Original Article

PHYTOTOXICITY OF ETHANOLIC EXTRACT OF TURNIP LEAVES (*Raphanus sativus* L.)

FITOTOXICIDADE DO EXTRATO ETANÓLICO DE FOLHAS DE NABO (Raphanus sativus *L.*)

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ABSTRACT: The study of allelopathic activity has been aim of research that evaluates mainly species used in green fertilization. *Raphanus sativus* L. stands out among these species, because it shows high capacity for nutrient recycling, specially nitrogen and phosphorus, what makes it an advantageous cover plant in crop rotation systems. Considering the exposed, the present study had as objective the evaluation of the allelopathic and phytotoxic potentials of different concentrations of the *R. sativus* leaves ethanolic extract by mean of seeds germination analyses and development of lettuce seedlings, evaluating the phytotoxicity by determination of the mitotic index of lettuce root cells, realizing the phytochemical profile and investigating the antioxidant activity. It was possible to verify that the *R. sativus* extract interferes in the germination index, decreasing the germinability (5 $mg.mL^{-1}$ = 9.84%; 10 $mg.mL^{-1}$ = 11.91% and 20 $mg.mL^{-1}$= 57.51%). In the lettuce seedlings growth, the extract of this species affected the roots and hypocotyls growth. It was possible to observe phenols and total flavonoids in the extract for the concentration of 1000$\mu g.mL^{-1}$(161mg and 83.57 mg, respectively). It was also observed, higher antioxidant activity for the concentration of 1000 $\mu g.mL^{-1}$ (89.76%). In the phytotoxicity assay was observed a dose dependent effect in the mitotic index and in the cellular events during cellular division. In this study it was possible to conclude that this species has allelochemical compounds which are able to interfere directly on the stabilization and development of other species.

KEYWORDS: Allelopathy. Development. Germination. Antioxidant. Lettuce

Introduction

Green fertilization is an efficient practice to improve chemical, physical and biological conditions of the soil (TEJADA et al. 2008). Among the species that are used in the agriculture and horticulture in the southern and midwest parts of the state of São Paulo (Brazil), the turnip (*Raphanus sativus* L. var. oleiferus Metzg.) stands out. It is a cruciferous, annual, allogamous, herbaceous and upright species (DERPSCH; CALEGARI 1992), and has been used as winter green fertilizer or cover plant in conservation tillage systems (CRUSCIOL et al. 2005; LIMA et al. 2007).

Allelochemical capacity, which is the interaction between plant species in a determined environment, has been aim of research that evaluates mainly species used in the green fertilization (INDERJIT et al. 2006). The allelopathic effects can increase or lower the biological activity depending on the allelochemicals produced and released in the environment (INDERJIT 2006). Studies of allelochemicals and their allelopathic activities have become an important tool in the search for new active compounds, as their synthetic derivatives, with the ability to act as natural pesticides, considering its specificity and the ability to cause smaller impacts at the environment, then contributing to the aim of sustainable agriculture (MEDEIROS 1990; SMITH; MARTIN 1994; MACÍAS et al. 1998; CHOU 1999; FERREIRA; ÁQUILA 2000; ZHAO et al. 2010). In Brazil, there are farmer's reports showing that the *R. sativus* L. can procuce active compounds with allelopathic potential, and it have been used to decrease the use of glyphosate in crops (GIACOMINI et al. 2004; RIZZARDI; SILVA 2006).

Raphanus sativus L. has shown high capacity for nutrient recycling, specially nitrogen and phosphorus, what makes it an advantageous cover plant in crop rotation systems (DERPSCH and CALEGARI 1992; ANDREOLA et al. 2000). Researches indicate that corn crop planted after turnip, increases up to 20% of the corn yield, besides reducing the number of weeds at the subsequent crops (HERNANI et al. 1995; CAMARGO et al. 2011).

Given such a situation, this research had the objective of evaluating the allelopathic potential of

Received: 10/10/12
Accepted: 05/06/13

different ethanolic extract concentrations of the *R. sativus* leaves, through bioassay tests of the seeds germination and development of *Lactuca sativa* L. seedlings. As well as evaluating the phytotoxicity by determination of the mitotic index in the meristematic cells of *L. sativa* root and to quantify the total phenols and flavonoids of the extract.

MATERIAL AND METHODS

Experimental procedure

The bioassays were conducted at the Laboratório de Fisiologia Vegetal e Fitoterápicos do Departamento de Ciências Biológicas, Faculdade de Ciências e Letras de Assis (FCLA), Universidade Estadual Paulista (UNESP), Assis-SP, Brazil.

Plant material

The leaves of *R. sativus* were collected from specimens cultivated at the greenhouse of the Departamento de Ciências Biológicas da Universidade Estadual Paulista, Campus Assis/SP (22º32'20''S and 50º22'60''W). A voucher specimen was deposited in the herbarium of the Instituto Florestal de São Paulo (register: SPSF71371). After collection, the leaves were selected and dried in a forced-air oven at an average temperature of 40°C for 24h, and shortly after, they were ground and the resulting powder was stored in dark plastic bottles.

Ethanolic extract

The ethanolic extract was prepared by mechanical maceration of the powdered plant material in with ethanol PA (IMPEX, Brazil) (at a ratio of 1:10 [w/v]) for 24h at room temperature. The extract was then filtered at low pressure under vacuum, a methodology similar to the used by Rutherford and Powrie (1993), Hajhashemi et al. (2003) and Boligon et al. (2009). The extraction was performed three times with the same plant material. The extracts were pooled and concentrated on a rotary evaporator (model: MA120, Marconi, Brazil), at an average temperature of 60 ^{o}C and the dry residue was used in the bioassays, according to Aqüila (2000) and Sadraei et al. (2003).

Germination Bioassay

The bioassay was conducted in Petri dishes (60x15mm) lined with germination paper, which was moistened with 1 mL of the extract diluted in distilled water to concentrations of 5, 10 or 20mg mL^{-1}. Fifty achenes of *Lactuca sativa* L. cv. 'Grand Rapids' (lettuce) were sown per dish, separated into experimental and control (distilled water) groups, and incubated for 96h in a growth chamber (model: 411/FPD, Nova Ética, Brazil) at 23±2°C (Alves et al. 2004). The experimental design was completely randomized, with four replicates of each concentration of treatment or control. Germination was monitored every 6h, with the protrusion and geotropic curvature of the root being the evaluation criterion of germination, as described by Ferreira and Aqüila (2000), Ferreira et al. (2008) and Maraschin-Silva and Aqüila (2006a). From the data obtained, the following indexes were calculated:
Germination: G% = [∑ni/A].100

Mean germination time:

$$\bar{t} = \frac{\sum_{i=1}^{k} niti}{\sum_{i=1}^{k} ni}$$

Mean germination speed:

$$\bar{v} = \frac{CV}{100} = \frac{1}{\bar{t}}$$

Synchronism of germination:

$$\overline{E} = -\sum_{i=1}^{k} fi \log_2 fi$$

Where, A: Total number of achenes put to germinate; ni: Number of germinated achenes at each time point (ti); ti: Time between the start of experiment and the i-th hour of observation; fi: Relative frequency of germination and *k*: Last day of observation (LABOURIAU, 1983; SANTANA; RANAL, 2004; PEREIRA et al. 2009).

Tetrazolium assay

The seeds that did not germinate during the germination assay were incubated in a tetrazolium solution (2,3,5 triphenyl tetrazolium chloride, 0.5%) for 6 h at 30°C in the dark, as described by Delouche et al. (1976) and the Rules for Seed Analysis (BRASIL, 2009). The viability and the number of dead or dormant seeds were determined in order to characterize the metabolic state of the ungerminated seeds (SOUZA, 1994; ALMEIDA et al. 2002; PINHEIRO; BORGHETTI 2003).

Physicochemical characterization (pH and osmotic potential)

The pH of the diluted *R. sativus* extract was determined directly in the treatment solutions using a pH meter (Tecnopon®, model MPA210, Brazil). Measurement of the osmotic potential of the extracts was performed using dilutions of polyethylene glycol (PEG- 6000) to produce the osmotic potentials of -0.02 to -1.0 MPa, as described by Villela et al. (1991) and Mazzafera (2003). The measurement of the refractive Brix for each concentration of PEG-6000 and the extract was determined by an ABBE refractometer and the values were used to calculate the water potential, as described by Bakke et al. (2006).

Root and hypocotyl length

In the growth bioassay, thirty lettuce seedlings (with approximately 2mm of primary root growth) were placed in the Petri dishes (60x15 mm) with 1mL of extract (5, 10 or 20 mg mL^{-1}) or distilled water, as described above. The dishes were placed in a B.O.D. (Biological Oxygen Demand) (model: 411/FPD, Nova Ética, Brazil) growth chamber under the same conditions of the germination assay, as described by Ferreira, Medeiros and Soares (2008) and Maraschin-Silva and Aqüila (2006b). The experimental design was completely randomized with four replicates of each treatment concentration and the control. Monitoring was conducted at 24h and 48h and the length of the primary root and hypocotyl were measured with a digital caliper (model: IP65, DIGIMESS®, Brazil).

Mitotic Index

For the mitotic index's analysis, the primary roots of *L. sativa* seedlings (approximately 2 mm of primary root growth) were collected and prepared by the squash technique (Guerra and Souza 2002; Mahajan and Sharma 2008). Firstly, the roots were fixed in Carnoy's solution (ethanol: glacial acetic acid, 3:1) for 2h, hydrolyzed in 5N HCl for 15min at room temperature, washed with distilled water and stained with 5% acetic carmine. Cells were observed under a light microscope with 100x magnification, and 2000 cells were analyzed for each treatment to verify the number of cells in each phase of mitosis. The mitotic index (MI) was obtained from the equation, MI=(m/T)x100, where m= the number of cells in mitosis and T= total number of cells (Pires et al. 2001; Tabur and Oney 2009).

Total phenols and flavonoids quantification

The quantification of total phenols and flavonoids was performed at extract diluted in ethanol concentrations of 25, 50, 75, 100, 250, 500, 1000, 3000, 5000 and 10000 $\mu g.mL^{-1}$. For the determination of total phenols, the Folin-Ciocalteu (1927) method was performed. For each 0.5 mL of extract at the different concentrations was added 5mL distilled water and 0.25 mL *Folin-Ciocalteu* reagent. After 3 minutes, was added 1mL of saturated Na_2CO_3 solution at 10% and the mixture was stored for 1 hour. The absorbance was measured at 725nm using a UV-Vis spectrophotometer (model: SP220, BIOSPECTRO, Brazil). All the tests were performed in triplicate and the results were expressed in mg gallic acid per gram of extract.

For the total flavonoids quantification of extract was performed the determination by UV-Vis spectrophotometer and the samples were prepared as described by Zhishen, Mengcheng and Jianming (1999), based on flavonoids complexation with $AlCl_3$. An aliquot of 250 µL extract at the different concentrations was mixed with 1.25 mL distilled water and 75 µL $NaNO_2$ solution at 5%. After 6 minutes, a 150 µL $AlCl_3/H_2O$ solution at 10% was added. After 5 minutes, 0.5 mL NaOH 1M solution was added and then the total volume was completed by adding 2.5 mL distilled water. The samples were shaken in a vortex mixer and the absorbance was measured at 510 nm. All the tests were performed in triplicate and the results were expressed in mg rutin per gram of extract.

DPPH radical scavenging activity

The sTable 1.1-diphenyl-2-picrylhydrazyl (DPPH, Sigma, USA) radical scavenging activity was determined according to the methodology proposed by Blios (Manian et al. 2008). The dry ethanolic extract of each sample was dissolved in ethanol (75%) at different concentrations (25, 50, 75, 100, 250, 500, 1000, 3000, 5000 and 10000 $\mu g.mL^{-1}$) and then mixed with 5 mL of DPPH solution (1.5x10-4M). The extract reacted with the DPPH radical for a period of 30 minutes in a low luminosity, and then they were submitted to the UV-Vis spectrophotometer (model: SP220, BIOSPECTRO, Brazil) at 517 nm wave length. The calculation of the antioxidant activity was performed according to the formula: I%=[(control-sample)/control]x100. Gallic acid (Vetec – Química Fina, Brazil) was employed as the reference. Triplicates were made to the analyses.

Statistical analysis

The data was analyzed by ANOVA and Tukey (α=0.5) parametric tests. These tests were performed using the SISVAR software (version: 5.0,

Lavras, MG, Brazil), according to Santana and Ranal (2004) and Pereira et al. (2009). For the analysis of the mitotic index, the Chi-squared Test was performed to identify a positive response between the experimental and control groups, according to the analysis proposed by Ribeiro et al. (2003).

RESULTS

The effect of *R. sativus* ethanolic extract at *L. sativa* seeds germination

In Table 1, the germination indexes evaluated at the germination bioassay are presented. The percentage and the mean time of germination for lettuce seeds treated with 5 and 10 mg.mL^{-1} did not show significant difference between them (germination percentage of inhibition: 5 mg.mL^{-1}= 9.84%; 10 mg.mL^{-1} = 11.91%), but they presented values statistically different comparing to the ones treated with 20 mg.mL^{-1}(germination percentage of inhibition: 20 mg.mL^{-1}= 57.51%), and all the treatment concentrations showed results that differed statistically from the control groups. As for speed of germination the treated groups did not present difference between them, differing just from the controls, which germinated with a high speed. Regarding the synchronism, just the treatment with 10 mg.mL^{-1} differed statistically comparing to the other treatments and the controls, showing an increase of mean synchronism of germination.

Table 1. Germinability (G%), mean germination time (*t*), mean germination speed (*v*), and synchronism of germination (*E*) of seeds of *L. sativa* subjected to different concentrations of ethanolic extract of *R. sativus* leaves (5, 10 or 20 mg.mL^{-1}).

Extract (mg.mL^{-1})	G%±SD	*t*±SD (h)	*v*±SD (seeds/h)	*E*±SD
5	87.00±8.87a	35.56±4.16a	0.028±0.0033a	2.90a ±0.16a
10	85.00±6.22a	37.90±0.76a	0.026±0.0005a	1.95b±0.69b
20	41.00±7.64b	43.28±2.59b	0.023±0.0013a	2.22c ±0.43c
Water	96.50±4.73c	23.41±0.64c	0.042±0.0011b	2.89a ±0.10a

Data presented as mean±standard deviation; Means sharing the same letter in a column do not differ significantly by Tukey's test (α=0.05).

Physicochemical characterization (pH and osmotic potential)

The physicochemical characterization of the ethanolic extract of *R. sativus* leaves revealed that the pH showed an average acidity value of 5.00. The values of osmotic potential showed variation between -0.052 and -0.060 MPa (Table 2).

Table 2. Physico-chemical features (pH and osmotic potential) of the ethanolic extract of R. sativus in different concentrations (5, 10 and 20 mg.mL-1).

Concentration (mg.mL^{-1})	pH	OP*(MPa)
5	5.0	-0.060
10	5.0	-0.060
20	5.0	-0.052
Water	5.3	-0.060

*OP (osmotic potential)

The effect of *R. sativus* ethanolic extract on *L. sativa* root and hypocotyl length

In figure 1, the average length of primary root of lettuce seedlings are presented. The root length, after 24 hours, did not differ significantly between the treatments with 10 and 20 mg.mL^{-1}, however both treatments differed statistically from the group treated with 5 mg.mL^{-1} (Figure 1). The experimental groups, independent of the concentrations, differed significantly from the control groups. After 48 hours of observation, the root length of the seedlings treated with 5 and 10mg.mL^{-1} did not show difference between them, but presented values with significant difference comparing to the ones treated with 20 mg.mL^{-1} and all the groups treated showed results that differed statistically from the control groups. The average length of lettuce roots was concentration-dependent inhibition in obtaining an average size of 16.75% to 5 mg.mL^{-1}, 27.44% to 10mg.mL^{-1} and 38.27% to 20 mg.mL^{-1} compared with control (water).

Similar results were observed in the hypocotyls lenght, experimental groups did not differ significantly from each other, but differed in comparison to control. The results demonstrated a significant reduction in the development of aerial parts of the recipient plant (5 mg.mL^{-1} = 44.44%; 10 mg.mL^{-1} = 58.00% and 20 mg.mL^{-1}= 68.10%) (Figure 1).

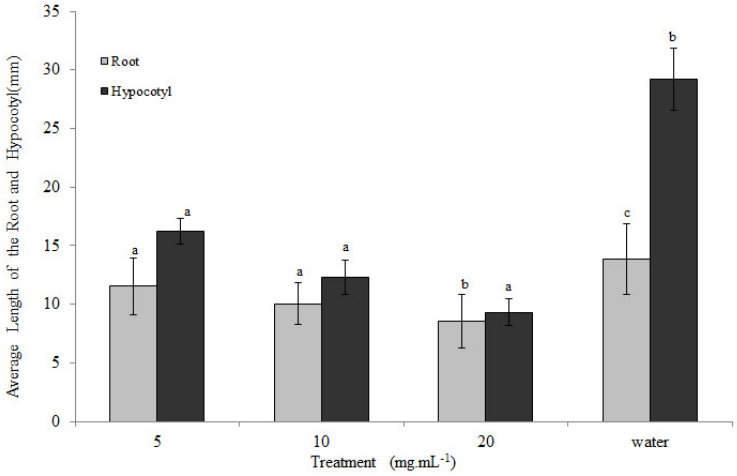

Figure 1. Average length of the root and hypocotyl of *L. sativa* seedlings subjected to different concentrations of ethanolic extract of *Raphanus sativus* after 48h of exposure. Means sharing the same letter in a column do not differ significantly by Tukey`s test (α=0.05) (F= 37,527; p=0,001).

Determination of the mitotic index in the meristematic root cells of *L. sativa*

The values of mitotic index (MI) are shown in Table 3. They show a significant MI increase (31.07%) in the treatment 5mg.mL^{-1} compared with the control. The treatment with the concentration of 10mg.mL^{-1} did not show significant difference compared with the control and 20mg.mL^{-1} decreased (47.74%) significantly the MI. The treated groups showed a decrease of mitotic index while the extract concentration was increased, featuring a possible dose-dependent action.

The amount of interphases found increased while the extract concentration was increased on the treated groups. The smaller amount of prophases was observed at the groups treated with 20mg.mL^{-1}. The cells analysis evidenced that the control groups contained bigger quantity of anaphases, followed by the groups treated with 5, 10 and 20mg.mL^{-1}. The number of telophases found in the treated group was 5 mg.mL^{-1} =60, 10mg.mL^{-1}=29 and 20 mg.mL^{-1}=10, while the control presented water=40. On the treated groups, the quantity of cells in telophase was smaller while the extract concentration increased (Table 3).

Table 3. Mitotic index of meristematic root cells of lettuce treated with different concentrations (5, 10 and 20 mg.mL^{-1}) extract of *R. sativus* and control water.

Treatment (mg.mL^{-1})	Stages of Cell Division					Mitotic Index[a]
	Interphase	Prophase	Metaphase	Anaphase	Telophase	
5	4536	212	148	44	60	9.28a
10	4651	229	64	27	29	6.98b
20	4815	137	24	14	10	3.70c
Water	4646	153	110	51	40	7.08b

aMitotic Index = (n° total number of dividing cells / n° total of cells analyzed x 100), Values sharing the same letter in a column do not differ significantly by Chi-squared Test, ($\chi 2 < 0,05$).

DPPH radical scavenging activity and total phenols and flavonoids

In Table 4, the values of quantification of total phenols and flavonoids of *R. sativus* ethanolic extract are presented. These results demonstrate that the 10000 µg.mL^{-1} extract concentration presented 161mg total phenols (expressed in mg gallic acid per gram of extract). Regarding the total flavonoids level, all the concentrations presented flavonoids, but the biggest value was found at 10000 µg.mL^{-1} concentration (83.57 mg expressed in mg of rutin per gram of extract).

The antioxidant activity showed similar results, the highest activity was obtained with 10000 µg mL^{-1} (89.76%). The IC50 of the ethanol extract of *R. sativus* is 4920 µg.mL^{-1}, which can be obtained

through the equation of the line y = 0.0092 x + 4.7662 (R^2 = 0.9614).

Table 4. Radical scavenging activity and total phenol and flavonoids contents in do extract of *Raphanus sativu*.

Concentration (µg.mL^{-1})	Ethanolic extract		
	Phenol content[a]	Flavonoids content[b]	% Antioxidant activity
25	02.30	1.59	1.27
50	02.93	1.60	3.98
75	03.47	1.94	4.14
100	03.94	2.15	5.28
250	06.96	2.68	6.09
500	10.80	3.68	7.15
1000	20.60	9.79	10.27
3000	61.98	32.11	45.30
5000	101.18	62.72	59.37
10000	161.00	83.57	89.76

amg gallic acid equivalent/g of extract, bmg quercetin equivalent/g of extract

DISCUSSION

According to Dal Magro et al. (2010), *R. sativus* presents superior competitive skills to many cultures besides quickly realizing the coverage of the occurring area. This peculiar characteristic of the species makes it develop necessary strategies to conquer the environment, being liable of having allelochemicals substances, as confirmed by bioassays applied in this study.

Although most of the tests performed with ethanol extracts are intended to phytochemical studies, the present study aimed only to evaluate its allelopathic potential, and the results indicate that the extract of *R. sativus* contains compounds capable of interfering in the germination index, in the root growth and in the mitotic index of lettuce root meristematic cells.

According to Ferreira and Aqüila (2000), the variation in the germination standard may result in many effects caused in primary level. Among them, highlights variations in membrane permeability, in transcription and translation of the DNA, in the operation of secondary messengers, breathing, enzyme conformation and receptors, or even by the combination of these factors.

The pH and the osmotic potential are factors that could interfere in the germination process. They were evaluated and these maintained their values within the accepTable standards of what is considered proper to germination and initial growth (AQÜILA, 2000) (Table 2). Gatti et al. (2004) recommend that the osmotic potential of extracts involving germination tests doesn't exceed values of -0.2Mpa, which is in accordance with observed values in this study. Such evaluations are necessary, because ethanolic extract can present specific solutes that may change the properties of the water, resulting in an osmotic pressure different of zero in the solution (VILLELA et al. 1991). Solutes such as sugars, amino acids and organic acids may mask the allelopathic effect of the extracts by interfering in the pH and being osmotically active (FERREIRA; AQÜILA, 2000).

For the activity of the extract on the root length of lettuce seedling, Aqüila et al. (1999) shows that the allelochemicals may act on different ways depending on the environment and the life cycle stage in which the target plant is, since both reflect different physiological states. Besides, the effects can also vary considering in which plant organ they are acting on. Although, in studies realized by Miró et al. (1998), Jacobi and Ferreira (1991) and Aqüila (2000), was observed the higher allelopathic effect on the initial development of target seedling (length of the root and hypocotyl) when compared to germination, since this last process uses reserves from the own seed. However, the results in the present study show effects on the vegetative development and the germination (Table 1 and Figure 1).

The results obtained in the initial development of seedling, in the end of 48 hours of experimentation, show significant reduction (F=37,527; p=0,001) in the root and hypocotyl growth of lettuce seedling treated with the three concentrations of extracts when compared to a control group (Figure 1). This data show that the extract, besides presenting cytotoxic characteristics in the germination process, presented phytotoxic action also to the seedling development, corroborating the results of studies realized by Azambuja et al. (2010).

The mitotic index evaluation in meristematic cells of lettuce root show that the R. sativus extract is capable of interfering in the cell division, in the different mitosis phase, reflecting directly on the mitotic index, as in the growth and development of the test plant. This interference was dose dependent, however the concentration of 5mg.mL^{-1} presented higher mitotic index (IM=9.28) when compared to the control (IM water= 7,08) (Table 4). This data collaborates to show the relation between the allelopathic effects and the cytogenetic, because the number of works that consider and evaluate such association are rare, however Pires et al. (2001) verified that plant extracts reduce the mitotic index in the corn root, compromising its normal length. On the other hand, Souza et al. (2005) verified the existence of cellular disturbs (anaphasic bridges) in lettuce root cells, whose seeds were submitted to aqueous extracts of *Maytenus ilicifolia* M.

Studies realized by Inderjit (1996) showed that the plant originated extracts appointed in bioassays to preliminar diagnostics of allelopathy are a mixture of many substances, which exercise inhibitory effects and additive or synergistic, making the analysis of the action of each substance important. Studies about allelochemicals compounds realized by Vyvyan (2002) and Ahmad et al. (2011) showed that the main substances with allelopathic potential, that act on pre-emergency, post-emergency and in the phytotoxicity, are the benzoquinones, cumarins, flavonoids, terpenoids, lactones, mucilages and alkaloids that may be associated to the effects on the germination, vegeTable development and possibly changing the cellular division, as demonstrated in this study.

Considering the possible mechanisms of action resulted by the different allelochemicals, the free radicals scavenging activity has become target of the assay. Appel (1993) demonstrated that the formation of reactive oxygen species have an important role in the interactions between plant and its pathogens, mutualists and competitors. However, Huckelhoven and Kogel (2003) demonstrated that Reactive Oxygen Species (ROS) not only has implicated in the transduction of signal and the defense mechanisms of plants, as the hypersensitivity response, but also ROS accumulated in the plant cells, in response to pathogens infections damaging the cells, and frequently conducting to cellular death. Testa (1996) and Hammondkosak and Jones (1995) demonstrated that the toxicity of many phenols can be largely attributed to the formation of radicals that donate electrons to the molecular oxygen, forming superoxide (O^2). These can undergo a series of additional reactions to become a more reactive allelochemical [hydroxyl radical (OH) or hydroperoxyl (HO2)]. Subsequentially, these radicals can affect the membrane's permeability, cause damage to DNA and proteins. Devi (1996), Yu et al. (2003) and Zeng et al. (2001) affirmed in their studies that rapidly some allelochemicals depolarize the cellular membrane, rising its permeability, inducing the lipid peroxidation, and causing a generalized cellular interruption that leads to cellular death. Nevertheless, Bradley et al. (1992), Grant e Loake (2000) and Weir et al. (2004) demonstrated that the H_2O_2 production besides being directly related with toxicity to microbes, also contributes to the structural reinforcement of the cell wall, coordinate the activation of defense genes and genes that cause production of phytoalexins.

With the presented information, this assay evaluated the presence of phenols and total flavonoids just as the antioxidant activity of the *R. satuvis* leaves extract, obtaining 89.76% of antioxidant activity for the 10000 µg.mL^{-1}, in this same concentration it was observed 161.00 equivalent grams of gallic acid per gram of total phenol extract and 83.57 equivalent grams of rutin per gram of total flavonoids extract. According to this results and in agreement with the assays cited above, it is possible to suggest that the free radical scavenging activity just as the high level of polyphenols found in the extract can be correlated with the action mechanism characterizing the allelopathic effect of *R. satuvis.*

According to Viles et al. (1996) and Blanco (2007), the production of allelochemicals in a process under genetic control is subject to natural selection, mainly because of the heterogeneity of the population, the relation with the competitors and its stabilization on the environment. Despite the results of this study show that the ethanol extract of *R. sativus* presents compounds with possible allelopathic potential, it is still necessary to use other experimental approaches (for example, a phytochemical screening and field test) to a greater understanding these results as well as to confirm if this potential allelopathic is also expressed in natural conditions, thus testifying with greater certainty the allelopathy of *Raphanus sativus.*

ACKNOWLEDGEMENTS

The authors acknowledge Laboratory Technician José Gilberto Millani for their help in conducting the experiments. This work was

supported by Universidade Estadual Paulista (UNESP).

RESUMO: O estudo da atividade alelopática tem sido alvo de investigação, avaliando principalmente espécies usadas na adubação verde. *Raphanus sativus* L. destaca-se entre as espécies, porque mostra alta capacidade de reciclagem de nutrientes, nitrogênio e fósforo, especialmente, o que faz com que seja uma planta de cobertura vantajosa em sistemas de rotação de culturas. Considerando o exposto, o presente estudo teve como objetivo a avaliação dos potenciais alelopáticos e fitotóxico de diferentes concentrações do extrato etanólico de folhas de *R. sativus* por meio de análises de germinação de sementes e desenvolvimento de mudas de alface, avaliar a fitotoxicidade por determinação do índice mitótico de células da raiz de alface, investigar o perfil fitoquímico e a atividade antioxidante. Foi possível verificar que o extrato de *R. sativus* interfere no índice de germinação, diminuindo a capacidade de germinação (5 $mg.mL^{-1}$ = 9,84%; 10 $mg.mL^{-1}$ = 11,91% e 20 $mg.mL^{-1}$= 57,51%). No crescimento de mudas de alface o extrato desta espécie afetou o crescimento de raízes e hipocótilos. Foi possível observar uma considerável quantidade de fenóis e flavonóides totais no extrato para a concentração de 1000 $\mu g.mL^{-1}$ (161 mg e 83.57 mg, respectivamente). Observou-se, também, a atividade antioxidante mais elevada para a concentração de 10000 $\mu g.mL^{-1}$ (89,76%). No ensaio de fitotoxicidade foi observado um efeito dose dependente no índice mitótico e nos eventos celulares durante a divisão celular. Neste estudo foi possível concluir que esta espécie tem compostos aleloquímicos capazes de interferir diretamente na estabilização e desenvolvimento de outras espécies.

PALAVRAS-CHAVE: Alelopatia. Development. Germinação. Antioxidant. Lettuce.

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