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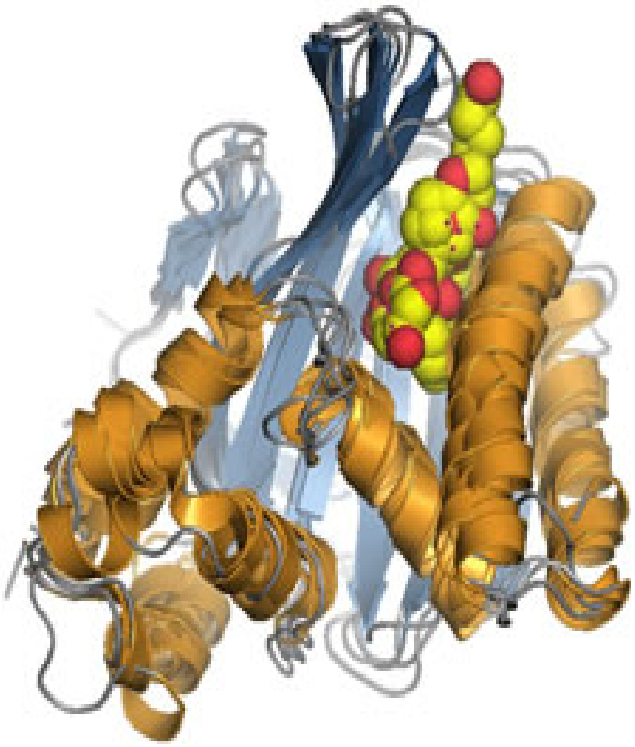

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Allelopathic potential of some Algerian plants

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Abstract. *Allelopathic activity study of 15 plant's aqueous extracts was evaluated on the germination and growth of two experimental models, Lactuca sativa and Rhaphanus sativus. The seeds tested were germinated in petri dishes with increasing concentration: 0.25; 0.5 ; 0.75 and 1 % of plant extract. Seasonal variation of allelopathic activity of Tetraclinis articulata was studied. Fractionation assay by liquid-liquid partitioning was also undertaken for the same species. In parallel of this biological activity test, phytochemical screening of the main phytoconstituents was established by TLC with quantification of phenolics and flavonoïds contents.*
Inhibitory effects with variable intensities were observed on the germination and growth of L. sativa. Aqueous extract of T. articulata was exhibited the more inhibitory effect on L. sativa germination while aqueous extract of Peganum harmala showed the more growth inhibitory effect with all concentrations tested, for the two experimental models. Investigation of seasonal variation revealed that June and November samples of T. articulata presented the most important inhibitory effect on L. sativa germination.
Phytochimical screening by TLC identified that these active extracts contain phenolic acids, flavonoïds, cardiotonic glycosids, sesquiterpens lactons and saponins. Phenolics and flavonoïds contents quantified by spectrophotometry were very important in some active extracts like Globularia alypum, Pistacia lentiscus, Acacia raddiana and Haloxylon scoparium.

***Key Words*:** *Allelopathic activity, aqueous extracts, Algerian medicinal plants, germination, growth, flavonoïds, phenolics, phytochemistry, seasonal variation*

Introduction

The secondary metabolites of the plants are famous since antiquity for their pharmacological properties. For a few decades, the man has also been interested in their other biological activities. In particular, these secondary compounds are often regarded as being a

means of defense of the producing plant against various organizations like the pathogenic ones and the ravagers. Indeed, the compounds of plant origin are used to fight against the insects and weeds because they are accessible and effective and also they are less toxic for the human than the majority of insecticides and synthetic herbicids because they are present in nature. The products of synthesis used in agriculture prove to be responsible for the pollution of the majority of the biotopes, as well as of an impoverishment of the biodiversity and rarefaction of pure water necessary to the human life. Their harmful effect is increasingly important not only on agriculture but on the general ecology of planet and, in the long run, on the harmonious survival of the Man. The interaction between plants via chemical molecules, or allelopathy, currently arouses a growing interest. A better knowledge of this phenomenon could offer prospects interesting for management for the spontaneous flora of the cultivated pieces and thus contribute to decrease the use of synthetic herbicids.

Materials and Methods

Experimental models and plant tested. The seeds of *Lactuca sativa* L. and *Rhaphanus sativus* L. are of commercial origin. The plant materials, relating to freeze-dried aqueous extracts used in the treatments are regrouped in table1.

Table 1: Extracts used in allelopathic activity assay.

Plants	Familly	Harvested date	Used part
Acacia raddiana Savi.	Fabaceae	November 2006	Leaves
Ajuga iva ssp. pseudo-iva L.	Lamiaceae	March 2007	Aerial part
Cassia ascherk Foressk.	Fabaceae	January 2008	Leaves
Citrullus colocynthis L.	Cucurbitaceae	November 2006	Fruit flesh
Globularia alypum L.	Globulariaceae	November 2006	Roots
Haloxylon scoparium Pomel.	Chenopodiaceae	March 2008	Leaves
Juniperus phoenicea L.	Cupressaceae	March 2008	Leaves
Osyris quadripartita Salzm.	Santalaceae	January 2008	Leaves
Peganum harmala L.	Zygophyllaceae	February 2008	Seeds
Pergularia tomentosa L.	Asclepiadaceae	November 2006	Leaves
Pistacia lentiscus L.	Anacardiaceae	June 2007	Leaves
Rhus pentaphylla L.	Anacardiaceae	November 2007	Leaves
Ruta chalepensis L.	Rutaceae	February 2008	Leaves
Tetraclinis articulata Vahl	Cupressaceae	June 2007 November 2007 January 2008 April 2008	Leaves
Wittania frutescens L.	Solanaceae		Leaves

Preparation of the aqueous extracts. For each species, the collected parts are dried with the drying oven during 24 h at 50 °C. After the crushing, the plant powder obtained (10 g) is put in 100 ml of distilled water, then, extracted by heat reflux for 3 times during 30 mn each. After filtration, the aqueous extract is freeze-dried and preserved at - 20°C until use.

Bioassay. Bioassays are realized in Petri dishes at 25 °C in regulated drying oven. For each test, 4 concentrations were used (0.25; 0.50; 0.75 and 1 %) compared to a control (distilled water).The counting of the percentage of germination is carried out every day, during 5 days. Biometric measurements were noted only on the seedlings where the aqueous extract presented a notable effect on the growth. The results represent the means of 4 repetitions of 25 seeds for each treatment.

Phytochemical analysis

1. Identification of the phytoconstituents by thin layer chromatography (TLC)

The identification of the phytoconstituents of the active extracts is made on plates of thin layer in normal phase where the stationary phase used is the silica gel not hydrated (Silicagel 60 F 254, 0.25 mm thickness) on an aluminum support (Merck). Systems of solvents employed differ from one identification to another, according to their polarity. The chromatograms are evaluated under UV at 254 and 365 nm and they are visualized before and after revelation to detect phenolic acids, coumarins, flavonoïds, lignans, quinones, saponins, terpens, sesquiterpens lactones, alkaloids and cardiotonic glycosides.

Total phenolics and flavonoïds content assay

Total phenolic assay. The content is estimated by the method of Folin-Ciocalteu (**Singleton and Rossi, 1965**) with some minor modifications. To 100 µl of extract 500 µl of diluted Folin-Ciocalteu reagent (1/10 dissolves in distilled water) are added. The mixture is agitated by vortex and is left at the obscurity during 5 mn in room temperature. Then 1.5 ml of saturated sodium carbonate (2 % dissolves in distilled water) is added with agitation. After incubation during one hour, the reading of the absorbance is carried out with the spectrophotometer UV-Visible (8500 P Double-BEAM spectrophotometer) at 765nm. The quantities of total phenolics are expressed out of mg of gallic acid equivalents per gram of freeze-dried extract (mg GA/g DW) starting from a linear calibration curve prepared using gallic acid with various concentrations (4.76-9.52-14.2-19 and 23.8 µg /ml) under the same conditions as the samples.

Flavonoïds content assay. Flavonoïds contents determination assay of the different samples was carried out according to the colorimetric method described by Kim et al. (2003) with little modifications. This assay is carried out according to the following steps:
The extract (500 µl) was diluted with 1500 µl distilled water. At Zero time, 150 µl $NaNo_2$ 5 % (dissolved in distilled water), are added. After 5 mn, 150 µl of 10 % of $AlCl_3$ solution (in MeOH) are added. After 11 mn, 500 µl NaOH (1M, in distilled water) are added. The mixture is agitated with the vortex and the absorption was reading immediately at 510 nm. The results are expressed as mg of catechin equivalent/g DW from a calibration curve, carried out with increasing concentration (5-10-15, and 25 µg/ml).

Stastistical analysis. Stastistical computations were performed using SPSS Software (version17.0). The data of extracts effects were subjected to one-way analysis of variance (ANOVA).

Results

Effect of the aqueous plant extracts on the germination of *Lactuca sativa* L and *Rhaphanus sativus* L.

From 15 different aqueous extracts tested, only 9 have exhibited remarquable activity.

The aqueous extract of *Globularia alypum* L. presents a strong inhibition of the germination of *Lactuca sativa* L. for all the concentrations tested. The inhibition of germination is noticed starting from the concentration 0.25 %; the inhibiting effect is maximum for the 1 % treatment (**Fig 1A**). This effect is much less net on the germination of *Rhaphanus sativus* L. (**Fig 1B**)

All concentrations of the aqueous extract of *Pistacia lentiscus* L. (leaves) (except 0. 25 %) are inhibiting germination of *Lactuca sativa* L. (**Fig 1A**); but one notes no effect on germination of *Rhaphanus sativus* L. (**Fig 1B**)

The inhibiting effect of the aqueous extract of *Rhus pentaphylla* L. on the germination of seeds of *Lactuca sativa* L. is visible only at the concentration 1 % of the extract (**Fig 1A**). On the other hand, null effect is observed on germination of *Rhaphanus sativus* L. (**Fig 1B**)

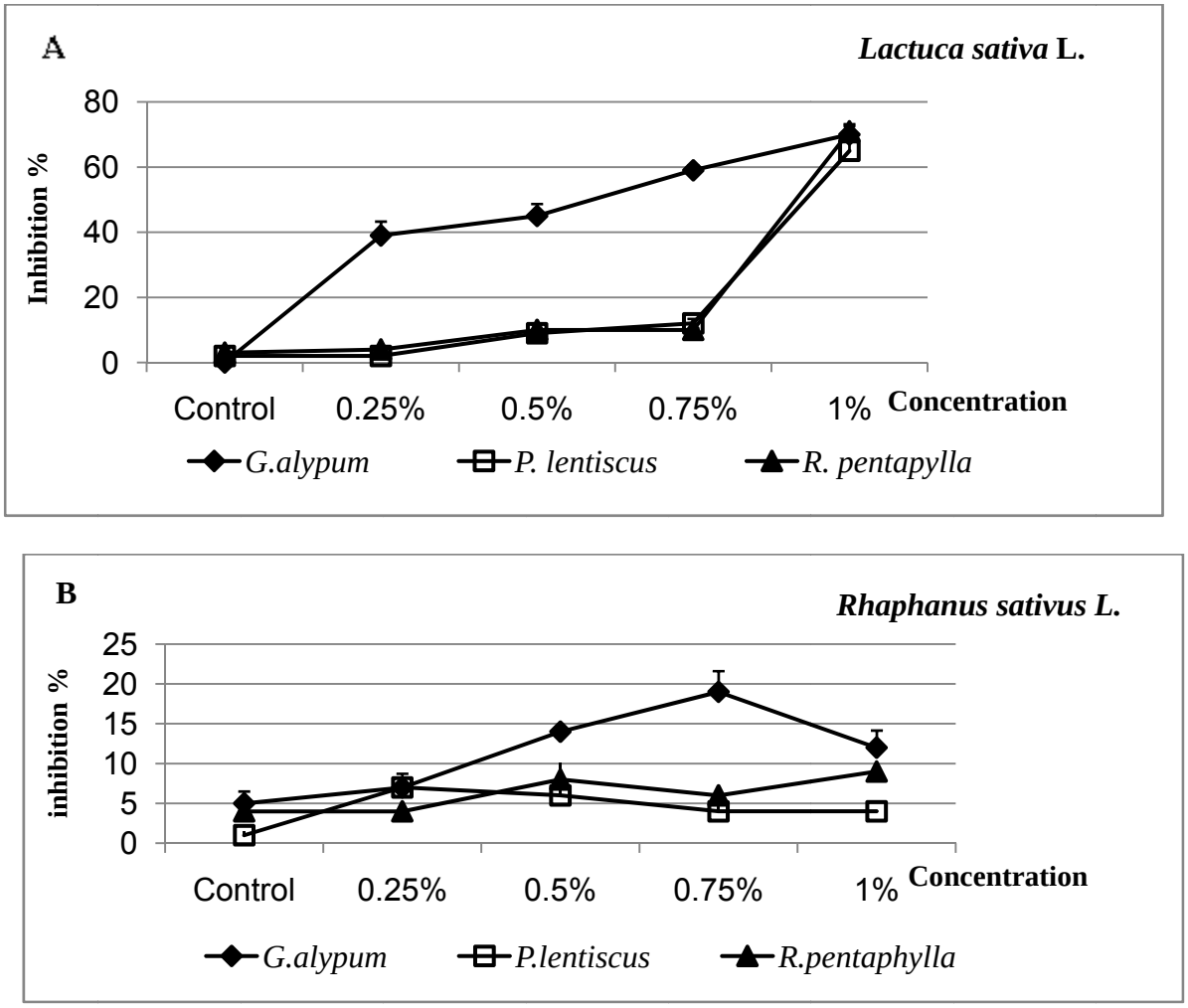


Fig.1: Effect of *Globularia alypum* L., *Pistacia lentiscus* L. and *Rhus pentaphylla* L. aqueous extracts on germination of *Lactuca sativa* L. and *Rhaphanus sativus* L.

These results were statistically significant. **(Table 06, Table 07)**

Effect of the plant extracts on the growth of *Lactuca sativa* L. and *Rhaphanus sativus* L.
The aqueous extract of *Peganum harmala* L. presents a very important inhibiting effect on the growth of the seedlings of *Lactuca sativa* L. and *Rhaphanus sativus* L. This effect increases gradually with the concentrations increase. For the two experimental models, an important necrosis on roots (for all the concentrations) occurs. (**Fig. 2; Photograph 1**). The aqueous extract of *Ruta chalepensis* L. presents an inhibiting effect on the growth of the seedlings of the two experimental models and that for all the concentrations tested. It is also noticed that this inhibiting effect is more important on the seedlings of *Lactuca sativa* L. (**Fig. 2A**)
According to the results obtained, the aqueous extract of *Haloxylon scoparium* presents an inhibiting effect of the growth on the seedlings of *Lactuca sativa* L. and of *Rhaphanus sativus* L with the presence of important necrosis on the roots for all the concentrations used. This inhibiting effect is more net on the seedlings of *Lactuca sativa* L than on those of *Raphanus sativus*. (**Fig. 2**)

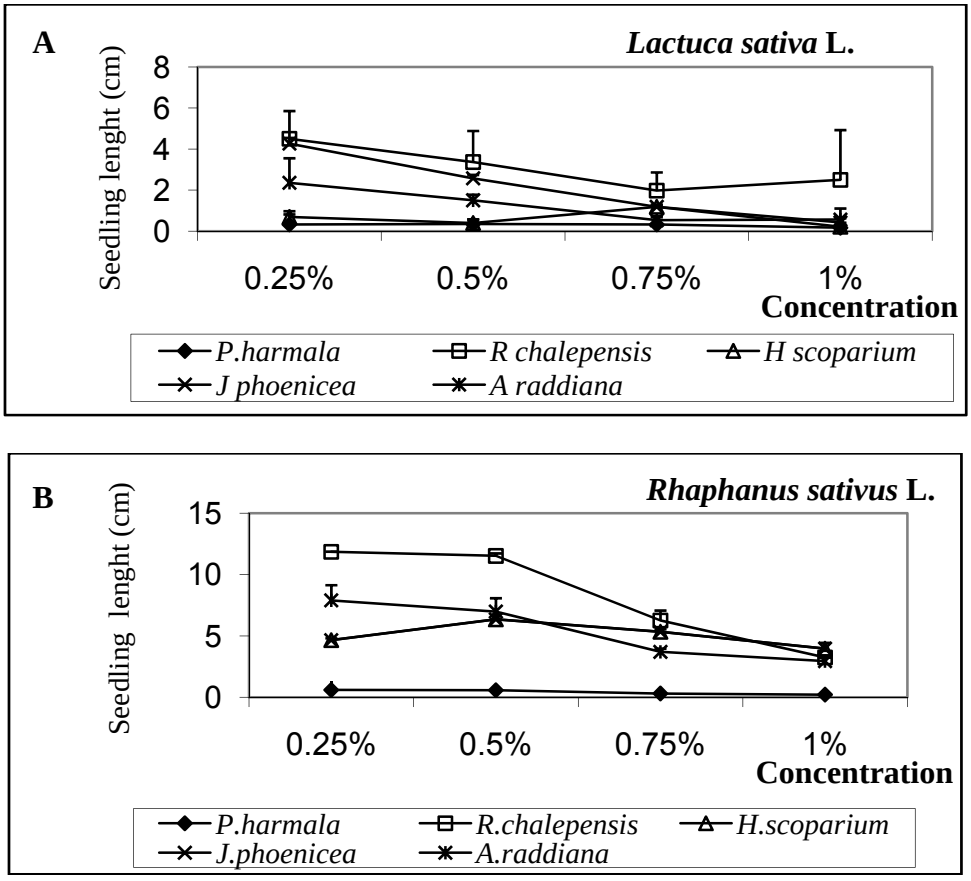
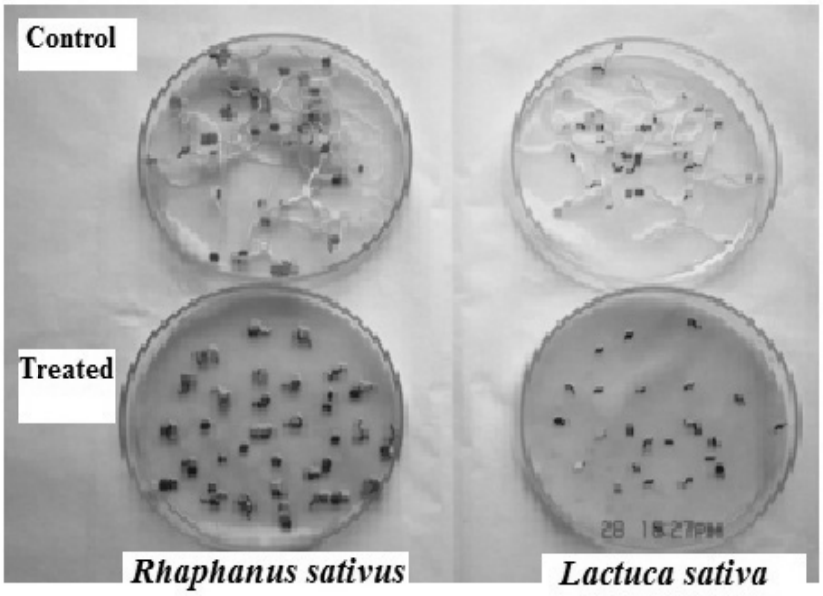


Fig.2: Effect of plant extract on growth of *Lactuca sativa* L. and *Rhaphanus sativus* L.



Photograph1: Effect of aqueous extract of *P. harmala* on growth of *Lactuca sativa* L. and *Rhaphanus sativus* L.

According to the table (1), it noticed that the length of the roots of the two experimental models decreased with the concentration of *Juniperus phoenicea* L extract. (**Fig.2A**). On the seedlings of *Lactuca sativa* L. , it noted a weak germination at the concentrations 0.75 % and 1 % with an important inhibition of the growth of the seedlings reaching a value of 95 % at the maximum concentration of the extract. An important necroses were also noticed for all the concentrations tested.

A low level of growth of the seedlings of *Lactuca sativa* L. is noted starting from the concentration 0.25% with a inhibiting percentage value of 67 % and reaching a value of 92% at the concentration 1% accompanied by important necrosis on the roots(**Fig. 2A**). On the other hand; the inhibition of the growth of the seedlings of *Rhaphanus sativus* L. is noticed only starting from the strong concentrations in aqueous extract of *Acacia raddiana* (0.75; 1 %) with the presence of strong necrosis at the concentration 1% (**Fig. 2B**).

These results were statistically significant. **(Table08)(Table09)**

Seasonal variation of allelopathic activity of *Tetraclinis articulata* (Vahl.) Mast

The aqueous extract of the leaves of *Tetraclinis articulata* (Vahl) Mast collected in June and November gives the strongest inhibiting action of the germination of seeds of *Lactuca sativa* L. and that for all the concentrations tested. **(Photograph 2)** This action is maximum at the concentration 1 % (97 and 99 % of inhibition respectively), whereas it is weak for *Rhaphanus sativus* L. **(Fig 3B)**

For January month, the germination inhibition remains weak for the both experimental models; except of *T. articulata* at the extract that with the concentration 1 % where it reaches 56 % for *Lactuca sativa* L. **(Fig 3A)**

The germination inhibition is very weak for the both experimental models treaties by the aqueous extract of *T. articulata* collected in April and that for all the concentrations tested. **(Fig 3)**

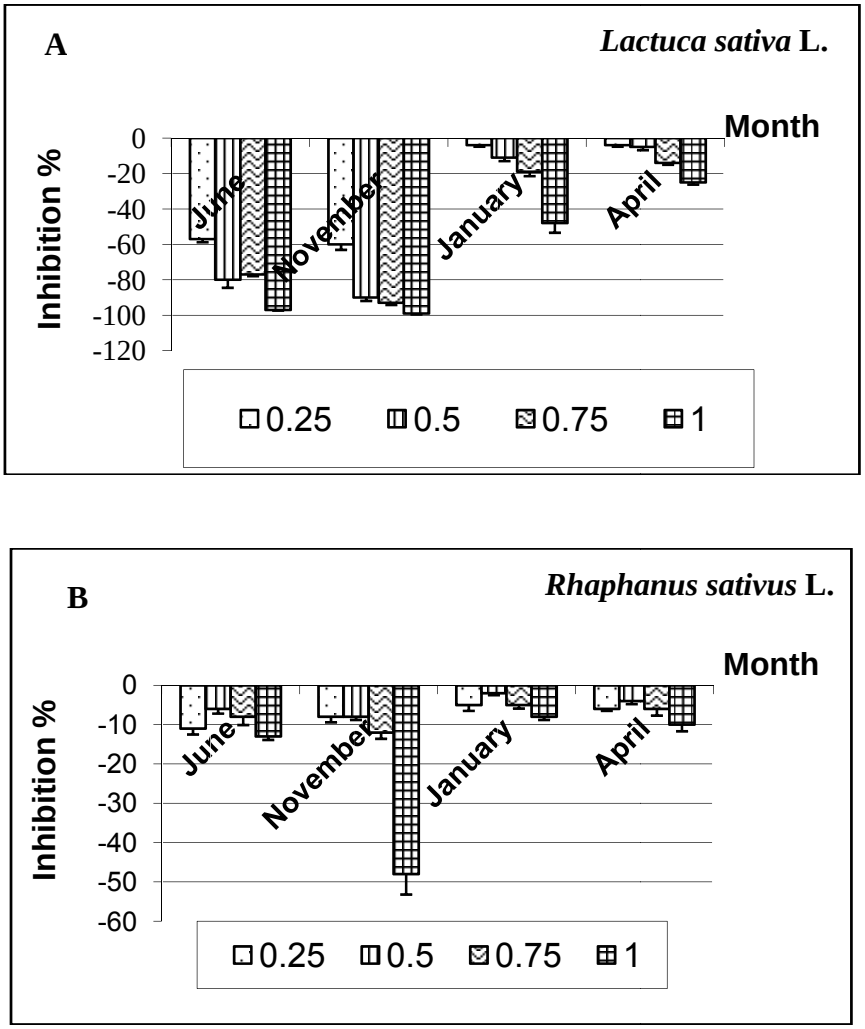


Fig.3: Seasonal variation of germination inhibitory activity of *Tetraclinis articulata* (Vahl) Mast. on *Lactuca sativa* L. and *Rhaphanus sativus*.

Photograph 2: Effect of aqueous extract of *Tetraclinis articulata* leaves on germination of *Lactuca sativa* L.

These results were statistically significant. **(Table 10, Table 11)**

Phenolics and the flavonoïds Content

Table 2: Phenolics and the flavonoïds contents (mg/g DW) of the extracts used (the results are expressd as means ± SD, n=3 for each species).

Species	Used part	Total phenolic content (mg/g)	Flavonoïds content (mg/g)
Acacia raddiana	Cortex	266.821±9.142	115.366±11.283
Globularia alypum	Roots	210.798±9.222	66.828±6.568
Haloxylon scoparium	Leaves	163.169±7.053	39.334±7.421
Juniperus phoenicea	Leaves	199.663±7.327	49.258±10.651
Peganum harmala	Seeds	72.783±2.518	15.244±1.769
Pistacia lentiscus	Leaves	349.843±21.796	68.443±1.663
Rhus pentaphylla	Leaves	94.227±7.220	35.555±0.726
Ruta chalepensis	Leaves	66.954±6.840	16.693±0.23

Table 3: Phenolics and the flavonoïds Content (mg/g DW) of *Tetraclinis articulata* (Vahl) Mast leaves according to the seasons (the results are expressed as means ± SD, n=3 for each sample).

Sample	Total phenolics contents	Flavonoïds contents
June 2007	163.691±3.208	49.455±1.714
November 2007	155.166±3.637	57.449±7.831
January 2008	111.103±6.248	27.480±4.133
April 2008	206.187±16.612	66.389±7.242

Phytochemical analysis

Table 4: Recapitulatory results of phytochemical screening of aqueous extract with have germination effect.

	Species			
phytoconstituants	*G. alypum*	*P .lentiscus*	*R. pentaphylla*	*T.articulata*
Phenolic acid	-	-	-	-
Alkaloids	-	-	-	-
Anthrones and anthranols	-	-	+	-
Coumarins	+	+	+	+

Anthracenic derived	-	-	+	-
Flavonoïds	+	+	+	-
Cardiotonic glycosides	+	-	-	-
Lignans	+	-	-	-
Quinones	-	-	-	-
Saponins	+	-	-	-
lactones Sesquiterpenes	-	+	-	-
Triterpenes	-	-	-	-

(-): the absent of compounds, (+): the presence of compounds.

Table 5: Recapitulatory results of phytochemical screening of aqueous extract with have growth effect.

	Species				
phytoconstituants	*A. raddiana*	*H. scoparium*	*J. phoenicea*	*P. harmala*	*R. chalepensis*
Phenolic acids	+	-	-	-	-
Alkaloids	-	-	-	-	-
Anthrones and anthranols	-	-	-	-	-
Coumarins	+	+	-	+	+
Anthracenic derivatives	-	-	-	-	-
Flavonoïds	+	-	-	+	+
Cardiotonic glycosides	+	-	-	-	-
Lignans	-	-	-	-	-
Quinones	-	-	-	-	-
Saponins	-	-	-	-	-
lactones Sesquiterpenes	+	-	-	-	-
Triterpenes	-	-	-	-	-

(-): the absent of compounds, (+): the presence of compounds.

Discussion

This work determines the existence of allelopathic phenomenon in experimental conditions. It provides the proof that the plant contains allelochimic compounds whose action can be potentially exerted in natural conditions.

The aqueous extract of *Tetraclinis articulata* presents a strong effect of inhibition on the germination of *Lactuca sativa* L. and weak on *Rhaphanus sativus* L. but the aqueous extracts of *Peganum harmala* L., *Haloxylon scoparium* and *Ruta chalepensis* exert a negative impact very marked on the viability and the growth of the seedlings for both experimental models.

Viles and Reese (1996) reported that aqueous extracts of *Echinacea angustifolia*, have the possibility of preventing the germination of seeds and the growth of the seedlings of *Lactuca sativa.*

According to Serghini *et al.* (2001) the extract of sunflower roots (*Helianthus annuus* L) has an effect on germination of the *Orobanche ramosa* but does not have any effect on germination of the *Orobanche cernua*. On the other hand, other species are able to prevent the germination of *Orobanche cernua* such as: *Nicotiana tabacum*, *Helianthus tuberosus*, *Solanum tuberosum*.

Among the aqueous extracts of *T. articulata* resulting collected in different periods from the year 2007-2008, only those of November and June present a very strong allelopathic activity which results in an inhibiting action of the germination of seeds of *Lactuca sativa* L for all the concentrations tested and more particularly at 1 % (97 % and 99 % of inhibition). The inhibiting effect of these extracts on the germination of *Rhaphanus sativus* L. is always less important.

The factors of the environments such as the geography, the temperature, the length of the day and food etc play a main role and important in the composition of the allelochemical substances, and affect their production in plant. (Robles *et al.*, 1999)

According to Perrot and Paris (1971), the content of active ingredients of a medicinal plant varies with the part's plant, the age of the plant and the time of harvest like with the varieties or races.

Eman and Salama (2013) indicated that cold and hot aqueous extracts of *Lantana camara* L (donor species) exhibit strong inhibitory allelopathic effect on the germination process of *Phalaris minor Retz.* and *Sorghum bicolorL. (Moench)* (Recipient species).

Germination is not the only developmental stage of the plant which can be affected by the allelopathic substances. The aqueous extracts of *P. harmala* (seeds), *R. chalepensis* (leaves), *H. scoparium* (leaves), present a very important negative effect on the growth of the seedlings of *Lactuca sativa* and *Rhaphanus sativus.*

The phytochemical analysis of our extracts revealed a certain number of secondary metabolites: phenolic acids, flavonoïds, lactones sesquiterpenes and saponins. The germination inhibiting effect may be due to these substances.

Several works showed the toxic action of various substances. Abdelgaleil and Hashinaga (2007) reported that sesquiterpenes of the extract of leaf of *Magniolia grandiflora* cause the inhibition of the germination of wheat (*Triticum aestivum*), lettuce (*Lactuca sativa* L), radish (*Rhaphanus sativus* L) and onion (*Allium cepa*). The lettuce seeds are most sensitive compared to seeds of wheat, radish and onion which are affected only by a strong sesquiterpens concentration.

Kil and Lee (1987) noted that the aqueous extracts of *Chrysanthemum morifolium* L. prevent the germination of seeds of several species in experimental conditions; phenolic acids of this species, identified by gas chromatography, can be responsible for the allellopathic effect

observed. Indeed, Beninger *et al.,* (2003) showed that the phenolic acids and the flavonoïds of *Chrysanthemum morifolium* L are responsible for the allelopathic activity of the extracts of leaves.
Ben Hammouda *et al.* (1995) noted that the inhibition of the growth of the wheat radicals varies with the various concentrations of total phenolic compounds in the various parts of Sorghum
Einhellig and Eckrich (1984) showed that the p-coumaric acid has an inhibiting effect on the growth of *Vicia faba* L. Vaughan and Ord (1990) observed that the p-coumaric acid and the hydroxybenzoic acid inhibit the growth of the roots of *Pisum sativum* L. the ferulic acid inhibits the growth of the roots of cucumber, tomato, broad bean (Blum *et al.,* 1999) and maize (Devi and Prasad, 1996). Another phenolic compound such as the catechine inhibits the germination and the growth of various plants (Weir *et al.,* 2003**).**
Studies on phytotoxic extracts of Cynodon dactylon showed that the weed extracts contained several phenolic compounds such as ferulic, vanillic, p-hydroxybenzoic and p-coumaric acids (Homa, 2010). As allelochemicals, long fatty acids and esters were identified in many species, like the wheat (Dong *et al.,* 2005), *Echinochloa crusgalli* (Xuan *et al.,* 2006) and *Cucumis sativus* L. (Yu *et al.,* 1994).

Conclusion

This study showed the allelopathic potentialities of the various aqueous extracts of plants tested.
The effects of the aqueous extracts of *Tetraclinis articulata, Globularia alypum, Pistacia lentiscus, Rhus pentapylla, Peganum harmala, Ruta chalepensis, Haloxylon scoparium, Juniperus phoenicea* and *Acacia raddiana* are relative to the species.
Some extracts studied have inhibiting effect on germination like *T. articulata* (the strongest effect), *G. alypum*, *P. lentiscus* and *R. pentapylla* as well on seeds of *Lactuca sativa* and *Rhaphanus sativus*. Others cause an inhibiting effect on their growth like: *P. harmala* (the most important effect*), R. chalepensis, H. scoparium, J. phoenicea* and *A. raddiana*.
From the follow-up of the seasonal variation of the allelopathic activity of the aqueous extract of *T. articulata* we observe that this one is more important in November and June.
The phytochemical compounds identified in these plants belong to the secondary metabolism: phenolic acids, lignans, flavonoïds, coumarins, cardiotonic glycosides, sesquiterpenes, saponins, anthracenic derivatives (anthrones and anthranols) and phenolic acids. These substances may be responsible for the allelopathic effect observed.
The selective allelopathic effects can be of considerable interest for the control of weeds in the crops cultures. Indeed, the allelopathy may replace nefast phytosanitary products for the environment. Contrary to the weedkillers which must be applied regularly and which see their concentration in the soil decreasing during time, the natural allelopathic substances are continuously released in the soil. The incorporation of the allelopathic characteristics of the species wild or cultivated in the crop plants by the traditional crossings or the methods of genetic modifications could induce the allelochemicals biosynthesis and the release of these compounds in the soil. The species with the allelopathic capacity can also be planted with the cultivated variety (this late is insensitive to the introduced plant) in order to protect it from wild weeds.

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Supplement materials: Stastistical analysis

Table 6: Effect of the aqueous plant extracts on the germination of *Lactuca sativa* L.

	Somme des carrés	ddl	Moyenne des carrés	F	Signification
Inter-groupes	7572,000	19	398,526	7,804	,000*
Intra-groupes	3064,000	60	51,067		
Total	10636,000	79			

* significant at α=0,05

Table 7: Effect of the aqueous plant extracts on the germination of *Rhaphanus sativus*

	Somme des carrés	ddl	Moyenne des carrés	F	Signification
Inter-groupes	92087,000	19	4846,684	65,319	,000*
Intra-groupes	4452,000	60	74,200		
Total	96539,000	79			

* significant at α=0,05

Table 8: Effect of the plant extracts on the growth of *Lactuca sativa* L., Variance analysis:

	Somme des carrés	ddl	Moyenne des carrés	F	Signification
Inter-groupes	618,833	24	25,785	128,242	,000*
Intra-groupes	15,080	75	,201		
Total	633,913	99			

* significant at α=0,05

Table 9: Effect of the plant extracts on the growth of *Rhaphanus sativus* L., Variance analysis:

	Somme des carrés	ddl	Moyenne des carrés	F	Signification
Inter-groupes	1786,169	24	74,424	78,880	,000*
Intra-groupes	70,763	75	,944		
Total	1856,932	99			

* significant at α=0,05

Table 10: Allelopathic activity of *Tetraclinis articulata* seasonal variation on *Lactuca sativa*, Variance analysis

	Somme des carrés	ddl	Moyenne des carrés	F	Signification
Inter-groupes	109820,800	19	5780,042	73,475	,000*
Intra-groupes	4720,000	60	78,667		
Total	114540,800	79			

* significant at α=0,05

Table 11: Allelopathic activity of *Tetraclinis articulata* seasonal variation on *Rhaphanus sativus* , Variance analysis

	Somme des carrés	ddl	Moyenne des carrés	F	Signification
Inter-groupes	7204,800	19	379,200	7,966	,000*
Intra-groupes	2856,000	60	47,600		
Total	10060,800	79			

* significant at α=0,05

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