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Phytotoxicity of *Tridax procumbens* L.

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ABSTRACT

Phytotoxicity is the term used to describe the toxic effect of chemical compounds on growth and development of plants. Phytotoxic compounds vary in their chemical composition, and may be found in natural environments due to their biosynthesis and active release (allelochemicals). These compounds have the potential to be used as natural pesticides for the control of agricultural plagues. Therefore, this study aimed to evaluate the phytotoxic potential of different extracts and fractions of *Tridax procumbens*, using pre and post-emergence bioassays of *Lactuca sativa* L. seeds and mitotic index of *Allium cepa* L. root cells. Furthermore, the chemical compounds present in the extracts were phytochemically elucidated and the most bioactive fraction determined. Germination and development of tested seedlings were inhibited by the different extracts and ethyl acetate fraction of the ethanolic extract. There were notable alterations in the mitotic index of exposed *A. cepa* root cells compared to those of the negative control. Phytochemical identification was performed by HPLC-PAD, which showed that a considerable amount of flavonoid compounds were present in the ethanolic extract and its ethyl acetate fraction. According to the results obtained, it can be concluded that *T. procumbens* possesses phytotoxic compounds that are probably of flavonoid origin. The possession of these bioactive compounds may be related to its invasive nature and weed capacity.

1. Introduction

Phytotoxicity is the term used to describe the toxic effects of a variety of compounds on the growth and development of plant species. These compounds can cause molecular alterations, which include modification of gene transcription and/or duplication of the DNA molecule. These alterations lead to chromosome mutation and aberration, and subsequent damage to vital processes of target plants (Inderjit et al., 1999; Gniazdowska and Bogatek, 2005). Phytotoxicity can be caused by a vast variety of compounds such as heavy metals, pesticides and allelochemical substances (Martins et al., 2007; Guimaraes et al., 2007; Carvalho et al., 2009). Compounds produced by plants can only be termed allelochemicals, if they are actively produced and released into the environment to directly or indirectly interfere with the growth and development of surrounding species (Chou and Kuo, 1986; Costa and Menk, 2000; Souza Filho and Alves, 2002).

Research related to monitoring bioactivity of extracts, fractions and isolated compounds from plants, has been frequently used to identify potentially toxic substances, especially those with allelopathic characteristics (Inderjit and Callaway, 2003; Noldin et al., 2003). Therefore, allelopathic activity of extracts can be evaluated from their effect on germination of pre-selected sensitive species, mitotic index and chromosomal aberrations (Morel and Guillemain, 2004). Species like lettuce, tomatoes, onion, radish and cucumbers are frequently used for testing the phytotoxic effects of allelochemicals/bioactive compounds (Mazzafera, 2003; Morel and Guillemain, 2004).

Recent research has demonstrated the applicability of allelochemicals as an important means of controlling weeds, insects, algae and microorganisms, that directly and/or indirectly prejudice agricultural production and human health (Leflaive and Ten-Hage, 2007; Hajimahmoodi et al., 2010; Bártová et al., 2011; El Marsni et al., 2011; Zak et al., 2012; Kato-Noguchi et al., 2012).

Brazil is the fifth largest consumer of pesticides worldwide. More than 150,000 tons of pesticides with herbicides representing 33% of this amount is used per year in Brazil (IBGE, 2009). Chemical control is the most common means of handling invasive plants and plagues in Brazil. However, this causes ecosystem disequilibrium, changing the physical and chemical properties of water and soil, and presenting agro-ecological management challenges (Mascarenhas, 1999; Lorenzi, 2000; Modesto Júnior and Mascarenhas, 2001; Macías et al., 2007).

Tridax procumbens L. (Asteraceae) popularly known as "erva-de-touro", is an invasive perennial plant, occurring mainly in tropical and subtropical environments, and occupying pastures, meadows, fields,

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side of highways and degraded areas (Kissmann and Groth, 1999). It has therapeutic, hypotensive (Salahdeen et al., 2004), immunomodulatory, antibiotic, antioxidant, anti-inflammatory and anticancer properties (Tiwari et al., 2004; Sharma and Kumar, 2009; Agrawal et al., 2009; Jachak et al., 2011). Phytochemical assays performed with this species have confirmed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins (Jude et al., 2009; Agrawal and Talele, 2011).

The present study evaluated the phytotoxic potential of different extracts and fractions of *T. procumbens*, using pre- and post-emergence bioassays of *Lactuca sativa* L. seeds and mitotic index of *Allium cepa* L. root cells. Furthermore, chemical compounds present in the most bioactive extracts and fractions were phytochemically elucidated.

2. Materials and methods

2.1. Plant material and extracts preparations

The plant parts (branches, leaves and flowers) of *T. procumbens* were collected from specimens found in the Universidade Estadua Paulista — SP (22°39′42″S e 50°24′44″W, Altitude: 546 m). The species voucher is deposited in the scientific collection of the Plant Systematics laboratory, FCL, UNESP — Assis (HASSI) sobnumber 113. For preparation of the extracts, the plant parts were pooled, washed, dried in stove (40 °C) and pulverized. The aqueous extract was obtained by mechanical agitation, in distilled water (proportion 1:10 (w:v) for 24 h at 24 °C). Right after, it was vacuum filtered, frozen and lyophilized (model: L101, Líotop. Brazil) to obtain a dry extract. The hydroethanolic extract was obtained by mechanical agitation in a ethanol:water (70:30) solution in the proportion 1:10 (w:v) for 24 h. The process was repeated 3 times with the same plant material. Then, the obtained extract was filtered and taken to a rotary evaporator (model: MA120, Marconi, Brazil) at 60 °C for the ethanol removal and later frozen and lyophilized to obtain a dry extract. The same was performed to the ethanolic extract, where only the ethanol:water (70:30) solution was substituted by absolute ethanol (IMPEX, Brazil), which had the dry extract obtained by rotary evaporator concentration followed by desiccation chamber at room temperature.

2.2. Extract fraction

The crude ethanol extract of *T. procumbens* parts was subjected to fractionation as it had the highest allelopathic activity in trials of pre- and post-emergence, for this reason a chromatographic column was set with about 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 of solvents were performed 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 the pre-and post-emergence bioassays.

2.3. Bioassay for pre-emergence

The pre-emergence bioassay was performed with *L. sativa* L. cv. Grand Rapids (Lettuce) seeds by control of seeds germination of this plant in Petri dishes (60 mm × 15 mm) and germination paper, with relative humidity, temperature and luminosity controlled artificially in germination greenhouse type BOD (Biological Oxygen Demand) (model: 411/FPD, Nova Ética, Brazil). Moreover, an experiment was set with an completely randomized design (CRD), where petri dishes were separated in experimental groups and control, containing 50 lettuce seeds in each plate, with six repetitions to each experimental group, treated with 1 mL of the different extracts of *T. procumbens* (in the concentrations of 5, 10 and 20 mg mL^{-1}), and a negative control group (water). As the germination evaluation criteria it was used the protrusion and the geotropic curvature of the radicle, as indicated by Labouriau (1983). The seeds that presented fake germination by imbibition were not accounted in the results. The monitoring of the species germination was performed every 6 h, during 48 h.

For the results obtained in the assays, different indexes were calculated: average percentage of germination ($[\sum ni / A] \cdot 100$), average time of germination ($Tm = [\sum ni \cdot ti] / \sum ni$) and average germination speed ($Vm = 1 / Tm$) where ni = number of seeds germinated at each instant "ti"; A = total number of seeds put to germinate; and ti = time between the begin of the experiment and the time i-th of observation (Santana and Ranal, 2004; Pereira et al., 2009).

2.4. Bioassay for post-emergence

The bioassay was performed according to the methodology suggested by Soares and Vieira (2000) and Alves et al. (2004) and adapted to our laboratory conditions. The lettuce seeds were previously germinated in Petri dishes, covered with germination paper and dampened with distilled water. After 24 h in BOD greenhouse conditions, the seedlings that presented an average length of 2 mm were used in the bioassay, which was set with a completely randomized design (CRD) with Petri dishes containing as substrate germination paper, dampened with 1 mL of different *T. procumbens* extracts solution. These were separated in experimental and control groups, containing 25 seedlings in each plate, with four replicates for each treatment and control (water).

The evolution process of the treatments was performed by observation and measurement of seedlings radicles and hypocotyl, using a digital caliper rule (model: IP65, DIGIMESS®, Brazil), at each 24 h until it completed 48 h of exposition (MIRÓ et al., 1998; PROCOPIO et al., 2005).

2.5. Statistical analysis for pre- and post-emergence testing

For the statistical analysis of pre- and post-emergence tests were performed the normality tests and homogeneity. The transformed data did not present normality and its variances were not homogeneous, therefore the results were analyzed by Kruskal–Wallis and Dunn ($\alpha = 0.05$) with the support of the BioEstat 5.3 software, according to Santana and Ranal (2004).

2.6. Determination of osmotic potential, pH and electrical conductivity

The osmotic potential determination was performed according to the technique described by Villela et al. (1991). The treatment was evaluated by osmotic solutions obtained using Polyethylene Glycol 6000 (PEG 6000). The values obtained in the PEG 6000 osmotic potential solutions were compared to the values found in the *T. procumbens* extracts.

The pH of the different *T. procumbens* extracts was determined using a pH meter (Tecnopon® model MPA210). Similarly, the electrical conductivity was measured with a conductivity meter (Conductivity Meter Instrutherm®, model CD860).

2.7. A. cepa root cell mitotic index

A. cepa (onion) seeds were germinated in Petri dishes, when the roots of the seedlings reached 1 cm in length they were exposed to extracts at concentrations that presented highest activity in pre- and post-emergence experiments, for a period of 48 h. After this period the seedlings were replaced in Petri dishes containing distilled water until they reached 5 cm in length average (recovery period). The entire experiment was conducted in a BOD germination greenhouse conditions. The roots were fixed in Carnoy (absolute ethyl alcohol and glacial acetic acid, 3:1). Following for setting and analysis of the roots, they were hydrolyzed in hydrochloric acid (HCl) 1 N at 60 °C for 8 min, and right after, were stained with Schiff Reactive for 2 h in the dark.