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Original article

First Record of *Cladonia portentosa* (Dufour) Coem. (Cladoniaceae) in Libya and Its Association with *Juniperus phoenicea* L. (Cupressaceae) at Al-Gabal Al-Akhdar Area

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Abstract
Juniperus phoenicea L. is one of the most important Libyan plants, accounting for approximately 80% of the vegetation cover in the Al-Jabal Al-Akhdar region. This region is characterized by a higher richness and diversity of cryptogams, especially lichens, compared to other Libyan areas. During the last decades, this species has been exposed to invasion by lichens, and for this, we studied the colonization of fruticose lichen that appeared on juniper trees at the Al-Gabal Al-Akhdar area. Three different locations were selected, and the results from the field study showed that the lichen invaded the trees more on the southern-facing slopes when compared with those of the northern-facing slopes. In addition, the second location revealed more invasion compared to the other two study locations. it was clear that the abundance of the invasion was very high and formed a large scale in the area of the study site number two. This lichen is commonly spread on dead plants, especially J. phoenicea and, to a different degree, on other plant species, such as *Pistacia lentiscus, Olea europaea, and Rhamnus oleoides.* According to the British Society, references, and morphological characteristics, the results showed that the lichen species belongs to the Cladoniaceae family of lichenized fungi in the order Lecanorales, genus *Cladonia*, species *portentosa.* This species was recorded for the first time in Libya.
Keywords. Libya, Green Mountain, *J. phoenicea,* Fruticose Lichen, *Cladonia.*

Introduction

The Green Mountain, which is locally known as “Al-Gabal Al-Akhdar”, is located in the northeastern part of Cyrenaica, Libya, in the Mediterranean Basin (Figure 1). Climatically, it has a rainy winter, with the highest value (about 600 mm) in Libya, in contrast, a hot and dry long period summer. El-Barasi and Saaed [1] reported that this region consists of about 1% of the Libyan territory but contains more than 50% of the country’s flora.

Juniperus phoenicea L. Section *Juniperus sect. Sabina* [2] (local name “Sha'ara”) is a member of the cypress family (Cupressaceae) and one of the most important Libyan flora [3]. It is an evergreen, aromatic, coniferous high shrub or tree, up to 10 m high. It has a dense and conical crown. The bark is grey- brown or white-grey in ageing plants. Leaves are small, opposite, scale-like. The male cones are yellow and the female blackish-violet. They appear between February and April. The fruit is a berry-like globe cone, lustrous, dark reddish-brown, up to 1.5 cm in diameter, with 3-6 seeds; it constitutes about 80% of the total vegetation cover of this area [4]. On the other hand, it is rare in the western lands (Tripolitania).

Lichens are symbiotic organisms that consist of a mycobiont (fungi) and a photobiont (green algae or cyanobacteria, or both) [5]. Because of this structure, taxonomical point of view, it is difficult to classify these organisms [6]. Lichens grow on rocks, soils, and trees (bark, wood, leaves) and can tolerate harsh conditions such as freezing or intense heat. Some lichens are dry out for long periods, especially in hot conditions, and subsequently, become active when rehydrated.

The distribution and abundance of *Juniperus phoenicea* L. in Libya are exposed to danger because of human impacts and climate change. It was classified as threatened in the Red List of IUCN (2016). During the last decades, a symptom like burning appeared on juniper trees. This led researchers and institutions (beginning of the 2000s) to find out the reasons and causes of this phenomenon. Unfortunately, the results were not clear enough to solve this problem, while more influential trees appeared; in addition, more areas were significantly increased during the last few years. This study aimed to define and classification of the colonized fruticose lichen found on juniper plants at Al-Gabal Al-Akhdar area, and to determine out there is any relationship between lichen growth, soil moisture, and affected trees.

Methods

Study area

The study area is located on the Mediterranean coast of the north eastern part of Libya with three terraces: the first is up to 112m above sea level, the second up to 624m, and the third one, which is described as the highest in Libya, is up to 855.5m “Sidi Ahmad Al-Hemery” [7,8]. It is characterized by a Mediterranean climate, and edaphic factors include types of soil that differ in their chemical and mechanical characteristics, such as Terrarossa, Randozina, Calcareous soil, and others [9].

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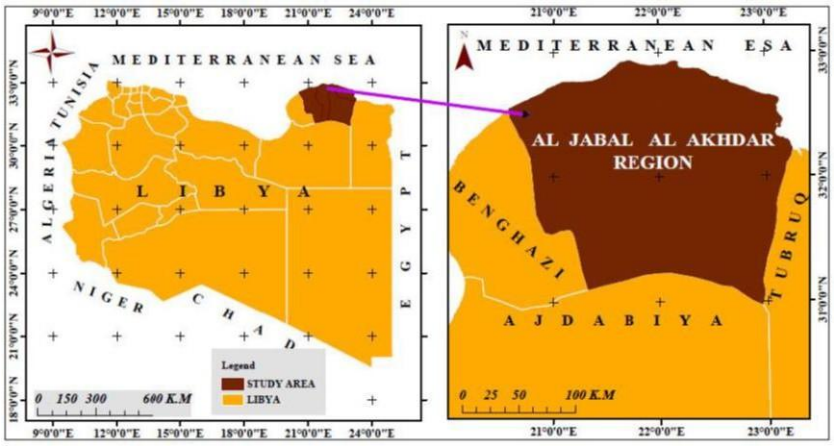

Figure 1. The site of Al-Jabal Al-Akhdar in the North East of Libya

Plant material

Thallus on trunks and branches of *Juniperus phoenicea* L. (Cupressaceae) examined material has been collected during several field trips in drought and rainy seasons in 2022and 2023. Many trees of Juniper have been analyzed to produce a complete epiphytic lichen. Samples were collected from the trunk and branches and kept at the Laboratory until they were used. In addition, three quadrats of 5*5 m. were used to find out the percentage of infected Juniper trees.

Morphology and identification of the lichen samples

The lichen morphological characteristics were studied at the Botany department, Faculty of Sciences, Benghazi University, using a microscopic device with a camera (Bender 4203). Spores of the lichen were also studied and counted. the branches of the lichen body were also noticed. Samples of lichen were taxonomically identified and classified according to different references by using the standards of the British Society of Lichens.

Assessment of lichen biomass

Samples of lichens collected from branches and stems of *Juniperus phoenicea* L. were transported in plastic bags to the laboratory and evaluated for their biomass (size and weight). The lichenometry method, as described by McCarthy [10]. was used as a technique to determine these features.

Soil moisture determination

Soil moisture content was gravimetrically determined according to Rashed et al. [11]. The soil around *Juniperus phoenicea* L. was sampled in plastic bags and transferred to the laboratory. The individual original weights were recorded, samples were dried in an oven at 105 c˚ overnight, then their weight were recorded, the soil moisture content calculated by the following formula:

*Moisture content % = (Original weight –Dry weight) *100*
Original weigh

Isolation and cultivation of lichen spores

This step of the experiment was carried out according to Downes and ItO [12]. Spores of growing lichen were isolated from the surface of plant material using a sterile needle and placed in the center of a Petri dish containing Sabouraud dextrose agar media, which was supplemented with chlorophenicole as a bacteriostatic agent. They were incubated for 48 h in an incubator at 38 °C.

Statistical analysis

Variables were displayed as means and standard deviations. The statistical analysis was performed using SPSS (Statistical Package for Social Sciences, version 26). The study was analyzed by one-way analysis of variance (ANOVA) to find out if there were statistical differences in the means of the parameters.

Results

Distribution and abundance of lichen

The results from the field study showed that the lichen invaded junipers located on southern-facing slopes compared than those on northern-facing slopes. In addition, the second location revealed denser conditions when compared to the other two study locations. The quadrat method reflected that more than 90% of unhealthy trees were colonized by lichens.

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It was clear that the abundance of the invasion was very high and formed a large scale in the area from "Astata" to "Alwardia" as study site number two. This type of lichen is commonly seen on dead trees and shrubs, especially *Juniperus phoenicea* L. and, to a relatively lesser degree, on other species, such as *Pistacia lentiscus* L., *Olea europaea* L., and *Rhamnus oleoides* L. (Figure 2).

Figure 2. Lichen growth on J. phoenicea (Lift) and Olea europea (Right) trees in the study area

Identification and classification of the lichen

According to the British society standards, references and morphological characteristics, our taxonomic results confirmed that lichen specimen belongs to the Cladoniaceae family of lichenized fungi in the order Lecanorales, genus *Cladonia*, (The reindeer moss and cup lichens). The species is *Cladonia portentosa*, which can be found in different habitats in the Mediterranean region. Here, according to our knowledge, it was not recorded before in Libya.

Morphology and structure of the lichen body

The results appeared the lichen as primary fruticose thallus, grey-green in spring and rainy season, and often with a yellowish green tinge in the summer (Figure 3), the surface often roughened, areolate particularly towards the base, richly branched; terminal branches mostly erect, not orientated in one direction, forming broccoli-like, rounded heads (Figures 4), the branching predominantly trichotomous at the apices, often with perforated axils. Pycnidial jelly is colorless. forming mats of neat tufts amongst juniper vegetation, sometimes with distorted main branches; inflated-bloated and grossly modified morphs can occur in other situations. The light greenish grey or pale cream thallus is richly branched with a slightly woolly surface. The branch can be divided into three, but two branches develop sooner than the third, giving the appearance of dividing into two at the tips. The tips often have small holes. Overall, the lichen forms a compact, delicately tufted mat (Figure 3).

Figure 3. Above (A), Primary fruticose thallus in spring and rainy season. Down (B), the growth of lichen with a yellowish-green tinge in the summer

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Figures 4. terminal branches forming broccoli-like, rounded heads. Left, in natural habitat at the study area, right under microscopic investigation

Evaluation of lichen growth parameters and soil moisture content

In the three locations of Al-Jabal Al-Akhdar, lichens weight ranged between (1.48-180g), with mean weight (55.102 ± 42.169g), their size ranged from (10 to 60 mm^2), with mean size (26.63 **±** 11.153mm^2), The moisture content of the soil in term of percentage ranged from (5.64-70.07%) with mean moisture (25.508 **±** 15.954%). The results are presented in (Table 1).

Table 1. Evaluation of lichen growth parameters and soil moisture content

Parameters	N	Minimum	Maximum	Mean ± Std. Dev.
Weight	30	1.48	180.00	55.102± 42.169
Size	30	10.00	60.00	26.63 ± 11.153
Moisture %	27	5.64	70.07	25.508 ± 15.954

Evaluation of lichen biomass and growth

Lichens' weight: Lichens' weight was evaluated and compared using an analysis of variance (ANOVA) test. The results showed that there were high statistically significant differences in the weights of lichens (p-value > 0.001). The least significant differences test (LSD) showed that Lichens at the second location were heavier than those recorded in other locations, followed by the first location (Table 2).

Lichen size: The results showed that there was a statistically significant difference in the sizes of lichens (p-value > 0.05). The least significant differences test (LSD) showed that Lichens at the first location recorded more sizes than the compared to other locations, followed by the second location (Table 2).

Effect of the soil moisture content on the growth parameters of lichens

The results of the correlation between the soil moisture content and growth parameters of lichens grown on *J. phoenicea* L. revealed no association between moisture content and weight (p-value > 0.05), but significant strong positive association between lichens size and moisture content of the soil, lichens size increases as soil moisture content increase (r = 0.476, p-value 0.016) (Table 3).

Table 2. Evaluation of lichen growth on Juniperus phoenicea* L. *at three locations of Al-Jabal Al-Akhdar

Parameters		N	Mean ± Std. Dev.	ANOVA (Sig.)
Weight	Location1	12	42.92 ± 31.37	0.000
	Location2	8	104.75± 38.190	
	Location3	10	30± 17.423	
	Total	30	55.10± 42.169	
Size	Location1	12	31.08± 13.84	0.022
	Location2	8	29.5± 5.099	
	Location3	10	19 ± 6.928	
	Total	30	26.63± 11.153	

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Table 3. Effect of the soil moisture content on the growth parameters of lichens.

Moisture %	Weight	Size
Correlation	0.288	0.476*
Sig.	0.163	0.016
N	25	25

Lichen and spore growth in the Laboratory

To detect the presence of fungal hyphae by applied the microscopic characteristics of fungal structure were applied to identify genera and species by using a microscope and Methyl cotton blue. Growth of fungal hyphae in the plates was not observed, which ensures the season is not suitable for the growth of this lichen.

Discussion

Juniperus phoenicea L. is one of the most important Libyan florae, which consists of 1800 species [3]. During the last decades, the dieback phenomenon associated with a dense growth of lichens on *Juniperus phoenicea* L. has rapidly increased. According to Hagan (2004) [13], many factors, like anthropogenic pressure and increasing drought periods, caused damage to plants, which might lead to critical invasion by lichens. Therefore, the fast lichen growth on *Juniperus phoenicea* L. and other trees and shrubs in Al-Jabal Al-Akhdar is probably a sign of vegetation degradation due to prolonged drought and climate change [14].

Cladoniaceae is one of the largest families of lichens, which includes about 560 species. The reindeer moss and cup lichens (Cladonia) belong to this family [15]. Many Cladoniaceae lichens grow on soil, but others can use decaying wood or tree trunks. They grow in places with high humidity and cannot tolerate aridity. In contrast, the lichen species found in the area, *Cladonia portentosa,* can tolerate the long drought period that characterizes Al-Jabal Al-Akhdar, as described previously. it can reproduce itself and form new colonies even though the precipitates are not enough during the rainy season. Unfortunately, the spores used in this study were not germinated. This could be due to immaturity of spores, germination season, and/or death or dormancy of spores. Several studies [16-18] have taxonomically developed many Cladoniaceae genera, especially in the Mediterranean region [17], with exceptional of Libya. Cladonia lichen, also known as reindeer moss or cup-moss, is a species that has a unique appearance due to its branching structure, which makes it easily recognizable in the wild [18].

The lichen flora of Libya has more than one hundred identified species belonging to different genera [19]. This includes a wide range of ecological niches and morphological adaptations. They can grow on tree barks, sandy soils, limestone stones, and rocks with a variety of growth forms, including crustose, foliose, and fruticose types. Genera like *Xanthoria, Peltigera, Usnea, Physcia,* and *Cladonia,* with a number of species, were identified. But, according to our knowledge, *Cladonia portentosa* was not included; therefore, this study revealed this species as a new record of Libyan lichen flora.

Conflicts of Interest

The authors declare no conflicts of interest.

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