Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v39i4.32603

# Cashew nut shell liquid and formulation: toxicity during the germination of lettuce, tomato seeds and coffee senna and seedling formation

**Rosemary Matias[1], Ana Carolina Rosa[1], Ademir Kleber Morbeck Oliveira[1*], Kelly Cristina Lacerda Pereira[1], Elvia Silvia Rizzi[1] and Alexandre Alves Machado[2]**

*[1]Programa de Pós-Graduação em Meio Ambiente e Desenvolvimento Regional, Universidade Anghanguera-Uniderp, Rua Alexandre Herculano, 1400, 79037-280, Campo Grande, Mato Grosso do Sul, Brazil. [2]Universidade Católica Dom Bosco, Centro de Ciências da Saúde, Campo Grande, Mato Grosso do Sul, Brazil. *Author for correspondence. E-mail: akmorbeckoliveira@gmail.com*

**ABSTRACT.** Cashew (*Anacardium occidentale*) nut shell liquid (CNSL) has been successfully used in trials as an *Aedes aegypti* larvicide, but little is known about its environmental effects. In this study, the potential effects of CNSL and a CNSL-based phyto-product formulation on the germination and growth of *Lactuca sativa* (lettuce), *Lycopersicon esculentum* (tomato) and *Senna obtusifolia* (coffee senna) were assessed. The pH of CNSL and the formulation were 6.4 and 6.8, respectively; the electrical conductivities were 2.89 µS $cm^{-1}$ (CNSL) and 2.21 µS $cm^{-1}$ (formulation), respectively, and both contained anacardic acid (53.2%) and degradation products. In bioassays for germination and growth, CNSL (25, 50, 100, 150, and 200 mg $mL^{-1}$), the formulation (100 mg $mL^{-1}$) and the control were used in a completely randomized design. The results demonstrated the chemical effects of CNSL, which negatively affected the germination and vigor of lettuce and tomato and the vigor of coffee senna; for growth, it negatively influenced both the root and aerial parts of lettuce and tomato, but only the roots of coffee senna. The formulation had negative effects on the vigor of coffee senna and the growth of tomato and lettuce seedlings (roots and aerial parts). The results indicate the phytotoxicity of CNSL and the formulation for the plant species tested.

**Keywords:** Anacardiaceae, *Anacardium occidentale*, CNSL, anacardic acid, allelopathy.

## Líquido da casca da castanha de caju e formulação: toxicidade sobre sementes de alface, tomate e fedegoso e formação de plântulas

**RESUMO.** O líquido da casca da castanha de caju (LCC) de *Anacardium occidentale* está sendo utilizado com sucesso em ensaios larvicidas para *Aedes aegypti*; porém pouco se sabe sobre seus efeitos ambientais. Neste trabalho, o objetivo foi avaliar os efeitos do LCC e de um fitoproduto a base de LCC na germinação e no crescimento de *Lactuca sativa* (alface), *Lycopersicon esculentum* (tomate) e *Senna obtusifolia* (fedegoso). O pH do LCC e formulação é 6,4 e 6,8, respectivamente; a condutividade elétrica 2,89 *µS* $cm^{-1}$ (LCC) e 2,21 *µS* $cm^{-1}$ (formulação) e ambos contêm ácido anacárdico (53,2%) e produtos de degradação. Nos bioensaios de germinação e de crescimento, utilizou-se LCC (25, 50, 100, 150 e 200 mg $mL^{-1}$) e a formulação (100 mg $mL^{-1}$) e controle, com delineamento inteiramente casualizado. Os resultados demonstraram efeito do LCC, afetando negativamente a germinação e vigor da alface e tomate e o vigor do fedegoso; no crescimento, influenciou negativamente o crescimento da raiz e caule de alface e tomate e no fedegoso, apenas da raiz. A formulação exerceu efeito negativo apenas para sementes de fedegoso e afetou negativamente o crescimento das plântulas de alface e tomate, raiz e parte aérea. Os resultados obtidos indicaram fitotoxicidade do LCC e da formulação para as espécies testadas.

**Palavras-chave:** Anacardiaceae, *Anacardium occidentale*, LCC, ácido anacárdico, alelopatia.

## Introduction

The arboreal species *Anacardium occidentale* L. (Anacardiaceae) is popularly called the cashew tree; of the 11 species of the genus *Anacardium*, it is the best known. Although the cashew tree is a plant native to Brazil that is abundant in the Northeast and Midwest, it is also cultivated in various tropical regions in Asia and Africa and in countries such as India, Mozambique and Tanzania (Kamath & Rajini, 2007).

The easy reproduction and extensive cultivation area of the cashew tree are of great socioeconomic importance in Brazil. Among other uses, this tree is used in agroforestry systems, in family agriculture (Vieira, Rosa, Vasconcelos, Santos, & Modesto,

2007) and for reforestation (Salomão, Brienza Júnior, & Rosa, 2014). Among the pioneer species in Mato Grosso, the cashew tree has proven to be one of the native trees of the Cerrado indicated for the consortium with manioc for influences on productivity (Martinotto, Martinotto, Coelho, Azevedo, & Albuquerque, 2012). The fruit, especially the nut, has great social and economic importance in the Northeast due to the large volume exported.

As the nut is obtained, cashew nut shell liquid (CNSL) is a by-product that represents approximately 25% of the weight of nut and is considered to be a low value-added product (Mazzetto, Lomonaco, & Mele, 2009). CNSL is an oil resin with caustic properties (Lorenzi & Matos, 2008) and until recently, was employed exclusively in the automotive industry for friction powder formulations, electrical insulations, and waterproofing, among other uses (Lomonaco et al., 2009).

CNSL it is not pure and contains approximately 90% anacardic acid, a phenolic compound biosynthesized from fatty acids that can be phytotoxic (Correia, David, & David, 2006; Chaves et al., 2010). Anacardic acid is readily decarboxylated at high temperatures and generates degradation products such as cardanol, cardol and 2-methil-cardol (Rodrigues, Feitosa, Ricardo, França, & Carioca, 2006; Mazzetto et al., 2009).

CNSL has also been reported to have several medicinal properties, including anti-inflammatory, analgesic and antitumoral effects, in addition to indications as a molluscicide, fungicide and bactericide (Lomonaco et al., 2009). CNSL has been used as a potential larvicide targeting *Aedes aegypti* (Linnaeus, 1762) (Shaalan, Canyon, Younes, Abdel-Wahab, & Mansour, 2005; Porto, Roel, Machado, Cardoso, & Oliveira, 2013; Dourado et al., 2015), a vector of dengue and other arboviruses of relevance that is considered to be a serious public health problem.

As a larvicide, various concentrations of CNSL have been employed. Mendonça et al. (2005) described the lethal concentration ($CL_{50}$) to be 14.5 mg $L^{-1}$; Shaalan et al. (2005) suggested 10 mg $L^{-1}$; Asogwa, Mokwunye, Yahaya, and Ajao (2007) cited 100% mortality at 60, 80, and 10 mg $mL^{-1}$ concentrations; and Porto et al. (2013) recently used a base formulation of CNSL in concentrations of 0.002 to 10 mg $mL^{-1}$.

Unfortunately, the reforestation and forestry programs of *A. occidentale* in various regions of Brazil do not consider some inherent family characteristics. According to Ceruks, Romoff, Fávero, and Lago (2007), this group has different species with high allelopathic activity, the action of which is mainly due to phenolic constituents with toxic actions.

The allelopathy occurs through the release of chemical substances called allelochemicals that can act as "natural herbicides", reducing competition for resources; the release occurs through the decomposition of leaves and stems and direct exudation into the soil by the roots, among other ways. This type of process currently constitutes a potential source for new structural models of herbicides, which may act more specifically and with greater potential than those currently used in agriculture, causing less damage to the environment (Zeng, Mallik, & Luo, 2010).

Thus, allelopathy can provide different methods of integrated weed control and new generations of phytotoxins.

The resistance or tolerance to secondary metabolites is a species-specific characteristic, resulting in higher sensitivity in *Lactuca sativa* L. (lettuce) and *Lycopersicon esculentum* Miller (tomato), which are considered to be indicator plants of allelopathic activity. To be indicated as a test plant, the species must present rapid and uniform germination and a degree of sensitivity that enables the expression of results at low concentrations of allelopathic substances (Soltys, Bogatek, & Gniazdowska, 2012). However, it is also necessary to test the metabolites in wild species, such as coffee senna, *Senna obtusifolia* (L.) H. S. Irwin & Barneby, an annual subshrubby species that is considered invasive in pasture and crop areas.

As CNLS and its formulations are indicated for use as an alternative for the larval control of *A. aegypti* (Porto et al., 2013; Dourado et al., 2015) and the use of these products occurs in the environment where the larvae develop, it is necessary to evaluate allelopathic effects. Therefore, the aim of this study is to evaluate the effects of commercial CNLS and a CNLS-based phyto-product formulation on the germination and development of *L. sativa*, *L. esculentum* and *S. obtusifolia* and to determine the quantitative and qualitative chemical composition of CNLS and the evaluated formulation.

## Material and methods

### Study material

The CNSL (Cashol®) component extracted from the *A. occidentale* endocarp used in the experiment was provided by Resibras Company, São Paulo, São Paulo State, Brazil.

The CNSL-based formulation was prepared by a spray-drying method, which involved the formation of small and uniform sized particles from a liquid sample that was nebulized in a chamber for drying in pre-warmed medium by dispersing CNSL in a hydro-alcoholic solution followed by absorption of 10% w/w corn starch. The powder was screened (200 mesh) for grain size calibration and stored at ambient temperature protected from light (Oliveira & Petrovick, 2010).

CNSL and the obtained powdered formulation were submitted for chemical analysis to determine the anacardic acid content and degradation products (cardanol, cardol and methyl-cardol) since the anacardic acid is thermolabile (Risfaheri, Anwar Nur, & Sailah, 2009) and were subsequently employed in bioassays of germination and growth.

### Research development

The research was conducted in the Research Laboratory of Environmental and Biodiversity Systems, University Anhanguera-Uniderp, Campo Grande, Mato Grosso do Sul State, Brazil.

### Chemical analysis

The detection of anacardic acid in CNSL and the formulation (10 mg $mL^{-1}$) was performed by thin-layer chromatography (TLC) in aluminum chromatoplates (Merck $GF_{254}$); the mobile phase was chloroform ($CHCl_3$): hexane (Hex): ethanol (EtOH) (5.0:5.0:0.4 mL). The visualization of these substances in CNSL was conducted with an ultraviolet lamp camera ($\lambda_{max}$ = 254 and 365 nm) that scanned each 10 mg $mL^{-1}$ sample between 200 to 700 nm with ultraviolet–visible spectroscopy.

CNSL and the formulation were analyzed for pH (pH DM-20, Digimed) and electrical conductivity (DM3, Digimed); anacardic acid in CNSL and the formulation (0.004 g 100 $mL^{-1}$ hexane) was quantified by interpolating the absorbance values of the samples against a constructed calibration curve ($y = 0.1088 + 0.03102\ x$; $r^2 = 0.9762$) of known anacardic acid concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg $mL^{-1}$) obtained as reported by Agostini-Costa et al. (2004).

### Bioassays

According to Rizzi et al. (2016) 5 mL of extracts were added to Petri dishes (7.0 cm diameter) and 10 mL in transparent plastic boxes (11.0 cm x 3.5 cm of height), both lined with two Germitest paper sheets that were not moistened again. The dishes and boxes were maintained in a germination chamber at 20°C (lettuce) and 25°C (tomato and coffee senna). The concentrations used were based on Asogwa et al. (2007).

### Germination

CNSL extracts of 25, 50, 100, 150, and 200 mg $mL^{-1}$ were used for lettuce and tomato and formulation concentrations of 100 and 200 mg $mL^{-1}$ were used for coffee senna, lettuce and tomato; these, along with a distilled water control (0 mg $mL^{-1}$), were all setup in Petri dishes. The experimental design was completely randomized, with six treatments for lettuce and tomato and three for coffee senna, and four replications of 25 seeds for each treatment.

The dishes were evaluated every 24 hours for 7 days and considered germinated seeds when they showed a 2 mm primary root (Rizzi et al., 2016). The coffee senna seed dormancy was overcome by soaking seeds in sulfuric acid for 20 minutes and then washing in running water for five minutes (Pozitano & Rocha, 2011).

For the germination test, the percentage of germination and seed vigor was measured indirectly by average germination time in days (AGT) (Labouriau & Agudo, 1987) and the germination speed index (GSI) (Maguire, 1962).

### Growth

CNSL extracts of 25, 50, 100, 150, and 200 mg $mL^{-1}$ were used for tomato and lettuce seedlings, 100 and 200 mg $mL^{-1}$ for coffee senna, formulation for lettuce and tomato (in transparent plastic boxes), with a distilled water control (0 mg $mL^{-1}$). The design was completely randomized with six treatments for lettuce and tomato and three for coffee senna with four replications containing 10 pre-germinated seeds for each species. An evaluation was performed 10 days after the beginning of the experiment and measured plant height (mm) from base to apex and root length (mm) from base to apex of the meristematic root system using a digital pachymeter (Rizzi et al., 2016).

### Statistical analyses

The averages were initially tested for normality presupposition of residues (Shapiro-Wilk test) and homogeneity of the variances (Bartlett's test), and it was not necessary to transform the data. The data of the characteristics evaluated were submitted to polynomial regression and posteriorly to analysis of variance. When there was significance, there was a comparison of means by Tukey test (5%) with statistical analysis processed using the Assistat 7.7 beta program. We present the data in tables (analysis of variance) and figures (polynomial regression) to better understand the results.

## Results and discussion

### Chemical analysis of CNSL

Quantification of the anacardic acid in CNSL was 53.2%, which demonstrates the degradation of the CNSL used in this study. Mazzetto et al. (2009), studying the CNSL and thermo-oxidative degradation, affirmed that it contains the most anacardic acid, followed by cardol, cardanol and 2-methyl-cardol. According to Lomonaco et al. (2009), the anacardic acid is thermolabile and undergoes decarboxylation, losing the carboxyl and degrading it completely to generate structures such as cardanol (70%–75%), cardol (10%–20%), polymerized material (10%) and traces of 2-methyl-cardol.

The degradation was evident in the absorption spectra of CNSL and the formulation, which both presented the same maximum absorption at 280 and 320 nm (Figure 1C and D) corresponding to the cardol and anacardic acid, respectively. These same wavelength values were described by Agostini-Costa et al. (2004) for CNSL. The bands 3 and 4 (Figure 1C) most likely represent methyl-cardol and cardanol, also by-products of CNSL. Degradation was also observed in chromatographic analysis (TLC) with registration of 4 bands (Figure 1A) with average retention factors (Rfs) of 0.22 for anacardic acid and 0.49 and 0.82 for cardol and cardanol, respectively. The Rfs values are consistent with those described by Agostini-Costa, Dos Santos, Garruti, and Feitosa, (2000) for CNSL composed of different clones of the cashew tree.

These results indicate that degradation of anacardic acid to 3 other substances has occurred in the CNSL; conversely, for the formulation based on CNSL (10 mg $mg^{-1}$), in addition to anacardic acid, the presence of only 2 other bands (Figure 1B) indicates a lower degradation and consequently greater stability of the CNSL present in the formulation.

The CNSL and the formulation had a pH of 6.4 ± 0.3 and a pH of 6.8 ± 0.2, respectively. Agostini-Costa et al. (2004) indicated that the pH of pure anacardic acid isolated from cashew nuts shells was between pH 4.2 to 4.7. Andrade, Oliveira, Innecco, and Silvas (2008) found similar pH values for CNSL, which were pH 4.4 to 4.6. The acidic pH is directly influenced by the concentration of organic acids in the sample, including the predominance of anacardic acid (Agostini-Costa et al., 2004), which would be responsible for the acidity of CNSL because the decomposition releases $H^+$ ions.

The pH values of the solutions do not interfere with the germination process, only extreme values of acidity and alkalinity may have negative effects. The same is true for the electrical conductivity, where the values obtained for CNSL (EC = 2.89 $\mu S\ cm^{-1}$) and the formulation (EC = 2.21 $\mu S\ cm^{-1}$) did not have a negative influence on the germination and growth of seedlings similar to the findings of Gonzales, Paula, and Valeri (2009).

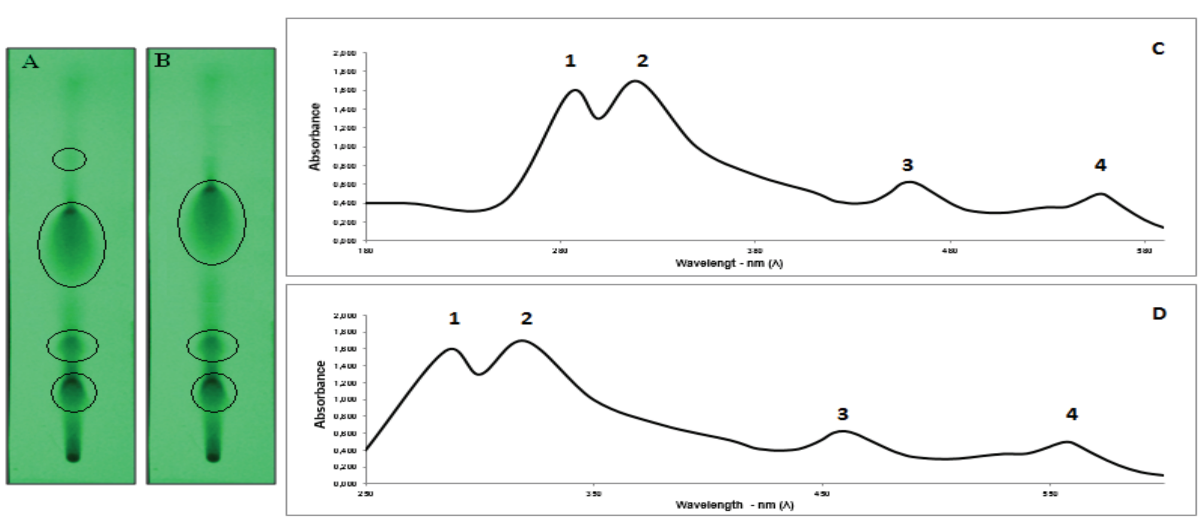


**Figure 1.** Chromatoplate A corresponds to CNSL, a component of *A. occidentale*, and Chromatoplate B to the formulation. Figures 1C and 1D represent CNSL and formulation spectra UV-vis absorption; absorption at 280 nm corresponds to cardol (1) and at 320 nm to anacardic acid (2).

### Germination and growth bioassays (CNSL)

CNSL affected the germination of the lettuce seeds only at a concentration of 200 mg $mL^{-1}$, while for tomatoes, 100 mg $mL^{-1}$ (Figure 2, Table 1) had an effect and for coffee senna, seed germination interference did not occur (Figure 2, Table 2). For AGT, the concentration of 100 mg $mL^{-1}$ decreased the rate of lettuce and tomato germination, while for GSI, 150 mg $mL^{-1}$ (Figure 2, Table 1) decreased germination. For coffee senna seeds, only 200 mg $mL^{-1}$ interfered with germination in both AGT and GSI (Figure 2, Table 2). With regards to the formulation, there was no interference with any of the parameters as they were statistically equal to the control (Figure 2, Table 1).

Recent studies show that, although the final germination percentage is not significantly affected by the action of chemical compounds, the germination pattern can be modified when evaluating major differences in the speed and synchrony of the germination of seeds subjected to such compounds (Oliveira, Matias, Lopes, & Fontoura, 2014a). These changes were observed in lettuce, tomato and coffee senna seeds subjected to CNSL as germination was less affected than seed vigor. For the formulation, only the size of the seedlings was negatively affected, which differs from the general observations regarding vigor.

It should also be noted that the tomato seeds were more affected than the lettuce seed extracts. This species, which belongs to the family Solanaceae, demonstrated the highest sensitivity to secondary metabolites identified in the extracts. Alternatively, lettuce, family Asteraceae, had lower sensitivity. The difference in the germination process is probably related to the physiology of the species tested because different families respond differently to the presence of metabolites, according to Rizzi et al. (2016).

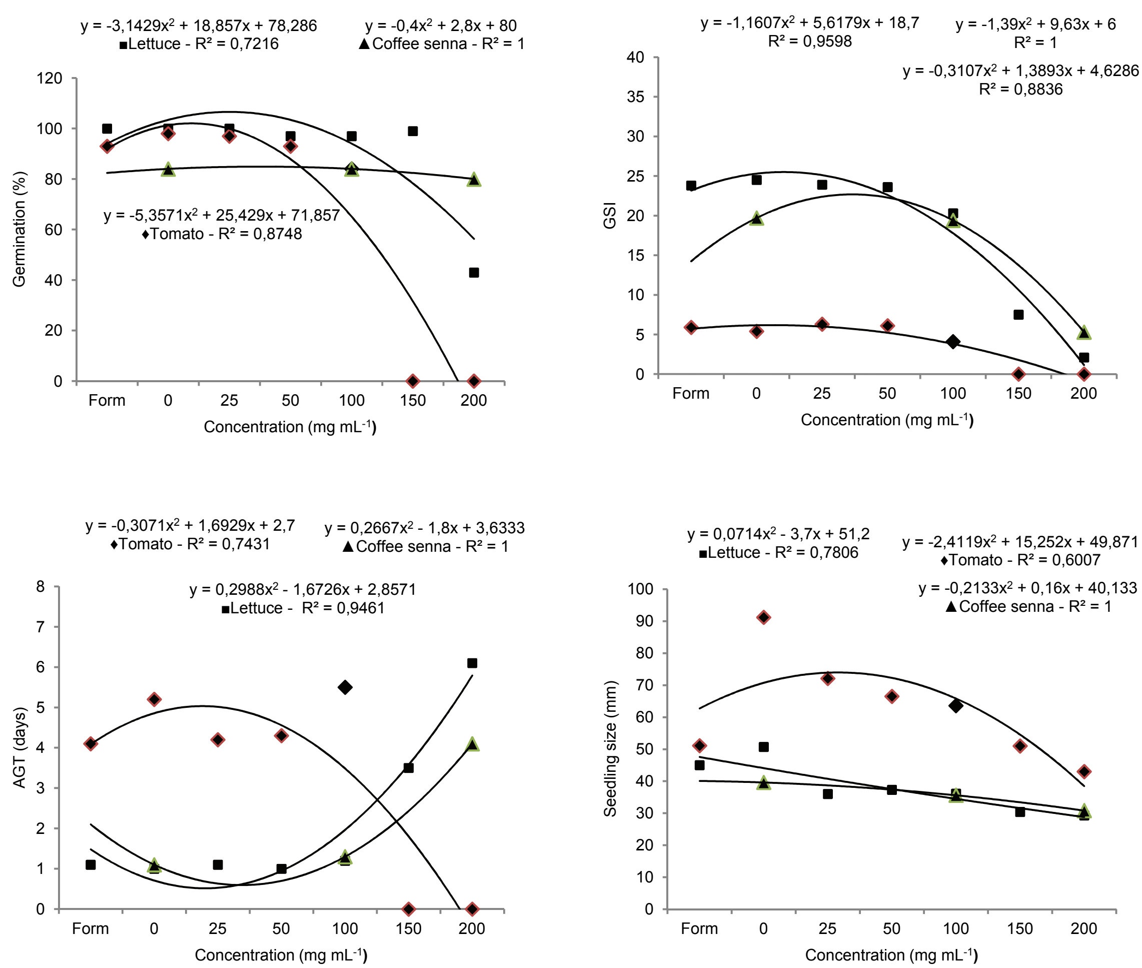


**Figure 2.** Germination (%), germination speed index (GSI) and average germination time in days (AGT) of lettuce, tomato and coffee senna seeds and the size (mm) of seedlings (root and aerial part) in the presence of 0, 25, 50, 100, 150, or 200 mg $mL^{-1}$ CNSL or the formulation, both from *A. occidentale*.

**Table 1.** Germination (%), germination speed index (GSI) and average germination time in days (AGT) of lettuce (lett) and tomato (tomt) seeds and size (mm) of seedlings (root and aerial part) in the presence of 0, 25, 50, 100, 150, or 200 mg $mL^{-1}$ CNSL or the formulation, both from *Anacardium occidentale*

| Extract | Germination (%) | | GSI | | AGT (days) | | Size | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | lett | tomt | lett | tomt | lett | tomt | lett-root | lett-stem | tomt-root | tomt-stem |
| Formulation | 100 a | 93 ab | 23.8 a | 5.9 a | 1.1 a | 4.1 a | 42.1 b | 2.9 b | 31.6 bc | 19.5 c |
| 0 | 100 a | 98 a | 24.5 a | 5.4 a | 1.0 a | 5.2 a | 47.5 a | 3.2 b | 59.7 a | 31.5 a |
| 25 | 100 a | 97 a | 23.9 a | 6.3 a | 1.1 a | 4.2 a | 32.2 b | 3.8 b | 38.4 b | 33.7 a |
| 50 | 97 a | 93 ab | 23.6 a | 6.1 a | 1.0 a | 4.3 a | 32.0 b | 5.3 a | 31.8 bc | 34.7 a |
| 100 | 97 a | 84 b | 20.3 b | 4.1 b | 1.2 a | 5.5 a | 30.5 b | 5.6 a | 27.0 c | 36.6 a |
| 150 | 99 a | 0 c | 7.5 c | 0 c | 3.5 b | 0 b | 24.9 c | 5.5 a | 19.8 d | 31.2 a |
| 200 | 43 b | 0 c | 2.1 c | 0 c | 6.1 c | 0 b | 23.9 c | 5.4 a | 17.6 d | 25.4 b |

*Means followed by the same letter in a column are not statistically different from each other by Tukey test 5% probability.

**Table 2.** Germination (%), germination speed index (GSI), average germination time in days (AGT) of coffee senna seeds and the size (mm) of seedlings (root and stem in the presence of 0, 100, or 200 mg $mL^{-1}$ CNSL)

| Extract (mg $mL^{-1}$) | Germination (%) | GSI | AGT (days) | Root | Stem |
|---|---|---|---|---|---|
| 0 | 84 a | 19.7 a | 1.1 a | 26.2 a | 13.4 a |
| 100 | 84 a | 19.4 a | 1.3 a | 23.5 a | 12.1 a |
| 200 | 80 a | 5.3 b | 4.1 b | 17.5 b | 13.3 a |

*Means followed by the same letter in the column are not statistically different from each other by Tukey test 5% probability.

Zamberlam et al. (2012) described long side chain phenolic compounds and hydrophobic (phenolic lipids), which are natural compounds found in Anacardiaceae that have attracted the interest of several research groups searching for natural herbicides; among the compounds tested, several demonstrated remarkable chemical activity, including cytosporone A that inhibited germination and growth of seedlings.

A study by Tuan Noorfatiehah et al. (2011) with *A. occidentale* (leaf and root aqueous extracts at 10, 50, and 100 g $L^{-1}$) on the effects on the germination and growth of *Zea mays* L. and *Cucumis sativus* L. indicated that with the increasing concentrations, the percentage of germinations and primary root lengths were reduced.

Nwokeocha and Ezumah (2015) also identified negative effects on the germination of *Z. mays* using leaves and stem barks extracts of *A. occidentale*, and the rate of inhibition increased with increases in the concentration of the extracts of 25%, 50%, and 75%, revealing that the leaves and stem bark of the cashew plant contained substances that could inhibit the sprouting of seeds.

These results demonstrate that the presence of allelopathic compounds is capable of adversely affecting other species.

The results also indicated that the coffee senna, despite being considered an invasive species, is sensitive to chemical compounds present in the extracts, which increased the germination time at 200 mg $mL^{-1}$ CNSL (Figure 2, Table 2). Thus, the use of these products at high concentrations would cause indirect damage to other species in the environment, which requires cautious use

Periotto, Gualtieri, and Lima (2012) evaluated the allelopathic potential of 0, 4, 8, 12, and 16% (w/v) aqueous extracts from *Anacardium humile* Mart. stems and leaves on the germination of radish and lettuce. Only the radish seeds were affected by the 8% aqueous extracts. The rate of germination in lettuce seeds was significantly reduced using leaf extracts at all concentrations. Leaf extracts (8, 12, and 16%) and stems extracts (8%) also caused significant reductions in the rate of germination of the radish seeds.

The results demonstrated the allelopathic potential of species of this genus, indicating the presence of allelochemicals with a capacity to affect the germination of the target species.

Other researchers, such as Silva, Martim, Silva, Young, and Ladeira (2006), identified the allelopathic potential in the leaves of an arboreal species of Cerrado [*Ouratea spectabilis* (Mart.) Engl., *Qualea grandiflora* Mart., *Pouteria ramiflora* (Mart.) Radlk., and *Stryphnodendron adstringens* (Mart.) Coville] on germination and concluded that the inhibitory activity was the result of a mixture of substances with different polarities.

Regarding the development of the lettuce and tomato seedlings, both root systems were negatively affected at the 25 mg $mL^{-1}$ concentration of CNSL, and the formulation also damaged root development of the lettuce and tomato (Figure 2, Table 1). The coffee senna root system was affected by 200 mg $mL^{-1}$ CNSL (Figure 2, Table 2).

Again, the results indicated that coffee senna, a weed species, was sensitive to chemical compounds that negatively affected the growth of the root system.

Similar results presented by Periotto et al. (2012) evaluated the allelopathic potential of aqueous extracts from *A. humile* on the seedling growth of radish and lettuce. Lettuce and radish demonstrated a significant inhibition of total length when stem extracts were used. Leaf extracts (4%

and 12%) significantly inhibited growth in radish seedlings, demonstrating the potential use of species in this genus.

Reigosa, Gomes, Ferreira, and Borghetti (2013) described the negative effects of chemical compounds that were observed during germination and seedling growth as germination was less sensitive than the growth of the seedlings and root necrosis, one of the more common symptoms, which were partially observed in the tests carried out.

The data obtained indicated that the root systems of the species tested were more affected than the aerial part. This behavior pattern was described by Hagemann et al. (2010), who wrote that the root system is more sensitive to chemical metabolites due to its rapid stretching, which depends on the cell division process that is affected by the presence of metabolites. Additionally, the roots receive a longer exposure to metabolites as they are the first structure to emerge, and the compounds must be transferred through the roots before reaching the aerial parts, which is another factors that negatively influences development.

Similar results were reported by Oliveira, Pereira, Muller, and Matias (2014b) with greater effects on the growth than the germination using extracts from *Ocotea odorifera* (Vell.) Rohwer.

Data from Hagemann et al. (2010) indicated that the use of extracts of aerial parts of *Avena sativa* L. and *A. strigosa* Schreb caused a reduction in both germination and growth of roots and hypocotyl using *Lolium multiflorum* Lam. and *Euphorbia heterophylla* L.

According to Reigosa et al. (2013), certain chemical compounds inhibit germination and growth by interfering with cell division, membrane permeability and the activation of enzymes. In the case of CNSL and the formulation, the effect on the tested species was related to the phenolic lipids (anacardic acid and decomposition products). Phenolic lipid action mechanisms are not yet understood, but Guimarães, Momesso, and Pupo (2010) and Dourado et al. (2015) described these effects on bacteria and *Aedes aegypti* larvae, respectively.

Based on this information, the effect of phenolic lipids occurs by presenting an aliphatic side chain with nonpolar (hydrophobic) and polar (hydrophilic) characteristics, which corresponds to the hydroxyl and carboxyl groups, respectively (Mazzetto et al., 2009). The aliphatic chain promotes the permeation of phenolic lipids by the lipid bilayer of the seed (lipoprotein membrane), affecting the permeability. Inside the cell, the polar groups of the phenolic lipids can act on protein amino acid residues, deactivating them (Guimarães et al., 2010).

Physiological and morphological responses of seeds or seedlings to chemical compounds are secondary manifestations resulting from molecular and cellular alterations, whose mechanisms still remain unclear (Reigosa et al., 2013). Likewise, the chemical profiles of most species tested in bioassays are also not available in the literature. Thus, the physio-chemical characterization of plant extracts used in these bioassays is important to inform the biological effects observed.

Periotto et al. (2012) also wrote that bioassays are important for understanding and demonstrating allelopathy and to evaluate the phytotoxicity of active fractions of allelopathic compounds. The same authors also wrote that the presence of allelopathic compounds in plants does not necessarily imply allelopathic potential because allelopathic action in the field depends on additional factors, such as soil microorganisms. The action of microorganisms can cause the degradation of phytotoxins or render nontoxic compounds toxic.

However, authors such as Parayil, Honey, and Abhilash (2013) indicated that the allelopathic potential of this species was very large, recommending that other cultures, such as *Vigna unguiculata* (L). walp should be maintained near *A. occidentale* crop fields. The researchers indicated that by increasing the concentration of leaf extract there was a decrease in germination, shoot and root length, leaf area, fresh and dry weight, and stomatal index.

## Conclusion

CNSL and the formulation had negative effects on the seeds of the species tested, to various degrees, indicating its chemical potential due to anacardic acid and cardol constituents and demonstrating that the use of these compounds under natural conditions may affect the survival of other species. Therefore, for use in the field, more studies are required to evaluate its environmental effects.

## Acknowledgements

The authors are grateful to the National Council for Scientific and Technological Development (CNPq) for providing the Scientific Start-up Grant (PIBIC) and the present research grant (PQ2) and to the Coordination for the Improvement of Higher Education Personnel (Capes) for the master and doctoral level scholarships. We would also like to thank the

Pantanal Research Centre (CPP), National Institute of Science and Technology in the Wetlands (INAU), National Council for Scientific and Technological Development (CNPq/MCT), Foundation to Support the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT) and the University Anhanguera-Uniderp for funding the GIP project (Interdisciplinary Research Group).

## References


Agostini-Costa, T. S., Dos Santos, J. R., Garruti, D. D. S., & Feitosa, T. (2000). Caracterização, por cromatografia em camada delgada, dos compostos fenólicos presentes em pedúnculos de caju (*Anacardium ocidentale* L.). *Boletim do Centro de Pesquisa de Processamento de Alimentos*, *18*(1), 129-137.

Agostini-Costa, T. S., Jales, K. A., Garruti, D. S., Padilha, V. A., Lima, J. B., Aguiar, M. J., & Paiva, J. R. (2004). Teores de ácido anacárdico em pedúnculos de cajueiro *Anacardium microcarpum* e em oito clones de *Anacardium occidentale* disponíveis no Nordeste do Brasil. *Ciência Rural*, *34*(4), 1075-1080.

Andrade A. P. S., Oliveira, V. H., Innecco, R., & Silvas, E. O. (2008). Qualidade de cajus-de-mesa obtidos nos sistemas de produção integrada e convencional. *Revista Brasileira de Fruticultura*, *30*(1), 176-179.

Asogwa, E. U., Mokwunye, I. U., Yahaya, L. E., & Ajao, A. A. (2007). Evaluation of cashew nut shell liquid as a potential natural insecticide against termites (soldiers and workers caste). *Research Journal of Applied Sciences*, *2*(9), 939-942.

Ceruks, M., Romoff, P., Fávero, O. A., & Lago, J. H. G. (2007). Constituíntes fenólicos polares de *Schinus terebinthifolius* Raddi (Anacardiaceae). *Química Nova*, *30*(3), 597-599.

Chaves, M. H., Citó, A. M. G. L., Lopes, J. A. D., Costa, D. A., Oliveira, C. A. A., Costa, A. F., & Brito Júnior, F. E. M. (2010). Total phenolics, antioxidant activity and chemical constituents from extracts of *Anacardium occidentale* L., Anacardiaceae. *Revista Brasileira de Farmacognosia*, *20*(1), 106-112.

Correia, S. J., David, J. P., & David, J. M. (2006). Metabólitos secundários de espécimes de Anacardiaceae. *Química Nova*, *29*(6), 1287-1300.

Dourado, D. M., Rosa, A. C., Porto, K. R. A., Roel, A. R., Cardoso, C. A. L., Favero, S., & Matias, R. (2015). Effects of cashew nut shell liquid (CNSL) componente upon *Aedes aegypti* Lin. (Diptera: Culicidae) larvae´s midgut. *African Journal of Biotechnology*, *14*(9), 829-834.

Gonzales, J. L. S., Paula, R. C., & Valeri, S. V. (2009). Teste de condutividade elétrica em sementes de *Albizia hassleri* (Chodat) Burkart. Fabaceae-Mimosoideae. *Revista Árvore*, *33*(4), 625-634.

Guimarães, D. O., Momesso, L. S., & Pupo, M. T. (2010). Antibióticos: importância terapêutica e perspectivas para a descoberta e desenvolvimento de novos agentes. *Química Nova*, *33*(3), 667-679.

Hagemann, T. R., Benin, G., Lemes, C., Marchese, J. A., Martin, T. N., Pagliosa, E. S., & Beche, E. (2010). Effect of extracts from the above-ground part of oat genotypes on ryegrass and wild poinsettia. *Bragantia*, *69*(3), 509-518.

Kamath, V., & Rajini, P. S. (2007). The efficacy of cashew nut (*Anacardium occidentale* L.) skin extract as a free radical scavenger. *Food Chemistry*, *103*(2), 428-433.

Labouriau, L. G., & Agudo, M. (1987). On the physiology of seed germination in Salvia hispanica L. I - Temperature effects. *Anais da Academia Brasileira de Ciências*, *59*(1), 37-50.

Lomonaco, D., Santiago, G. M. P., Ferreira, Y. S., Arriaga, Â. M. C., Mazzetto, S. E., Mele, G., & Vasapollo, G. (2009). Study of technical CNSL and its main components as new green larvicides. *Green Chemistry*, *4*(11), 31-33.

Lorenzi, H., & Matos, F. J. A. (2008). *Plantas medicinais no Brasil: nativas e exóticas* (2nd ed.). Nova Odessa, SP: Plantarum.

Maguire, J. D. (1962). Speed of germination aid in selection and evaluation for seedling and vigour. *Crop Science*, *2*(2), 176-177.

Martinotto, F., Martinotto, C., Coelho, M. D. F. B., Azevedo, R., & Albuquerque, M. C. F. (2012). Sobrevivência e crescimento inicial de espécies arbóreas nativas do cerrado em consórcio com mandioca. *Pesquisa Agropecuária Brasileira*, *47*(1), 22-29.

Mazzetto, S. E., Lomonaco, D., & Mele, G. (2009). Óleo da castanha de caju: oportunidades e desafios no contexto do desenvolvimento e sustentabilidade industrial. *Química Nova*, *32*(3), 732-741.

Mendonça, F. A. C., Silva, K. F. S., Santos, K. K., Ribeiro Júnior, K. A. L., & Sant'Ana, A. E. G. (2005). Activities of some Brazilian plants against larvae of the *Aedes aegypti*. *Fitoterapia*, *76*(7), 629-636.

Nwokeocha, O. W., & Ezumah, B. S. (2015). Effect of cashew leaves and stem-bark extracts on the germination of maize. *International Journal of Plant & Soil Science*, *4*(5), 494-498.

Oliveira, A. K. M., Matias, R., Lopes, S. S., & Fontoura, M. F. (2014a). Alelopatia e a influência de folhas de *Palicourea rigida* (Rubiaceae) na germinação de sementes e na formação de plântulas de alface. *Bioscience Journal*, *2*(2), 938-947.

Oliveira, A. K. M., Pereira, K. C. L., Muller, J. A. I., & Matias, R. (2014b). Análise fitoquímica e potencial alelopático das cascas de *Pouteria ramiflora* na germinação de alface. *Horticultura Brasileira*, *32*(1), 41-47.

Oliveira, W. O., & Petrovick, P. R. (2010). Secagem por aspersão (*spray drying*) de extratos vegetais: bases e aplicações. *Revista Brasileira de Farmacognosia*, *20*(4), 641-650.

Parayil, S. P., Honey, & Abhilash, E. S. (2013). Study on the effect of aqueous leaf extract of *Anacardium* *occidentale* on seed germination and seedling growth of *Vigna unguiculata*. *Asia Pacific Journal of Environment Ecology and Sustainable Development*, *1*(1), 10-14.

Periotto, F., Gualtieri, S. C. J., & Lima, M. I. S. (2012). Allelopathic potential of *Anacardium humile* Mart. and their effects on seed germination and early development of seedlings. *Revista Eletrônica Científica Inovação e Tecnologia*, *2*(6), 2-14.

Porto, K. R. A., Roel, A. R., Machado, A. A., Cardoso, C. A. L., & Oliveira, J. M. (2013). Atividade inseticida do líquido da castanha de caju sobre larvas de *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). *Revista Brasileira de Biociências*, *11*(4), 419-422.

Pozitano, M., & Rocha, S. C. S. (2011). Caracterização física e germinação de sementes de *Senna macranthera*. *Revista Brasileira de Sementes*, *33*(4), 777-784.

Reigosa, M., Gomes, A. S., Ferreira, A. G., & Borghetti, F. (2013). Allelopathic research in Brazil. *Acta Botanica Brasilica*, *27*(4), 629-646.

Risfaheri, T. T., Anwar Nur, M., & Sailah, I. (2009). Isolation of cardanol from cashew nut shell liquid using the vacuum distillation method. *Indonesian Journal of Agriculture*, *2*(1), 11-20.

Rizzi, E. S., Pereira, K. C. L., Abreu, C. A. A., Silva, B. C. F. L., Fernandes, R. M., Oliveira, A. K. M., & Matias, R. (2016). Allelopathic potential and phytochemistry of cambarazinho (*Vochysia haenkeana* (Spreng.) Mart.) leaves in the germination and development of lettuce and tomato. *Bioscience Journal*, *32*(1), 98-107.

Rodrigues, F. H. A., Feitosa, J., Ricardo, N. M., França, F. C. F. D., & Carioca, J. O. B. (2006). Antioxidant activity of cashew nut shell liquid (CNSL) derivatives on the thermal oxidation of synthetic cis-1, 4-polyisoprene. *Journal of the Brazilian Chemical Society*, *17*(2), 265-271.

Salomão, R. P., Brienza Júnior, S., & Rosa, N. A. (2014). Reforestation dynamics in areas of restoration after mining in unit of forest conservation in the Amazon. *Revista Árvore*, *38*(1), 1-24.

Shaalan, E. A. S., Canyon, D., Younes, M. W. F., Abdel-Wahab, H., & Mansour, A. H. (2005). A review of botanical phytochemicals with mosquitocidal potential. *Environment International*, *31*(8), 1149-1166.

Silva, G. B., Martim, L., Silva, C. L.; Young, M. C. M. & Ladeira, A. M. (2006). Potencial alelopático de espécies arbóreas nativas do Cerrado. *Hoehnea*, *33*(3), 331-338.

Soltys, D., Bogatek, R., & Gniazdowska, A. (2012). Phytotoxic effects of cyanamide on seed germination and seedling growth of weed and crop species. *Acta Biologica Cracoviensia. Series Botanica*, *54*(2), 87-92.

Tuan Noorfatiehah, T. K., Nashriyah, M., Hasbullah, M., Raja Danial, R. I., & Muhamad Azhar, A. W. (2011). Allelopathic effects of *Anacardium occidentale* Linn of Terengganu and Kelantan on the growth of maize and cucumber. *International Research Journal of Applied and Basic Science*, *2*(5), 175-180.

Vieira, T. A., Rosa, L. D. S., Vasconcelos, P. C. S., Santos, M. M. D., & Modesto, R. D. S. (2007). Sistemas agroflorestais em áreas de agricultores familiares em Igarapé-Açu, Pará: caracterização florística, implantação e manejo. *Acta Amazonica*, *37*(4), 491-501.

Zamberlam, C. E. M., Meza, A., Leite, C. B., Marques, M. R. L., Dênis, P. L., & Beatriz, A. (2012). Total synthesis and allelopathic activity of cytosporones A-C. *Journal of the Brazilian Chemical Society*, *23*(1), 124-131.

Zeng, R. S., Mallik, A, U., & Luo, S. (2010). *Allelopathy in sustaintable agriculture and forestry*. New York, USA: Springer-Verlag.

*Received on July 6, 2016.*
*Accepted on December 7, 2016.*