



### Phytotoxic effects of *Schinus terebinthifolius* Raddi volatiles on *Arabidopsis thaliana* (L.) Heynh.

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*Schinus* L. (Anacardiaceae) essential oils (EO) show a wide spectrum of ecological interactions such as phytotoxicity (1,2). Initial evaluation of phytotoxic activity involves analysis of allelochemical interference on germination and initial growth of target species, processes that reflect effects on cellular levels. Plants have developed a complex signaling network that senses and protects them from different abiotic and biotic stresses (3). A research project has been started in order to investigate the phytotoxic effects of *S. terebinthifolius* EO on *Arabidopsis thaliana* germination, seedling growth and gene expression. Leaves samples from six individuals were harvested in natural vegetation in Porto Alegre, RS, Brazil. A voucher (registry: 164707) was deposited in the herbarium ICN of the Universidade Federal do Rio Grande do Sul. Sampled leaves were dried at room temperature, fragmented and subjected to hydrodistillation in a Clevenger apparatus for 4 h. Thirty *A. thaliana* Columbia wild type seeds were surface sterilized and sown in Petri dishes containing 3 % sucrose, 0.8 % (w/v) agar and 0.1x MS minerals (4). Seeds were stratified and EO (5, 10, 15, 20 and 25  $\mu$ L) were pipetted on filter paper attached to the inner face of the Petri dish. Control was a treatment without EO application. Parameters examined included germination rate (GR) and speed of accumulated germination (AS). In post-germination assays, EO was applied after emergence of the primary root. Ten seedlings remained exposed to the EO for 7 days. Photographs were taken to measure shoot length (SL) and root length (RT) using ImageJ 1.45s software. For quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis, 5  $\mu$ L of the EO were applied and seedlings remained exposed to volatiles during 24 h. Roots were collected (3 repetitions of 25 mg material), frozen in liquid nitrogen and stored at -80 °C until RNA isolation. Total RNA was prepared using Plant RNA Purification reagent and DNase I treatment. RNA quantification was carried out using a NanoDrop. First-strand cDNA synthesis was performed with reverse transcriptase (M-MLV) using 1  $\mu$ g of total RNA. Gene evaluated was ANP1 *Arabidopsis* NPK1-like protein kinase 1 (ANP1). Comparisons between treatments and control were performed using one way analyses of variance (ANOVA) and a post hoc Student-Newman-Keuls (SNK) or Tukey, using SPSS 17.0 software. Differences were considered significant at  $P \leq 0.05$ . EO inhibited all morphometric measurements. Amounts from 10  $\mu$ L reduced about 50 % the GR of *A. thaliana*. AS was reduced by 65 % and 75 % when 5 and 10-25  $\mu$ L were applied, respectively. Volumes equal or higher than 10  $\mu$ L of EO reduced SL by 60 %. Inhibitory effect on RL demonstrated a dose-dependent effect. EO reduced by 52 % and 81 % *A. thaliana* RT when 10  $\mu$ L and up to 15  $\mu$ L were applied, respectively. RT-qPCR results showed that ANP1 expression were not affected by EO. Results suggest that phytotoxic effects of *S. terebinthifolius* EO, in quantities studied seem to be explained in terms of cellular damage rather than by induction of stress-inducible genes.

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