

Full Length Research Paper

Allelopathic effects of aqueous extract of leaves and roots of *Luetzelburgia auriculata* (Allemão) Ducke on seeds germination and initial growth of lettuce (*Lactuca sativa* L.)

PINTO Érika do Nascimento Fernandes¹, SOUTO Jacob Silva¹, PEREIRA Leonardo Francisco de Assis¹, BORGES César Henrique Alves¹ and FERNANDES Tássia Rayane Nascimento²

¹Faculdade de Ciências Florestais, Departamento de Engenharia científica da Universidade Federal de Campina Grande, Brazil.

²Federal University of Rio Grande do Norte Technology Center, Brazil.

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The secondary metabolism of plants produces allelopathic substances which are able to interfere with germination and growth of other species, when released in the environment. In order to evaluate the allelopathic effects of *Luetzelburgia auriculata* on the germination and early growth of lettuce (*Lactuca sativa*), leaf extracts and roots were tested in lettuce seeds sowing. The *L. auriculata* plant material was collected from adults, subsequently washed, dried, weighed, crushed and thus used to preparing a raw extract. In a completely randomized design, six concentrations (0, 20, 40, 60, 80 and 100%) of the extracts of leaves and roots were tested separately with four replications on lettuce seeds in germitest paper. The experiment was conducted in a germination chamber TE 402 during seven days with photoperiod of 12 h light and a constant temperature of 20°C, to check the germination speed index (GSI), germination percentage (% G) and initial growth lettuce (radicle length and hypocotyl). The data were submitted to a variance analysis applying the F test at 1% probability and using the error bars model. It was observed that the results of the treatments with root extract and leaf extract, when compared to the control test, were negatively affected by the aqueous extracts of *L. auriculata*. The GSI and %G of seeds treated with roots extract were the parameters that presented most significant responses to the allelopathic effects of *L. auriculata* extract at 20% concentration, followed by GSI and %G seeds subjected to 20% leaf extract. Both extracts at other concentrations reduced GSI and %G in 85 and 90%, respectively. The length of the radicle and hypocotyl decreased by 32 and 15% respectively when the extract was used at a lower concentration, and 40% (radicle) and 30% (hypocotyl) in other concentrations. The aqueous extracts of leaves and roots *L. auriculata* caused negative allelopathic interference on the germination and growth of lettuce seedlings.

Key words: Secondary metabolites, Brazilian semiarid region, allelopathy.

INTRODUCTION

In nature living organisms interact naturally. This interaction, called interference, is responsible for the

occurrence of many phenomena that can happen between plants and microorganisms. The competition, indirect interference and allelopathy are examples of such phenomena, which can be generated in individuals of an ecosystem, have been largely investigated from the organisms behavior (donors and recipients) presents in the same environment perspective. It is important to remember that these interactions can have positive, negative or no effects.

The allelopathy phenomenon occurs, according Rice (1984), when an organism releases chemicals into the environment and these substances interact inhibiting or stimulating the growth and/or development of another organism present in the same environment. Allelopathic substances, phytotoxins, allelochemicals or secondary products are the names given to chemicals released by organisms in the environment that affect other community components (Oliveira et al., 2011), such chemicals are released from the plant tissue through volatilization, leaching, root exudate and the decomposition of plant residues and their allelopathic effects can be studied experimentally, by biological tests of plant extracts containing allelochemicals.

There are many species that presents allelopathic features; the effects on other species of these individuals have been studied by applying plant extracts donor (allelopathic) in seeds or seedlings of other plants (receptor). The techniques for preparing such extracts are varied, generally, the extract is obtained firstly from the milling of plant parts (roots, leaves, flowers or fruits), and secondly placed in contact with water or organic extractors (alcohol or ether, for example), and finally filtered and tested on test plants like tomato, lettuce and radish (most sensitive of all species used in such studies) (Medeiros, 1989).

Moreover, *Luetzelburgia auriculata* has drawn attention by the way it occurs in the landscape, usually in coppices, suggesting rusticity, developing in dry, rocky places, maintaining green foliage even in much of the dry season. Considering these features, it has aroused interest in the study of possible allelopathic effects that may be caused by these individuals.

As stated earlier, the use of biological tests is fundamental to the increase of the knowledge of allelopathic effects. According to Chou (2014), the study of allelopathic interactions is useful in the search for natural phytotoxins produced by plants or microorganisms, and its synthetic derivatives that can be used as natural herbicide to have more specific and less harmful to the environment compounds. Therefore, this study aimed to investigate the allelopathic effects of aqueous extracts of leaves and *L. auriculata* roots

(Allemão) Ducke on germination and growth of lettuce seedlings (*Lactuca sativa* L.).

MATERIALS AND METHODS

Leaves and roots were collected manually and randomly of adult individuals of a population of *L. auriculata*, located in Cachoeira Farm in São Porfírio, Várzea-PB city. The plant material was packaged separately in plastic bags and sent to the Plant Mineral Nutrition Laboratory at the Center for Health and Rural Technology Federal University of Campina Grande, Campus of Patos, where two different experiments (leaves and roots) were installed.

In order to obtain the aqueous extracts, leaves and roots separately, were weighed, washed, rinsed with distilled water, minced in broken (fresh still) in a household mixer for 5 and 10 min, respectively, in a proportion of 200 g of plant material to 800 ml of distilled water, and finally allowed to rest for a period of 30 min. Later, the extract was filtered through a sieve with 2.0 mm mesh to give crude extracts (concentration 100%).

Soon after, 25 lettuce seeds were equidistant distributed on two sheets of *germitest*, 25 x 30 cm size overlapped; a third sheet was also placed over the seeds distributed and 250 ml of the extract already diluted was applied to each of the experimental units, totaling a 1000 ml per treatment. Once the application of the extract is finished, paper rolls were confectioned maintaining the seeds, conditioned inside plastic bags (capacity 1.0 kg), and placed in a germination chamber TE 402 for 7 days with a photoperiod adjusted to 12 h light and constant temperature of 20°C.

Each experiment was installed in a completely randomized design with six treatments (concentrations), four replications and twenty-four experimental units; treatment (control) was prepared only with distilled water. Evaluations were made daily, looking at seed germination submitted to treatments with the aqueous extracts of the leaves and roots, separately, adopting as a criterion, the germination radicle protrusion (2.0 mm).

Once the data were collected, the germination speed index (GSI), germination percentage (% G) and checked the radicle length and hypocotyl in seedlings emerged in both experiments were determined. The collected data were transformed into $\sqrt{x + 1}$ and subjected to analysis of variance, applying the F test at the 1% probability level. For the complete presentation of the answers, the error bars of the averages were included.

RESULTS AND DISCUSSION

Aqueous extract of leaves *L. auriculata*

In the analysis of the results, regarding the GSI, %G, radicle length and hypocotyl of lettuce seeds submitted the leaf aqueous extract of *L. auriculata*, it has been found from the determination coefficient value that the most appropriate model to explain the relation between ratio concentrations of the extracts and the parameters analyzed was the regression model until the second order. For the germination speed index, it is possible to checks that the increase in concentrations of aqueous

*Corresponding author. E-mail: erikafdes@hotmail.com.

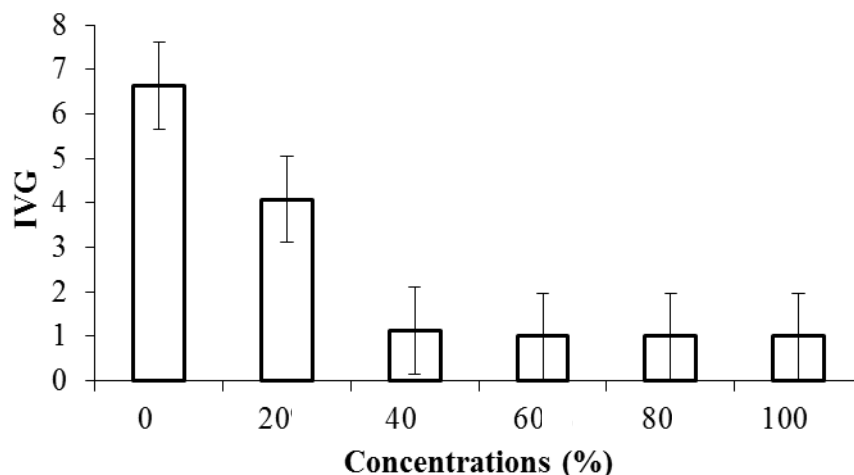


Figure 1. Speed index of lettuce seeds germination under aqueous extract treatment of *L. auriculata* in different concentrations.

extract of *L. auriculata* leaves promoted reduction of this parameter in relation to the attestant test in the order of 43, 72, 88, 95 and 81% of the concentrations of 20, 40, 60, 80 and 100%, respectively (Figure 1). The result suggests that the extract of *L. auriculata* exerted negative effect allelopathic on this parameter. Pelegrini and Cruz-Silva (2012) when using aqueous extracts of fresh leaves of *Coleus barbatus* (A.) Benth on lettuce seeds, observed that the GSI was reduced as the extract concentration increased, occurring what authors call dose dependent response. Borges et al. (2007) also identified this negative relationship between GSI lettuce seed and plants extract. For Aoki et al. (1997), the intensity of allelopathic effects is dependent on the concentration of allelopathic substances present in a species.

Souza and Furtado (2002) observed negative allelochemical effect of rye extracts on lettuce seed GSI as a result of the tested extract concentrations. In a study of aqueous extracts of umbu (*Phytolacca dioica* L.) on lettuce seeds, Borella and Pastorini (2010) observed that the germination rate index also decreased when the seeds were submitted to extracts with concentrations starting from 4%.

Teixeira et al. (2004) observed that there was a 90% reduction in lettuce seed GSI submitted to the aqueous extract of *Crotalaria juncea* and found out that often the extract influences more the speed of germination than any other parameter.

Aquila (2000), using *Ilex paraguariensis* extracts in lettuce seeds also found a loss of seed vigor, so the author says this is an economically important parameter to be evaluated. According to Rodrigues (2012), a late germination may mean losses for the farmer, especially in the case of species with short life cycle.

It is expressed in Figure 2, the percentage of germination of lettuce seeds submitted to different

concentrations of aqueous extracts of *L. auriculata* leaves. It can be perceived from the data that a negative effect of the extracts related to the different concentrations did happened. In the treatment where the concentration of the extract was 20%, seed germination was reduced by 25%; the extract where the concentration was 40% had a reduction of 85%, and to the other concentrations, the decrease was also high (approximately 90%).

Conducting laboratory tests, Al-Sherif et al. (2013) have identified that the germination of weed seeds was reduced even when used at lower concentrations (1%) of aqueous allelopathic extract, and totally inhibited when used at higher concentrations (4%). França et al. (2008) found out that neem extracts (*Azadirachta indica*) also was responsible for reducing the percentage of lettuce seed germination and beggartick.

Pelegrini and Cruz-Silva (2012) observed that the use of aqueous extracts of fresh leaves of *Coleus barbatus* (A.) Benth on lettuce seeds significantly inhibited the germination percentage, which presented a considerable difference from the control of this study. Corsato et al. (2010) observed that there was a significant reduction in the percentage of germination of conventional soybeans when applied the highest concentrations of aqueous extract of sunflower (80 and 100%).

In a study on the allelopathic effects on weeds *Annona Crassiflora*, Inoue et al. (2010) reported that extracts of leaves from the donor species reduced by more than 50% germination of *Brachiaria* seeds. According to Souza Filho and Duarte (2007), the biological activity of an allelochemical depends directly on the response limit of the receiving species, because it is closely related to its sensitivity. In an experiment conducted by Corsato et al. (2010), it was observed that there was a reduction in germination of beggartick seeds, caused by fresh

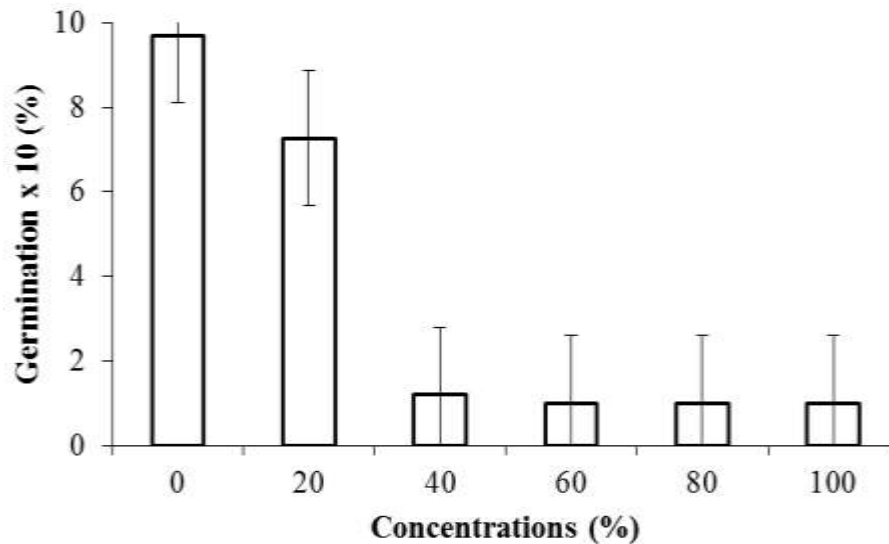


Figure 2. Lettuce germination under different concentrations of aqueous *L. auriculata* extract.

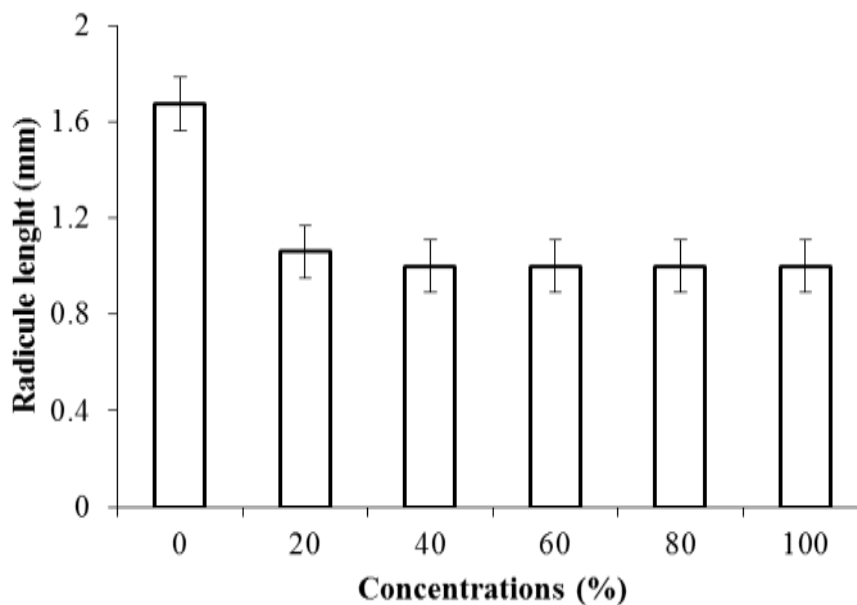


Figure 3. Lettuce radicle length under different concentrations of aqueous *L. auriculata* leaves extract.

leaves of sunflower (*Helianthus annuus* L.), species considered as allelopathic.

The results obtained in relation to the length of radicle seedling grown under different concentrations of aqueous extracts of *L. auriculata* leaves' is as shown in Figure 3; it displays that the seedlings emerged in the extract with a concentration of 20%, have developing 32% less than the control. Moreover, other treatments have a reduction of radicle length by almost 40%, compared to the control.

Thus, results indicate that there was a negative allelopathic effect of the donor species on the development of seedlings. This study corroborates with the results of Tur et al. (2010), which while testing aqueous extracts of fresh leaves of *Duranta repens* on the germination and early growth of lettuce, found that there was significant reduction in root length of this vegetable seedlings. Lima and Moraes (2008) also observed a significant reduction in the growth of radicle,

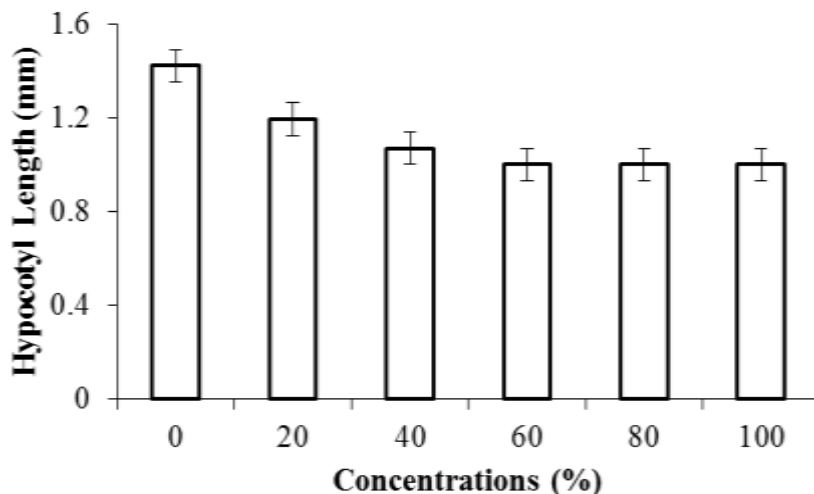


Figure 4. Lettuce hypocotyl length under different aqueous extract concentrations of *L. auriculata* leaves.

while studying the effect of aqueous extract of *Ipomoea fistulosa* on the germination and early growth of lettuce. Peres et al. (2004) observed that the extract obtained from *Adiantopsis radiata* leaves' and *Adiantum tetraphyllum* significantly inhibited the growth of lettuce radicle at all concentrations used (250, 500, and 1000 mg.L⁻¹). In these cases, also, it was noticed that the increase in the concentration resulted in higher inhibition and morphological abnormalities apex oxidation radicle and the absence of absorbents structures.

Aumonde et al. (2012), by using extract of *Zantedeschia aethiopica* leaves in lettuce seeds obtained similar results, as there was a negative interference of the extract on the radicle and a more evident effect, as the concentration increased. According to Chung et al. (2001), the effect of the extract on the radicle can be attributed to increased sensitivity of the organ and by direct contact of the extract with the tissue.

Utilizing error bars (Figure 4), the hypocotyl length of lettuce seedlings was analyzed and subjected to different concentrations of *L. auriculata* leaves aqueous extract. A reduction of 15% the hypocotyl length of seedlings emerged in the extract concentration with 20 and 30% in seedlings of other treatments (concentrations 40, 60, 80, 100%) was found as the main result.

In a similar study, Maraschin-Silva and Aquila (2006) tested plant extracts of native species on the initial growth of lettuce and found that *Psychotria leiocarpa* provided a negative allelopathic effect on hypocotyl size of lettuce seedlings treated with the extract. Formagio et al. (2012) evaluated the allelopathic effect of *Tropaeolum majus* leaves extract on the initial growth of beggarticks and observed that the hypocotyl length of seedlings was also negatively affected under the treatment when compared to the control treatment. However, negative

effects suffered by radicle were more significant than those suffered by the hypocotyl.

Evaluating the allelopathic potential of aqueous extract of oat leaves, Hagemann et al. (2010) observed that *Avena sativa* and *Avena strigosa* caused a reduction in the growth of radicle and hypocotyl on *Lolium multiflorum* and *Euphorbia heterophylla*, respectively.

Oliveira et al. (2012) found that the allelopathic effects of Mulungu aqueous extract also affected the development of lettuce seedlings. While applying leaf extract of *Annona crassiflora* on weed seeds, Inoue et al. (2010) detected that there was a significant inhibition in the development of hypocotyl compared to the control, however, this inhibition was less intense than that affecting the radicle.

Garsaball and Natera (2013) stated that the allelopathic effects of aqueous extracts of *Tithonia diversifolia* leaves negatively altered the overall growth of lettuce seedlings, especially the growth of root and hypocotyl.

It is believed that the allelopathic effects of aqueous extracts of *L. auriculata* leaves on lettuce germination may have occurred due to the presence of flavonoids. This substances that in preliminary studies (phytochemical) conducted at the Federal University of Rio Grande do Norte (data not published), were detected in extracts from leaves and roots of *L. auriculata*. According to Alves et al. (2004), flavonoids are bioactive metabolites derived from the secondary metabolism. Chaves et al. (2001) stated that these substances, showing phytotoxic activity, are present in various structures of *Cistus ladanifer* plants and also found in the soil.

Aqueous extract of roots *L. auriculata*

It was found from the value of the coefficient of

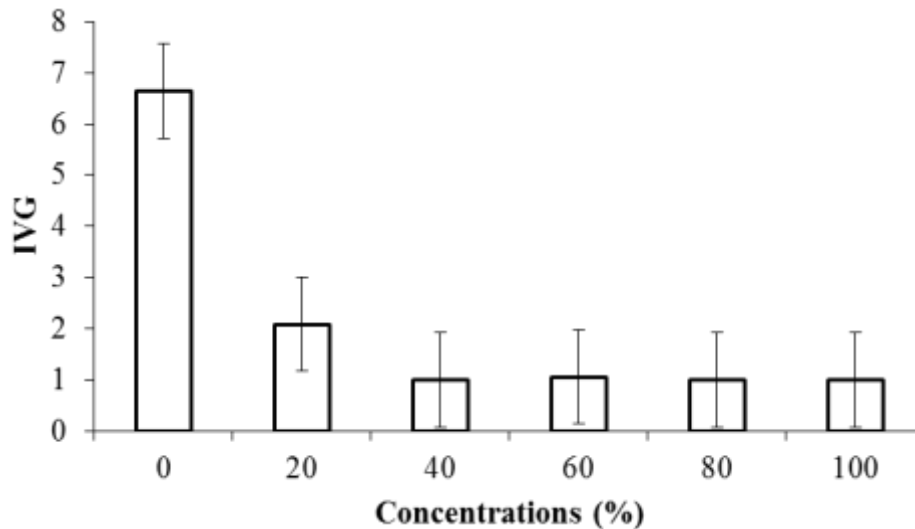


Figure 5. Lettuce germination speed index (GSI) under aqueous *L. auriculata* extract under different concentrations.

determination of each of the parameters considered (GSI, % G, length of the radicle and hypocotyl), that the relationship between the concentrations of the root aqueous extract of *L. auriculata* on lettuce seeds and such parameters had quadratic regression as the most suitable model to explain the relationship.

Lettuce seeds imbibed with the aqueous extract of *L. auriculata* roots 20% had GSI almost 70% lower when compared with the control (without application of aqueous extract), since, increase in extract concentrations made GSI lettuce seeds' decrease, reaching a reduction of 85% to 40-100% concentrations. Thus, these data suggest that the aqueous extract of *L. auriculata* roots exerted negative allelopathic effects on lettuce germination. Gatti et al. (2004) also found similar results (Figure 5), while testing aqueous extract of marcela (*Aristolochia esperanzae* O. Kuntze) on the germination and growth of lettuce. This negative relationship between GSI and concentration of the plant extract is also reported by Borges et al. (2007).

The aqueous root extracts of *L. auriculata* also influenced negatively vigor of lettuce seeds. According to Ferreira and Borghetti (2004), the greater the GSI, the greater the force of seeds. In tests with extracts allelopathic species (40 to 100% concentration), seeds had the GSI six times lower than the control treatment. Similar data were found in Carvalho et al. (2014), where the use of aqueous extracts of six allelopathic species acted decreasing vigor of lettuce seeds.

According to Tur et al. (2010), GSI has shown sensitivity to the allelopathic effects to be an important parameter to be evaluated. Rodrigues (2012) believes that the delay or reduction in the time taken for germination can be reversed as profits or losses in the field, especially when it comes to species that have short

life cycle.

It is noticed that there were differences between the extracts and the control treatment, even when the extract was used at lower concentrations of 20%. In a study by Sartor et al. (2009) on allelopathic effect of Loblolly pine extracts on germination and development of *Avena strigosa* seedlings, the authors also observed variations in the receiving species development patterns, being the extract effect perceived in lower concentrations and enhanced as concentration was increased.

It is possible to check on Figure 6, the results of the lettuce seeds germination rate, when submitted to different concentrations of *L. auriculata* roots extract, where a decrease in the rate was found.

The aqueous extract of *L. auriculata* roots, in relation to the control treatment, reduced germination by approximately 60% when used at a concentration of 20%; and at a concentration of 40 to 100%, the germination was reduced to 90%. According to Rodrigues (2012), when the control treatment is above 90%, the result is consistent with the germination recommended by seed producers. According to Corsato et al. (2010), there is a significant reduction in the percentage of germination of the conventional soybean seed when the highest concentrations of the aqueous extract are applied (80 to 100%).

Formagio et al. (2012), in a similar study, found that the seeds of beggartick treated under root extract of *Tropaeolum majus* L., reduced their germination in 32%. In tests with extracts of *Hovenia dulcis* Thunb roots. on *Parapiptadenia rigida* (Benth.) Brena seeds, Araldi (2011), noticed that the viability of the seeds was changed.

For the radicle length of the emerged plants under different concentrations of aqueous extracts of *L.*

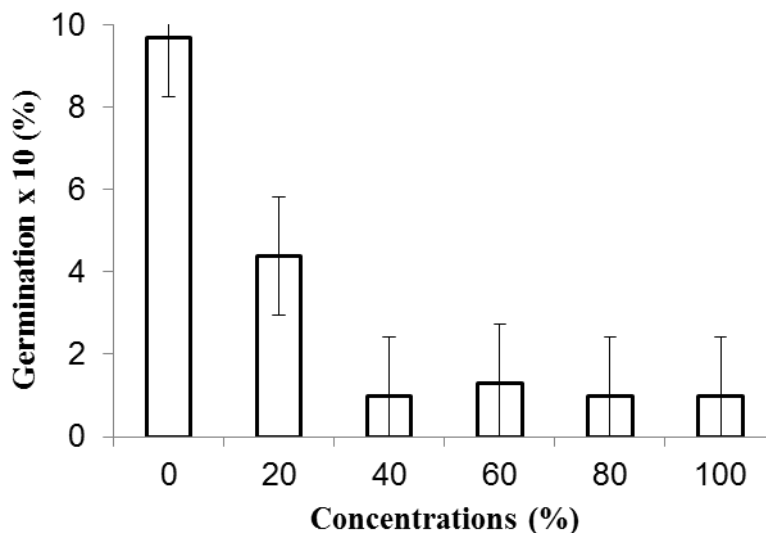


Figure 6. Lettuce germination under different concentrations of aqueous *L. auriculata* roots extract.

auriculata roots, it was observed that, compared to the control, there was a 31% reduction in the length of sprouts from seeds treated with the extract at 20% and almost 40% in those which emerged from other treatments, which suggests that there was an adverse allelopathic effect from the aqueous extract (Figure 7).

The radicle of the emerged plants in the concentrations used was the most affected structure by the aqueous extract of *L. auriculata* root, because 100% showed reduced size, deformation and/or necrosis. Grisi et al. (2011), in a study conducted on the allelopathic effect of *Sapindus saponaria* morphology, found that the reduction in size and necrosis were the most common symptoms in the roots of the seedlings treated with the donor plant extract. In the same study, the authors found that lettuce seedlings showed reduced growth of the primary root, reaching zero values when subjected to the concentration of 7.5%.

Evaluating the initial growth of lettuce under the effect of aqueous extracts of five species of Gleicheniaceae, there was a significant reduction in the length of sprouts of this vegetable. Even plants which have grown, suffered from a toxic effect on its structures growth showing a similar damage found detergents effects in other plants, characterized by reduced size and necrotic aspect of structures (Soares and Vieira, 2000). To Maraschin-Silva and Aquila (2006), many phytotoxins are able to affect the morphology and anatomy of seedlings, which can be evidenced by hardening and darkening of the root apex, fragility and increased branching.

Similar data for the present study were found by Grisi et al. (2013), while verifying that the negative allelopathic effect of aqueous *S. saponaria* roots extract on barnyardgrass and rope-glory was significant on the

growth of seedlings of recipients species, particularly, evidenced by the decrease in the length of the radicle. Rosado et al (2009) observed that there was a significant reduction in root growth of lettuce, compared to the control treatment, when submitted to the aqueous extract basil extract.

The hypocotyl length of lettuce seedlings also responded negatively to the allelopathic effect of aqueous *L. auriculata* roots extract; however, showing less intensity compared to results found for the length of the radicle. In the control test, there was a reduction of 15% in structures growth when applied the extract with a concentration of 20%, and almost 30% when applied to the concentration of 40, 60, 80 and 100% of the aqueous extract (Figure 8), respectively. According to Silva and Aquila (2006), in tests with extracts of native species of *Erythroxylum argentinum*, *Divaricata luehea*, *Myrsine guianensis* and *Ocotea puberula* on the initial growth of lettuce, all caused reduction in the size of the hypocotyl-root axis with the hypocotyl presenting a low inhibition.

Silveira et al. (2014) studied the allelopathic effects of aqueous extract of *Araucaria angustifolia* on lettuce initial growth and found that the final growth of seedlings was affected, as the extract concentration increased; however, the length of hypocotyl showed no significant difference for different concentrations of the extract. The allelopathic effect of aqueous extract of *S. saponaria* root on barnyard grass seeds and rope-glory, significantly, reduced the hypocotyl of seedlings of these weed species (Grisi et al., 2013).

In tests with five *Erythroxylum* species extracts on tomato and onion seedlings growth, Taveira et al. (2013) observed that there was a significant reduction in hypocotyl development of the receiving plants. In the

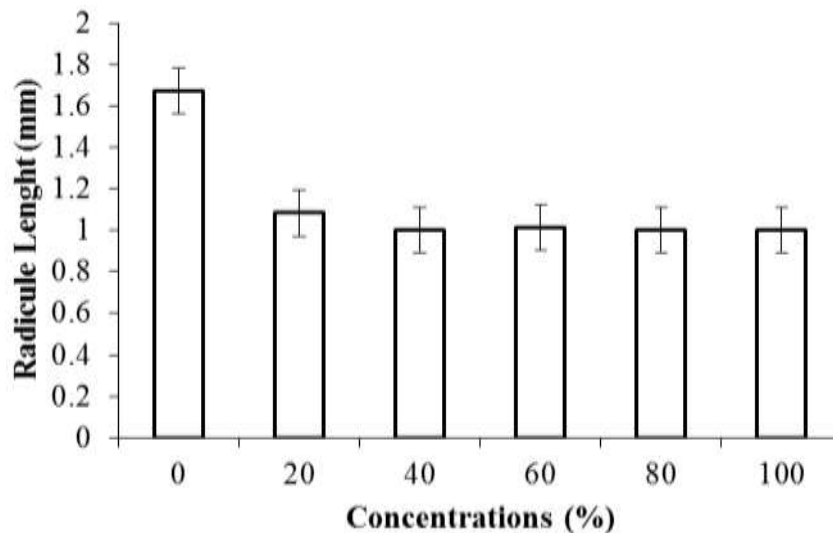


Figure 7. Lettuce radicle seedlings length under different concentrations of aqueous *L. auriculata* extract.

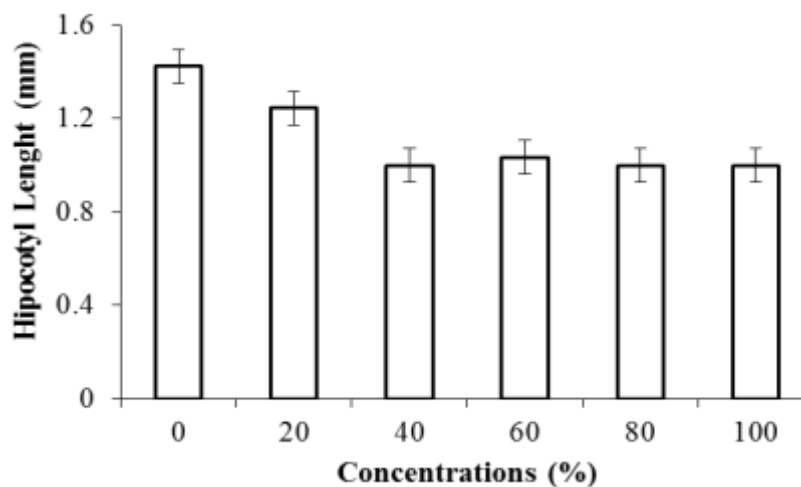


Figure 8. Lettuce hypocotyl length under different aqueous *L. auriculata* extract.

evaluation of allelopathic effects on the aqueous *S. saponaria* extract in onion seedlings development, it was found that the length of hypocotyl also was reduced (Grisi et al., 2011).

Conclusion

The aqueous *L. auriculata* leaves extract caused negative allelopathic interference on the germination and growth of lettuce seedlings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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