Allelopathic effect of essential oils of medicinal plants in *Bidens pilosa* L.

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ABSTRACT: We determined the inhibitory allelopathic effects of the volatile extracts of *Cinnamomum zeylanicum* Ness*, Lippia sidoides* Cham*.* and *Cymbopogum nardus* L. on seed germination and root growth of seedlings of *Bidens pilosa*. The experiment was conducted at the Seed Analysis Laboratory of the Department of Plant Science, Federal University of Ceará. For this end, we used oils at the concentrations of 0.01, 0.02, 0.04 and 0.08% (v/v). Five treatments were used for each of the oils arranged in a completely randomized design with four replications of 25 seeds. The seeds were sown in Petri dishes lined with filter paper moistened with distilled water and, aiming at the indirect contact with each oil, two sheets of filter paper were placed on top of the lid, in which three (3) mL of each oil solution were added. Then, the dishes were incubated in a germination chamber at 25°C. The pH did not contribute to alter the results; the volatile extracts of essential oils of *C. zeylanicum*, *L. sidoides* and *C. nardus* inhibited seed germination and root growth of seedlings of *B. pilosa*, which shows allelopathic potential; and the concentration of 0.08% of oils caused the overall deterioration of the roots and death of seedlings of *B. pilosa*.

Key words: allelopathy, *Cinnamomum zeylanicum* Ness, *Cymbopogum nardus* L., herbicides, *Lippia sidoides* Cham.

RESUMO: Efeito Alelopático de oleos essencias de plantas medicinais em *Bidens pilosa* L. Determinou-se o efeito alelopático inibitório dos extratos voláteis de *Cinnamomum zeylanicum* Ness, *Lippia sidoides* Cham. e *Cymbopogum nardus* L., sobre a germinação de sementes e o crescimento de raiz de plântulas de *Bidens pilosa*. O experimento foi conduzido no Laboratório de Análises de Sementes do Departamento de Fitotecnia da Universidade Federal do Ceará. Para isso, utilizaram-se óleos essenciais nas concentrações 0,01, 0,02, 0,04 e 0,08%. (v/v). Foram utilizados cinco tratamentos para cada óleo dispostos em delineamento inteiramente casualizado com quatro repetições de 25 sementes. As sementes foram semeadas em placas de Petri forradas com papel de filtro umedecido com água destilada e, visando o contato indireto com cada óleo, foram colocadas duas folhas de papel de filtro na parte superior interna da tampa, onde foi adicionado 3(três) mL da solução de cada óleo. Em seguida, as placas foram incubadas em câmara de germinação a 25ºC. O pH não contribuiu para alterar os resultados; os extratos voláteis de óleos essenciais de *C. zeylanicum*, *L. sidoides* and *C. nardus* inibiram a germinação de sementes e o crescimento da raiz de plântulas de *B. pilosa*, evidenciando potencial alelopático; a concentração de 0,08% dos óleos causaram a deterioração total das raízes e morte das plântulas de *B. pilosa*.

Palavras-chave: alelopatia, *Cinnamomum zeylanicum* Ness, *Lippia sidoides* Cham., herbicidas*, Cymbopogum nardus* L.

INTRODUCTION

The presence of weeds in crops is always a concern of farmers, attributing the damage they have caused by competition and disease problems. Thus the use of synthetic herbicides has become a very common practice for the control of weeds herbs in Brazilian pastures. Nonetheless, these procedures have been demonstrated to be ineffi cient (Almeida, 1988; Souza Filho et al, 2006). Thus,

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synthetic herbicides currently used in agriculture, despite its importance for the control of weeds, there is extensive discussion of the damage they cause to both man and the environment (Fonseca et al., 2012).

Furthermore, the indiscriminate use of herbicides and excessive increasingly contribute to the weeds develop different mechanisms of resistance to synthetic products, making increasing dependence on these inputs. Currently, there are 249 species worldwide, with biotypes resistant to herbicides. In Brazil, to date, seven species were found to resistance among these *Bidens pilosa* L. (Vidal & Merotto, 2001).

The use of chemical herbicides, second Pleban et al. (1998), has caused world concern over the environment and public health. Accordingly it is important to alternatives for controlling weeds. An alternative suggested by the author, to control weeds, is the development and application of control measures with plant products. However, the control of these species should not be made with the intention to eradicate them completely, since several studies have confirmed its beneficial effects, both in agriculture (allelopathy), and human health (Gevilanes et al. 1988; Almeida, 1988).

Several medicinal plants are mentioned in the literature, because they have an allelopathic effect on seed germination and seedling growth of weed species, among which we mention *Raphanus sativus* L. (Gianfrancisco et al. 1998; Cruz et al. 2000), *Ocotea odorifera* (Vell.) Rohwer (Carmo et al. 2007), *Azadirachta indica* A. Juss. (France, et al. 2008); *Joanesia princeps* (Vell.), *Casearia sylvestris* Sw (Capobiango et al. 2009).

The *Bidens pilosa* is considered a weed, widely distributed throughout the world, being a cosmopolitan tropical. It is a dicotyledonous plant, difficult to control because it presents the annual short cycle, with several generations during the year. An alternative that has been used to assist conventional control methods is the use of allelopathy (Vidal et al. 2001).

Thus, this study aimed to determine possible allelopathic effects of volatile extracts of essential oils of *Cinnamomum zeylanicum* Ness (Lauraceae), *Lippia sidoides* Cham. (Verbenaceae) and *Cymbopogon nardus* (L.) Rendle (Poaceae) on germination and radicle length of seedlings of *B. pilosa* L.

MATERIALS AND METHODS

The test was conducted at the Seed Analysis Laboratory of the Center for Agrarian Sciences, Federal University of Ceará, in Fortaleza, using seeds *B. pilosa* and submitted to the effect of essential oils from three medicinal species: *Cinnamomum zeylanicum* Nees, *Lippia sidoides* Cham and *Cymbopogon nardus* L. Rendle.

The collection of seeds *B. pilosa* was held for existing plants in the garden of the Centre for Agrarian Sciences, Federal University of Ceará in Fortaleza. After processing in South Dakota type blower for removing deteriorated seeds were empty, the seeds were sterilized with sodium hypochlorite, 1% (v/v) for fifteen minutes and then treated with Captan (250 g/100 kg seed).

The leaves of *L. sidoides* Cham. and *C. nardus* grass were collected in the morning (between 8 and 9 AM) at the Experimental Farm Valley Curu, municipality of Pentecoste-CE. Meantime, the oil of *C. zeylanicum* bark was acquired in commerce of Fortaleza. The essential oils were extracted from leaves of adult plants, through the process of extraction by steam distillation of water, following the methodology described by Alencar et al. (1990). The oils obtained were placed in dark bottles and stored in a refrigerator at 5 º C. Thereafter, each oil was emulsified with Tween 80, at the ratio 1:1 and dissolved in distilled water to obtain solutions at concentrations of 0.01, 0.02, 0.04 and 0.08% (v/v). These concentrations constituted the treatments. For the concentration of 0.0, the solution Tween 80 was used at 1.0% v/v. The pH of each solution was calculated (Table 1).

The allelopathic potential of volatile extracts of concentrations of each oil on the *B. pilosa* the seedlings was subjected to the following determinations:

a) Germination - Four replications of 25 seeds were sown on Petri dish (9 cm diameter) and as substrate three sheets of filter paper moistened with a quantity of distilled water equal to three times the mass of the dry paper. Soon after seeding, 3 mL of each oil in their respective concentrations were divided into two filter papers, glued to the cover plate, to promote indirect contact. Then these plates were placed in a germination chamber at 25°C with a photoperiod of 8 hours of light and germination percentage assessed on the fourth day after installation. Were considered germinated seeds that showed radicle protrusion;

b) inhibition of germination - obtained by subtracting the number of germinated seeds of the control treatment and the number of germinated seeds of each treatment, divided by the number of germinated seeds of control according to the formula below, the results being expressed as a percentage.

$$IG = \frac{G - TG}{G} \text{ x } 100 \qquad \text{Where:}$$

IG → Inhibition of germination;
GC → Number of seeds germinated

in control treatment;

GT → Number of seeds germinated in each treatment.

c) Radicle Length - Before starting the test of the root length, proceeded to break dormancy of seeds, putting them to germinate on Petri dish 9 cm in diameter on three filter papers moistened with 3mL of solution of GA3 (200 ppm) and conditioned at a 0^{o}C for 24 hours. Later they were transferred to a germination chamber under constant temperature of 25 ° C and a photoperiod of 8 hours of light. Installed in a similar manner to germination, after 48 hours was selected sixty seedlings (4 replicates of 15) showed 0.5 cm radicle. Then these seedlings were transferred into Petri dish containing the solutions related to treatment and returning to a germination chamber. After four days of incubation, the length of the primary root of each seedling was measured and the average results expressed in centimeters per plant.

The experimental design was completely randomized, with four repetitions. The data were subjected to analysis of variance and when the effects of treatments showed significant differences (P <0.01), The averages were compared using the non-linear regression test, according with Ferreira (1996).

RESULTS AND DISCUSSION

The evaluation of pH and osmotic potential of essential oils is fundamental when ignores its incorporation into sugars, amino acids, organic acids, ions and other molecules, for extreme values of both pH and the osmotic potential of the oils can act on the seeds and / and mask, or seedling allelopathic effect (Ferreira & Aquila, 2000).

The pH of the tested essential oils ranged from 5.52 to 6.93 (Table 1) had low acidity. Similar results were obtained by Silva et al. (2013), when used alcoholic extracts of *Piper hispidinervium* in laboratory bioassays. According Aquila (2000), the pH remained within acceptable standards in what is considered suitable for germination and early growth.

The allelopathic of *C. zeylanicum* oil on seeds *B.pilosa* can be seen in Figure 1. Note that the concentration of 0.01% of *C. zeylanicum* oil inhibited germination of 75.7% in *B. pilosa* (Figure 1b). Regarding the growth of the root, there is shown in Figure 1c, the result being inversely proportional to the concentration to 0.08% of the oil showed necrotic roots, possibly due to tissue deterioration and no increase in length. In Figures 1a and 1b it appears that *C. zeylanicum* oil causes much stronger inhibitory effect on seed germination, and root length. Similar results were observed by Borges et al. (2007), where there was also a reduction of the germination and root growth of *Mimosa pudica*, with increasing concentration Surinamesina (compound obtained from leaves *Virola surinamensis*).

It was observed that, when subjected to a 0.02% concentration of oil of *C. zeylanicum* roots *B. pilosa* presented with elongated, but with abnormal development, necrotic and without the secondary roots. Wandscheer (2008) noted that lettuce seeds submitted to treatments with leaf extracts of wild radish, there was a visible darkening of rootlets and consequently the degradation of their tissues. Some allelopathic substances can induce the appearance of abnormal seedlings, and radicle necrosis of the most common (Ferreira & Borguetti, 2004).

Although the literature on this information is uncommon, it can be inferred from the results that the inhibitory effect of germination and root growth is possibly due to the presence of *C. zeylanicum*, requiring further studies.

Results of percentage of germination, seeds do not germinate and inhibitions of seed germination *B. pilosa* subjected to *L. sidoides* oil are shown in Figures 2. It was found that the concentration of 0.01% (Figure 2b) provided a percent inhibition greater than 50% compared to control, and that the progressive increase in this percentage was up to a concentration of 0.08% to 84% inhibition. Regarding the growth of the root (Figure 2c), there was started to decrease from the concentration of 0.01% compared to control (1.00cm and 1.38 cm, respectively) to values close to zero for 0,04% (0.04 cm) from oil of *L. sidoides*.

Looking at Figures 2b and 2c, it is noted that the *L. sidoides* oil, shows the inhibitory effect on seed germination and root growth of *B. pilosa*. According Craveiro et al. (1981) *L. sidoides* oil, shows a return of 60% of thymol, a monoterpene. The data suggest that lead to the thymol, probably have the

TABLE 1. pH of the solutions of essential oils according to the different concentrations.

	CONCENTRATION (%)			
OIL	0,01	0,02	0,04	0,08
Cinnamomum zeylanicum	6,32	6,59	5,68	5,52
Lippia sidoides	6,93	6,41	6,03	5,93
Cymbopogon nardus	5,61	5,65	5,67	5,80

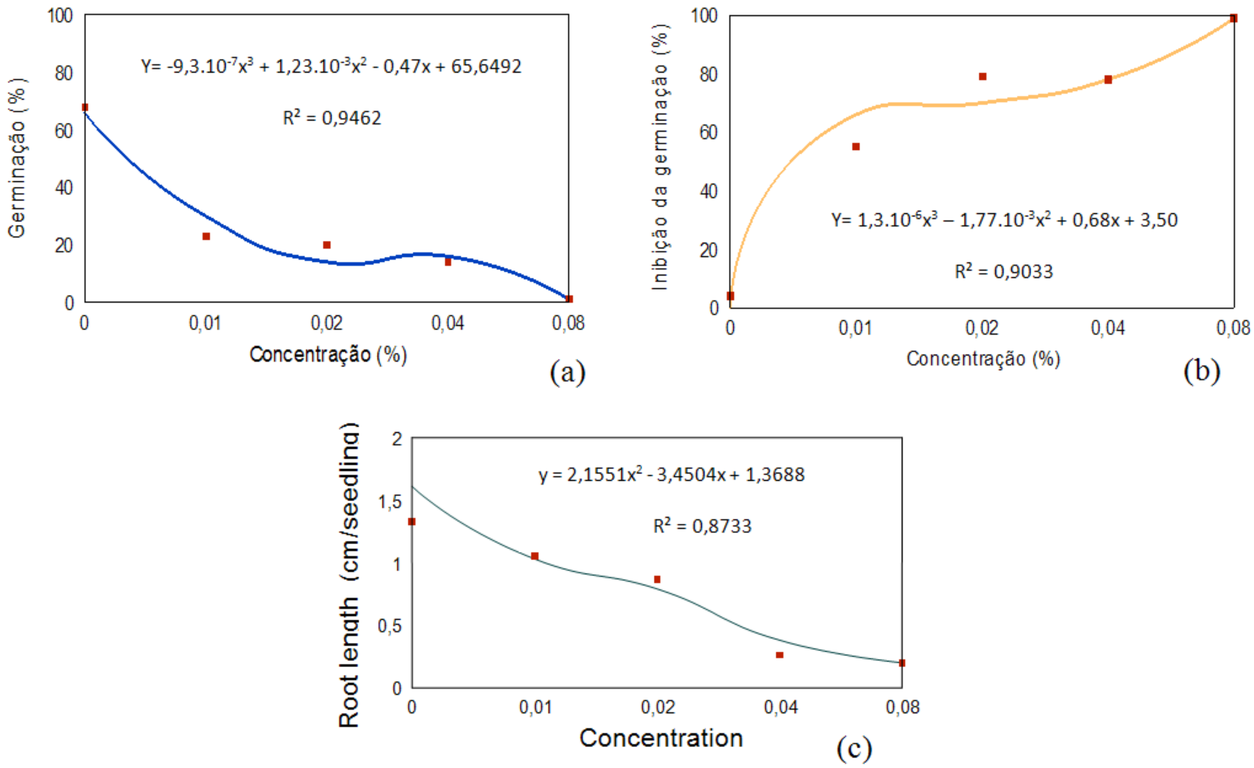

FIGURE 1. Percentage of germination (a), inhibition of germination (b) and root length (c) of *B. pilosa* for different concentrations essential oil of *C. zeylanicum*.

inhibitory effect. Souza Filho et al. (2009), working with essential oils of plants *Piper hispidinervium* C.DC. and *Pogostemon heyneanus* Benth, observed the allelopathic activity at low concentrations of oils on weeds such as Malice (*Mimosa pudica*) and forest-pasture (*Senna obtusifolia*), apparently the allelopathic activity of essential oils may be related to the presence of monoterpenes, oxygenated monoterpenes and sesquiterpenes.

In figure 3, shows the allelopathic effect of essential oil of *C. nardus* on seed germination and seedling growth of *B. pilosa*. When analyzing the inhibitory effect of essential oil of *C. nardus* , it was found that the concentration of 0.08% was higher than the other treatments inhibiting the germination of *B. pilosa* (Figure 3a). It was observed that the percentage of inhibition was higher than 50% compared to control at a concentration of 0.01% (Figure 3b). However, when comparing the effect of the oil concentrations in the root length, it was found that this superiority occurred starting from the concentration of 0.04% only (Figure 3c).

In Figure 3c, it is observed that *C. nardus* oil showed an inhibitory effect on root length *B. pilosa* at concentrations of 0.01% and the effects were more intense with increasing the oil concentration. It was also found that the concentration of 0.08% was necrosis of roots, probably due to tissue degradation, preventing root growth and consequently causing the death of the seedling. Souza Filho et al. (2009) also observed that the inhibitory effects were positively associated with the concentration of the oils as much as looked at the effects on the germination and radicle *Mimosa pudica* being the maximum inhibition observed at a concentration of 1% of the oil *Piper hispidinervum* C. DC.

Juan Jimenez-Osornio et al. (1996) observed that the essential oil of *Chenopodium ambrosioides* L. at concentrations between 0.8 and 1% inhibited germination and growth hypocotyl of *Amaranthus hipocondriacus*. This author also reported that the major component is the monoterpene (ascaridol), which is responsible for phytotoxicity. When analyzed in isolation ascaridol, observed that the concentration of 0.50 microlitres / plate was the inhibition of germination and 0.55 microlitres / plate was inhibition of growth of the hypocotyl. These results find that the monoterpenes are responsible for the inhibition of germination and early seedling growth.

In general, the concentration of 0.08% of the allelopathic effects of essential oils were more drastic on root growth than on germination of *B. pilosa*. The roots of this plant showed 100% inhibition, completely oxidized and spoiled. Maraschin-Silva & Aquila (2006), Abreu (1997) and Souza Filho et al. (1997)

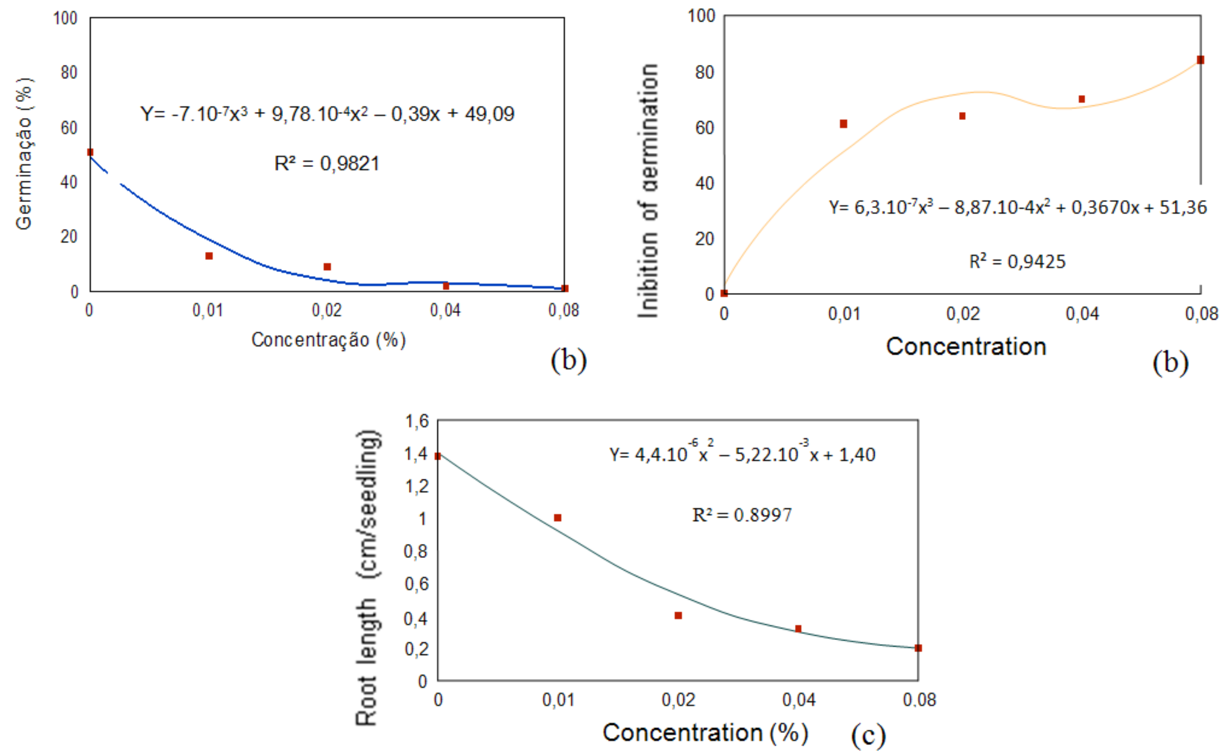

FIGURE 2. Percentage of germination (a), inhibition of germination (b) and root length (c) of *B. pilosa* for different concentrations of the essential oil of *L. sidoides.*

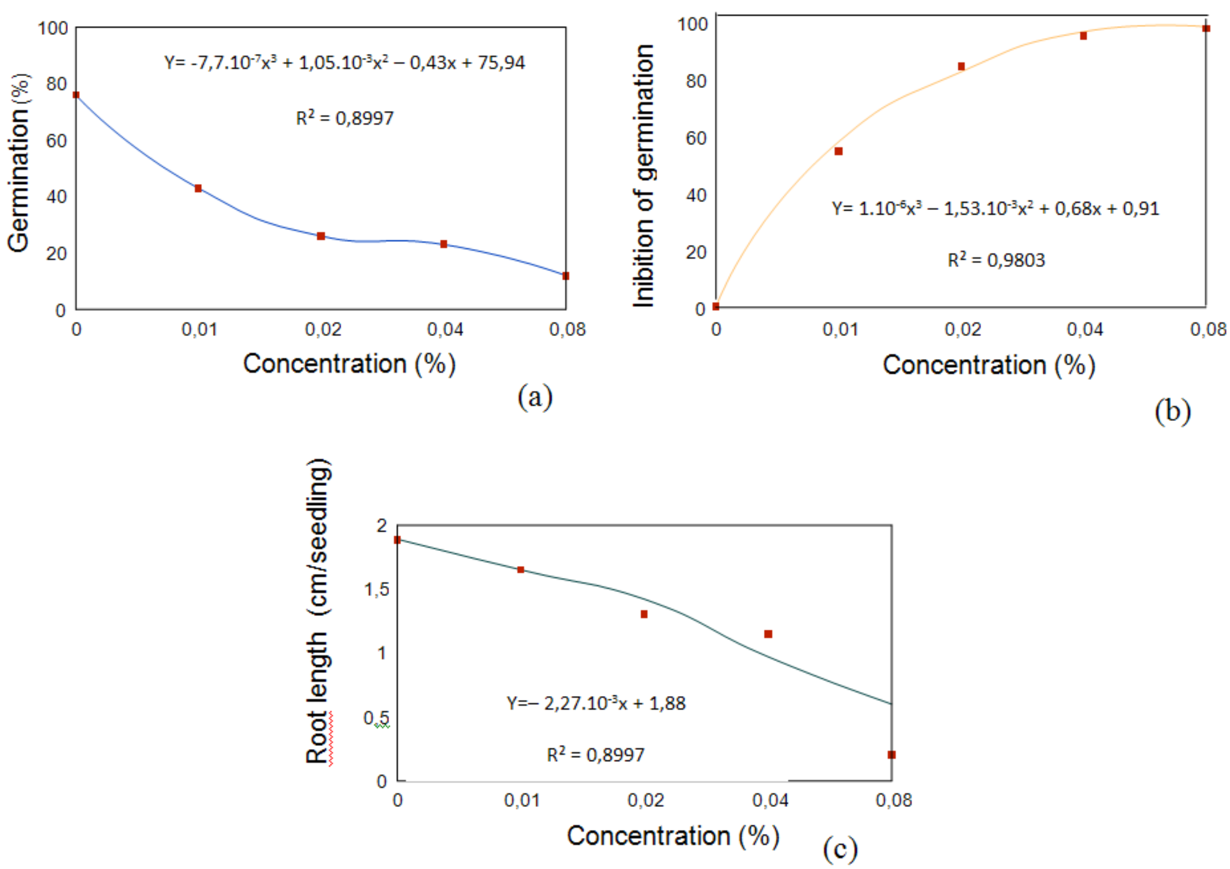

FIGURE 3. Percentage of germination (a), inhibition of germination (b) and root length (c) of *B. pilosa* for different concentrations of the essential oil of *C. nardus*

reported similar results in their experiments showing that the growth and / or seedling development are more sensitive to allelochemicals than germination. These results do not agree, however, those found in concentrations of 0.01 to 0.02% of oil of *C. zeylanicum* and *C. nardus* , where germination was more sensitive than root growth. Therefore the allelopathic effect of allelochemicals can influence a step further life cycle of the plant than in another, depending on the concentration or species donor or recipient.

It was concluded that: a) the volatile extracts of essential oils of *C. zeylanicum* , *L. sidoides* and *C. nardus* inhibit seed germination and root growth of seedlings of *B. pilosa*, showing allelopathic potential; b) the concentration of 0.08% of the oils of *C. zeylanicum* , *L. sidoides* and *C. nardus* cause overall deterioration and death of roots of seedlings of *B. pilosa*.

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