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Comparative allelopathic effects of *Cryptocarya moschata* and *Ocotea odorifera* aqueous extracts on *Lactuca sativa*

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ABSTRACT. The aim of this work was to compare the allelopathic effects of aqueous leaf extracts from *Cryptocarya moschata* and *Ocotea odorifera* on the seed germination and initial growth of *Lactuca sativa* (lettuce). The extracts were prepared by cold maceration, which was accomplished by adding distilled water to the dried plant material 1:10 (w v^{-1}) and grinding. The treatment concentrations (0, 0.25, 0.5, 0.75 and 1%) were obtained by dissolving the dried crude extract in distilled water. The allelopathic potential of the dried *Ocotea odorifera* and *Cryptocarya moschata* leaves on *Lactuca sativa* was observed through reduction of the percentage of seeds that germinated, the index of germination and the length of the roots as well as structural abnormalities in *L. sativa* seedlings. For both extracts, the extract concentration and allelopathic effect were highly correlated. However, the *C. moschata* extract had no effect on caulicle length at the different tested concentrations.

Keywords: allelopathy, allelochemical, Lauraceae, germination.

Efeito alelopático comparativo de extratos aquosos de *Cryptocarya moschata* com *Ocotea odorifera* sobre *Lactuca sativa*

RESUMO. Neste trabalho foi avaliado o efeito alelopático comparativo do extrato aquoso de folhas de *Cryptocarya moschata* e *Ocotea odorifera* sobre a germinabilidade e desenvolvimento inicial de *Lactuca sativa* (alface). Os extratos foram preparados por maceração a frio, adicionando o material vegetal seco e moído à água destilada 1:10 (m v^{-1}). As concentrações tratamento de 0; 0,25; 0,5; 0,625; 0,75; 0,875 e 1% foram obtidas por meio da solubilização do extrato bruto seco em água destilada. Observou-se potencial alelopático das folhas secas de *Ocotea odorifera* e *Cryptocarya moschata* sobre *Lactuca sativa* pela redução do percentual de germinação, do índice de velocidade de germinação e do comprimento das raízes, bem como pelas anormalidades estruturais encontradas nas plântulas. Observou-se alta correlação entre a concentração de extrato e efeito alelopático com ambos os extratos. O extrato de *C. moschata* não apresentou efeito sobre o comprimento do caule nas diferentes concentrações avaliadas.

Palavras-chave: alelopatia, aleloquímico, Lauraceae, germinação.

Introduction

The term allelopathy refers to the capacity of either superior or inferior plants to release a substance to their immediate environment that modulates the development of other plant species (FRITZ et al., 2007; GATTI et al., 2004; RICE, 1984). Seed germination bioassays in the presence of vegetable extracts provide a starting point to investigate allelopathic effects because seeds constitute biological units and because germination may be initiated through intra- and inter-specific competition processes, seed colonization of new habitats and native vegetable regeneration (CARMO et al., 2007; GRISI et al., 2012; NÓBREGA et al., 2009).

When absorbed by the roots and transported throughout the whole plant, the allelochemical substances may act on cell division; the stretching, permeability and ultra-structure of cells; membrane permeability; hormone-driven mechanisms of growth induction; protein synthesis; and lipid and organic acid metabolism. Physiological phenomena such as the opening and closing of the stomata, photosynthesis and respiration may also be affected by allelopathic compounds. These compounds, which can be found in the soil, might also interfere in nutrient absorption (FERREIRA; ÁQUILA, 2000; GOLDFARB et al., 2009).

Currently, due to the well-established and advanced understanding of the chemistry of natural compounds, many natural compounds can now be identified and classified as allelopathic substances. Allelopathic compounds can be found across several classes of natural products, including phenolic acids, coumarins, terpenoids, flavonoids, alkaloids, cyanogenic glycosides, saponins and tannins. These compounds, once hydrosoluble, are responsible for the direct and indirect allelopathic effects that can be observed under natural conditions (AMBIKA, 2002; LARCHER, 2000). Allelopathic compounds have been investigated worldwide using aqueous or alcoholic extracts of both cultivated and medicinal plants; therefore, identification becomes essential in assisting the creation of genotypes that are more resistant to a variety of bacteria, fungi and insects (FERREIRA; ÁQUILA, 2000; MEDEIROS, 1990).

Cryptocarya moschata Nees & Mart. ex Nees is an arboreal plant endemic to Atlantic Forest, but it is also found in the Pernambuco and Rio Grande do Sul states. This plant is popularly known as 'canela-fogo', 'canela-de-porco', 'canela-pimenta', 'canela-amarela' and 'canela-branca'. It is commonly used in urban and rural afforestation, and its fruits are consumed by several animal species, which makes it ideal for use in restoring degraded areas and promoting the sustainability of such ecosystems (LORENZI, 2000; NEHME et al., 2002).

Canela sassafrás (*Ocotea odorifera* (Vell.) Rowher) is an arboreal species native to Brazil; it is known for containing high concentrations of safrole, an essential oil. This oil is used as a substitute for North American sassafrás oil, which can be extracted from *Sassafras albidum* as well as from the pau-rosa oil of the Amazonian plant *Aniba roseodora*. Safrole is often used in sudorific, anti-rheumatic, anti-syphilitic and diuretic medicinal preparations as well as in insect repellents and perfume fixatives (LORENZI; MATOS, 2002). The various compounds that are present in this species may harbor interesting chemical properties; for example, the plant may possess phenolic and terpenic substances with allelopathic properties. The allelopathic effect of *Ocotea odorifera* was evaluated by Carmo et al. (2007) and Gatti et al. (2004), who showed the allelopathic potential of this species on the germination and rooting of *Sorghum bicolor* (sorghum), *Raphanus sativus* (radish) and *Lactuca sativa* (lettuce).

In contrast to the extensive literature on medicinal plants, little information is available regarding the characterization of native allelopathic arboreal species (whether or not they are under the threat of extinction) for potential utilization in agroforestry systems (SOUZA-FILHO et al., 2005). For this reason and taking into account the high rate of vegetable extinction, investments in research focusing on the understanding of diverse plant-plant chemical interactions may be easily justified and become urgent (LARCHER, 2000).

Investigating the allelopathic behavior effects of native species on germinating seeds is justified because seeds constitute biological units through which ecological processes, such as intra- and inter-specific competition for natural resources, new niche invasion by non-native species, colonization of new habitats and regeneration of native vegetation, may occur. The synthesis and release of secondary metabolites capable of negatively affecting the germination of seeds around the producer is a competitive and robust inference strategy that confers advantages to the species found in natural environments; however, this strategy has gone underexplored in the organization of agroforestry and cultures (CARMO et al., 2007).

Utilizing plants with proven allelopathic potential may help reduce the environmental impact caused by the uncontrolled use of pesticides and hence favor the well-balanced development of crops with a positive reflex to their productivity and longevity. Taking into account the lack of work on the allelopathic properties of *C. moschata*, this work is aimed at comparing the allelopathic effects of *Cryptocarya moschata* and *Ocotea odorifera* aqueous leaf extracts on the germination and initial development of *Lactuca sativa* (lettuce).

Material and methods

Plant material

Fresh leaf samples of *Cryptocarya moschata* and *Ocotea odorifera* were collected in Marcelino Ramos (27º28'9" S and 51º54'5" W) and Erechim (27°37'47" S and 52°16'12" W), Rio Grande do Sul State, Brazil. After collecting and identifying the leaves, the tissues were dehydrated to a constant weight in an oven with forced air at 35 ± 1°C and then submitted to knife milling. Vegetable extracts were prepared by cold maceration over 48 h, using a 1:10 (wt v^{-1}) ratio of raw material to distilled water. Afterward, the extract was filtered and oven-dried with forced air at 25°C for 72h. The crude extract was then diluted in water at treatment proportions of the following percentages: 0, 0.1, 0.25, 0.5, 0.625, 0.75, 0.875 and 1%. The solution pH for the different extract solutions was determined with a potentiometer calibrated at pH 4 and 7.

Bioassays

The bioassays were set up in Petri dishes with a layer of filter paper wetted with 5 mL of the treatment extracts at a pre-established concentration.

For the blank tests, filter papers were wetted with distilled water at the same proportion as the treatment experiments and placed in Petri dishes; these controls were considered to have a concentration of zero. All treatment experiments were replicated 4 times at each concentration and applied to 50 commercially available lettuce seeds. All Petri dishes were randomly distributed inside the germination chamber at 25 ± 1°C with a photoperiod of light for 8 hours followed by dark for 16 hours. The assays were run for seven days; the germination percentage (G%), germination speed index (GSI) and the values from the first counting test were determined.

Counting was performed at the end of each 24h interval, considering the germinated seeds to be those with broken integument and germination of the root apex. The first germination counting was performed on the basis of the results obtained on the fourth bioassay day (BRASIL, 2009). To evaluate the initial seedling development, as measured by the root and stalk length (mm), seeds were previously germinated with water and then transferred to a different concentration to be tested under identical experimental conditions. The initial development was assessed through the stalk and root measurements of 60 randomly chosen lettuce seedlings at each concentration.

The germination values (G%) were obtained using the following expression: G (%) = (N/A) x 100, where N denotes the total number of germinated seeds and A is the total number of seeds put to germination (IGANCI et al., 2006) on the seventh bioassay day (BRASIL, 2009). The germination speed index (GSI) was calculated using the following expression: $GSI = G_1/N_1 + G_2/N_2 \ldots G_n/N_n$, where G_1, G_2 and G_n represent the number of normal germinated seeds up to the sixth day and N_1, N_2 and N_n denote the number of days used to evaluate germinations G_1, G_2 and G_n (MACULAN et al., 2007). The data were submitted to a variance analysis (Student *t* test) and regression analysis, all to a 5% significance level ($p < 0,05$).

Results and discussion

The pH control of the extracts is of fundamental importance in the allelopathic tests when the extract composition is unknown (ÁQUILA, 2000). In the present case, the extract concentrations tested did not present low acidity; instead, the pH values varied within the range of 6.45 to 7.6, which favors biochemical processes and plant nutrition. These pH values are suitable for germination and initial plant development (BRASIL, 2009; FERREIRA; ÁQUILA, 2000; LARCHER, 2000). Similar pH values were found by Maraschin-Silva and Aquila (2006a and b) when testing the extracts of *Erythroxylum argentinum*, *Luehea divaricata*, *Myrsine guianensis* and *Ocotea puberula*. Furthermore, similar pH values were also obtained by Periotto et al. (2004) for the extracts of *Andira humulis* and by Borella and Pastorini (2009) for aqueous extracts of *Phytolacca dioica* over *Lycopersicum esculentum* (tomato) and *Bidens pilosa* (picão-preto) seeds.

Lettuce germination was significantly affected by the aqueous extracts of *C. moschata* and *O. odorifera*. In relation to the control, the *O. odorifera* extract caused a significant negative effect even at the lowest concentration tested (0.1%); the *C. moschata* extract caused a significant negative effect at a concentration of 0.25% (Figure 1).

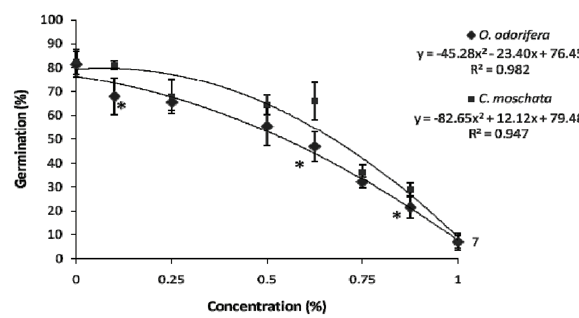

Figure 1. Average values and standard deviation of germination seedling of *L. sativa* submitted to *O. odorifera* e *C. moschata* extracts. *significant difference by t student test ($p < 0.05$).

A correlation between the extract concentration and the germination percentage for both species, *O. odorifera* (0.98) and *C. moschata* (0.94), was observed. *O. odorifera* tends to exhibit a greater allelopathic effect on the germination of lettuce seeds in relation to *C. moschata*; significant differences were verified for dosages of 0.1, 0.625 and 0.875%. Using an aqueous extract (leaves, roots and barks from stalk) of *O. odorifera*, Carmo et al. (2007) have also observed a significant reduction in the germination of sorghum seeds.

In Figure 2, the extract effect on the germination speed index (GSI) of lettuce seeds can be clearly observed. In fact, a linear correlation shows that as the extract concentration increases, the GSI decreases. The extract of *C. moschata* presented a significant effect at lower concentrations (0.1%) in relation to the *O. odorifera* extract, which exhibited effects at higher concentrations (0.25%).

Maraschin-Silva and Aquila (2006a) observed the effects of a variety of aqueous extracts from different native species (*Erythroxylum argentinum*, *Luehea divaricata*, *Myrsine guianensis* and *Ocotea puberul*) on the GSI of lettuce seeds. Oliveira et al. (2009) noted a reduction in GSI (100, 98.75 and 82.5%) of lettuce seeds with increasing concentration (50, 75, 100%, respectively) of fruit extracts from *Ziziphus joazeiro*. Borella and Pastorini (2009) have also noted a reduction in the GSI for tomato and *Bidens pilosa* seeds due to the action of aqueous extracts from *Phytolacca dioica* leaves and that such reductions were intensified with increasing extract concentrations.

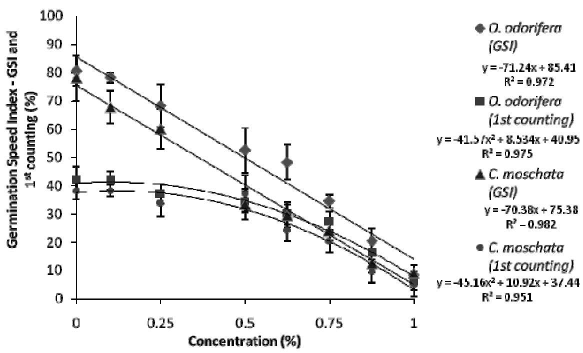

Figure 2. Average values and standard deviation of germination speed index (GSI) and 1st counting of seeds of *L. sativa* submitted to *O. odorifera* and *C. moschata* extracts.

According to Ferreira and Borghetti (2004), frequently, the allelopathic effect may not occur on the germinability, but instead, it may affect the germination velocity or other process parameters. The germinability is a frequently used index, although it does not reveal other aspects of the germination process (e.g., delays) because it considers only the final results. Hence, this index does not take into account inactive germination periods during the bioassay (CHIAPUSO et al., 1997).

In fact, allelochemical compounds may be selective regarding their action on the plant, and additionally, the plant may be selective with respect to its output, making the physiological understanding of such compounds difficult (GATTI et al., 2004). Some researchers suggest the occurrence of a more remarkable effect on the roots in relation to the shoot, due to the greater contact with allelochemical solutions (CHUNG et al., 2001). Generally, the effects caused tend to be dependent on the concentration of the allelochemical substances (i.e., higher concentrations usually lead to greater effects, as observed in the growth bioassays). In the present work, data from the first counting (Figure 2) indicate that both germination and the germination rate decreased with increasing extract concentration.

Figure 3 presents the results of initial root development (mm) of *L. sativa* after subjection to *O. odorifera* and *C. moschata* extracts, where a linear reduction in the root length (17.09 ± 0.52 and 16.14 ± 1.17 mm to 2.49 ± 0.18 and 2.88 ± 0.38, for *O. odorifera* and *C. moschata*, respectively) can be observed as a function of increasing extract concentration. Significant differences ($p < 0.05$) were observed between the extracts at concentrations of 0.5 to 0.875%.

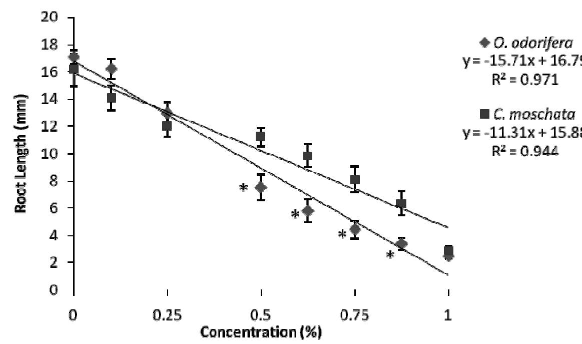

Figure 3. Average values and standard deviation of root length (mm) in the initial development of *L. sativa* submitted to *O. odorifera* and *C. moschata* extracts.
*significant difference by t student test ($p < 0.05$).

For most of the concentrations tested, a significant inhibitory effect of the *O. odorifera* extract was observed when compared with the of *C. moschata* extract. Qualitatively, the observed atrophy of the radicle and oxidation of root cap was noted by tissue darkening.

Ferreira and Áquila (2000) noted that germination is less sensitive than seedling growth to allelochemical substances because these substances may induce the appearance of atypical seedlings, with necrosis of the radicle being one of the most common effects. The presence of abnormalities in the roots and seedlings seem to occur more frequently because these organs are more susceptible to allelopathic action than the aerial parts of the plant (GATTI et al., 2008; PIRES; OLIVEIRA, 2001).

In this work, the stalk length of *L. sativa* was significantly affected by the *O. odorifera* extract at a concentration of 0.25%, while the extract of *C. moschata* did not present any effect on the stalk growth, as shown in Figure 4. Nevertheless, alterations in seedling growth, mainly on the dimension of leaves, were observed in both extracts.

Borella and Pastorini (2009) tested the effect of aqueous extracts from *Phytolacca dioica* leaves on the germination of tomato and *Bidens pilosa*. Notably, the concentrations that presented the greatest interference in germination standards were 4 and 8%, where the latter concentration completely inhibited seedling germination. In the present case, lower germination rates were observed at low aqueous extract concentrations from both species, indicating that *B. pilosa* and tomato presents high allelopathic affects when compared to other species.

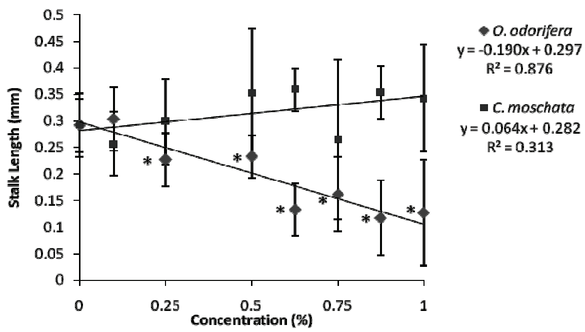

Figure 4. Average values and standard deviation of stalk length (mm) in the initial development of *L. sativa* submitted to *O. odorifera* and *C. moschata* extracts.
*significant differences by t student test ($p < 0.05$).

According to Einhellig (1995), allelopathic effects result from the joint action of several substances because allelochemical compounds are usually found at a very low concentration in the natural environmental. It should also be considered that the conditions used to study the effects of allelochemical substances *in vitro* may differ from actual soil conditions. Obviously, the methods employed to demonstrate the allelopathic effects of certain extracts cannot conclude more than the existence of allelopathic substances in the vegetable material. Consequently, it is not possible to infer or even extend such findings to field conditions because of the simultaneous occurrence of the biotic and abiotic factors that may influence this phenomenon.

Conclusion

The results presented in this work clearly indicate the existence of allelopathic properties in the dried leaves of *Cryptocarya moschata* and *Ocotea odorifera* over *Lactuca sativa*. The effect was verified by a reduction in the percentage of seed germination, the germination speed index and the root length as well as through the structural abnormalities found in the seedlings. A high correlation between the extract concentration and the allelopathic effect was observed for both extracts. Nevertheless, the extract of *C. moschata* presented a low allelopathic effect in relation to the stalk length.

Acknowledgements

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