

The importance of microorganisms and their effects on medicinal plants and their various applications.

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Abstract:

As photosynthesis is the basic process during which light energy is absorbed and converted into organic matter, the importance of the plant pigment chlorophyll (a and b forms) as an intermediary in the transformation of the absorbed solar energy and its activity in the process of photosynthesis and synthesis of organic substances in plants is crucial. Therefore, this paper provides an overview of methods for monitoring the optical activity of chlorophyll molecules and methods (non-destructive and destructive) for quantification of chlorophyll in plants. These methods are used to estimate the effects of different stress factors (abiotic, biotic and xenobiotic) on the

efficiency of photosynthesis and bioproductivity, aiming to assess the impact that these limiting factors have on the yield of various cultivars. Besides, those methods for the analysis of chlorophyll optical activity and/or content are appropriate for assessing the reaction of weed species to different agricultural practices (mineral nutrition, treatment by herbicides, etc.) and studies of different aspects of weed ecophysiology and their influence on crop harvest.

Drought stress is one of the most important factors affecting plant growth. Plant growth under drought stress may be enhanced by the application of microbial inoculation including plant growth-promoting Rhizobacteria. This research was conducted as a factorial experiment in a completely randomized design. The first factor included the bio-fertilizer (A) and control (without bio-fertilizer). The results showed that treatment microbial inoculation level had the highest effect on increasing photosynthetic pigments ($p < 0.01$). While the lowest amount of photosynthetic pigments occurred in treatment A. The highest and lowest shoot fresh weight was belonged to the treatments respectively ($p < 0.01$). The results showed that the use of bio-fertilizers separately had more positive effects on the nutrients uptake of *Juniperus phoenicea*. In general, the results of this study suggest that growth-promoting bacteria as biofertilizers have a greater effect on growth, photosynthesis pigments and nutrient uptake of *Juniperus phoenicea*. The use of microbial inoculation indicated that the use of microbial isolates in agriculture increases plant productivity and its resistance to diseases and other influences such as drought, stress and lack of nutrients and provides it with important needs.

Key words: Microbial isolates, *Juniperus phoenicea*., Green plant, Medicinal plants, pigments and Chlorophyll.

Introduction :

The most important problems of arid and semiarid rangelands are drought and water shortage that affect plants growth and development. Given that the majority of the world's rangelands are located in these areas, the effect of drought stress on these areas' plants is of great importance (Zandi Esfahan and Azarnivand, 2012). Drought stress is one of the main environmental factors limiting the growth and yield of vegetation cover (Nazar et al., 2015; Sagdeghi and Rostami, 2016), the most common cause is the increase in temperature and reduced available water to plants (Nazar et al., 2015). Water scarcity as a limiting factor of plants' growth prevents seed germination and plants' development and reduces the plant productions around the world (Yan, 2015). Nowadays, application of microorganisms in the soil as bio-fertilizer is considered as the most natural and desirable solution for maintaining live and active soil system (Zahir et al., 2004; Nadeem et al., 2014). In addition, the supply of nutrients quite fitting the normal plants' feeding contributing to biodiversity, improving the status and maintaining the health of the environment is one of the most important benefits of bio-fertilizers (Delshadi, 2015). Bacteria and fungi, especially growth-promoting bacteria and materials derived from their activity, are the most important biofertilizers. The fertilizers according to the growth and development of plants are commonly called yield promoting bacteria (Zahir et al., 2004; Nadeem et al., 2014). The mechanism of the plant growth-promoting bacteria has not been fully understood to increase the plant growth, but the bacteria are capable of producing some growth-promoting hormones especially a variety of cytokinin, gibberellic acid and auxin, fixing nitrogen, and phosphorus (Vacheron et al., 2013). *Azotobacter* spp. and *Pseudomonas* spp. are the most important bacteria that increase soil mineral elements, with the production of matters regulating growth and they affect development and yield of plants (Zahir et al., 2004; Hayat et al., 2010). The use of

bio-fertilizers such as nitrogen fixation bacteria of the genus *Azotobacter* and bacteria dissolving phosphate such as *Pseudomonas*, provide nutrients needed by the plant such as nitrogen and phosphorus and thus improves plants' growth and yield in addition to increasing useful soil microorganisms' population (Arancon et al., 2004). It should be noted that the effect of growth-promoting bacteria depends on the yield of the host plant and soil environment as well as the inherent capabilities of bacteria (Nadeem et al., 2014). However, growth-promoting bacteria play an important role in maintaining soil fertility and improving the plant growth and development, but some concerns have been also reported in some studies (eg. Saharan and Nehra, 2011; Vacheron et al., 2013). For example, the production of cyanide is a well-known feature of *Pseudomonas* (Martínez-Viveros et al., 2010). Cyanide in fact as an environmental controller can increase the growth and on the other hand has negative impacts on the plant growth (Martínez-Viveros et al., 2010). Also, the production of auxin by the bacteria at low concentration increased the plant growth and at high concentration reduced the plant growth (Patten and Glick, 2002; Vacheron et al., 2013). Although growth-promoting bacteria are very effective on the plant growth and development, specific bacterial species may reduce specifically the growth and the negative role may occur under certain conditions. Therefore, it is necessary to choose the appropriate species to obtain maximum plant production (Nadeem et al., 2014). *Avena sativa* L. (Gramineae family) is a species of cereal grain grown for its seed. It is suitable for human consumption and livestock feed (Achleitner et al., 2008; Nirmalakumari et al., 2013). Although this plant can grow in cold and wet weather and low fertility soil (Ren et al., 2007; Buerstmayr et al., 2007), it is sensitive to drought stress (Frey et al., 1986).

Materials:

1.1. Plant material:

The samples of *Juniperus phoenicea* collected from the EL-Gabal EL-Akhdar area (Asulntah area) ((Sample1= plant with microbial inoculation (biofertilizer) naturally grow, sample2= plant without (microbial isolates). All samples were collected from the same location.

1.2. Chemicals:

1, 1-Diphenylpicrylhydrazyl (DPPH[•]), methanol, ethanol and acetone were supplied by Sigma and Merck company. Ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloroacetic acid, sodium carbonate, anhydrous sodium sulfate and pyrogallol.

Results:

1. The results of morphological and growth characteristics are given in table (1).

From this table, the sample number (1) reflected the highest mean value of plant height and stem diameter. They were 222.67 cm and 16.42 cm, respectively.

Table (1): Growth in term of plant height (cm), stem diameter (cm) and leaf dry weight (mg) of *J. phoenicea* L. in EL-Gabal EL-Ahkdar area.

Sample Name	Plant height (cm)		Stem diameter (cm)		Leaf dry weight (mg)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Healthy sample	222.67	(21.35)	16.42	(5.90)	3.0234	(0.19)
Semi healthy	216.55	(17.93)	11.22	(3.14)	3.2058	(0.23)

sample						
Sample sick	214.23	(23.47)	9.89	(2.05)	3.8240	(0.11)

2. The results obtained in this study as shown in table (2) indicate that the healthy sample contains a high amount of chlorophyll A, chlorophyll B and carotenoids as compared with the semi healthy sample and Sample disease.

Table(2): Estimation of photosynthetic pigments of *J. phoenicea* L. leaves in EL-Gabal EL-Ahkdar area

Sample	Chlorophyll A (mg/ml)	Chlorophyll B (mg/ml)	Carotenoids (mg/ml)
Healthy sample	5.7708	4.894	6.323
Semi healthy sample	5.670	4.721	5.126
Sample sick	4.346	2.915	3.503

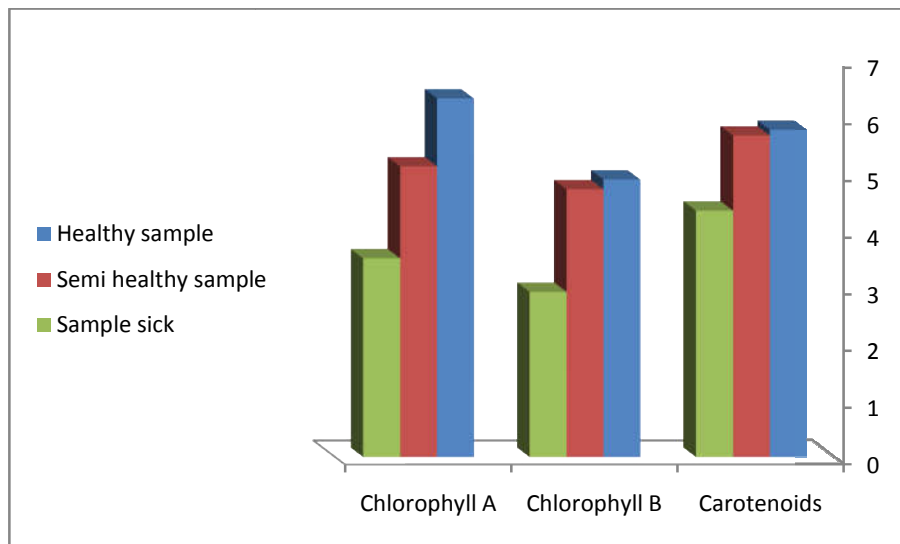


Fig. (1) Estimation of photosynthetic pigments of *J. phoenicea* L. leaves in EL-Gabal EL-Ahkdar area

2.3. Growth characteristics of *J. phoenicea*:

The study samples were divided into lots of different trees, depending on the appearance the exterior of the tree with three replicates in each tree was made to find out the quantitative and morphological differences.

2.4. Estimation of photosynthetic pigments:

The photosynthetic pigments were extracted from a known fresh weight of leaves in 85% aqueous acetone to a certain concentration for spectrophotometric measurements. The photosynthetic pigments (chlorophyll a, b and carotenoids) were determined by, the spectrophotometric method as described by Metzner *et al.* (1965). The pigments extract was measured against a blank of pure 85% aqueous acetone at three wavelengths of 452.5, 644 and 663 nm. Taking into consideration the

dilution factor, it was possible to determine the concentration of pigment fractions (chl. a, b and carotenoids) as mg/ml using the following equations:

$$\text{Chlorophyll a} = 10.3 E_{663} - 0.918 E_{644} = \text{mg/ml}$$

$$\text{Chlorophyll b} = 19.7 E_{644} - 3.87 E_{663} = \text{mg/ml}$$

$$\text{Carotenoids} = 4.2 E_{452.5} \left\{ \begin{array}{l} 0.0264 \text{ chl. a} \\ + \\ 0.4260 \text{ chl. b} \end{array} \right. = \text{mg/ml}$$

Finally these pigment fractions were calculated as mg/g fresh matter.

2.5. The Estimation of microbial number:

2.5.1. Nutrient Agar medium

23 g of nutrient agar medium were suspended in 1 liter ml distilled water and then heat to boiling to dissolve the medium completely, then sterilized by autoclaving at 15 lbs pressure (121C) for 15 min.

2.5.2. Sample preparation:

2.5.2.1. Plant:

leaves (brown and green) were collected from *Juniperus phoenicea*. 2 grams of each part was put in a test tube containing 8 ml of distilled water and shaken for 30 seconds. 1 ml of the sample was put in a petri dish and then poured by nutrient agar medium under sterile conditions and incubated at 37.5°C for three days. Total number of microbial colonies of each plate was estimated.

2.5.2.2. Soil:

Two samples collected from sifting soil. 1 gram of each sample from different sites (under the sieve and above the sieve) was added to a test tube containing 9 ml of distilled water and shaken for 30 seconds. 1 ml of each sample was put in a petri dish and then set up the nutrient agar medium incubated at 37.5 °C for three days. The total number of microbial colonies of each plate was estimated.

2.5.3. pure Culture:

A single bacterial colony from each mixed culture was taken by loop and streaked on nutrient agar medium and incubated at 37.5 °C for three days.

2.5.4. Gram stain

Differential stains (Gram stain) was carried out to confirm that the bacteria were gram-negative and rod shaped. Smears from single colony bacteria grown on EMB at 37 °C and 44.5°C were prepared and covered by primary stain (Crystal violet) for 1 min and then Mordant (Iodine) for 1 min to make CV-I complex after that decolourized agent (70% ethanol) was washed the slide to remove the CV-I complex in the thin layer of peptidoglycan of gram-negative bacteria. wall. Drops of counterstain (safranin) were added for a half minute. The slide then washed in the distilled water and dried by filter

paper after that examined by microscope using an oil immersion lens.

In summary, gram-positive cells retain the dye and remain purple, Gram-negative cells did not retain the dye; they were colorless until counterstained with a red dye (pink cells).

2.5.5. Endospore staining:

A film was made on a clean slide by emulsifying part of a colony in loopful of distilled water. The film was then air-dried and stained with malachite green for 4-5 min, using a flame. The smear was rinsed rapidly with water and stained with safranin solution for 30 sec. The slide was washed with water and allowed to dry. On microscopic examination the endospores appeared green and the cells were pink (Abualdhab and Gorani, 1983).

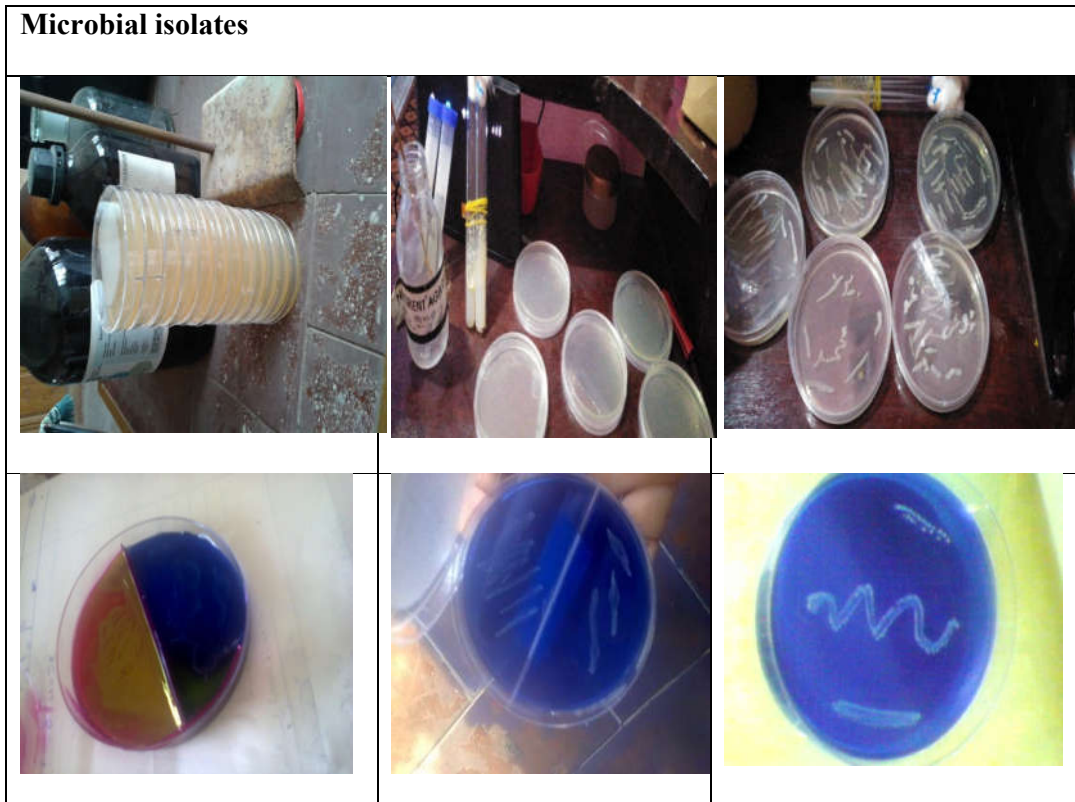
2.5.6. Motility test:

The test used to distinguish between motile and non-motile bacteria, using the hanging-drop preparation.

First, a little oil immersion was placed around the edge of the slide, then with a wire loop, a small loopful of the culture was transferred to a clean dry covered slip. After that, the cover slide was inverted over the coverslip so that the drop was in the centre of the cavity and the slide was pressed down gently but slimly so that the oil seals the coverslip in position.

The slide was inverted quickly and smoothly and the drop of culture was placed in the form of the hanging-drop, and the preparation was examined quickly.

It is necessary to distinguish between Brownian movement (a continuous agitation of very small particle suspended in a fluid which is called unbalanced impacts with molecules of the surrounding fluid) or drift in one direction caused by the slide being slightly tilted and true motility (Abualdhab and Gorani, 1983).



Conclusion:

The results showed that growth-promoting bacteria individually had a greater effect on increasing the plant growth, photosynthesis and nutrient uptake in the root of *Juniperus phoenicea*, compared with

the combined use of growth-promoting bacteria. The remarkable note of the results of this study was that growth-promoting bacteria under irrigation conditions increased studied traits and had a role in mitigating the effects of drought stress. The results of this study showed that growth-promoting bacteria can be used in the restoration and improvement of rangelands, but it should be considered the effect of Biofertilizers depends on the plant species, climate and soil condition. However, the question as to what extent can the Rhizobacteria promote the host plant's resistance to drought effects needs further research so that appropriate strains of each region and plant can be known and used, given that growth-promoting Rhizobacteria include a wide range of soil microorganisms. Therefore, a more comprehensive and accurate survey and study in the field are recommended. The current study was divided into two types of experiments, one of which is using microbial isolates as an important type of fertilization and the other without adding microorganisms. Because drought stress is one of the most important factors of

reduced plant growth, the present study was conducted to aim to study the effect of growth-promoting bacteria by microorganisms (with microbial isolates) under drought stress on growth and photosynthetic pigments of *Juniperus phoenicea*. To find out the effect of microorganisms on the root nutrient uptake in *Juniperus phoenicea*. Under drought stress. The role and efficiency of microorganisms in supplying the plant with the nutrient uptake it needs and increasing plant growth.

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