



## ***Arbutus Pavarii* and *Ceratonia Siliqua* as Bioindicators of Plant Community Structures in Al Jabal Al Akhdar, Libya (Allelopathic Study)**

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### **Abstract**

*Arbutus pavarii* Pamp, an evergreen plant of the family Ericaceae, is vernacularly known as Shmari in the Libyan society. Carob tree *Ceratonia siliqua* L. of the legume family, Caesalpiniaceae, is an evergreen shrub or tree. They are found naturally in the Al Jabal Al Akhdar region, located immediately south of the coastal belt in the north-eastern part of Libya. The main objective of this study was to determine the impact of allelopathic potentials of *Arbutus pavarii* and *Ceratonia siliqua* leaf powder on germination and metabolite accumulation in *Vicia faba*. Leaves were collected from Blkhana village to represent the leaves of the two species. The experimental design was completely randomized with three replicates. *Arbutus pavarii* and *Ceratonia siliqua* dry leaf powder were mixed with sandy loam soil in ratios to get different concentrations (0, 1, 2, 3 and 4%) in addition to the soil without leaf powder as control. The dry weight (g seedling<sup>-1</sup>) and shoot length (cm) of *Vicia faba* achieved their reductions at higher concentration percentages (3 and 4%). On the other hand, the *Arbutus pavarii* leaves exhibited more root length reduction (cm) than the *Ceratonia siliqua* leaves. The concentration of different nutrient elements varied concerning the element, organ, and powder type. Data also showed a decrease in the recipient species' total carbohydrates and total protein, and the decline was more noticeable under *Arbutus pavarii*.

**Keywords:** *Allelopathic potentials, Arbutus pavarii* Pamp., *Bioindicators, Ceratonia siliqua* L., *Physiological and metabolic parameters*

## Introduction

Plant-to-plant relationships are one of the most fundamental driving forces in forest ecology and eventually in forest dynamics and biodiversity (Escudero et al., 2000; Brooker, 2006). Allelopathy in plants can be defined as the effects of one plant on other plants through the release of chemical compounds in the environment (Mallik, 2008).

*Arbutus pavarii* Pamp. (Ericaceae), is an endemic species in Al Jabal Al Akhdar -Libya, currently considered an endangered species of shrub or tree and grows in mountainous sites that are relatively high above sea level, where it is widely spread and densely located in the west of Lamlouda, at an altitude of about 675 meters above sea level and has attracted public attention about its conservation (OMU, 2005). In addition to its ecological importance in honey production, *A. pavarii* has been used in traditional Libyan medicine to treat various human ailments including gastritis and kidney diseases (Al Groshi et al., 2021). The genus *Arbutus* (Ericaceae) has been traditionally used in folk medicine due to its phytomedicinal properties, especially *Arbutus pavarii* Pamp. However, this plant has not been evaluated for its efficacy, quality, and consistency to support the traditional uses, potentially in treating diabetes. Despite previous studies that revealed the biological activities of *A. pavarii* as the antioxidant and  $\alpha$ -glucosidase inhibitory agents, scientific reports on the bioactive compounds that contribute to its health benefits are still scary. Fruits of *A. pavarii* are an excellent source of minerals, nutrients, carbohydrates and most importantly, vitamin C. The uses of its leaves, fruits and bark in the tanning process and branches as a fuel are among other economic importance of this plant (Buzgaia et al., 2020; Jadallah et al., 2020).

*Ceratonia siliqua* is an evergreen tree belonging to the Caesalpinaceae family in the Caesalpinioideae sub-family and cultivated mainly in the Mediterranean basin (**Durazzo et al., 2014**). The tree is one of the most important species used locally to produce honey and Carob syrup. Carob is used in several industries: food, pharmaceuticals, and cosmetics (**Durazzo et al., 2014**). Diverse studies have shown that Carob has several biological activities, i.e., antioxidant, antitumor and antibacterial (**Custódio et al., 2009; Meziani, 2015**). Carob pods and seeds are often used in traditional medicine as analgesic, anti-constipation, antiabsorptive of glucose, gastrointestinal propulsion and antidiarrhea.

The Food and Agriculture Organization of the United Nations (FAO) in 2018 reported that the production of *Ceratonia siliqua* beans in Tunisia decreased from 1000 tons in 2000 to 847 in 2017 (**Ben Ayache et al., 2020**). In the Tunisian flora, the Carob tree is also considered a vulnerable species whose transition to endangered species (**Ben Ayache et al., 2020**).

Although the distribution of *Ceratonia siliqua* is large, the population size is decreasing due to numerous low to medium-impact threats. These include the threat of ruthless collection for domestic uses. Locals and collectors heavily collect it for wood, fodder, food, medicinal and domestic uses, and trade (e.g., in Morocco, 7 dirhams/kg for the fruit) (**El Bouzdoudi, 2017**). The species is threatened by the growing pressures from collection practices, overgrazing, deforestation and habitat loss, tourism expansion in the Mediterranean region, agricultural intensification, and soil erosion. The new management of public forests also threatened it, the poorly recognised traditional sustainable forest management, the erosion of the species' genetic diversity, and the replacement of the existing Carob by more intensive crop

varieties (**Reigosa et al., 2006**). *Ceratonia siliqua* is threatened more generally by long periods of drought and climate change (**Issam et al., 2021**). The species is also impacted to some degree by minor threats, including the infection by several pests and diseases and the predation of Carob orchards by small rodents. However, the contribution of this loss to wild populations is unknown. Plants may affect others growing in their vicinity in a stimulatory or inhibitory manner through released biologically active compounds often called allelocompounds or allelochemicals. Recently, the phenomenon receiving increased attention and is considered to be applied in practice for weeds and pest management (**El-Darier, 2002**).

It is crucial indeed to understand the role of the allelopathy phenomenon, and such importance should be a priority in regions where natural vegetation is degraded like what we have in Libya. Studies about the role of allelopathy in forest ecosystem development are, in fact, rare (**Elshatshat, 2010**). For these reasons, we present this study to examine the effect of the allelopathic potential of *Arbutus pavarii*, and *Ceratonia siliqua* leaves on germination efficiency, growth, and some physiological parameters of the *Vicia faba* crop plant.

## Material and Methods

### Sampling and Analysis of Plant Materials

*Arbutus pavarii* Pamp and *Ceratonia siliqua* L. were selected as they are found naturally in the Al Jabal Al Akhdar region, located immediately south of the coastal belt in the north-eastern region of Libya. The samples were washed thoroughly with running tap water and then distilled water to remove dust particles from leaf surfaces. All samples were dried in an oven at 60°C till constant weight and then powdered in an electrical mill.

A pot experiment was carried out to assess the effect of different levels of leave crude powder (w/w) mixed with sandy loam soil on some growth [seedling shoot and dry root weights (g seedling<sup>-1</sup>) and lengths (cm)]. Physiological and metabolic parameters (photosynthetic pigments, some nutrient elements, protein profile, as well as ultrastructure of the leaf) of the *Vicia faba* plant were tested. To accomplish this aim, five pots of 10 seeds were used for each tested crude powder level (0, 1, 2, 3, and 4%). After 21 days, the homogenous seedlings were carefully collected from each treatment; washed with tap water to remove the adhering soil particles, and then by distilled water; and gently blotted with filter paper. Determination of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids (mg g<sup>-1</sup> fresh wt. of leaves) were determined spectrophotometrically according to **Metzner et al. (1965)**. Total carbohydrates in the dry shoot and root were determined by using a colorimetric method (**Herbert, 1971**), and total protein content was determined by the method described by **Lowry et al. (1951)**. Concentrations of some nutrient elements in the dried shoot and root were determined using the method of **Cottenie et al. (1982)**.

## Results

Generally, the results in **Table (1)** showed a significant reduction in dry weight ( $\text{g seedling}^{-1}$ ) and length (cm) of *Vicia faba* shoot as affected with *Arbutus pavarii* and *Ceratonia siliqua* leaf powders compared to control. The two parameters achieved their reductions at higher concentration percentages (3 and 4%). Additionally, the two parameters mentioned for the root decreased progressively with the increase in *Arbutus pavarii* powder percentage at all concentrations relative to control. Generally, the *Arbutus pavarii* leaves exhibited more reduction than the *Ceratonia siliqua*.

**Table 1.** Variation in dry weights ( $\text{g seedling}^{-1}$ ) and length (cm) of the shoot and root of *Vicia faba* seedlings germinated in soil supplemented with different concentrations (w/w) *Arbutus pavarii* (A) and *Ceratonia siliqua* (C) leaves

Treatment (%)	Dry weight ( $\text{g seedling}^{-1}$ )				Length (cm)			
	Shoot		Root		Shoot		Root	
	C	A	C	A	C	A	C	A
Control	0.26	0.26	0.9	0.9	29.93	29.93	16.80	16.80
1	0.14	0.15	0.06	0.07	28.0	29.0	12.95	14.5
2	0.05	0.23	0.02	0.07	24.7	28.2	11.35	13.2
3	0.12	0.09	0.03	0.05	25.67	24.1	10.60	11.1
4	0.11	0.02	0.04	0.04	22.5	20.0	8.0	8.80
<b>Treatment</b>	F=244.962* (<0.001*)		F=7.375*(0.001*)		F=129.828*(<0.001*)		F=657.062*(<0.001*)	
<b>Concentrations</b>	F=11.308*(0.003*)		F=4.723*(0.042*)		F=0.107(0.747)		F=73.429*(<0.001*)	
<b>Interaction</b>	F=116.885*(<0.001*)		F=1.509(0.237)		F=15.625*(<0.001*)		F=9.544*(<0.001*)	

Interaction (Treatment vs Concentrations)

F: value for **two-way ANOVA test**.

\*: Statistically significant at  $p \leq 0.05$ .

Pigments (chlorophyll a, b, and carotenoids) were decreased for all concentrations compared to control, and the decrease was remarkable for *Arbutus pavarii* rather than *Ceratonia siliqua* leaves powder, as shown in **Table (2)**.

**Table 2.** Variation in total photosynthetic pigments (mg g<sup>-1</sup>fresh weight) of *Vicia faba* seedlings germinated in soil supplemented with different concentrations (w/w) of *Arbutus pavarii* (A) and *Ceratonia siliqua* (C) leaves

Treatment (%)	Photosynthetic pigments (mg g <sup>-1</sup> fresh weight)							
	Chl a		Chl b		Carot.		Total	
	C	A	C	A	C	A	C	A
Control	105.90	105.90	48.30	48.30	14.90	14.90	169.10	169.10
1	96.3	89.0	52.7	38.7	16.1	22.8	165.1	150.5
2	88.2	74.5	41.6	30.1	15.2	27.1	145.0	131.7
3	80.7	56.0	37.0	27.6	15.9	30.5	133.6	114.1
4	72.4	44.8	31.3	22.3	16.8	43.8	125.3	110.9
Treatment	F=9006.057*(<0.001*)		F=9453.231*(<0.001*)		F=3615.991*(<0.001*)		F=90791.819*(<0.001*)	
Concentrations	F=6829.945*(<0.001*)		F=11118.519*(<0.001*)		F=20513.434*(<0.001*)		F=36527.755*(<0.001*)	
Interaction	F=855.273*(<0.001*)		F=808.904*(<0.001*)		F=2857.443*(<0.001*)		F=2558.181*(<0.001*)	

Interaction (Treatment vs Concentrations)

F: value for **two-way ANOVA test**

\*: Statistically significant at  $p \leq 0.05$

Data in **Table (3)** also showed a decrease in total carbohydrates and total protein contents in shoot and root of the recipient species, and the reduction was more noticeable under *Arbutus pavarii* compared to *Ceratonia siliqua* leaves powder.

**Table 3.** Variation in total carbohydrates and total protein contents (mg g<sup>-1</sup>dry weight) of *Vicia faba* seedlings germinated in soil supplemented with different concentrations (w/w) of *Arbutus pavarii* (A) and *Ceratonia siliqua* (C) leaves

Treatment (%)	Total protein (mg g <sup>-1</sup> dry weight)				Total carbohydrate (mg g <sup>-1</sup> dry weight)			
	Shoot		Root		Shoot		Root	
	C	A	C	A	C	A	C	A
Control	199.0		115.57		227.11		126.57	
1	175.1	175.3	91.1	111.0	197.5	221.4	108.5	132.7
2	160.1	120.6	72.8	103.0	191.6	230.1	93.6	131.0
3	90.8	171.7	66.3	106.1	133.5	196.6	87.1	117.6
4	78.0	151.8	45.1	95.5	116.3	173.1	71.7	101.4
Treatment	F=75469359*(<0.001*)		F=12129306*(<0.001*)		F=34666623*(<0.001*)		F=5180791*(<0.001*)	
Concentrations	F=42839784*(<0.001*)		F=41242338*(<0.001*)		F=47450089*(<0.001*)		F=15344280*(<0.001*)	
Interaction	F=43756115*(<0.001*)		F=3906309*(<0.001*)		F=4667248*(<0.001*)		F=1075074*(<0.001*)	

Interaction (Treatment vs Concentrations)

F: value for **two-way ANOVA test**

\*: Statistically significant at  $p \leq 0.05$



The variation in the concentration (%) of some nutrient elements of shoot and root of *Vicia faba* seedlings germinated in soil supplemented with different concentrations (w/w) of *Arbutus pavarii* (A) and *Ceratonia siliqua* (C) leaves were illustrated in **Table (4)**. Notably, for the shoot, data showed that the two types of leaves significantly increased the concentration of Na, P, Fe and Ni while the concentrations of Zn and Cu were decreased. For Ca, the concentration was increased and decreased as affected by the addition of *Ceratonia siliqua* and *Arbutus pavarii* leaves powder, respectively. Concerning root, the concentration of Na and Cu was increased upon applying the two types of leaves powders, while that of Ca was decreased. Regarding Mg and Fe, an increase and decrease were exhibited as affected by *Ceratonia siliqua* and *Arbutus pavarii* powders.

**Table 4.** Variation in the concentration (%) of some nutrient elements of *Vicia faba* seedlings germinated in soil supplemented with different concentrations (w/w) of *Arbutus pavarii* (A) and *Ceratonia siliqua* (C) leaves

Treatment (%)	Cu	Zn		Ni	Fe	P	Mg	Ca	Na
<b>Concentration (%) Shoot</b>									
Control	5.97	3.90		0.30	0.20	9.5	1.60	3.90	0.10
C	1.20	0.70		0.70	0.50	12.5	2.30	4.60	2.30
A	0.80	0.70		0.60	0.50	14.4	1.60	1.80	0.60
F	455.286*	384.0*		7.753*	2.063	610.333*	24.500*	637.000*	238.922*
P	<0.001*	<0.001*		0.022*	0.208	<0.001*	0.001*	<0.001*	<0.001*
<b>Concentration (%) Root</b>									
Control	1.10	0.20		-	2.90	3.40	2.30	14.6	1.20
C	1.70	0.20		-	4.30	6.90	2.80	12.3	2.20
A	4.20	0.40		-	2.50	3.40	1.80	10.6	3.67
F	405.500*	2.400		-	57.429*	408.333*	13.235*	3627.0*	218.737*
P	<0.001*	0.171		-	<0.001*	<0.001*	0.006*	<0.001*	<0.001*

Interaction (Treatment vs Concentrations)

F: value for **two-way ANOVA test**

\*: Statistically significant at  $p \leq 0.0$



## Discussion

Allelopathy is a physiological phenomenon with ecological implications (**Vinterhalter & Vinterhalter, 1992**) and the allelopathic potential impact on secondary metabolism is dependent on the species studied some species activate some biosynthetic pathways, while others are affected in different ways. Allelopathy also plays an essential role in the life of other components of the forest ecosystem, the introduction of forest tree species and the ecology of the forest (**Caboun & John, 2015**). Limited phytochemical studies of *A. pavarii* revealed the presence of simple phenolics, e.g., arbutin, gallic acid and polyphenolics, flavonoids and tannins, e.g., apigenin, epicatechin, hesperidin, kaempferol, naringin, quercetin and rutin, and some triterpenes and sterols (**Cottenie et al., 1982; Buzgaia et al., 2020**). Rutin was the most abundant flavonoid, while kaempferol was the least in the aerial parts of *A. pavarii*. Arbutin appears to be the chemotaxonomic marker compound of the genus *Arbutus* as this compound is present in other species of this genus.

Forest ecosystems are very complex, with numerous tree species and organisms dominated by trees in forestry systems, and allelopathy affects many aspects of plant ecology (**Caboun & John, 2015**).

The determination of metal traces is crucial because they are involved in biological cycles (**Dhiman et al., 2011**). The present investigation exhibited that the concentration of Na, P, Fe and Ni increased as affected by the two species of *Arbutus pavarii* and *Ceratonia siliqua* leaves, while that of Zn and Cu were decreased. On the contrary, Ca increased and decreased as affected by the addition of *Ceratonia siliqua* and *Arbutus pavarii* leaves powder, respectively. The concentration of Na and Cu in roots was increased upon applying the two types of leaves powders, while that of Ca was decreased.

Additionally, an increase and decrease in attitude for Mg and Fe. **El Darier (2002)** reported that Eucalyptus crude leaves powder significantly increases the accumulation of phosphorus and potassium in leaves of *V. faba* and *Zea mays* while nitrogen was not affected. The allelopathic compound released from *Arbutus pavarii* leaves significantly suppressed the accumulation of Zn, Cu and Ca in the shoot and Mg and Fe in the root. Many polyphenols at higher concentrations (4%) can chelate divalent and trivalent metal ions (**Crawley, 1997**), which may be coincided with the reduction of growth and development in *V. faba* plant. **Puri & Khara (1991)** obtained the same results.

In the present study, the ultrastructural responses of palisade cells in *V. faba* leaves increase the proportion of chloroplasts showing abnormal shape, swelling or disarrangement of the lamellae and distorted thylakoids associated with a high density of stroma. Moreover, the cell wall becomes thick, and marked dispersion in chromatin materials leads to suppressing nucleic acid biosynthesis. These alterations were prominent in *Arbutus pavarii* leaves compared with *Ceratonia siliqua*. In this context, **Lovett (1982)** observed increased vacuolation and other apparent disruptions in the root tip cells of flax to which allelochemicals had been applied. Phenolic acids also retarded hypocotyl growth in *Phaseolus aureus* not through structural damage but by interference with mitochondrial respiration (**Koch & Wilson, 1977**). Leaf cells of *Sorghum bicolor* were less damaged than those of *Zea mays* at low water potentials (**Othman et al., 2018**) and strengthen the hypothesis that maintenance of membrane structure is an essential factor in the ability of plants to withstand severe water stress.

## Conclusion

Maquis vegetation in Al Jabal Al Akhdar is also an excellent source for wild animals. It faces a decline in the production of seedlings despite the massive number of seeds produced by mother trees in natural habitats. Over-grazing could be a significant reason for the loss of fresh seedlings, but in some inaccessible locations, it is hard to notice any natural regeneration. Hence, the present study examined the possible role of allelopathy on this species and further understood the action of the Allelopathic effect of *Arbutus pavarii* and *Ceratonia siliqua* on plant community structure in nature.

*Arbutus pavarii* and *Ceratonia siliqua* were empirically selected. *Arbutus pavarii* and *Ceratonia siliqua* cultivation remain traditional and sporadic in the agroforestry systems, with no grafting nor silviculture treatment or fertilization. Trees are only in rainfed conditions. The present study may indicate that *Arbutus pavarii* and *Ceratonia siliqua* may consider a bioindicator as they are susceptible to different plant community structures in Al Jabal Al Akhdar. Field experiments are needed because, under natural conditions, the variety of *Arbutus pavarii* and *Ceratonia siliqua* interactions with the physical environment and other organisms can intensify or decrease its allelopathic effects. An extrapolation of these findings may be the key to the beginning of adopting new management tools in forest ecology.

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