Chemical composition of Schinus lentiscifolius March. essential oil and its phytotoxic and cytotoxic effects on lettuce and onion

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1. Introduction

Schinus L. (Anacardiaceae) is a flowering woody shrub or small tree native to South America. Some species of this genus are commonly known as “pepper trees.” Schinus species are well known due to the production of several secondary metabolites, mainly essential oils and phenolic derivatives (Santana et al., 2012; Zahed et al., 2011). Schinus lentiscifolius March. is a tree with opaque, smooth, and glabrous leaves found in grasslands, both alone and in groups, especially in stony soils. This species is recognized to present a pleasant scent in the leaves and is used in folk medicine (Fleig, 1989; Klamt and Schneider, 2004; Stumpf et al., 2009).

Essential oils are natural, complex, and multicomponent mixtures of volatile terpenes in addition to some other non-terpene components as phenylpropanoids (Baser and Buchbauer, 2010). These oils may exhibit several biological attributes and can be cytotoxic (Pawlowski et al., 2012; Schmidt-Silva et al., 2011), mutagenic (Bakkali et al., 2012), antimicrobial (Adam et al., 1998; Burt, 2004), or amoebicidal (Sauter et al., 2012). Terpenes are also involved in a variety of ecological interactions, including allelopathy (Ding et al., 2010), broadly defined as a mechanism of biochemical interaction between plants (Rice, 1984).

Germination and seedling growth bioassays are primary tools for determining the allelopathic potential of secondary metabolites. The growth-limiting effects of various kinds of stresses have been related to their influence on the regulation of shoot elongation and cell division (Sánchez-Moreiras et al., 2008). Although S. lentiscifolius belongs to a genus that produces several compounds of biological importance, little is known about the chemical composition of this species or the biological activity of its compounds. Therefore, this work studied the composition of essential oil from S. lentiscifolius and its potential phytotoxic effects on onion (Allium cepa L.) and lettuce (Lactuca sativa L.), including its effects on germination, seedling growth, and mitotic activity.

2. Material and methods

2.1. Plant material

Leaves of S. lentiscifolius were collected in Encruzilhada do Sul City (30° 31’ 36.67” S, 52° 31’ 6.17” W), in the State of Rio Grande do Sul, Brazil. Samples were identified and a voucher (164708) was deposited in the herbarium of the Universidade Federal do Rio Grande do Sul (ICN).
2.2. Essential oil extraction

Air-dried leaves of *S. lentiscifolius* were subjected to hydrodistillation in a Clevenger-type apparatus (Brazilian Pharmacopeia, 2010) until no significant increase occurred in the volume of oil collected (3 h). The oil was dried over anhydrous sodium sulfate, covered with N₂ in a sealed vial, and stored in an ultrafreezer (−80 °C) until required.

2.3. Compound identification

Chromatographic analysis was performed using a Shimadzu gas chromatograph coupled to a mass spectrometer detector (GCMS-QP2010 Plus) and a Shimadzu 17A gas chromatograph with a flame ionization detector (GC-FID). Two capillary columns were used under the following conditions: DB-5 (30 m × 0.25 mm × 0.25 mm) with an initial oven temperature of 60 °C, raised by 3 °C/min until reaching a final temperature of 220 °C; and Carbowax (30 m × 0.25 mm × 0.25 mm) with an initial oven temperature of 40 °C, increased by 3 °C/min until a final temperature of 220 °C was reached. Injector and detector temperatures were kept at 220 °C for DB-5 and 250 °C for Carbowax. Helium flow rate was 1.0 ml/min, and desorption occurred in the split mode.

Linear temperature programmed retention indexes (LTPRIs) were determined from the retention data of an n-alkane solution (C₅–C₃₂), along with the retention data of volatile compounds from *S. lentiscifolius* samples. All of the components were tentatively identified through a comparison of their LTPRI with those registered in the literature databases (Wiley, 6th edition). The relative percentage of each component was obtained directly from chromatographic peak areas, assuming the sum of all eluted peaks totaled 100%.

2.4. Germination and growth assays

For germination assays, 50 *Allium cepa* and *Lactuca sativa* diaspores were soaked in 5 ml of distilled water on one layer of filter paper in Petri dishes and incubated in a germination room (average temperature: 20 °C; photoperiod: 12 h; illumination was provided by 20-W fluorescent lamps). The essential oil (0.1 ml) was dropped onto a cotton ball attached to the inner face of the Petri dish lid to avoid direct contact between diaspores and the essential oil. A negative control group was treated with distilled water, and four replicates of each treatment were tested. We evaluated the percentage of germination and accumulated germination (Anjum and Bajwa, 2005). For growth assays, diaspores were germinated and the essential oil was applied after the emergence of the primary root. The seedlings remained exposed to the volatiles for 72 h, and then 10 seedlings per replicate were evaluated by measuring root and shoot length.

2.5. Cytogenetic assay

Diaspores were germinated in Petri dishes under the same conditions used for the germination assays. A negative control group was treated with distilled water and a positive control group was treated with N-(4-hydroxyphenyl)ethanamine (Garbulli and Bashasha, 2008) at 0.50 mg/ml for lettuce and 0.75 mg/ml for onion. After 48 h for lettuce and 144 h for onion, root tips were cut and subsequently fixed in a freshly prepared mixture of ethanol and acetic acid (3:1, v/v). The fixed root tips were stained using the Feulgen reaction. Four replicates were evaluated for each treatment and scoring was performed for four roots per replicate totaling 8000 cells per treatment. The parameters evaluated were mitotic and metaphase indices, frequency of each mitotic phase, and the percent of chromosomal aberrations.

2.6. Statistical analysis

Comparisons between groups were performed using t-test or one-way analyses of variance (ANOVA) and a post hoc Tukey test at a 5% significance level whenever the data satisfied presuppositions of normality and homogeneity of variance. Nonparametric Kruskal–Wallis tests followed by Tamhane’s T2 test for multiple comparisons were used if the data did not follow a normal distribution. All statistical analyses were performed using SPSS 17.0 software.

3. Results

The chemical composition of the essential oil studied is shown in Table 1. *Schinus lentiscifolius* essential oil is a complex mixture of terpenes. The most abundant chemical category was sesquiterpene hydrocarbons (41.52%), followed by monoterpane hydrocarbons (27.68%), oxygenated sesquiterpenes (26.16%), and oxygenated monoterpenes (4.64%). The major compound was α-cadinene (14.43%), a sesquiterpene. Other terpenes present in high quantities in the essential oil were limonene (8.26%), τ-murolol (5.70%), sabine (5.16%), α-cadinol (4.98%), α-pinene (4.87%), terpinen–4-ol (3.91%), and α-calamodore (3.38%). This essential oil affected both germination and seedling growth in the target species (Fig. 1). However, the germination of lettuce was more affected than that of onion. Compared to the control group, treated groups showed a significant reduction in germination, onion by 27% and lettuce by 38%. The speed of accumulated germination (AS) was also affected. The essential oil reduced the AS of onion by 30% and lowered that of lettuce by 58%. Exposure to *S. lentiscifolius* essential oil resulted in different inhibitory effects on seedling growth of both onion and lettuce. Compared to the control group, the essential oil affected shoot length more than root length in onion, whereas the reverse was true for lettuce. *Schinus lentiscifolius* essential oil reduced the shoot and root length of onion by 61% and 45%, respectively, whereas in lettuce, the respective parameters were reduced by 48% and 53%.

Table 2 presents an analysis of the mitotic behavior and the percentages of dividing cells in prophase, metaphase, anaphase, and telophase for both the controls and treatment groups. *Schinus lentiscifolius* essential oil affected the mitotic index of meristematic cells of both target species. Compared with the negative control, volatiles reduced the mitotic index of onion by 19.35% and that of lettuce by 25.14%. The essential oil also reduced the metaphasic index of lettuce root meristems by 35.17%, but no significant difference was observed in the metaphasic index of onion. The proportion of mitotic phases did not differ between essential oil treatments and negative controls in onion or lettuce root meristems, except for the proportion of lettuce cells in telophase, which was increased by exposure to the essential oil.

The essential oil induced a variety of genotoxic effects in both onion (Table 3) and lettuce root meristems (Table 4). This damage was evident through the occurrence of chromosomal abnormalities. In onion assays, it was observed as a threefold increase in the percentage of abnormalities compared to the control group. In onion, volatiles induced a higher incidence of micronuclei (58% of total abnormal cells) and induced aneugenic effects (33%), which could be explained by the high incidence of metaphase with chromosome adherence (sticky metaphase) and c-mitosis. In lettuce assays, the observed effect was more pronounced, and volatiles increased the percentage of abnormal cells by 24 times. In this case, about 60% of the abnormal cells corresponded to cells with a micronucleus and 22% displayed clastogenic effects (chromosome breaks and bridges at different mitotic phases).

4. Discussion

Few studies have examined the micromolecular chemistry and biological activity of *S. lentiscifolius* essential oil. Rossini et al. (1996) verified sesquiterpene hydrocarbons as the most abundant chemical
### Table 1

#### Chemical composition of *Schinus lentiscifolius* essential oil.

| Compound                      | LTPRI*  | LTPRI Area (%) |
|-------------------------------|---------|----------------|
| **Monoterpene hydrocarbons**  |         |                |
| Tricyclene                    | 927     | 0.18           |
| α-Thujene                     | 930     | 0.23           |
| α-Pine                         | 939     | 4.80           |
| Camphene                       | 954     | 1.38           |
| Sabine                       | 975     | 5.08           |
| β-Pine                       | 979     | 1.82           |
| Myrcene                       | 991     | 1.39           |
| α-Phellandrene                | 1003    | 0.12           |
| α-Terpine                     | 1017    | 0.70           |
| Para-cymene                   | 1025    | 1.08           |
| Limonene                      | 1029    | 8.14           |
| 1,8-Cineole*                  | 1031    | 0.10           |
| cis-Ocime                     | 1037    | 0.09           |
| trans-Ocime                   | 1050    | 0.10           |
| γ-Terpine                     | 1060    | 1.15           |
| Terpinolene                   | 1089    | 0.12           |
| **Oxygenated monoterpenes**   |         |                |
| Linalool                      | 1109    | 0.06           |
| Menth-2-en-1-ol < cis-para-  | 1122    | 0.13           |
| Terpinol                       | 1134    | 0.08           |
| Terpinen-4-ol                 | 1177    | 3.85           |
| α-Terpineol                   | 1189    | 0.40           |
| Isobornyl acetate             | 1286    | 0.03           |
| α-Terpinen                    | 1448    | 0.18           |
| N.I. 1443                     | 0.08     |
| N.I. 1433                     | 0.36     |
| Germacrene A                  | 1510    | 0.52           |
| N.I. 1497                     | 0.92     |
| N.I. 1494                     | 0.15     |
| Isobornyl acetate             | 1286    | 0.03           |
| **Sesquiterpene hydrocarbons**|         |                |
| α-Cubebene                    | 1351    | 0.37           |
| α-Copaene                     | 1377    | 1.21           |
| β-Bourbonene                  | 1388    | 2.37           |
| N.I.                           | 1391    | 0.14           |
| N.I.                           | 1391    | 0.58           |
| β-Elemene                     | 1409    | 0.07           |
| α-Gurjunene                   | 1410    | 0.59           |
| β-Caryophyllene               | 1419    | 2.41           |
| N.I.                           | 1433    | 0.36           |
| N.I.                           | 1443    | 0.08           |
| N.I.                           | 1448    | 0.18           |
| trans-Muurola-3,5-diene       | 1454    | 0.30           |
| α-Humulene                    | 1455    | 0.66           |
| Altoaromadendrene             | 1460    | 0.38           |
| cis-Muurola-4,14,5-diene      | 1467    | 0.24           |
| N.I.                           | 1471    | 0.07           |
| γ-Gurjunene                   | 1477    | 1.12           |
| α-Amorphene                   | 1485    | 0.94           |
| Germacrene D                  | 1485    | 1.65           |
| β-Selinene                    | 1490    | 0.47           |
| N.I.                           | 1494    | 0.15           |
| N.I.                           | 1497    | 0.92           |
| Bicyclogermacrene             | 1500    | 2.15           |
| α-Murolene                    | 1500    | 2.28           |
| Germacrene A                  | 1510    | 0.52           |
| γ-Cadinene                    | 1514    | 1.04           |
| N.I.                           | 1522    | 0.28           |
| δ-Cadinene                    | 1523    | 14.21          |
| trans-Cadinole-1(2),4-diene   | 1535    | 0.40           |
| α-Cadinol                     | 1539    | 0.38           |
| α-Calacorene                  | 1546    | 3.33           |
| N.I.                           | 1554    | 0.08           |
| N.I.                           | 1550    | 0.11           |
| N.I.                           | 1563    | 0.02           |
| Bourbonanone < 1-nor>         | 1563    | 0.17           |
| N.I.                           | 1568    | 0.34           |
| Ledol                         | 1569    | 0.50           |

**Oxygenated sesquiterpenes**

| Compound                        | LTPRI#  | LTPRI Area (%) |
|--------------------------------|---------|----------------|
| Spathulenol                    | 1578    | 1.39           |
| Globulol                       | 1585    | 2.11           |
| N.I.                           | 1591    | 2.16           |
| Viridiflorol                   | 1593    | 1.19           |
| N.I.                           | 1611    | 0.58           |
| N.I.                           | 1616    | 0.60           |
| N.I.                           | 1619    | 0.10           |
| N.I.                           | 1624    | 1.70           |

### Table 1 (continued)

#### Oxidized sesquiterpenes

| Compound                        | LTPRI#  | LTPRI Area (%) |
|--------------------------------|---------|----------------|
| N.L.                           | 1628    | 0.19           |
| N.L.                           | 1635    | 2.75           |
| α-Cadinol                      | 1654    | 4.91           |
| N.L.                           | 1653    | 0.87           |
| N.L.                           | 1663    | 5.61           |
| N.L.                           | 1666    | 0.05           |
| N.L.                           | 1672    | 0.04           |
| N.L.                           | 1677    | 0.23           |
| Cadalene                       | 1677    | 0.18           |
| N.L.                           | 1685    | 0.05           |
| N.L.                           | 1695    | 0.15           |
| N.L.                           | 1700    | 0.63           |
| N.L.                           | 1714    | 0.24           |
| N.L.                           | 1807    | 0.23           |
| N.L.                           | 1821    | 0.20           |

**LTPRI**, linear temperature programmed retention indexes, tabulated (Adams, 2001).

**N.L.**, not identified.

*Oxygenated monoterpenes.*

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category in *S. lentiscifolius* essential oil and δ-cadinene as its main component. In the present study, about 80% of the peaks detected were identified. The identification of terpenes is difficult because some of these compounds present similar mass spectra (especially sesquiterpenes) and many co-elutions may occur, preventing a correct identification and quantification process. Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC × GC/TOFMS) is a powerful analytical tool for the analysis of complex matrices due to its high peak capacity, selectivity, and sensitivity (Shellie and Marriott, 2003). This technique shows potential for the separation and tentative identification of the components of essential oils that exhibit moderate complexity in one-dimensional gas chromatography/mass spectrometry (1D-GC/MS). When GC × GC/TOFMS is employed, co-elutions in the first chromatographic dimension (1D) can be resolved in the second chromatographic dimension (2D). Therefore, the use of this technique can help to clarify components that may make a relevant contribution to the biological activity of essential oils.

The allelopathic potential of natural products is often verified by testing their influence on seed germinability and seed viability (Gniadowska and Bogatek, 2005). Accumulated germination speed is a measure of seedling vigor; higher values express higher seedling vigor in one sample compared to another (Ranal and Santana, 2006). A reduction in seedling vigor can cause a progressive loss of productive capacity and a reduction in the uniformity of germination. Consequently, *S. lentiscifolius* volatiles affect both the percentage of germination and seedling vigor in onion and lettuce.

Terpenes typically suppress seed germination and cause injury to seedling growth (Singh et al., 2002; Volou et al., 2003; Zhao et al., 2009). Some volatile terpenes detected in the essential oil of *S. lentiscifolius* have been cited previously as being phytotoxic. Considering monoterpenes, β-pinene significantly reduced the root and coleoptile length of rice (Chowhan et al., 2011), and limonene affected the germination and radicle elongation of radish and garden cress (De Martino et al., 2010). Terpenes-4-ol, an oxygenated monoterpene, completely inhibited the seed germination and seedling growth of *Amaranthus retroflexus* L., *Chenopodium album* L., and *Rumex crispus* L. (Kordali et al., 2007). Among sesquiterpenes, β-caryophyllene inhibited the seed germination, and root and shoot growth of *Brassica campestris* L., *Raphanus sativus* L., *L. sativa,* and *Mikania micrantha* Kunth (Wang et al., 2010), and δ-cadinene was found in high concentrations in the root extract of the bitou bush (*Chrysanthemoides monilfera* spp. *rotundata* (DC.) *T. Norl*) and exhibited phytotoxic activity on *Isolepis nodosa* (Rott.) R. Br. (Ens et al., 2009).
Important effects of allelopathy have been identified at the plant organismal, cellular, and molecular levels, and alterations in the last two levels can reflect deleterious effects on normal plant organ functions (Blanco, 2007). Plant growth is driven by the process of cell division, coupled with subsequent expansion and differentiation of the resulting cells. Cell growth in plants depends on a normal mitotic process (Teerarak et al., 2010). In this study, the essential oil tested induced a decrease in the mitotic index in the root meristem cells of both onion and lettuce. Such a reduction in the mitotic index suggests that exposure to *S. lentiscifolius* essential oils led to cell cycle disturbances, decreasing the number of cells entering mitotic division and causing the observed inhibitory effects on root length in target species.

Beyond mitodepressive activity, *S. lentiscifolius* volatiles induced genotoxic effects. A positive control used in cytogenetics analysis, N-(4-hydroxyphenyl)ethanamide, commercialized as Paracetamol®, is a recognized substance that induces genotoxicity (Garbulli and Bashasha, 2008). In the present study, *S. lentiscifolius* essential oil caused a similar number of abnormalities in lettuce as the positive control. The positive control caused more pronounced genotoxic effects in onion assays than in lettuce assays because Paracetamol® was used at a lower concentration in the lettuce assays (0.75 mg/ml for onion and 0.50 mg/ml for lettuce). This was necessary because a higher concentration of the positive control caused seedling death in lettuce (data not shown).

Chromosomal abnormalities are characterized by changes in chromosome structure (clastogenic effects) or by changes in the total number of chromosomes (aneugenic effects), both of which may occur simultaneously as a result of exposure to physical agents or natural and synthetic chemical agents (Leme and Marin-Morales, 2009). In the onion seedlings treated with *S. lentiscifolius* essential oil, the most common abnormalities were the development of micronuclei and sticky metaphase, whereas in lettuce seedlings the most frequent abnormality was micronuclei. The micronucleus is a structure that can be derived fromacentric fragments, involving clastogenic activity, or from entire chromosomes, involving aneugenic activity (Türkoğlu, 2012). At telophase, a nuclear envelope forms around fragments and/or whole chromosomes, which are unable to travel to the spindle poles during mitosis. These chromosomes then uncoil and gradually assume the morphology of an interphase nucleus, except that they are smaller than the main nuclei in the cell (Fenech, 2000).

Stickiness is considered a chromosome aberration type and its formation can be diverse. It can be formed due the effect of a chemical or physical agent: on the physicochemical properties of DNA, protein, or both; on the formation of complexes with phosphate groups in DNA; on DNA condensation; or on the formation of inter- and intra-chromatid cross-links (El-Ghamery et al., 2003; Türköğlu, 2012). The stickiness, an irreversible chromosome abnormality, commonly gives rise to cell death (Fernandes et al., 2009) and likely contributed to the observed decline in the mitotic index.

The biological effects of essential oils are often explained in terms of the presence of some main constituents. In this study, *S. lentiscifolius* essential oil was found to be a complex mixture of numerous molecules. The activity of the main components of this mixture can be modulated by the minor molecules (Franzios et al., 1997). Moreover, several components of the essential oil likely play a role in defining cell penetration, lipophilic or hydrophilic attraction, fixation to cell walls and membranes, and sticky metaphase, whereas in lettuce seedlings the most frequent abnormality was micronuclei. The micronucleus is a structure that can be derived fromacentric fragments, involving clastogenic activity, or from entire chromosomes, involving aneugenic activity (Türkoğlu, 2012). At telophase, a nuclear envelope forms around fragments and/or whole chromosomes, which are unable to travel to the spindle poles during mitosis. These chromosomes then uncoil and gradually assume the morphology of an interphase nucleus, except that they are smaller than the main nuclei in the cell (Fenech, 2000).

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### Table 2

|                     | Allium cepa | Lactuca sativa |
|---------------------|-------------|----------------|
|                     | Negative control | Positive control | Essential oil |
| Cells in mitosis    | 1224        | 854            | 987           |
| Mitotic index       | 15.30 ± 0.27* | 10.68 ± 0.58*  | 12.34 ± 0.59* |
| Metaphasic index    | 2.76 ± 0.46* | 1.36 ± 0.41*   | 2.44 ± 0.34*  |
| Mitotic phase       |             |                |               |
| % Prophase          | 39.37 ± 0.57* | 64.35 ± 3.82*  | 38.33 ± 3.28* |
| % Metaphase         | 18.34 ± 2.93* | 12.65 ± 3.18*  | 19.71 ± 2.12* |
| % Anaphase          | 14.08 ± 3.24* | 7.76 ± 1.06*   | 16.87 ± 2.07* |
| % Telophase         | 29.51 ± 0.59* | 15.24 ± 1.96*  | 25.08 ± 2.71* |

Means within a column followed by different capital or lower-case letters are significantly different according to Tamhane’s T2 or Tukey’s test, respectively (p < 0.05).
studies will contribute to a greater understanding of the mode of action of natural products with allelopathic potential and aid in clarifying ecological relationships among species.

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