

## Full Length Research Paper

**Allelopathic activity of extracts of *Citharexylum spinosum* L. from Tunisia**

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**Allelopathic potential of *Citharexylum spinosum* L. (Verbenaceae) an exotic tree introduced in Tunisia many years ago was evaluated. Organic extracts using hexane, ethyl acetate and methanol solvents together with aqueous extracts at different concentrations were prepared from different parts of the plant (roots, stems, leaves and flowers). Yields in the 12 organic extracts together with their phenol contents were reported. Leaves methanol extract showed the highest yield and amount in total phenols (6.43%; 617.93±1.12 mg gallic acid equivalent/100 g MS, respectively). All extracts were tested on germination and early growth of two crops: (*Lactuca sativa* L.) and (*Triticum aestivum* L.) and two weeds: (*Peganum harmala* L.) and (*Silybum marianum* L.). Twelve parameters were established and used to the principal components analysis (PCA) and the hierarchical clusters (HCA) analysis. Three groups of extracts were separated according to their allelopathic potentiality. Almost of organic extracts were totally opposed to seed germination of peganum and thistle.**

**Key words:** Allelopathy, bioherbicides, *Citharexylum spinosum* L., crops, extracts, polyphenols, weed management.

**INTRODUCTION**

The overuse of herbicides has provoked increasing incidences of herbicide resistance in weeds (Valverde et al., 2000) and so disappearance of some susceptible species, which affect biodiversity (Itoh, 2004). Moreover, herbicides cause environmental pollution, unsafe agricultural products and human health concerns (Kohli et al., 1998; Xuan et al., 2004, 2005; Khanh et al., 2005). In response to this problem; the adverse effect of herbicides on people and environment, and the interest in environmentally friendly alternatives for weed control have rapidly increased in recent years (Amossé et al., 2013; Dommagnet et al., 2014; Kruidhof et al., 2014).

This research mainly focused on strategies of Integrated Weed Management System. Among the possible new strategies, agronomic solutions based on the use of plant natural compounds have been suggested (Dudai et al., 1999; Tworokski, 2002; Campiglia et al., 2007). This approach would mainly rely on the exploitation of allelopathic effects. Allelopathy is defined as “any process that involves secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of biological systems” (IAS, 1996). Chemicals that impose allelopathic influences are called allelochemicals or allelochemicals (Einhelling, 1996).

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**Table 1.** Aqueous and organic extracts used tested for allelopathic tests and their abbreviations.

Aqueous and organic extracts for allelopathic test	Abbreviation
Control for aqueous extracts	AC
Aqueous Root extracts at 1, 2, 3 and 4 g/l	AR1, AR2, AR3, AR4
Aqueous Shoot extracts at 1, 2, 3 and 4 g/l	AS1, AS2, AS3, AS4
Aqueous Leaves extracts at 1, 2, 3 and 4 g/l	AL1, AL2, AL3, AL4
Aqueous Flowers extracts 1, 2, 3 and 4 g/l	AF11, AF12, AF13, AF14
Control for organic extracts	OC
Root, stem, leaf, flower hexane extracts	Rhex, Shex, Lhex, Flhex
Root, stem, leaf, flower ethyl acetate extracts	Reac, Seac, Leac, Fleac
Root, stem, leaf, flower methanol extract	Rmet, Smet, Lmet, Flmet

These chemicals are largely classified as secondary plant metabolites (Rice, 1984). Allelochemicals are present practically in all plant tissues. They may be released from plants into their immediate environment (El-Khawas and Shehata, 2005; Bulut et al., 2006). These chemicals may exert their phytotoxic effect directly or indirectly as they selectively inhibit the growth of other plants, soil microorganisms or both (Lorenzo et al., 2013; Saraf et al., 2014). Originally, classified as waste products, allelochemicals more recently have been investigated extensively by ecologists and pharmacologists, and many complex biological functions have been discovered (Hadacek, 2002). Now it has been established that allelopathic properties of plants can be exploited successfully as a tool for weed control (Mahajan and Chauhan, 2013). The advantage of utilizing natural compounds in sustainable agriculture patterns such as organic farming depends on their rapid decomposition in the environment (Tworkoski, 2002; Campiglia et al., 2007). *Citharexylum spinosum* L is one tree among many that produces sufficient biomass with allelopathic extracts that can be exploited for weed control purposes.

*Citharexylum spinosum* (syn. *Citharexylum quadrangular* Jacq. and *Citharexylum fruticosum* L.) (Verbenaceae Family) (Wagner et al., 1999) is native to the Caribbean (Turner and Wasson, 1997) introduced in Tunisia for many years and cultivated along the roadsides and in gardens. This tree possesses medicinal properties and was useful in the treatment of various ailments. A decoction of young twigs was used for children thrush and bark decoction for treating colds (Cordero, 1978; Lachman-White et al., 1992). The leaves were used as a source of an antiallergic and as an alternative in hepatic disorders (Balázs et al., 2006). *C. spinosum* was used with other plants as anthelmintic (Lans, 2007).

Research information on the allelopathic potential of *C. spinosum* is relatively paltry. So, the aim of this present study was to determine the allelopathic potential of *C. spinosum* aqueous and organic extracts on the seed germination and early growth of four target seeds; wheat (*Triticum aestivum* L.), lettuce (*Lactuca sativa* L.), harmful

(*Peganum harmala* L.) and milk thistle (*Silybum marianum* L.).

## MATERIALS AND METHODS

### Plant material

*C. spinosum* different organs (roots, stems, leaves and flowers) were collected in the garden of the High Institute of Biotechnology of Monastir (latitude 35° 46' 0"N, longitude 10° 59' 0" E, coastal region, East of Tunisia, with a sub humid climate). A voucher specimen (CQV 12) was deposited at the Herbarium of the Laboratory of Botanic in the Institute. Roots were cleaned with tap water, and all the plant parts were air-dried in a shaded area at ambient temperature. Dried material was ground into a powder to pass through a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and stored at 4°C until use.

### Preparation of aqueous and organic extracts

One hundred grams of powder from each dried plant part (roots, stems, leaves and flowers) were separately extracted by soaking in 1 L distilled water at ambient temperature for 24 h (Khanh et al., 2005). The aqueous extracts were filtered through a double layered muslin cloth followed by Whatman no. 1 filter paper and then passed through 0.22 µm micro-filter pore size to remove bacteria. Filtrates were preserved at 4°C. Each crude aqueous extract at 10% (m/v) was diluted with sterile distilled water to give final concentrations of 1, 2, 3 and 4% (m/v) (Table 1). The 16 extracts were used freshly within a week (Omezzine et al., 2011; El Ayeb et al., 2013).

Sequential extraction was carried out in organic solvents with rising polarity: hexane, ethyl acetate and methanol. One hundred grams of powder were immersed in the appropriate solvent for 7 days at room temperature. The 12 organic extracts (Table 1) were evaporated to dryness under reduced pressure in a rotary evaporator at 45°C, to remove the solvent. After determination of the yield the extracts were stored at 4°C until use.

### Determination of the total polyphenol content

The content in total polyphenol in each organic extract was measured by spectrophotometric method based on a colorimetric oxidation/reduction reaction. The oxidizing agent used was of the Folin-Ciocalteu's phenol reagent (Merck) (Singleton and Rossi, 1965; AOAC, 1984). 50 µL of the diluted extract (1 mg/1 mL of

methanol) was added to 750  $\mu\text{L}$  of distilled water/Folin-Ciocalteu solution (28:2 v:v). After 3 min, 200  $\mu\text{L}$  of sodium carbonate solution (20% in distilled water) was added and the test tubes were properly shaken before incubating in a boiling water bath for 1 min. The tubes were then allowed to cool in the dark at ambient conditions for 30 min to complete the reaction. For the control sample, 50  $\mu\text{L}$  of methanol was used. The absorbance was measured at 765 nm. Tests were carried out in triplicate. Quantification was obtained by reporting the absorbance in the calibration curve prepared with gallic acid solutions ranging 0.01 to 0.1 mg/ml, results are expressed as mg of gallic acid equivalent (GAE) per gram of extract.

### Allelopathic bioassays

#### Bioassays with aqueous extracts

Four target species; two crops, lettuce and wheat and two weeds; milk thistle and harmful were used to test germination and early growth responses. Lettuce has been used as a test plant because it was too sensitive to chemicals at low concentration (Olofsdotter, 2001). Wheat has been used because it was one of the most important agricultural foods and feed crops worldwide (Högy and Fangmeier, 2008) and milk thistle is a well known competitive weed for crops.

Five millilitres of each diluted aqueous extract was added onto three layers of Whatman no.1 sterilized filter paper, lined on the bottom of a sterile Petri dish (90 mm) and allowed to dry under reduced pressure. All target seeds were surface sterilized by immersing in 0.525 g  $\text{L}^{-1}$  of sodium hypochlorite for 5 min, rinsed in sterile deionised water four times and soaked in the last water bath at 22°C for 4 h. Preliminary essays prove that the bleach did not inhibit germination. Thirty swollen seeds of each species were sown in each Petri dish where the filter paper was moistened with 5 mL of sterile distilled water and kept in a growth chamber to germinate in the dark with an average temperature of  $23 \pm 2^\circ\text{C}$  for 7 days. Distilled water was the control (AC) (Table 1). The experimental design was a randomized complete block replicated three times. A seed was considered germinated when the radical protruded  $\geq 2$  mm.

#### Bioassays with organic extracts

The 12 dried organic extracts were dissolved in methanol to compare their phytotoxic effects. Five millilitres of each extract dissolved at 6000 ppm (6 mg  $\text{mL}^{-1}$ ), were added to 3 sheets of filter paper displayed in a Petri dish (90 mm) and evaporated to dryness for 24 h at 24°C. The filter paper was moistened with 5 mL of sterile distilled water and then thirty imbibed seeds from each target species were arranged in each Petri dish and allowed to grow in a growth chamber in the dark at  $23 \pm 2^\circ\text{C}$  for 7 days. Treatments were arranged in a completely randomized design with three replications. Test conditions were identical to the previous bioassay. Control Petri dishes contained only methanol and distilled water (OC) (Table 1).

### Statistical analysis

Percentage of germinated seeds was recorded and the root and shoot lengths were measured for all seedlings in each Petri dish on day 7 after placing the seeds on the medium. The data were transformed to percent of control for analysis. Data from the experiments were transformed using arcsin-square root (arcsine  $\sqrt{x}$ ) to conform with assumptions of normality for analysis of variance (ANOVA) using SPSS 12.0, for Windows program. The significance of the differences between means was determined at  $P < 0.05$  using *Duncan's* multiple range tests. We evaluated whether the

type of extract (or group of extracts) was useful in reflecting its phytotoxic effect on the germination and the early growth of each target seed species. The data obtained for all parameters in accordance with all extracts tested were subjected to Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA) using SPSS 12.0 software (SPSS Inc. Chacago, IL, USA).

## RESULTS

### Yields and total polyphenol contents in the organic extracts

Yields in the 12 organic extracts together with their polyphenol contents were reported in Table 2. Leaves have the highest yield with the three organic solvents; hexane, ethyl acetate, and methanol (0.91, 1.31 and 6.43%, respectively) followed by flowers then stems and finally roots. On the other hand, methanol gave the highest yield in all the plant parts analyzed. According to Folin Ciocalteu test, the different extracts from the different organs contained phenols. Leaf, flower, root and stem methanol extracts showed the highest amount with  $617.93 \pm 1.12$ ,  $346.85 \pm 2.38$ ,  $134.95 \pm 0.69$  and  $94.77 \pm 0.78$  mg GAE/g extract. For all the other extracts contents in total phenol were low and varied between  $3.72 \pm 0.02$  and  $32.51 \pm 1.21$  mg GAE/g.

### Allelopathic activity of aqueous and organic extracts

To evaluate the allelopathic effects of organic and aqueous *C. spinosum* extracts, data recorded were subjected to Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). The results indicated that, extracts have varying degree of inhibitory or stimulatory effect on germination and seedling growth. Effects of organic and aqueous extracts on germination and early growth of the 4 target seeds tested were reported in Table 3.

The HCA (data not given) based on the *Euclidean* distance between groups indicated three groups of extracts; Groups 1, 2 and 3, identified by the parameters with which they correlate. Data were reported in Table 3 according to the HCA analysis. The 3 groups were separated according to their allelopathic activity. Those groups clearly stand out forming separate groups and responses for tested parameters were different from one group to another. The group 3 was divided into 2 subgroups 3A and 3B, 3A was divided into subgroups 3Aa, 3Ab, 3B was subdivided into subgroups 3Ba and 3Bb. The later (3Bb) still divided into subgroups 3Bb1 and 3Bb2.

#### Group 1 and subgroups 1A and 1B

The group 1 represented by controls (AC, OC) and root

**Table 2.** Yields and total polyphenol contents in organic extracts from the different organs of *C. spinosum* L.

Organ and solvent extract	Total phenols (mg gallic acid equivalent/100 g MS)	Yield (%)
*Rhex	3.72±0.02	0.27
Reac	5.33±0.96	0.32
Rmet	134.95 ±0.69	1.60
Shex	4.92±0.04	0.27
Seac	18.27±0.00	0.88
Smet	94.77±0.78	2.05
Lhex	22.33±1.73	0.91
Leac	29.28±6.03	1.31
Lmet	617.93±1.12	6.43
Flhex	14.37±0.21	0.53
Fleac	32.51±1.21	1.23
Fimet	346.85±2.38	5.94

\*For abbreviation see Table 1.

aqueous extracts at 1 and 2% (AR1 and AR2) correlated with all parameters related to germination and seedlings growth. We note however that the germination percentage of harmful seeds was between 88.3 to 100%, for milk thistle it was equal to 90 and 85%, in AC and OC, respectively. In contact with AR1 and AR2 extracts, the germination percentages of target seeds were slightly reduced compared to control (93.3-95, 100, 83.3-93 and 83.3, respectively for lettuce, wheat, harmful and milk thistle seeds). Root elongation was also close to control for lettuce (92.6 to 100.7% of control), wheat (93.6 to 95.6% of control), and milk thistle (82 to 90% of control). Root elongation of harmful was more reduced (67.8 to 69.3% of control). Those extracts highly correlate with the elongation of lettuce, wheat and harmful shoot seedlings and so stimulate their development in percentages exceeding the control (126.8-138.3, 109.3-115.4 and 148.7-156.7, respectively). Nevertheless development of milk thistle was slightly less than the control (74.4-95.5% of control).

### **Group 2 (Rmet, Smet, Fimet)**

The group 2 consisting of the three methanol extracts from roots (Rmet), stems (Smet) and from flowers (Fimet), was totally opposed to the germination of harmful and milk thistle seeds and so there is no seedling development. On the other side, those extracts correlated with the germination of wheat seeds (90-100%) and least for the germination of lettuce seeds (88.3-96.7%) and with shoot and root lettuce seedlings elongation. The development of these seedlings (16.5-41.8% of control for root, 23.8-31.9% of control for shoot), was more important than that of wheat (7.2-15.4 and 11.4-22.9% of control).

### **Group 3Aa (Rhex, Reac, Shex)**

Ethyl acetate and hexane root extracts and hexane stem extract were totally opposed to seed germination of milk thistle and harmful (0%) but correlated with wheat and lettuce seeds germination (95-96.7 and 100%, respectively). The development of lettuce and wheat root seedlings was moderately reduced (93.9-74.0% of control, and 53.7-71.6% of control, respectively). Unlike the shoot of wheat seedlings was more elongated than this of lettuce ones (99.2-99.5% of control and 60-79% of control, respectively).

### **Group 3Ab (Lmet, Fleac, Seac)**

Germination of lettuce and wheat seeds were weakly inhibited (75-88.3%) in contact with those extracts, which were almost totally opposed to germination of milk thistle seeds (0-6.7%). Germination of harmful seeds was correlated with Fleac and Seac extracts and reached 90 and 83.3%, respectively, but was weakly correlated with Lmet extract (12.2%), solely all harmful seedlings grow slightly. The development of lettuce seedlings was highly reduced in Lmet and Fleac extracts (14.4-27.8% of control for roots; 16.7-49.1% of control for shoots) compared to those of wheat (39.3-49.4% of control for roots 89.6-97.5% of control for shoots). Seedlings of the two target plants (lettuce and wheat) were less sensitive to Seac extract.

### **Group 3B**

All extracts (17) from this group correlate with germination percentages of lettuce, wheat and harmful

**Table 3.** Percentages germination (%G) of *L. sativa* (Lac), *T. aestivum* (Tri), *P. harmala* (Peg), and *S. marianum* (Sil) seeds tested in presence of *C. spinosum* organic (6 mg/ml) and aqueous extracts (1, 2, 3 and 4%; m/v) and root (Re) and shoot (Se) elongation of their seedlings in percent of control. The table was established according the Hierarchical Clusters Analysis (HCA).

Groups/ subgroups		Aq/Org.	<i>Lactuca sativa</i> (Lettuce)			<i>Triticum aestivum</i> (Wheat)			<i>Peganum harmala</i> (Harmal)			<i>Silybum marianum</i> (Milk thistle)			
		Ext.	%GLac	LacRe	LacSe	%GTri	TriRe	TriSe	%GPeg	PegRe	PegSe	%GSil	SilRe	SilSe	
Group 1	1A	*AC	100±0.0 <sup>h</sup>	100±0.0 <sup>k</sup>	100±0.0 <sup>g-i</sup>	100±0.0 <sup>d</sup>	100±0.0 <sup>n</sup>	100±0.0 <sup>cd</sup>	88.3±1.3 <sup>cde</sup>	100±0.0 <sup>m</sup>	100±0.0 <sup>f</sup>	90±0.0 <sup>g</sup>	100±0.0 <sup>i</sup>	100±0.0 <sup>h</sup>	
		OC	100±0.0 <sup>h</sup>	100±0.0 <sup>k</sup>	100±0.0 <sup>g-i</sup>	100±0.0 <sup>d</sup>	100±0.0 <sup>n</sup>	100±0.0 <sup>cd</sup>	100±0.0 <sup>f</sup>	100±0.0 <sup>m</sup>	100±0.0 <sup>f</sup>	85±0.0 <sup>g</sup>	100±0.0 <sup>i</sup>	100±0.0 <sup>h</sup>	
	1B	AR1	95.0±0.0 <sup>e-h</sup>	100.7±6.4	126.8±3.7 <sup>l-m</sup>	100±0.0 <sup>d</sup>	95.6±0.6 <sup>k-n</sup>	109.3±5.3 <sup>c-e</sup>	83.3±0.5 <sup>cd</sup>	67.8±7.7 <sup>hi</sup>	148.7±5.2 <sup>j</sup>	83.3±0.1 <sup>i</sup>	90±0.7 <sup>h</sup>	95.5±4.6 <sup>h</sup>	
Group 2	Rmet	AR2	93.3±2.8 <sup>d-g</sup>	92.6±8.3 <sup>jk</sup>	138.3±9.9 <sup>mn</sup>	100±0.0 <sup>d</sup>	93.6±6.0 <sup>j-n</sup>	115.4±7.7 <sup>i</sup>	93.3±1.2 <sup>de</sup>	69.3±7.6 <sup>hi</sup>	156.7±3.5 <sup>kl</sup>	83.3±5.8 <sup>j</sup>	82±1.2 <sup>g</sup>	74.4±3.7 <sup>g</sup>	
		Smet	96.7±0.1 <sup>fh</sup>	41.8±6.6 <sup>de</sup>	31.9±1.6 <sup>a</sup>	100±0.0 <sup>d</sup>	9.3±1.5 <sup>a</sup>	17.0±3.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0.5±1.0 <sup>bc</sup>	0.3±0.5 <sup>a</sup>	0±0.0 <sup>a</sup>
	Flmet	Rhex	88.3±0.5 <sup>b-d</sup>	32.6±1.8 <sup>cd</sup>	23.8±3.3 <sup>a</sup>	100±0.0 <sup>d</sup>	7.2±1.9 <sup>a</sup>	22.9±3.4 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0.0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>
		3Aa	90±0.0 <sup>b-e</sup>	16.5±2.1 <sup>ab</sup>	29.2±5.7 <sup>a</sup>	90±0.0 <sup>d</sup>	15.4±4.5 <sup>a</sup>	11.4±2.6 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0.0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>
	3A	3Ab	Rhex	95.0±0.1 <sup>e-h</sup>	93.9±7.2 <sup>jk</sup>	75.4±6.3 <sup>d-f</sup>	100±0.0 <sup>d</sup>	58.9±1.5 <sup>de</sup>	99.5±0.3 <sup>cd</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>
			Shex	96.7±1.6 <sup>fh</sup>	88.9±0.1 <sup>h-k</sup>	79.0±2.5 <sup>ef</sup>	100±0.0 <sup>d</sup>	71.6±5.5 <sup>fg</sup>	99.3±0.1 <sup>cd</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>
Reac		95.0±2.1 <sup>e-h</sup>	74±4.1 <sup>fg</sup>	60±9.7 <sup>b-d</sup>	100±0.0 <sup>d</sup>	53.7±7.5 <sup>cd</sup>	99.2±0.3 <sup>cd</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>		
Lmet		88.3±3.3 <sup>b-d</sup>	27.8±0.5 <sup>bc</sup>	49.1±1.6 <sup>b</sup>	100±0.0 <sup>d</sup>	49.4±2.1 <sup>cd</sup>	97.5±1.0 <sup>cd</sup>	12.2±0.2 <sup>b</sup>	0.6±0.4 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>		
Seac		86.7±2.8 <sup>bc</sup>	14.4±2.3 <sup>a</sup>	16.7±6.1 <sup>a</sup>	100±0.0 <sup>d</sup>	39.3±0.5 <sup>b</sup>	89.6±2.1 <sup>bc</sup>	90±0.0 <sup>de</sup>	2.5±0.8 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>		
AR3		75.0±5.1 <sup>a</sup>	90.0±2.3 <sup>i-k</sup>	67.4±3.2 <sup>c-e</sup>	100±0.0 <sup>d</sup>	70.1±2.0 <sup>fg</sup>	97.4±0.2 <sup>cd</sup>	83.3±2.3 <sup>cd</sup>	5.7±2.1 <sup>ab</sup>	4.9±4.5 <sup>a</sup>	6.7±0.6 <sup>ab</sup>	0.4±0.3 <sup>a</sup>	2.5±4.2 <sup>a</sup>		
Group 3	3Ba	AFI1	95±0.0 <sup>e-h</sup>	86.6±4.4 <sup>g-k</sup>	150.2±7.0 <sup>n-p</sup>	100±0.0 <sup>d</sup>	84.1±4.3 <sup>h-j</sup>	117.7±6.2 <sup>f</sup>	95.0±0.3 <sup>ef</sup>	82.3±8.1 <sup>jk</sup>	190.9±1.2 <sup>b</sup>	53.3±2.9 <sup>f</sup>	37.2±9.9 <sup>e</sup>	42.6±1.1 <sup>e</sup>	
		3Ba1	AS1	95±5.0 <sup>e-h</sup>	94.9±0.5 <sup>jk</sup>	131±2.1 <sup>k-m</sup>	96.7±5.7 <sup>b-d</sup>	99.6±9.6 <sup>mn</sup>	98.7±0.6 <sup>cd</sup>	95.0±5.2 <sup>ef</sup>	89.6±8.2 <sup>kl</sup>	183.1±2.3 <sup>no</sup>	70.0±9.9 <sup>h</sup>	45±5.5 <sup>f</sup>	53.5±4.2 <sup>f</sup>
	3Ba2	AS2	93.3±5.7 <sup>d-g</sup>	84.2±2.2 <sup>ji</sup>	144.2±2.2 <sup>m-o</sup>	93.3±3.3 <sup>a-d</sup>	96.6±2.6 <sup>j-n</sup>	98.6±5.2 <sup>cd</sup>	93.3±0.8 <sup>ef</sup>	93.3±5.7 <sup>lm</sup>	167.3±3.2 <sup>k-m</sup>	63.3±5.8 <sup>gh</sup>	24.1±2.7 <sup>d</sup>	42.8±3.4 <sup>e</sup>	
		AS3	91.7±0.3 <sup>c-f</sup>	77.9±0.5 <sup>fi</sup>	163.9±1.4 <sup>p</sup>	92.2±5.0 <sup>a-c</sup>	88.7±3.6 <sup>h-m</sup>	88.2±2.9 <sup>bc</sup>	83.3±0.3 <sup>cd</sup>	66.7±8.5 <sup>hi</sup>	130.5±2.9 <sup>h</sup>	40±0.0 <sup>e</sup>	18.4±2.0 <sup>c</sup>	39.6±0.6 <sup>de</sup>	
	3Bb	3Bb1	AR4	88.3±1.2 <sup>b-d</sup>	75.9±4.2 <sup>fh</sup>	158.1±7.7 <sup>op</sup>	100±0.0 <sup>d</sup>	78.6±4.6 <sup>gh</sup>	117±1.2 <sup>f</sup>	95±0.0 <sup>de</sup>	86.1±3.4 <sup>kl</sup>	208.2±9.3 <sup>p</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>
			AL2	95.0±0.5 <sup>e-h</sup>	54.1±5.1 <sup>e</sup>	117.2±6.2 <sup>l</sup>	92.2±6.9 <sup>a-c</sup>	71.3±1.6 <sup>fg</sup>	110±0.5 <sup>c-e</sup>	95.0±0.0 <sup>ef</sup>	74.2±1.7 <sup>ij</sup>	172.7±4.1 <sup>n</sup>	6.7±5.8 <sup>ab</sup>	3.2±1.6 <sup>a</sup>	5.4±5.3 <sup>a</sup>
		3Bb2	AL3	90.0±1.6	49.9±2.7 <sup>e</sup>	113±3.5 <sup>h-j</sup>	93.3±0.0 <sup>a-d</sup>	64.7±5.9 <sup>ef</sup>	100.8±5.2 <sup>c-e</sup>	95.0±0.0 <sup>ef</sup>	55.8±9.5 <sup>g</sup>	162.9±5.2 <sup>kl</sup>	6.7±5.8 <sup>ab</sup>	1.2±0.5 <sup>a</sup>	2.3±4.0 <sup>a</sup>
			AL1	95.0±0.8 <sup>e-h</sup>	51.2±3.6 <sup>e</sup>	106.9±5.7 <sup>g-i</sup>	91.1±9.6 <sup>ab</sup>	91.4±2.8 <sup>i-n</sup>	113.8±1.5 <sup>ef</sup>	100±0.6 <sup>f</sup>	93.8±4.3 <sup>lm</sup>	178.7±5.5 <sup>m-o</sup>	30±0.0 <sup>d</sup>	5.3±0.6 <sup>a</sup>	14.7±3.2 <sup>b</sup>
		3Bb2	AL4	86.7±1.6 <sup>bc</sup>	33.8±3.8 <sup>cd</sup>	92.3±4.2 <sup>fg</sup>	88.9±5.7 <sup>a</sup>	44.7±2.3 <sup>bc</sup>	93.1±3.6 <sup>bc</sup>	90.0±0.6 <sup>de</sup>	52.6±6.7	140.6±7.3 <sup>hi</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>
			AS4	90±2.8 <sup>b-e</sup>	33.8±3.8 <sup>cd</sup>	92.3±4.2 <sup>fg</sup>	91.1±1.9 <sup>ab</sup>	82.4±2.6 <sup>hi</sup>	87.4±3.3 <sup>bc</sup>	80.0±0.0 <sup>c</sup>	61.8±6.6 <sup>gh</sup>	83.7±2.5 <sup>e</sup>	16.7±5.8 <sup>c</sup>	17.2±0.1 <sup>bc</sup>	36.7±7.2 <sup>d</sup>
	Leac		93.3±2.5 <sup>d-g</sup>	74.4±1.6 <sup>fg</sup>	80.4±2.8 <sup>ef</sup>	100±0.0 <sup>d</sup>	85.4±2.9 <sup>h-k</sup>	93.6±3.1 <sup>bc</sup>	83.3±0.6 <sup>cd</sup>	18.5±5.6 <sup>c</sup>	24.5±8.6 <sup>b</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	
	Flhex		91.7±2.8 <sup>c-f</sup>	50.7±9.5 <sup>e</sup>	53.4±7.7 <sup>bc</sup>	100±0.0 <sup>d</sup>	65.5±1.0 <sup>ef</sup>	99.7±1.0 <sup>cd</sup>	90±1.1 <sup>de</sup>	11.8±2.3 <sup>cd</sup>	35.2±3.3 <sup>bc</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	
3Bb2	Lhex	98.3±2.6 <sup>gh</sup>	99.3±2.3 <sup>k</sup>	98.4±1.2 <sup>gh</sup>	100±0.0 <sup>d</sup>	97.2±2.1 <sup>i-n</sup>	89.3±0.1 <sup>bc</sup>	93.3±0.5 <sup>ef</sup>	35.3±1.1 <sup>d</sup>	59.3±7.8 <sup>d</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>		
	AFI2	90.0±1.1 <sup>b-e</sup>	68.5±4.1 <sup>f</sup>	132.8±6.1 <sup>n</sup>	95±8.6 <sup>a-d</sup>	79.1±7.2 <sup>gh</sup>	109.5±0.9 <sup>c-e</sup>	95.0±0.0 <sup>ef</sup>	50.5±4.7 <sup>ef</sup>	79.5±0.5 <sup>e</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>		
3Bb2	AFI3	88.3±0.3 <sup>b-d</sup>	47±7.7 <sup>e</sup>	114.4±7.1 <sup>h-k</sup>	98.3±2.8 <sup>cd</sup>	69.2±6.2 <sup>e-g</sup>	107.8±0.1 <sup>c-e</sup>	93.3±2.8 <sup>ef</sup>	44.6±3.6 <sup>e</sup>	86.4±9.6 <sup>e</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>		
	AFI4	85.0±2.1 <sup>b</sup>	26.5±1.4 <sup>a-c</sup>	91.2±7.2 <sup>fg</sup>	96.7±2.8 <sup>b-d</sup>	50.7±6.0 <sup>cd</sup>	81.8±0.6 <sup>b</sup>	86.7±5.7 <sup>cde</sup>	19.3±3.6 <sup>c</sup>	47.0±5.8 <sup>cd</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>	0±0.0 <sup>a</sup>		

Means ±SE followed by different letters differ significantly at P < 5%, as established by Duncan's test. \*For abbreviation see Table1.

seeds and with the development of their seedlings. We note also that several extracts stimulate the development of shoots;

percentages of shoot elongation exceed that of the control. On the other side, the percentages germination of thistle seeds and the

development of seedlings were low or equal to zero, with the exception for extracts for the subgroup 3Ba1.

### **Subgroups 3Ba1 and 3Ba2 (AR3,4, AFI1, AS1-3)**

When grown in contact with those extracts, germination percentages for lettuce, wheat, and harmful seeds reached 88.3-95.0, 92.2-100 and 83.3-100, respectively. The development of root seedlings was important (75.9-94.9, 78.6-99.6 and 66.7-93.3% of control, respectively). The development of shoot seedlings was higher than that of control and harmful shoot was more stimulated (113.6-208.2% of control), than this for lettuce (131-163.9% of control). The shoot of wheat seedlings was stimulated by AR3 and AR4 and by AFI1 (from 111.3 to 117.7% of control). Although, AR4 extract was highly correlated with shoot elongation of harmful seedlings which reached 208.2% of control.

Extracts from subgroups 3Ba1 and 3Ba2 were less correlated with thistle seed germination and with their seedling development. In presence of AR3, AFI1, AS1-3 (3Ba1 subgroup), milk thistle seed percentage germination was between 36.7 and 70% and root and shoot elongation varied from 12.4 to 45.0% of control and from 24.6 to 53.5% of control, respectively. Germination of this target seeds was completely inhibited by AR4, the only representative of 3Ba2 subgroup.

### **Subgroup 3Bb1 and 3Bb2**

Group 3Bb, was shared in 3Bb1 and 3Bb2, and all extracts reduced the development of lettuce, wheat and harmful more than those from 3Ba group. The subgroup 3Bb1 consisted of extracts AL1-4, AS4 which correlated with lettuce, wheat and harmful seeds germination (86.7-95%, 88.9-93.3 and 80-100%) but less with milk thistle seeds germination (6.7-30%). More, AL4 inhibited milk thistle seed germination. Extracts from 3Bb1 subgroup were moderately correlated with the development of wheat root and harmful seedlings (44.7-91.4% of control; 52.6-93.8% of control, respectively) and less with that of lettuce (33.8-54.1% of control). Contrary to the results obtained for wheat and harmful, a low correlation was reported between those extracts and the root elongation of milk thistle seedlings (1.2-17.2% of control).

In presence of AL1-3 extracts from 3Bb11 subgroup, the development of lettuce, wheat and mainly harmful shoots was enhanced (106.9-117.2, 100.8-113.8 and 162.9-178.7% of control). Milk thistle seedling shoot elongation was reduced in the presence of those extracts (2.3-14.7% of control). In AL4 the shoot elongation of harmful was also highly stimulated (140.6% of control). AS4, the only representative of subgroup 3Bb12, stand out of the group 3Bb1 by its moderate effect on milk thistle seedling development (17.2-36.7%).

All extracts from the subgroup 3Bb2 (Leac, Flhex, Lhex, AFI2, AFI3 and AFI4) inhibited the germination of milk thistle seeds. Nevertheless, percentages of germination of lettuce and wheat seeds were close to the

effect reported in presence of extracts from subgroup 3Bb1. When seeds were in contact with Leac, Flhex, Lhex (3Bb21 group extracts), the development of roots (50.7-99.3 and 65.5-97.2% of control, respectively) and of shoots (53.4-98.4 and 89.3-99.7% of control) of lettuce and wheat seedlings was important but less than the control. The development of harmful seedlings was less important (for the root 11.8-35.3% of control; for the shoot 24.5-59.3% of control). When grown in contact with AFI2-4% (subgroup 3Bb22), elongation of lettuce and wheat root seedlings varied from 26.5 to 68.8% and from 50.7 to 79.1% of control, respectively. Nevertheless, the development of their respective shoot was important (91.2-132.8, 81.8-109.5% of control) and was higher than the control. On contrary, the development of the harmful seedlings was reduced (for the root 19.3-50.5% of control, for the shoot 47-86.4% of control).

## **DISCUSSION**

Our findings were supported by previous reports that demonstrated the allelopathic effects of many other trees such as *Melia azedarach* (Hong et al., 2003, 2004), *Azadirachta indica* (Neem) (Al-Charchafchi et al., 2007; Ashrafi et al., 2008; Abdus Salam and Kato-Noguchi, 2010), *Sesbania sesban* (Mubarak et al., 2009), *Acacia cyanophylla* (El Ayeb et al., 2013), and more recently for six trees from South Africa (Sunmonu and Staden, 2014). For *C. spinosum*, to the best of our knowledge, findings that indicate about its allelopathic effects are not available. Hence, it was for the first time that we have systematically evaluated and demonstrated the allelopathic inhibitory effects of *C. spinosum* on seed germination and early growth of milk thistle and harmful.

Phenol compounds are well known as potential phytotoxins (Seal et al., 2004). In our study we demonstrate that the inhibition of seed germination and the reduction of seedling elongation were not only related to the content in polyphenols of the extract (Table 2) but probably with the presence in all plant parts of one or more of phenol compound responsible for the activity (Inderjit, 1996; Khan and Siddique, 2012). In fact, we demonstrate that extracts containing low quantities in total phenol show a great inhibitor power and that the toxic metabolites are distributed in all plant parts in various concentrations (Harborne, 1977).

The effect of *C. spinosum* extracts varied with the kind of organ, concentration and target species. Roots of the different target species appeared to be more sensitive to *C. spinosum* extracts than shoots, presumably because of their more ultimate contact with the treated filter paper (Ahn and Chung, 2000). Similar results were reported with other crops by Maharjan et al., 2007, Ashrafi et al. (2008) and Wakjira et al., (2009). Abdus Salam and Kato-Noguchi (2010) reported that the extracts of allelopathic plants had more inhibitory effect on root growth than on

shoot growth because the root is the first organ to absorb allelochemicals from the environment. Germination inhibition would be attributed to those allelochemicals (Bulut et al., 2006). Furthermore, the permeability of allelochemicals to root tissues was reported to be greater than that to shoot tissues (Nishida et al., 2005) due to the direct contact between the root and phytotoxic compounds present in extract. Those compounds might inhibit or reduced rate of cell division (Wang et al., 2002; Qin et al., 2006) which is highly active at meristematic tissue of the growing root tip.

In all organic extracts germination of lettuce and wheat seeds was not or weakly reduced and germination percentages were proximate to control in the majority of cases (75.0-96.7 and 88.9-100%, respectively). Harmal seeds germination was strongly reduced (12.2%) or totally inhibited by all methanol extracts from stems, roots, leaves and flowers, hexane extract from roots and stems and ethyl acetate extract from roots. However, the two weeds: harmal and milk thistle were more sensitive than lettuce and wheat and the flowers extracts were the most toxic and milk thistle was more susceptible to extracts, than harmal. Organic extracts were more toxic than aqueous ones. Those later extracts, at high concentrations and all organic extracts reduced strongly or inhibited totally germination of milk thistle seeds. Contrariwise, the aqueous extracts at 1-3% (from leaves, stems and roots) stimulated elongation of lettuce, wheat, and harmal shoot seedlings in percentages exceeding control.

## Conclusion

The present study demonstrated that aqueous and organic extracts of *C. spinosum* possess allelopathic potential and contain inhibitory substances. Allelopathic substances present in *C. spinosum* under favourable conditions should release into the environment and likely act synergistically to affect the growth of weed plants. These results suggest that *C. spinosum* could be one of the useful natural resources for developing bioherbicides for weed management and crude extracts of this tree could be a cost effective way for crops protection against weeds. Further research in order to know the growth inhibitory substances from *C. spinosum* organs are underway.

## Conflict of Interest

The authors have not declared any conflict of interest.

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