Instituto de Estudios Vegetales "*Dr. A. R. Sampietro*".

Facultad de Bioquímica, Químicay Farmacia.

Universidad Nacional de Tucumán. España 2903. CP 4000,

Tucumán, Argentina.

June 28, 2006

Dear Professor Sampietro,

I am pleased to inform you that your manuscript, "Effects of allelochemicals from sugarcane straw on growth and plant physiology of weeds (Ms No. 502-06)", has been accepted for publication in Allelopathy Journal.

Any queries concerning your manuscript should now be addressed to the Editorial Office at: allelopathy1947@yahoo.com. If further revisions are needed, you will be contacted by editorial office.

Sincerely yours

Professor Chuihua Kong

Regional Editor, Allelopathy Journal

Institute of Applied Ecology, Chinese Academy of Sciences

P. O. Box 417, Shenyang 110016, P. R. China

Tel: +86-24-83970452, Fax: +86-24-83970300

E-mail: kongch@iae.ac.cn

1

2   Running title: **Sugarcane straw allelochemicals**

3

4   **Effects of allelochemicals from sugarcane straw on growth and plant physiology of weeds**

5   D. A. Sampietro*, M. A. Sgariglia, J. R. Soberón and M. A. Vattuone

6 Instituto de Estudios Vegetales "*Dr. A. R. Sampietro*". Facultad de Bioquímica, Química y Farmacia. Universidad

7   Nacional de Tucumán. España 2903. CP 4000, Tucumán, Argentina.

8   E-mail: sampietro@tucbbs.com.ar

9

10   **ABSTRACT**

11   Studies have showed that sugarcane (*Saccharum officinarum* L.) straw leachates inhibited weed growth. The

12 aim of this work was to identify the phytotoxins from sugarcane straw leachate through a bioassay-guided isolation.

13 The phytotoxic activity of the isolated compounds was evaluated on seedling growth of *Lactuca sativa* L. and four

14 weeds. Physiological action of these compounds was also evaluated on *L. sativa*. Three compounds identified were

15 ferulic (FA), syringic (SA) and vanillic (VA) acids. VA had the strongest inhibitory effect on plant root elongation,

16 following by FA and SA. These phytotoxins increased root membrane permeability and depressed root metabolic

17 activity in *L. sativa*. VA and FA inhibited mitotic index while SA increased cell division. These compounds also

18 affected root morphology of *L. sativa* seedlings. Vanillic acid inhibited root branching while FA and SA stimulated

19 secondary root proliferation. As phenolic acids were often found spatially concentrated in the top soil layers under

20 plant straw, more studies are needed to establish the participation of these compounds in natural settings.

21

22 **Key word:** bioassay-guided isolation, phenolic acids, physiological effects, sugarcane straw leachate.

23

24   **INTRODUCTION**

25   Sugarcane (*Saccharum officinarum*) straw is left on the field after harvest in the northwest of Argentina. This

26 management practice showed to prevent soil erosion and environmental pollution (15). Straw retention on soil

27 surface also suppresses the growth of noxious plants reducing the need for chemical herbicides (12). Otherwise,

28 greenhouse experiments showed that sugarcane straw leachates inhibit weed growth (11). Although some

29 compounds related to specific biological activities have been isolated from sugarcane (9,13), phytotoxins were

30 rarely identified. Recently, 2,4-dihydroxi-1,4-benzoxazin-3-one (DIBOA) and 2-benzoxazolinone (BOA) were

31 isolated from sugarcane leaves (16). It is possible that an important amount of unknown phytotoxins remain to be

32 isolated from sugarcane straw. The purpose of this study was: 1) to isolate and identify the phytotoxic compounds of

33 a sugarcane straw leachate, 2) to determine the relative phytotoxic activity of the isolated compounds on receptor

34 plants (weeds) and on physiological processes of *Lactuca sativa*, and 3) to determine the concentration of the

35 isolated compounds in the sugarcane straw leachate.

36   **MATERIALS AND METHODS**

37 **General:** Column chromatography (CC) was performed on Sephadex LH 20 (800 x 27 mm, Amersham Pharmacia

38 Biotech) and Silica gel 60 (0.2–0.5 mm, 240 x 16 mm, Merck). HPLC operations utilized IB-SIL RP 18 (5 µm, 250x

39 10 mm and 4.6 mm, Phenomenex) columns and a Gilson 118 UV detector. GC–MS analysis were carried out on a

40 GC-HP 6890 with a quadrupole mass selective detector HP 5973, source 70 eV, fitted with a HP-5 MS column (5%

41 phenylmethylsiloxane, 30 m, 0.25 mm id, 0.25 µm film thickness) using helium as carrier gas (1.0 mL min^{-1}). UV–

1   Vis absorption spectra were recorded (200-400 nm) using a spectrophotometer Beckman DU-650. Known

2   compounds were identified by comparison of HPLC retention times, MS and UV-Vis absorption spectra with

3   literature data and analytical grade standards. The identified compounds were quantified by HPLC using external

4   standards.

5   **Plant material:** *Saccharum officinarum* L. var Tuc (CP) 77-42 straw was collected from Las Talitas (Tucuman,

6   Argentina). Then, straw was dried at 60 ℃ for 48 h in a forced draft oven.

7   Seeds of *Amaranthus quitensis* L., *Bidens subalternans* L., *Brassica campestris* L. *Lactuca sativa* L. cv

8   Grand Rapids and *Sida rhombifolia* L. were sterilized with 1% sodium hypochlorite for 30 min, rinsed with sterile

9   distilled water, dried with sterile filter paper and germinated in water. The germinated seeds with a root elongation

10 of 1 mm were used in bioassays.

11 **Straw leachate preparation:** Dry straw (70 g) was soaked in 1 l of sterile distilled water and shaken at 25 °C in the

12 dark for 4 h. The leachate was vacuum filtered through a filter paper (Whatman #1) and centrifuged at 23428 g for

13 15 min at 4 °C. The supernatants were stored at –20 °C until use. Leachate dilutions were prepared with sterile

14 distilled water and filtered through 0,22 µm nylon filter membranes (Millipore) before use.

15 **Isolation and identification of phytotoxic compounds:** A leachate (70 g of dry straw l^{-1}) was lyophilized to

16 dryness. The residue (776.8 mg) was dissolved in 50 mL of water and successively extracted with diethyl ether

17 followed by ethyl acetate. The organic fractions were dried in a rotary evaporator at 50 °C, dissolved in 3 mL

18 methanol (MeOH) and clarified by centrifugation.

19 The diethyl ether fraction (8.74 mg) was fractionated by gel filtration CC using MeOH as eluent. Fractions

20 were monitored at 280, 310 and 350 nm. Five fractions were separated (L1 – L5). Fraction L3 (4.85 mg) was loaded

21 on a Silica gel 60 column and eluted with chloroform – acetic acid (100 mL 90:10; 100 mL 80:20; 50 mL 70:30

22 and 50 mL 50:50, v/v). Six fractions (S1-S6) were obtained and concentrated under reduced pressure.

23 Fraction S1 (0.04 mg) was injected via a Rheodyne valve with a 500 µl loop into a micropreparative HPLC

24 column. The elution was performed at a flow rate of 2 mL min^{-1} with solvent A (2% acetic acid in water) and B (2%

25 acetic acid in MeOH) in the ratio of 35 % B for 5 min, increasing to 90 % B in 15 min; after 5 min at 90% B, the

26 column was cleaned-up decreasing to 35% B in 5 min. Re-equilibration was done at 35% B for 20 min. Compounds

27 were detected at a wavelength of 266 nm. The retention times (min) were 24.77 (**1**), 28.38 (**2**), 31.28 (**3**) and 32.27

28 (**4**). The detected peaks were collected and analyzed by TLC and analytical HPLC. The UV-Vis absorption spectra

29 of each isolated compound were recorded. The compounds and standards were permethylated with diazomethane

30 before injection to GC- MS according to Fieser and Fieser (7). GC-MS conditions were as follows: 2 µl of the

31 sample were injected to the capillary column and the temperature gradient was: from 90 to 270°C, 90 °C (2 min), 90

32 to 150°C (3 min), 150 °C (1 min), 150 to 270°C (9 min) and 270°C (1 min).

33 **Leachate content of the isolated phytochemicals:** The isolated compounds were quantified in the leachate using

34 external standard method by HPLC. Calibration curves were built with authentic vanillic (VA), ferulic (FA) and

35 syringic (SA) acids (> 99%, Aldrich). A gradient elution was performed to separate phenolic acids in the leachate at

36 a flow rate of 1 mL min^{-1} with solvent A (2% acetic acid in water) and B (2% acetic acid in MeOH) as follow: 0% B

37 to 30% B in 30 min; 30% B to 42% B in 12 min; 42% B to 100% B in 4 min. Compounds were detected at 266 nm.

38 **Phytochemical screening:** The components of the straw leachate and column fractions were separated on silica gel

39 60 F_{254} plates (0.25 mm layer, Merck). Ethyl acetate – formic acid – acetic acid - water (100:11:11:26, v/v) and

40 benzene - dioxane – acetic acid (90:25:4, v/v) were used as development solvents. The separated components were

41 visualized under UV light (254 and 366 nm, UV Lamp Model UV 5L-58 Mineralight Lamp) and sprayed with 1%

1 ferric chloride in MeOH.

2 **Determination of total phenolics:** Total phenolic compounds were estimated using the method of Singleton et al.

3 (17) with Folin-Ciocalteau reagent. Ferulic acid was used as standard.

4 **pH:** Aqueous solutions and water controls assayed were buffered with 5 mM 2-[*N*- morpholino] ethanosulfonic acid

5 (MES) adjusted to pH 5.7 with 1 N sodium hydroxide.

6 **Bioassay in 24-well plates:** The biological activity of column fractions was assayed as previously reported for *L.*

7 *sativa* in 24-well plates (14). The amounts of fractions assayed were 0.02, 0.10, 0.20, 0.30, 0.40 and 0.50 µmol of

8 total phenolic compounds per well in 200 µl of 5 mM MES (pH 5.7). Mannitol solutions prepared in MES (pH 5.7)

9 with the same osmotic potential as the assayed samples were also assayed. Five *L. sativa* seeds were placed on a

10 filter paper (Whatman #1) set at the bottom of each well. The plates were sealed with parafilm and placed in the

11 growth chamber at 25 ± 1 °C with a 12 h photoperiod at 400 µmol m^{-2} s^{-1} photosynthetically active radiation. Root

12 length of *L. sativa* was measured on 5 d-old-seedlings. Each treatment consisted of 4 replications and was repeated

13 twice. HPLC analysis confirmed the isomerization of FA in the assayed growth conditions in the ratio 2:1 (*trans*:

14 *cis*).

15 **Bioassay in Petri dishes:** Aqueous solutions of vanillic, ferulic and syringic acids with concentrations between 0.1

16 and 4.0 mM and leachate dilutions with concentrations of total phenolic compounds between 0.1 and 4.0 mM were

17 assayed in 6 cm Petri dishes. Mannitol solutions with the same osmotic pressure as the dilutions of straw leachate

18 (25-75 mmol kg^{-1}, SemiMikro osmometer, Knauer) and distilled water were also assayed**.** In each Petri dish, 17

19 germinated seeds of a test plant were placed on a layer of filter paper and watered with 3 mL of a test solution. The

20 dishes were sealed with parafilm and placed in a growth chamber outlined previously. After 5 d root and shoot

21 length were measured.

22 **Changes in root morphology:** A set of *L. sativa* seedlings were grown in solutions of the phenolic acids with

23 concentrations of 0.1 to 1.0 mM in the Petri dish bioassay previously indicated. After 5 d the seedlings were

24 transplanted to unsterile soil and grown in the growth chamber for 30 d. Then, the elongation of the primary root

25 seedlings and the number of secondary roots were determined. The experiment was performed in triplicate and

26 twice-repeated.

27 **Leakage test:** Changes in membrane permeability due to the identified phenolic acids were assayed by measuring

28 leakage of solutes and ultraviolet absorbing materials. Samples of excised radicles (0.3 g fresh weight) from 5 day-

29 old *L. sativa* seedlings were suspended in 20 mL of aqueous solutions (pH 5.7) of FA, SA and VA at concentrations

30 between 0.1 and 1 mM after 48 h at 25 ºC. After three washings in distilled water, roots were suspended in 15 mL of

31 deionized water for 4 h at the same temperature. Then, electric conductivity was analyzed (DDS – 11A). The UV-

32 absorbing materials (e.g. amino acids, nucleotides, polypeptides, etc) were determined by measuring the absorbance

33 at 260 nm. The experiment was performed in triplicate and twice-repeated.

34 **Root reduction activity test:** *L. sativa* roots (100 mg of fresh weight) grown in the phenolic acids solutions (pH

35 5.7) were washed, blot-dried, weighed and soaked in 5 mL of 2 g L^{-1} of 2,3,5-triphenyl tetrazolium chloride (TTC)

36 dissolved in a 50 mM PO$_4$ buffer (pH 7) at 37 ºC for 4 h in the dark. Then, 0.5 mL of 1 M sulfuric acid was added

37 to stop the reaction. Roots were removed, washed with distilled water, blot-dried, and ground with two portions of 3

38 to 4 mL ethyl acetate in a mortar and pestle. The extract was filtered through filter paper. The volume was made up

39 to 7 mL and the optical density of the formazan production was read at 485 nm. The experiment was performed in

40 triplicate and twice-repeated.

41 **Mitotic index test:** Inhibition of *L. sativa* root cell division (mitotic index) was measured according to Armbruster

1   et al. (2) with some modifications. A filter paper (Whatman #1) and 17 surface sterilized L. *sativa* seeds were placed

2   in 6-cm Petri dishes. Three mL of aqueous solutions of ferulic, vanillic and syringic acids were added in each Petri

3   dish. The phenolic acids were assayed at the concentrations of 0.1, 0.5 and 1 mM (pH 5.7). Dishes were sealed with

4   Parafilm and placed in the growth chamber outlined previously. After 6 days, root tips were fixed in glacial acetic

5   acid / absolute ethanol (1:3) for 24 h and stored in 70% ethanol at 5 ℃ until use. Root tips were then hydrolyzed in 2

6   N chloride acid for 15 min at 60 ℃ and then repeatedly washed with distilled water. Root tips were stained in the

7   dark for 15 min using Schiff's reagent (8) and then squashed in 15 g L^{-1} carmine dissolved in 45% glacial acetic

8   acid. Mitotic stages were observed in at least 1000 cells per slide at a magnification of 40x with a Olympus CH

9   microscope.

10   **Statistical analysis:** Data in tables and figures were presented as means ± standard error. Data of the measured

11   physiological processes previously indicated were subjected to analysis of variance (ANOVA) with significant

12   differences between means identified by GLM procedures. The results were given in the text as probability values,

13   with $P < 0.05$ adopted as the criterion of significance. Differences between treatment means were established with

14   Dunnet T3. Non-linear regression procedures were needed to analyze root elongation response against phenolic acid

15   concentrations. Complete statistical analysis was performed with SPSS 7.5 for Windows.

16

17   **RESULTS AND DISCUSSION**

18   The components extracted in diethyl ether fraction inhibited root elongation of *L. sativa* more than those

19   extracted by ethyl acetate. Separation of the diethyl ether components by a Sephadex LH 20 yielded five peaks (L1

20   to L5, Figure 1). L3 peak was the most active on plant growth. The constituents of L3 were loaded on a silica gel

21   column and separated in six fractions (S1 to S6). S1 was the most phytotoxic and its constituents were separated in

22   four peaks (1,2,3 and 4), by micropreparative HPLC. Each peak comprised a pure compound. The UV-Vis and MS

23   spectra of these compounds indicated that they were hydroxybenzoic acids (vanillic and syringic acids) and isomers

24   of a hydroxycinnamic acid (*trans*-ferulic and *cis*-ferulic acids) (Table 1). These compounds are commonly present in

25   angiosperms (19). Furthermore, plant residues localized on soil surface are often the main source of these

26   phytotoxins in managed ecosystems (10).

27   <u>**Fig 1**</u>

28   <u>**Table 1**</u>

29   **Effect of the isolated compounds on seedling growth**

30   Only root elongation was shown because leachate dilutions and their isolated constituents did not affect shoot

31   growth of the test plants (Table 2). Mannitol solutions with the same osmotic potential and pH as the leachate

32   dilutions had no effect on seedling growth of the test plants (not shown) suggesting that leachate organic

33   constituents were responsible of the observed growth inhibition.

34   <u>**Table 2**</u>

35   The effect of the isolated phenolic compounds was concentration dependent and also varied with the test

36   plant. In general, VA inhibition on root elongation of the test plants was stronger than FA and SA (Table 2). Low

37   concentrations of FA and SA (0.1 and 0.5 mM) significantly stimulated root elongation of *B. subalternans* and *L.*

38   *sativa* (not shown). SA was the less phytotoxic isolated compound. Previous studies indicated that root length could

39   decline with the increase of phenolic acid concentration in a linear or a curvilinear fashion (5). In this study, root

40   inhibition of *L. sativa* and the assayed weeds was best described as a curvilinear response over the range of phenolic

41   acids and straw leachate concentrations assayed (Table 3).

1   **Table 3**

2

3   **Physiological effects of the isolated compounds on *L. sativa***

4   Membrane is usually suggested as the initial action site of phenolic compounds, triggering subsequent

5   changes in plant physiology (6). As shown in Figure 2A and 2B, the isolated compounds increased the root cell

6   leakage of electrolytes and non-electrolytes. Vanillic acid induced the highest loss of electrolytes, following by FA

7   and SA. On the other hand, the highest loss of non electrolytes was observed on lettuce roots treated with FA,

8   following by VA and SA. The efflux of organic and inorganic cell constituents indicated that phenolic acids

9   damaged root membrane integrity (3).

10   **Fig 2**

11   Lettuce roots grown in the control (MES buffer solution) produced more formazan than those grown in

12   solutions of phenolic acids. When compared with VA and FA, roots treated with SA significantly produced more

13   formazan (Figure 1C). Formazan production is an indicator of the mitochondrial dehydrogenase activity (18). The

14   decrease in dehydrogenase activity may associate with a diminution in ATP production. Low ATP production may

15   induce the inhibition of root growth.

16   Phenolic acids affected cell division in *L. sativa* root meristems (Figure 3). Vanillic acid significantly reduced

17   mitotic index with respect to MES buffer solution. Concentration of 1 and 0.5 mM FA reduced cell division while

18   0.1 mM FA significantly stimulated mitotic activity. Otherwise, 0.1 and 0.5 mM SA promoted cell division while 1

19   mM SA had no effect on mitosis. SA reduced root elongation at concentrations higher than 1 mM implying that SA

20   may inhibit root cell division at high concentrations. Blum et al. (5) suggested that the curvilinear decline of plant

21   root elongation with the increase of phenolic acids concentration could be due to an inhibition in root cell division

22   and expansion. Accordingly, our results indicate that the identified phenolic acids affected root elongation, at least

23   partially, through an inhibition in cell division of the meristematic root zone.

24   **Fig 3**

25   Primary roots pretreated with VA and FA were shorter than those pretreated with MES buffer (Figure 4).

26   Roots were less elongated and had less secondary roots as VA concentration increased. Seedlings pretreated with

27   FA and SA increased branching of primary roots (Figure 5A and B). Many natural monophenols can stimulate auxin

28   decarboxilation (6). Inhibition of auxin activity could be responsible for the observed increase in secondary roots.

29   The observed response suggests that exposure to the isolated phenolic acids was able to modify the normal root

30   growth pattern of *L. sativa* and that these modifications further persisted in the subsequent seedling growth.

31

32   **CONCLUSIONS**

33   Leachate concentrations of VA, FA and SA were 1.01 ± 0.05 mmol l^{-1} (66 % *trans*; 34% *cis*), 0.82 ± 0.02 mmol

34   L^{-1} and 0.10 ± 0.01 mmol L^{-1}, respectively. In general, root elongation of the test plants and the evaluated

35   physiological processes on *L. sativa* indicated that VA was the strongest inhibitory phytotoxin, following by FA and

36   SA. The relative phytotoxic potency of these compounds was different but their effects on the evaluated

37   physiological processes suggest that, in general, these compounds have essentially the same action mode. As

38   phenolic acids are often found spatially concentrated in the top soil layers under plant straw (1,4), more studies are

39   needed to establish the participation of these compounds in natural settings.

40   **Fig 4 Fig 5**

41

REFERENCES

1. Abenavoli, M. R., Sorgonà, A., Albano, S. and Cacco, G. (2004). Coumarin differentially affects the morphology of different root types of maize seedlings. Journal of Chemical Ecology **30**: 1871-1883.

2. Armbruster, B. L., Molin, W. T. and Bugg, M. W. (1991). Effects of the herbicide dithiopyr on cell division in onion root tips. *Pesticide Biochemistry and Physiology* **39**: 110-120.

3. Baziramakenga, R., Leroux, G. D. and Simard, R. R. (1995). Effects of benzoic and cinnamic acids on membrane permeability of soybean roots. *Journal of Chemical Ecology* **21**: 1271-1285.

4. Blum, U. (1996). Allelopathic interactions involving phenolic acids. Journal of Nematology **28**:259-267.

5. Blum, U., Dalton, B. and Rawlings, J. O. (1984). Effects of ferulic acid and some of its microbial metabolic products on radicle growth of cucumber. *Journal of Chemical Ecology* **10**: 1169-1191.

6. Einhellig, F. A. (2004). Mode of allelochemical action of phenolic compounds. In: *Allelopathy: Chemistry and mode of action of allelochemicals*, (Eds, F. A. Macías, J. C. G. Galindo, J. M. G. Molinillo and H. G., Cutler), pp. 217-38. Boca Ratón, CRC Press.

7. Fieser, L. F. and Fieser, M. (1967). Reagent for Organic synthesis. John Wiley and Sons, New York.

8. Jensen, W. A. (1962) *Botanical Histochemistry*. W. H. Freeman & Co., San Francisco, CA.

9. Godshall, M. A. and Lonergan, T. A. (1987). The effect of sugarcane extracts on growth of the pathogenic fungus *Colletotrichum falcatum. Physiology and Molecular Plant Pathology* **30**:299-308.

10. Kuiters, A. T. (1990). Role of phenolic substances from decomposing forest litter in plant-soil interactions. *Acta Botanica Neerlandica* **39**: 329-348.

11. Lorenzi, H. J., Gandini, M. O. and Gazon, A. L. (1989). Trash blankets: the potential to control weeds and the effect on ratoon cane development. In: *Proceedings of the 20th International Sugarcane Technologists Congress.* pp 571-576. Sao Paulo, Brazil.

12. Manechini, C. (2000). The impact of cane trash on weed control. *International Cane Energy News*. Winrock International, Arlington VA.

13. Legaz, M. E., De Armas, R., Piñón, D., Vicente, C. (1998). Relationships between phenolics-conjugated polyamines and sensitivity of sugarcane smut (*Ustilago scitaminea*). *Journal of experimental botany* **49**: 1723-1728.

14. Rimando, A. M., Dayan, F. E., Czarnota, M. A., Weston, L. A. and Duke, S. O. (1998). A new photosytem II electron transfer inhibitor from Sorghum bicolor (L.). *Journal of Natural Products* **61**: 927-930.

15. Scandaliaris, J., Perez Zamora, F., Rufino, M., Romero, E. and Morandini, M. (2002) La cosecha verde como estrategia para disminuir el impacto ambiental de la caña de azúcar. *Avance Agroindustrial* **1**: 14-17.

16. Singh, P., Suman, A. and Shrivastava, A. K. (2003). Isolation and identification of allelochemicals from sugarcane leaves. *Allelopathy Journal* **12**:71-79.

17. Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other

oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Methods in Enzymology* **299**: 152-178.

18. Stowe, R. P., Koenig, D. W., Mishra, S. K., Pierson, D. L (1995). Nondestructive and continuous spectrophotometric measurement of cell respiration using a tetrazolium-formazan microemulsion. *Journal of Microbiological Methods* **22**: 283-92.

19. Souto, C. X., Bolaño, J. C., González, L. and Santos, X. X. (2001). HPLC techniques - Phenolics. In: *Handbook of Plant Ecophysiology Techniques*, (Ed., Reigosa, M. J.). pp. 251-282. Kluwer Academic Publishers. Netherlands.

1
2

Table 1. Absorption maxima and retention times of the isolated compounds.

Compound isolated	Absorption maxima (UV-Vis spectra)	Retention time (micropreparative HPLC)
Vanillic acid (**1**)	208, 255 y 288 nm	24,77 min
Syringic acid (**2**)	212 y 262 nm	28,38 min
cis-Ferulic acid (**3**)	217, 233, 321 y 324 nm	31,28 min
trans-Ferulic acid (**4**)	217, 233, 321 y 324 nm	32,27 min

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

Table 2. Effect of the straw leachate dilutions and their identified phytochemicals on radicle elongation of weed plants and *Lactuca sativa* L. Data are shown as mean ± SE.

	Treatment	*Amaranthus quitensis*	*Bidens subalternans*	*Brassica campestris*	*Lactuca sativa*	*Sida rhombifolia*
EC_{50} (mM)	Straw leachate[b]	0.80±0.01	0.97±0.02	0.43±0.04	0.42±0.03	0.88±0.03
	Vanillic acid	0.80±0.01	1.80±0.04	0.40±0.02	0.45±0.01	0.70±0.03
	Ferulic acid	1.25±0.01	2.00±0.02	1.90±0.04	1.20±0.03	2.00±0.01
	Syringic acid	3.50±0.01	2.90±0.02	3.60±0.02	2.75±0.05	1.50±0.02

EC_{50} – Concentration needed to reduce 50% root elongation of the test plants, b Leachate concentrations are expressed as total phenolic compounds in equivalents of ferulic acid.

Table 3. Partial regression coefficients and R^2 values for root elongation (% of control) of the

test plants in presence of phenolic acids (ferulic, vanillic and syringic) and straw leachate.

Test plant	Phenolic acid	Line intercept	Linear	Quadratic	R^2 (Quadratic regression)
Amaranthus quitensis	Vanillic (VA)	2.15	-1.67	0.51	0.92
	Ferulic (FA)	2.24	-1.31	0.35	0.96
	Syringic (SA)	2.15	-0.88	0.30	0.88
	Straw leachate (SL)	2.02	-1.44	0.41	0.81
Bidens subalternans	VA	1.53	-0.81	0.24	0.92
	FA	1.64	1.21	-0.55	0.84
	SA	1.84	0.64	-0.24	0.81
	SL	2.02	-1.44	0.41	0.98
Brassica campestris	VA	2.82	-2.72	0.85	0.89
	FA	3.17	-1.48	0.61	0.91
	SA	3.15	0.26	-0.50	0.94
	SL	2.91	-3.10	0.95	0.95
Lactuca sativa	VA	2.34	-2.76	0.93	0.94
	FA	2.77	-1.57	0.24	0.85
	SA	2.61	0.91	-0.54	0.79
	SL	2.15	-2.38	0.78	0.98
Sida rhombifolia	VA	2.34	-1.86	0.53	0.95
	FA	2.60	0.30	-0.48	0.83
	SA	2.60	-0.50	-0.14	0.99
	SL	2.61	-1.89	0.52	0.96

1
2
3
4
5
6
7
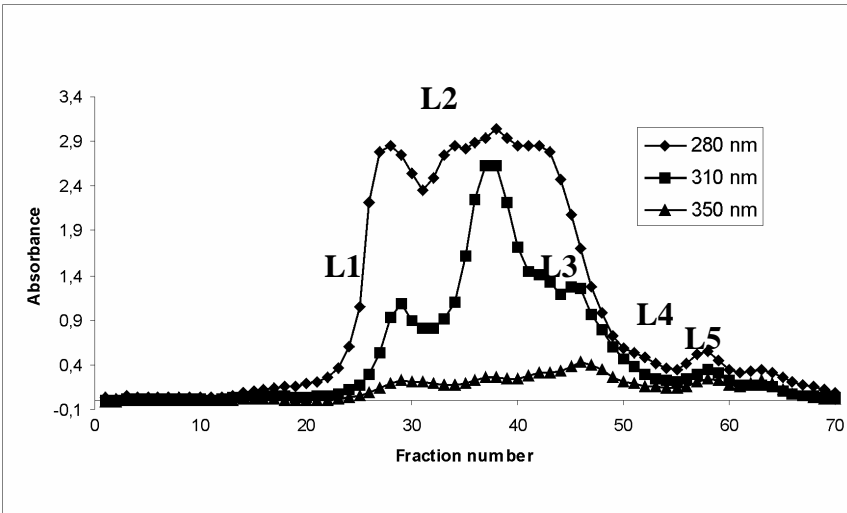8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

Figure 1. Elution profile on Sephadex LH 20 of the ether fraction extracted from sugarcane (*Saccharum officinarum* L.) straw.
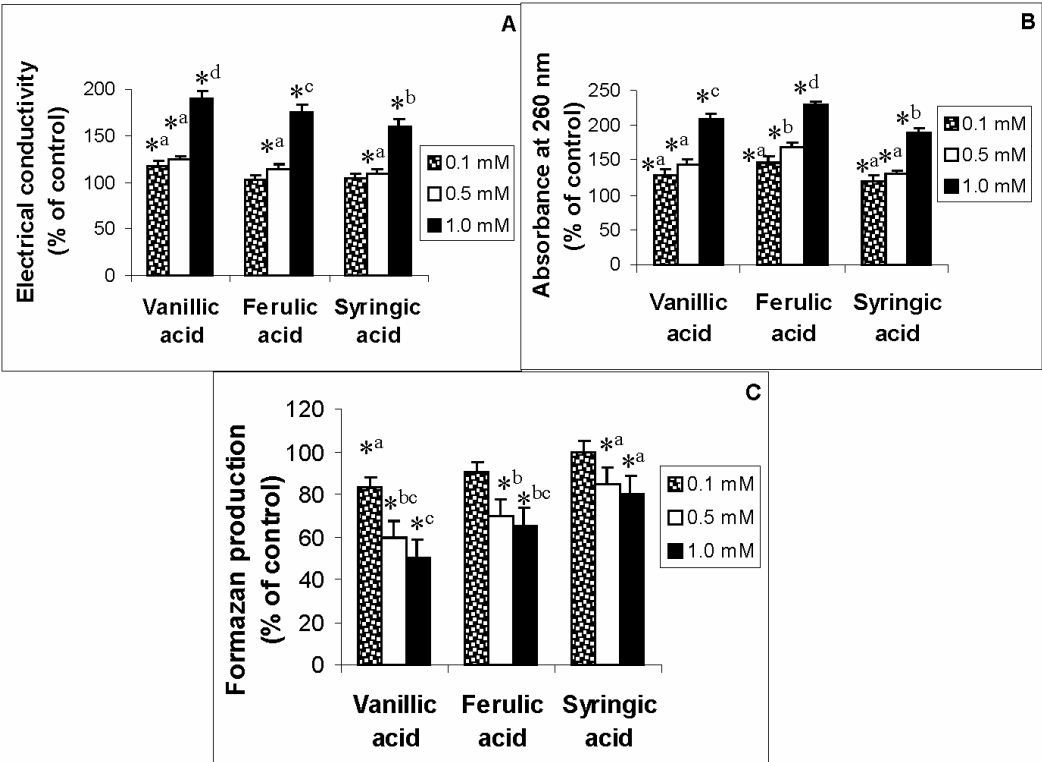

Figure 2. Effects of compounds isolated from a sugarcane (*Saccharum officinarum* L.) straw leachate on roots of *Lactuca sativa* L.: leakage of (A) electrolytes and (B) non electrolytes; (C) formazan production. Bars represent the standard error of each mean and asterisks indicate significant differences at level 0.05 as compared with controls. Different letters indicate significant differences between means at level 0.05.
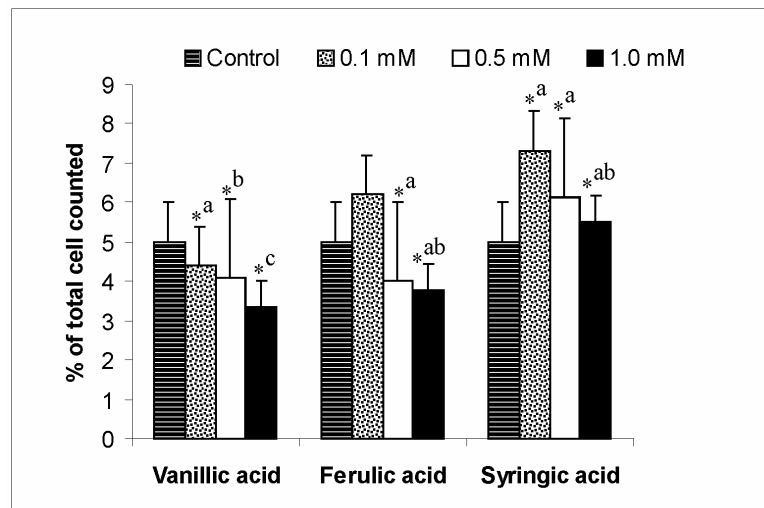
Figure 3. Mitotic Index of lettuce tips exposed to phenolic acids isolated from a sugarcane (*Saccharum officinarum* L.) straw leachate. Bars represent the standard error of each mean. Asterisks indicate significant differences at level 0.05 as compared with the control. Different letters indicate significant differences between means at level 0.05.
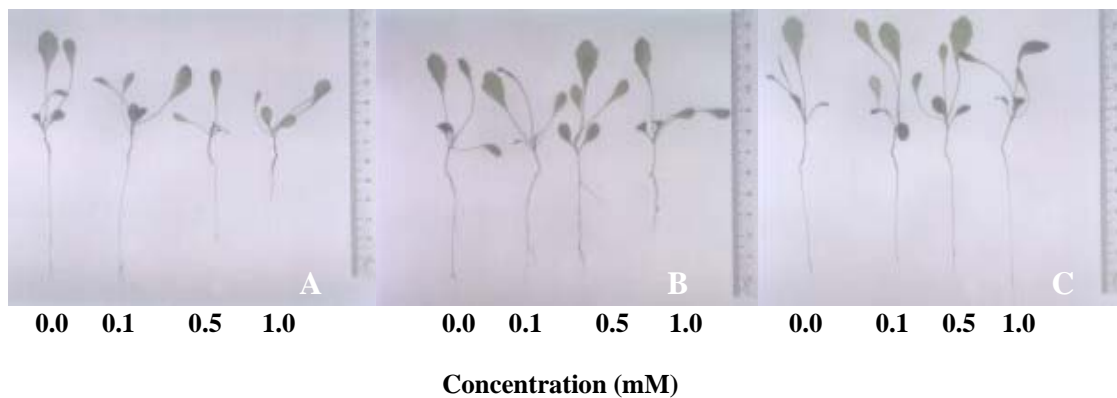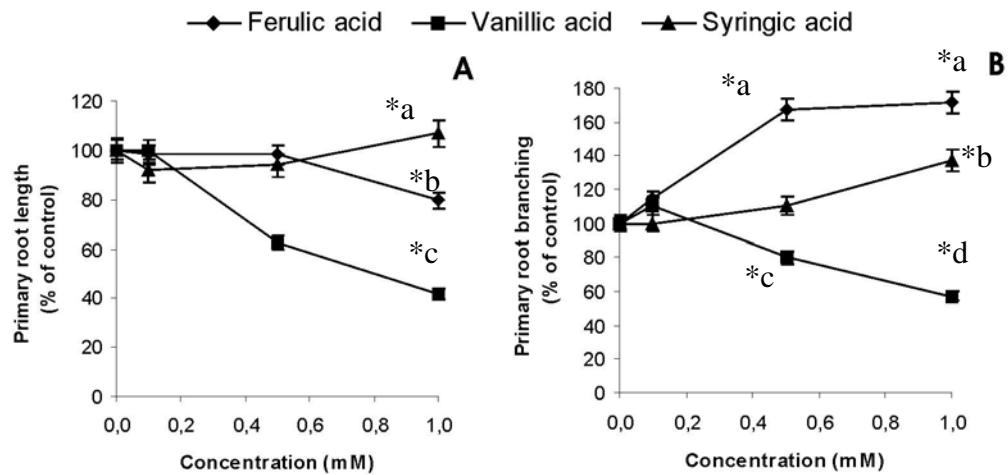

Figure 4. Seedling growth of *Lactuca sativa* L. after 30 days pretreated with (A) vanillic acid, (B) ferulic acid and (C) syringic acid of under unsterile soil.

Figure 5. Elongation of primary roots (A) and number of secondary roots (B) of *Lactuca sativa* L. after 30 days pretreated with the phenolic acids isolated from sugarcane (*Saccharum officinarum L.*) straw leachate under unsterile soil. Different letters indicate significant differences between means at level 0.05.