME - solid phase microextraction, SI - similarity index.

# Declarations

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

### CONSENT FOR PUBLICATION

Not applicable.

### AVAILABILITY OF DATA AND MATERIALS

All data generated or analysed during this study are included in this published article and its supplementary information file
at https://docs.google.com/document/d/1UkNm1xKbzdzuPdlO2wOIlgstW5JHqHPWR3JEoPZmz7A/edit?usp=sharing

### COMPETING INTEREST

The authors declare that they have no competing interests.


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### AUTHORS' CONTRIBUTIONS

**Ritielly M. G. Guerino:** Elaboration of all stages of construction of the article, elaboration of the project, bibliographic review, definition of the methodology, collection and treatment of data, discussion of the results and conclusion. **Junilson A. P. Silva:** Methodology. **Débora de Jesus Pires:** Methodology, Writing - original draft. **Rafael A. C. Souza:** Methodology, Writing - original draft. **Raquel M. F. Sousa:** Funding acquisition, Supervision,

Conceptualization, Writing - review e editing. **Alberto de Oliveira:** Funding acquisition, Supervision, Conceptualization, Writing - review e editing. **Isa Lucia de Morais**: Supervision, elaboration of the project, bibliographic review, definition of the methodology, discussion of the results and conclusion, conceptualization, writing - review e editing.

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# Figures

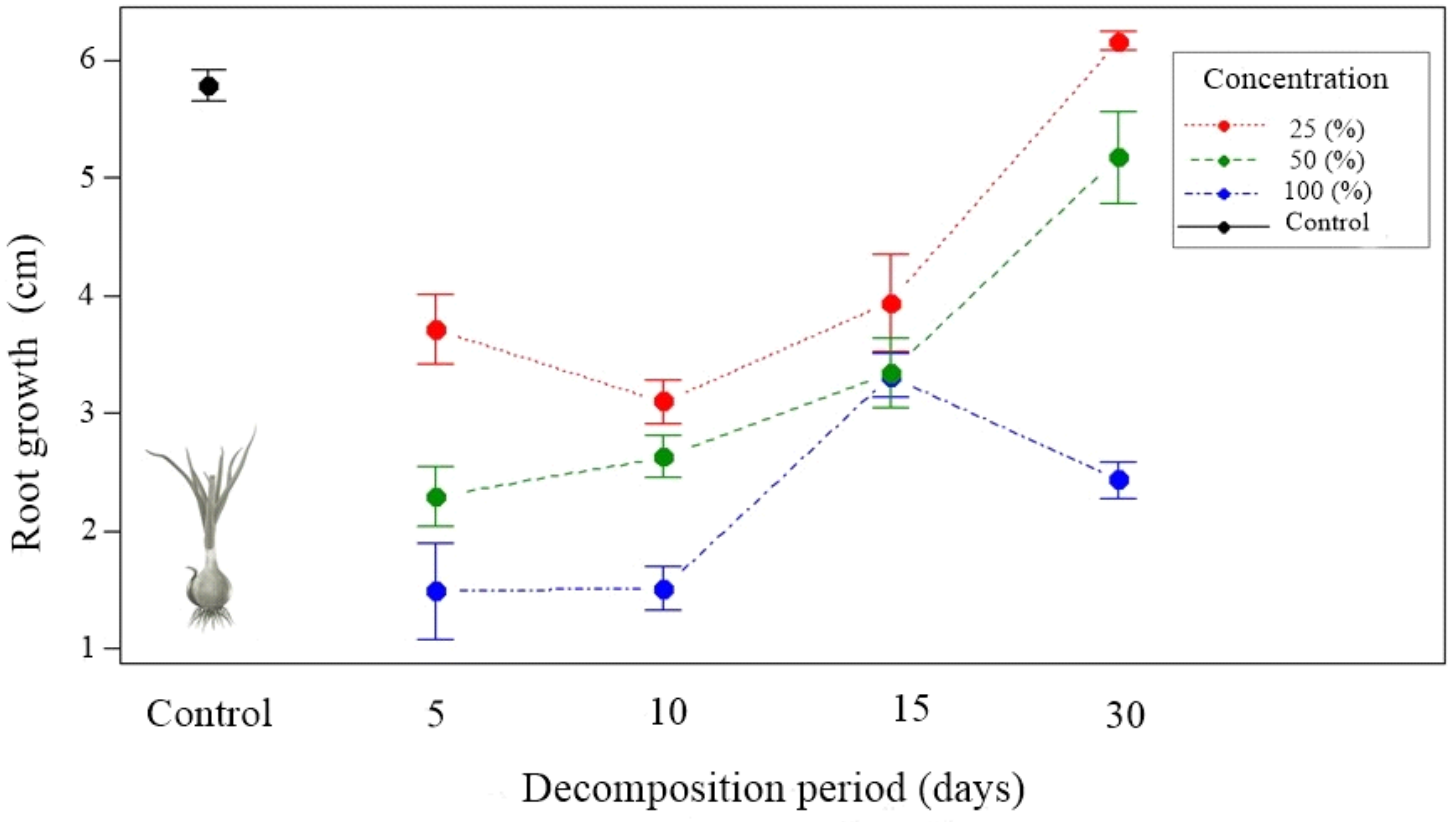


**Figure 1**

Influence of the concentration (%) of leaf decomposition water and decomposition time (days) on root growth (cm) of A. cepa (ANOVA Factorial test).

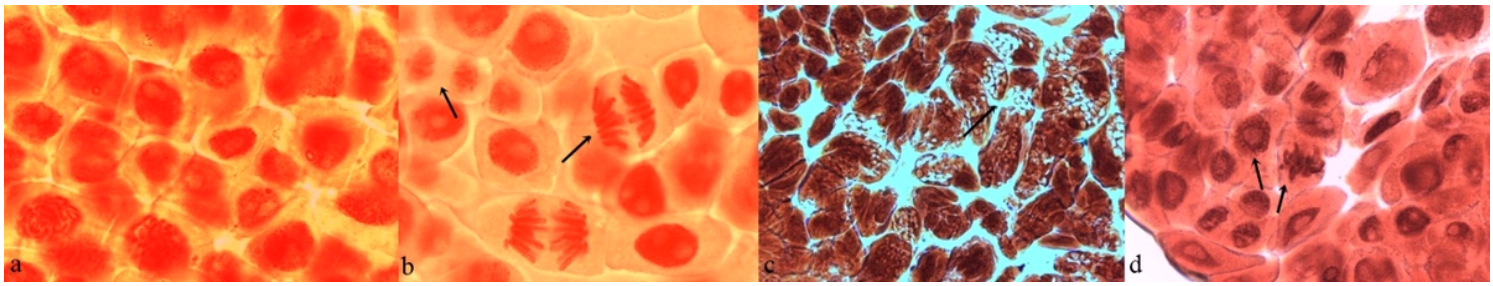


**Figure 2**

Meristematic cells of A. cepa root in the mitotic division. The arrows indicate: a) Cells from the negative control histological section; b) cells dividing the negative control into telophase and anaphase; c) root cells of treatment with decomposition water of leaves at 30 days at a concentration of 100% with signs of cell death; and d) root cells of treatment with decomposition water of leaves at 15 days at a concentration of 25% in irregular metaphase and anomalous interphase.

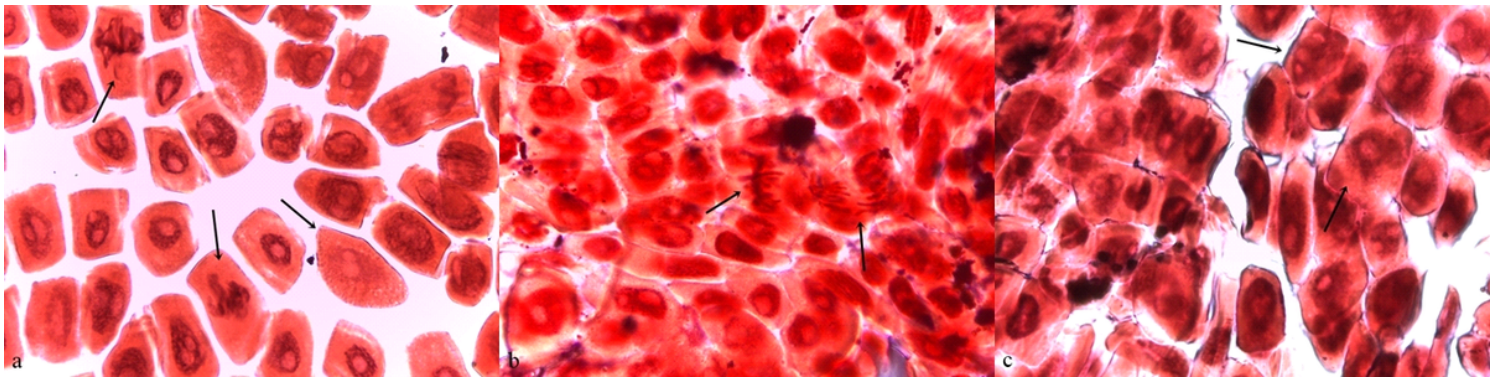


**Figure 3**

Meristematic cells of A. cepa root exposed to decomposition water of leaves of E. urophylla at 10 days. The arrows indicate a) Cells of the histological cut of the root of treatment with decomposition water of leaves at a concentration of 50% with the compartmented nucleus and anomalous metaphases; b) root cells of treatment with decomposition water of leaves at a concentration of 100% with abnormal metaphase and bridged telophase; and c) root cells of treatment with decomposition water of leaves at a concentration of 100% with signs of cell death.

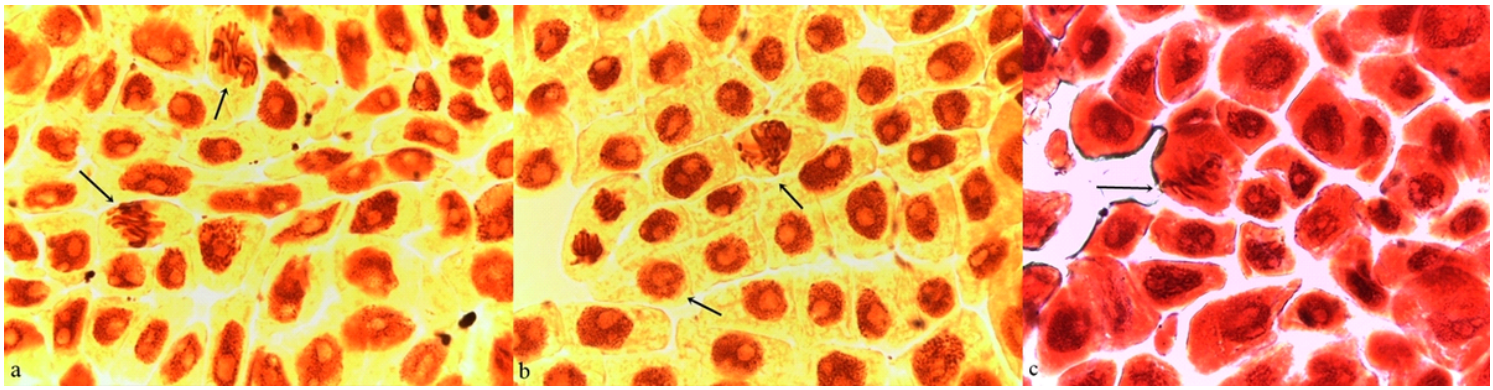


**Figure 4**

Meristematic cells of the A. cepa root exposed to decomposition water of leaves of E. urophylla at 5 days. The arrows indicate a) Cells of the histological cut of the root of treatment with decomposition water of leaves at a concentration of 100% with abnormal prophase and compartmented nucleus; b) root cells of treatment with decomposition water of leaves at a concentration of 100% with abnormal prophase and compartmented nucleus; and c) root cells of treatment with decomposition water of leaves at a concentration of 25% with anomalous metaphase and signs of cell death.

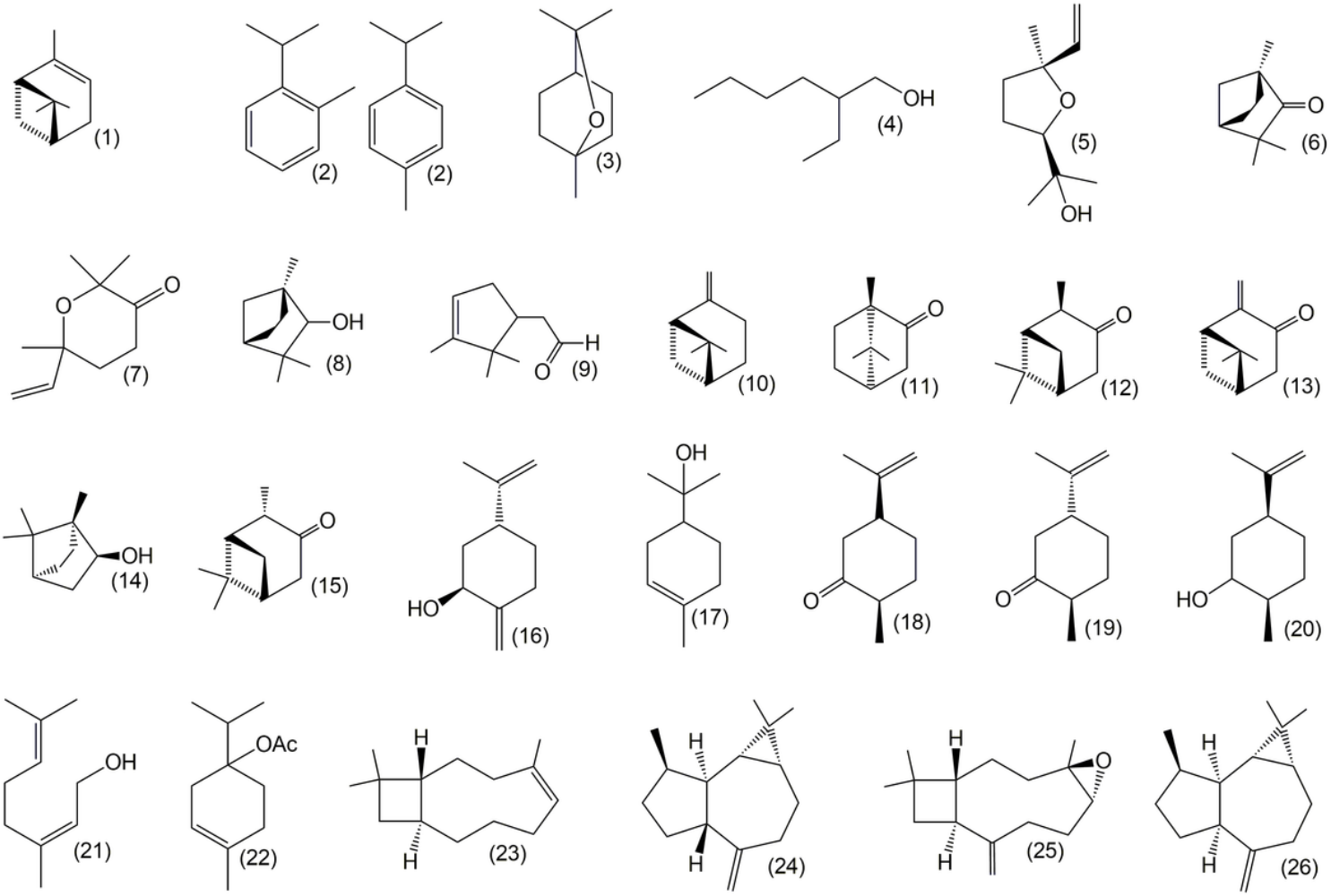


**Figure 5**

Structures of the compounds identified in the leaf decomposition water at different periods (days) and in the aqueous extract of the leaves of the litter of E. urophylla.

## Supplementary Files

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