Libyan Journal of Ecological & Environmental Sciences and Technology
(LJEEST)
www.srcest.org.ly/jou

Effect of Mastic Shrub (*Pistacia lentiscus* L.) Aqueous Extract on Early Growth Stages of Radish and Fenugreek

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ARTICLE INFO

Vol. 1 No. 1 June, 2019
Pages (19 - 24)

Article history:
Revised form 30 April 2019
Accepted 29 Mayy 2019

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Keywords:
Allelopathy; Aqueous extract; Germination percentage; Pistacia lentiscus L.; Raphanus sativus L.

ABSTRACT

The effects of the aqueous extracts of aerial parts of mastic shrub (Pistacia lentiscus L.) at different concentrations (20, 40, 80%) were evaluated on germination and seedling growth of (Radish) Raphanus sativus L. and (Fenugreek) Trigonella foenum-graecum after 7 days in "in vitro condition ". Pistacia had strong allelopathic effects, it reduced the germination and seedling growth of Radish and Fenugreek, in all tested concentrations. Results obtained showed significant inhibition in germination percentage (GP), plumule length (PL) and radicle length (RL) of both Radish and Fenugreek seeds, and the degree of inhibition was in concentration dependent. At 20,40 and 80% Pistacia lentiscus L aqueous extracts the germination percentage of Radish seed was reduced to (81.67, 71.67 and 55%) respectively, while the germination of Fenugreek seeds was reduced to (75, 56.67 and 45%) respectively, comparative to control which was 100% and 98.3% for Radish and Fenugreek respectively.

الملخص العربي

تم تقييم آثار المستخلصات المائية للأجزاء الهوائية من شجيرة المصطكى (*Pistacia lentiscus* L.) بتركيزات مختلفة (20 ، 40 ، 80٪) على إنبات ونمو الشتلات لنبات الفجل .*Raphanus sativus* L ونبات الحلبة *Trigonella foenum-graecum* بعد 7 أيام "في المختبر". كان لنبات البطم *Pistacia* آثار اليلوباثية قوية ، فقد قلل من نمو وإنبات شتلات الفجل والحلبة ، في جميع التركيزات المختبرة. وأظهرت النتائج التي تم الحصول عليها تثبيط كبير في نسبة الإنبات (GP) ، وطول الرويشة (PL) وطول الجذير(RL) فى كل من بذور الفجل والحلبة ، وكانت درجة التثبيط تعتمد على التركيز, حيث وجد انه عند التراكيز 20،40 و 80٪ من المستخلصات المائية لنبات البطم ، تم تقليل نسبة إنبات بذور الفجل إلى (81.67 ، 71.67 و 55٪) على التوالي ، في حين تم تقليل إنبات بذور الحلبة إلى (75 ، 56.67 و 45٪) على التوالي ، مقارنة مع السيطرة التي كانت 100 ٪ و 98.3 ٪ للفجل و الحلبة على التوالي.

INTRODUCTION

Allelopathy is a physiological process with ecological implications (Reigosa, Pedrol & Gonzalez, 2006). Also, allelopathy is generally accepted as a significant ecological factor in determining the plant growth, succession, dominance, distribution, species diversity, structure and composition of plant communities (Scrivanti, Zunino & Zygadlo, 2003). The term allelopathy refers to any method involving secondary metabolites (allelochemicals) created by plants, or microorganisms such as, bacteria, viruses and fungi that influence the growth and development of agricultural and biological systems as well as positive and negative effects. Allelochemicals from plants are produced by any organ of the plant and discharged into the environment by volatilization and exudation from roots, leaching from stems and leaves or decomposition of plant material (Rizvi, Tahir, Rizvi, Kohli, & Ansari,1999) Allelochemicals are mainly secondary metabolites which usually associated with plant defense against herbivores and pathogens, these distinctive compounds may be linked to wide range of ecological functions (Hussain & Reigosa, 2011). Allelochemicals that may affect the physiological processes of the plants such as respiration, cell division, water and nutrient uptake, oxidative stress and others. Most plant species, including wild plants, crops and trees are capable of producing such molecules into the environment to inhibit the development of neighboring plants (Wilson & Agnew,1992; Weir, Park & Vivanco,2004; Callaway & Vivanco,2006). Phenolic compounds are one in every of the biggest group of secondary metabolites, consisting of four main groups divided in keeping with the number of phenol rings and the structural parts that bind those rings, including flavonoids, phenolic acids, tannins, saponins, cinnamic acid coumarins, terpenoids, quinones, and lignans (Balasundram, Sundram, & Samman, 2006). Allelopathic effects can be stimulatory or inhibitory, depending on the identity of the active compound on the static and dynamic availability, persistence and fate of organics in the environment and on the particular target species (Keating,1999). *Pistacia lentiscus* L. is an evergreen shrub or small tree growing to 1–8 m tall belonging to the Anacardiaceae family which grows in many Mediterranean countries (Iauk, Ragusa, Rapisarda, Franco & Nicolosi,1996). *Pistacia,* a genus of flowering plants contains about twenty species, among them five species are more popular including *P. vera, P. atlantica, P. terebinthus, P. khinjuk,* and *P. lentiscus*, that are native to all of Africa, and southern Europe, warm and semi desert areas across Asia (Alma *et al*., 2004). The aerial part has traditionally been used as a stimulant, for its diuretic properties, and to treat hypertension, coughs, sore throats, eczema, stomach aches, kidney stones and jaundice2,3. Mastic gum from *Pistacia* has been used by traditional healers for the relief of upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer (Al-Habbal, Al-Habbal & Huwez, 1984). Various types of compounds like terpenoids, phenolic compounds, fatty acids, and sterols have been identified from different parts of *Pistacia* species. According to previous researches, wide pharmacological activities had been showed from various parts of Pistacia species such as anti-inflammatory,antitumor, antioxidant, antimicrobial, antiviral and their effects in gastrointestinal disorders improvement (Benhammou, Bekkara & Panovska,2008). *Pistacia lentiscus* L. distributed in the Mediterranean region up to700 m above sea level. In Libya, it is an important medicinal plant grownin several regions geographical in Al-Jabal AlAkhdar province (El-Gali, 2016). The study was aimed to evaluate the allelopathic effect of (mastic shrubs) *Pistacia lentiscus* L. on germination and seedling growth of (Radish) *Raphanus sativus* L. and (Fenugreek) *Trigonella foenum-graecum."*

MATERIALS AND METHODS

1.*Sources of the seeds*:

The seeds of Radish and Fenugreek were obtained from the local market. Al Bayda- Libya, and was defined the type of seeds through the Herbarium in the Department of Botany, and were kept in the containers which they were supplied, then stored in the laboratory at room temperature until required for sowing.

2.*Plant Material Collection*:

Pistacia lentiscus L. samples were collected from Shahat region the north of Al-Jabal Al-Akhdar – Libya, in October 2018, (aerial parts). The plant were classified and authenticated according to Jafri, & El-Gadi (1983) through the Herbarium in the Department of Botany, Faculty of Science, Omar Al-Mukhtar University, EL-Bayda, Libya.

3.*Preparing the aqueous extract of Pistacia lentiscus L.*:

Anumber of fresh samples from the aerial parts of the donor species were collected from the natural habitats in the study area during the vegetative stage. The samples were air- dried then ground in a Wiley Mill to fine uniform texture and stored in glass jars until use. Stock aqueous extract was obtained by soaking 50 g air-dried plant material in 500 ml of cold distilled water (10% w/v) at room temperature (20 ± 2^o C) for 24 hours with occasional shaking. The mixture was left on shaker (heidolph titramax 101) for 24 h in room temperature at speed of 120 rpm four-folded cotton fabric was used as filter to separate rough solid particles from solution. The contents were then filtered with Whatman filter paper, and then it was centrifuged in laboratory centrifuges (Thermo Electron Corporation, Sorvall RC 6 Plus) with the speed of 2000 rpm for 15 minutes (Bajalan, & Rezaee, 2013).

4.*Preparing the aqueous extract concentrations*:

Three concentrations (20, 40, and 80 %) of the solutions were prepared from the stock solution based on volume/volume percent (v/v)% (Elshatshat, 2010). In addition to the distilled water as control (0 %). To prepare solutions of different concentrations, doses of *Pistacia lentiscus* L. aqueous extract (20, 40 and 80 ml) were taken. Then, volume was completed to100 ml by adding distilled water to obtain (20, 40, and 80 %).

5.*Treatment of seeds with plant extracts* :

Sixty seed of each species (Radish and Fenugreek) were distributed in 12 petri dishes (5 seed in each dish) on two layers of Whatman filter paper No.1. Five ml of each prepared aqueous extract (20, 40, and 80 %) or distilled water as control (0%) were added in petri dishes (added daily).Three replicates were incubated in randomized complete block design at 20^o C in an incubator. Before sowing, the seeds were surface sterilized with 2% sodium hypochlorite for 2 minutes then rinsed four times with distilled water (Nesrine, El-Darier & EL-Taher,2011). The sterilized seeds were soaked in distilled water for 24 hours. The germination percentage (GP), plumule length (PL) and radicle length (RL) were recorded after one week at the end of the experiment (Nesrine *et al*., 2011).

A variety of parameters were used in this work to assess the effects of extracts on seed germination and seedlings development of test species. These parameters include:

1.Germination Percentage (GP): was calculated according to the following equation (Scott, Jones, & Williams, 1984).

$$\% \, Germination = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds} \times 100$$

2.Plumule Length (PL) and radicle length (RL): Length of plumule and radicle system was measured in cm using a ruler.

Statistical Analysis :

Statistical analysis was performed using a computer run program (Minitab software). One way ANOVA followed by Tukey, s HSD test was performed to show the statistical significance among the means of the groups. Results were expressed as mean ± Standard Division (SD). P-value below 0.05 was considered to be statistically significant (Ross, 2004).

RESULTS

A. *The allelopathic effect of Pistacia on Radish seeds*:

1.*Germination Percentage (GP)* : The germination percentage of Radish seeds was significantly affected by the increase at different concentration levels of *Pistacia* aqueous extract after seven days of germination. *Pistacia* aqueous extracts have inhibitory effect on germination and seedling growth of *Raphanus sativus* L. and considerably suppressed the germination compared to control treatment. The total percentage of Radish seeds germination was decreased by increasing the concentration of *Pistacia* aqueous extract, at control (0%) GP value was about (100%). While the percentage was reduced to (81.67%) and to (71.67%) at concentrations 20 and 40% *Pistacia lentiscus* L. aqueous extract, respectively. The maximum allelopathic effect was recorded in 80% *Pistacia lentiscus* L. aqueous extract concentration, which was 55% (Table1, Figure1)

2.*Plumule Length (PL):* Findings of PL of *Raphanus sativus* L. the inhibitory effect of the allelopathic substances on seedling stage. Evidently, PL was significantly reduced due to each main effect as treatment. Additionally, the value of PL was 4.2 cm at control level. While the percentage was reduced to 3.9 cm at concentration 20 %, and to 3.4 cm at concentration 40 %, the maximum effect was recorded at 80% *Pistacia* aqueous extract concentration, which was 3 cm (Table1, Figure1).

3.*Radicle Length (RL):* A decrease was observed among Radish RL assessment in seeds culture, compared to control value which was 6.1cm. Elevated *Pistacia* aqueous extracts concentrations have possessed a significant inhibitory effect on radical growth. At 20 % *Pistacia lentiscus* L. aqueous extracts concentration, it was 5.2 cm. Upon applying the highest *Pistacia* extracts concentration (80%), RL was 2.9 cm (Table1, Figure1).

Table1. Allelopathic effect of different concentrations of aqueous extract of *Pistacia lentiscus* L. on germination percentage (GP) and radicle (RL)and plumule (PL) length (cm) of *Raphanus sativus* L. (after 7 days).

Extract concentration %	**Seed germination Mean ± SD**	**GP %**	**RL(cm) Mean ± SD**	**PL(cm) Mean ± SD**
0	$20^{a} \pm 0$	100	$6.1^{a} \pm 0.09$	$4.2^{a} \pm 0.16$
20	$16.33^{b} \pm 0.47$	81.67	$5.2^{b} \pm 0.12$	$3.9^{b} \pm 0.08$
40	$14.33^{c} \pm 0.94$	71.67	$3.7^{c} \pm 0.16$	$3.4^{c} \pm 0.09$
80	$11^{d} \pm 0.82$	55	$2.9^{d} \pm 0.08$	$3^{d} \pm 0$

Data are expressed as mean ± SD of three replicate. Within each row, means with different superscript (a, b, c or d) were significantly different at p<0.05. Where means superscripts with the same letters mean that there is no significant difference (p>0.05).

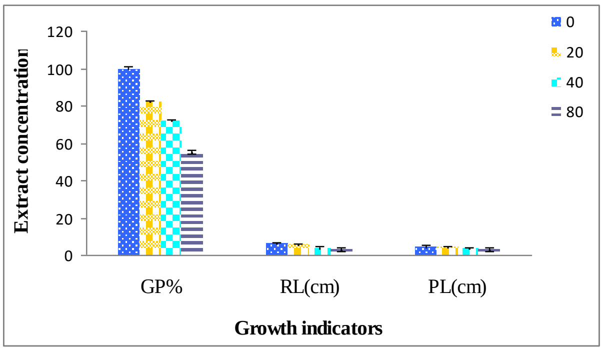


Figure1. Allelopathic effect of different concentrations of aqueous extract of *Pistacia lentiscus* L. on germination percentage (GP) and radicle (RL)and plumule (PL) length (cm) of *Raphanus sativus* L. (after 7 days).

B. *The allelopathic effect of Pistacia on Fenugreek seeds:*

1.*Germination Percentage (GP):* The allelopathic effects of *Pistacia lentiscus* L aqueous extracts on Fenugreek seeds was evaluated, the total percentage of Fenugreek seed germination was decreased by increasing the concentration of *Pistacia* aqueous extract, at control (0%) GP value was about (98.33%). The percentage was reduced to (75%) and to (56.67 %) at concentrations 20 and 40% *Pistacia lentiscus* L. aqueous extract, respectively. The maximum allelopathic effect was recorded in 80 % *Pistacia lentiscus* L. aqueous extract concentration, which was 45% (Table2, Figure2).

2.*Plumule Length (PL):* Evaluation of PL correlated with higher *Pistacia* aqueous extract concentrations has demonstrated their inhibitory influence on *Trigonella foenum-graecum* growth process (Table2, Figure2). The plumule elongation was not completely inhibited by the extract but it was less at higher concentration levels.

Obviously, all allelopathic concentrations have reduced PL. at control level PL of Fenugreek was 6.3 cm. On the other hand, 20, 40 and 80 % concentrations were considered as inhibitory concentrations (5, 3.7 and 2.5 cm) respectively.

3.*Radicle Length (RL):* Compared to control, a gradual decrease in RL of Fenugreek seed was observed along gradual increase in *Pistacia lentiscus* L. aqueous extracts concentrations. RL implication was significantly affected by the treatment. At control, the value of RL was 4.6 cm. And applying higher concentrations of *Pistacia lentiscus* aqueous extracts were notably active inhibiting radicle emergence. At 20 and 40 % concentrations, RL decreased to 3.9 and 2.8 cm respectively. Constantly, it continues reduction till it attained a value of about 1.8 cm at 80 % concentration level (Table 2, Figure2).

Table 2. Allelopathic effect of different concentrations of aqueous extract of *Pistacia lentiscus* L. on germination percentage (GP) and radicle (RL)and plumule (PL) length (cm) of *Trigonella foenum-graecum* (after 7 days).

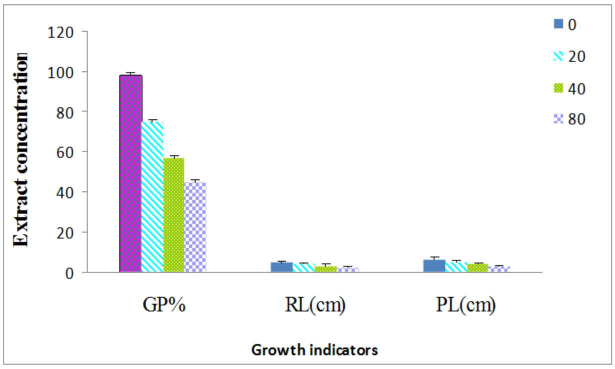
Extract concentration %	Seed germination Mean ± SD	GP %	RL(cm) Mean ± SD	PL(cm) Mean ± SD
0	19.67^{a} ± 0.47	98.33	4.6^{a} ± 0.09	6.3^{a} ± 0.16
20	15^{b} ± 0.82	75	3.9^{b} ± 0.08	5^{b} ± 0.05
40	11.33^{c} ± 0.94	56.67	2.8^{c} ± 0.17	3.7^{c} ± 0.2
80	9 ^{d}± 0	45	1.8^{d} ± 0.05	2.5^{d} ± 0 .12

Data are expressed as mean ± SD of three replicate. Within each row, means with different superscript (a, b, c or d) were significantly different at p<0.05. Where means superscripts with the same letters mean that there is no significant difference (p>0.05).

Figure2. Allelopathic effect of different concentrations of aqueous extract of *Pistacia lentiscus* L. on germination percentage (GP) and radicle (RL)and plumule (PL) length (cm) of *Trigonella foenum-graecum* (after 7 days).

DISCUSSION

Germination is an important indicator which depicts the plants response to changes in the environment resources or any allelopathic stress induced as a result of allelochemicals released from the donor plants (Hussain, Ahmad & Ilahi, 2010). Germination indices are generally



used to detect potential stimulatory or inhibitory allelopathic activity of the test plant (Hussain & Reigosa, 2014). The present work was carried out as a study to investigate any possible allelopathic activity *Pistacia lentiscus* L. aqueous extract on germination and seedling growth of *Raphanus sativus* L. and *Trigonella foenum-graecum*, the results showed severe toxicity at high extract concentration and moderate toxicity at low concentration. The highest germination rate of Radish and Fenugreek seeds was obtained from distilled water treatment (Control) which was 100% and 98.3% for Radish and Fenugreek respectively, and seed germination decreased with increasing concentrations of extract. In general the results showed that when concentration of extract increases, traits significantly decrease, the degree of inhibition was in concentration dependent. At 20,40 and 80% *Pistacia lentiscus* L aqueous extracts the germination percentage of Radish seed was reduced to (81.67, 71.67 and 55%) respectively, while the germination of Fenugreek seeds was reduced to (75, 56.67 and 45%) respectively, this can result from the increase in amount of allelochemicals and the toxicity characteristics (Batish, Singh, Kaur, Kohli & Yadav,2008). Aqueous extract of plants may contain phenolics such as ferulic acid P-coumaric, vanillic, caffeic, chlorogenic and others (Hussain & Khan,1988; Habib & Rehman,1988). These phenolics inhibit the germination process (Williams & Hoagland,1982; Al-Charchafchi, Redha & Kamal,1987). *Pistacia lentiscus* L. aqueous extract may contain some phytotoxic substances that inhibit germination and growth of Radish and Fenugreek. These results correlated with the findings that Allelochemicals presented in the aqueous extracts of different plant species commonly identified as allelopathic agents, which have inhibitory and/or lethal effects on seed germination growth and development , reduction in seedling growth and have been reported to effect on different physiological processes through their effects on enzymes responsible for phytohormone synthesis and were found to associate with inhibition of nutrients and ion absorption by affecting plasma membrane permeability (Batish, Kaur, Singh & Kohli,2007; Fateh, Sohrabi & Gerami,2012). Therefore, *Pistacia lentiscus* L. might release some soluble phenolic allelochemicals to the environment, which has a growth inhibitory effect on new seedling of both *Raphanus sativus* L. and *Trigonella foenum-graecum* or other plant species (Xu *et al.*, 2003). Our results are agreed with Escudero, Albert, Pita & Pérez-García, (2000) ; Periotto, Perez & Lima (2004) who reported that seeds of some species can be suppressed using water extracts from plants, and theses extracts can effect on germination behavior too. Various phytochemical compounds have been identified in Pistacia species, phenolic compounds, catechin, epicatechin, and gallic acid (Bozorgi *et al.*, 2013). The obtained results for the allelopathic activity of *P. lentiscus* confirm the allelopathic properties of Anacardiaceae family reported by other reports (Amri *et al.*, 2012; Zahed, Hosni, Brahim, Kallel & Sebei, 2010).

Thus, our results are in agreement with Huda, Laila, Amina & Fathia (2017) which reported the allelopathic effects of aerial parts, of *P. lentiscus***.**

CONCLUSION

Based on this study: the leaves of *P. lentiscus* species contain biologically active compounds soluble in water which have allelopathic potential must be examined for their selective action on other specific plants including weeds and crops under field conditions. There is possibility of using these allelochemicals as environment friendly herbicides to control weeds. Analysis of possible allelochemicals in this plant is also required.

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