

# Phytotoxicity of organic extracts of *Turnera ulmifolia* L. and *Turnera diffusa* Willd. ex Schult. in cucumber seeds

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## ABSTRACT

We evaluated the phytotoxic effects that the hexane, ethyl acetate and methanol extracts from leaves and branches of the species *Turnera ulmifolia* L. and *Turnera diffusa* Willd. ex Schult. (Turneraceae), at concentrations of 1.25, 2.50, 3.75 and 5.00 mg ml<sup>-1</sup>, have on seed germination and seedling development in cucumber (*Cucumis sativus* L.). None of the extracts tested prevented germination, although the ethyl acetate extracts of *T. diffusa*, at 3.75 and 5.00 mg ml<sup>-1</sup>, reduced the mean germination speed and time to germination. Hexane extracts of both species reduced the main root length, number of secondary roots and hypocotyl length. In the ethyl acetate and methanol extracts, the number of secondary roots and hypocotyl length varied by species and concentration. Ethyl acetate extracts of *T. ulmifolia* at  $\leq 2.50$  mg ml<sup>-1</sup> stimulated growth of the main root and hypocotyl, as did *T. ulmifolia* methanol extracts at 1.25 and 3.75 mg ml<sup>-1</sup>; all other extract/concentration combinations had an inhibitory effect on those parameters. The hexane and ethyl acetate extracts of *T. diffusa* inhibited the formation of secondary roots and of the main root, although significant inhibition of hypocotyl growth was observed only at  $\geq 2.50$  mg ml<sup>-1</sup>. Comparatively, *T. diffusa* extracts inhibited development to a greater degree, thus presenting greater allelopathic potential, than did *T. ulmifolia* extracts.

**Key words:** Allelopathy, *Cucumis sativus*, germination, development

## Introduction

Allelopathy can be defined as any effect, direct or indirect, which a plant or microorganism has on another plant or organism. It occurs through the production of chemical compounds, which are released into the environment, in an aqueous phase via the substrate (soil), or by gaseous substances volatilized into the air (Rice 1984). Allelopathy has been recognized as a major mechanism of ecological dominance, plant succession, and plant community formation, as well as having applications in agriculture, to increase productivity and otherwise manage crops (Hierro & Callaway 2003). It has been used as an alternative means of controlling plant pests (insects and nematodes) and as a substitute for synthetic herbicides (Ferreira & Aquila 2000). For testing the allelopathic potential of one species on the germination or development of another (*i.e.*, the test species), it is common to use plants that are highly sensitive to allelochemicals, are affordable and are widely available (Ferreira & Aquila 2000). Cucumber (*Cucumis sativus* L.) is one such plant. The prospecting of plants for their allelopathic or phytotoxic potential typically involves the use of aqueous, hydroalcoholic extracts or partitioning with solvents of increasing polarity (Souza-Filho *et al.* 2010).

The allelopathic effects differ greatly depending on the plant to be studied, the part of the plant to be studied and the concentration of the extract (Souza-Filho *et al.* 2010).

The Turneraceae family occurs in Africa and in the Americas. In Brazil, there are two genera and approximately 80 species. *Turnera ulmifolia* and *T. diffusa* are shrubs usually found in gardens and also used in folk medicine. Distributed throughout Brazil, *T. ulmifolia* is popularly used to treat asthma, rheumatism, indigestion and bronchitis (Kumar *et al.* 2005). It has antioxidant and anti-inflammatory properties (Nascimento *et al.* 2006; Galvez *et al.* 2006). *Turnera diffusa* (var. *aphrodisiaca*) is found mainly in Mexico, where it is considered an aphrodisiac and is used as an astringent, diuretic, expectorant, purgative, stimulant or tonic (Kumar *et al.* 2005).

Although there have been some pharmacological studies involving species belonging to the Turneraceae family, there have been no studies of their allelopathic or phytotoxic potential. Considering that some *Turnera* species are considered invasive and many form dense and dominant populations, it is important to investigate such potential. Therefore, the main objective of the present work was to determine whether extracts of *T. ulmifolia* and *T. diffusa*, at different concentrations, have phytotoxic effects.

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## Material and methods

Leaves and branches of *T. ulmifolia* were collected in the municipality of Assis (22°38'39"S; 50°25'57.1"W), in the state of São Paulo, Brazil. A voucher specimen was deposited at the Dom Bento Pickel Herbarium of the São Paulo State Forestry Institute (code, SPSF; voucher specimen no. 41,584), in the city of São Paulo. This material was dried in an oven at 60°C before grinding. Pre-ground leaves and branches of *T. diffusa* were acquired from a commercial supplier (Santosflora, São Paulo, Brazil).

The plant material (10 g) was extracted by "dynamic" maceration (*i.e.*, maceration with agitation) in 100 ml of hexane for 2 h, and this step was repeated. The extract was filtered and concentrated under reduced pressure to produce a hexane extract yield of 1.36% for *T. ulmifolia* and 1.16% for *T. diffusa*. The plant residue obtained from this procedure was extracted three times with ethyl acetate in the same proportion as used above, resulting in a yield of 2.24% for *T. ulmifolia* and 0.812% for *T. diffusa*. The plant residue resulting from the extraction with ethyl acetate was extracted three times with methanol to yield 12.97% for *T. ulmifolia* and 12.48% for *T. diffusa*.

The bioassay was performed in Petri dishes (14 cm diameter) covered with two sheets of filter paper. The filter paper was moistened with 10 ml of extract, at different concentrations (5.00, 3.75, 2.50 and 1.25 mg ml<sup>-1</sup>), and allowed to dry. Distilled water was used as a negative control. The filter papers were then moistened with 10 ml of water, and 25 cucumber seeds were placed on each dish (n = 4 dishes per condition). The experiments were carried out in a seed germinator (FC 102; Eletrolab, Mumbai, India), at 25±1°C on a 12/12-h light/dark cycle. The criterion for germination was the protrusion of the radicle. We assessed germination as described by Santana & Ranal (2004), determining, for

each experimental condition, the mean percentage of seeds germinated (G), the mean speed of germination (v) and the mean time to germination (t), comparing the results with those obtained for the negative control. Germination was monitored every 12 h over a 72-h period. At 120 h, we evaluated the effects of each extract on the growth of the main root, the length of the hypocotyl and the number of secondary roots. In order to evaluate the effects of any solvent residue remaining after drying, a second control was performed by replacing the distilled water with the solvents. The solvents had no significant effect on germination or development when compared with the negative control (p>0.05, data not shown). The experimental design was completely randomized, each treatment being applied in quadruplicate. In our statistical analysis of the data, we applied the nonparametric Kruskal-Wallis test followed by Dunn's test (p<0.05), using the BioEstat program, version 5.0 (Sociedade Civil Mamirauá, Belém, Brazil). The organic extracts were compared, among themselves and with the negative control. The effect of increasing concentrations of the extracts on cucumber seedlings was also examined, and each extract was assessed individually by regression.

## Results and discussion

Germination is usually the first assay selected when studying allelopathy. In the present study, the *T. diffusa* and *T. ulmifolia* extracts were found to have no allelopathic effects on cucumber seed germination percentage (data not shown). However, other aspects of the germination process, such as delays, have been studied (Gusman *et al.* 2008). Therefore, we also analyzed the parameters of mean speed of germination and mean time to germination. For those parameters, only the ethyl acetate extract of *T. diffusa* at the

**Table 1.** Effects that extracts of *Turnera diffusa* Willd. ex Schult. (at 3.75 and 5.00 mg ml<sup>-1</sup>) had on the germination of cucumber seeds (n = 4 sets of 25 seeds per experimental condition).\*

Treatment	Parameter**		
	G (%)	t (hours)	v (hours <sup>-1</sup> )
Negative control (distilled water)	98±4.00 (a)	24.98±0.53 (b)	0.0400±0.0008 (a)
Extracts at 3.75 mg ml <sup>-1</sup>			
Hexane	94±5.00 (a)	25.04±0.11 (ab)	0.0399±0.0002 (ab)
Ethyl Acetate	96±2.00 (a)	28.00±1.54 (a)	0.0357±0.0020 (b)
Methanol	98±5.00 (a)	26.80±1.25 (ab)	0.0373±0.0018 (ab)
Extracts at 5.00 mg ml <sup>-1</sup>			
Hexane	98±4.00 (a)	25.68±0.73 (ab)	0.0389±0.0011 (ab)
Ethyl Acetate	100±1.00 (a)	28.16±0.84 (a)	0.0355±0.0010 (b)
Methanol	96±1.00 (a)	26.81±0.97 (ab)	0.0373±0.0013 (ab)

G – germination (percentage); t – time to germination; v – speed of germination.

\*Values presented as median and standard deviation; \*\*medians followed by same letter in the same column indicate that there was no significant difference (p>0.05) among treatments at the same concentrations or between each treatment and the control.

concentrations 3.75 and 5.00 mg ml<sup>-1</sup> caused a significant delay in cucumber germination in comparison with that observed for the control (Tab. 1). It has been reported that allelopathy affects germination less than it does seedling development (Ferreira & Aquila 2000). The effects that the extracts of *T. ulmifolia* and *T. diffusa* had on cucumber seedling development in the present study are shown in Tab. 2 and Tab. 3, respectively.

Tab. 2 shows the growth parameters for the control seedlings and for the seedlings germinated in the extracts of *T. ulmifolia*. The median number of secondary roots was significantly lower among the seedlings germinated in the hexane and methanol extracts, at all concentrations, as well as among those germinated in the ethyl acetate extract at  $\geq 2.50$  mg ml<sup>-1</sup>, than among the control seedlings. The median length of the main root was significantly shorter among the seedlings germinated in the hexane extract, as it was among those germinated in the ethyl acetate extract at  $\leq 2.50$  mg ml<sup>-1</sup>, although not among those germinated in the ethyl acetate extract at  $\geq 3.75$  mg ml<sup>-1</sup>. Among the seedlings germinated in the methanol extract at  $\geq 3.75$  mg ml<sup>-1</sup>, the growth of the main root was inhibited. Among the seedlings germinated in the hexane extract, the median length of the hypocotyl was significantly shorter than it was among the control seedlings. The same was true for the seedlings germinated in the ethyl acetate extract at  $\geq 2.50$  mg ml<sup>-1</sup>, although not at  $\geq 3.75$  mg ml<sup>-1</sup>. Among the

seedlings germinated in the methanol extract, the median length of the hypocotyl, in comparison with that observed for the control seedlings, was significantly longer at 1.25 mg ml<sup>-1</sup>, not statistically different at 2.50 mg ml<sup>-1</sup>, significantly longer at 3.75 mg ml<sup>-1</sup> and not statistically different at 5.00 mg ml<sup>-1</sup> (Tab. 2).

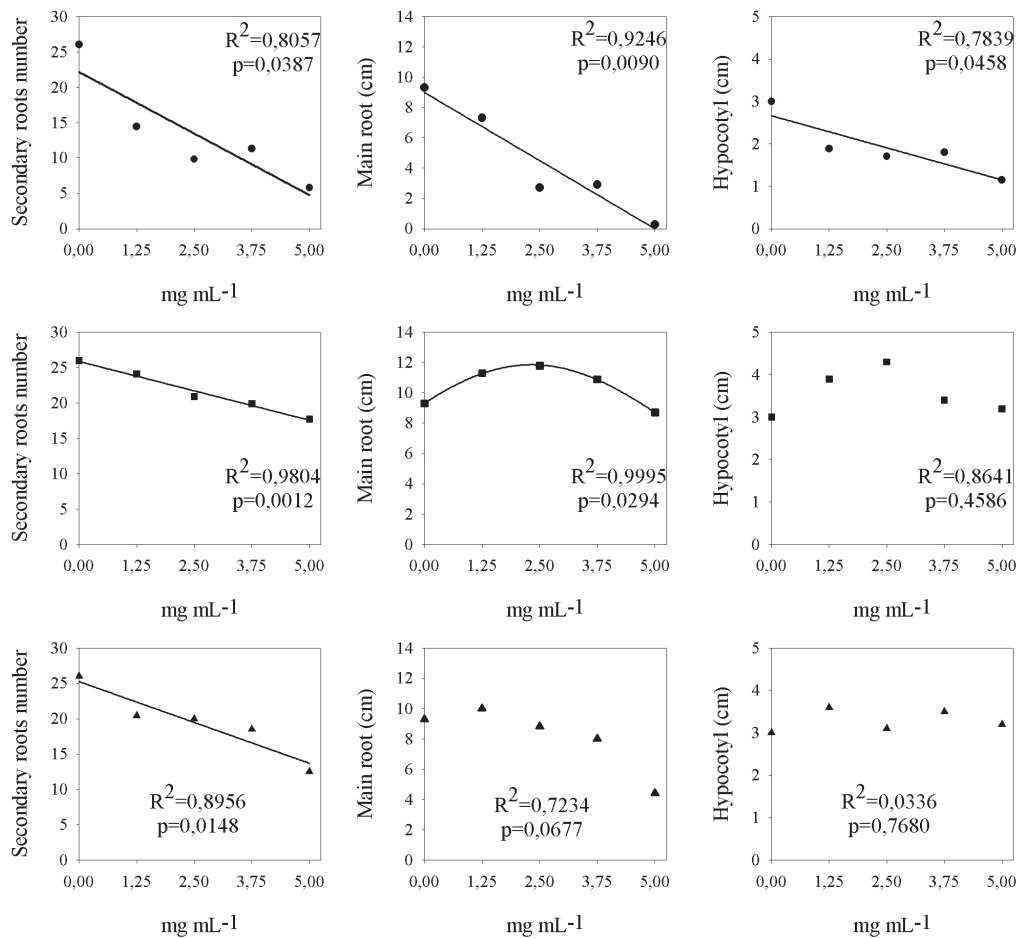
Apparently, many organic compounds with allelopathic activity are stimulants when present at lower concentrations (Rice 1984). It should also be borne in mind that the chemical nature of allelochemicals is extremely diverse and that some only operate in the presence of others, in certain combinations and proportions; therefore, it is difficult to distinguish the individual effects, making allelopathic inhibition a complex process (Pereira *et al.* 2008). This could explain, at least in part, the seemingly contradictory results obtained under the various conditions tested here.

Studying the effects that the hexane extract of *T. ulmifolia* had on the growth of cucumber seedlings, we noted that the decreases in the values of all three growth parameters (number of secondary roots, length of the main root and length of the hypocotyl) were directly proportional to the increases in the concentrations of the extract (Fig. 1), as was the decrease in the median number of secondary roots among the seedlings germinated in the ethyl acetate extract. However, the ethyl acetate extract stimulated growth of the main root at the lower concentrations and inhibited that growth at the higher concentrations. For the length of the

**Table 2.** Phytotoxic effects that *Turnera ulmifolia* L. extracts, at different concentrations, had on the initial development of cucumber seedlings (n = 4 sets of 25 seeds per experimental condition).\*

Treatment	Parameter**		
	Secondary roots (n)	Main root (cm)	Hypocotyl (cm)
Negative control (distilled water)	26±6.50 (a)	9.9±3.40 (b)	2.9±0.80 (b)
Extracts at 1.25 mg ml <sup>-1</sup>			
Hexane	14.5±6.25 (c)	7.5±2.20 (c)	1.8±0.65 (c)
Ethyl Acetate	25.0±6.00 (ab)	11.5±2.65 (a)	4.0±1.02 (a)
Methanol	21.5±7.25 (b)	10.5±2.85 (ab)	3.6±0.42 (a)
Extracts at 2.50 mg ml <sup>-1</sup>			
Hexane	9.5±4.00 (c)	1.4±4.28 (c)	1.8±0.42 (c)
Ethyl Acetate	22.5±8.25 (b)	12.3±3.25 (a)	4.2±1.30 (a)
Methanol	20.0±4.25 (b)	9.3±2.82 (b)	3.1±0.52 (b)
Extracts at 3.75 mg ml <sup>-1</sup>			
Hexane	11.0±5.25 (c)	2.2±5.32 (d)	1.8±0.42 (c)
Ethyl Acetate	21.0±9.00 (b)	11.1±3.10 (b)	3.3±0.65 (ab)
Methanol	18.5±5.50 (b)	8.2±3.38 (c)	3.6±1.02 (a)
Extracts at 5.00 mg ml <sup>-1</sup>			
Hexane	7.0±7.00 (d)	0.2±0.10 (d)	1.1±0.35 (c)
Ethyl Acetate	17.0±11.00 (b)	8.5±4.12 (b)	3.1±0.80 (b)
Methanol	12.0±5.00 (c)	4.3±2.18 (c)	3.2±0.72 (b)

\*Values presented as median and standard deviation; \*\*medians followed by same letter in the same column indicate that there was no significant difference (p>0.05) among treatments at the same concentrations or between each treatment and the control.



**Figure 1.** Relationships between growth parameters in cucumber seedlings (number of secondary roots, main root length and hypocotyl length) and the concentrations of hexane extracts (●), ethyl acetate extracts (■) and methanol extracts (▲) of the leaves and branches of *Turnera ulmifolia* L.

hypocotyl, the effect of the ethyl acetate extract was not concentration-dependent (Fig. 1). Among the seedlings germinated in the methanol extract, only the effect on the number of secondary roots was found to be concentration-dependent, the degree of inhibition increasing in parallel with increasing concentrations of the extract (Fig. 1).

In the cucumber seedlings, the hexane and ethyl acetate extracts of *T. diffusa* both inhibited the development of secondary roots, as well as the growth of the main root, at all concentrations tested, whereas the methanol extract had such effects only at the higher concentrations (Tab. 3). Compared with the control seedlings, those treated with the hexane and ethyl acetate extracts presented a significant decrease in median length of the hypocotyl at  $\geq 2.50$  mg ml<sup>-1</sup>. For that same parameter, there were no significant differences between the control seedlings and those treated with the methanol extract, at any of the concentrations tested (Tab. 3).

We found that the hexane extract of *T. diffusa* had an inhibitory effect that was directly proportional to the concentration of the extract, for all three parameters of

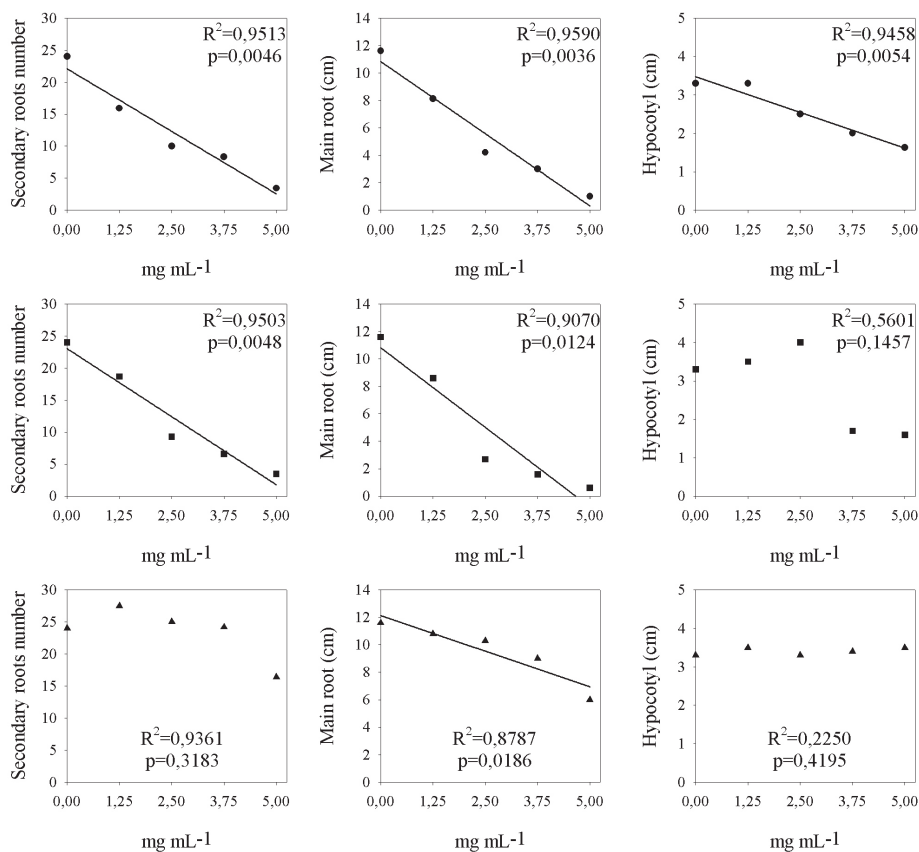
cucumber seedling growth (Fig. 2). The inhibitory effect of the ethyl acetate extract was also found to be concentration-dependent, increasing in parallel with increasing concentrations of the extract, although only for the number of secondary roots and the length of the main root (Fig. 2). The same was true for the methanol extract, although only for the length of the main root (Fig. 2).

In allelopathic studies, the concentration of the extracts analyzed is an important factor and allelopathic effects can be concentration-dependent. In the present study, the *T. diffusa* methanol extracts had inhibitory effects on the length of the main root and number of secondary roots only at higher concentrations (Tab. 3). The same was noted by Almeida (1991) in a study of the allelopathic effect that aqueous extracts of the fruit of *Eucalyptus saligna* have on the emergence of weeds. The author reported that only the higher concentrations of this extract inhibited the emergence of *Cenchrus echinatus* (southern sandbur). In the present study, we observed concentration-dependent effects on all growth parameters evaluated for the hexane extract of *T. ulmifolia*, as well as on the number of secondary roots for

**Table 3.** Phytotoxic effects of *Turnera diffusa* Willd. ex Schult. extracts, at different concentrations, had on the initial development of cucumber. (n=4 sets of 25 seeds per replication).\*

Treatment	Parameters**		
	Secondary roots (n)	Main root (cm)	Hypocotyl (cm)
Negative control (distilled water)	24.0 ± 5.00 (a)	11.6 ± 3.00 (a)	3.2 ± 0.72 (a)
Extracts at 1.25 mg mL <sup>-1</sup>			
Hexane	16.0 ± 7.25 (b)	7.9 ± 4.70 (b)	3.3 ± 0.48 (a)
Ethyl Acetate	18.0 ± 7.25 (b)	8.5 ± 2.42 (b)	3.5 ± 1.00 (a)
Methanol	30.0 ± 17.00 (a)	11.3 ± 3.38 (a)	3.5 ± 0.70 (a)
Extracts at 2.5 mg mL <sup>-1</sup>			
Hexane	10.0 ± 4.00 (b)	3.8 ± 2.08 (b)	2.5 ± 0.42 (b)
Ethyl Acetate	9.0 ± 3.00 (b)	3.5 ± 2.50 (b)	2.6 ± 0.70 (b)
Methanol	24.0 ± 14.20 (a)	10.6 ± 2.13 (a)	3.2 ± 0.50 (a)
Extracts at 3.75 mg mL <sup>-1</sup>			
Hexane	8.5 ± 4.00 (b)	3.0 ± 1.45 (b)	2.1 ± 0.25 (b)
Ethyl Acetate	7.0 ± 3.00 (b)	1.1 ± 1.85 (b)	1.6 ± 0.95 (b)
Methanol	23.0 ± 14.50 (a)	9.5 ± 4.68 (a)	3.5 ± 0.80 (a)
Extracts at 5.0 mg mL <sup>-1</sup>			
Hexane	3.0 ± 5.00 (c)	0.7 ± 0.50 (c)	1.6 ± 0.72 (b)
Ethyl Acetate	4.0 ± 4.25 (c)	0.3 ± 0.20 (c)	1.6 ± 0.90 (b)
Methanol	16.0 ± 8.00 (b)	5.7 ± 3.48 (b)	3.5 ± 0.52 (a)

\*Values represent the median and interquartile deviation; \*\*medians followed by same letter in the same column indicate that there was no significant difference (p>0.05) among treatments at same concentrations or between each treatment and the control.



**Figure 2.** Relationships between growth parameters in cucumber seedlings (number of secondary roots, main root length and hypocotyl length) and the concentrations of hexane extracts (●), ethyl acetate extracts (■) and methanol extracts (▲) of the leaves and branches of *Turnera diffusa* Willd. ex Schult.



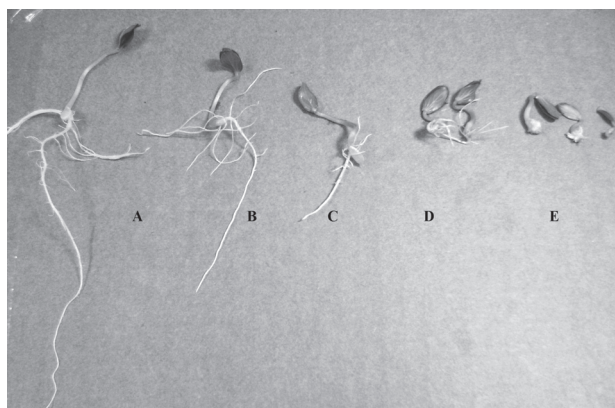
the ethyl acetate and methanol extracts of the species. For *T. diffusa*, concentration-dependent effects were observed for the hexane extract (on all growth parameters evaluated), for the ethyl acetate extract (on the length of the main root and number of secondary roots) and for the methanol extract (only on the length of the main root). Silva *et al.* (2006) also noted that the inhibitory effect that the extract of *Amburana cearensis* had on the growth of the main roots and shoots of sorghum seedlings increased in parallel with increases in the concentration of the extract.

Another factor to be considered is the polarity of the organic extracts used. In our study, considering the more concentrated extracts (5.00 mg ml<sup>-1</sup>), we observed that the hexane extracts of both *Turnera* species inhibited the development of cucumber seedlings (roots and hypocotyls), as did the ethyl acetate extracts, especially those of *T. diffusa*, whereas the methanol extracts inhibited only root development and did so to a lesser degree in comparison with the other two types of organic extracts (Tab. 2 and 3). It is accepted that extracts obtained with polar solvents can provide active constituents, such as phenolic compounds (Carmo *et al.* 2007). Belinelo *et al.* (2008), studying *Arctium minus* extracts of different polarities, found that the allelopathic effect was greatest for the ethanol extract, which had the highest polarity of the extracts evaluated. However, the use of non-polar extracts cannot be ignored, because they could be a source of active terpenes. Souza-Filho *et al.* (2010) described inhibition of the root development of *Mimosa pudica* by the hexane extract of *Copaifera duckei*. Silva *et al.* (2011) found that the allelopathic effect of *Pyrostegia venusta* was independent of polarity, the hexane and methanol extracts being the most active.

The leaves of the *Turnera* species studied here contain phenolic compounds and terpenes (Galvez *et al.* 2006; Zhao *et al.* 2007; Pino 2010). It is therefore possible that the allelopathic activity observed is related to these compounds, terpenes being preferentially extracted by hexane, whereas phenolic compounds, mostly flavonoids, are preferentially extracted by ethyl acetate and methanol. Candido *et al.* (2010a) performed bioassays with commercial herbicides and obtained germination and growth results similar to those observed for the use of semi-purified hexane and ethyl acetate fractions of *Senna occidentalis*. Using thin layer chromatography, the authors identified terpenes in the hexane fraction and phenolic compounds, mostly flavonoids, in the ethyl acetate fraction.

Both of the *Turnera* species tested in the present study had a more pronounced allelopathic effect on development than on germination, the root being the most affected organ. At the highest concentration tested, the ethyl acetate extract of *T. diffusa* induced the formation of abnormal cucumber seedlings, in which the main root was markedly atrophied (Fig. 3), suggesting inhibition of cell division and elongation.

In general, root development is more sensitive to substances in the extracts than is shoot development (Borella



**Figure 3.** Effects of ethyl acetate extracts of *Turnera diffusa* Willd. ex Schult., at different concentrations, on cucumber roots: A, control; B, 1.25 mg ml<sup>-1</sup>; C, 2.50 mg ml<sup>-1</sup>; D, 3.75 mg ml<sup>-1</sup>; E, 5.00 mg ml<sup>-1</sup>.

*et al.* 2009; Candido *et al.* 2010b; Silva *et al.* 2011). That is because the roots come into direct contact with the allelochemical solutions (Chung *et al.* 2001). In addition to affecting growth, allelochemicals can induce the appearance of abnormal seedlings with atrophied roots (Gusman *et al.* 2008; Borella *et al.* 2009), as was observed in this study.

Changes in the pattern of germination and development can result from various effects at the primary level. Such effects include changes in membrane permeability; in transcription and translation of DNA; in the operation of secondary messengers; in respiration; in conformation of enzymes and receptors; and in cell division (Rizvi and Rizvi 1992; Ferreira & Aquila 2000).

The results of the present study demonstrate the allelopathic potential and phytotoxicity of both of the *Turnera* species evaluated. This effect was manifested mainly by the impairment or inhibition of the germination of cucumber seeds, as well as by structural abnormalities in the resulting seedlings. The extracts showing the greatest allelopathic potential were the hexane extracts of both species and the ethyl acetate extract of *T. diffusa*. Fractionation and isolation of substances in these extracts will elucidate which compounds are responsible for inhibiting growth, as well as allowing the identification of any synergistic or antagonistic interactions among the compounds.

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