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Allelopatic effect of *Prosopis juliflora* (Sw.) DC. On seed germination growth and development of *Vicia faba*

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ABSTRACT

The current study is an endeavor to recognize the allelopathic interactions of *P. juliflora*. Generally, a significant inhibition of some growth parameters and pigments by all concentrations of pods and leaves extracts of *P. juliflora* was recognized in this study. Aqueous extracts of *P. juliflora* pods and leaves were prepared at concentrations of (2%, 4%, 8%, 16% and 32%) the control was distilled water. The allelopathic effect of *P. juliflora* pods and leaves extract as a donor species on *Vicia faba* as a recipient species was demonstrated with respect to its dry weight, length, leaf area and pigment fractions. Remarkably, the effect of pods extract on dry weight and both shoot and root lengths of *V. faba* seedlings were significantly higher than the effect of leaves extract at different concentrations. Conversely, there no significant variation in leaf area as affected with the two extracts. Regarding pigment content, the lowest values of Chl.a, Chl.b, B-carot as well as total pigments were attained at 2% concentration of pods extract.

INTRODUCTION

The genus *Prosopis* L. belongs to the family Fabaceae and subfamily Mimosoideae. It encloses 44 species, of which 40 are native to Americas, three to Asia and one to Africa. *Prosopis juliflora* (Sw.) DC. is considered as an invasive weed in numerous countries, where it was introduced. It is a noxious invader in Ethiopia, Hawaii, Jamaica, Kenya, India, Nigeria, Senegal, Somalia, southwestern United States Sri Lanka, and Sudan, as well as the Middle East and Southern Africa. It is difficult and expensive to get rid of these weeds, where the plant can regenerate from the roots (**Gedyon, 2017)**. *Prosopis juliflora* has demonstrated to be a promising species for the driest zones of the Northeast's Semi-Arid Region, being one of the rare species which occur spontaneously in aridtropicalzones with annual rainfall below 100 mm **(Roy, 2011)**.It is either a shrub or a small tree which is highly documented for their usages as windbreakers, sand stabilizers, soil binders as well as their capability to grow in the poorest soils and to survive in areas where others trees cannot survive. In addition, it act as a weed controler and manager, antibacterial and in primary health care. It also serves as one of the main sources of fuel for the rural and urban poor in the country. It has high calorific value and the wood does not need long periods of storage and drying **(Prasad and Tewari,2016)**. It improves soil physiochemical and biological properties, generating fertility islands or resource islands beneath its canopy. Its pods can be used as a

livestock feed and for making human foods **(Khandelwal *et al*., 2015)**. The different species from genus *Prosopis* has important applications in medicinal products for human use and in veterinary medicine, include antidiabetic, antiinflammatory,anticancer, and antimicrobial activities. **(Santhaseelan *et al*., 2017)**. The allelopathic effects of *P. juliflora* on the associated flora depends significantly on the density and size of the canopy. Large individuals and great densities have significantly negative impact on the associated plants. The annuals were suppressed more than perennials. The annuals number with significant decreases in density and/or frequency under *P. juliflora* canopies was considerably greater than the number of perennials. Thus, the density of more than 50% of the associated annuals was significantly repressed under *P. juliflora* canopies. *P. juliflora* has little or no auto-inhibition because under field condition the density of *P.juliflora* seedlings was greater under the canopy of the same species than away from them **(El-Keblawy and Al-awai, 2006)**.

Allelopathic Effects

The plant foliage has allelopathic effects on seed germination and seedling growth of Bermuda grass (*Cynodon dactylon*), three cultivars of *Zea mays* L. (R 796, Gohar, EV 1081**)**, four cultivars of *Triticum aestivum* L. (Inqalab, Chakwal, Pak 81, Rohtas), and *Albizia lebbeck* L. **(Homa, 2013)**.The leaves contain various chemicals including tannins, flavonoids,steroids,hydrocarbons, waxes and alkaloids **(Pasiecznik *et al.*,` 2001)**. These are known to have effects on the germination and growth of other plant species. As a result of this, the plant diversity (both the number of individual plants of a species and the number of species around *P. juliflora*) will be affected by the allelochemicals. Low light under *P. juliflora* canopy also make other plant species' survival difficult **(kumar, 2014)**. In addition, many reported suggested that L-tryptophan may play an important role in allelopathy of *P. juliflora* leaves **(Raghavendra *et al.*, 2009)**. The negative impact of the plant could be through light deprivation, competition for water and nutrient, or leaching of allelopathic compounds **(Getachew *et al.*, 2012).**

MATERIALS AND METHODS

Plant Material

The plant material was collecting at two locations; Garawla (two sites) and El-Kasr (one site) at Matruh region along the western Mediterranean costal region of Egypt. The area is located about 300Km west of Alexandria. The coordinates were 29.56969,26.41939 (Garawla) 31.35861, 27.22089 (El-Kasr)

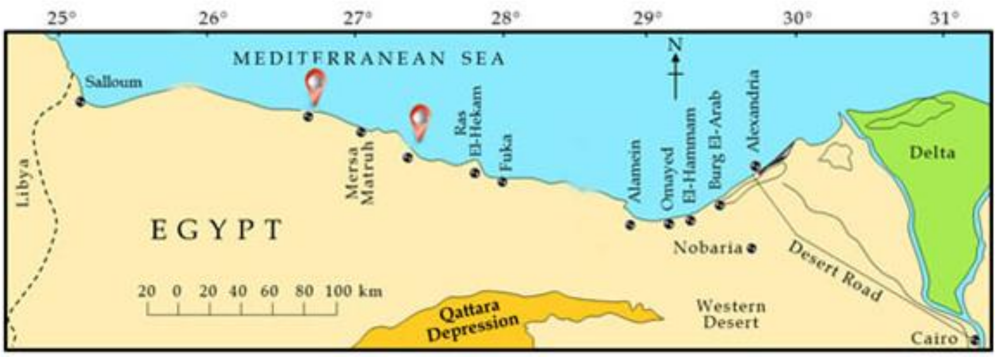

Figure (1): Map of the Western Mediterranean coastal region of Egypt indicating the location of the study sites (Garawla and El-Kasr).

(**Figure 1) Preparation of *P. juliflora* pods and leaves aqueous extracts for germination bioassay experiment (allelopathy)** Collected pods and leaves of *P. juliflora* from El-Kasr region were washed and dried in an electric oven at 45 °C. The dried pods and leaves were ground to fine powder. 75 g was transferred to labeled bottles and then 100 mL of sterile deionized distilled water were added to each bottle. The mixture was shaken and the bottles were left for 48 hours at refrigerator, then filtered to get extracts of 2, 4, 8, 16 and 32%, the control was distilled water. The procedures were performed according to **El-Rokiek *et al*. (2010), Algandaby *et al*. (2014)** and **El-Darier *et al*. (2018)**

Calculation of growth parameters

Growth experiment was performed to test the allelopathic effect of different concentrations (2, 4, 8, 16 and 32%) of *P. juliflora* pods and leaves aqueous extract with sandy clay soil (2:1) on some growth parameters (seedling dry weight, shoot and root lengths, single leaf area) as well as leaf pigments.

The soil samples were finally sterilized at (90º C for 48 h) to remove any microorganisms and weed seeds. Seven seeds of *Vicia faba* (recipient species) were sown in plastic pots (7.5 cm in diameter and 11 cm height) with about 160 g of sandy clay soil (2:1). Fifty ml of *P. Juliflora* aqueous extracts from different concentrations (0, 2, 4, 8, 16 and 32%) were added daily to three replicates in a randomized complete block design.

The experiment was performed under normal laboratory conditions (20±2º C temperature, 75±2% relative humidity, and 14/10 h/dark photoperiod) **(El-Darier and Zein El-Dien, 2011)**. After twenty-one days, the homogenous seedling were carefully collected then washed with tap water to remove the adhering soil particles, and then, by distilled water, gently blotted with filter paper. The samples were separated into shoots and roots for determination of growth parameters. Other samples were dried at 65º C till constant weight to determine the dry weight. A part of fresh samples were used for determinaion of pigment content and leaf area.

1. **Seedling dry weight (mg/seedling)**

Two homologous seedlings of *V. faba* were taken and each individual was separated into shoot and root then the oven dry weight was determined after drying in an oven at 60 °C till constant weight.

2. Shoot length (cm)

3. Root length (cm)

4. Area of a single leaf (cm2)

Leaf width and length were measured to the nearest cm, and then leaf area was calculated using the following equation **(Cain and Castro, 1959)**.

LA= 0.667*L*W

Where **L** is the leaf length, **W** is the leaf width, and **0.667** is a correction factor used to convert the rectangular product of leaf length and width into the area of the leaf.

5. Determination of photosynthetic pigments

The photosynthetic pigments chlorophyll a, b (**chl. a, chl. b**) and carotenoids (**carot**.) were determined following N, N-dimethylformamide (DMF) method described by **Inskeep and Bloom (1985)**. A known weight of the dissected plant leaves (60mg) were incubated in 12ml of DMF reagent and kept at 4oC for hr. in dark, The extract –containing pigments was decanted and the absorbance was measured at the following wavelengths 647, 665 and 453nm using spectrophotometer (IENWAY, 6305, UK). Formula and extinction coefficients used for determination of **chl. a** and **chl. b** were:

Chl. a= 12.70 A665 – 2.79 A647

Chl. b = 20.70 A647 – 4.62 A665

The carotenoids were estimated according to **Lichtenthaler (1987)**.

Carotenoids = 4.2 A453 – (0.0264 chl. a + 0.426 cg. b)

The values were then expressed as mg g-1f.w.

RESULTS

Allelopathy Marker

The allelopathic effect of *P. juliflora* pods and leaves extract as a donor species on *Vicia faba* as a recipient species is demonstrated with respect to its dry weight, length, leaf area and pigment fractions.

Data in **Table (1)** illustrates the allelopathic effect of *P. juliflora* on dry weight (g/individual) of shoot and root of *V. faba*. It was noticed that the highest values of shoot and root dry weight (0.82 and 0.43, respectively) were attained at concentration control level of pods and leaves extract. While in treatment with pods extract, the lowest value of *V. faba* shoot dry weight (0.52) was attained at concentration 16%, but the lowest value of root dry weight (0.16) was attained at concentrations (8 and 32%). However, in treatment with leaves extract, the lowest value of shoot and root dry weight (0.44 and 0.26, respectively) were attained at concentration 32%.

Table (1):Variation in dry weight (g/individual) of shoot and root of *Vicia faba* as affected by pods and leaves aqueous extracts of *Prosopis juliflora*.

Concentration (%)	Dry weight (g/individual)					
	Shoot		p_1	Root		p_1
	Pods extract (n = 2)	Leaves extract (n = 2)		Pods extract (n = 2)	Leaves extract (n = 2)	
0 (control)	$0.82^{a} \pm 0.04$	$0.82^{a} \pm 0.03$	0.982	$0.43^{a} \pm 0.04$	$0.43^{a} \pm 0.02$	0.937
2	$0.63^{b} \pm 0.02$	$0.59^{bc} \pm 0.01$	0.124	$0.20^{b} \pm 0.01$	$0.32^{bc} \pm 0.01$	0.018*
4	$0.69^{b} \pm 0.02$	$0.65^{b} \pm 0.02$	0.195	$0.21^{b} \pm 0.01$	$0.37^{ab} \pm 0.02$	0.016*
8	$0.53^{c} \pm 0.01$	$0.48^{d} \pm 0.02$	0.077	$0.16^{b} \pm 0.0$	$0.28^{c} \pm 0.03$	0.102
16	$0.52^{c} \pm 0.02$	$0.57^{c} \pm 0.01$	0.156	$0.21^{b} \pm 0.01$	$0.27^{c} \pm 0.01$	0.051
32	$0.60^{bc} \pm 0.02$	$0.44^{d} \pm 0.02$	0.015*	$0.16^{b} \pm 0.01$	$0.26^{c} \pm 0.03$	0.082
F	45.054*	102.963*		65.281*	19.671*	
P	<0.001*	<0.001*		<0.001*	0.001*	

Data was expressed with **Mean ± SD**

Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant)

F: **F for ANOVA test**, Pairwise comparison bet. Each 2 groups was done using **Post Hoc Test (Tukey)**

p: p value for comparing between the different **concentrations** in each group

p_1: p value for comparing between **Pods** and **Leaves extract**

*: Statistically significant at $p \leq 0.05$

Data in **Table (2)** showed the allelopathic effect of *P. juliflora* on length (cm) of shoot and root of *V. faba*. It was noticed that the highest values of shoot and root length (27.5 and 12.5, respectively) were attained at concentration 0% of pods and leaves extract. Although, in treatment with pods extract, the lowest value of *V. faba* shoot length (24.5) was attained at concentration 16%, but the lowest value of root length (10.5) was attained at concentrations 2%. Nevertheless, in treatment with leaves extract, the lowest value of *V. faba* shoot length (25.8) was recorded at concentration 8%, and then the lowest value of root length (9.6) was reached at concentrations 4%.

Table (2): Variation in length (cm) of shoot and root of *Vicia faba* as affected by pods and leaves aqueous extracts of *Prosopis juliflora*.

Concentration (%)	Length (cm)					
	Shoot		p_1	Root		p_1
	Pods extract (n = 3)	Leaves extract (n = 3)		Pods extract (n = 3)	Leaves extract (n = 3)	
0 (control)	27.50[a] ± 2.50	27.50[a] ± 2.50	1.000	12.50[a] ± 0.50	12.50[a] ± 0.50	1.000
2	27.33[a] ± 2.08	33.33[a] ± 5.51	0.152	10.50[a] ± 2.0	10.33[a] ± 2.08	0.925
4	26.50[a] ± 3.91	30.17[a] ± 4.75	0.360	13.0[a] ± 2.60	9.67[a] ± 2.08	0.158
8	24.83[a] ± 2.02	25.83[a] ± 1.26	0.507	11.33[a] ± 1.53	10.67[a] ± 2.02	0.672
16	24.50[a] ± 1.50	30.17[a] ± 3.75	0.072	11.33[a] ± 2.08	13.50[a] ± 2.18	0.281
32	26.67[a] ± 3.01	31.33[a] ± 4.54	0.212	11.0[a] ± 1.80	10.83[a] ± 1.26	0.902
F	0.701	1.362		0.771	1.953	
P	0.633	0.305		0.588	0.159	

Data was expressed with **Mean ± SD**
Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant)
F: **F for ANOVA test**
p: p value for comparing between the different **Concentrations** in each group
p_1: p value for comparing between **Pods** and **Leaves extract**

Data in **Table (3)** showed the allelopathic effect of *P. juliflora* on pigment fractions (mg/g fw) in shoot of *V. faba*. These pigments were Chl.a, Chl.b, B-carot and total pigments. It was postulated that the highest values of all pigment fractions were attained at control level of pods and leaves extracts. At first, in treatment with pods extract, the lowest values of Chl.a (11.06), Chl.b (4.78), B-carot (2.63) and total pigments (18.47) were attained at concentration 2%. However, in treatment with leaves extract, the lowest values of Chl.a (11.02), Chl.b (4.43), B-carot (2.37) and total pigments (17.8) were recorded at concentration 32%.

Table (3): Variation in pigment fractions (mg/g fw) of *Vicia faba* leaves as affected by pods and leaves aqueous extracts of *Prosopis juliflora*.

Concentration (%)	Pigment fraction (mg/g fw)											
	Chl.a		p_1	Chl.b		p_1	B–carot		p_1	Total		p_1
	Pods extract (n = 2)	Leaves extract (n = 2)		Pods extract (n = 2)	Leaves extract (n = 2)		Pods extract (n = 2)	Leaves extract (n = 2)		Pods extract (n = 2)	Leaves extract (n = 2)	
0 (control)	16.49^{a}±1.0	16.49^{a}±1.0	1.000	6.08^{a}±0.35	6.08^{a}±0.35	1.000	4.16^{a}±0.40	4.16^{a}±0.40	1.000	26.73^{a}±1.73	26.73^{a}±1.73	1.000
2	11.06^{a}±0.34	14.67^{a}±3.94	0.418	4.78^{a}±0.23	5.30^{a}±1.35	0.682	2.63^{a}±0.10	3.29^{a}±1.06	0.473	18.47^{a}±0.47	23.26^{a}±6.36	0.479
4	13.36^{a}±1.52	13.13^{a}±1.90	0.906	5.09^{a}±0.47	5.14^{a}±0.57	0.939	3.23^{a}±0.35	3.12^{a}±0.55	0.844	21.67^{a}±2.32	21.38^{a}±3.03	0.925
8	13.94^{a}±3.64	15.87^{a}±3.79	0.655	5.50^{a}±1.51	5.64^{a}±1.25	0.932	3.29^{a}±0.85	3.63^{a}±0.96	0.744	22.73^{a}±6.0	25.14^{a}±6.01	0.727
16	15.28^{a}±2.45	13.31^{a}±0.06	0.460	5.76^{a}±0.83	4.60^{a}±0.01	0.299	3.72^{a}±0.73	3.11^{a}±0.10	0.446	24.76^{a}±4.01	21.01^{a}±0.04	0.317
32	15.70^{a}±1.10	11.02^{a}±0.94	0.045*	5.48^{a}±0.20	4.43^{a}±0.09	0.021*	3.46^{a}±0.23	2.37^{a}±0.18	0.040*	24.63^{a}±1.53	17.81^{a}±1.20	0.038*
F	1.927	1.373		0.758	1.207		1.982	1.682		1.594	1.354	
P	0.224	0.352		0.610	0.406		0.215	0.271		0.292	0.357	

Data was expressed with **Mean ± SD**
Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant)
F: **F for ANOVA test**
p: p value for comparing between the different **concentrations** in each group
p_1: p value for comparing between **Pods** and **Leaves extract**
*: Statistically significant at $p \leq 0.05$

Data in **Table (4)** showed the allelopathic effect of *P. juliflora* on leaf area (cm) of *V.faba*. It was recorded that in treatment with pods extract, the maximum value of *V. faba* leaf area (232.6) was attained at concentration 4%, while the minimum one (222.7) was attained at concentrations 8%. However, in treatment with leaves extract, the maximum value of *V. faba* leaf area (243.2) was recorded at concentration 16%, while the minimum value (223.4) was reached at the highest concentration (32%).

Table (4): Variation in leaf area (cm^2) of *Vicia faba* as affected by pods and leaves aqueous extracts of *Prosopis juliflora*.

Concentration (%)	Leaf area		p_1
	Pods Extract	Leaves Extract	
0 (control)	226.7^{a} ± 99.76	231.8^{a} ± 110.7	0.955
2	224.8^{a} ± 104.2	226.7^{a} ± 97.76	0.982
4	232.6^{a} ± 98.15	226.9^{a} ± 103.2	0.948
8	222.7^{a} ± 101.3	230.5^{a} ± 106.5	0.931
16	228.9^{a} ± 103.5	243.2^{a} ± 112.5	0.879
32	226.1^{a} ± 104.3	223.4^{a} ± 96.10	0.975
F	0.003	0.013	
P	1.000	1.000	

Data was expressed with **Mean ± SD**

Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant)

F: **F for ANOVA test**

p: p value for comparing between the different **concentrations** in each group

p_1: p value for comparing between **Pods** and **Leaves extract**

DISCUSSION

In the present study, the allelopathic potential of *P. juliflora* pods and leaves extract (donor species) on *Vicia faba* (recipient species) is established with respect to its dry weight, length, leaf area and pigment fractions. Noticeably, the effect of pods extract on dry weight and both shoot and root lengths of *V. faba* were significantly higher than the effect of leaves extract at different concentrations. On the other hand, there no significant variation in leaf area as affected with pods and leaves extracts. Regarding pigment content, the lowest values of Chl.a, Chl.b, B-carot as well as total pigments were attained at concentration 2% of pods extract growth bioassays are more sensitive than germination bioassays **(Hatata and El-Darier, 2009)**. Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids **(Weston and Duke, 2003)** might be determined by interactions of all these compounds, not just a single chemical **(Xuan *et al*., 2003)**. These allelochemicals impede plant growth (dry weight, shoot and root lengths and leaf area) by blocking of nutrient reserve, cell differentiation, cell division, both ion and water uptake, water stress, phytohormon metabolism, photosynthesis and respiration, as well as, enzyme function, signal transduction and gene expression **(El- Darier *et al*., 2011)**. Thereby, those allelochemicals caused a significant decrease in the growth of plumule and radicle of many crops **(Belz and Hurle, 2004)**.

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