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Allelopathy of *Miconia* spp. (Melastomataceae) in *Lactuca sativa* L. (Asteraceae)

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Abstract

The aim of this work is to study the effects of the brute aqueous extract (BAE) of seven species of the genus *Miconia* occurring in the Chapada do Araripe-CE on *Lactuca sativa*, in addition to identifying the classes of secondary metabolites present in the extracts. The treatments consisted of four concentrations of leaf extract (25, 50, 75 and 100%), in addition to a control (0%) of distilled water, with five replicates each in a totally randomized experiment design layout for seven days. The following were assessed: number of germinated seeds, germination speed index (GSI), caulicle and radicle root length of the seedlings, occurrence of radical root necrosis, changes in the mitotic index and occurrence of chromosomal aberrations. The classes of secondary metabolites present in the extracts were identified through color changes and/or formation of precipitation. The brute aqueous extract of the leaves of *M. albicans*, *M. alborufescens* and *M. stenostachya* inhibited the germination of lettuce. All the BAEs of *Miconia* spp. had an adverse effect on the growth of the caulicle and radicle root of *L. sativa*. The extracts of *M. albicans*, *M. ibaguensis*, *M. lingustroides* and *M. stenostachya* were cytotoxic for the lettuce seedlings. Chromosomal aberrations were observed in all tested extracts. The metabolites found were hydrolysable and condensed tannins, flavonoids, flavones, flavanonols, chalcones, aurones and alkaloids. The tested species of *Miconia* showed inhibitory allelopathic activity within the parameters tested. The classes of secondary metabolites found could be responsible for the allelopathic and cytotoxic effects observed.

Keywords: allelopathic activity, chromosomal aberrations, cytotoxicity, secondary metabolites

1. Introduction

Allelopathy, defined as the positive or negative interference of one plant on other plants, fungi, insects and algae (Aires, 2007), is provoked by chemical substances called allelochemicals. These allelochemicals are products of secondary metabolism which inhibit or promote the germination and/or growth of the receptor organism (Aires, 2002) and are released into the environment by leaching, volatilization, radicle exudation and decomposition of plant parts.

Derived from different metabolic pathways, the allelochemicals are products which originate in glucose metabolism and whose intermediate products are acetate and shikimic acid. Some allelochemicals result from a unit of shikimate and one or more units of acetate, or from derivatives such as anthraquinones, flavonoids and condensed tannins (Brito, 2010). According to Pessotto and Pastorini (2007), the allelochemicals cause interference in seed conservation, dormancy and germination, seedling growth and adult plant vegetative vigor when they affect vital respiration functions, photosynthesis, cell division, nutrition and reproduction. The allelochemicals affect the seed germination or the seedling growth of the receptor plants, thus making impossible the development of species which are intolerant to these compounds (Azevedo Neto, 2010). However, these effects could be considered secondary manifestations caused by primary events which take place at cellular and molecular level (Aires, 2007).

Nevertheless, the plants which produce allelochemicals are acting in self-defense and the release must be

continuous in order for it to be effective (Borella et al., 2010). Comiotto (2006) argues that the allelopathic compounds protect the plant from the attacks of compounds produced by other plants, fungi, nitrifying bacteria and pathogens. This happens through a mechanism which has been acquired over the course of evolution and is ecologically extremely important as it directly and indirectly influences adjacent plants.

In natural ecosystems and agro-ecosystems, the allelochemicals are those principally responsible for the failure of the establishment and persistence of plant species (Rezende et al., 2003), functioning as a control mechanism.

Due to the large decrease in productivity of crops caused by invasive plants, the study of allelopathy has mainly attracted interest in the field of agriculture; it constitutes a control of undesirable species, resulting in more balanced environments with positive effects on the productivity and longevity of the farmed species (Haida et al., 2010). In natural ecosystems, allelopathy is an important ecological mechanism, having a strong influence on plant dominance, succession, local biodiversity (Brito, 2010), the formation of plant communities and plant climax (Sousa et al., 2007).

The use of synthetic herbicides is detrimental to agricultural production, as it harms the human population and food production. One of the greatest concerns of various countries is the indiscriminant use of these herbicides which incur grave environmental damage (Carvalho et al., 2002). The principal motive for research on allelopathy is the large interest in substituting commercial herbicides for natural substances in agro-ecosystems, since the research results can be used to improve the sustainability of production systems, as well as contributing to the conservation of natural or semi-natural plant species by offering an alternative which is biologically more specific and less harmful to the environment (Borella et al., 2010).

The study of the allelopathic potential of native species is an important tool in the understanding of interaction between plants in an ecosystem; knowledge of these relations allows a view of the species which can be used in the recovery of degraded areas and as a source of bio-herbicides. Although various plant species of natural environments have been covered through the increase in studies mentioned earlier, many more still need to be studied. Among the species little known for their allelopathic action is the *Miconia* Ruiz & Pavon genus, with few entries in specialized literature. The studies of Gorla and Perez (1997) and Gatti (2008) are examples of these entries. They have recorded the inhibitory allelopathic activity of *Miconia albicans* (SW.) Triana extract on the germination, radicle growth and germination speed of lettuce, tomato, cucumber and sesame seeds. The small number of studies involving *Miconia* species demonstrates the necessity to extend research to other species of the genus.

In work on allelopathy, it is quite common to see the effects of various plant species extracts on the morphological aspects of the receptor plant, without considering the cellular events and the physiological and genetic changes. These can be diagnosed through cytogenetics, using mitotic index analysis and the occurrence of chromosomal alterations (Pires et al., 2001; Cuchiara et al., 2007; Almeida et al., 2008).

Considering the scarcity of information on the allelopathic activity of the *Miconia* species, the objective of this work is to investigate the effect of the aqueous extract of different *Miconia* species found in the Chapada do Araripe-CE on the germination, development and mitotic index of *Lactuca sativa* L., in addition to diagnosing the secondary metabolite classes present in these extracts.

2. Materials and Methods

The plant material was collected in the Chapada do Araripe from Cerrado and humid forest areas during the period of January-December, 2011. The collected species of *Miconia* were treated according to usual plant collection methods, identified and sent to a specialist for identification confirmation. The collected exsiccates were placed in the Cariri Dárdano de Andrade-Lima Herbarium of the Cariri Regional University – URCA under the following entry numbers: *Miconia albicans* (SW.) Triana (4861), *Miconia alborufescens* Naudin. (5249), *Miconia ciliata* (Rich.) DC. (2099), *Miconia ibaguensis* (Bonpl.) Triana (2837), *Miconia lingustroides* (DC.) Naudin (7202), *Miconia minutiflora* (Bonpl.) DC. (1697) and *Miconia stenostachya* DC. (6928).

The bioassays were carried out in the Laboratory of Applied Botany-LBA of the Department of Biological Sciences in the Cariri Regional University-URCA during the period of January to December 2011.

The brute aqueous extract (BAE) was prepared by triturating 200 g of fresh leaves of *Miconia* species in an industrial blender with distilled water. The quantity of water to be added was established according to the ratio of the weight of fresh matter (FMW) and the weight of dry matter (DMW). To do this, 100 grams of fresh matter were put in a dryer at a temperature of 100 °C for 24 hours. After this, the leaves were weighed in order to determine the weight of the dry matter (DMW). With the FMW/DMW ratio, an index was obtained which was multiplied by the weight of fresh matter (100 g) corresponding to the volume of distilled water in ml to be used

in the extract preparation (Medeiros, 1989).

After trituration, the matter was filtered using a glass funnel and cotton. The resulting liquid was centrifuged at 3000 rpm per minute for 10 minutes to obtain the brute aqueous extract (100%) from which the dilutions of 75%, 50%, 25% were made. The pH of the *Miconia* species extracts in various concentrations was measured with a pH meter. The extracts ranged between 3.47 and 4.46 and an adjustment was made to 6.0 with solutions of KOH 0,1mol/L and HCl at 5%, as recommended by Macias et al. (2000). These authors recommend that the pH of the aqueous extracts be adjusted to 6.0, as this is the ideal pH range for seed germination and observation of allelopathic effects.

The bioassays consisted of 4 treatments in concentrations of 100, 75, 50 and 25%, as well as a control group (0%) of only distilled water, applied in completely randomized experimental design (CRD). Each treatment featured five replicates of 20 seeds of *Lactuca sativa* L. each, making a total of 100 seeds per treatment.

The experiments were conducted in petri dishes lined with two discs of filter paper on which the lettuce seeds were placed. 3 ml of each extract concentration was placed in each dish, and 3 ml of distilled water for the control. The experiments were conducted in a BOD germination chamber at a temperature of 25 °C and a photoperiod of 12 hours for 7 days.

The analyzed variables were number of germinated seeds, germination speed index (GSI), caulicle and radicle root length, occurrence of radical root necrosis, mitotic index and presence of chromosomal aberrations.

The germination speed index (GSI) was assessed every 24 hours, being calculated through the sum of the ratio between the number of germinated seeds on day i (ni) and the number of days (i) (Fernandes et al., 2007) and using the formula below:

$$GSI = \sum_{i=1}^{n}\left(\frac{ni}{i}\right) \quad (1)$$

For the analysis of the mitotic index, five radicles per replicate were collected on the fourth day following the germination of the lettuce seeds, making a total of 25 per treatment. The radicles were fixed in Carnoy solution (3 parts ethanol: 1 part glacial acetic acid) for 24 hours and then stored in a freezer in this solution.

For analysis, the radicles were washed twice with distilled water for five minutes each time, hydrolyzed in HCl 5N (hydrochloric acid-5N) for 20 minutes and washed again in distilled water for 5 minutes. The radicle tips were put in polished microscope slides; crushed in acetic acid at 45%, covered with glass slips which were removed by freezing in liquid nitrogen, air dried and stained with Giemsa at 2% for 20 minutes and mounted with Entelan according to the methodology proposed by Guerra and Sousa (2002). Five slides were prepared per treatment.

In the mitotic index analysis, five fields per slide were analyzed and the total number of cells per field was counted. On average 200 cells were seen with the help of an optic microscope with a magnification of 400X. The cell count was carried out according to the methodology proposed by Pires et al. (2001), with modifications.

The mitotic index was obtained by dividing the number of cells in mitosis (prophase + metaphase + anaphase + telophase) by the total number of observed cells (interphase + mitosis) and multiplying by 100, according to the equation proposed by Pires et al. (2001). The result was expressed as a percentage.

$$MI = \frac{m}{T} \times 100 \quad (2)$$

Where, m = number of cells in mitosis; T = total number of cells observed.

Qualitative analysis was conducted on all the mounted slides in the search for chromosomal aberrations such as: anaphase and telophase bridges, c-mitosis, micronuclei, chromosome adherence, chromosome breaks, metaphase and anaphase losses. These were photographed by a microscope with a photographic camera with 1000X enlargement.

The phytochemical analysis was carried out in the Laboratory of Research of Natural Products-LPNN of the Cariri Regional University-URCA. Concerning the research on secondary metabolites, ethanolic extracts were used, produced with 500 g of fresh leaves of the species of *Miconia*. For this, the leaves were triturated and immersed in ethanol P.A. for a period of seven days under periodic agitation. Next the material was filtered to take out the leaves, which were dried for subsequent weighing in order to obtain the dried mass after the extraction. The diluted extract was taken to a rotary evaporator for complete distillation of the solvent. The excess ethanol was evaporated in bain-marie until the brute ethanol extract was obtained. The phytochemical research tests were carried out according to the methodology proposed by Matos (2009), based on color change

and precipitate formation through the addition of specific reagents.

The statistical analysis of germination data, caulicle and radicle root length and occurrence of necrosis was performed using variance analysis (ANOVA) and means comparison using the Tukey test at 1% and 5% probability. Regarding the statistical analysis of the mean values of the mitotic index and as recommended by Banzatto and Kronka (1989) and Storck et al. (2011), the data expressed as a percentage underwent a transformation with $arcsin\sqrt{X/100}$ and analyzed by polynomial regression. The analyses were performed with the help of the program ASSISTAT version 7.6 beta.

3. Results

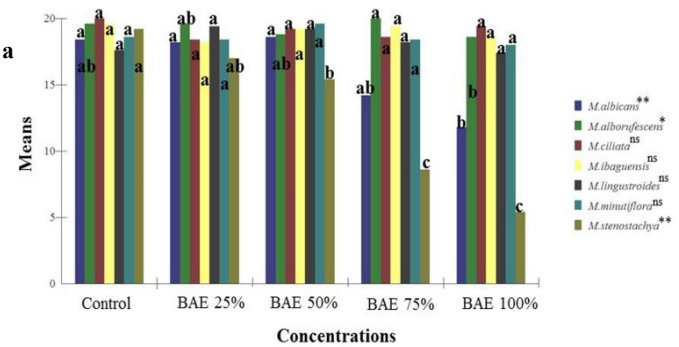
The leaf extracts of *M. albicans*, *M. alborufescens* and *M. stenostachya* significantly inhibited lettuce germination. All concentrations of the leaf extract of *Miconia stenostachya* caused a reduction in the number in the number of germinated lettuce seeds and the results were significantly different from the control, whereas the extract of *M. albicans* inhibited lettuce seed germination only at concentrations of 75% and 100%. The extract of *M. alborufescens* at 100% caused germination inhibition while at 75% it provoked an increase in the number of germinated seeds in relation to the control (Figure 1a). The leaf extracts of *M. ciliata*, *M. ibaguensis*, *M. lingustroides* and *M. minutiflora* did not inhibit lettuce seed germination; it is possible that the compounds present in the extracts of these species may not have a phytotoxic effect on germination.

Regarding the GSI of the receptor species, the extracts of the *Miconia* species interfered in both a significant and differentiated manner. The extracts of *M. albicans* and *M. stenostachya* at a concentration of 50% and upwards inhibited the germination speed index of lettuce seeds (Figure 1b). It has already been shown that the extract of *M. alborufescens* at 100% caused a delay in the GSI of lettuce. The extract of *M. ciliata* at concentrations of 25, 50 and 75% delayed the GSI, while *M. lingustroides* and *M. minutiflora* provoked a delay in GSI of *L. sativa* at a concentration of 100%.

As can be seen in Figure 1c, at 25% concentration the extracts of the species *M. albicans*, *M. minutiflora*, *M. alborufescens* and *M. ciliate* provoked an increase in the length of the lettuce caulicle. However, this was without statistical difference in comparison to the control for *M. albicans* and *M. minutiflora*, but with statistical difference regarding *M. alborufescens* and *M. ciliata*. Furthermore, it can be observed in the same table that the extracts of *M. ciliata* and *M. minutiflora* at 50% concentration caused an increase in caulicle length of *L. sativa*, while at 75 and 100% concentration they provoked a decrease. Regarding *M. ibaguensis*, it can be observed that the brute aqueous extract of its leaves caused a gradual decrease in the length of the lettuce caulicle at the various concentrations tested, leading to the belief that the phytotoxic compounds present in this species impede the extension of this structure.

The extracts of *M. lingustroides* and *M. stenostachya* inhibited the length of the lettuce seedling caulicles at all the concentrations tested.

In relation to the radicle length of the seedlings of *Lactuca sativa* (Figure 1d), the extracts of all the *Miconia* species were capable of significantly inhibiting growth and promoting necrosis (Figure 1e). In this figure it can be observed that the extracts of *M. alborufescens*, *M. ciliata* and *M. minutiflora* at 75% concentration affected the largest quantity of radicles compared to the control.



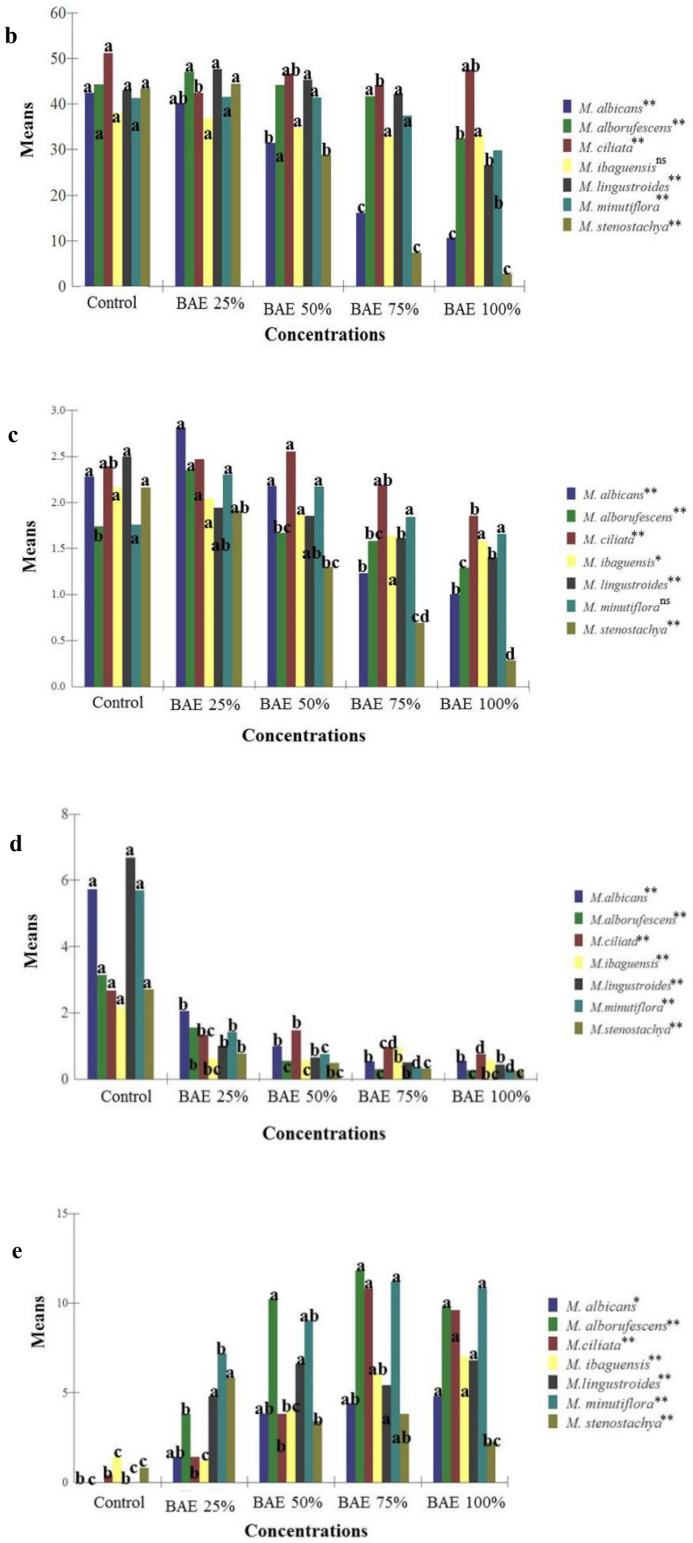


Figure 1. Number of germinated seeds (a), Germination Speed Index (b), Mean length of caulicles (c), radicles (d) and number of radicles with necrosis (e) under the effect of different concentrations of brute aqueous extracts of *Miconia* spp.

Note. (**) significance at level of 1% probability ($p < 0.01$), (*) significance at level of 5% probability ($0.01 \leq p < 0.05$), (ns) not significant ($p \geq 0.05$). Equal letters do not differ statistically by the Tukey test at 5% probability.

In Table 1, the data of the variance analysis regarding the mitotic index (MI) of the meristematic cells of the

lettuce seedlings radicles exposed to the brute aqueous extract of *Miconia* species at various concentrations is displayed. It can be observed that *M. ibaguensis*, *M. lingustroides*, *M. albicans* and *M. stenostachya* had a significant effect on the mitotic index of lettuce. In addition, the first two species provoked inhibition of the mitotic index in the radicle cells of the test plant, while *M. albicans* and *M. stenostachya* provoked stimulus in the cellular division of *L. sativa*. The extract of the species *Miconia alborufescens*, *Miconia ciliata* and *Miconia minutiflora* neither inhibited nor stimulated the cellular cycle of the radicles of *L. sativa*.

Table 1. Analyisis of variance of mitotic index (MI) of lettuce meristematic cells exposed to different concentrations of BAE of *Miconia* spp. *Miconia albicans* (*M. alb*.); *Miconia alborufescens* (*M. albor*.); *Miconia ciliata* (*M. cil*.); *Miconia ibaguensis* (*M. ibag*.); *Miconia lingustroides* (*M. ling*.); *Miconia minutiflora* (*M. minut*.); *Miconia stenostachya* (*M. sten*.)

Causes of variance	**GL**	**Mean Square**						
		M. alb.	*M. albor.*	*M. cil.*	*M. ibag.*	*M ling.*	*M. minut.*	*M. sten.*
Linear regression	1	93,748554 ^{ns}	1,03488 ^{ns}	0,02473 ^{ns}	1,75464 ^{ns}	27,69191 ^{ns}	20,03011 ^{ns}	53,43721*
Quadratic regression	1	71.98716 ^{ns}	22.28476 ^{ns}	4,54606 ^{ns}	33,91708*	3,54267 ^{ns}	0,94220 ^{ns}	17,35411 ^{ns}
Cubic regression	1	312, 37623**	9.53590 ^{ns}	12,79722 ^{ns}	1,85511 ^{ns}	0,00499*	16,96608 ^{ns}	12,25020 ^{ns}
Regression error	1	6.57975 ^{ns}	3.52205 ^{ns}	21,69600 ^{ns}	0,29550 ^{ns}	49,83039 ^{ns}	1,45741 ^{ns}	5,07984 ^{ns}
Residual	20	30,55712	11.99994	8,25466	5,57238	15,58273	7,87706	10,97626
CV(%)		25,48	19.78	15,85	16,36	24,57	16,78	22,41

Note. (**): significance to level of 1% probability ($p < 0,01$); (*):significance to level of 5% probability ($0.01 \leq p < 0.05$); (ns): not significant ($p \geq 0,05$); (CV): Coefficient of variation in %.

In the radicle of the lettuce seedlings exposed to the extract of *M. alborufescens* at 25% concentration the presence of metaphase with chromosome loss was observed. In those exposed to 50% concentration, interphases and telophases with micronuclei were found, at 75%, binucleated cells and anomalous nucleus and at 100%, chromosome loss in anaphase, anaphase bridge, chromosome break and rupture of mitotic fusion.

Regarding the lettuce seedling radicles exposed to the extract of *M. ciliate*, the following were observed: chromosomes not lined up at the equatorial plate, chromosome loss in anaphasic cells, C-metaphases, multipolar anaphases, chromosomal adherence, metaphase delay, micronuclei in interphase, broken metaphases.

Chromosomal adherence and chromosomal delay in metaphase were observed in the radicles exposed to the extract of *M. minutiflora* at 25% concentration. At 50%, micronuclei in telophase cells and premature separation of chromatids were observed. At 75% concentration chromosome breaks and C-metaphase were noted and at 100%, chromosomal aberrations of micronuclei in prophase and anaphase break. From this, it can be inferred that the *Miconia* species may have genotoxic and mutagenic effects.

The extracts of *M. ibaguensis* and *Miconia lingustroides* provoked negative alterations in the mitotic index of the lettuce radicle, while the extracts of *M. albicans* and *Miconia stenostachya* caused positive alterations. The graphs of these results, with curves of polynomial regression, are illustrated in Figures 2, 3, 4 and 5.

In Figure 2, the cubic model provided the best representation of the relation between the extract concentrations and the analyzed variable (MI), making it apparent that all concentrations of the BAE of *M. albicans* caused an increase in the mitotic index of the radicle cells of lettuce. This effect is most clear at 25 and 100%.

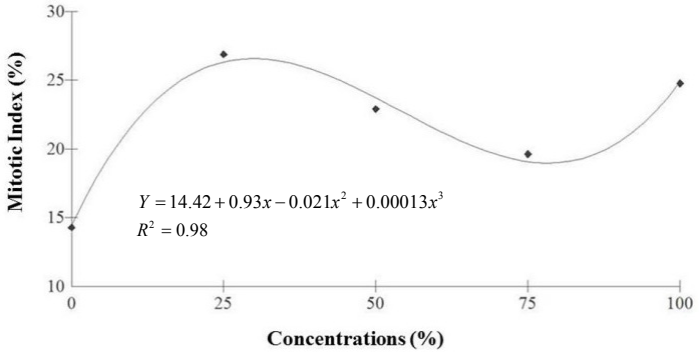


Figure 2. Average mitotic index of lettuce cells when grown in the presence of brute aqueous extract of *Miconia albicans*

The brute aqueous extract of *M. albicans* caused anomalies of the following type: chromosome adherence, chromosome loss and chromosome delay in metaphase, anaphase breaks, metaphase disorganization and C-metaphase (Figure 6).

The extract of *M. ibaguensis* proved to be cytotoxic for the lettuce radicle cells, the quadratic model adapting itself best to showing the effects of the concentration in relation to the mitotic index (Figure 3).

Cells with adherent chromosomes and trinucleotide cells were observed in the radicles exposed to the extract of *M. ibaguensis* at 50% concentration. Anaphase and telophase bridges were observed at 75% and the occurrence of chromosome delay in metaphase and chromosome break was seen at 100%.

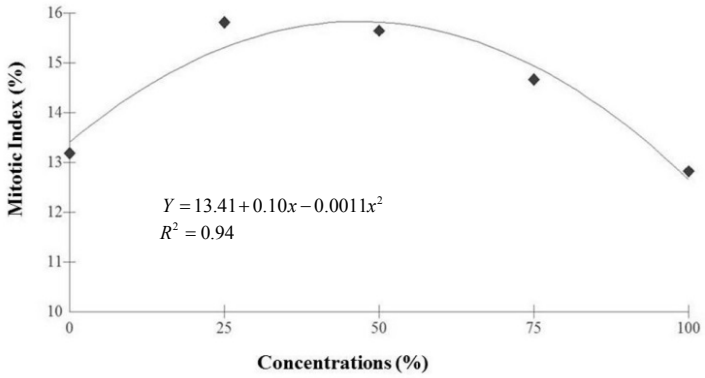


Figure 3. Average mitotic index of lettuce cells when grown in the presence of brute aqueous extract of *Miconia ibaguensis*

In addition, the brute aqueous extract of *M. lingustroides* was cytotoxic for the cells of *L. sativa*, with the cubic model proving to be the most appropriate model of polynomial regression (Figure 4). It also shows that the mitotic index of *L. sativa* went down in accordance with the increase in BAE concentration of *M. lingustroides,* being most regressive at the maximum concentration of 100%.

The chromosome alterations observed in the cells exposed to the extract of *M. lingustroides* at 25% concentration were of the prophase type with chromosome loss. At 50% there were cells with chromosome adherence, while at 75% formation of metaphases with micronuclei, loss of various chromosomes and anaphase and telophase bridges were caused. At 100%, there were cells with micronuclei and broken metaphase (Figure 6).

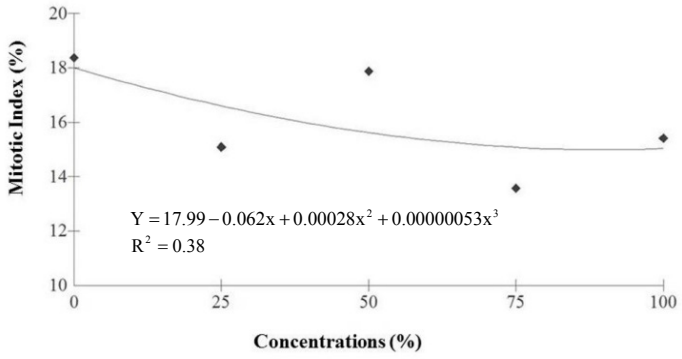


Figure 4. Average mitotic index of lettuce cells when grown in the presence of brute aqueous extract of *Miconia lingustroides*

In Figure 5, it can be seen that the BAE of *Miconia stenostachya* stimulated the mitotic index of the radicle cells of *L. sativa* and in this case the regression curve was the best suited model, being linear. The cells exposed to the extract at 25% concentration underwent a reduction in the number of cell divisions, whilst at higher concentrations these divisions were greater, with the clearest increase in the cells exposed to 100% extract. This demonstrates a stimulatory effect on the mitotic index of the plant tested.

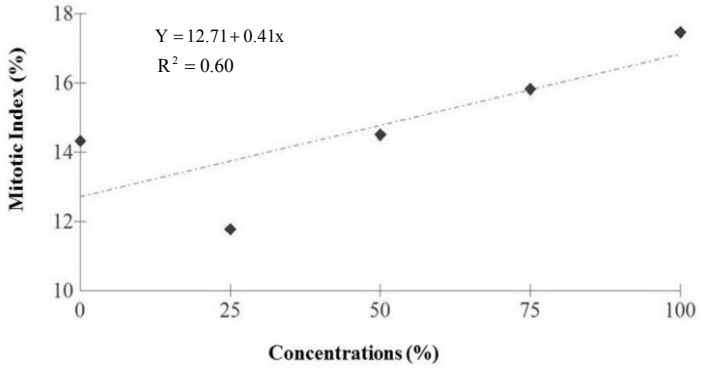


Figure 5. Average mitotic index of lettuce cells when grown in the presence of brute aqueous extract of *Miconia stenostachya*

Furthermore, this last concentration caused anomalies such as anaphase bridges, micronuclei, shortening of chromosomes in anaphase, chromosome breaks and broken metaphases. The concentrations of 50% and 75% were those that most provoked chromosome alterations such as cells with nuclear buds, c-metaphase with full chromosome spread and metaphases with chromosome loss. All these abnormalities are shown in Figure 6.

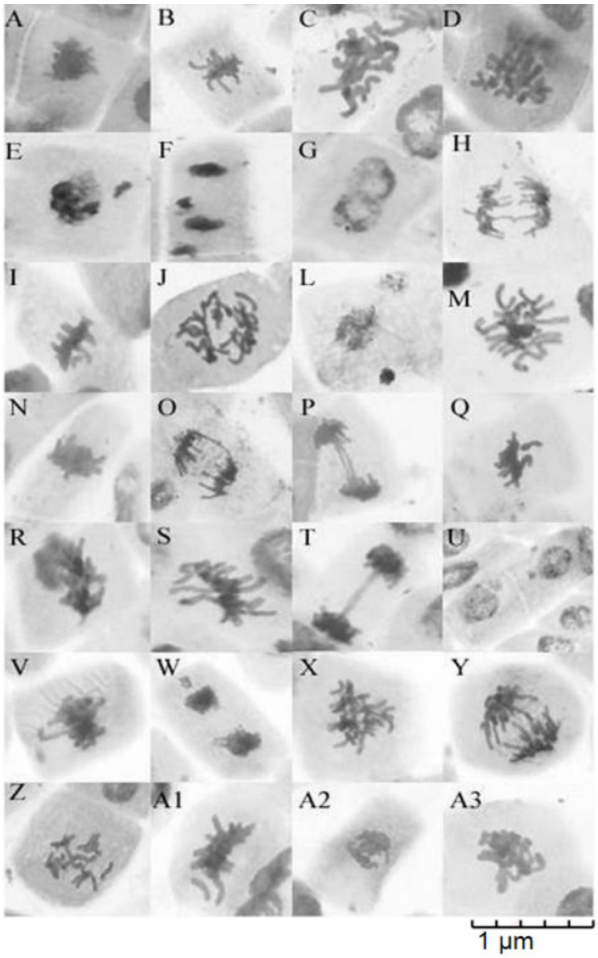


Figure 6. Principle chromosomal abnormalities observed in the brute aqueous extracts of *Miconia* spp.

Note. A-D: *Miconia albicans*; E-H: *Miconia alborufescens*; I-M: *Miconia ciliata*; N-Q: *Miconia ibaguensis*; R-U: *Miconia lingustroides*; V-Y: *Miconia minutiflora*; Z-A3: *Miconia stenostachya*. A: Chromosome adherence at 25% concentration. B: Chromosome loss. C: Metaphase disorganization. D: C-metaphase present at 100%. E: Interphase micronucleus carrier cell in 50% concentration. F: Telophase cell with micronucleus. G: Binuclear cell at 75% concentration. H: Anaphase bridge. I: Metaphase cell displaying chromosome break in 25% concentration. J: Cell in C-metaphase. L: Peripheral micronuclei. M: Broken metaphase at 100% concentration. N: Cell in metaphase with adhered chromosomes. O: Anaphase bridge. P: Telophase bridge in 75% concentration. Q: Chromosome delay in metaphase at 100% concentration. R: Cell with chromosome adherence at 50% concentration. S: Cell in metaphase with loss of various chromosomes. T: Telophase bridges at 75% concentration. U: Micronuclei carrier cells at 100% concentration. V: Chromosome delay in metaphase at 25% concentration. W: Telophase cell with a micronucleus at 50% concentration. X: Premature separation of chromatids in 50% concentration. Y: Anaphase bridges in 100% concentration. Z: Cell in C-metaphase with total chromosome spread. A1: Metaphase with chromosome loss. A2: Anaphase with chromosome shortening. A3: Chromosome break at 100% concentration.

In the phytochemical tests, the metabolites found in the species of *Miconia* were tannins, flavonoids and alkaloids (Table 2).

Table 2. Classes of secondary metabolites found in ethanolic extracts of species of *Miconia* genus

Species	Classes of secondary metabolites			
	Tannins	**Phenols**	**Flavonoids**	**Alkaloids**
Miconia albicans (SW.) Triana	+	-	+	-
Miconia alborufescens Naudin.	+	-	+	+
Miconia ciliata (Rich.) DC.	+	-	+	-
Miconia ibaguensis (Bonpl.) Triana	+	-	+	-
Miconia lingustroides (DC.) Naudin	+	-	+	+
Miconia minutiflora (Bonpl.) DC.	+	-	+	+
Miconia stenostachya DC.	+	-	+	-

4. Discussion

In literature, it can be seen that allelopathic activity has been established in few species of *Miconia*. This is the case of the work carried out by Gatti et al. (2007), in which the extract of *Miconia albicans* at 10% did not affect the germination of the lettuce seeds. The same applies to the work of Isaza et al. (2007), where fourteen species of Melastomataceae, among them *Miconia minutiflora*, were tested and shown to not interfere with the germination of various receptor species, including *L. sativa*. In this study, the brute aqueous extract at concentrations of 25% and 50% also did not interfere with the germination of lettuce seeds, whereas the higher concentrations of 75 and 100% did inhibit germination.

Work carried out by other authors also gave negative results for germination of the receptor species when exposed to the higher concentrations. The work of de Gatti et al. (2004) on *Aristolochia esperanzae* O. can be cited, as can Kuntze, Candido et al. (2010) on *Amaranthus viridis* L., *Acanthospermum hispidum* DC, *Bidens pilosa* L., *Conyza canadensis* L. Cronquist, *Galinsoga parviflora* Cav., *Parthenium hysterophorus* L., *Commelina benghalensis* L., *Euphorbia heterophylla* L., *Leonurus sibiricus* L., *Digitaria insularis* L. Fedde, *Eleusine indica* L. Gaert and *Nicandra physaloides* (L.) Pers.

The allelopathic effect can cause alterations in the germination distribution curve, ranging from less complex situations such as normal distribution going into a kurtosis, to distribution involving lengthening along the curve through the time axis or a complex distribution model of the germination of the seeds (Labouriau & Agudo, 1987). These alterations can have effects on the permeability of membranes; DNA transcription and translation; functioning of secondary messengers; respiration, by sequestering oxygen (phenols); formation of enzymes and receptors; or even a combination of these factors (Ferreira & Aquila, 2000).

The fact is that researchers such as Borghetti and Pessoa (1997) and Rodrigues et al. (1999) have argued for decades that in many cases allelopathic action may not influence the germination of the receptor species and that the action has greater effect on germination speed or any other variable.

Ferreira and Aquila (2000) stress that allelopathic action does not affect germination or the final germination percentage, but rather germination speed; this is confirmed in our results, where interference of all the *Miconia* extracts, with the exception of *M. ibaguensis*, reduced the GSI.

The caulicle and radicle lengths are within the parameters most used to evaluate allelopathic effect upon seedling growth (Jacobi & Ferreira, 1991; Inderjit & Dakshini, 1995; Pratley et al., 1999). Undoubtedly, the emergence of the seedling and its growth are the most sensitive phases in the ontogeny of the individual (Blum, 1999). This validates the present study in which the extracts of *Miconia* interfered with the development of the lettuce seedlings to a greater or lesser extent, depending on the concentration.

In the phytochemical screening tests, the occurrence of phlobaphene tannins was identified in *M. stenostachya*. According to Souza Filho and Alves (2002), various phenolic allelochemicals have the ability to alter the biosynthesis of the main constituents of plants and this is reflected in their growth. Thus the tannins found in the leaf extract of *M. stenostachya* could be responsible for the strong inhibition of the growth of the lettuce caulicle. It is worth highlighting that in order to certify the activity of these compounds, it is necessary to carry out bioassays on the isolated substances (Taveira, 2011).

Effects similar to those obtained during our research have been observed by other authors regarding the *Miconia* species. Gorla and Perez (1997) and Gatti (2008) analyzed the effect of the brute aqueous extract of *Miconia*

albicans on tomato seedlings and noted that the 25% concentration of the extract interfered negatively with the development of the tomato radicle. Compounds such as pyrogallol tannins present in the extract of *M. albicans* could be responsible for the allelopathic effects on the roots of the plant tested. Hui Li et al. (2010) have confirmed that phenolic allelochemicals can inhibit the length of the radicle by paralyzing cell divisions. In the phytochemical screening tests performed on *M. ciliata* and *M. ibaguensis*, pyrogallol tannins, flavones, flavonols, xantones, flavanonols, chalcones, aurones, catechins and flavanols were found. This confirms the assertion of Cassiano et al. (2010) that the plants of the Melastomataceae family are characterized by the presence of flavonoids, hydrolysable tannins and anthocyanins.

In the view of Souza Filho and Alves (2002), the flavonoids are those principally responsible for the allelopathic phenomena. They can cause alterations in the permeability of the chloroplast membrane, thus inhibiting the growth of the seedlings. In addition, these substances can be used as herbicides due to the variety of their strong biological activities. Therefore, it can be concluded that the phytotoxic effects of the *M. ciliata* and *M. ibaguensis* extracts are due to flavonoids.

M. alborufescens, *M. lingustroides* and *M. minutiflora* tested positive for alkaloids, as well as for the other secondary metabolite classes of the analysis. According to Henriques et al. (1999), alkaloids are toxic and this toxicity can affect the radicle cells. Hoffmann et al. (2007) states that the radicle system is the most sensitive to allelochemicals and that its growth depends on cell division. Hence the presence of these alkaloids, or a combination of substances in the species of *Miconia*, could have been responsible for the marked inhibition of the growth of the lettuce radicles.

According to Ferreira and Aquila (2000) in their studies of allelopathic effects, the morphological norms of the seedlings are a valuable tool, since the allelopathic substances can cause the emergence of abnormal seedlings. Radicle necrosis is one of the most common symptoms.

One explanation for the high incidence rate of radicle necrosis in lettuce following exposure to 75% concentration of the extracts of the three species (*M. alborufescens*, *M. ciliata* and *M. minutiflora*) is that it could be the result of the production and accumulation of reactive oxygen species in the radicle cells. According to Almeida et al. (2008), the accumulation of these substances damages the cells and leads to their death. This is due to the rapid depolarization of their membranes which increases permeability, thus causing lipid peroxidation and leading to general cell disruption. Another factor which could also cause the death of cells by oxidation stress is the degradation of DNA by endonucleases; this cleaves the chromatids of the chromosomes, leading to programmed cell death.

The changes to the mitotic index and the aberrations observed in the *Miconia* species that were researched can be attributed to chromosomal alterations which developed in the presence of allelochemicals. This resulted in abnormal physiological processes, leading to the inhibition of seedling development (Almeida et al., 2008).

Alkaloids were detected in the phytochemical screening tests on *Miconia lingustroides*. According to Henriques et al. (1999), alkaloids are cytotoxic; they are principally responsible for the inhibition of plant growth when they alter the cellular cycle of the organism.

In this study, the brute aqueous extract of *M. stenostachya* reduced the length of the lettuce radicle. This inhibition is probably not related to the high incidence of mitotic divisions in the radicles, since the radicle underwent stimulation on exposure to the higher concentrations, but to the occurrence of chromosomal aberrations which seemingly led to the reduction in radicle length.

5. Conclusions

Therefore it can be concluded that the extracts of *M. stenostachya*, *M. albicans* and *M. alborufescens* had a negative effect on the germination of lettuce seeds, depending on the concentration. However, the extracts *M. ciliata*, *M. ibaguensis*, *M. lingustroides* and *M. minutiflora* did not affect the germination of the receptor species. Moreover, the germination speed index was negatively affected on exposure to all the concentrations of *Miconia*, with the exception of *M. ibaguensis* at 50, 75 and 100% concentration.

The extracts of *Miconia* affected the caulicle development of *L. sativa* in both a positive and negative way; positive when exposed to the extracts of *M. albicans*, *M. minutiflora*, *M. alborufescens* and *M. ciliata* at 25% concentration and negative when exposed to *M. lingustroides* and *M. stenostachya* at all concentrations.

All the BAEs of *Miconia* spp. were capable of interfering negatively with the growth and length of the lettuce radicles in concentrations of 50% and upwards. This is probably due to their secondary compounds being toxic for the radicle tissue, causing necrosis of the radicle tips.

The brute aqueous extracts of *M. ibaguensis* and *Miconia lingustroides* had a cytotoxic action, causing chromosome and cellular aberrations. The extracts of *M. albicans* and *M. stenostachya* stimulated the mitotic index of the lettuce cells. The extract of *M. stenostachya* at 75% concentration caused C-metaphase abnormalities.

The species of *Miconia alborufescens*, *Miconia ciliata* and *Miconia minutiflora* are not capable of inhibiting the mitotic index of lettuce, but all the concentrations which were tested cause chromosome abnormalities by interfering with the growth of the lettuce seedling radicle.

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