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Allelopathic Potential of *Inula viscosa* against Crops and Weeds

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ABSTRACT

Aqueous (10, 20, 30 & 40 g L^{-1}) and organic (hexane, chloroform & methanol, at 3 & 6 mg mL^{-1}) extracts of *Inula viscosa* L. (roots, stems, leaves & flowers) were evaluated for their allelopathic activities on radish, lettuce, peganum and thistle. In addition leaf and flower powder was incorporated in soil at 1.25 and 2.5 g kg^{-1}. PEG (Polyethylene glycol) solutions, with similar osmotic potentials of aqueous extracts, at the highest concentration, were without effect on target species, this eliminates the extracts osmotic effect. Germination index was not affected by root and stem extracts and significantly decreased by the two other ones. For growth, leaves and flowers extracts had the most significant toxicity, inducing up to total inhibition. Leaves leachates strongly inhibited seedling growth of lettuce (93.5%) and it was more toxic than leached-leaves extract (43%); however, toxicity of unleached-leaves extracts was slightly lower (90.5%). For organic extracts, the three fractions of leaves and flowers were more toxic; thistle was more sensitive especially to chloroform and methanol fractions of various organs. The two organs residues incorporation caused (at 2.5g kg^{-1}) an average reduction between 34 and 100% for root and shoot length of target species. Irrigating soil with leaves and flowers aqueous extracts decreased seedlings length by 100% for peganum and 82% for thistle shoot. Results show that *I. viscosa* allelopathic potential seems to be attributed mainly to leaf leachate, which indicates the facility of providing this product and its use in irrigation for sustainable weed management.

Key Words: *Inula viscosa*; Allelopathic potential; Inhibitory response

INTRODUCTION

Increase in use of synthetic herbicides for weed management in recent years has raised the environmental and health concerns. The researchers are now looking for alternate ways of weed management in field crops (Jamil *et al*., 2009). Application of allelopathy has shown tremendous scope in agricultural pest management (Farooq *et al.*, 2011). A number of higher plants were observed to possess allelopathic potential (Kohli *et al.*, 1998).

Several species of family *Asteraceae* have allelopathic effects on other species, reducing seed germination and emergence of subsequent small-grain crops when grown in rotation (Muehlchen *et al.*, 1990). *Inula viscosa* L. Aiton (syn. *Dittrichia viscosa* L. Greuter) is evergreen widespread plant of family *Asteraceae* in the Mediterranean region. It has been used as medicinal plant (Susplugas *et al.*, 1980) with anti-inflammatory (Hernández *et al.*, 2007), antidiabetic (Yaniv *et al.*, 1987), antipyretics, healing, antiseptic, antiphlogistic (Lauro & Rolih, 1990), anti-viral (Abad *et al.*, 2000), anti-fungal (Cafarchia *et al.*, 2002) and antibacterial properties (Squalli *et al*., 2007).

Leaves of *I. viscosa* L. bear sessile and stalked glandular hairs, which secrete a resinous mixture of secondary metabolites throughout the leaf life span (Werker & Fahn, 1981). These exudates consist of several flavonoid aglycones (Wollenweber *et al.*, 1991) and terpenoids (Grande *et al.*, 1992), all possessing strong allelopathic potential (Stavrianakou *et al.*, 2004), suppression potential against phytopathogenic microorganisms (Stavrianakou *et al.*, 2001). Levisou *et al.* (2002), for example, observed that *I. viscosa* leaf exudates are water soluble and suppress the lettuce germination, while germination of *Malcolmia maritima* and *Phlomis fruticosa* is considerably delayed (the latter species having the same habitat as that of *I. viscosa*) (Stephanou & Manetas, 1995; Levisou *et al.*, 2002). Apart from a negative effect on final percentage of seed germination, the material also reduced the root length and frequency of cell divisions in the meristematic zone, induced abundant lateral roots and completely suppressed the formation of root hairs. Moreover, the presence of statocytes was rare and their internal polarity strongly perturbed. A strong biological activity of the rinsate may enhance the competitive ability of *I. viscosa* by interfering with resource acquisition of germinating neighbors (Levisou

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et al., 2002). A phytochemical study of the aerial parts of *I. viscosa* resulted in the isolation of 16 flavonoïdes (Grande *et al.,* 1985), flavonoid aglycones (Wollenweber *et al.,* 1991), 10 triterpenoids as free alcohols, acetates or fatty esters (Grande *et al.,* 1992), three new esters of 9-hydroxynerolidol and two new eudesmane acids (Sanz *et al.,* 1991), sesquiterpenoids (Abu Zarga *et al.,* 1998), sesquiterpene lactones and sesquiterpene acids (Hernández *et al.,* 2001) as viscic acid and viscosic acid (Ayhan *et al.,* 1987).

Levisou *et al.* (2002) investigated the allelopathic potential in *I. viscosa* leaf leachates. This study was conducted to investigate whether *I. viscosa* allelopathic potential is limited to its leaf exudate. Aqueous and organic extracts of different parts (leaves, flowers, stems & roots) of *I. viscosa* were tested against two crops (*Raphanus sativus* L., *Lactuca sativa* L.) and two weeds (*Silybum marianum* L., *Peganum harmala* L.). Aqueous extract of leaves were compared to leaf leachates and to leached leaves extract. In addition allelopathic potential of its leaf and flower powder and aqueous extracts (used for irrigation) were also investigated.

MATERIALS AND METHODS

Plant material: *Inula viscosa* plants were collected at the flowering stage from Monastir (latitude 35°46'0''N, longitude 10°59'0'' E). A voucher specimen was collected, dried and deposited at the herbarium (Asteraceae 24) of the Higher Institute of Agronomy of Chott Meriem, University of Sousse Tunisia.

Aqueous extracts: Fresh *I. viscosa* plants were rinsed and separated into roots (R), stems (S), leaves (L) and flowers (F). Different organs were then oven-dried at 60°C for 72 h and grinded. Forty grams of each dried material were soaked in 1 L distilled water at room temperature for 24 h (Chon *et al.,* 2005). The extracts were filtered several times and kept at 4°C in the dark until use.

To see if the allelopathic potential is limited to leaf leachates, a supplementary experiment was conducted, (i) preparation of leaf leachates following Stephanou and Manetas (1997), *I. viscosa* leaves were immersed for 3 h in deionized water with gentle shaking, (ii) leaves used for leachates preparation (leached leaves) and (iii) leaves not leached (unleached leaves) were oven-dried at 60°C for 72 h, grinded and used to prepare aqueous extracts.

Organic extracts: Sequential extraction was done with organic solvents of increasing polarity: hexane, chloroform and methanol. A 40 g dried powder of roots, shoots and flowers were immersed in organic solvent for 7 d at room temperature. Organic extracts were evaporated to dryness under reduced pressure at 45-50°C, using rotavapor R-114 (Buchi, France). The residue was weighed and yield was determined. Dry fractions were stored at 4°C until use. The extracts were tested at two concentrations (3 & 6 mg mL^{-1}) in bioassays.

Laboratory bioassays: To eliminate the possible effect of aqueous extracts pH and osmotic potential, conductivity and pH of all aqueous extracts at the highest concentration (40 g L^{-1}) were measured and solutions of PEG 4000 with the same pH and osmotic potential were prepared and applied to lettuce and *peganum* seeds in Petri plates.

Aqueous extracts (of R, S, L & F) were diluted to give final concentrations of 10, 20, 30 and 40 g L^{-1} (Chon *et al.,* 2005). They were tested on two crops (*Raphanus sativus* L., *Lactuca sativa* L.) and two weeds (*Silybum marianum* L., *Peganum harmala* L.). Seeds were surface sterilized with 0.525 g L^{-1} sodium hypochlorite for 15 min, then rinsed four times with deionized water, imbibed in it at 22°C for 12 h and carefully blotted (Chon *et al.,* 2005). Twenty imbibed seeds of target species were separately placed on the filter paper in 9 cm Petri dishes, 5 mL of each extract were applied as per treatment. Seedlings watered with distilled water were used as control. The Petri plates were then placed in a growth chamber with 400 µmol photons $m^{-2}s^{-1}$ photosynthetically active radiation (PAR) at 24/22°C for 14/10 h light and dark periods, respectively. Treatments were arranged in a completely randomized design with three replications.

Germinated seeds were counted at 24 h intervals during 6 days. Shoot and root length of receiver species were measured seven days after sowing. Data were transformed to percent of control for analysis. The index of germination GI was determined using the following formula (Chiapuso *et al.,* 1997):

$$GI = (N_1) * 1 + (N_2-N_1) * 1/2 + (N_3-N_2) * 1/3 + ... + (N_n-N_{n-1}) * 1/n$$

Where, N_1, N_2, N_3, ….,N_n: proportion of germinated seeds observed afterwards 1, 2, 3,…., n-1, n days. This index shows the germination delay induced by the extract (Delabays *et al.,* 1998). The inhibitory or stimulatory percent was calculated using the following equation given by Chung *et al.,* (2001):

$$\text{Inhibition (-)/stimulation (+) \%} = [(\text{extract} - \text{control})/\text{control}] \times 100$$

Where extract: parameter measured in presence of *I. viscosa* extract and Control: parameter measured in presence of distilled water.

For organic extracts studies, four residues concentrated from hexane, chloroform and methanol were dissolved in methanol and two concentrations were prepared 3 and 6 mg mL^{-1}, to estimate their effect on germination and early growth of target species. Two controls were considered, distilled water and methanol, to eliminate the organic solvent effect. Filter paper placed in Petri dish, were soaked with distilled water, methanol or various organic extracts. Organic solvent was evaporated for 24 h at 24°C, then 5 mL distilled water was added and 20 soaked seeds were put to germinate for seven days. Germination, shoot and root length of target species were estimated as before and expressed in percent of the control. Treatments were arranged in a completely randomized design with three

replications and data were transformed to percent of control for analysis.

Activity in Soil

Pot trials with biomass: The nursery trays (7 × 11 grids, each square 3 cm by 3 cm) were filled with sandy soil. Powder of *I. viscosa* leaves and flowers were thoroughly mixed with soil (1.25 & 2. 5 g kg^{-1} of soil on dry weight basis). Soil without *I. viscosa* material incorporation, was the control. Subsequently, the nursery trays were irrigated with tap water. After that target seeds (lettuce, radish, peganum & thistle) were sown in each pot, 5 seeds per square. Nursery trays were placed in growth room at 25°C under 12 h photoperiod for seven days and then transferred to open sunlight. Pots were irrigated daily to keep the soil moisture level at field capacity. Plants were harvested four weeks after sowing and data regarding root/shoot length were recorded. Treatments were arranged in a completely randomized design with three replications and data were transformed to percent of control for analysis (Javaid *et al.*, 2008).

Irrigation with aqueous extract: To determine whether the phytotoxic effects of *I. viscosa* extract would be maintained in 'soil', additional trial was carried out in an incubator set at 20°C with 14/10 h, day/night. In this study, the medium used was sand. Nursery trays (7×11 grids, each square 3 cm×3 cm) were filled with sand. Five pre-germinated seeds (lettuce, radish, peganum & thistle) were planted per square just under the soil surface then sprayed with distilled water to moisten the soil. Three days later, 5 mL of each extract treatment of leaves and flowers at 40 g L^{-1} were added per square. Distilled water was the control. Treatments were arranged in a completely randomized design with three replications and data were transformed to percent of control for analysis (Seal *et al.*, 2010).

Statistical analysis: The laboratory bioassays and pot culture were conducted in a completely randomized design with three replications. ANOVA and a post hoc LSD tests were performed with PASW Statistics 18, for Windows program, to analyze treatment differences. The means were separated on the basis of least significant differences at the 0.05 probability level.

RESULTS

Preliminary experiments were conducted to eliminate the possible effect of aqueous extracts pH and osmotic potential. Conductivity and pH of all aqueous extracts at the highest concentration (40 g L^{-1}) were measured and solutions of PEG 4000 with same values of these two parameters were prepared and tested on lettuce and peganum. Results showed that all PEG solutions had no effect on germination index and growth of the two target plants (Table I). Indeed, all results were comparable or improved relative to the control. This test allowed us to attribute observed effects of plant extracts to their allelochemicals.

Effect of *I. viscosa* Aqueous Extracts on Germination and Growth of Test Species

Germination: *Inula viscosa* leaves and flowers extracts were the most toxic for all the target species (Table II). Although thistle showed a germination index of 66.71% at the highest concentration, there was no significant effect of root extract on test species germination. The same result is registered with stem extract with all species, except thistle, which germination delay was concentration dependent and reached to 27.19% of the control. However, leaves and flowers extracts slowed the germination rate, compared to the control and this effect increased significantly with concentration. In presence of leaves extract, GI reached to 52.23, 43.37, 62.71 and 14.42% of the control for respectively lettuce, radish, peganum and thistle, these values were 6.96, 53.12, 72.6 and 27.93% in presence of flowers extract (Table II).

Growth: Effect of *I. viscosa* extracts effect varied with the kind of organ and target species. Leaves (L) and flowers (F) extracts were more toxic than those of roots (R) and stems (S), generally toxic effect increased with extract concentration (Fig. 1). Extracts of R and S showed a significant stimulation of seedling growth for all target species and percentage stimulation varied between 12 and 92%, except for thistle, for which seedling growth was affected only in presence of S extract at 40 g L^{-1}, where seedling length was reduced by 34% of the control (Fig. 1). Extract of *I. viscosa* leaves and flowers were the most toxic and substantially suppressed the roots growth, generally the degree of suppression increased with increasing extract concentration (Fig. 1). Weeds were more sensitive to leaves extract, their roots showed an average inhibition of 94% at 40 g L^{-1}, while crops showed an average inhibition of 81% at the same concentration (Fig. 1). However, crops were more sensitive to flowers extract; which caused an inhibition of 98 and 99% for lettuce and radish roots, respectively at 40 g L^{-1}. The inhibition varied between 43 and 91% for the two weeds at all the concentrations (Fig. 1).

Effect of *I. viscosa* leaves leachates and aqueous extracts of leached and unleached leaves on growth of lettuce: To see if allelopathic potential of *I. viscosa* is limited to epicuticular exudates, we compared the effects of leaves leachates, aqueous extracts of leached-leaves and unleached-leaves. Leachates of *I. viscosa* leaves significantly suppressed the lettuce seedling growth (Fig. 2). Reduction growth was respectively 97 and 90% for roots and shoots. The same effect was observed from unleached leaves aqueous extract, which induced 91 and 90% reduction for the two organs. However, leached-leaves aqueous extract was less toxic as it caused an inhibition of 61 and 25% for roots and shoots, respectively (Fig. 2).

Effect of Organic Extracts of *I. viscosa* on Germination and Growth of Target Species

Yield of organic extracts: Leaves, have the highest yield with the three organic solvents (3.418%, 4.48% & 6.77% in

Table I: Germination index and roots and shoots length (expressed in % of control) of lettuce and peganum in presence of aqueous extracts (at 40 gL^{-1}) of *I. viscosa* plant parts (R: roots, S: stems, L: leaves and F: flowers) and polyethylene glycol 4000 (PEG) solutions at the same pH and the same osmotic potential (-bars)

	R extract	PEG solution	S extract	PEG solution	L extract	PEG solution	F extract	PEG Solution
pH	6.12	6.12	5.88	5.88	5.98	5.98	5.61	5.61
Osmotic potential	0.000777	0.000547	0.00123	0.0006	0.00182	0.00082	0.00091	0.000547
Germination Index								
Lettuce	91.99±7.9	97.97±14.1	82.41±1.3	105.75±2.5	52.23±2.1	113.92±3.2	6.96±1.2	102.62±15.3
Peganum	93.64±12.6	102.39±6.4	96.91±4.6	109.89±6.3	62.71±7.7	99.68±9.9	72.6±9.1	107.09±8.5
Roots length								
Lettuce	86.72±6.7	89.17±11.3	76.56±6.5	111.64±7.9	8.87±13.6	105.32±9.8	2.26±2.3	114.49±10.6
Peganum	80.84±7.3	108.22±9.3	110.42±8.3	109.05±14.4	6.2±1.3	103.55±9.9	9.18±4.4	88.71±18.1
Shoots length								
Lettuce	163.55±3.5	100.5±12.6	83.93±7.1	109.93±9.5	9.21±8.4	89.39±10.1	0.07±0.1	121.76±2.3
Peganum	108.38±6.1	96.32±11.1	153.8±6.2	96.66±14.3	4.38±0.7	76.66±14.9	23.1±4.9	83.36±12.4

presence hexane, chloroform & methanol, respectively) followed by flowers, stems then roots. In the other hand, methanol gave the highest yields for all parts of *I. viscosa* (Table III).

Germination: Germination index (GI) expressed in % of control, of test species in the presence of different organs organic extracts of *I. viscosa* differed. The organic residues were dissolved in methanol, this solvent had no effect on germination and the effects would be attributed to allelochemicals contained in organic extracts. Root extracts generally were without significant effect on seed germination at 3 mg mL^{-1} for the two crops tested. However, at 6 mg mL^{-1} crop seeds germination was delayed, and the lowest values of GI were, respectively 68.43% and 62.99% of the control in presence of hexane and chloroform extracts. The behavior of weeds was different; peganum showed a greater sensitivity to the lowest dose in the presence of the three extracts and its GI varied between 51 and 74%. Conversely, thistle seed germination was strongly delayed in all cases at 6 mg mL^{-1} and GI was ranged between 3.33 and 10.43% of control. At 3 mg mL^{-1}, the most toxic organic extract for thistle was the methanol (GI=18.29%) (Table IV). Stem extracts showed a moderate toxicity especially chloroform fraction, which caused for crops germination an average delay of 53% at the two concentrations. Hexane fraction produced the same delay at the two concentrations for lettuce and at 6 mg mL^{-1} for radish. Methanol fraction effect was significantly only with radish at 3 mg mL^{-1}. Tested weeds showed greater sensitivity against all organic fractions of stems. Averages of germination index were 45%, 35% and 58% for peganum and 11%, 6% and 9% for thistle in presence of hexane, chloroform and methanol, respectively at the two concentrations (Table IV). Organic leaves extracts have shown more toxic and this toxicity increased with concentrations for all target species. For seed crops, the greater sensitivity was registered with chloroform fraction, which gave an average of 12.77% for the two species at 6 mg mL^{-1}. Nevertheless, all organic fractions were very toxic for germination of the two weeds. Hence GI was between 5.34 and 49.03% at 3 mg mL^{-1} and between 1.66 and

Table II: Germination index (GI), expressed in % of control, of test species: *Lactuca sativa, Raphanus. Sativus, Peganum harmala and Sylibium marianum* in presence of aqueous extracts (at 10, 20, 30 & 40 gL^{-1}) of *I. viscosa* plant parts (R: roots, S: stems, L: leaves & F: flowers)

Extracts	concentration gL^{-1}	GI (% of control)			
		L. sativa	*R. sativus*	*P.harmala*	*S. marianum*
R. extract	10	100.03 ab	101.71 ab	100.68 a	92.29 a
	20	103.47 b	99.94 a	93.09 a	68.41 a
	30	99.76 ab	89.53 a	97.93 a	86.76 a
	40	91.99 a	93.75 a	93.64 a	66.71 a
S. extract	10	93.94 a	91.04 a	96.1 a	60.95 c
	20	96.38 a	90.46 a	92.8 a	72.28 c
	30	89.16 a	91.57 a	99.16 a	48.13 b
	40	82.41 a	86.64 a	96.91 a	27.19 a
L. extract	10	87.84 b	91.64 c	84.14 b	34.01 b
	20	67.72 a	77.86 bc	84.89 b	13.67 a
	30	61.45 a	65.88 b	84.13 b	10.36 a
	40	52.23 a	43.37 a	62.71 a	14.42 a
F. extract	10	69.86 d	84.52 b	86.23 a	33.99 a
	20	44.67 c	76.82 b	82.02 a	20.42 a
	30	15.54 b	71.02 ab	71.75 a	36.42 a
	40	6. 96 a	53.12 a	72.6 a	27.93 a

Means with the same letters in a column are not significantly different at $P<0.05$

Table III: Residues yields (% of dry matter) after successive extraction in three organic solvents (Hexane, Chloroform, Methanol) of *I. viscosa* different organs. Value = Average ± S.E., n=3

	Roots	Stems	Leaves	Flowers
Hexane	0.506±0.79	0.784±2.1	3.418±1.1	2.773±0.98
Chloroform	0.827±1.28	1.084±0.86	4.481±0.05	3.658±1.52
Methanol	3.118±1.56	2.214±1.14	6.773±1.07	2.848±1.63

13.33% at 6 mg mL^{-1} for the two weeds in presence of the three fractions. It was noted that thistle was more sensitive than peganum (Table IV). Likewise, flowers extracts were very toxic and their toxicity was localized especially in chloroform and methanol fractions at the highest concentration. Indeed, chloroform extract have led to reduction of germination with GI values reaching to 2.43 and 3.70% for lettuce and radish respectively, and to 9.09

Table IV: Germination index (GI) expressed in % of control, of test species: *Lactuca sativa, Raphanus sativus, Peganum harmala and Sylibium marianum,* germed in presence of organic extracts (at 3 & 6 mg.mL^{-1}) of *I. viscosa* plant parts (R: roots, S: stems, L: leaves & F: flowers).

Extract concentration (mg.mL^{-1})			GI (% of control)			
			L. sativa	*R. sativus*	*P. harmala*	*S. marianum*
R. extract	Hexane	3	91.95 ab	98.64 d	51.21 a	89.49 b
		6	68.43 a	95.51 cd	85.98 ab	10.43 a
	Chloroform	3	85.57 ab	75.15 b	74.66 ab	88.06 b
		6	78.96 a	62.99 a	87.6 ab	3.33 a
	Methanol	3	100.77 b	88.78 c	61.70 ab	18.29 a
		6	77.89 ab	94.06 cd	98.77 b	7.68 a
S. extract	Hexane	3	62.95 ab	97.86 c	51.21 ab	0 a
		6	52.43 ab	63.32 a	40.23 ab	21.35 b
	Chloroform	3	56.85 ab	55.91 a	41.5 ab	3.53 ab
		6	44.14 ab	55.67 a	30.32 a	9.20 ab
	Methanol	3	91.48 c	65.73 ab	69.92 b	8.88 ab
		6	76.45 bc	81.84 bc	50.81 ab	10 ab
L. extract	Hexane	3	89.04 c	56.86 c	14.99 a	5.34 a
		6	21.62 a	48.5 bc	4.27 a	5.34 a
	Chloroform	3	54.39 b	26.57 ab	8.46 a	15.53 a
		6	12.76 a	12.78 a	10.95 a	9.78 a
	Methanol	3	81.23 c	36.98 abc	49.03 b	9.13 a
		6	57.41 b	45.78 bc	13.33 a	1.66 a
F. extract	Hexane	3	81.93 c	77.24 b	12.36 a	59.11 c
		6	25.23 ab	67.11 b	17.67 ab	53.3 bc
	Chloroform	3	50.93 bc	58.64 b	24.25 ab	40.65 bc
		6	2.43 a	3.79 a	9.09 a	33.84 b
	Methanol	3	74.75 c	63.23 b	36.53 b	13.38 a
		6	19.06 a	16.89 a	12.54 a	0 a

Means with the same letters in a column are not significantly different at P<0.05

and 33.84 for peganum and thistle. Methanol fraction caused a total inhibition for thistle and a germination delay with an average value of GI equal to 16.16% for the three other target species at 6 mg mL^{-1}. Hexane fraction of flowers was toxic especially for peganum, the seed germination of which was delayed and GI was reached to an average of 15% with the two concentrations whereas for the other species its toxicity was moderate (Table IV).

Growth: Seedlings growth varied with organ extract, concentration and species. Overall, *I. viscosa* organic leaves and flowers extracts were the most toxic for all target species (Fig. 3). In presence of root extracts, roots and shoots lengths of crops were between 43% and 117% of control in all cases. Though, growth of peganum roots was reduced by an average of 88, 92 and 62% in presence of hexane, chloroform and methanol extracts at the two concentrations, respectively and growth shoots was reduced by 38%, 49% and 73% in the same conditions. Thistle seedlings were more sensitive; they showed an average inhibition of 92% in all cases (Fig. 3). Effects of stems extracts were similar to the roots one. Lettuce and radish showed more or less important inhibition depending on extract origin. Thus roots growth of lettuce showed a significant inhibition of 88% in presence of the chloroform fraction at 6 mg mL^{-1} however, aerial parts which were more resistant, showed an inhibition of 71% at the same conditions. Seedling growth of radish did not register an important inhibition with all extracts and the two concentrations. Peganum roots were more sensitive especially to hexane and chloroform extracts, which induced an average inhibition of 94% at 6 mg mL^{-1}, while shoots was inhibited by an average of 56% in all cases. Thistle roots were more sensitive especially to hexane extracts which induced a total inhibition from 3 mg mL^{-1} and were reduced by an average of 95% in presence of chloroform and methanol extracts at 3 and 6 mg mL^{-1}, while shoots growth was inhibited by an average of 94% in all cases (Fig. 3). Leaves extracts were most inhibitory for seedling elongation for all target species and in presence of the two concentrations. Though, weeds were more sensitive than crops, and toxic effect increased with concentration. A total inhibition of growth was recorded at 6 mg mL^{-1} with the three extracts for peganum and with hexane and methanol fraction for thistle seedling. Seedling growth of lettuce was strongly affected with hexane and chloroform fractions at 6 mg mL^{-1}, where we registered an average inhibition of 97%. For radish, seedling growth showed a significant decrease with averages inhibition of 83% and 78% at 3 mg mL^{-1} and 6 mg mL^{-1}, respectively (Fig. 3). The three fractions of flowers extract, at 6 mg mL^{-1}, induced a total inhibition of peganum seedling. While thistle seedling showed an average length of 7% and 15% of the control in presence of methanol fraction at 3 and 6 mg mL^{-1}, respectively and maximum length was 50% of the control in presence of

Fig. 1: Effects of aqueous extract of *I. viscosa* root, stem, leaf and flower on root and shoot length of test plant spp. Value=Average±S.E., n=3. Different letters in columns indicate significant differences among concentrations at P<0.05 (LSD test)

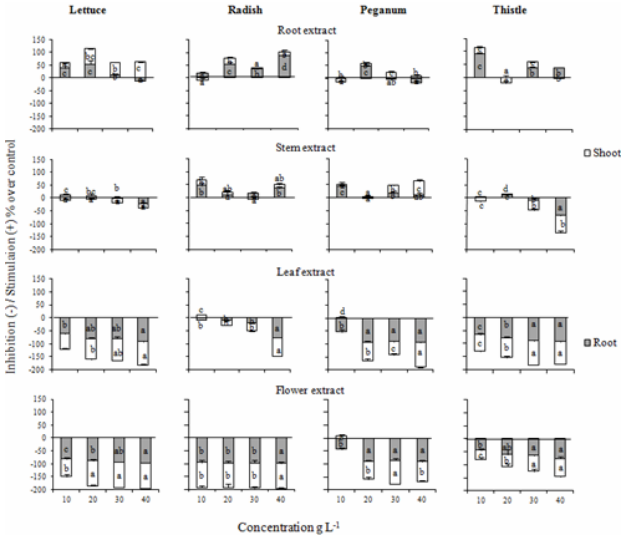


Fig. 2: Roots and shoots length (% control) of lettuce, 7 days after germination, in the presence of leaves leachates and aqueous extracts of leached and unleached leaves of *I. viscosa*. Value=Average±S.E., n=3. Different letters in columns indicate significant differences among treatments at P<0.05 (LSD test)

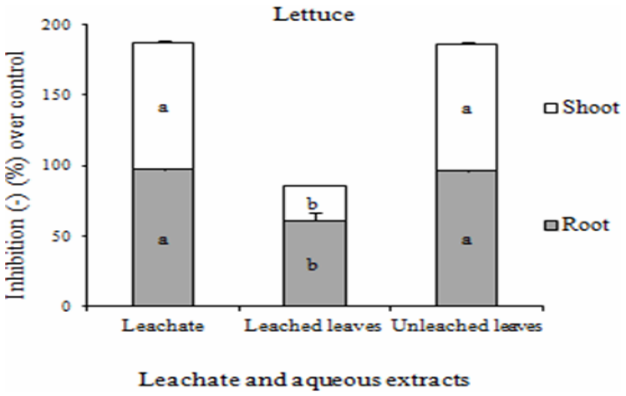


Fig. 3: Effects of organic extracts of *I. viscosa* root, stem, leaf and flower, prepared with three solvents: Hexane (H), Chloroform (C) and Methanol (M), applied at 3 mg mL^{-1} (H3, C3, M3) and 6 mg mL^{-1} (H6, C6, M6), on root and shoot inhibition (%) over control of test spp. Value=Average±S.E., n=3. Different letters in columns indicate significant differences among treatments at P<0.05 (LSD test)

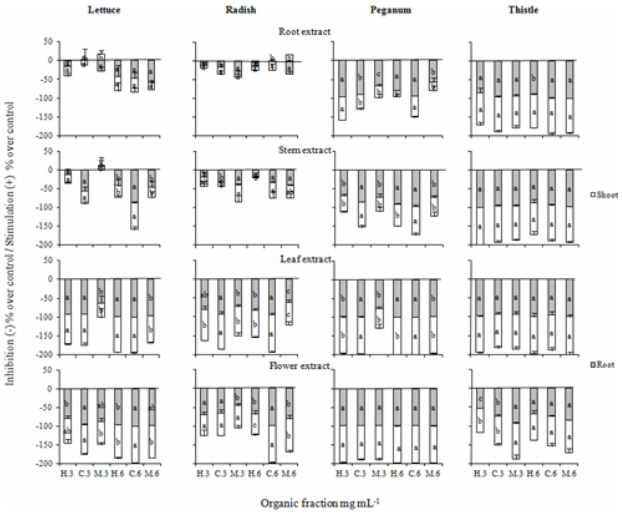
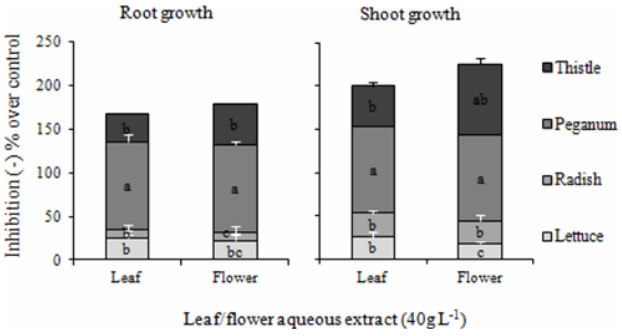


Fig. 4: Inhibitory effects of residues (leaf, flower) incorporation of *I. viscosa* in soil, at 1.25 and 2.5 g.Kg^{-1}, on root and shoot growth over control of test spp. 30 days after germination. Value = Average ± S.E., n=3. Different letters in columns indicate significant differences among target species at P<0.05 (LSD test)



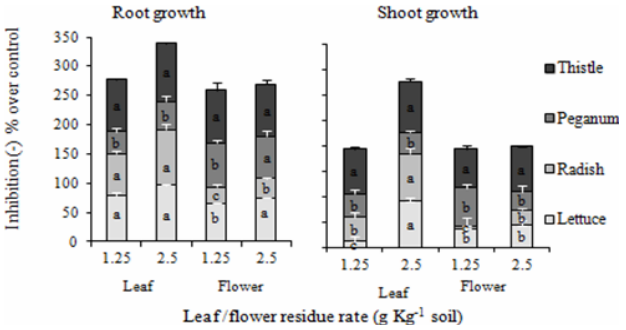
hexane fraction at 3 mg mL^{-1}. For crops we registered a total growth inhibition of the two species in presence of chloroform fraction at 6 mg mL^{-1}. Percentages inhibition varied between 62% and 98% for lettuce and between 45% and 89% for radish, in the other cases (Fig. 3).

Activity in Soil

Powder incorporation in soil: Residue addition to the soil affected seedling growth and thistle was the most sensitive species and leaves residue was the most toxic especially at dose 2 (Fig. 4). Thus in presence of L residue, growth reduction of lettuce and radish seedlings was an average of 94% at dose 2. Thistle seedlings were reduced by 89% and 100% at dose 1 and dose 2, respectively. Peganum seedling showed some resistance since the percentage inhibition have an average of 44% at the two doses. In the presence of F residue, thistle was the most sensitive species its seedling growth showed an average inhibition of 83 and 90% at dose 1 and 2, respectively. Growth of lettuce and radish was inhibited moderately as averages inhibition of 58% at two doses for roots and 38% for shoots were registered. While growth peganum was more reduced at first (76% inhibition) compared to the second dose (53% inhibition) (Fig. 4).

Irrigation with aqueous extract: Irrigation with aqueous extracts of *I.viscosa* prepared from leaves and flowers were

Fig. 5: Effects of aqueous extract (at 40 g.L^{-1}) of *I. viscosa* leaf and flowers, on root and shoot inhibition (%) over control of test spp. 30 days after germination. Value=average±S.E., n=3. Different letters in columns indicate significant differences among target species at P<0.05 (LSD test)



more or less inhibitory of target species seedlings length. Extract of L and F caused a total inhibition of peganum growth (Fig. 5). While, flowers extract caused a significant inhibition of thistle root and shoot growths which were reduced by 47 and 82%. Extracts of *I. viscosa* significantly reduced the growth of the two target crops but the inhibition percentage did not exceed 19% (Fig. 5).

DISCUSSION

This study was conducted to investigate if allelopathic potentialities of *I. viscosa* are limited to their leaf exudates. So aqueous and organic extracts, of different *I. viscosa* organs were tested using two crops (lettuce & radish) and two weeds (peganum & thistle) as test species. A complementary experiment was conducted to assign the power allelopathic leaves; hence, the allelopathic potential of leached leaves was compared to that of unleached leaves.

Test species showed different responses to *I. viscosa* extracts. Germination of either species was not affected by R and S aqueous extracts, nonetheless it was reduced and delayed by L and F extracts in all the test species (Table II). Stephanou and Manetas (1995, 1997) showed that *I. viscosa* possess strong allelopathic potential on lettuce seed germination and radicle growth of *Phlomis fruticosa*, which often occupies the same habitat. The most common explanation for this variation in response would be the selectivity of allelochemicals for target species (Inderjit & Duke, 2003). Generally it is accepted that the degree of inhibition increases with increasing the extract concentration (Laosinwattana *et al.,* 2007). Inhibition of seed germination was attributed to the (i) disruption of "dark" or mitochondrial respiration (Podesta & Plaxton, 1994) and (ii), disruption of the activity of metabolic enzymes involved in glycolysis and in oxidative pentose phosphate pathway (OPPP) (Muscolo *et al.,* 2001). Some allelopathic compounds interact with the mitochondrial membrane and directly impair the mitochondrial respiration (Abrahim *et al.,* 2003).

Root aqueous extract stimulated the root growth of test species and percentage stimulation varied between 12 and 92% (Fig. 1). However, this extract showed an inhibition of lettuce and peganum roots by an average 16% at 40g L^{-1}. Likewise, shoot extract significantly stimulated lettuce, radish and peganum roots by 13, 44 and 47%, respectively at 10 g L^{-1}, nonetheless thistle was more sensitive, their roots and shoots showed an average inhibition of 67% at 40 g L^{-1}. Allelochemicals inhibiting the growth of some species at certain concentration may stimulate the growth of the same or different species at different concentrations (Narwal, 1994). On the other hand, L and F extracts were found to be the most toxic at higher concentrations. The most remarkable inhibition (total inhibition) was recorded with F extract at a concentration greater or equal to 30 g L^{-1} for lettuce and radish seedlings (Fig. 1). Reduction of seedlings length may be attributed to the reduced of cell division rate and cell elongation due to the presence of allelochemicals in the aqueous extracts (Javaid & Anjum, 2006). In presence of leaves extract, weeds were more sensitive their roots showed an average inhibition of 94% at 40 g L^{-1}, while this value was 81% for the crops. Aerial parts of target species showed sensitivity almost similar to that of roots, except for peganum roots, which appeared more sensitive to allelochemicals than shoots (Fig. 1). Werker and Fahn (1981) observed that leaves of *I. viscosa* bear abundant glandular trichomes and the phytotoxicity was directly proportional to higher concentrations of leachates (Hashim *et al.,* 2005).

To see if allelopathic potential of *I. viscosa* is limited to epicuticular exudates, we compared the effects of leaves leachates, aqueous extracts of leached-leaves and unleached-leaves. In a previous study, Levisou *et al.* (2002) showed that the leaf epicuticular exudate of *I. viscosa* reduced lettuce root length, frequency of cell divisions in the meristematic zone, induced abundant lateral roots and completely suppressed the formation of root hairs. Moreover, the presence of statocytes was rare, and their internal polarity was strongly perturbed (Levisou *et al.,* 2002). In the present study, leachates of *I. viscosa* leaves strongly and significantly inhibited the lettuce seedling growth. Percentage reduction growth was respectively 97 and 90% for roots and shoots. Similar effect was obtained in presence of unleached-leaves aqueous extract; however, leached-leaves aqueous extract was less toxic (Fig. 2). Leachate allelochemicals effects were slightly lessened, when mixed with active allelochemicals. This difference in toxicity may be due to possible antagonism between the compounds of leaf internal tissues and those of epicuticular glands of leaves. However, effect of aqueous extract of leached leaves was much less toxic, suggesting that the majority of allelochemicals have extracted in the leachate. In addition, most part of allelochemicals inhibiting root length are located in leaf internal tissues, while those inhibiting shoot length seem to belong to epicuticular glands of leaves (Fig. 2).

To determine the chemical group to which bioactive molecules of *I. viscosa* belonged, we conducted a fractional extraction in three organic solvents. Seeds germination of

target species showed no significant differences with R organic extracts, but the S, L and F organic extracts significantly affected germination and the leaves organic extracts were the most toxic (Table IV). It is reported that organic extracts contain different types of allelochemicals, which explains their differential effects. Previous works showed that leaves of *I. viscosa* bear abundant glandular hairs (Werker & Fahn, 1981) excreting a complex, viscous mixture in which numerous sesquiterpene acids (Ceccherelli *et al.,* 1985) and flavonoid aglycones (Wollenweber *et al.,* 1991) have been detected. One of the suggested explanations for the allelopathic effect of flavonoïds is the modification in mitochondrial respiration after the decreased supply of ATP for all processes, which reduced the seedling growth (Gniazdowska & Bogatek, 2005). Accordingly, Stephanou and Manetas (1997) and Levisou *et al.* (2002), the extend of allelopathic interference was positively correlated with phenolic concentration in the leaf leachates. Some concentrations of an allelochemical severely reduce the seed germination, while others only depress or delay the germination (Hoagland & Williams, 2003). The delay in seed germination can have important biological and ecological implications, because it can affect the ability of the seedling to establish themselves in natural conditions (Chaves *et al.,* 2001).

Effects of organic extracts on plant growth are largely reported in literature. In the presence of the hexane extract obtained from L and F of *I. viscosa* at 6 mg mL^{-1} we recorded for seedling growth an average inhibition ranged between 69 and 100%. However, chloroform fraction induced the greatest toxicity, since we recorded an average inhibition of 95% for all target seedlings (Fig. 3). This indicates that, molecules with higher polarity, extracted by hexane, *I. viscosa* L and F contained other bioactive molecules which were extracted by chloroform. Methanol fraction induced an inhibition ranged between 33% and 98%. Wollenweber *et al.* (1991) have identified, in acetone fraction of *I. viscosa* dried aerial parts, 4 compounds of flavones, 6 flavonoles, 3 flavonones and 7 dihydroflavonoles. In other study, several secondary metabolites have been reported from *I. viscosa* with acetone such as 17 compounds of flavonoides and 10 triterpenoides (Grande *et al.,* 1992). This data could explain the effect of methanol extract, which has a similar polarity than acetone, and we can say that *I. viscosa* aerial parts contain compounds having a toxic allelochemicals such as flavonoid compounds. Flavonoids and other secondary compounds have been frequently implicated in allelopathic reactions, usually inhibiting seed germination and root growth (Rice, 1979). Phenolics and flavonoids yet have a variety of other ecologically important roles (Dakora, 1995), and epicuticular compounds of *I. viscosa* in particular, are highly toxic against seedling development of other species (Stephanou & Manetas, 1995).

Pot cultures were conducted in order to indicate the effects that could be reproduced under natural conditions (Corrêa *et al.,* 2008) and to evaluate the biological activity of allelochemical compounds released from the plant residues. Among the two residues, leaves exhibited the highest phytotoxicity (Fig. 4). Effects of residues varied with recipient species (Khanh *et al.,* 2005) and the inhibitory effect was attributed to their phytotoxicity (Chon *et al.,* 2005). Although the presence of allelopathic compounds was found in all plant organs, but the vegetative parts, particularly leaves are richer in these compounds (Politycka & Lipinska, 2005). Plant residues and their decomposition products could be involved in virtually all biochemical processes taking place in the soil and most affects plants directly or indirectly. Toxicity symptoms varied from complete inhibition of seed germination to reduced seedlings growth (Qasem, 1995). In the soil incorporated with residues, the elimination of phytotoxic activity of *I. viscosa* residues over time is generally due to chemical decomposition or microbial degradation of organic compounds (Kobayashi, 2004). The phytotoxic activity of *I. viscosa* in soil depends on the concentration of active compounds released into soil from residues, even though phytotoxic activity is also influenced by soil factors, absorption on the soil solids and degradation by microorganisms (Kobayashi *et al.,* 2008). Seal *et al.* (2010) were showed that results from the soil trials also suggest that irrigation with aqueous extract of Wollemi pine could be a feasible source of natural herbicides against ryegrass seeds (ARG).

In conclusion, *I. viscosa* extracts from different plant parts show a phytotoxic influence on crops (lettuce & radish) and weeds (peganum & thistle). The phytotoxic effect was differential and tissue specific: leaf > flower > stem> root. The degree of inhibition was largely dependent on the concentration of the extracts being tested. Residue (mainly leaves & flowers) of *I. viscosa* had potent herbicidal activity on seedling growth of both weed species and may be favorably used for incorporating in agricultural systems for weed management. In addition, results show that the allelopathic potential is attributed mainly to leaf leachate, which indicates the facility of providing this product and its use in irrigation. *I. viscosa* residue release allelopathic substances, which accumulate in bioactive concentrations and adversely affect seed germination and seedling growth.

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