Comparison of photosynthetic pigments and carbohydrate contents in fungus-infected juniper plant (Juniperus phoenicea L.) and the uninfected plant

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Abstract:
This study aimed to compare the physiological characteristics of a fungus-infected juniper plant with another healthy plant, these characteristics include photosynthetic pigment content, soluble sugars and sucrose content. The present study revealed the presence of 4 genera of fungi belonging to Ascomycetaceae and Deuteromycetes isolated from the leaves of Juniperus phoenicea L. and identified by morphological and microscopic characteristics. The species of fungi isolated from the juniper plant are Alternaria alternate, Aspergillus niger, Fusarium oxysporum, Penicillium crustaceum. The affected juniper plant contained 0.306, 0.296 and 0.351 mg g\(^{-1}\) FW chlorophyll a and b and a carotenoid, respectively. The observed decrease in photosynthetic pigment content in the affected plant compared to the healthy plant was 0.713, 0.695 and 0.874 mg g\(^{-1}\) FW, respectively. The content of soluble sugars in juniper plants was measured and found to be 2.26 mg g\(^{-1}\) FW in healthy plants and 1.937 mg g\(^{-1}\) FW in infected plants. The sucrose content was 2.938 mg g\(^{-1}\) FW in the pathogen infected plant and 3.28 mg g\(^{-1}\) FW in the healthy plant. There was a very significant decrease because the sugars are attached to photosynthetic pigments, and as the amount of pigments decreases, the amount of sugars decreases.

Keywords: Alternaria alternate, Aspergillus niger, Fusarium oxysporum, Penicillium crustaceum, Juniperus Phoenicea.
وزن طازج كلوروفيل أ وب وكاروتين، على التوالي. كان الانخفاض الملحوظ في محتوى صبغة التمثيل الضوئي في النبات المصاب مقارنة بالنبات الصحي.

0.713 و 0.695 و 0.874 مجم/جم وزن طازج على التوالي. تم قياس محتوى السكريات الذائبة في نبات العرعر، ووجد أنه 2.26 مجم/جم وزن طازج في النبات السليم و1.937 مجم/جم وزن طازج في النبات المصاب. بلغ محتوى السكروز 2.938 مجم/جم وزن طازج في النبات المصاب بالممرض و3.28 مجم/جم وزن طازج في النبات السليم.

كان هناك انخفاض كبير جدًا لأن السكريات مرتبطة بأصباغ التمثيل الضوئي، ومع انخفاض كمية الأصبغ، تنخفض كمية السكريات.

الكلمات المفتاحية: الترنيت المائي، اسبرجليس نيجري، فيوزاريوم أوكسيسبوريوم، بنيسليوم كريستيكوم، نبات العرعر.
1. Introduction

Vegetation cover is an essential component of biodiversity, and plants are considered to be the most important ecological habitat for all other living things, so they are of the greatest importance worldwide, and their density, distribution, influences diversity. Forests are a renewable natural resource and have become the main focus of many global concerns [10].

The natural vegetation of the Al-Jabel Akhdar (Green Mountains) region contains the majority of Mediterranean vegetable crops. One of the most important components of this vegetation is the *Juniperus phoenicea* L. tree. It accounts for about 80% of the total number of trees and evergreen shrubs found in the eastern part of Libya at El-Gabel El-Akhdar (Cyrenica) [7, 22]. *Juniperus phoenicea* is a plant of the Cupressaceae family that thrives in dry climates with low winter temperatures and comprises a total of 1800 species [17]. It is a fragrant coniferous tree that grows up to 10 meters and has high summer irradiation [33].

*Juniperus phoenicea* L. play important ecological, scenic and economic roles: they increase water resources and prevent soil erosion [37]. Juniper berries are used as a spice to flavor dishes [29]. Their hardiness and drought tolerance make junipers a good choice for ornamental plants [19, 31]. The pharmaceutical properties of *J. phoenicea* and its use in traditional medicine are well documented. Extracts from the leaves and berries are used to treat diarrhea, rheumatism, broncopulmonary symptoms and diabetes [5]. Also, it can improve liver and kidney function and may be useful in curing hepatotoxicity [4]. Antioxidant activity is also reported in extracts of *J. phoenicea*, possibly due to its content of phenols, sterols and flavonoids [33].

The forests of *Juniperus phoenicea* L. in the Libyan region of Jabal Al Akhdar are in a state of severe degradation in some places. Symptoms appear on infected plants, the broad crowns fall off, the leaves on the affected branches turn yellow, then pale red, and finally brown. The leaves of the trees can last two months and then they will start to fall off and the tree will die. These plant species are threatened with extinction due to various factors, including their lack of regenerative capacity and infection with various plant pathogens such as fungi and bacteria [20], as well as the impact of climatic changes in the region, particularly high temperatures and low rainfall, additionally human impacts such as overgrazing and agricultural Expansion and collect medicinal plants, fires, etc. [33].
Many plants interact with soil fungi in a variety of ways, ranging from mutualism to parasitism [16]. Fungi have a powerful impact on plant community structure, limiting the abundance of some species while promoting the abundance of others [25]. Interactions between fungi and plants can have a major impact on plant growth, either directly or indirectly through physiological processes. Fungi can have indirect effects on plant communities by altering the outcome of competitive and facilitating interactions between plants [16]. Acremonium sp., Alternaria sp., A. alternata., Botryodiplodia juniperina, Cercospora sp., Cladosporium sp., Cylindrocarpon sp., Fusarium spp., F. avenaceum, F. chlamydosporium, F. equiseti, F. moniliforme, F. oxysporum, F. semitichatum, F. solani, Phoma sp., Phoma eupyrena, Pythium sp., Rhizoctonia solani, Stigmina juniperina, Theilaviopsis sp. were the most common fungi found on roots and branches of juniper trees [34]. The aim of this study was to determine whether the symbiotic relationship between the host plant (Juniperus phoenicea L.) and the fungi affects the physiological state of the plants. This is done by comparing a healthy plant that has not formed a relationship with fungi and a heavily infested plant.

2. Materials and Methods

2.1. Plant material and preparation of extract

The plant was identified and authenticated as J. phoenicea by botanists at the Department of Botany (Silphium Herbarium), Faculty of Science, Omar Al-Mukhtar University, Al Bayda Libya.

2.2. Identification of Fungal Species of Leaf tissue

The fungi are grown and isolated in vitro according to a method of [1]. The fungi were identified based on mycelial and spore characteristics, and identification of fungal genera and species was based on previous studies [3,12].

2.3. Estimation of photosynthetic pigment contents

Leaf samples (0.2 g) harvested from two plants were homogenized in acetone 85% (v/v) according to the method [9]. The extract was centrifuged at 5000 rpm for 15 minutes and absorbance was recorded at 646 and 663 nm for chlorophyll determination (a and b) and at
470 nm for carotenoids. The pigment content was calculated according to the following formulas as reported by [24].

$$\text{Chlorophyll a} = 12.25 \text{ A}_{663} - 2.79 \text{ A}_{646}$$
$$\text{Chlorophyll b} = 21.21 \text{ A}_{646} - 5.1 \text{ A}_{663}$$
$$\text{Carotenoids} = (1000 \text{ A}_{470} - 1.8 \text{ Chl a} - 85.02 \text{ Chl b}) / 198$$

2.4. soluble sugars, and sucrose extraction and estimation:

2.4.1. Sugars extraction:

For the extraction of soluble sugars, the sucrose method [32] was followed with minor modifications. Freshly weighed leaf samples (0.3 mg) of junipers each macerated in 20 ml of 80% (v/v) ethanol. The tube was held in a boiling water bath with bolts for 20 min and allowed to cool to room temperature. After placing the tube in a centrifuge apparatus at 10,000 rpm for 10 minutes, the supernatant was decanted. Again 20 ml of the previous ethanol was added to the remaining residue in the tubes, stirred, boiled, cooled and centrifuged as previously described and the supernatant was collected and added to the first extract.

2.4.2. Total soluble sugars estimation:

The anthrone method is a colorimetric method for determining total sugar concentration. Sugars react with the anthrone reagent under acidic conditions to give a blue-green color that was linearly related to the amount of sugar in the sample. This method determines both reducing and non-reducing sugars due to the presence of strongly oxidizing sulfuric acid [38].

2.4.3. Total soluble sugar estimation:

Total soluble sugars and sucrose were determined using the method [38], in which the alcoholic extract (0.5 mL) was carefully added to 3.0 mL of freshly prepared anthrone (150 mg anthrone and 100 mL 72% H$_2$SO$_4$) in a test tube, and placed in a boiling water bath until stable color development. The color was read at 625 nm and the standard sample of glucose (0.1 mg/ml) alone was treated with treatments for calculations.
2.4.4. Sucrose estimation:

The sucrose content was determined after hydrolyzing 1.0 ml of the alcoholic extract by adding 1.0 ml of 5.4 N KOH and heating it in the water path at 100 °C for 10 minutes [14]. After cooling, 3.0 mL of freshly prepared Anthrone Reagent was then added and boiled until color developed. The color was read at 620 nm. Sucrose (0.2 mg/ml) was used as a standard.

3. Results and Discussion

In the current study, the fungal species isolated from juniper leaves were identified by light microscopy based on the fungal characteristics. The results showed the presence of two genera of fungi belonging to Ascomycetae Aspergillus niger, Penicillium crustaceum and two genera of fungi belonging to Deuteromycetes Alternaria alternate, Fusarium oxysporum.

The study [1] showed that fungal species isolated from roots of Juniperus phoenicea L. in Al-Jebal Al-Akhdar Libya were identified, where 24 fungal species belonging to 14 genera including 2 Zygomycetes, 7 Ascomycetes and 5 Deuteromycetes.

The content of photosynthetic pigments (chlorophyll a, b and carotenoids mg g⁻¹ FW) in the leaves of Juniperus phoenicea L. was high and significantly decreased in the affected plant compared to a healthy plant (Figure 1). The levels of chlorophyll a, b and carotenoids in Juniperus phoenicea L. were 0.713, 0.695 and 0.874 mg g⁻¹ FW, respectively, in a healthy plant. The decrease in photosynthetic pigment in the affected plant was recorded at 0.306, 0.296 and 0.351 mg g⁻¹ FW, respectively. Pathogenic fungi have negative effects on the rate of photosynthesis and photosynthetic pigment. The present results showed that a healthy plant contains chlorophyll a, chlorophyll b and carotenoids, which play an important role in photosynthetic light absorption [26].

This study is consistent with several studies that showed that the ratio of chlorophyll a/b was reduced in plants infected with fungal pathogens, as [27] reported that the reduction in chlorophyll a, b and carotenoid resulted in a reduction in the photosynthetic rate of infected Phaseolus vulgaris plants caused by Colletotrichum lindemuthianum. Also [35] reported that a similar chlorophyll a/b ratio was significantly reduced in the sunflower leaves infected with Alternaria helianthi. While chlorophyll was higher in healthy leaves. This study confirms the
results of [30] showing a decrease in photosynthesis after *Uromyces appendiculatus* and *Colletotrichum lindemuthianum* infections of *Phaseolus vulgaris* plants. Chlorophyll decrease in *Blumeria graminis*-infected *Hordeum vulgare* plants [2]. The negative effects were also found when two cultivars of *Vitis vinifera* were infected with *Phaeoacremonium angustius* and *Phaeomoniella chlamydospora* [39]. Furthermore, according to [21], *Colletotrichum lindemuthianum* caused leaf damage and cell death in *Phaseolus vulgaris* plants.

The amount of total chlorophyll in inoculated plants decreased significantly by two factors, mainly loss of the photosynthetic area of the leaves, which promotes lower light absorption, and chloroplast disruption during pathogen infection [36]. The rate of photosynthesis in inoculated plants was reduced in all time periods evaluated, pigment reduction and the resulting lower light absorptivity favoring a decrease in the rate of photosynthesis. A low rate of photosynthesis in leaves is the reason for the lower yield of fungus-infested plants [18]. The reduction in photosynthetic water use efficiency of infected plants is a consequence of the negative effect of fungal infection on gas exchange in the leaf, since this parameter is dependent on photosynthesis and transpiration [41]. Changes in pigment concentration were viewed as changes in photosynthesis. Since pigment concentration and damage are negatively related, they can be used as a method for selecting tolerant and sensitive varieties in crops [11]. On the other hand, changes in pigment content have been found to be influenced by exposure time and degree of injury, as well as plant species or genotypes. Carotenoid levels showed a significant reduction as a result of the spread of infection and consequent cell death caused by the pathogen in leaf tissue [42]. It is an accessory pigment involved in stabilization of the lipid membrane in chloroplasts [15] and protection of the photosystem [23]. In addition, other secondary factors of infection can occur when the pathogen causes the accumulation of reactive oxygen species (ROS) that cause inactivation/oxidation of already present pigments in chloroplasts [6, 8, 43].

The present study demonstrated a decrease in carbohydrate content of the *Juniperus phoenicea* plant as shown in Figure 2, where 2.26 and 1.937 mg g\(^{-1}\) FW for soluble sugars were recorded in healthy and affected plants, respectively. The sucrose content in pathogen-infected plants was 2.938, in healthy plants 3.28 mg g\(^{-1}\) FW were measured. Which agrees
with [28] who reported that *Phaseolus vulgaris* plants had lower levels of carbohydrates and sucrose as a result of the reduction in photosynthesis, since the transport and distribution of carbon compounds from the leaf to other parts of the plant is dependent on photosynthesis. In addition, lower rates of photosynthesis mean a low carbohydrate source for apical and root meristems, flowers and seeds.

This result differed from previous studies showing an increase in total soluble carbohydrate content in resistant and susceptible *Phaseolus vulgaris* L. plants infected with *Colletotrichum lindemuthianum*. [8] found similar results in sunflower (*Helianthus annuus* L.) infected with sunflower chlorotic mottle virus. [40] reported a reduction in chlorophyll *a*, *b* and accumulation of all soluble carbohydrates in *Theobroma cacao* plants infected with *Crinipellis perniciosa*. The results of the study [35] indicate a significant increase in the level of soluble sugars in the sunflower plant infected with *Alternaria*. It was observed that the soluble sugar content was more infected than the healthy leaves of the sunflower. This may be an infection-related process, which may require the infected plants to divert more carbon skeletons into the TCA cycle to balance energy demands [13]. The amount of reducing carbohydrates in infected plants increased, probably due to increased activity of the sucrose synthase enzyme. This activity could lead to a small change in the sucrose content of infected plants.

![Figure (1): Photosynthetic pigment and carotenoid contents in the leaves of the studied plants.](image-url)
Conclusion

Forests cover 31 percent of the world's land surface, store an estimated 296 gigatons of carbon, and are home to most of the world's terrestrial biodiversity. Forests are a source of fiber, fuel, food and fodder and provide livelihoods for millions of people, including many of the world's poorest. Around 2.4 billion people use energy from wood for cooking. Forests contribute to climate protection and improve the quality of soil, air and water. When managed sustainably, forests are also a source of renewable raw materials that make a crucial contribution to the development of circular economies. Juniper trees are one of the main components of the Green Mountain, which suffer greatly from fungal growth and the harmful relationship that is driving them to extinction, according to what is mentioned in our study and confirmed by the Red List. Therefore, many future studies and researches in this regard should be considered in order to protect this medicinally, ecologically and economically important plant.

Acknowledgments

The authors would like to thank the Botany Department of Omar AlMukhtar University, Al Bayda Libya for our support and for providing laboratory work facilities.
References


