ALLELOPATHIC POTENTIAL OF *Anacardium humile* MART. AND THEIR EFFECTS ON SEED GERMINATION AND EARLY DEVELOPMENT OF SEEDLINGS

POTENCIAL ALELOPÁTICO DE *Anacardium humile* MART. E SEUS EFEITOS SOBRE A GERMINAÇÃO DE SEMENTES E NO DESENVOLVIMENTO INICIAL DE PLÂNTULAS

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ABSTRACT

In this research it was evaluated the allelopathic potential of aqueous extracts from Anacardium humile stems and leaves in seed germination and seedling growth of radish and lettuce. In the germination tests the aqueous extracts were used at 0, 4, 8, 12 and 16% (w/v) concentration. Four replicates of thirty seeds of lettuce and radish were distributed in Petri dishes covered with filter paper moistened with 5 mL different extracts, kept at 27 °C and light absence. The counting of germinated seeds was performed daily, being calculated speed and the percentage of germination. In tests measuring the germination percentage, only radish‘s seeds were affected by the aqueous extracts of leaves 8%. The speed's germination in lettuce seeds was significantly reduced using leaves extract at all concentrations. Leaf extracts 8, 12, 16% and stems extracts 8% also caused significant reductions in the speed germination of radish seeds. Growth experiments were carried out with four replicates of eight germinated seeds of lettuce and radish, at 27 °C, ten days after the germination and having filter paper as substratum, moistened with 0%, 4% and 12% of aqueous extracts. Both, lettuce and radish, presented a significant inhibition of total length when stem extracts were used. Leaves's extracts 4% and 12% also produced significant growth inhibition in radish seedlings. The extracts interference on seed germination and seedling growth of lettuce and radish was disassociated of pH and osmotic potential influence, indicating an allelopathic potential of the species studied.

Key words: allelopathy; *cerrado*; seeds germination; seedling.

RESUMO

Nesta pesquiSa, foi avaliado o potencial alelopático de extratos aquosos de caules e folhas de Anacardium humile na germinação de sementes e crescimento de plântulas de rabanete e alface. Nos testes de germinação dos extractos aquosos foram usados ??nos tempos 0, 4, 8, 12 e 16% (w / v) da concentração. Quatro repetições de 30 sementes de alface e rabanete foram distribuídos em placas de Petri forradas com papel de filtro umedecido com 5 ml diferentes extratos, mantidas a 27 oC e ausência de luz. A contagem de sementes germinadas foi feita diariamente, sendo calculada a velocidade ea porcentagem de germinação. Nos testes que medem a porcentagem de germinação, apenas sementes de rabanete `s foram afetadas pelos extratos aquosos de folhas de 8%. A velocidade de germinação de sementes de alface foi significativamente reduzida utilizando extracto de folhas em todas as concentrações. Extratos de folhas 8, 12, 16% e caules extrai 8% também provocou reduções significativas na velocidade de germinação de sementes de rabanete. Experimentos de crescimento foram realizados com quatro repetições de oito sementes germinadas de alface e rabanete, a 27 oC, 10 dias após a germinação e em papel-filtro como substrato, umedecido com 0%, 4% e 12% dos extratos aquosos. Ambos alface, rabanete e, apresentaram uma inibição significativa de comprimento total, quando extratos de caule foram utilizados.

Extratos de folhas da 4% e 12% também produziu inibição do crescimento significativo em mudas de rabanete. A interferência extratos na germinação de sementes e no crescimento de mudas de alface e rabanete foi desassociada de pH e influência potencial osmótico, indicando uma potencial alelopático das espécies estudadas.

Palavras-chave: alelopatia; cerrado; germinação de sementes; mudas.

INTRODUCTION

Anacardium humile Mart. is a native species from Anacardiaceae family occurring in *cerrado* vegetation with distribution restricted to Bahia, Mato Grosso, Mato Grosso do Sul, Minas Gerais, São Paulo states and Federal district. *A. humile* is a hermaphrodite shrub with 80 cm height, presenting calix and corolla covered by hairs. There are several lateral branches emerging from a developed xylopod that allows this species a drought tolerance (Almeida *et al*., 1998). The branches grows from September to June, in the rainy season and the species development is characterized by many old branches under ground, and the new ones, recovering the aerial part, above the ground (Figure 1).

Allelopathy involves plant chemical interactions in both natural and agro-ecosystems. These interactions are due to the release of secondary metabolites from producing plants to the environment and interfere with the metabolism of other plants (Dakshini and Inderjit, 1999; Romero-Romero *et al*., 2005).

All the plants species release allelochemicals by exudation, leaching or plant tissue decomposition. Allelochemicals are released into the environment by the plant through foliar leachate, root exudation, residue decomposition, leaf litter or volatilization (Inderjit and Nilsen, 2003) and influence various physiological processes in different plant species (Inderjit and Dakshini, 1998). These compounds can enter or affect another plant directly by uptake or indirectly by the effects of the allelochemicals on soil microorganism that increases or inhibits plant growth. The world needs for research and development of allelopathy in forestry, ecology and agriculture will be outlined. The world agricultural and forestry production as well as ecological dimensions related to population calls for global changes to be brought by allelopathy.

Allelopathic effects are typically the result of an interaction of a mixture of compounds rather than a single compound and their interactions are based primarily on the production of secondary chemicals by higher plants that produce a wide array of biochemical compounds, creating biological changes (Inderjit, 2003).

As the Brazilian flora presents a high biodiversity, the data about the allelopathic properties of *cerrado* species are still necessary and not available until now. Besides, *cerrado* vegetation areas are being quickly reduced since the last century, due to several agricultural and animal husbandry activities, and only isolated small dots are preserved. Researches about the allelopathics effects of cerrado species are also important to isolate and identify the substances that may act as natural herbicides and pesticides.

In nature, many factors are involved in an allelopathic interaction, linked either simultaneously or sequentially and the overall dynamic process. Due to the difficulty to separate competitive from allelopathic interactions, under field conditions, studies have been based upon biological assays conducted under laboratory or controlled conditions.

There are at least, three fundamental reasons to study allelochemicals. First of all, the identification of allelochemicals may help explain plant or microbial allelopathic interactions in ecological systems. A second reason is to identify allelochemicals which may have potential as herbicides or plant growth regulators or that could be used as templates for the design of other synthetic herbicides or phytoregulators. The last one, the use and/or development (breeding or genetic transformation) of crops that produce allelochemicals may be used to suppress weed growth as an alternative management practice.

germination and seedling development of lettuce confirming the hypothesis of the presence of allelopathic agents. Thus, in the present work was questioned the occurrence of allelopathic effects by aqueous extracts of aerial organs, stems and leaves of *A. humile* on seed germination and seedling growth of radish and lettuce, both used as bioindicators.

2 MATERIAL AND METHODS

2.1 BIOLOGICAL MATERIAL

Stems and leaves of *Anacardium humile* Mart. (Anacardiaceae) (Figure 1) were collected from healthy and mature plants, growing in cerrado vegetation belonging to the *campus* of São Carlos Federal University, São Paulo state, Brazil, (21°58‘S, 47°53‘W at 868 m sea level). The experiments were conducted at the seed germination laboratory of the Botany Department.

Figure 1. *Anacardium humile* Mart. is a native from *cerrado* vegetation reserve, UFSCar, São Carlos *campus*, SP state.

Field observations in the *cerrado* reserve areas of UFSCar indicate that *A. humile* Mart. form homogenous groups and preclude the establishment of others. Furthermore, results obtained in preliminary tests using aqueous extracts of leaves and steams of the species resulted in inhibitory effects on seed

In the bioassays were used seeds of *Raphanus sativus* L. cv. "Crimson Giant" (radish) and *Lactuca sativa* L. cv. "Grand rapidis" (lettuce). Preliminary tests were performed in the laboratory to verify the viability and vigor, underscoring that preliminary tests showed photoblastism neutral for both varieties tested.

2.2 EXTRACTS PREPARATION

Four kilograms of each aerial organ, stems and leaves of *Anacardium humile* were collected during February 2002, at the morning, during sunny days. The plant material was stored at -10 ºC until the moment of the extraction. The aerial portions were tested, so that the xylopodium, the underground portion of the stem, wasn't tested because they belong anatomically to the stem.

These extracts were triturated in a mixer, according to the ratio 33.3 g to 200 mL of distilled water. The trituration was made during three minutes, at room temperature. After the filtration, the extract obtained was considered the most concentrated 16% (w/v).

From this extract a dilution was made using distilled water, producing the following concentrations: 12, 8 and 4% (w/v). The extract yield was also calculated, using 5 mL of 16% (w/v) extract to moist the filter paper. The moistened filter paper remained inside a chamber during 24 hours at room temperature (±28 ºC). After this period, the filter paper was weighted and the initial weight was deduced.

2.3 MOLAR CONCENTRATION AND PH

Determinations of molar concentration were done using an *Automatic Osmometer Model 5004 ìOsmette™ Precision Systems INC* and the pH measurements with a *pH/mVMeter UB-10*. After the osmotic potential measurements of the higher concentration extract (16% w/v) were registered, PEG 6000 solutions were prepared, according to Villela *et al.* (1991) at the same osmotic potentials of the plant extracts, as a way to isolate the osmotic from allelopathic effects on seed germination.

2.4 GERMINATION TESTS

The bioassays were carried out with vigorous seeds of *Raphanus sativus* L. cv. "Crimson giant" (radish) and *Lactuca sativa* L. cv. "Grand rapids" (lettuce). Four simultaneous replicates of 30 seeds of lettuce and radish were distributed in Petri dishes with 9 cm of diameter, with the bottom covered with two sheets of filter paper moistened with 5 mL of extracts from different plant organs and concentrations, plus the control group (distilled water).

The Petri dishes were maintained in a growth chamber, at 27 ºC during ten according to Brazil (2009). The seeds were considered germinated when radicle is 2 mm longer and with positive gravitropism. The speed and germination percentage were calculated according to Labouriau and Valadares (1976).

2.5 SEEDLING GROWTH

Lettuce and radish seeds were previously germinated in Petri dishes with filter paper moistened with distilled water at 27 ºC. Homogeneous seedlings were selected and distributed in Petri dishes with 9 cm of diameter, with filter paper moistened with different organ extracts. Due to the higher sensitivity of seedling growth observed in preliminary tests, we used the concentrations 0, 4 and 12% (w/v). The test was conducted in BOD chambers (NT 708-AT model) under light absence at 27 ºC. The biometric evaluations, total length of the seedlings, were made with a digital paquimeter in *Lactuca sativa* and *Raphanus sativus*, ten days after the germination (Benincasa, 1988). The experiments were carried out with four replicates of eight seedlings.

2.6 DATA ANALYSIS

The data obtained, the mean parameters of seed germination and seedling growth, separately, were submitted to ANOVA, compared by Tukey test at 5% probability using the GraphPad InStat software – Scientific graphing, curve fitting and nonlinear regression statistics. The experimental design used in the bioassays of seed germination tests and seedling growth was totally randomized.

3 RESULTS AND DISCUSSION

3.1 MOLAR CONCENTRATION AND PH

When the osmotic potential of the solution is lower to the cells of the embryo occurs at reduced speed germination and/or seedling growth (Carvalho and Nakagawa, 2000). This occurrence has been reported for seeds of several species, such as *Senna occidentalis* (Delachiave and Pinho, 2003) *Chorisia speciosa* (Fanti and Perez, 2003), *Mimosa tenuiflora* (Bakke *et al*., 2006), *Daucus carota* (Lopes and Dias, 2004) and *Ruta graveolens* (Yamashita *et al.*, 2009).

Table 1. Physical and chemical characteristics and yield of aqueous extracts of stems and leaves of *Anacardium humile* Mart. in the bioassays.

Aqueous extract (*Anacardium humile*)	pH	Molar concentration (mOsm)	Yield (mg.mL^{-1})
Leaves 16% (w/v)	4,39	52	10,33
Stems 16% (w/v)	5,10	33	14,34

According to the results on Table 1, the osmotic potential and pH, didn't affect the germination and seedling growth. Leather and Einhellig (1985) affirmed that seed germination is sensitive to solutions over 100 mOsmol, so suggesting an allelopathic interference on both process without interference from the osmotic potential of aqueous extracts.

With the data obtained osmotic potential, tests were made of seed germination and seedling growth of lettuce and radish in molar concentrations equivalent to their osmotic potential. The molar concentrations found in extracts of stems and leaves of *A. humile* 16% were converted to values of osmotic potential (Table 1).

In these tests, in molar concentrations of extract equivalent to 16%, where the values were 52 mOsm to leaves and 33 mOsm to aqueous extracts of stems (Table 1), no inhibition could be due this factor investigated. Thus we can say that the aqueous extracts of stems and leaves of *A. humile* used in this study were not osmotically active.

Verification of pH is too important since the extracts contain solutes such as sugars, organic acids and amino acids that can mask the effect of allelopathic extracts (Ferreira and Aquila, 2000). The pH analysis of the aqueous extracts showed slight acidity and low variation as being between 4.39 to 5.10 (Table 1). Roy (1986) reports that germination and seedling growth were affected when the pH was extremely alkaline or strongly acidic, so deleterious effects were observed under conditions of pH below 4 and above 10.

Thus, the pH values found in this study would not have affected the results, or, this analysis allowed rule out the possibility of interference of this factor in the process of seed germination and seedling growth of lettuce and radish.

After investigating these factors, strengthens the idea that the changes recorded in the germination and growth of lettuce and radish can be probably caused by allelopathic substances present in extracts of *A. humile*.

3.2 SEED GERMINATION BIOASSAYS

All the extracts concentrations from stems and leaves of *A. humile* did not affect the germination percentage of lettuce seeds (Figure 2), but the velocity was significantly reduced, when all concentrations of leaves extracts were used.

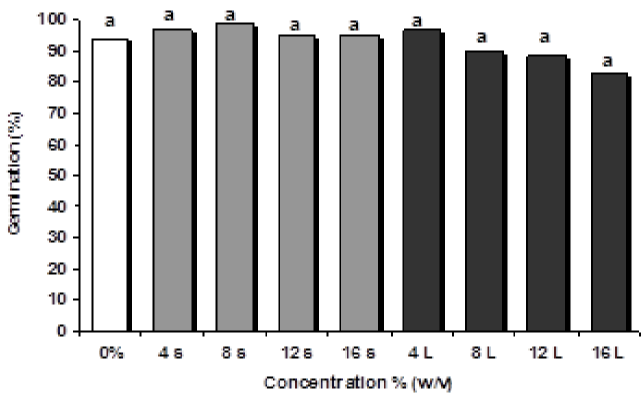

Figure 2. Germination percentage of *Lactuca sativa* L. under the effect of different concentrations of aqueous extracts of stems (s) and leaves (L) of *Anacardium humile* Mart. Same letters indicate that values do not differ significantly at 5% probability by Tukey test.

The stems extracts did not produce significant effects on the speed germination (Figure 3).

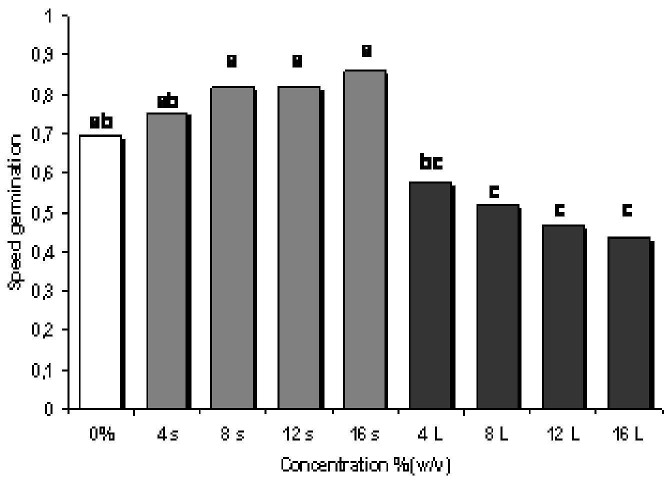

Figure 3. Speed germination (days^{-1}) of *Lactuca sativa* L. seeds exposed to different concentrations of aqueous extract of stems (s) and leaves (L) of *Anacardium humile* Mart. Same letters indicate that values do not differ significantly at 5% probability by Tukey test.

Inhibition of seed germination and early seedling growth stage is the most affected, and the physiological effects caused by allelopathic interactions are secondary responses of primary effects that occur in plant metabolism (Pedrol *et al*., 2006).

Inhibitory effects on seed germination and plant growth are often associated with allelopathy. In fact, this process is of paramount importance in understanding the interactions vegetables in the natural systems (Fritz *et al*., 2007).

Studies in *cerrado* vegetation indicate *Miconia albicans* (Sw.) Triana (Career and Zaidan, 2007), *Andira humilis* Mart. ex Benth (Periotto et al., 2004) and *Solanum lycocarpum* (Aires et al., 2005) as species with allelopathic potential. The authors mention that field observations indicate the possibility of an allelopathic effect on other plant species, considering that plants of the same species, form homogeneous groups and can inhibit germination of some seeds to establish other.

Moreover, the response of seedling growth is quite used to confirm the allelopathic effects in the laboratory, and thus help to understand the biological and physiological characteristics that involve the mechanism of action of allelopathic. The root system was touted as being one of the most sensitive responses indicative of seedling growth receptor (Miró *et al*., 1998).

In relation to radish seeds, the germination percentage was negatively affected as compared to control only when the leaves extracts to 8% (w/v) was used (Figure 4).

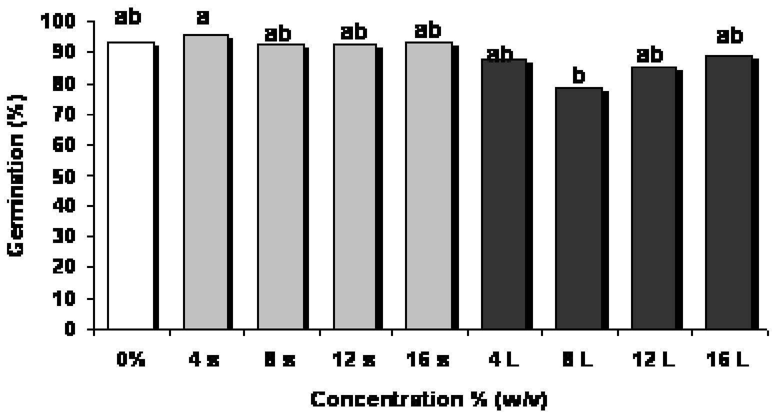

Figure 4. Germination percentage of *Raphanus sativus* L. under the effect of different concentrations of aqueous extracts of stems (s) and leaves (L) of *Anacardium humile* Mart. Same letters indicate that values do not differ significantly at 5% probability by Tukey test.

Necrosis dots in lettuce and radish cotyledons were registered. Besides it the oxidative process that makes the root tips dark brown and seedling growth was suppressed. Seed germination bioassays are probably the most common tests reported in the allelopathy literature. Seeds from tests plants were placed in Petri dishes with several concentration of extracted plant material and know allelochemicals on filter paper, flowed by incubators at controlled temperature.

In investigative experiments on allelopathy the speed germination is a widely used test, so that you can see by this parameter significant effects of the

However, the speed germination of radish was significantly reduced as compared to control using stems extracts at 8% and leaves extracts at 8, 12 and 16% (w/v) concentrations (Figure 5).

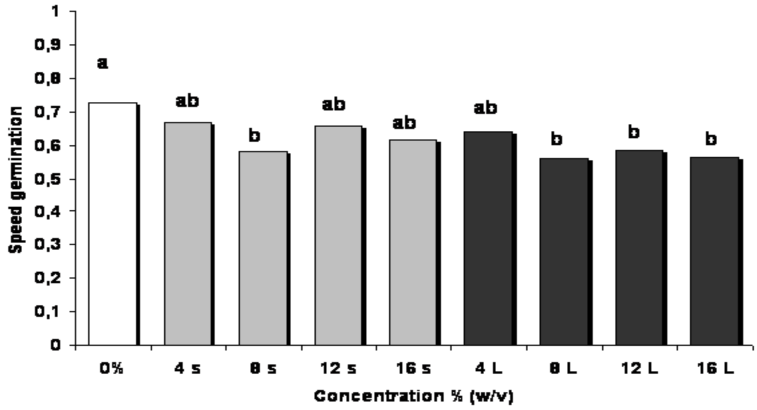

Figure 5. Speed germination (days^{-1}) of *Raphanus sativus* L. seeds exposed to different concentrations of aqueous extract of stems (s) and leaves (L) of *Anacardium humile* Mart. Same letters indicate that values do not differ significantly at 5% probability by Tukey test.

extracts on mean germination time entropy germination and differences in germination, compared to control (Ferreira and Aquila, 2000).

Such changes in patterns of germination of each species tested can result from several effects on the primary level. Among them, Ferreira and Aquila (2000) highlight changes in the permeability of membranes, the transcription and translation of DNA, the functioning of secondary messengers in breathing due to sequestration of oxygen in the formation of enzymes and receptors or a combination of these factors.

Indirect effects include interference in plant productivity, biodiversity in agroecosystems and local, to cause changes in plant succession, the structure and composition of plant communities and the dominance of certain plant species (Rizvi *et al.*, 1992).

The changes at the cellular level, fitormonal, photosynthesis and respiration, protein synthesis, lipid metabolism and organic acids, stimulation or inhibition of specific enzymatic activity, effects on the relationship of water and effects on the synthesis of DNA or RNA comprise direct effects caused by allelochemicals (Borella *et al.*, 2011).

These factors, in cellular levels, may have been responsible for delays in speed germination caused by all concentrations of aqueous extracts of leaves in lettuce and radish and by 8% concentration in aqueous extracts of stems in radish.

Silva et al. (2006) found the allelopathic potential in leaves of four arboreal species of cerrado on the germination of lettuce seeds, *Ouratea spectabilis*, *Qualea grandiflora*, *Pouteria ramiflora* and *Stryphnodendron adstringens*. The authors found that the inhibitory activity was the result of a mixture of substances with different polarities.

Anyway, in nature, these species can influence the development of other if the amount of leaves that are being degraded produce high levels of the active compounds, otherwise this effect is not observed.

In any case it is important to remember that in plant communities the allelopathic inhibition are not the result of the action of only one metabolite, but the result of the action of various agents allelochemicals. Thus, it may be considered that the biological activity of a mixture of these agents will be determined not only by their concentration when combined, but also by the various interactions between them resulting (Souza Filho, 2006).

3.3 SEEDLING GROWTH

The lettuce seedlings were significantly affected by stems extracts only at 4% and 12% (w/v) and the leaves extracts did not produce growth inhibition (Figure 6).

All concentrations of aqueous extracts from stems and leaves of *A. humile* produced a significant reduction in seedlings development and the seedling length was reduced as a contrast with the control group (Figure 7).

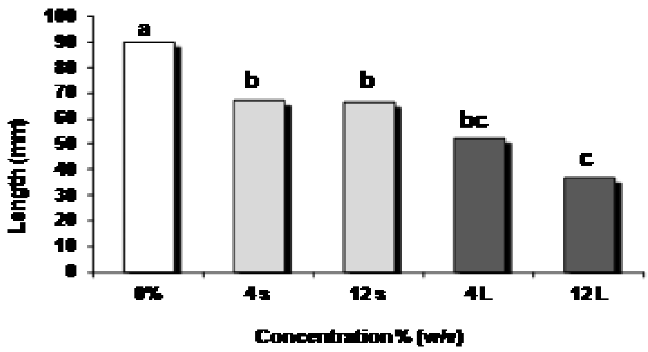

Figure 7. Total length of *Raphanus sativus* L. seedlings exposed to different concentrations of aqueous extract of stems (s) and leaves (L) of *Anacardium humile* Mart. Same letters indicate that values do not differ significantly at 5 % probability by Tukey test.

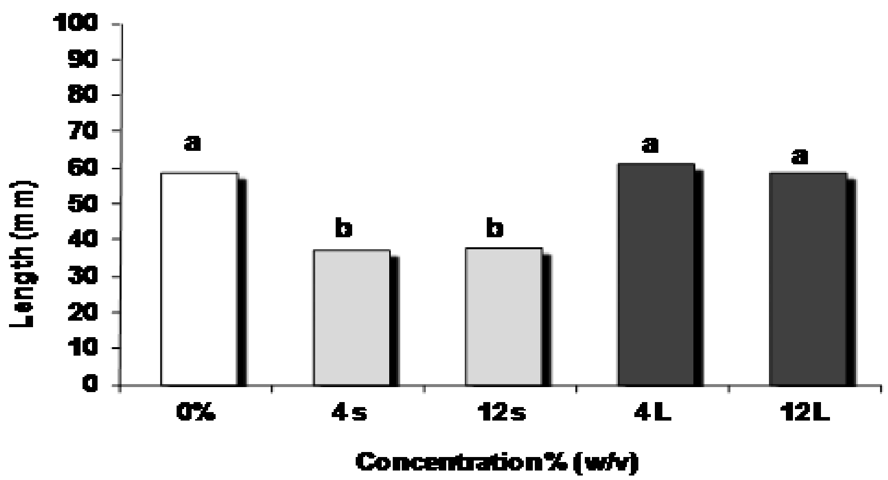

Figure 6. Total length of *Lactuca sativa* L. seedlings exposed to different concentrations of aqueous extract of stems (s) and leaves (L) of *Anacardium humile* Mart. Same letters indicate that values do not differ significantly at 5% probability by Tukey test.

esides, the aqueous extracts of *A. humile* reduced the hypocotyls length of lettuce and radish seedlings. The root tips presented necrosis and no secondary roots or root hairs were developed. The presence of abnormal seedlings, tissues necrosis and growth suppression may suggest allelopathic interference on the seedling development.

Laboratory, greenhouse and growth chamber bioassays conducted under controlled conditions are very important to sort out such allelopathic phenomena when suspected in nature, has been well documented in literature (Hoagland and Williams, 1985). Bioassays with seeds and seedlings are important for the understanding and the demonstration of allelopathy and to follow the phytotoxicity of active fractions of allelopathic compounds during isolation, purification and identification.

According to Einhellig (2001) the allelochemicals act first on biomembrane functions, affecting ion uptake and balance, action of hormones, respiratory metabolism, several enzyme functions. Also, they affect water status stomatal action, photosynthesis and carbon flow and finally, a modification in plant growth pattern is evident.

Loss of plasma membrane integrity could be a primary effect of allelochemical phytotoxins and mitotic inhibitions may be the secondary (Einhellig, 2001). The synthesis of these compounds, as example, the xantines, may act as the powerful growth inhibitors that could remain in the soil, producing auto toxicity and affecting another species, and so, *A. humile* is probably affects seed germination and seedling growth of species situated in the neighborhood, due the products from secondary metabolism.

Plants may be passive, only responding to fluctuations in the resources they require, or they may directly interact with other plants, microbes, or animals by transmitting, receiving, and responding to chemicals and other signals in their environment, independent of resources. As understood as microbial and animal interactions, interactions stimulated by signals may ultimately determine resource acquisition, but whether or not resources are acquired and defended by passive or active processes, it is important for understanding how plants interact with each other. How plants interact with each other is pertinent to how communities are organized. If plants react differently to chemicals released from particular neighbors, then the identity of neighbors may have a substantial effect on species coexistence and community composition. If such species-specific mechanisms were common, then diffuse co-evolution would be more likely (Inoye and Stinchcombe, 2001).

The presence of allelopathic compounds in plant tissues does not necessarily imply allelopathic potential in an ecological system. Allelopathic action in field depends on factors including the effects of released plant compounds on soil microorganisms or surrounding of plant species that may decrease or increase the effects of growth-promoting microorganisms on plants and/or could increase pathogenic microbial activity. Microbial action could result in the degradation of released phytotoxics or may transform nontoxic allelochemicals into active phytotoxins.

Another consideration, that may affect some aspects of allelopathy, has arisen over the past fifty years, with the introduction and the use of a xenobiotic compound as insecticides, fungicides, plant growth regulators, harvest aids and herbicides. These compounds have been and continue to be used on a world scale. Some of these chemicals and or their transformations products are present in soils and water. Researches including these aspects would contribute to the knowledge of a complex interactions plant.

Anyway the allelopathy's evolution resulting from changes in the plant environment factors such as competition for oxygen, sources of nutrients, space and light has led to the production of secondary metabolites that serve as allelopathy (Inderjit *et al*., 2011).

The investigation of these factors reinforces the idea that the aqueous extract of stems and leaves of *A. humile* has an allelopathic potential that reflected in changes in the patterns of seed germination and seedling growth of the species tested.

However, with the obtained results it can be inferred that allelopathic compounds possibly present in aqueous extracts studied caused the observed effects on seed germination and early development of seedlings of species bioassays. It is essential to note that the results obtained in the laboratory for allelopathy can not be confirmed under natural conditions, because of the simultaneous occurrence of various abiotic and biotic factors that surely affect the final results observed here.

CONCLUSION

The significant changes that aqueous extracts of stems and leaves of Anacardium humile resulted in percentage, speed of germination and growth of seedlings of lettuce and radish suggest their allelopathic potential. This finding suggests that future studies should be made as the investigation of the chemistry of these molecules promoting effects.

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