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Allelopathic effect of *Tetraclinis articulata* (vahl) mast (from Algeria) on germination and growth of *Lactuca sativa* L.

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The present study deal with an endemic medicinal plant from western Algeria extract allelopathic activity (Tetraclinis articulata) on germination and growth for lactuca sativa L. The tested seeds were germinated in Petri dishes. Increasing concentrations (0.25, 0.50, 0.75 and 1%) extracts effect was tested on germination and growth of Lactuca sativa L. Germination test of different fractions from liquid- liquid partition of Tetraclinis articulata (chloroform, ethyl acetat and butanolic fractions). At the same time, three T. articulata extracts at different concentrations (10%, 30% and 50%) were prepared and tested on Lactuca sativa seeds in pots. We also tested these extracts at different concentrations 1.5 and 3% on Lactuca sativa seedlings (foliar spray). Inhibitory effects with variable intensities were observed on L. sativa the seeds germination and growth. The aqueous extract of T. articulata exhibits the strongest inhibition activity on L. sativa seeds at all tested concentrations. T. articulata ethyl acetate and chloroform fractions exhibit Lactuca sativa germination inhibition while butanolic fraction exhibits growth inhibition. Germination in pots mixed with crude extract of T. articulata for all tested concentrations shows inhibition on germination and for the foliar spraying, growth inhibition is observed for different fractions and all the concentrations tested. It is noted that Tetraclinis articulata contains allelopathic compounds that can replace synthetic bioherbicides 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.

Keywords: Allelopathy activity, Tetraclinis articulata, germination, growth, Lactuca sativa.

INTRODUCTION

The term allelopathy was coined by Prof. Hans Molisch in 1937, combining two Greek words "allelo" and "pathos" literally meaning "mutual suffering". Based on that concept, Rice (1984) defined allelopathy as the direct or indirect harmful or beneficial effects of one plant or another through the production of chemical compounds that escape into the environment. Allelopathy is chemical interference in which a plant releases chemicals that exert an effect on associated plants. These are phytochemicals synthesized in plants as secondary metabolites that appear to have no direct functions in their growth and development but serve for defensive adaptation. The significance of their synthesis seems to be an interaction between the plant and its environment and is called allelochemicals. Allelopathy offers the potential for biorational weed control through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots of living or decomposing plant materials (Weston, 1996). Also, allelopathy is generally accepted as a significant ecological factor in determining the structure and composition of plant communities (Scrivanti et al., 2003).

Tetraclinis articulata (Vahl) Mast. belongs to the family *Cupressaceae* and is native plant of the South-Western Mediterranean, mainly North Africa. It is an important element of the Maghreb vegetation (Morocco, Algeria and Tunisia). (Bourkhiss et al., 2010).

In Algeria, *T. articulata*, popularly known as "*Al'Araar*", is one of the most popular herbs, used extensively in folk medicine (Montanari, 2014). It is considered as an important flavoring agent, commonly used in the herbal tea, and very much appreciated for its smell as well as for its antiinflammatory and antioxydant properties (Bellakhdar et al., 1998).

MATERIALS AND METHODS

Experimental models and plant tested

The seeds of *Lactuca sativa* L. are of commercial origin. The plant materials used in the treatment is *Tetraclinis articulata* (Vahl) Mast. (Leaves), harvested in June 2014 at Misserghin Oran, Algeria. The botanical identification confirmation was performed by Marouf Abderrazak, Professor at the University Centre of Naama, Algeria.

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.

Partitioned successive liquid liquid extraction

This method was used for the purpose of separating the compounds from the aqueous extract of *T. articulata* using four solvents of different polarities. The solvents used are *n*-hexane, chloroform, ethyl acetate and *n*-butanol.

Petri-dish experiment

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.

Pot experiment

Pot experiment was performed to test the effect of different levels of the donor species crude powder mixed with potting and sandy soils on some growth parameters and germination of *Lactuca sativa*. Five seeds of each of the recipient species were sown in plastic pots with about 100 g of each potting and sandy soils thoroughly mixed (w/w) with 10, 30 and 50% of crude powder of the *T. articulata*.

Foliar spray bioassay

Plastic pots of 7 cm diameter and 8 cm depth were filled with sandy loam soil at 100 g soil per pot. Aqueous extracts of 1,5 and 3% of different fraction of *T. articulata*. These extracts were sprayed on pot grown *Lactuca sativa* plants after 0, 10 and 20 days of germination. Plants in the control treatment were sprayed with distilled water. Plants were harvested after 30 days and data regarding root and shoot biomass were determined.

Statistical analysis

Stastistical computations were performed using SPSS Software (version20). The data of extracts effects were subjected to one-way analysis of variance (ANOVA)

RESULTS AND DISCUSSION

Germination and early seedling growth bioassays

The aqueous extract of *T. articulata* present a strong inhibition of the germination of *Lactuca sativa* L. for all concentrations tested. The inhibition of germination is noticed starting from the concentration 0, 25%; the inhibiting effect is maximum for the 1% treatment. (Fig.1)

The results from bioassay tests showed that aqueous extracts from terrestrial Saururaceae contained water-soluble substances inhibiting seed germination and growth of *L. sativa*, *Echinochloa* and *Monocharia*. Also, the study suggested that the inhibitory natural substances



present in Saururaceae plants could be used as a

potential natural herbicide (Lin et al., 2006).

Figure1. Effect of aqueous extract of *Tetraclinis articulata* leaves on germination of *Lactuca sativa*



Figure. 2 Effect of the fractions of chloroform, ethyl acetate and butanolic of *T. articulata* on germination of *Lactuca sativa*.

Additionally, Kil et al., (2002) reported that an inhibitory effect of *Tagetes minuta* extracts on germination and growth of *L. sativa* was also recorded.

Allelochemicals presented in aqueous extract of different plant species have been reported to affect different physiological processes through their effects on enzymes responsibles for plant hormone synthesis and were found to associate with inhibition of nutrients and ion absorption by affecting plasma membrane permeability (Qasem and Foy, 2001; Qasem and Hassan, 2003). The inhibitory or stimulatory effects of leachates may be due to certain chemicals released from foliage parts including allelochemicals, amino acids, carbohydrates and phytohormones (Tukey, 1969). Incorporation of dried shoot residues of certain medicinal species in the soil showed varied effects on weed seed germination and seedlings growth, depending on source of residue used and weeds tested (Qasem and Hassan, 2003).

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.

At the seven day of the treatment, germination percentage was calculated and it was observed that, chloroform and ethyl acetate fractions of *T. articulata* significantly reduced the germination; confirming the presence of the bioactive allelochemicals in the fractions. Chloroform extract of *T. articulata* were highly toxic to germinating lettuce and for ethyl acetate extract the inhibition of the germination is noticed for all concentrations tested. (Fig.3) For the butanol extract of *T. articulata* (leaves), present a very important negative effect on the growth of the seedlings of *Lactuca sativa*.

Data of the present study demonstrated that seedling length was significantly affected due to the apparent allelopathic action of butanol extract concentrations. (Fig.3)



Figure 3 Effect of the butanolic fraction of *T. articulata* on growth of *Lactuca sativa*.

Ali Khan et al., (2011) Water, methanolic and butanolic fractions showed marked growth inhibition of root and shoot while *n*-hexane and ethyl acetate fraction of both plants showed moderate effects.

Pot experiment

The allelopathic effects of the crude powder of *T. articulata* on shoot and root lengths of *Lactuca sativa* is represented in Table1.

Generally, shoot length decreased with the increase in treatment concentrations. Compared to control, root length of T. articulata exhibit a significant reduction along gradual concentrations. Generally, the effect on leaf number (LN) is significant with the increase in treatment concentrations. Fresh weight of T. articulata was significantly affected by different concentrations. (Fig 4). The crude powder of *T. articulata* affect some growth parameters like shoot length, root length, number of leaves and fresh weight of lettuce. Leaf and root lengthening inhibited effectively. Similar results are obtained by Khan et al., (1999) on wheat and maize and also in many similar studies with eucalyptus. The workers reported similar finding through their work on weed and crop species. Travlos et al., (2018) reported that velvet bean leaf and chia inflorescence tissues had a significant allelopathic activity on seedling emergence and growth parameters.

Reduction in shoot and root growth of *Lactuca* sativa may be due to the effect of certain allelochemicals on cell division and elongation, resulted in a short root system and small shoot growth.

Foliar spray bioassay

Foliar spray with both 1,5 and 3 % *T. articulata* extracts significantly reduced root and shoot lengths of *Lactuca sativa*. We notice a weak growth for both shoot and root length. The root and shoot biomass of *Lactuca sativa* plant was significantly affected by sprays of both 1,5 and 3% aqueous extracts. Similar inhibitory effects of foliar sprays of *T. articulata* butanolic and ethyl acetate extracts have also been reported. (Table2, Fig.5)

 Table 1 : Effect of crude powder of *T. articulata* on germination and early seedling growth of Lactuca sativa.

Species	Treatement (%)	Germination (%)	Shoot lenght (cm)	Root length (cm)	Fresh weight (g)	Number of leaves
Control	0	86.67 ±23.09 a	4.4 ±1.082 a	9.12±1.76 a	2.737±0.37 a	3.67±0.58 a
T. articulata	10	80±20 a	1.2±0.87 b	4.93±5.52 a	0.163±0.029 b	1.33±0.58 b
	30	66.67±23.09 a b	1.23±0.67 b	3.93±3.76 a	0.477±0.38 b	1.67±0.58 b
	50	16.67±15.27 b	0.3±0.43 b	1.1±1.82 a	0.03±0.03 b	0.67±0.58 b



Figure .4 Effect of a crude powder of *Tetraclinis articulata* on germination and growth of *Lactuca sativa*

Lactuca Sativa.										
	Treatment (%)	Shoot length (cm)	Root length (cm)	Fresh shoot weight (mg)	Fresh root weight(mg)	Number of the leaves				
control	0	2.27 ±0.15a	13.17±9.5a	0.29±0.1	0.27±0.071ab	4±0a				
<i>T.articulata</i> aqueous extract	1.5	1.3±0.2b	2.82±1.14a	0.117±0.03b	0.06±0.06c	2.67±0.57b				
	3	1.7±0.17ab	11.8±8.83a	0.22±0.02ab	0.39±0.02b	3.67±ab0.58ab				
<i>T.articulata</i> Butanol extract	1.5	1.5±0.17b	8.3±6.23a	0.19±0.006ab	0.23±0.06abc	3±0ab				
	3	1.17±0.05b7	6.5±6.22a	0.15±0.005b	0.19±0.06abc	2.67±0b				
<i>T.articulata</i> Ethyl acetate extract	1.5	1.367±0.40b	5.8±3.64a	0.15±0.03b	0.11±0.011ac	3±0ab				
	3	1.63±0.41ab	7.86±5.44a	0.22±0.02ab	0.27±0.106ab	3±0ab				

 Table 2: Effect of aqueous, butanolic and etyl acetat extracts of *T.articulata* on seedling growth of *Lactuca sativa*.



Figure.5 Effect foliar sprays of aqueous, butanolic and ethyl acetate extracts of *T. articulata* on growth of *Lactuca sativa*.

Foliar spray bioassay

Foliar spray with both 1,5 and 3 % *T. articulata* extracts significantly reduced root and shoot lengths of *Lactuca sativa*. We notice a weak growth for both shoot and root length. The root and shoot biomass of *Lactuca sativa* plant was significantly affected by sprays of both 1,5 and 3% aqueous extracts. Similar inhibitory effects of foliar sprays of *T. articulata* butanolic and ethyl acetate extracts have also been reported. (Table2, Fig.5)

The root and shoot biomass of *Lactuca sativa* was significantly decreased by sprays of both 1,5 and 3% extracts. Similar inhibitory effect of foliar sprays of sorghum extract has also been reported against other weed species (Javaid et al., 2006). Cheema et al., (2000) found that two foliar sprays of sorghum extracts significantly reduced weed density and biomass in cotton.

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).

CONCLUSION

The result of present study indicated that *Tetraclinis articulata* (Vahl.) Mast. had a significant allelopathic activity on germination and seedling growth of *Lactuca sativa*.

From these data, it was inferred that, *Tetraclinis articulata* chloroform, ethyl acetate and butanolic fractions have significant herbicidal potency, which might be the presence of allelochemicals. Therefore, further study on the isolation and purification of these allelochemicals are suggested. The crude powder of different concentrations of *Tetraclinis articulata* affect some growth parameters like shoot length, root length, number of leaves and shoot and root fresh weight of *lactuca sativa*. For foliar spraying with *T. articulata* extracts significantly reduced root and shoot lengths of *Lactuca sativa*. It provides that *T. articulata* extract has significant herbicidal effects on the germination and growth of lettuce.

The indicated allelopathic activity of *T. articulata* (Vahl.) Mast. could be further exploited in future studies, by using either allelochemicals as models for future herbicides or the plants as useful cover crops or mulches in terms of an integrated weed management system.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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