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A calorimetric study of plant–plant and plant–soil interactions of extracts from *Ixorhea tschudiana*

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ABSTRACT

The effect of the $CHCl_3$ and MeOH extracts from aerial parts from the Northwestern Argentina endemic species *Ixorhea tschudiana* Fenzl. on germination of the dicot tomato and the monoct onion and on soil microbial activity was studied by calorimetric and calorespirometric experiments. The $CHCl_3$ extract inhibited seedling growth of tomato which increased with increasing concentrations. Seeds imbibed with 250 mg dm^{-3} extract germinated 24 h later than control and those imbibed with 100 and 500 mg dm^{-3} extract. This was reflected in the calculated low specific seedling growth rate ($p_{SG}\Delta H_B$) as determined from calorespirometric experiments. On the other hand, seedlings obtained in 500 mg dm^{-3} extract showed shorter and thicker hairy roots than control with significantly higher $p_{SG}\Delta H_B$. Probably, the extract induces inhibition of water uptake by seedling roots. Germination was reduced 50% in onion seeds imbibed with 500 mg dm^{-3} $CHCl_3$ extract which apparently is due to higher rate of imbibition during the first stages as determined for calorimetry. On the other hand, 83 and 250 mg kg^{-1} of the MeOH and $CHCl_3$ extract seem to selectively inhibit the growth of certain microorganisms and to enhance the activity of soil actinomycetes. Two actinobacteria were isolated from soil treated with these concentrations of both extracts: *Kocuria* sp. and *Kocuria rosea*; this latter species is known as a keratinolytic agent and seems to degrade complex carbon compounds of the soil and those incorporated by the MeOH extract. Both *Kocuria* specie seem to be beneficial for the soil converting substrate into biomass and thus *I. tschudiana* could be used as a soil phytoremediator.

1. Introduction

Plant invasion is an important area of ecological research and has received much attention from ecologists during the last decade. Invasive plant species can have significant impact on the native community and ecosystem and also, on biodiversity and global change [1]. It was reported [2] that about 20–30% of the non-indigenous species of plants, animals and microbes out of a total number of 50,000 are considered as major environmental problems. Exotic invasive species have the potential to affect the structure of native plant communities. They can also change the composition of the soil microbial community. Invasive species may displace native ones through competition, changes in ecosystem processes, or allelopathy among other mechanisms [3]. Allelochemicals play an important role in plant–plant interactions (allelopathy) but also, they might be present in the soil influencing their biotic and abiotic components. Is in this context where calorimetry may play a key role to help to understand the nature of the interactions occurring between plants, plant–soil and plant–microorganisms and thus, to aid in the problematic of weed control. Two aspects of this problem can be approached by using calorimetry as a tool. One implies the search for bioactive compounds with herbicidal properties and the other, the study of the weed itself and its influence on the soil. In both cases, different doses of extracts or pure compounds of the plant species under study could be used for experiments of seed germination and soil microbial activity as well as for other chemical and microbiological conventional studies.

In this context, we made a search for natural herbicides by germinating the dicot tomato, *Solanum lycopersicum* and the monocot onion, *Allium cepa* in the presence of different plant extracts. Three plant specie (spp.) were tested: *Sicyos poliacanthus*, *Brachiaria platyphylla* and *Ixorhea tschudiana*. Allelopathic activity was observed in the MeOH and EtOAc extracts of *B. platyphylla* and the $CHCl_3$ extract of *I. tschudiana* Fenzl. In this work we present results of the interaction of the $CHCl_3$ and MeOH extracts of *I. tschudiana* with soil and of the former with tomato and onion.

I. tschudiana is an endemic species (sp.) to the provinces of Tucumán and Salta in Northwestern Argentina that belongs to the

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Boraginaceae family. Nothing was found in the literature about this species except for its taxonomic description.

2. Experimental

2.1. Plant material

$CHCl_3$ and MeOH extracts of aerial parts of *I. tschudiana* were obtained from the Organic Chemistry group of the Faculty of Agronomy and Zootechny, Nacional University of Tucumán, Argentina. Briefly, ground dried plant material was subsequently treated with $CHCl_3$ (twice × 3 days) and MeOH (twice × 3 days). The $CHCl_3$ extract was further partitioned with petroleum ether and $CHCl_3$ after taking it up with an EtOH–H_2O, 4:3 mixture. This $CHCl_3$ sub-extract was used for this work. Seeds of the dicot tomato (*S. lycopersicum*) and the monocot onion (*A. cepa*) were acquired in the market with 88% germinability.

2.2. Soil material

Loam soil samples were collected in Los Pizarros, La Cocha, Tucumán Province, Argentina (27°45′S, 65°39′W). Ten sites were randomly chosen and sub-samples were collected up to depth of 15 cm (A horizon = 20 cm), after removing the top layer. After combining the sub-samples, they were sieved (2 mm × 2 mm) to remove the root residues and coarse material and stored in polyethylene bags at 5 °C until used. To treat soil with extracts a gardening plug containing 25 pots was used. An aliquot of 110.0 g dry weight (bulk density: 1.03 g cm^{-1}) was placed in each pot (105 cm^3, 9 cm deep) at field capacity humidity (FCH = 25.5%). The extracts were dissolved in dimethylsulfoxide (DMSO) or MeOH (for $CHCl_3$ or MeOH extract, respectively), mixed with sterile distilled water, and used to adjust soil water content to FCH. Final solvent concentration was 0.3% for control and 500 mg kg^{-1} extracts and 0.05 and 0.15% for 83 and 250 mg kg^{-1} extracts. The plug with treated soil samples (three replicates per treatment, each one used as replicate for chemical, microbiological and calorimetric experiments) was wrapped with polyethylene to avoid humidity losses and left at room temperature from December 2006 till February 2007 (30–40 °C). Then, the three replicates per treatment were air dried and stored individually in polyethylene bags at 5 °C until analysed.

2.3. Chemical and microbiological soil analysis

Water content (WC) was determined by drying an aliquot (10 g) until constant weight at 105 °C [4]. Bulk density and field capacity humidity (FCH) were determined by the graduated cylinder method [5]. The pH was measured with a glass electrode on a suspension of soil in deionised water (1:1) [5]. Organic carbon (OC) was determined by wet oxidation with $K_2Cr_2O_7/H_2SO_4$ [6]. Extractable phosphorus (P) was photometrically determined by the Olsen extraction method [7]. The Folin Ciocalteu's method was used to determine total polyphenol content (TPC) [8] of soil extracts and the results are expressed as microgram of gallic acid equivalent (GAE) per gram of dry soil. Total carbohydrate content (CHC) in soil extracts was determined by the phenol–sulfuric method [9] and results are expressed as microgram of glucose equivalent (GE) per gram of dry soil. Colony formation units (CFU g^{-1}) were determined by the serial dilution method by using trypteine soy agar (TSA) as culture media.

2.4. Isolation and identification of Kocuria specie (spp.)

From the TSA media, colonies with a distinctive colour were isolated and further identified at molecular level using 16S DNA_r. The 16S DNAr gene sequences was aligned using the CLUSTAL W software, version 1.7 [10], and corrected manually.

Phylogenetic analyses were performed using the neighbor-joining (NJ) [11] method.

2.5. Soil extraction

Soil extracts were produced by sonicating soil suspended in MeOH (1:1.5, w/v) during 10 min. Then, soil extracts were filtered through Whatmann No. 1 filter paper and evaporated to dryness in rotary evaporator. An aliquot (about 8 mg) extract was re-suspended in 1 ml MeOH for TPC and CHC determinations.

2.6. Germination assays

Preliminary germination assays in chamber were performed at 25 °C by placing ten seeds on Petri dishes containing 1% agar with 0 (control), 100, 250 and 500 mg dm^{-3} MeOH or $CHCl_3$ extract of *I. tschudiana*. To avoid a solvent effect, 1 g agar was thoroughly mixed with the extract solution in either $CHCl_3$ or MeOH. This agar was placed 24 h at 40 °C and further placed under vacuum another 24 h to eliminate solvent. Then, aliquots were weighed and mixed with the 1% agar solution to achieve the desired extract concentration in the Petri dish. The end of germination was considered when cotyledons were differentiated (14 days) and then, number of germinated seeds, seedlings weight, length and dry weight (24 h at 60 °C) or biomass were evaluated.

Percentage of inhibition was calculated by the expression: $I\,(\%) = [(\text{control} - \text{treatment})/\text{control}] \times 100$.

2.7. Calorimetric analysis

A twin heat conduction type calorimeter (Lund University, Sweden) was used [12,13].

2.7.1. Plant material

Three seeds were placed in the calorimeter ampoule containing 1% agar with the desired concentration of extract [14]. Power–time curves of germination were recorded at 25 °C after 30 min of thermal equilibration of the system.

2.7.2. Soil

Soil sample (3.0–4.0 g dw) was stabilized during 24 h at 25 °C in a polyethylene bag. Then, an appropriate amount of water containing glucose as to get FCH (25.5%) and 1.5 mg glucose per g of dry soil was added. The soil was thoroughly mixed by hand and then, the equivalent to 1.0–1.5 g (dw) was weighed in the calorimeter ampoule (8.0 cm^3). The ampoule was hermetically closed and after the 30 min needed to equilibrate the calorimetric system, thermal power (P)–time (t) curves of microbial growth were recorded at 25 °C. An empty ampoule was used as reference. Blank experiments were performed with soil at FCH to correct the P–t curves of microbial growth for other thermal effects than those of glucose. By using Microsoft Excel 2002 (Microsoft corporation) and the Origin 4.0 program (Microcal, Inc.) the curves obtained were converted into mass specific thermal power (p)–time (t) curves and integrated to obtain the specific heat (q) associated with the glucose degradation. From the semi-logarithmic conversion of the portion of the curve that indicates exponential microbial growth ($\log p = \log p_0 + \mu t$) the rate of microbial growth constant, μ, was calculated as well as the value of p at $t = 0$, p_0. Replicate curves were then averaged and the average curve of blank experiments was subtracted to obtain the average p–t curve of microbial growth due to glucose degradation. The value of μ together with the value of peak time (t_p) allows quantification of the increment in CFU g^{-1} (ΔCFU g^{-1}) by applying the

equation of microbial growth [15]. Results are reported as an average of the three replicates ± SD (standard deviation). This SD was determined from the curves without correction and recalculated for the corrected average curve.

2.7.3. Calorespirometry

Calorespirometric assays were performed by using soil (1–1.5 g dw) at FCH or a seedling previously germinated in chamber (14 days) at 25 °C. Once the system was equilibrated and the values of thermal power were constant (P_1), a vial containing a solution of 0.4N NaOH (trap of CO_2) was introduced and values of P were collected again (P_2). After collecting data for 2–3 h (soil) or 0.5 h (seedling), the vial was removed, and metabolism was measured again (P_3) [16]. As our calorimeter does not allow a simultaneous manipulation of both ampoules (reference and sample), several blank experiments were run by introducing a vial with water instead of NaOH into the ampoule containing soil or agar. In both cases an exothermic shift occurred. The average values obtained were 4.0 ± 1.0 μW for soil and 3.6 ± 0.5 μW for agar that were subtracted from P_2 to obtain the value of CO_2 evolution. Then, the values of P were converted into specific values (p) by dividing by the mass of the sample. The specific rate of CO_2 evolution, rCO_2, was calculated by using the expression: $rCO_2 = \{p_2 - [(p_1 + p_3)/2]\}/108.5m$. The value of −108, 5 kJ mol^{-1} is the heat of reaction of CO_2 with NaOH to produce CO_3^{2-}, and 'm' is the dry weight of soil. Specific mass seedlings growth rates were calculated from the expression: $p_{SG}\Delta H_B = rCO_2(1 - \gamma/4)\Delta HO_2 - p$ [17]. Where the value of ΔHO_2 is the Thornton constant of 455 ± 15 kJ mol^{-1} O_2 [17]; then, $455rCO_2(1 - \gamma/4)$ gives the total rate of energy available from respiration; p is the specific metabolic thermal power that represents the rate of respiratory energy loss to the surroundings; ΔH_B is the enthalpy change for the formation of biomass and γ is the oxidation state of the substrate. As these measurements in soil are performed without the addition of glucose, $p_{SG}\Delta H_B = 0$ and thus, γ of the soil can be calculated.

Results are reported as the mean of at least three measurements (one for each replicate per treatment in soil experiments) (±SD) on dry weight (dw) basis. One way ANOVA was used to determine differences between treatments by means of the computer program Origin 4.0 (Microcal Inc., 1995).

3. Results and discussion

3.1. Plant–plant interaction

Our work started with the evaluation of allelopathic activity of plant extracts. We used the dicot tomato and the monocot onion due to their fast germination. Results from the preliminary germination assays with extracts of *I. tschudiana* are shown in Table 1.

The $CHCl_3$ extract of *I. tschudiana* produced the largest effect either by reducing seedling weight and length of tomato at all concentrations tested or by reducing germination of onion at 500 mg dm^{-3}. Roots of tomato seedlings germinated in the $CHCl_3$ extract were hairy in contrast to control. On the other hand, only seedlings obtained in 250 mg dm^{-3} had a significantly lower biomass (dry weight) than control. To understand this behavior, calorespirometric assays with tomato seedlings germinated in the three extract concentrations tested and calorimetry of seed germination with onion seeds and 500 mg dm^{-3} $CHCl_3$ extract were performed.

Table 1
Percentages of Inhibition with respect to control of tomato seedlings for: germination (G), seedlings weight (m_s), seedlings length (l_s), roots length (l_r) and biomass (BM).

Plant species – extract (mg dm^{-3})	G	m_s	l_s	l_r	BM
Tomato					
$CHCl_3$ – 100	−16	−25**	−33**	−36**	4
$CHCl_3$ – 250	−16	−43**	−36**	−32**	−21**
$CHCl_3$ – 500	0	−46**	−53**	−59**	−4
Onion					
$CHCl_3$ – 500	−50**	−27	−28	−25	7

** Significantly different from control.

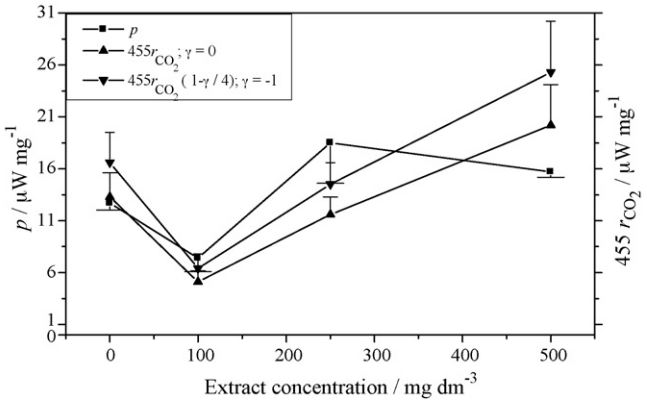

Fig. 1. Values of specific metabolic thermal power, p, and $455rCO_2$ (calculated for an oxidation state of substrate, $\gamma = 0$ and $\gamma = -1$) for tomato germinated with water (control), 100, 250 and 500 mg dm^{-3} $CHCl_3$ extract of *I. tschudiana*.

Fig. 1 shows specific metabolic heat rates, p, and specific CO_2 production rates, rCO_2, measured at the different extract concentrations for tomato seedlings. A significantly lower p value is observed for seedlings germinated in 100 mg dm^{-3} extract with respect to control whereas these values for seedlings germinated in 250 and 500 mg dm^{-3} are significantly higher. In the same figure, the values of $455rCO_2(1 - \gamma/4)$ are represented. The value of γ for carbohydrates is taken as $\gamma = 0$ [17]. Seedlings were placed in the calorimeter after 14 days of the start of experiment. Meanwhile, they were kept in germination chamber with no light periods and thus, carbohydrate content was most probably exhausted. Therefore, we also calculated this parameter for a more reduced value of γ (i.e. $\gamma = -1$). Note a very similar trend of p and $455rCO_2(1 - \gamma/4)$ values for seedlings control and those germinated with 100 and 250 mg dm^{-3}. Those seedlings germinated in 500 mg dm^{-3} extract present significantly higher value of $455rCO_2(1 - \gamma/4)$ than the corresponding p. In Fig. 2, the values of p/rCO_2 and of $p_{SG}\Delta H_B$ are plotted as a function of concentration. A value of p/rCO_2 not sig-

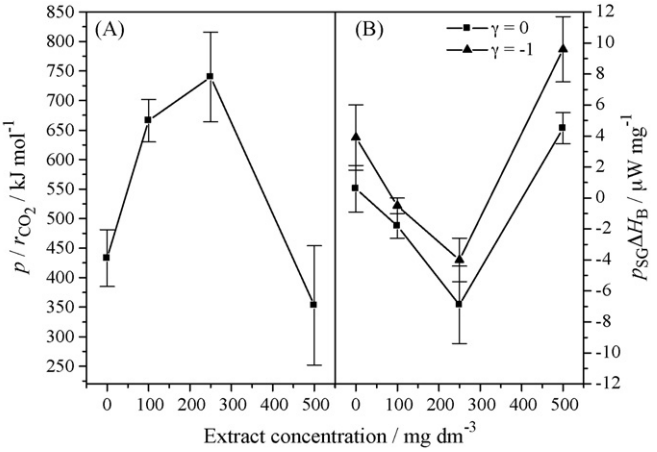

Fig. 2. Values of (A) p/rCO_2 and (B) $p_{SG}\Delta H_B$ (calculated for an oxidation state of substrate, $\gamma = 0$ and $\gamma = -1$) for tomato germinated with water (control), 100, 250 and 500 mg dm^{-3} $CHCl_3$ extract of *I. tschudiana*.

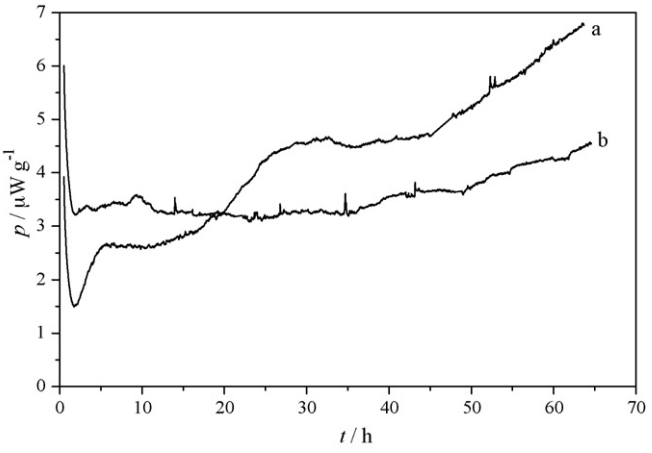

Fig. 3. Specific thermal power–time curve of onion seed germination in: (a) water and (b) 500 mg dm^{-3} $CHCl_3$ extract of *I. tschudiana*.

nificantly different from the Thornton's constant was determined for control seedlings and slightly lower for those germinated in 500 mg dm^{-3} extract. Values of $p_{SG}\Delta H_B$ for these seedlings indicated capability for growth being significantly higher for seedlings germinated in 500 mg dm^{-3} extract. Latter seedlings had hairy roots which in turn were shorter and thicker than those of control. Thus, the calculated $p_{SG}\Delta H_B$ values might reflect the thickening of roots due to a defense mechanism and not the potential for further growth and production of tomato in the presence of this concentration of $CHCl_3$ extract of *I. tschudiana*. It is worth to note the negative $p_{SG}\Delta H_B$ value determined for seedlings germinated in 250 mg dm^{-3} extract. These seeds germinated 24 h later than those in the other treatments. Roots were similar in length to those of control but hairy.

Preliminary germination assays showed the same biomass content of seedlings control and those germinated in 100 and 500 mg dm^{-3} extract but biomass for seedlings germinated in 250 mg dm^{-3} extract was significantly lower than control (see Table 1). However, seedlings weight decreased with increasing extract concentration. The relation obtained when plotting the $\log_{10}$ values of seedlings weight against those of concentration was: $\log m_s = 1.576 - 0.096 \log c$ ($R^2 = 0.90$). This clearly shows blockage of water uptake during seedling growth produced by the compounds contained in the extract which could explain the hairy roots.

Fig. 3 shows specific thermal power (p)–time curves of onion seeds germination. Note the difference in the shape of the curves control and in 500 mg dm^{-3} of $CHCl_3$ extract (Fig. 3, curves a and b, respectively). The higher p values for seeds imbibing in the extract during the first 15 h of imbibition would indicate a higher rate of imbibition which in turn might have caused anoxia in some seeds and thus, the decrease in germinated seeds observed. This is supported by the fact that seeds imbibed in the extract germinated (78% against 100% control) 10 h earlier than control (43.5 ± 10.6 and 53.3 ± 6.5 h, respectively).

As the negative effect observed increases with concentration of the extract, the metabolite or metabolites responsible of the allelopathic activity must be in low concentration in the plant. However, isolation and identification of this/these compound/s could indicate their potentiality as models for synthetic herbicides less harmful for the environment.

3.2. Plant–soil interaction

Table 2 shows chemical and microbiological characteristics for soil samples incubated with $CHCl_3$ and MeOH extracts of *I. tschudiana* as well as for the soil previous to incubation (OS). It is worth to note the low pH of the incubated soils with the $CHCl_3$ and MeOH extracts. It is known that carbonic acid is always a contributor to soil acidic pH [18]. Carbon dioxide (CO_2) in the soil atmosphere dissolves in the soil solution to yield H_2CO_3. In our experiments the time of incubation was during summer (30–45 °C) and humidity of soil was at its FCH which is reckoned to produce the highest microbial activity. Under these conditions, most probably the CO_2 produced did not diffuse completely through the polyethylene and great part dissolved in the soil. The same effect was observed in other experiments with extracts of *B. platyphylla* with a similar temperature and humidity of soils during incubation (results not shown). On the other hand, the much lower pH observed with soil treated with the $CHCl_3$ extract was due to the added DMSO. Separate experiments performed by incubating soil with different concentrations of DMSO showed that pH decreases with increasing concentrations of the solvent (results not shown) as occur in these experiments. This effect was not observed with soil incubated with different concentrations of MeOH.

It is worth to note in Table 2 an increase of CFU g^{-1} in soils incubated with 83 and 250 mg kg^{-1} $CHCl_3$ extract with respect to control whereas a decrease is observed for soil incubated with 250 mg kg^{-1} MeOH extract. From the microbiological cultures performed with soil samples incubated with 83 and 250 mg kg^{-1} of both extracts, an actinomycete species was isolated which was identified as *Kocuria* sp. (yellow colonies). From the soil treated with 250 mg kg^{-1} MeOH extract, beside *Kocuria* sp., an additional actinobacteria was isolated which was identified as *Kocuria rosea*

Table 2
Values of pH, organic carbon (OC), extractable phosphorus (P), total phenolic content (TPC) expressed as μg of galic acid equivalent (GAE), total carbohydrate content (CHC) expressed as μg of glucose equivalent (GE) and colony formation units (CFU g^{-1}) as determined in trypteine soy agar (TSA) for: original soil (OS), control (CS), 83, 250 and 500 mg kg^{-1} $CHCl_3$ and MeOH extracts of *I. tschudiana*.

	pH	OC (%)	P (mg kg^{-1})	TPC (μg GAE g^{-1})	CHC (μg GE g^{-1})	10^{-6} CFU g^{-1}
OS	6.13 ± 0.01	1.57 ± 0.01	39.5 ± 1.3	ND	ND	1.33 ± 0.02
$CHCl_3$ extract						
CS	4.31 ± 0.01*	1.75 ± 0.04*	41.5 ± 0.2*	88.4	62.5	1.63 ± 0.11*
83	4.94 ± 0.01**	1.63 ± 0.04**	16.9 ± 0.0**	90.9	46.2**	2.21 ± 0.29**
250	4.76 ± 0.01**	1.40 ± 0.01**	15.3 ± 0.1**	69.7**	39.2**	1.91 ± 0.13**
500	4.25 ± 0.01	1.25 ± 0.01**	29.6 ± 0.1**	42.0**	46.2**	1.44 ± 0.13**
MeOH extract						
CS	5.73 ± 0.05*	1.29 ± 0.03*	48.3 ± 1.9*	24.9	11.5	1.94 ± 0.05*
83	5.67 ± 0.01**	1.33 ± 0.01	43.4 ± 0.0**	69.2**	34.1**	1.88 ± 0.04
250	5.62 ± 0.01**	1.19 ± 0.02**	28.4 ± 0.8**	93.0**	53.3**	1.71 ± 0.03**
500	5.47 ± 0.03**	1.27 ± 0.01	19.3 ± 0.2**	33.6	16.5	1.54 ± 0.11**

* Significantly different from OS.
** Significantly different from control (CS).

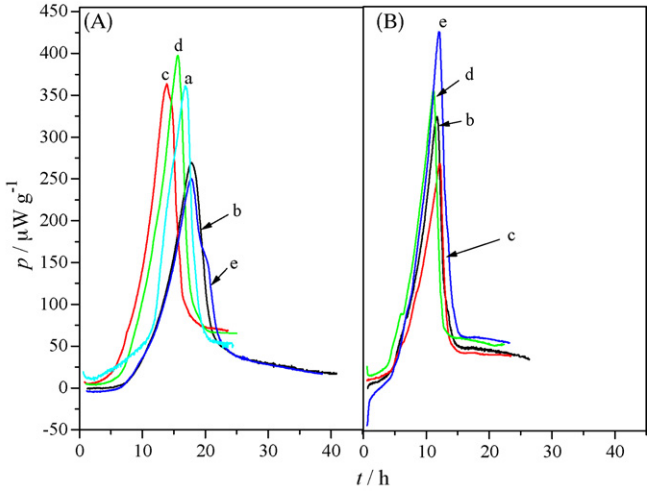

Fig. 4. Average specific thermal power–time curves of the degradation of 1.5 mg glucose by the microorganisms contained in: (a) Original soil (OS), (b) control (CS), (c) 83 mg kg^{-1}, (d) 250 mg kg^{-1} and (e) 500 mg kg^{-1} extract: (A) $CHCl_3$ and (B) MeOH.

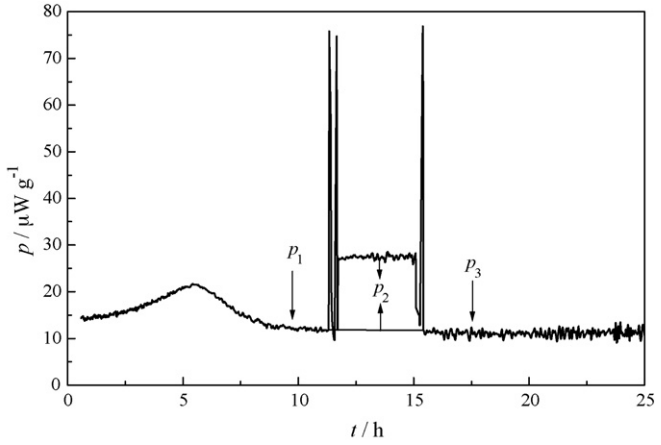

Fig. 5. Specific thermal power–time curve as obtained in a typical calorespirometric experiment where p_1 and p_3 represent basal respiration and p_2 is the specific thermal power due to CO_2 evolution.

(red colonies). It seems as if 83 and 250 mg kg^{-1} of the MeOH and $CHCl_3$ extract promote the growth of *Kocuria* sp. Besides, 250 mg kg^{-1} MeOH extract seem to selectively inhibit the growth of certain microorganisms and promotes the growth of *K. rosea*.

Fig. 4 shows average *p*–*t* curves of glucose degradation by microorganisms contained in the soil samples treated with $CHCl_3$ (Fig. 4A) and MeOH (Fig. 4B) extracts of *I. tschudiana*. In addition, in Fig. 4A, the *p*–*t* curve due to the original soil (OS) is shown (Fig. 4, curve a). The curves show the typical kinetics of microbial exponential growth.

Table 3 shows the thermodynamic parameters as determined from the curves in Fig. 4. Values of t_p for CS and that containing 500 mg kg^{-1} $CHCl_3$ extract were the same. However, a linear increase of t_p values with increasing concentrations of extract was found according to the equation: $t_p = 13.6 + 8.2 \times 10^{-3} C_{ext}$ (correlation coefficient, $R^2 = 1$). Values of t_p for soils treated with MeOH extract did not significantly differ among them. All concentrations tested of both extracts, negatively affected the growth of microorganisms (see values of ΔCFU g^{-1} in Table 3) as well as turned them into less energetically efficient (see values of heat yield, Yq/ΔCFU g^{-1}, in Table 3) to convert the glucose into biomass. The values of ΔCFU g^{-1} for soils treated with the $CHCl_3$ extract were positively correlated with phosphorus content of the soil ($R^2 = 0.81$). Phosphorus is essential for the growth of microorganisms and thus, its deficiency is limiting for growth [19]. Note in Table 2 the much lower value of extractable phosphorus for soils containing 83 and 250 mg kg^{-1} extract. However, it was reported that for restoration of ex-arable lands, low available phosphorus is needed [20] and if that is not the case it should be lowered by some means. Therefore, this species aerial part could be used for soil restoration purposes. Besides, *Kocuria* species are well known soil actinobacters. *K. rosea* is a gram (+) microorganism that belongs to the Micrococcaseae family. It is used as a keratinolytic agent to produce fermented feather meal [21] and thus, it is possible that these actinomycetes are extremely inefficient to mineralize simple carbon compounds but very efficient to degrade complex ones. Therefore, the higher Yq/ΔCFU g^{-1} values obtained in soils treated with extracts that activated them. This must be the reason for the higher total phenolic content (TPC) and carbohydrates content (CHC) of soils samples treated with 83 and 250 mg kg^{-1} MeOH extract (see Table 2) containing *Kocuria* spp. The MeOH extract incorporates complex metabolites that contain phenols and sugars. This would indicate that these microorganisms have degraded complex compounds into more simple units.

Fig. 5 shows a typical calorespirometric curve as obtained from experiments where not glucose amendment was used and CO_2 evolution was determined by introducing a vial with NaOH into the sample containing ampoule.

Table 4 shows the calorespirometric results as determined for the studied soil samples. An increase of oxidation state (γ) values

Table 3
Values of peak time (t_p), specific thermal power (p_0) when no glucose was added, specific thermal power at peak time (p_t), heat due to the degradation of 1.5 mg g^{-1} glucose (q), rate constant of microbial growth (μ), the increase in colony formation units per gram of dry soil during exponential growth (ΔCFU g^{-1}) and the heat yield per unit CFU formed (Yq/ΔCFU g^{-1}) for: original soil (OS), control soil (CS), 83, 250 and 500 mg kg^{-1} of $CHCl_3$ and MeOH extracts of *I. tschudiana*.

Soil	t_p (h)	p_0 (μW g^{-1})	p_t (μW g^{-1})	$-q$ (J g^{-1})	μ (h^{-1})	10^{-7} ΔCFU g^{-1}	Yq/ΔCFU g^{-1} (μJ CFU^{-1})
OS	16.8 ± 0.0	15.0 ± 3.0	361.3 ± 30.6	7.8 ± 0.3	0.073 ± 0.001	2.1	0.4
$CHCl_3$ extract							
CS	17.8 ± 0.5*	2.8 ± 0.4*	269.7 ± 37.2*	7.2 ± 0.7	0.116 ± 0.002*	18.7	3.8
83	14.3 ± 0.9**	8.6 ± 0.7**	363.5 ± 15.2**	8.2 ± 0.2	0.123 ± 0.005**	12.5	6.5
250	15.6 ± 0.3**	8.7 ± 1.0**	397.5 ± 70.5**	8.4 ± 1.7	0.109 ± 0.005**	9.4	8.9
500	17.7 ± 0.3	2.5 ± 0.4	250.6 ± 11.9	7.7 ± 0.2	0.117 ± 0.004	16.8	4.5
MeOH extract							
CS	11.7 ± 0.3*	11.3 ± 1.2*	324.1 ± 8.0	5.7 ± 0.0*	0.128 ± 0.001*	5.9	9.6
83	12.1 ± 0.1	14.6 ± 1.3**	268.0 ± 15.6**	4.7 ± 0.2**	0.106 ± 0.003**	3.4	13.8
250	11.1 ± 0.1	15.5 ± 1.1**	355.6 ± 8.3**	6.0 ± 0.1	0.126 ± 0.003	4.1	14.6
500	12.0 ± 0.1	14.7 ± 1.1**	425.0 ± 25.0**	7.1 ± 0.1**	0.124 ± 0.004	4.6	15.4

* Significantly different from OS.
** Significantly different from control (SC).

Table 4
Specific rate of CO_2 evolution (rCO_2), basal specific thermal power (p), the calorespirometric quotient (p/rCO_2), the oxidation state of the substrate (γ) and the rate of CO_2 evolution per CFU g^{-1} (rCO_2/CFU g^{-1}) for: original soil (OS), control soil (CS), 83, 250 and 500 mg kg^{-1} of $CHCl_3$ and MeOH extracts of *I. tschudiana*.

Soil	rCO_2 (pmol s^{-1} g^{-1})	p (μW g^{-1})	p/rCO_2 (kJ mol^{-1})	γ	10^6 rCO_2/CFU g^{-1} (pmol s^{-1} CFU^{-1})
OS	51.7 ± 5.1	41.9 ± 0.3	813 ± 87	−3.1	38.9 ± 4.4
$CHCl_3$ extract					
CS	134.7 ± 17.2*	13.5 ± 1.1*	100 ± 17*	+3.1*	82.6 ± 16.1*
83	67.7 ± 0.7**	12.6 ± 2.1	186 ± 38**	+2.4**	30.6 ± 4.3**
250	115.6 ± 1.3	15.3 ± 3.7	132 ± 30	+2.8	60.5 ± 4.8
500	122.6 ± 29.9	9.5 ± 1.8	77 ± 32	+3.3	85.1 ± 28.4
MeOH extract					
CS	239.2 ± 33.2*	19.4 ± 2.3*	83 ± 21*	+3.3*	123.3 ± 20.3*
83	89.3 ± 25.2**	31.2 ± 1.1**	349 ± 86**	+0.9**	47.5 ± 14.4**
250	279.7 ± 23.5	23.9 ± 1.3	85 ± 12	+3.3	163.6 ± 16.6
500	194.8 ± 25.7	24.0 ± 0.1	123 ± 15	+2.9	126.5 ± 25.7

* Significantly different from OS.
** Significantly different from control (SC).

upon incubation was observed. Among the incubated soils, those containing 83 mg kg^{-1} of both extracts and 250 mg kg^{-1} $CHCl_3$ extract are the less oxidized.

Fig. 6 shows the relation of the p/rCO_2 ratios with extract concentration. Note a respirometric quotient higher than their corresponding controls for soils incubated with 83 mg kg^{-1} of both extracts being that of the MeOH extract the highest. A better understanding of this behavior is acquired when one examines the rCO_2/CFU g^{-1} ratios. It was reported that soil microflora with an efficient metabolism releases less CO_2 to the atmosphere per unit cell [22]. The determined rCO_2/CFU g^{-1} ratio for 83 and 250 mg kg^{-1} $CHCl_3$ and 83 mg kg^{-1} MeOH extracts were the lowest indicating that the microorganisms contained in them were metabolically more efficient. These three soils contained *Kocuria* sp. and therefore, these results suggest that this species might be beneficial for the soil and thus, further studies will be conducted with it. On the contrary, the value for soil containing 250 mg kg^{-1} MeOH extract is not significantly different from control. This latter soil contained both isolated *Kocuria* specie. Thus, the rCO_2/CFU g^{-1} value obtained for this soil might be due to *K. rosea* degrading complex carbonaceous compounds which in turn seems to energetically overcome *Kocuria* sp. It is well recognized that many root exudates can enhance the growth of specific organisms [23] due to certain compounds contained in them. In this case we have used extracts from aerial parts of *I. tschudiana* which promoted the growth of both *Kocuria* specie. Further studies with this plant species are needed but as a first approximation one could think that aerial parts of *I. tschudiana* could be used to amend deteriorated soils to promote the mineralization of organic matter and reduce P content.

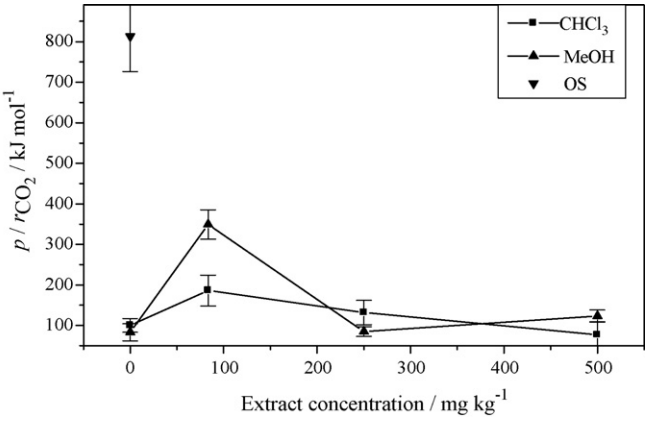

Fig. 6. Values of p/rCO_2 for the soil previous to incubation (OS) and soil samples incubated with 83, 250 and 500 mg kg^{-1} $CHCl_3$ and MeOH extracts of *I. tschudiana*.

4. Conclusions

The results presented here indicate that the $CHCl_3$ extract of *I. tcshudiana* seems to negatively affect seedlings growth of the dicot tomato as well as germination of the monocot onion. Also, lower ΔCFU g^{-1} (Table 3) in soil amended with both extracts with respect to control indicated a toxic effect. However, 83 and 250 mg kg^{-1} of both extracts tested promoted the activity of two isolated soil actinobacteria identified as *Kocuria* sp. and *K. rosea*. These specie seem to degrade complex carbon compounds which would explain the lower ΔCFU g^{-1}.

Therefore, *I. tschudiana* is worth of further investigations concerning a bioguided isolation of the metabolites responsible for such activity which could be used either as models for more friendly herbicides for the environment or to enhance activity of soil *actinomycetes* capable to recycle soil organic matter.

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