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BIOTECHNOLOGY

Allelopathic bioactivity of fresh and infused aqueous extracts of Brazilian cherry (*Eugenia uniflora* L.) on lettuce and maize

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ABSTRACT. This study aimed to analyse the allelopathic bioactivity of fresh and infused aqueous extracts of Brazilian cherry leaves on the germination of lettuce and the initial development of maize. Brazilian cherry leaves were used to prepare a fresh aqueous extract (200 g L^{-1}) and an infused extract (100 g L^{-1}), which were diluted to concentrations of 0.4, 0.8, 1.2, 1.6 or 2.0% w/v. The variables evaluated in lettuce were the germination rate (GR), germination time index (GTI), germination speed index (GSI) and root length (RL). To determine the effect on the initial development of maize, we measured the shoot (SL) and root (RL) length. The experimental design included a factorial 2 × 5 design (two extracts and five concentrations), in addition to a control treatment (no extract). In general, the allelopathic bioactivity differed between the fresh and infused extracts. The fresh extract was more phytotoxic for the GR and RL of lettuce. Some beneficial results were observed for the infused extract, including an increase in the RL of lettuce and SL of maize. These effects were dependent on the extract concentration. Thus, there is evidence that Brazilian cherry extracts have allelopathic bioactivity.

Keywords: *Lactuca sativa*; *Zea mays*; allelopathy; allelochemicals; chemical ecology.

Bioatividade alelopática de extrato aquoso fresco e infuso de pitangueira (*Eugenia uniflora* L.) sobre alface e milho

RESUMO. O presente trabalho tem por objetivo em analisar a bioatividade alelopática do extrato fresco e infuso de pitangueira sobre a germinação de alface e no desenvolvimento inicial de milho. Para tanto, utilizaram-se folhas frescas de pitangueira para preparação de extratos aquosos fresco (200 g L^{-1}) e infuso (100 g L^{-1}), diluídos em 0,4; 0,6; 1,2; 1,6 e 2,0% p/v de concentração. Os parâmetros avaliados em alface foram porcentagem (PG), tempo médio (TMG) e velocidade média de germinação (VMG) e comprimento de raiz (CR). Já no desenvolvimento inicial do milho, obteve-se o comprimento de raiz (CR) e parte aérea (CPA). Os experimentos foram conduzidos em esquema fatorial 2x5 + tratamento controle (ausência de extrato), com dois tipos de extratos e cinco concentrações. De modo geral, a bioatividade alelopática dos extratos frescos e infusos foi diferente, sendo que o extrato fresco mostrou-se fitotóxico sobre a PG e CR de alface. Alguns resultados benéficos foram observados com o extrato infuso como aumento no CR de alface e no CPA de milho. Os efeitos desses extratos dependem das concentrações onde são testados. Dessa forma, há evidência de que os extratos de pitangueira possuem bioatividade alelopática.

Palavras-chave: *Lactuca sativa*, *Zea mays*, alelopatia, aleloquímicos, ecologia química.

Introduction

Brazilian cherry or Surinam cherry (*Eugenia uniflora* L.) is a Brazilian tree commonly found in many South American countries (Bicas et al., 2011). In addition to its great economic importance in the production of juices, jams and jellies (Costa, Garcia-Diaz, Jimenez, & Silva, 2013), Brazilian cherry is also important for the recovery of degraded preservation in heterogeneous as in areas as agroforestry systems, by providing food for the avifauna.

Infusions containing Brazilian cherry leaves are commonly used in popular medicine (Santos, Fortes, Ferri, & Santos, 2011), due to its wide variety of chemicals, produced in the leaves and fruit, particularly phenolic compounds (Bagetti, Facco, Rodrigues, Bizzotto, & Emanuelli, 2009), terpenes, alkaloids and flavonoids (Bakr, Mohamed, & Waly, 2017). These substances are produced by secondary metabolism and contribute to a diverse number of functions in plant, such as defense, seed dormancy, and reproduction (Moore, Andrew, Kulheim, & Foley, 2014).

The release of these compounds can affect the development of other surrounding plants either a beneficial or harmful way, a phenomenon known as allelopathy (Inderjit, Wardle, Karban, & Callaway, 2011). This ecological characteristic is common in other Myrtaceae species, including *Myrcia guianensis* (Souza Filho et al., 2006), *Eugenia involucrata*, *Acca sellowiana* (Sausen, Lowe, Figueiredo, & Buzzarro, 2009), *Blepharocalyx salicifolius*, *Myrcia multiflora*, *Myrcia splendens* and *Myrcia tomentosa* (Imatomi et al., 2013; Imatomi, Novaes, Miranda, & Gualtieri, 2015).

Plants with allelopathic characteristics can be used in agricultural systems for natural weed control and for the evaluation of bioactive compounds, aimed at producing natural herbicides and insecticides (Silva, 2012). Preliminary identification of the allelopathic effects of a particular plant involves laboratory testing, in which the allelochemicals are extracted from plant material by aqueous extraction. This process replicates the natural process of allelochemical release by the plant (Reigosa, Gomes, Ferreira, & Borghetti, 2013). These extracts are then tested to determine their effects on seed germination and on the initial development of bioindicator plants such as lettuce and maize (Souza Filho, Guilhon, & Santos, 2010).

The effects observed in target plants result from the biological activity of the extract, and this activity varies depending on its method of preparation and concentration of allelochemicals (Faria, Gomes Júnior, Sá, & Cassiolato, 2009; Peres, Cândido, Bonilla, Faccenda, & Hess, 2010; Correa, Marco Junior, & Costa Junior, 2013). The mode of action of allelochemicals is related to its concentration. However, this does not always occur in a direct and proportional manner, as it can switch between having stimulatory and inhibitory peaks with the increasing extract concentration (Reigosa, Sanchez-Moreiras, & González, 1999).

It is recommended that the preparation of aqueous extracts have to be performed without heating so that the bioassays of allelopathy are more similar and representative to allelochemicals natural events of release (Reigosa et al., 2013). However, after their release into the environment, all chemical compounds can change based on entropy and microorganism metabolism (Albuquerque et al., 2011). Investigating different methods of extract preparation may assist in understanding the effects of chemical dynamics on these compounds in natural environments, and their potential effects on other plants.

Thus, the objective of this study was to evaluate the allelopathic bioactivity of fresh and infused aqueous extract of Brazilian cherry leaves, at different concentrations, on the germination of lettuce seeds and the initial development of maize.

Material and methods

The allelopathic tests were performed at the Laboratory of Plant Physiology of the Biological Sciences and Health Center at *Universidade Estadual do Oeste do Paraná* (UNIOESTE; Cascavel), Paraná State, Brazil.

Disinfection of stands and germination camera was performed prior to installation and before each evaluation, in order to avoid biological contamination and interference with the results. For these purposes, Nystatin was used as fungicide and Lyzoform® as a bactericide, both 10% weight/volume (w/v). The materials used in the experiment, including Petri dishes, filter paper, and Germitest paper, were autoclaved at 121°C for 20 min. prior to use.

Brazilian cherry (*Eugenia uniflora* L.) Myrtaceae leaves were obtained from three matrices in the vegetative stage of development, located in the UNIOESTE (24°59'16.7"S 53°26'52.6"W, 758 m) area and one sample was identified and recorded in UNOP Herbarium under the number 3671. Leaves were collected randomly from the top of the matrices, then brought to the laboratory where they were washed and any excess water was removed.

For the preparation of the fresh aqueous extract, the leaves were ground in a conventional blender with distilled water, to make a concentration of 200 g L^{-1}. The solution was then sieved to remove any solid waste, and the remaining liquid was diluted, based on the chosen concentrations.

The infused aqueous extract was prepared at a concentration of 100 g L^{-1}, as specified for infusions (Lorenzi, 2002). Brazilian cherry leaves were added to hot water (90°C, not boiling), which remained in a closed recipient until the temperature of the extract reached room temperature, at which time dilutions were performed.

The initial solutions of the two extracts, at the concentrations described above, were diluted to obtain concentrations of 0.4, 0.8, 1.2, 1.6 and 2.0%. Extracts at these concentrations were compared to a control treatment, consisting only of distilled water.

The allelopathic bioactivity of the extracts was tested in two separate trials, the first to determine its effect on lettuce seed germination, and the other on the initial development of maize. These species were chosen based on their sensitivity to chemical interference, easy availability, and fast development.

For the germination trials, lettuce seeds var Grand Rapids TBR were placed into Petri dishes containing three filter paper sheets (25 seeds per dish) onto which 7 mL of either fresh aqueous and infused extract was added, depending on the experimental treatment. The number of germinated seeds was counted every day for 5 days to calculate the germination rate (GR), which was expressed as a percentage (Hadas, 1976), in addition to the germination time index (GTI as proposed by Edmond and Drapalla (1958) and the germination speed index (GSI) obtained by the formula described by Labouriau (1983). On the final day of the experimental period, we measured the length of the longest primary root of five seedlings from each Petri dish, and the average root length (RL) was calculated.

To determine the effect of the Brazilian cherry extracts on the initial development of maize, Germitest paper was moistened with 2.5-times of its weight with either the fresh or infused aqueous extract, and 10 seeds of pre-germinated maize seeds were planted. The seeds were placed between the Germitest paper sheets, which were then rolled and placed upright in a transparent cylindrical recipient. To keep the Germitest rolls moist, the bottom of each recipient contained the same amount of solution used to initially moisten the paper, which was replaced every 60 hours to prevent oxidation.

Each Germitest roll corresponded to one repetition, and each recipient corresponded to one treatment (extracts at different concentrations). The initial development trials were conducted for 7 days, after which the shoots and roots were separated and the maize seedlings were measured to obtain the average shoot length (SL) and root length (RL).

Both the lettuce and maize trials were conducted in a germination chamber with a controlled temperature of 25 ± 2°C and a 12-hour of light photoperiod provided by fluorescent tubular 40 W light, as described by Brasil (2009). The experiments were conducted in a completely randomized 2x5 factorial design, consisting of four repetitions per treatment group, as well as a control group (distilled water only). One factor was the type of extract tested (two levels; fresh and infused extract) and the other was the concentration of extract tested (five levels; 0.4, 0.8, 1.2, 1.6 and 2.0%).

For each variable we performed descriptive, Anderson-Darling and analysis of variance (ANOVA) were performed for each variable. The statistical analysis of each variable was carried out according to significant results as determined by the ANOVA. Tukey's test was used to determine significant differences between the experimental treatments groups, and Dunnett's test was used to compare the treatments groups with the control group.

Regression analysis was used to evaluate the effect of the extract concentration on the significant variables. Where a significant interaction between factors was observed, we analyzed the effect of the extract type in the chosen concentrations and concentration effect in extracts. A 5% significance level was chosen for all statistical analyses. All statistical analyses were performed by using Minitab® 17 statistical software.

Results and discussion

The germination rate and root length of lettuce were found to be affected by the extract type and were further submitted to Tukey's and Dunnett's test. When the two extracts were compared, germination rate and root length of lettuce were lower in the presence of fresh extract (Table 1). When compared to the control, only the fresh extract had a significant effect on the germination rate, whereas both the extracts differed from control for the root length.

This suggests that the germination process and initial root growth are not equally affected by the chemical properties of the different extracts. Germination is generally recognised as being less sensitive to allelopathic substances than seedling growth, however, we found that the fresh Brazilian cherry extract negatively affected both of these parameters. There was also a difference observed for the effect of both extracts on root length. While the fresh extract reduced the root length, the infused extract had the opposite effect, stimulating cell growth and differentiation.

Table 1. Effect of the fresh and infused aqueous extracts of Brazilian cherry leaves on the germination rate (GR) and root length (RL) of lettuce seeds.

	Tukey's test[1]	
Treatment	GR (%)	RL (cm)
Fresh	$48.1^{(2.08)*}$ b	$2.16^{(0.18)}$ b
Infused	$94.2^{(1.10)}$ a	$3.84^{(0.04)}$ a
	Dunnet's test[2]	
Treatment	GR (%)	RL (cm)
Control	$96.0^{(1.07)}$ a	$3.12^{(0.05)}$ a
Fresh	$48.1^{(2.08)}$ b	$2.16^{(0.18)}$ b
Infused	$94.2^{(1.10)}$ a	$3.84^{(0.04)}$ b

[1] Averages followed by the same letter do not differ statistically from each other by Tukey's test (5%); [2] Averages followed by the same letter do not differ statistically from control by Dunnet's test (5%); *Standard error of mean.

Allelopathic stimulation events are rare and they may be associated with interference of allelochemicals with the production of plant growth regulators (Reigosa et al., 2013) in addition to the presence of nutrients such as sugars, minerals and amino acids in the extracts (Sausen et al., 2009).

In contrast to our results, Cruz-Silva, Nasu, Pacheco, and Nobrega (2015) reported an inhibitory effect of the infused extract of *Bidens sulphurea* L. on lettuce root length, while the unheated fresh extract increased the root length at some concentrations. These observations clear that the extract preparation may not be directly related to stimulatory or inhibitory effects.

The bioactivity of plant extracts is affected by the method of preparation, and consequently, the concentration of secondary metabolic products (Souza Filho, Guilhon, & Santos, 2010). Maraschin-Silva and Aquila (2006a; 2006b) reported differences in the allelopathic and stimulatory effects between the fresh and heated extracts from the native Brazilian trees *Luehea divaricata, Myrsine guianensis*, *Sapium glandulatum* and *Sorocea bonplandi* on lettuce.

The sensitivity levels of seeds to allelopathic effects can be determined by testing different extract concentrations. The regression models, shown in Figure 1, were used to evaluate the effect of extract concentration on lettuce root length. These analyses showed that there was a decreasing trend of lettuce root length with increasing concentrations of both the fresh (Figure 1-A) and infused (Figure 1-B) extracts of Brazilian cherry leaves.

This trend is normally expected since with increasing extract concentrations, a higher accumulation of allelochemicals in the solution is expected which was corroborated by Souza Filho et al. (2006) who studding the allelopathic effects of *Myrcia guianensis* (L.) (Myrtaceae) and Reichel, Bazaretti, Stefanello, Paulert, and Zonetti (2013), who studied the effect of different extracts of *Jatropha curcas* L. leaves.

In the present study, we observed that higher extract concentrations were associated with root necrosis. Necrosis and subsequent abnormal formation of plants are two of the most evident symptoms suggestive of an allelopathic effect (Grisi, Ranal, Gualtieri, & Santana, 2012).

The effects of extracts type at each concentration on the lettuce germination rate and root length are presented in Figure 2-A and C. The germination rate of the lettuce seeds was lower for the seeds treated with fresh Brazilian cherry extract than the infused extract, at all concentrations tested. When the treatments groups were compared with the control (Figure 2-B), the fresh extract differed from control at all concentrations. The average germination rate found for the treated lettuce seeds was lower than that obtained in the control treatment, which is characteristic of the phytotoxic allelopathic effects of the extract.

Eugenia incolucrata and *Acca Sellowiana* are two Myrtaceae trees that showed the same effect by significant inhibiting lettuce seed germination (Sausen et al., 2009) indicating that inhibitory allelopathic effects may be a common feature among members of the same family.

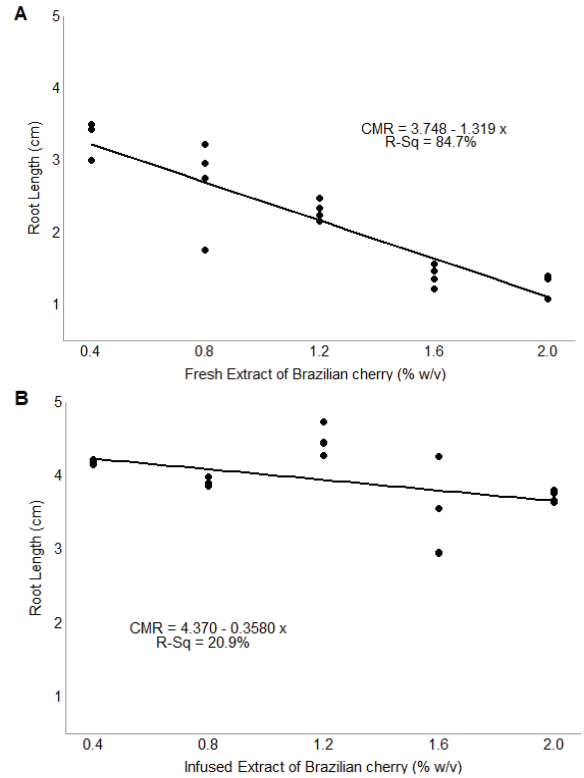

Figure 1. Regression model for the root length (RL) of lettuce seeds treated with different concentration of (A) fresh and (B) infused aqueous extracts of Brazilian cherry leaves.

Changes in the germination process indicate that the bioactive substances in the extract interfered with metabolic reactions at the primary physiological level. The secondary effects of these changes relate to an increase in the activity of amylase, that promoting greater mobilization of reserves and oxidative stress in the embryo and inhibition of water absorption by the tegument (Singh, Singh, & Singh, 2009), all of which affect the germination process.

The root length of lettuce seedlings treated with the fresh extract (Figure 2-C) differed from the infused extract at all concentrations tested. Differences in the allelopathic effects of the two extract types at different concentrations are presented in Figure 1-D. Reigosa et al. (1999) described that the mode of action of allelochemicals switches between peaks of activation and inhibition with increasing extract concentrations. It appears that treatment with 0.8 and 1.2%w/v fresh extract, and 0.4 and 0.8% w/v infused extract, resulted in phytotoxic effects on the root length of lettuce, whereas treatment with 1.8% w/v infused extract improved initial root development.

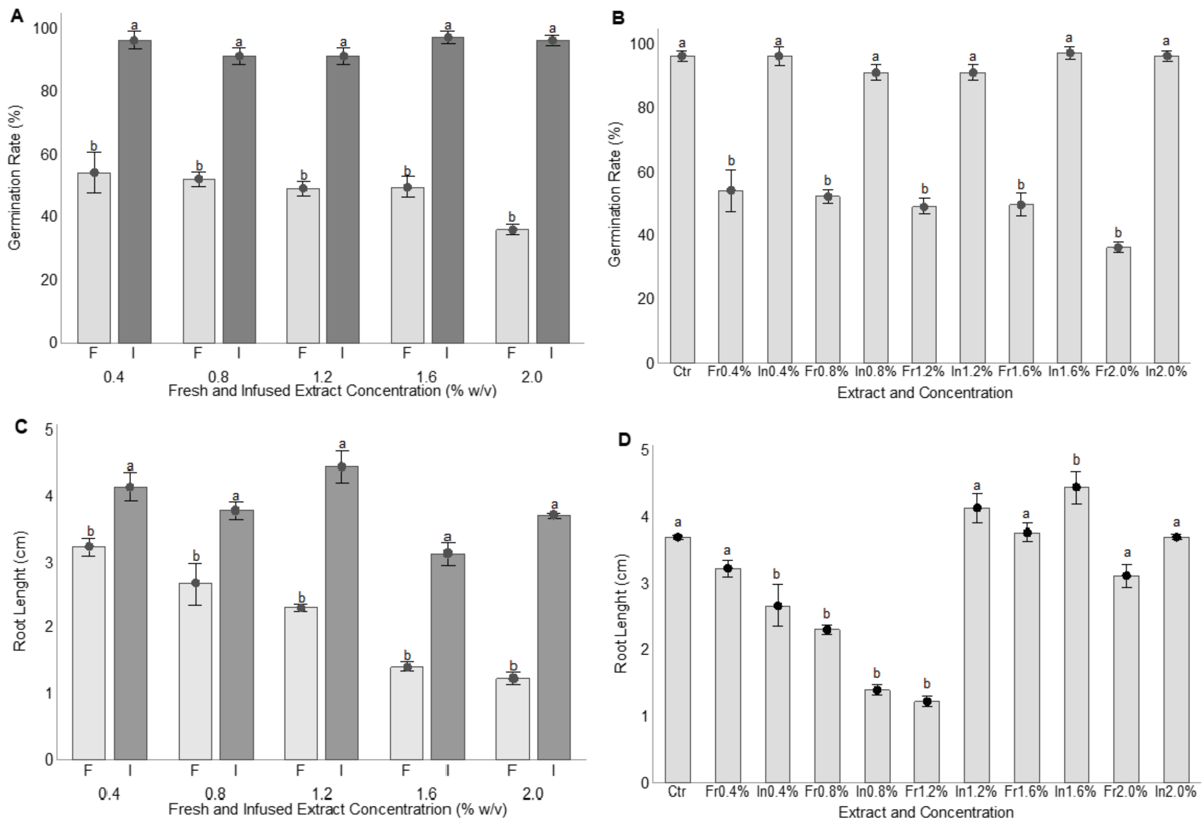

Figure 2. Average germination rate and root length of lettuce seeds for each concentration of fresh and infused aqueous extracts (A and C, respectively) compared by Tukey's test with a significance level of 5%, and all treatment groups compared by Dunnet's test with the control (B and D, respectively) with a significance level of 5% of significance according to extract (fresh – Fr and infused – In).

Peres et al. (2010) discuss that stimulatory or inhibitory effects may be caused by different concentrations. Fluctuation between stimulatory and inhibitory responses, depending on the extraction methods and the extract concentration, has also been observed by Pelegrini and Cruz-Silva (2012) in *Coleus barbatus* (A.) Benth extracts, and by Viecelli and Cruz-Silva (2009) in *Salvia officinalis* L. extracts. A stimulatory effect on the initial development of lettuce has also been reported as a result of treatment with *Eugenia dysenterica* Mart. ex Dc. Berg. extracts at some concentrations (Giotto, Oliveira, & Silva, 2007).

The ANOVA results from the maize initial development trials showed that there was a significant interaction between the type of extract and concentration for shoot and root length. Furthermore, the type of extract and the concentration had a significant effect on shoot length. Therefore, the type of extract and the relationship between the extract concentration and shoot length were evaluated separately.

The shoot length of maize did not differ from the control treatment; however, there was a significant difference between the two types of extracts on this variable. Similar to the effects observed for lettuce germination, the infused extract had a reduced phytotoxic effect compared to the fresh extract (Table 2). Correa et al. (2013) evaluated fresh and heated aqueous extracts of *Setarica italica* L. and reported more detrimental allelopathic effects from treatment with the infused extract.

Table 2. Effect of the fresh and infused aqueous extracts of Brazilian cherry leaves on the shoot length (SL) of maize seedlings.

	Tukey's test (5%)[1]
Treatment	SL (cm)
Fresh	$9.98^{(0.27)*}$ b
Infused	$11.90^{(0.48)}$ a
	Dunnet's test(5%)[2]
Treatment	SL (cm)
Control	$10.30^{(0.64)}$ a
Fresh	$9.98^{(0.27)}$ a
Infused	$11.90^{(0.48)}$ a

[1] Averages followed by the same letter do not differ statistically from each other by Tukey's test (5%); [2] Averages followed by the same letter does not differ statistically from control by Dunnet's test (5%); *Standard error of mean.

When the relationship between the concentration of the extracts and the length of maize shoots was analysed using the regression model, we observed a significant effect on root length only for the infused extract, establishing a trend of increased root length with higher extract concentrations (Figure 3). The same trend was also observed by Faria et al. (2009) for the effect of aqueous extract of millet and velvet bean on maize development. Some allelochemicals can stimulate the elongation of plant parts as they have a similar action to the auxin hormone (Gniazdowska & Bogatek, 2005) which may explain the observed stimulatory effect.

The interaction between the type and concentration of extract with the shoot and roots length of maize is shown in Figure 4. Concentrations up to 0.8% w/v for both extracts had no effect on shoot and root length, but the extracts differed at the 1.2% w/v concentration with lower averages observed for the fresh extract (Figure 4-A).

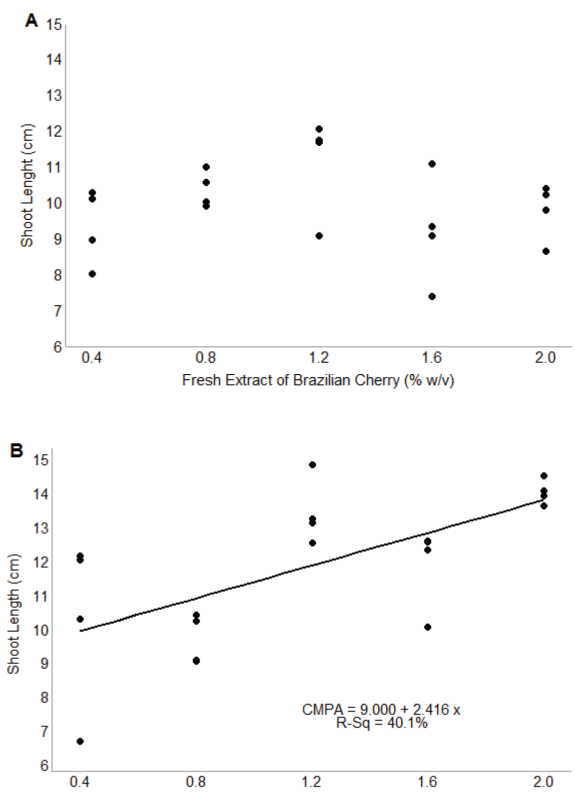

Figure 3. Regression model for the shoot length (SL) of maize seedlings treated with different concentration of (A) fresh and (B) infused aqueous extracts of Brazilian cherry leaves.

This result was expected as the allelopathic effect does not result from the isolated effect of a single chemical but from an interaction between all chemicals present in the extract mixture (Souza Filho, Guilhon, & Santos, 2010). These compounds can have inhibitory or stimulating bioactivities, depending on their functional group and on the concentration studied (Goldfarb, Pimentel, & Pimentel, 2009).

The data for the extract treatments were also compared with the control group using Dunnet's test (Figure 4-B). The only statistical difference was observed from treatment with 1.8% w/v fresh extract, which resulted in a lower average SL compared to the control group.

Unlike the observed for shoot length, root length of maize under fresh extract effect differed from infused extract only in the lowest concentration tested (Figure 4-C). When the effect of the type and concentration of the extracts were analysed, the results showed that the fresh and infused extracts differed from the control treatment at all concentrations (Figure 4-D).

Thus, the root development of maize was negatively influenced in a similar way by both types of Brazilian cherry extracts. Comparable effects on the root development of maize have also been observed for *Azadirachta indica* A. Juss (Rickli, Fortes, Silva, Pilatti, & Hutt, 2011) and Lupinus angustifolius L. (Gomes, Fortes, Silva, Bonamigo, & Pinto, 2013), at all concentrations tested. Imatomi et al. (2015) evaluated the allelopathic potential of extracts of 15 Brazilian Myrtaceae species, all of which had phytotoxic effects on root development of the target species.

The study of seedling growth is an interesting tool for studies on allelopathic effects, as allelochemicals can affect plant growth by interfering with cell division, synthesis and action of hormones, protein synthesis, stomatal conductance, CO_2 assimilation and transportation of electrons in photosynthesis (Silva, Fortes, Pilatti, & Boiago, 2012).

The results from this study indicate that the roots are more sensitive to Brazilian cherry extracts than the shoots. This effect appears to be common in allelopathic studies, supported by the results obtained by Capobianco, Vestena, and Bitencourt (2009) and Al-Sherif, Hegazy, Gomaa, and Hassan (2013) all of which demonstrated that the allelopathic effects vary according to the exposed part of the plant. Imatomi et al. (2015) suggested that this differential effect may be due to higher extract exposure of the roots, however, this does not apply in the current study as the shoots and roots were exposed to the extracts at the same intensity.

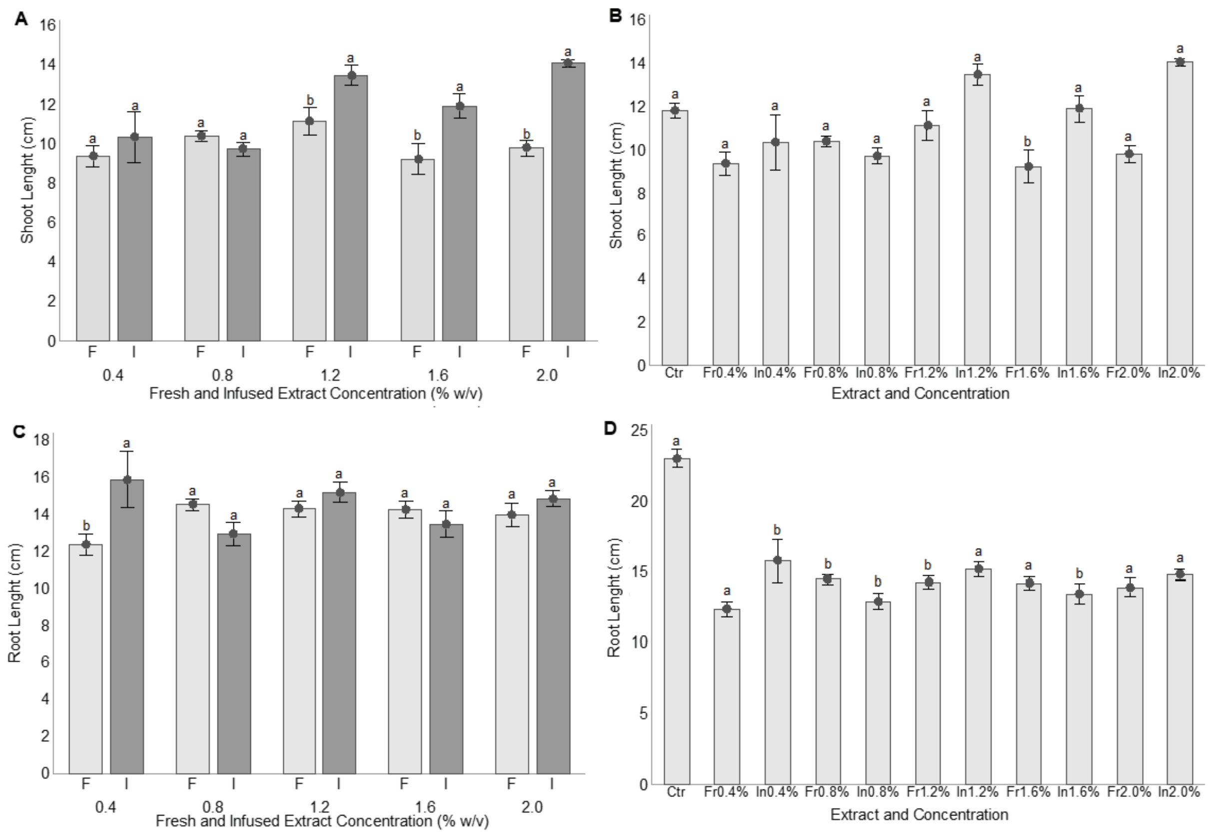

Figure 4. Average shoot and root length of maize seedlings for each concentration of fresh and infused aqueous extracts (A and C, respectively) compared by Tukey's test with a significance level of 5%, and all treatment groups compared by Dunnet's test with the control (B and D, respectively) with a significance level of 5% of significance according to extract (fresh – Fr and infused – In).

In general, treatment with Brazilian cherry extracts affected the target species in some way, but the phytotoxicity of the fresh extract was greater than the infused extract. It is important to note that, although heating the extract increases the solubility of bioactive substances (Azmir et al., 2013), the effects can be milder than the fresh extract. Some allelopathic substances may undergo chemical changes in biological activity as a result of heating during the extraction process.

In order to confirm the allelopathic effects of Brazilian cherry, the results should be applied to field experiments (Corsato, Fortes, Santorum, & Leszczynski, 2010). The results indicate that there is allelopathic potential, but there is no guarantee that *in vitro* observation will manifest in field conditions (Gusman, Bittencourt, & Vestena, 2008).

Conclusion

Brazilian cherry extracts showed allelopathic bioactivity on lettuce germination and the initial development of maize, and these effects depended on the method of preparation. We observed that the fresh aqueous extract of Brazilian cheery was more phytotoxic than the infused extract.

Furthermore, the infused extract exhibited potentially beneficial allelopathic effects on the initial root development of lettuce, and tended to be beneficial for the growth of maize shoots. The allelopathic effects varied depending on the concentration tested, and each variable analyzed responded differently to treatment with the extracts.

References

Albuquerque, M., Santos, R. C., Lima, L. M., Melo Filho, P., Nogueira, R., Câmara, C. A. G., & Ramos, A. R. (2011). Allelopathy, an alternative tool to improve cropping systems. A review. *Agronomy for Sustainable Development*, *31*(2), 379-395. doi: 10.1051/agro/2010031

Al-sherif, E., Hegazy, A. K., Gomaa, N. H., & Hassan, M. O. (2013). Allelopathic effect of black mustard tissues and root exudates on some crops and weeds. *Planta Daninha*, *31*(1), 11-19. doi: 10.1590/S0100-8358201 3000100002

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., ... Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, *117*(1), 426-436. doi: 10.1016/j.jfoodeng.2013.01.014

Bagetti, M., Facco, E. M. P., Rodrigues, D. B., Bizzotto, M., & Emanuelli, T. (2009). Antioxidant capacity and composition of pitanga seeds. *Ciência Rural, 39*(8), 2504-2510. doi: 10.1590/S0103-84782009005000172

Bark, R. O., Mohamed, S. A., & Waly, N. E. (2017) Phytochemical and biological investigation of *Eugenia uniflora* L. cultivated in Egypt. *Journal of Pharmacognosy and Phytotherapy*, *9*(5), 57-66. doi: 10.5897/JPP2017.0443

Bicas, J. L., Molina, G., Dionísio, A. P., Barros, F. F. C., Wagner, R., Maróstica, M. R., & Pastore, G. M. (2011). Volatile constituents of exotic fruits from Brazil. *Food Research International*, *44*(7), 1843-1855. doi: 10.1016/j.foodres.2011.01.012

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. (2009). *Regras para análise de sementes*. Brasília, DF: Secretaria de Defesa Agropecuária, Mapa/ACS.

Capobianco, R. A., Vestena, S., & Bitencourt, A. H. C. (2009). Alelopatia de *Joanesia princeps* Vell. e *Casearia sylvestris* Sw. sobre espécies cultivadas. *Brazilian Journal of Pharmacognosy*, *19*(4), 924-930. doi: 10.1590/S0102-695X2009000600023

Correa, D., Marco Junior, J., & Costa Junior, A. C. (2013). Efeito alelopático de extratos aquosos de *Setaria italica* L. sobre a germinação de sementes de soja e nabo forrageiro. *Cultivando o saber*, *6*(1), 1-7.

Corsato, J. M., Fortes, A. M. T., Santorum, M., & Leszczynski, R. (2010). Efeito alelopático do extrato aquoso de folhas de girassol sobre a germinação de soja e picão-preto. *Semina: Ciências Agrárias*, *31*(2), 353-360. doi: 10.5433/1679-0359.2010v31n2p353

Costa, A. G. V., Garcia-Diaz, D. F., Jimenez, P., & Silva, P. I. (2013). Bioactive compounds and health benefits of exotic tropical red-black berries. *Journal of Functional Foods*, *5*(2), 539-549. doi: 10.1016/j.jff.2013.01.029

Cruz-Silva, C. T. A., Nasu, E. G. C., Pacheco, F. P., & Nobrega, L. H. P. (2015). Allelopathy of *Bidens sulphurea* L. aqueous extracts on lettuce development. *Revista Brasileira de Plantas Medicinais*, *17*(4), 679-684. doi: 10.1590/1983-084X/14_09

Edmond, J. B., & Drapalla, W. J. (1958). The effects of temperature, sand and soil, and acetone on germination on okra seeds. *Proceedings of the American Society Horticultural Science*, *71*(2), 428-434.

Faria, T. M., Gomes Júnior, F. G., Sá, M. E., & Cassiolato, A. M. R. (2009). Efeitos alelopáticos de extratos vegetais na germinação, colonização micorrízica e crescimento inicial de milho, soja e feijão. *Revista Brasileira de Ciências do Solo*, *33*(3), 1625-1633. doi: 10.1590/S0100-06832009000600011

Giotto, A. C., Oliveira, S. C. C., & Silva, J. G. P. (2007). Efeito alelopático de *Eugenia dysenterica* Mart. ex DC. Berg. (Myrtaceae) na germinação e no crescimento de *Lactuca sativa* L. (Asteraceae). *Revista Brasileira de Biociências*, *5*(2), 600-602.

Goldfarb, M., Pimentel, L. W., & Pimentel, N. W. (2009). Alelopatia: relações nos agroecossistemas. *Tecnologia & Ciência Agropecuária*, *3*(1), 23-28.

Gomes, F. M., Fortes, A. M. T., Silva, J., Bonamigo, T., & Pinto, T. T. (2013). Efeito alelopático da fitomassa de *Lupinus angustifolius* L. sobre a germinação e desenvolvimento inicial de *Zea mays* L. e *Bidens pilosa* L. *Revista Brasileira de Agroecologia*, *8*(1), 48-56.

Gusman, G. S., Bittencourt, A. H. C., & Vestena, S. (2008). Alelopatia de *Braccharis dracunculifolia* DC. sobre a germinação e desenvolvimento de espécies cultivadas. *Acta Scientiarum Biological Sciences*, *30*(2), 119-125. doi: 10.4025/actascibiolsci.v30i2.3592

Grisi, P. U., Ranal, M. A., Gualtieri, S. C. J., & Santana, D. G. (2012). Allelopathic potential of *Sapindus saponaria* L. leaves in the control of weeds. *Acta Scientiarum Agronomy*, *34*(1), 1-9. doi: 10.4025/actascia gron.v34i1.11598

Hadas, A. (1976). Water update germination of leguminous seed under changing external water potential in osmotic solution. *Journal of Experimental Botany*, *27*(98), 480-489.

Imatomi, M., Novaes, P., Matos, A. P., Gualtieri, S. C. J., Molinillo, J. M. G., Lacret, R., … Macías, F. A. (2013). Phytotoxic effect of bioactive compounds isolated from *Myrcia tomentosa* (Myrtaceae) leaves. *Biochemical Systematics and Ecology*, *46*, 29-35. doi: 10.1016/j.bse. 2012.09.005

Imatomi, M., Novaes, P., Miranda, M. A. F. M., & Gualtieri, S. C. J. (2015). Phytotoxic effects of aqueous leaf extracts of four Myrtaceae species on three weeds. *Acta Scientiarum. Agronomy*, *37*(2), 241-248. doi: 10.40 25/actasciagron.v37i2.19079

Inderjit, Wardle, D. A., Karban, R., & Callaway, R. M. (2011). The ecosystem and evolutionary contexts of allelopathy. *Trend in Ecology & Evolution*, *26*(12), 655-662. doi: 10.10 07/BF02897150

Labouriau, L. G. (1983). *Seed germination*. Washington, DC: OEA.

Lee, M. I., Nishimoto, S., Yang, L. L., Yen, K. Y., Hatano, T., Yoshida, T., & Okuda, T. (1998). Two macrocyclic hydrolysable tannin dimmers from *Eugenia uniflora*. *Phytochemistry*, *44*(7), 49-52.

Lorenzi, H., & Matos, F. J. A. (2002). *Plantas Medicinais no Brasil*. Nova Odessa, SP: Instituto Plantarum.

Maraschin-Silva, F., & Aquila, M. E. A. (2006). Contribuição ao estudo do potencial alelopático de espécies nativas. *Revista Árvore*, *30*(4), 547-555. doi: 10.1590/S0100-67622006000400000

Maraschin-Silva, F., & Aquila, M. E. A. (2006). Potencial alelopático de espécies nativas na germinação e crescimento inicial de *Lactuca sativa* L. (Asteraceae). *Acta Botanica Brasil*, *20*(1), 61-69. doi: 10.1590/S0102-33062006000100007

Moore, B. D., Andrew, R. L., Kulheim, C., & Foley, W. J. (2014). Explaining intraspecific diversity in plant metabolites in an ecological context. *New Phytologist*, *201*(2), 733-750. doi: 10.1111/nph.12526

Pelegrini, L. L., & Cruz-Silva, C. T. A. (2012). Variação sazonal na alelopatia de extratos aquosos *Coleus barbatus* (A.) Benth. sobre a germinação e o desenvolvimento de *Lactuca sativa* L. *Revista Brasileira de Plantas Medicinais*, *14*(2), 376-382. doi: 10.1590/S1516-05722012000200019.

Peres, M. T. L. P., Cândido, A. C. S., Bonilla, M. B., Faccenda, O., & Hess, S.C. (2010). Phytotoxic potential of *Senna occidentalis* and *Senna obtusifolia*. *Acta Scientiarum. Biological Science*, *32*(3), 305-309, 2010. doi: 10.4025/actascibiolsci.v32i3.5833

Reichel, T., Bazaretti, J. F., Stefanello, S., Paulert, R., & Zonetti, P. C. (2013). Allelopathy of leaf extracts of jatropha (*Jatropha curcas* L.) in the initial development of wheat (*Triticum aestivum* L.). *Idesia*, *31*(1), 45-52. doi: 10.4067/S0718-34292013000100006

Reigosa, M., Gomes, A. S.; Ferreira, A. G., & Borghetti, F. (2013). Allelopathic research in Brazil. *Acta Botanica Brasilica*, *27*(4), 629-646. doi: 10.1590/S0102-330620 13000400001.

Reigosa, M. J., Sanchez-Moreiras, A., & González, L. (1999). Ecophysiological approach in allelopathy. *Critical Reviews in Plant Science*, *18*(5), 577-608. doi: 10.1080/07352689991309405

Rickli, H. C., Fortes, A. M. T.; Silva, P. S. S.; Pilatti, D. M., & Hutt, D. R. (2011). Efeito alelopático de extrato aquoso de folhas de *Azadiracha indica* A. Juss em alface, soja, milho, feijão e picão-preto. *Semina: Ciências Agrárias*, *32*(2), 473-484. doi: 0.5433/1679-0359.2011 v32n2p473.

Santos, R. M., Fortes, G. A. C., Ferri, P. H., & Santos, S. C. (2011). Influence of foliar nutrients on phenol levels in leaves of *Eugenia uniflora*. *Brazilian Journal of Pharmacognosy*, *21*(4), 581-586. doi: 10.1590/S0102-695X2011005000112

Sausen, T. L., Lowe, T. R., Figueiredo, L. S., & Buzarro, C. R. (2009). Avaliação da atividade alelopática do extrato aquoso de folhas de *Eugenia involucrata* DC. e *Acca sellowiana* (O. Berg) Burret. *Polibotanica*, *27*, 145-158.

Schmeda-Hirschmann, G. (1995). Flavonoids from *Calycorectes*, *Campomanesia, Eugenia* and *Hexachlamys* species. *Fitoterapia*, *66*(4), 373-374.

Silva, P. S. S. (2012). Atuação dos aleloquímicos no organismo vegetal e formas de utilização da alelopatia na agronomia. *Biotemas*, *25*(1), 65-74. doi:10.5007/ 2175-7925.2012v25n3p65

Silva, P. S. S., Fortes, A. M. T., Pilatti, D. M., & Boiago, N. P. (2012). Atividade alelopática do exsudato radicular de *Jatropha curcas* L. sobre plântulas de *Brassica napus* L., *Glycine max* L.; *Zea mays* L. e *Helianthus annuus* L. *Insula Revista de Botânica*, *41*(41), 32-41. doi: 10.5007/2178-4574.2012n41p32

Singh, A., Singh, D., & Singh, N. B. (2009). Allelochemical stress produced by aqueaous leachate of *Nicotiana plumbaginifolia* Viv. *Plant Growth Regulation*, *58*(2), 163-171. doi: 10.1007/s10725-009-9364-1

Souza Filho, A. P. S., Guilhon, G. M. S. P., & Santos, L. S. (2010). Metodologias empregadas em estudos de avaliação da atividade alelopática em condições de laboratório – revisão crítica. *Planta Daninha*, *28*(3), 689-697. doi: 10.1590/S0100-83582010000300026

Souza Filho, A. P. S., Santos, R. A., Santos, L. S., Guilhon, G. M. P., Santos, A. S., Arruda, M. S. P., & Arruda, A. C. (2006). Potencial alelopático de *Myrcia guainensis. Planta Daninha*, *24*(4), 649-656. doi: 10.1590/S0100-83582006000400005

Viecelli, A. C., & Cruz-Silva, C. A. T. (2009). Efeito da variação sazonal no potencial alelopático de sálvia. *Semina: Ciências Agrárias*, *30*(1), 39-46. doi: 10.5433/ 1679-0359.2009v30n1p39

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