# Allelochemicals of Neea theifera Oerst. (Nyctaginaceae) with phytotoxic potential on plant germination and growth

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***Abstract*****—** *Plant species able to produce phytotoxic substances are widely studied in agronomy because when identified and isolated can lead to herbicides or insecticides less toxic than current pesticides. Knowing the ecological characteristics of the Neea theifera species in cerrado biome, this study aimed to evaluate the phytotoxic effect of extracts and fractions of leaves and screen the secondary compounds in the methanol extract. The organic extracts and fractions showed phytotoxic potential in germination indexes, the initial growth of the root system and the mitotic index of L. sativa. In phytochemical screening performed by HPLC-PAD was possible to identify the presence of phenolic compounds, mainly flavonoids, a secondary class of compounds widely known in the literature for its medicinal and allelopathic actions. Therefore, according to the results it can be concluded that the species N. theifera is capable of producing phytotoxic compounds, since the leaf extracts and fractions changed the pattern germination indexes, root length and mitotic index of lettuce.*

***Keywords*****— germination indexes, HPLC-PAD, mitotic index, Phytotoxicity, Root length.**

## I. INTRODUCTION

The agricultural pest management occurs primarily through the use of chemical pesticides, which are often inadequate causing environmental damage. This situation may change physical and chemical properties of soil and water. However, it is common knowledge the importance of synthetic products for increased food production (Tigre et al. 2012). Given this reality, alternative methods of weed and pests control are required. With several professionals from different areas investigating different alternatives, it is possible to assure that the plant phytotoxic compounds can be highlighted as a good solution for the problem (Rice 1984; Inderjit et al. 2011).

Some plant species have the ability to produce chemical compounds which, when released into the environment, may interfere directly or indirectly with the growth and development of other nearby organisms. Such phytotoxic phenomenon is known as allelopathy (Inderjit and Duke 2003; Silva et al. 2012).

The allelochemicals indirect effects include changes in properties and nutritional soil characteristics, also changing the activity and different population dynamics. It is already known that the direct effects can cause alterations in growth and plant metabolism, involving changes at the cellular level, phytohormonal, photosynthetic, respiratory, protein synthesis, lipid metabolism and organic acids; inhibition or stimulation of specific enzymatic activity, effects on water relationship and on DNA or RNA the synthesis in target plants (Rizvi et al. 1992; Inderjit et al. 2011).

In the brazilian savanna, allelopathy is responsible for interspecific and intraspecific interactions in stabilization and maintenance of different life forms present in this biome (Durigan et al. 2004). Among numerous plants species occurring in this biome, *Neea theifera* (Nyctaginaceae) stands out by popular accounts, having a peculiar ecological characteristic: beneath its canopy does not occur the development of other plant species. *Neea theifera*, popularly known as "field-pink-cover" belongs to Nyctaginacea family.

It occurs in grassland phytophysiogenomies in the east and north of the São Paulo state, Brazil (Furlan 1996). It is classified as a small tree with rough branches, simple leaves, flowers arranged in panicles and reddish yellow oblong fruits (Durigan et al. 2004). In addition to their ecological traits, the species is also used in popular medicine to treat stomach ulcers and

inflammation, characteristics which show that the species has the ability to produce bioactive substances (Rinaldo et al. 2007).

From the ecological and medicinal characteristics reported in the literature about *N. theifera*, the aim of this study was to evaluate the phytotoxic potential and determine the secondary compounds present in the extracts and leaf fractions of this species.

## II. MATERIAL AND METHOD

### 2.1 Biological Material

The leaves of *Neea theifera* were collected from a specimen found in the cerrado region nearby Uberlândia city (Minas Gerais - Brazil) (218º58'29,14"S e 48°16'30,04"W). The species voucher is deposited in the scientific collection of the Plant Systematics laboratory, FCL, UNESP - Assis (HASSI) sob number 221. After collection, the leaves of *Neea theifera* were separated, washed and dried in a drying oven with an average temperature of 40°C. After drying were ground in a knife mill. The powder obtained was subjected to extraction by mechanical agitation with different organic solvents (n-hexane, ethyl acetate and methanol - IMPEX, Brazil), to obtain three different extracts, in proportion of 1:10 (w/v) by 24 hours, the process was repeated 3 times with the same plant materials. The filtered extracts were pooled and concentrated with the aid of a rotary evaporator (model L101: Líotop, Brazil) at an average temperature of 50 °C followed by drying chamber at room temperature.

### 2.2 Bioassay of Allelopathy for Pre-emergence

The pre-emergence bioassay was conducted with seeds of *Lactuca sativa* L. cv. Grand Rapids (lettuce) by controlling the germination of these plants in Petri dishes (60 mm × 15 mm) and germination paper with relative humidity, temperature and light artificially controlled in greenhouses of Germination type BOD (Biological Oxygen Demand) (model: 411/FPD, New Ethics, Brazil). This experiment was set up in a completely randomized design (CRD), where the Petri dishes were divided into experimental and control groups containing 50 seeds of lettuce on each plate, with four replicas for each experimental group treated with different organic extracts of *N. theifera* (at concentrations of 5, 10 and 20 mg $mL^{-1}$) and later with the fractions of the methanol extract and negative control group (water). The protrusion and geotropic curvature of the radicle was used as germination criteria as indicated by Labouriau (1983). The seeds that showed false germination by soaking were not considered in the results. The germination of the species was monitored every 6h over 48h.

From the resulting data obtained in the assay, different rates were calculated: germinability or germination percentage ($[\Sigma ni/A]\bullet 100$), germination mean time ($Tm = [\Sigma ni \bullet ti]/\Sigma ni$), and germination mean speed ($Vm = 1/Tm$) in which ni = the number of seeds that germinated in each time gap "ti"; A=the total number of seeds in the test; and ti = the time gap between the beginning of the experiment and the observation time (Santana and Ranal, 2004; Pereira et al. 2009).

### 2.3 Bioassay of Allelopathy for Post-Emergence

The bioassay was performed according to the methodology proposed by Soares and Vieira (2000) and Alves et al. (2004) and adapted to our laboratory conditions. Lettuce seeds were previously germinated in Petri dishes lined with germination paper moistened with distilled water. After 24h under BOD greenhouse conditions, the seedlings that showed an average of 2mm in length were used in the bioassay, which was set up in a completely randomized design (CRD) with Petri dishes containing germination paper moistened with 1mL of the solution from the different extract concentrations of *N. theifera* and later with the fractions of the methanol extract. These were divided into experimental and control groups, containing 25 seedlings on each plate with four replicas per treatment and for the control (water).

The evolution process of the treatments were observed and the measurement of roots and hypocotyls were performed using a digital caliper (model: IP65, DIGIMESS®, Brazil) every 24h up to 48h of exposure (Procópio et al. 2005).

### 2.4 Statistical analysis for Pre and Post-Emergence testing

For statistical treatment of pre and post-emergence tests, normality (Shapiro-Wilks) and homogeneity tests (Levene) were performed. The data did not present normality and its variances were not homogeneous. Therefore, the results were analyzed using the Kruskal-Wallis and Dunn test ($\alpha$=0.05) with the use of BioEstat 5.3 software according to the model proposed by Santana and Ranal (2004).

### 2.5 Fractionation of the methanolic extract

The methanolic crude extract from the leaves of *N. theifera* was subjected to fractionation because it showed the highest allelopathic activity in pre and postemergence trials. For this purpose, a chromatographic column was fitted with approximately 75% silica and 25% Silica Gel 60 (Sigma-Aldrich ®, USA) incorporated with 2.0 g of extract. The sequence of solvents for the elution was n-hexane, dichloromethane, ethyl acetate, ethyl acetate: methanol (70:30), ethyl acetate: methanol (50:50), ethyl acetate: methanol (30:70) and methanol. Changes in solvents were held whenever the fraction remained without evidence of separation. Filtered fractions were concentrated on a rotary evaporator at 40±2 °C. Then, they were subjected to bioassays for both pre and post emergence.

### 2.6 Determination of osmotic potential

The osmotic potential of organic and fraction extracts were determined according to the technique described by Villela et al. (1991). The treatment was evaluated by osmotic solutions obtained using polyethylene glycol 6000 (PEG 6000) in the amounts indicated to establish the osmotic potential of −0.01 to−1.0MPa. The values of osmotic potential obtained in PEG6000 solutions were compared with the values found in the different concentrations of the extracts of *N. theifera*.

### 2.7 Mitotic index

Seeds of *Lactuca sativa* were previously germinated in Petri dishes. Once the roots of the seedlings reached 1 cm length, they were exposed to extracts methanolic and ethyl acetate:methanol fraction (70:30) at concentrations of 2 mg $mL^{-1}$ and water control for a period of 48 h. After this period, the roots were replaced into a Petri dish containing distilled water until they reached an average length of 5cm (recovery period). The entire experiment was conducted in a greenhouse germination type BOD. The roots were fixed in Carnoy (absolute ethyl alcohol and glacial acetic acid, 3:1). For assembly and analysis, the roots were hydrolyzed in hydrochloric acid (HCl) 1N at 60°C for 6 min. The roots were placed on slides and a drop of 2% acetic Carmine was added, covered with cover slips and crushed with glass rod in a soft way and fixed. Analyzes of 5000 cells per treatment were performed using an optical microscope (100x), with four replicas per treatment. Phytotoxic effects of the extracts were determined by analysis of the mitotic index (the total number of dividing cells divided by the total number of cells analyzed, multiplied by 100). Statistical analysis of the results from the *L. sativa* assay was submitted to the nonparametric tests: Kruskal-Wallis and Mann–Whitney (analysis significance level of 5% and 1%) according to Guerra and Souza (2002).

### 2.8 HPLC-PAD analysis and instrumentation

The chromatographic profile of *N. theifera* MeOH leaves extract was obtained using a HPLC system with PU-2089S Plus (Jasco®) pump equipped with a MD-2015 Plus Photodiode Array Detector (PAD, Jasco®) and AS-2055 automatic injector (Jasco®) with Phenomenex® Luna C18 (250 × 4.6 mm i.d.; 5 μm) column and (Phenomenex®) 4 × 3 mm i.d., column guard. The chromatogram was monitored at 200-600 nm.

The *N. theifera* MeOH leaves extract was pretreated using solid phase extraction (SPE) cartridges (Strata-X, Phenomenex® - 500.0 mg of silica C18). An aliquote of 10mg was dissolved in 1mL of MeOH : H2O (1:1 v/v) and then eluted with 5mL of MeOH : H2O (8:2 v/v), yielding one fraction.

## III. RESULTS

### 3.1 Pre-emergence and post-emergence Test

In Table 1, the germination average percentage for the N-hexane extract showed statistical difference between its lower and other two concentrations, and these two did not differ from each other, but differed from the control group. As for the time and germination average speed, there was no difference between the experimental groups, but differed statistically when compared to the control.

The germination time and germination average speed rates for *L. sativa* seeds subjected to different concentrations of ethyl acetate extract have been verified that the 5mg $mL^{-1}$ concentration showed statistically significant differences from the other groups, but when compared to control group showed no significant difference (Table 1).

For the post emergence test, the treatments with different concentrations of methanol and ethyl acetate extracts showed a statistically significant difference between themselves and as compared with the water control, in radical measurement both at 24 and 48 hours. As for the N-hexane extract after 24 hours, was observed that the experimental groups were not statistically different from each other and neither when compared with control group. However after 48 hours of exposure, it

was observed that the concentration of 5mg $mL^{-1}$ was statistically different from treatments with 10 and 20mg $mL^{-1}$, and the two higher concentrations showed statistical difference when compared to the water control (Table 1).

**TABLE 1**
**DIFFERENT CONCENTRATIONS EFFECTS OF *NEEA THEIFERA* N-HEXANE, ETHYL ACETATE AND METHANOL EXTRACT ON *LACTUCA SATIVA* SEED GERMINATION AND GROWTH OF SEEDLINGS.**

| Treatment | Extract (mg $mL^{-1}$) | G±SD (%) | AT±SD (hours) | AS±SD (Seeds/hs) | Radicle 24h | Radicle 48h |
|---|---|---|---|---|---|---|
| n-Hexane | 5 | 66.00±4.54[a] | 35.37±3.64[a] | 0.028±0.003a | 7.97±0.53d | 15.86±0.94f |
| | 10 | 18.00±5.40b | 42.13±2.29[a] | 0.023±0.001a | 7.59±0.47d | 12.95±0.95g |
| | 20 | 15.00±2.18b | 37.43±7.57[a] | 0.027±0.005a | 7.33±0.40d | 11.43±0.35g |
| Ethyl Acetate | 5 | 87.00±1.48c | 24.70±2.55b | 0.040±0.004b | 6.19± 0.74a | 8.28±1.10d |
| | 10 | 15.00±1.37b | 34.82±5.10[a] | 0.029±0.004a | 5.22±0.86b | 7.84±1.58b |
| | 20 | 14.50±2.41b | 37.37±7.59[a] | 0.027±0.005a | 3.29±0.64c | 5.11±0.75e |
| Methanol | 5 | 74.00±3.90[a] | 35.01±1.52[a] | 0.028±0.001a | 6.10±0.87[a] | 9.68±1.05a |
| | 10 | 47.50±1.12d | 36.91±1.53[a] | 0.027±0.001a | 5.15±0.42b | 7.11±0.98b |
| | 20 | 14.50±0.75b | 32.41±6.75[a] | 0.031±0.005a | 3.78±0.65c | 6.43±0.81c |
| Water | | 99.00±1.15c | 19.66±0.68b | 0.050±0.001b | 7.44±0.88d | 15.09±1.07f |

***Averages with the same letter in the column do not differ, with α = 0.5 probability by Tukey test. Legend: G%= germination average percentage. AT= average germination time and AS= germination average speed***

### 3.1.1 Pre-emergence and post-emergence test with the methanol extract fractions

Table 2 shows the biological assays results, pre and post emergence with the different methanol extract fractions. For the germination rate was found that the fractions showed statistical difference when compared to the control group. However when the fractions were compared between themselves, the n-hexane and dichloromethane were not statistically different from each other, but were not statistically different compared to the other fractions, similar to that observed for fractions ethyl acetate and ethyl acetate: methanol (70:30). For groups treated with fractions of ethyl acetate:methanol (50:50), ethyl acetate: methanol (30:70) and methanol was not verified statistical difference between them, but they have become statistically different when compared with other.

As for the time and germination average speed, the fractions were not significantly different from each other, but all differed when compared to the control group. Regarding the post-emergence test conducted in the different fractions, it was found that after 24 hours of exposure, the dichloromethane fraction was the only treatment that showed no statistical difference when compared to the water control. However after 48 hours of exposure all treatments were statistically different when compared to control.

**TABLE 2. ACTIVITY OF FRACTIONS OF THE *NEEA THEIFERA* METHANOL EXTRACT ON SEED GERMINATION AND GROWTH OF *LACTUCA SATIVA* SEEDLINGS.**

| Treatment | Extract (mg $mL^{-1}$) | G±SD (%) | AT±SD (hours) | AS±SD (seeds/hs) | Radicle 24h | Radicle 48h |
|---|---|---|---|---|---|---|
| N-Hexane | 5 | 55.50±2.64a | 38.00±1.15[a] | 0.026±0.000a | 6.27±0.74a | 12.43±1.26[a] |
| Diclorometane | 10 | 52.50±7.91a | 36.03±1.30[a] | 0.027±0.001a | 7.61±1.41b | 12.65±0.89[a] |
| Ethyl Acetate | 20 | 38.00±1.70b | 36.93±1.28[a] | 0.027±0.000a | 4.07±0.74c | 7.15±0.48c |
| Acet/Met (70:30) | 10 | 35.00±6.29b | 38.31±2.48[a] | 0.026±0.001a | 3.52±1.01d | 5.60±0.75e |
| Acet/Met (50:50) | 10 | 67.50±4.91c | 36.03±2.71[a] | 0.027±0.002a | 3.79±0.83d | 7.21±0.70c |
| Acet/Met (30:70) | 10 | 60.00±4.40c | 36.21±1.99[a] | 0.027±0.001a | 5.90±0.50a | 9.41±0.62c |
| Methanol | 10 | 64.50±3.70c | 37.20±1.30[a] | 0.026±0.000a | 4.61±0.47c | 8.760±0.77c |
| Water | | 100.0±0.00d | 20.73±0.20b | 0.048±0.000b | 8.05±1.01b | 14.45±1.32g |

***Averages with the same letter in the column do not differ, with α = 0.5 probability by Tukey test. Legend: G%= germination average percentage, AT= average germination time and AS= germination average speed.***

### 3.2 Osmotic potential of organic extracts and methanol extract fractions

Table 3 shows the osmotic potential values measured for different organic extracts and methanol extract fractions. The variation of osmotic potential for organic extracts was -0.019 to -0.037, and in fractions -0.033 to -0.038 MPa.

**TABLE 3**
**OSMOTIC POTENTIAL OF ORGANIC EXTRACTS AND METHANOL EXTRACT FRACTIONS OF *NEEA THEIFERA***

| Extract | Concentration | Mpa |
|---|---|---|
| N-Hexane | 5mg $mL^{-1}$ | -0.035 |
| | 10mg $mL^{-1}$ | -0.035 |
| | 20mg $mL^{-1}$ | -0.037 |
| Ethyl Acetate | 5mg $mL^{-1}$ | -0.035 |
| | 10mg $mL^{-1}$ | -0.035 |
| | 20mg $mL^{-1}$ | -0.034 |
| Methanol | 5mg $mL^{-1}$ | -0.034 |
| | 10mg $mL^{-1}$ | -0.034 |
| | 20mg $mL^{-1}$ | -0.019 |
| **Fractions** | | |
| N-Hexane | 5mg $mL^{-1}$ | -0.038 |
| Diclorometane | 10mg $mL^{-1}$ | -0.037 |
| Ethyl Acetate | 20mg $mL^{-1}$ | -0.037 |
| Acet 70:30 Met | 10mg $mL^{-1}$ | -0.033 |
| Acet 50:50 Met | 10mg $mL^{-1}$ | -0.037 |
| Acet 30:70 Met | 10mg $mL^{-1}$ | -0.037 |
| Methanol | 10mg $mL^{-1}$ | -0.038 |
| | | |
| Water | | -0.038 |

### 3.3 Mitotic index in root meristem cells of L. sativa

The lettuce seeds treated with the methanol extract and ethyl acetate: methanol (70:30) fraction both at a 2mg $mL^{-1}$ concentration showed the mitotic index of 10.06 and 7.30 respectively, and only treatment with ethyl acetate: methanol (70:30) was statistically different from the control (9.50). The same was observed for the different phases of mitosis, in which the treatment ethyl acetate: methanol (70:30) fraction was the only one that reduced the cells number at these stages (Table 4).

**TABLE 4**
**MITOTIC INDEX OF LETTUCE ROOT MERISTEMATIC CELLS TREATED WITH THE CRUDE EXTRACT METHANOL, ETHYL ACETATE:METHANOL (70:30) FRACTION AT A CONCENTRATION OF 2 MG ML-1 AND WATER AS CONTROL.**

| Treatment | Interphase | Cell Division | | | | Mitotic Index |
|---|---|---|---|---|---|---|
| | | Prophase | Metaphase | Anaphaase | Telophase | |
| CE | 4497 | 374 | 71 | 20 | 38 | 10.06a |
| FE | 4635 | 289 | 22 | 18 | 36 | 07.30b |
| C | 4525 | 370 | 45 | 19 | 41 | 09.50a |

***5000 cells analyzed. Same letters column do not differ statistically average assessed with Kruskal-Wallis test (p <0.05). CE: methanolic crude extract; FE: ethyl acetate:methanol fraction of methanolic extract ; C: control with water.***

### 3.4 Identification of compounds by HPLC-PAD

Identification of all constituents in the *N. theifera* MeOH leaves extract was performed by HPLC-PAD analysis by comparing the retention time ($R_t$) and the UV-vis spectra data with literature (Rinaldo et al., 2007).

Seven peaks were characterized in the chromatogram (Fig. 1) of the *N. theifera* MeOH leaves extract. Peak **1** ( $R_t$ = 34.40, $\lambda_{max}$ = 272, 350 nm), Peak **2** ( $R_t$ = 35.44, $\lambda_{max}$ = 272, 350 nm), Peak **3** ( $R_t$ = 37.11, $\lambda_{max}$ = 272, 338 nm), Peak **4** ( $R_t$ = 37.87,

$\lambda_{max}$ = 272, 332 nm), Peak **5** ($R_t$ = 41.07, $\lambda_{max}$ = 260, 356 nm), Peak **6** ( $R_t$ = 58.81, $\lambda_{max}$ = 272, 338 nm) and Peak **7** ($R_t$ = 61.24, $\lambda_{max}$ = 272, 338 nm) were related to flavones derivatives (Andersen and Markham, 2006).

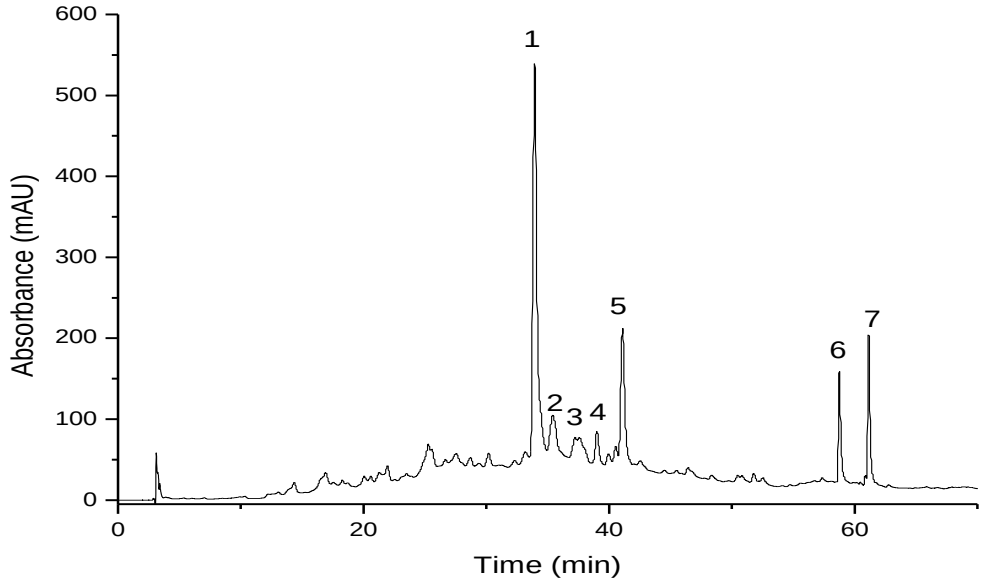


**FIGURE 1 - HPLC–PAD ANALYTICAL CHROMATOGRAM OF *N. THEIFERA* MeOH LEAVES EXTRACT WITH CHARACTERIZED PEAKS. EXPERIMENTAL CONDITIONS: ELUENTS A (MeOH + 0.1% Formic ac.) and B ($H_2O$ + 0.1% Formic ac.). Elution system: 5-100% of A in B in 60 min and 100% of A in 10 min. Column: Phenomenex® LunaC18 (250 × 4.6 mm i.d., 5 µm). Flow rate: 1 mL·min−1, λ = 254 nm. Injected volume: 20 µL.**

## IV. DISCUSSION

Plants with phytotoxic potential tend to act in different ways depending on the environment and the physiological stage of development that is the target individual. Due to the phytotoxic action of some classes of secondary compounds, plants capable of producing such substances are frequent targets of agronomic research, especially because they are potential alternatives to synthetic compounds with high environmental toxicity (Inderjit et al. 2011; Santos et al. 2015).

In the literature is common to find methodologies that test the phytotoxic potential of plant extracts by means of bioassays on the early development of seedlings target (Ferreira and Aquila 2000; Inderjit and Duke 2003; Silva et al. 2012). The results obtained in this study showed changes in germination indexes (Table 1 and Table 2) both in tests with organic extracts as with the fractions. According Labouriau (1983) and Maraschin-Silva et al. (2006), these changes indicate the interference of allelochemicals in metabolic reactions that culminate in germination.

Rice (1984) and Inderjit et al. (2006) reported that the changes in germination pattern can result from several effects in primary level. Among them are changes in permeability of membranes, in transcription and DNA translation, in the functioning of secondary messengers, in respiration due to the sequestration of oxygen, in the formation of enzymes and receptors, or a combination of these factors.

In relation to the root development, there was reduction in the size of the hypocotyl-root axis of seedlings subjected to organic extracts and fractions of *N. theifera.* (Table 1 and 2). Similar to those observed in studies by Carmo et al. (2007) and Ferreira et al. (2007), Miro et al. (1998) and Aquila (2000) observed in their studies that allelopathic effect is more pronounced on the initial development of a target seedling compared to germination, since the latter process utilizes own seed reserves. However, the results obtained in this study showed effects both on the vegetative development and on germination (Table 1 and 2).

Some of the effects caused by phytotoxic compounds are secondary reflections of changes that occur at the molecular level (Rizvi et al. 1992). Cruz-Ortega et al. (1998) reports that the hardening and darkening of the radicular apex are evidences of morphological and ultrastructural alterations caused by phytotoxins. Such aspects were observed in the experimental groups with the organic extracts and fractions of *N. theifera*.

Another factor that may interfere with germination and plant development process is the osmotic potential. In this study, the potential remained low and within appropriate germination patterns when compared with literature data (Table 3). To *Mimosa bimucronata* (DC.) OK., osmotic potential situated between -0.158 to -0.414 MPa are able to affect germination and growth (Astarita et al. 1996). Gatti et al. (2004) recommend that the osmotic potential germination and root development does not exceed -0,2MPa. The osmotic potential is an important variable to be evaluated in investigations of allelopathic and phytotoxic potential of plant extracts, since they can change the osmotic potential so that could prevent the germination and seedling development thus inducing misinterpretation of results. (Villela et al. 1991)

In the results of mitotic index, we observed that the ethyl acetate: methanol fraction (70:30) at a concentration of 2 mg $mL^{-1}$ was the treatment that showed a lower number of dividing cells (Table 4). The cytotoxic effect was observed in the intermediate concentration of the fraction in relation to other experimental groups and control, this may be due to the relationship extractor/secondary metabolite facilitating the extraction of a particular chemical component, since the saturation of extractor liquid or establishment of a diffusion balance between extractant and the into the cell does not allow the exhaustion of the raw plant (Sonaglio et al. 2003). In the literature is established some classes of phytotoxic compounds have the ability to control the production and accumulation of reactive oxygen (ROS), which when accumulated in the cell may cause cellular damage, such as changes in membrane permeability, thus leading to cell death (Silva et al. 2012; Silva et al. 2014). These results corroborate with studies conducted by Pires et al. (2001), which found that aqueous extracts of *Leucaena leucocephala* reduced the mitotic index in maize roots, compromising your normal stretching. Similar results were also reported by Lopes et al. (2007) and Mecina et al. (2014), which showed that different concentrations of *Brugmansia suaveolens* and *Ouratea spectabilis* respectively reduced the mitotic index of onion seeds. In turn, Souza et al. (2005) verified the existence of cell disorders (anaphase bridges) in roots cells of lettuce whose seeds were subjected to aqueous extracts of *Maytenus ilicifolia* M.

Many organic compounds are produced by higher plants that are considered phytotoxic, these substances may belong to different classes of compounds of the secondary metabolism (Inderjit 1996). According to Rice (1984) and Putnam (1988) the allelochemicals can exert inhibitory effects or additives and such metabolic changes may result from a synergy between these compounds, thus making important the analysis of the action of each substance alone. Studies with allelochemicals components carried out by Vyvyan (2002) showed that the main substances with allelopathic potential, that act in pre-emergence and post-emergence, are mainly secondary compounds belonging to the group of phenolic compounds, and within that large group we can highlight the class of flavonoids, which is the class most well studied due to its medicinal and agricultural effects as sought by companies in the industry.

The presence of phenolic compounds in the phytochemical screening carried out in HPLC can be one of the factors responsible for allelopathic activity observed in the germination test, root growth and cytotoxicity (Fig. 1). Due to its chemical characteristics, some classes of phenolic compounds are capable of capturing electrons, act as catalysts for the photochemical stage in photosynthesis, act as regulators of ion channels involved in oxidative phosphorylation, among other process (Carmo et al. 2007). Flavonoids participate in the processes related to ion absorption which may affect the electrochemical gradient of cell membranes of the roots (Glass and Dunlop 1974) and depending on the concentration may promote or inhibit the growth of roots (Macias et al. 1997). These results corroborate with studies by Maraschin-Silva et al. (2006) which showed that leaf extract *Cecropia pachystachya* and *Peltrophorum dubium* with a relative amount of flavones presented allelopathic effect in both pre and post-emergence testing.

Therefore, according with results obtained in this research, we can conclude that the species *Neea theifera* has the ability to produce phytotoxic compounds, since their leaf extracts and fractions caused changes physiological in the target plant as confirmed by the alteration in the patterns of seed germination, root length and mitotic index.

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