GROWTH OF *Pistacia lentiscus* L. SEEDLINGS AND TOLERANCE TO DROUGHT STRESS AND HYDROCARBON POLLUTION

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Abstract. The regions of the Mediterranean basin are not only exposed to environmental factors such as drought and sunshine aggravated by climate change but also to pollution of various kinds due to enthropological activities of all kinds. At service stations, there are various sources of fuel release to the environment such as releases from the vent pipes of storage tanks. Therefore, forest areas are subject to degradation. The objective of this work is to study the growth response of *Pistacia lentiscus* to water stress and hydrocarbon pollution. Two batches of fruit were harvested in Redjawna (Tizi ouzou) and Ifigha (Azazga); the first lot was used for the study of water stress while the second was used for the study of the effect of oil pollution. Soil used for the study of water stress was collected at redjaoua in the rhyzospheric region of *P. lentiscus* and the polluted soil was collected at the service station under the vent. The results show that *P. lentiscus* seedlings present high survival rate both under water stress and under soil pollution with hydrocarbons. However, the growth parameters were significantly reduced under the effect of the two types of stress: at the end of the treatment, seedling heights were 34 and 21 cm in well-watered and unwatered conditions respectively while shoot and root biomass were decreased 39.70 and 18% respectively. Under stress due hydrocarbon pollution, the shoot heights and aerial biomass were reduced by 72 and 86% while root length and root biomass were reduced by only 46 and 67% respectively. Although the growth parameters have been significantly reduced under the effect of drought and hydrocarbon pollution, the survival rate of *P. lentiscus* being high, this species could be considered in programs for the rehabilitation of degraded land.

Key words: *Pistacia lentiscus*; *hydric stress*; Growth Traits; RWC; hydrocarbon pollution.

INTRODUCTION

The Mediterranean climate is characterized by warm, dry summers and cool, wet winters [18, 55] and high temporal variability of climate at seasonal and inter-annual scales [56]. The southern regions of the mediterranean are particularly impacted by climate change and reduction of precipitation [34]. The ecosystems of this region are very prone to fires [33]. To all these ecological constraints, anthropogenic pressure like pollution and overexploitation of resources constitutes a threat leading to declin of these ecosystems [10]. Currently, environmental hydrocarbon pollution has disastrous and catastrophic consequences, on the human beings and all biotic components of the ecosystem [2, 30]. At service stations, there are various sources of fuel release to the environment such as releases from the vent pipes of storage tanks [27]. Therefore, the growth and development of plants are severely limited by these constraints, forest areas are subject to degradation [43].

To cope with environmental constraints such as drought and irradiation, Mediterranean species have developed a set of morpho-anatomical, biochemical and physiological traits allowing them to adapt and survive in these difficult conditions [20]. They can adjust their metabolism, structure and function quickly to optimize growth and reproductive capacity at any time in a constantly changing environment [4].

Pistacia lentiscus is an evergreen sclerophyllous shrub common throughout the mediterranean largely distributed in the Mediterranean basin [17]. It is commonly dispersed in Algeria ranging from the littoral to arid zones. *Pistacia lentiscus* presents a great ecophysiological intraspecific variability [15] well adapted to climatic hazards and can be used in the reforestation of eroded soils [19, 44]. Throughout ancient times, the populations around the Mediterranean, in particular those on the southern shore, used the different parts of *P. lentiscus* in traditional medicine [13].

Pistacia lentiscus is a species with strong ecological and economic potential in more than one respect. In the Mediterranean basin which considered the most vulnerable regions to climate change with a pronounced warming and a sharp decrease in precipitation in the spring and summer seasons [22], it seems to be less affected by drought stress [5]; It is also one of the most resistant shrubs to fire [25]. We report that just three months after the August 2021 fires that ravaged Kabylia, located in northern Algeria, *P. lentiscus* shrubs have regenerated well (Fig. 1). *P. lentiscus* possess relevant characteristics for phytostabilization projects [9]. It could thus be used in restoration programs for degraded maquis lands. The objectives of the present work were to investigate the

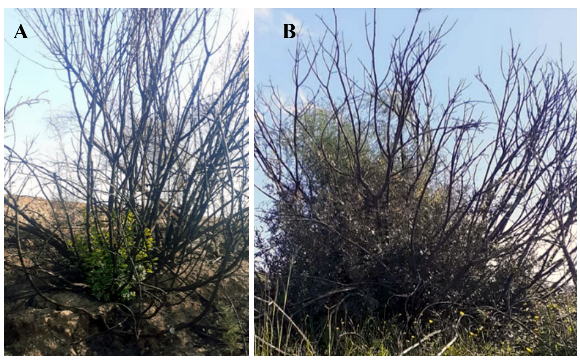

Figure 1. *P. lentiscus* regeneration after fire of august 2021, photos taken in October 2021 (A) and in April (B)

effects of drought stress and soil hydrocarbon pollution on germination and growth parameters of *P. lentiscus* as shoot height and aboveground biomass, root length and biomass, root length / shoot height and root biomass/ aboveground biomass rates.

MATERIAL AND METHODS

Soil sampling

To drought stress experimentation, soil sample was collected in Redjaouna land (Tizi-Ouzou, Algeria, at Latitude 36°43'32.32'' North, Longitude 4°03'44.26'' East and 372 m elevation) in the area of the *Pistacia lentiscus* L.; it was dried in the open air, debris and stones have been removed and then distributed in plastic bags of 2 kg approximately capacity. The sampling of the soil contaminated by hydrocarbons and that uncontaminated was carried out in the service station of Ifigha (region of Azazga, Algeria, at Latitude 36°40'46.30'' North, Longitude 4°23'51.15'' East and 402 m above sea level). The polluted soil has been taken under the vents; it's thus contaminated by the hydrocarbon vapors, which escape from the vents and condensed vapors falling to the ground after its condensation; uncontaminated soil was collected at approximately one and half meter away from the vent. In the laboratory, the soil samples were dried in the open air then sieved and distributed in sixteen plastic bags of 1 kg capacity approximately, eight of polluted soil and eight unpolluted. A sample of polluted soil has been reserved for the extraction and quantification of hydrocarbons, pH and conductivity measurements.

Plant material and experimental conditions

Pistacia lentiscus ripe fruits containing the seeds were harvested from 10 shrubs in November 2016 in Redjaouna land for drought stress experiment. Another batch of ripe fruits were also harvested from Ifigha land in Novembre 2019 for hydrocarbon pollution study. Al the fruits were separately soaked in water for 24 hours to remove the pulp. The floated seeds (non-viable) were removed and the remainder was scarified with concentrated sulfuric acid for 15 minutes and immediately rinsed and kept under tap water for 24 hours to remove all traces of sulfuric acid. For the drought experimentation, carried out in early January 2017, a total of 180 seeds were sown in 18 Petri dishes at the rate of 10 seeds per dish. After 15 days of incubation, the seeds germinated with 84.35±9.24%. At the two juvenile leaf stage, forty seedlings were planted in bags containing about 2 kg of soil collected in the area of growth of the species at the rate of one plant per bag. The bags were placed in a greenhouse of the ITMAS (Institute of Technology and Specialized Agricultural Means) of Boukhalfa (Tizi-Ouzou, Algeria), under natural conditions with temperatures reaching 45.6±3.84°C in average, and watered as needed, about 500 mL twice a week. since the start of treatment. At the beginning of drought treatment (mid-July), the seedlings were separated on two lots; the first one (W) continues to be watered regularly while the second (UW) was subjected to water stress by suspending watering. The drought treatment lasted two months, with watering once in the fourth week and then stopping watering again until mid-September. At the end of the treatment, the seedlings were harvested to analyze some physiological and growth traits. For hydrocarbon pollution experimentation, seeds were sown in sixteen bags at the rate of five seeds per bag. The bags were placed in a greenhouse at ITMAS, under natural conditions, and watered as needed, about 200 mL twice a week in summer until September.

Total soil hydrocarbon extraction and quantification

The soil total hydrocarbons were extracted by using Shaking method [49]. Briefly, 1 g of polluted soil was put in a glass vial with hermetic stopper containing 10 mL of acetone. The vials were sealed with a foil-lined cap and shaken on a VARIOMAG POLY 15 platform shaker at 120 cycles/min during 30 min. The extract was centrifuged for 10 min at 180g and the supernatant removed carefully. One other cycle was used on the soil used previously with 10 mL of clean acetone. Extracts were combined and stored at 4°C. The hydrocarbons were quantified by ultraviolet spectrophotometry method at 220 nm with diesel oil us standard diluted in cyclohexane [36]. Soil total hydrocarbons, pH and electrical conductivity (EC) were showed in table 1.

Table 1. soil total hydrocarbons, pH and EC of polluted and unpolluted soil sampled from gas station

Characteristic	Polluted soil	Unpolluted soil
Soil hydrocarbon content ($mg{\cdot}kg^{-1}$)	24.32	0
pH	7.767	8.071
EC ($mS{\cdot}cm^{-1}$)	0.148	0.168

Measurements under drought stress

Measurements of the growth traits

At the start of the experiment, the heights of all the plants were measured using a graduated ruler, then approximately every 15 days for two months. The length of the main stem was measured from the root collar to the ligule of the youngest fully expanded leaf and that of the first branch from the node to the ligule of the youngest fully expanded leaf. Fresh material (stems and roots) was dried at 60°C until constant weight was achieved at which point dry biomass was recorded.

Relative water content measurement

Relative water content (RWC) was measured according to the method described by Seelig et *al.* (2009) [50]. It was calculated following the expression: RWC=(FM−DM)/(TM−DM), where TM is the leaf turgid mass obtained at the beginning of the dehydration process, FM is the sample fresh mass measured at any moment of the process and DM is the leaf dry mass obtained after oven drying samples at 80°C for 24h.

Leaf pigment content

Frozen leaves samples (0.2 g) were analyzed for chlorophyll contents. The leaves were ground in 3 mL cold 80% acetone using a pestle and mortar. Acetone extracts were centrifuged at 3000g for 10 min and the resulting pellets were extracted in cold 80% acetone. This operation was repeated three times. The successive supernatants were pooled and centrifuged at 4000 g for 5 min for clarification. The absorbance of the acetone extracts was recorded at, 647 and 663 nm using UV/VIS spectrophotometer (Shimadzu UVmini-1240). The amounts of chlorophyll were calculated according to Porra (2002) [45].

Measurements under hydrocarbon pollution

Effect on seed germination and seedling survival

After two weeks of observation, the germination and mortality rates were calculated. After six months the seedlings were then carefully removed from the soil and the roots were washed under running water so as not to be damaged. The aerial parts were separated from the root parts and then, using a ruler, the root lengths and shoot heights were measured in cm. Both parts were dried at 40°C until dry weight constancy, shoot and root biomass.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). The values are analyzed using the student t-test. Statistical significance was set at $P<0.05$. Data were tested for normality by shapirowilk test and for homogeneity by Levene's test. Statistical analyses were performed using Statisica 7.1.

RESULTS

Response to drought stress

To investigate the physiological responses of *Pistacia lentiscus* seedlings to water deficit, some physiological and growth traits were evaluated. The daily average temperature was 51±8°C in the greenhouse.

RWC and chlorophyll contents

Drought stress treatment reduced significantly ($p=0.0000$) the RWC of *P. lentiscus* seedlings to 85.69±2.61% in comparison to the control ones with 91.57±1.41% (figure 2 A). The result presented in figure 2, B and C showed that no significant differences in chl a, chl b, chl a/b ratio and total chlorophyll contents between *P. lentiscus* seedling growth in control and drought conditions.

Growth traits and survival

During the first period of water stress, from the start until the 15th day, the heights of well-watered and unwatered plants do not show any significant difference ($p>0.05$). Their increases were 13.62±4.83% and 16.25±13.56% respectively (fig. 3 A). This difference becomes significant from the 23rd day ($p<0.05$). Well-watered plants showed a significant increase 52.13±16.06% while unwatered plants showed a low increase (7.13±4.30%). At the end of the treatment, seedling heights reached 33.50±5.98 and 20.88± 4.66 cm in well-watered and unwatered conditions respectively (Fig. 3 B). Furthermore, shoot and root biomass were significantly decreased under drought treatments compared to the control (39.70 and 18.20% respectively) (fig.4 A) however, no significant difference between the RB/AB ratio ($p=0.299$) (Fig. 4 B). In addition, after four weeks of watering suspension, two of the twenty seedlings perished probably due to the drought reflecting the low mortality rate, although the sample size does not allow this result to be generalized.

Response to hydrocarbon stress

After six months, all *P. lentiscus* seedlings have survived in both uncontaminated soil (NCS) and hydrocarbon contaminated soil (CS). Statistical analysis shows no significant difference between the rate of germination in contaminated and uncontaminated soil. In contrast, growth parameters were negatively impacted by hydrocarbon pollution (table 2). The shoot heights and aerial biomass were reduced by 71.5 and 86.47 respectively in polluted soil, while root length and root biomass were reduced by only 46.15 and 66.72% respectively. This was highlighted by the increased reports of these parameters (RL/SH and RB/SB were augmented by 179.95 and 120.92% respectively).

DISCUSSION

Relative water content is an important indicator of stress; it reflects the water status of the plant which provides key information about plant response to environment [39]. It is also positively correlated with the tolerance of the species to water stress [32, 58]. After two months of drought treatments, the seedling of *P. lentiscus* showed a statistically significant decrease in RWC under drought stress conditions comparatively to control conditions. However, this remains above 80%. Previous studies show slight decrease in RWC of this species seedling [6, 24, 38].

Photosynthetic pigments are essential for light harvesting and, hence, for photosynthesis and plant growth [35]. Chlorophyll content indicates tolerance or sensitivity to drought; while water deficit due to drought induces decrease in this content, some plants are able to maintain it [37]. Thus, cultivars of the olive tree for example have shown a decrease in chlorophyll content under the effect of stress conditions [8]. This could be linked to the redox status in the leaf [7] and dehydroascorbate reductase activity [16]. Moreover, Pourghayoumi et *al*. (2017) [46] who studied the biochemical and molecular responses of five pomegranate cultivars to severe water stress found that only the 'Ghojagh' cultivar did not show a significant reduction in chlorophyll (a+b) and suggested that it had better tolerance.

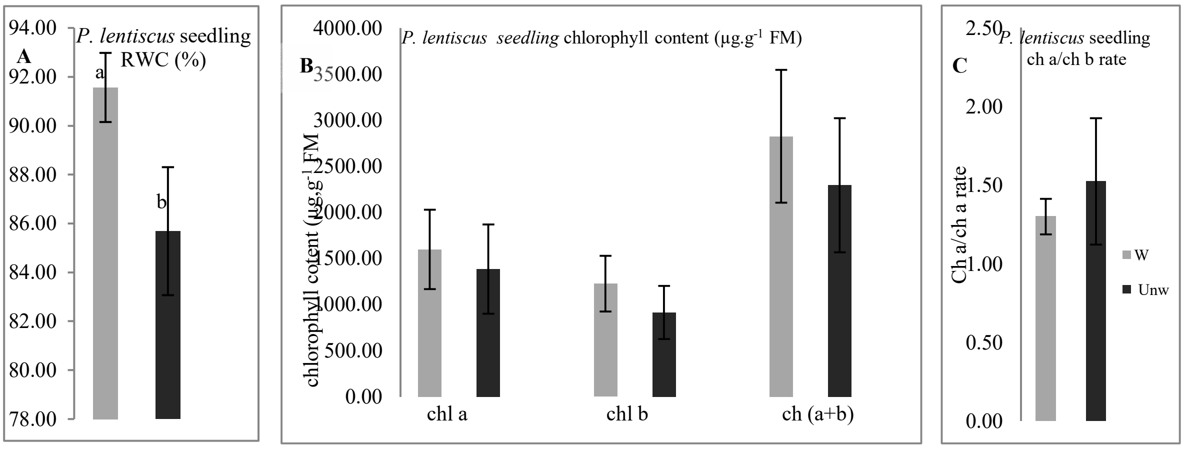

Figure 2. RWC (A), chlorophyll contents (B) and ch a/ch b rate of *P. lentiscus* seedlings under control (well-watered: W) and drought stress (unwatered: Unw) treatments. Means±SD, N ≥6, ***< 0.001

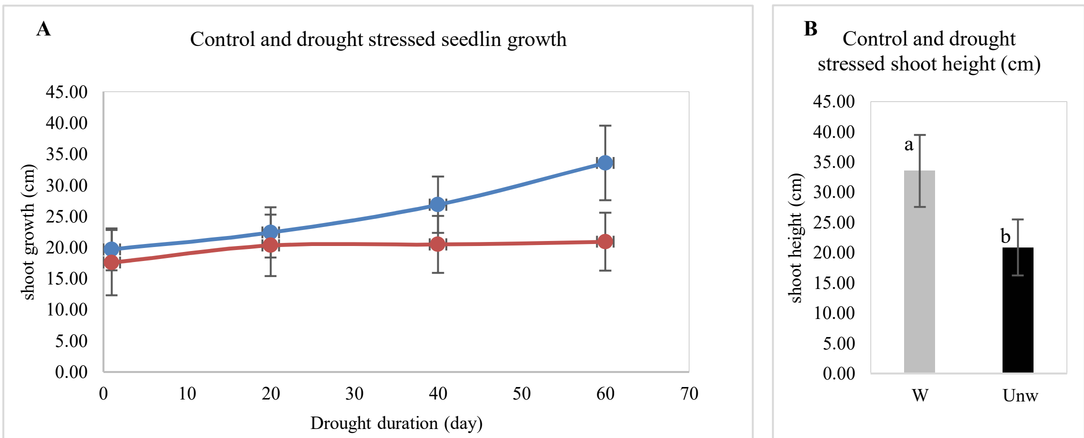

Figure 3. Shoot height and growth (cm) of *P. lentiscus* seedlings under control (well-watered: W) and drought stress (unwatered: Unw) treatments: shoot height (a), control and dought stressed plant growth (cm) (b)

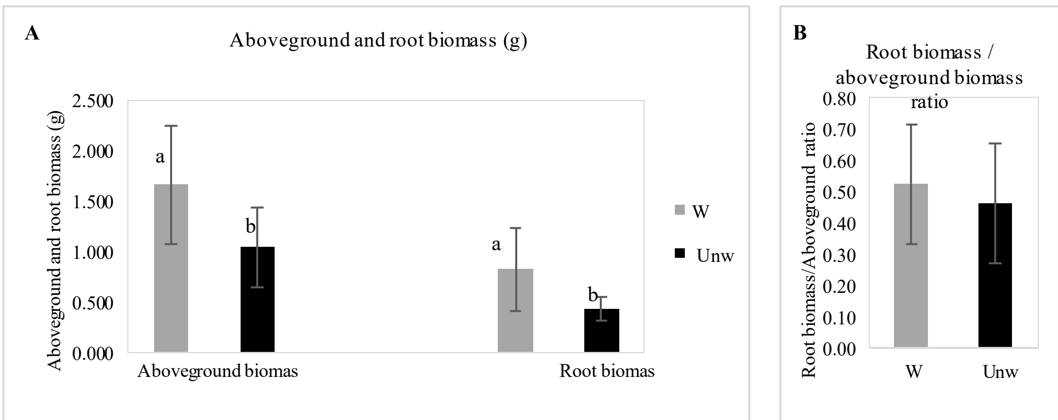

Figure. 4. Aboveground (AB) and root biomass (RB) (a) and root biomass/shoot biomass ratio (b) of *P. lentiscus* seedlings under control (well-watered: W) and drought stress (unwatered: Unw) treatments. Means ± SD. n=12

Table 2. Germination rate, mortality rate and growth parameters in uncontaminated soil (NCS) and contaminated soil by hydrocarbon

	Germination (%)	Mortality rate (%)	SH (cm)	R L (cm)	A B (g)	R B (g)	R L/SH	R B/AB
NCS	67.50±18.32	0	16.18±2 29	19.38±3.98	0 .25±0.04	0.04±0 .01	1.22± 0.28	0.18±0.11
CS	70.00±20.32	0	4.62±0 62	10.04±3.43	0.04±0.001	0.03±0.006	2.19±0.88	0.42 ±0.15

Note: (SH: shoot height, RL: root length, AB aerial biomass, RB: root biomass. The cover data are means ± SE, n=8

There were significant reductions in aboveground and root biomass (p=0.000) while the RB/AB ratio was non-significantly different (p=0.48). This could be due to the intra-individual variability of the response of seedlings to stress. Plants, which use drought avoidance mechanism, limit vegetative growth [1, 12]. During the first 15 days of the treatment, the heights of seedlings increase both for well-watered seedlings and for those under stress. Our results agree with those of several authors; seedling height of *Lycium ruthenicum* increases in first stage of treatment [26], *Erythrina velutina* seedling subjected to drought stress showed a growth decrease only after 21 days [52]. Water stress reduced the dry weight of leaf, stem, roots, and nodules in both soybean[and siratro [41]. Plant resistance to water stress depends on several factors such as soil type and plant species [14]. In the work of Valladares et *al.* (2006) [57], *P. lentiscus* was found to be sensitive to water stress. It was found to be water spender species since it suffers from water stress problems much earlier than do *Ceratonia siliqua* and *Quercus coccifera* [42]. However, Filella et *al.* (1998) [21] found it to be better adapted to drought.

The results on hydrocarbon pollution experiments show a non-significant difference between the germination rate of *Pistacia lentiscus* seeds in polluted soil and unpolluted soil. In previous studies, authors have reported negative effects of hydrocarbons on seed germination of many species [3, 29, 31]. However, in some species, germination is not reduced [47, 59]. This could be due to the intrinsic characteristics of the seed of the species. Indeed Seeds of *P. lentiscus* did not show dormancy [23]. Moreover, to facilitate germination, the seeds have previously undergone chemical scarification as recommended by Piotto et *al*. (2003) [44]. This could also be explained by the loss of volatile compounds during the drying of the soil; these compounds having an inhibiting effect on seed germination [54]. The results of the experiments showed that all seedlings were able to survive after six months in tested soils reflecting a great tolerance and adaptability to environmental stress [9]. These results were similar to those obtained on several species such as *Secale cereale* [53], *Sorghum bicolor*, *Linum usitatissumum* [51], *Zea mays* [40]. The effect of hydrocarbons on the germination and plant growth varies according to their concentration [29] in the soil and/or to the species [11]. According to Hu [28], hydrocarbons at soil concentrations above 1 $mg \cdot kg^{-1}$ inhibit plant growth by forming a layer of mucous membrane on the root system of the plant, which impedes the respiration and absorption of the root system. In addition, this reduction could be due to the direct toxic effect of hydrocarbons on the metabolism of the plant and to their indirect effects such as changes and alteration in the physicochemical and biochemical properties of the soil [30, 53] as well as the destruction of microorganisms in soil. On the other hand, the growth parameters are significantly reduced in the polluted soil. It is known the RB/AB ratios increase with unfavourable ecological conditions and where plant growth is limited by climatic and soil conditions [48]. Hydrophobicity of hydrocarbons can lead to reduced water and nutrient availability and, therefore, in plant growth [51]. Hydrocarbon soil pollution could therefore induce the same response of *P. lentiscus* by favoring the development of roots to search for water which is not directly available.

In conclusion, given the high survival rate in the two experiments, although the number of seedlings were reduced and these results cannot be generalized, we can consider *P. lentiscus* L. as a species tolerant to drought stress and more particularly to hydrocarbon pollution. It would be interesting to repeat this study by increasing the number of seedlings to draw a conclusion in order to be able to generalize. Furthermore, it would be interesting to analyze the microflora of the soil before and after cultivation and then evaluate the potential of *P. lentiscus* L. for phytoremediation of contaminated soils.

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