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RESEARCH PAPER

Influence of watering periods and organic fertilizer on seedling growth and development of two species of oak tree; *Quercus aegilops* and *Quercus libani*

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A B S T R A C T:

Raisin drought seasons and temperature at last decades are the maximum prominent threats for the world. It is predictable that there will be several regional influences by worldwide climate alteration, especially on freshwater reserves, energy, biodiversity, agriculture, human health, forest trees and sea level. This experiment was aimed for estimate the influence of watering periods and organic fertilizers on seedling growth and development of the two oak species*;* *Q. aegilops* L. and *Q. libani* L. The trial was conducted under three levels of water periods (once every 4 days, 8 days and once every12 days) and with three organic fertilizer levels (control, 25 gm, and 50 gm per pot) were used two times when seedlings age was 3 and 6 months. The result showed that the organic fertilizer with watering periods had a significant effect on seedling growth and physiological properties. The results indicated that the highest value Leaf number and seedling height increment (5.22 $plant^{-1}$, and 2.20 cm, respectively) were both recorded from (4 days watering $plant^{-1}$). The highest values of leaf area (10.95cm^2) were recorded from the (zero or control organic fertilizer $plant^{-1)}$ in *Q. aegilops*. The highest value moistures content was (63.52 %) were recorded from interaction between (8 days of watering*25gm organic fertilizer). Therefore, it is concluded that *Q. aegilops* and *Q. libani* seedlings could success under organic fertilizer and watering periods, especially seedlings were showed best performance under well water availability.

KEY WORDS: *Watering periods, Organic fertilizer, Q. aegilops, Q. libani, Morphological, and physiological parameters*
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1. INTRODUCTION:

Oak tree (*Quercus spp)* is a member of the Fagaceae family, which also includes other significant woody plants (Khwarahm, 2020). This family includes shrubs and trees with simple alternating leaves that are known for their wood, wind-pollinated blooms, acorns as fruits, and long lifespans. There are around 600 species of the genus *Quercus*, usually known as oaks, throughout the globe (Tantray *et al*., 2017).The number of native species decreases from southern to northern latitudes consistent with the tropical origin of the species (Nixon, 1993). The genus encompasses both deciduous and evergreen plants that are adaptable to a variety of habitats, including uplands and deep sands to seasonally flooded wetlands (Tyree and Ewers, 1991).

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Typically, the leaves are smooth and round with toothed borders, covered in star-shaped furs, and have soft yellow fur on the back. The fruit is elongated, semi-elliptical, and covered in a conical cup of white velvet, its fruit is called Gland that is placed in Acorn cup (Sabeti, 2003). Given this extensive variety, it should come as no surprise that each species responds to water stress differently. Recent reviews shown a range of characteristics of plant response to water stress (Hinckley *et al*., 1978). *Q. libani* is indigenous to the western Asian region of the eastern Mediterranean, encompassing Lebanon, eastern Turkey, northern Iraq (Kurdistan Region), and Iran. *Q. libani* is an 8-meter-tall deciduous tree 26 feet (Asouti *et al.*, 2014). The deciduous leaf has a circular base and a slightly pointed tip. It is thin, elongated, and frequently asymmetrical. The leaf's upper side is dark green and its underside is light green in the adult condition (Seigue, 1985). In the Kurdistan Region of Iraq, the oak tree (*Q. aegilops*) makes up over 70% of the oak woodlands. In addition to having ecological significance as the habitat for numerous unique and migratory species, *Q. aegilops* forest also provides socioeconomic benefits, such as those related to cattle feed, building materials, medicine, charcoal, and firewood (Khwarahm, 2020). Morphological adaptations are briefly examined to highlight traits of drought tolerance and drought avoidance as well as to give extra context for physiological responses, because both play variable degrees of roles in the mechanisms that different oak species have developed to survive water stress, morphological and physiological adaptations must be taken into account jointly (Seleiman, *et al*., 2021). The two biggest hazards to the planet in recent decades have been rising temperatures and drought seasons. A number of regional implications of global climate change are anticipated, particularly on agriculture, freshwater reserves, sea level, energy, human health, biodiversity, and forest trees (Le Provost *et al*., 2013). Three elements; nitrogen, phosphorous, and potassium make up the majority of organic fertilizers. Nitrogen affects leaf growth, phosphorus affects the growth of roots, flowers, and fruit, and potassium affects robust stem growth, water transport in plants, and the encouragement of flowering and fruiting. Although N: P ratios have been commonly utilized to estimate plant nitrogen and phosphorus limitation in wetlands (Güsewell *et al*., 2003). Several writers have researched the effects of organic fertilizer, other fertilizers, and manures in agricultural cultivation (Sultana *et al*., 2015). In many forestry services, fertilization has not been used. Numerous research conducted across the world have shown that fertilizing young trees improves their ability to flourish. Numerous earlier research demonstrated that the availability of nutrients, particularly N and P, is a key component in seedling growth (Lin and Stemberg, 2007). A successful plantation must be developed from healthy seedlings in a challenging environment, before the complete canopy closes, fertilizing newly planted trees can be very important for boosting future tree yields (Leech *et al*., 2012). Water is crucial for the development, growth, and productivity of plants (Emmanuel, 2014). According to Hartmann *et al.*, (2005), water stress represented by drought is the most important abiotic factor limiting plant growth and development. Water stress significantly reduced fresh and dry weight, leaf number, and overall leaf area (Vandoorne *et al*., 2012). According to Hsiao and Xu (2000), growth is easily stopped when there is a water shortage, and root growth is preferred over leaf growth. In a water-stressed habitat, leaves are frequently sparse in in number and size. In order to produce the healthy and strong oak seedlings in Erbil governorate, then transplant the seedlings in the northern of Erbil for better future achievement of afforestation/reforestation of oak trees in the northern of Erbil. Therefore, the objective of the study were conducted to investigate the Influence of watering periods and organic fertilizer on seedling growth and development of the two oak species; (*Quercus aegilops and Quercus libani)* seedlings.

2.Materials and methods:

2.1 Location and species under study:

This study was carried out in Hasar Organization for earth sciences, Cihan University-Erbil, Iraq, from October 2021 to July 2022 at an altitude 436 meters above sea level (Latitude North 36.16°, longitude East 44.03°). At the first, One-year-old seedlings of (*Quercus aegilops and Quercus libani)* were used in this study which provided and supported by Hasar Organization for earth sciences, from 1st May 2021. The seedlings were moved from polyethylene planted into larger plastic pots size (20 cm diameter, 20 cm depth and 25 cm High) filled with 10 Kg loamy soil for each pot. The seedlings were irrigated daily in the early morning. Samples of soil sent to laboratory at directorate of agriculture research center – Erbil for analysis chemical and physical properties as shown in (Table 1).

Table 1 Physical and chemical characteristics of the soil used in the study.

Soil properties		Units	Hasar Organization soil
Particle Size distribution	Sand	(g kg^{-1})	48.3
	Silt		30.1
	Clay		21.6
Texture name			Loamy soil
Soil PH			7.4
EC_E		dSm^{-1}	0.57
Organic matter content		(g kg^{-1} soil)	0.59
Potassium			235
Available Phosphorous		(µg g^{-1} soil)	6.9
Total nitrogen(N)		(Mg g^{-1}soil)	0.13

*****The soil properties examinations were directed on the Board of directors of Agriculture Research Center / Erbil.

2.2 The experimental design:

The experiment was arranged in a design with 18 treatments under three replications (2 species x 3 watering period x 3 level organic fertilizer x 3 replication = 54 unit of experiment). Two factors were used to investigate plant growth. The first factor has included three periods of irrigation: once per 4 days "W4", once per 8 days "W8", and once per 12 day "W12". The second factor has included fertilizer with organic fertilizer solution at three concentrations of (zero, 25 and 50) gm pot^{-1} modified from Demirkiran and Cengiz, (2010). Organic fertilizer nutrition was added as soil medium during a time. Seedlings were protected from rainfall in the upper by polyethylene covers. The results analyzed based on factorial complete randomized design (F-CRD) (Ferreira *et al.*, 2014).

2.3 Morphological parameter measurements:

At the start of the study (June, 2021), Measurements of stem length(cm), stem diameter(mm), root length and root diameter number of branch and leaf numbers to all seedlings were measured. The stem diameter was measured at 3cm above soil surface obtained by (digital vernier caliper) in millimeter units in addition to account number of leaves. The stem length was measured from the surface of soil for the maximum living apical stem using a measuring tape in cm units. The number of leaves for each seedlings was counted automatically. Also re-measured for this parameters to find number of leaves increment, stem diameter increment (mm) and stem length increment (cm) at the end of the experiment (November). For evaluate leaf area (cm^2), three seedlings were randomly selected from each treatment, then three leaves per seedling were collected and placed on a white paper and took photo by smart phone camera, then the photo transfer to the laptop and calculate by image J software for measures leaf area as illustrated by Ahmad *et al.*, (2015).

2.4 Physiological parameter measurements

The measurement of chlorophyll content% was obtained to completely plants under the study using a portable chlorophyll meter SPAD- 502 (Minolta Co. Ltd. Japan). Moreover, the mean of (3) chlorophyll content readings were obtained on the (3) leaves in (3) positions (bottom, middle and top) plant^{-1} by Westerman, (1990). To estimate the nitrogen(N) content, micro kjeldahl apparatus method was used, (30 mg) of dry leaves powder was laid in digestion tubes with (5.0 ml) of sulfuric acid (98 %) and (5.0 ml) hydrogen peroxide (36 %). In distillation method sodium hydroxide (40 %) and boric acid were used Bremner and Mulvaney, (1982). Phosphorus content was determined on the digested crud, using spectrophotometer at the wave length of (410 nm) as described by Olson *et al.*, (1986). Potassium content was determined, using flame-photometer method as described by Tandon, (1993). Protein content was calculated by multiplying total nitrogen value by (6.25) Govil and Kapur, (2000). Protein percentage % = N% * 4.64

2.5 Data analysis

Data of studied parameters were subjected separately for analysis of variance (ANOVA) by using statically analysis software (SAS vision 9.3). The significant different between treatment means were compared by Duncan multiple range Test at P ≤ 0.05 probability level.

3. Results and Discussion

3.1 Effects of species (*Q. aegilops and Q. Libani*), watering periods and organic fertilizer from leaf number increment, stem diameter increment (cm^2), seedling height increment (cm), number of branches increment seedlings.

Table 2, shows the significant effects of the species, watering period and organic fertilizer on the leaf number increment, stem diameter increment, seedling height increment, and number of branches increment per seedling. The highest values of leaf number, stem diameter and seedling height (4.33 per seedling, 1.10cm^2 and 1.91cm, respectively) were recorded in (*Q. aegilops)* under greenhouse condition. However, the lowest values of leaf number, stem diameter and seedling height (3.19 per plant, 0.10cm^2 and 1.03cm, respectively) were recorded from the (*Q. libani*). No significant different were founded in number of branches as showed in table (2). In regarding of different watering periods, results showed, the highest values of Leaf Number, stem diameter and seedling height (5.22 $plant^{-1}$, 0.86cm^2 and 2.20cm, respectively) was obtained on the application of (4 days watering $plant^{-1}$, 8 days watering $plant^{-1}$ and 4 days watering $plant^{-1}$, respectively) of watering period under greenhouse condition. However, the lowest values of leaf number, stem diameter and seedling height (0.89, 0.41cm^2 and0.34cm, respectively) were recorded from the (12 days watering $plant^{-1}$, 12 days watering $plant^{-1}$ and 12 days watering $plant^{-1}$, respectively) watering period. No significant different were founded in number of branches as showed in table 2. In the relevant to effectiveness of organic fertilizer, we found that the highest values of leaf number and seedling height (4.72 per plant and 2.39 cm, respectively) were both recorded at the (control or zero organic fertilizer) level organic fertilizer. Nevertheless, the lower values of leaf number and seedling height (2.5 per plant and 0.76 cm, respectively) were recorded at the (25gm organic fertilizer $plant^{-1}$ and 25gm organic fertilizer $plant^{-1}$, respectively). On the other hand, no significant different were founded in number of branches and stem diameter as presented in table 2. The impact of water stress on leaf growth could be clarified as a method of adaptation to the conditions of water deficit to limit the transpiration rate, in order to preserve the water amount in the soil around plant roots to increases the chance of plant survival. Yet, others parts of plant which did not affect during deficit irrigation period might be because of partial root drying reduce water losses by transpiration without affecting plant growth These are in consistent with the findings of (Maatallah *et al.*, 2010) as the drought may cause significant changes in laurel plants. Petropoulos *et al.*, (2008), reported that water stress due to drought is the most significant abiotic factor limiting plant growth and development. Water stress drastically decreased leaf number, and total leaf area (Vandoorne *et al*., 2012). Hsiao and Xu (2000) reported that under water deficiency, growth is readily inhibited and growth of roots is favored over that of leaves. Often, leaves of plants growing in water stressed environment are small both in number and size. This result is agreed with study of Petropoulos *et al.*, (2008) when stated that there was no significant increase in plant growth when the level of applied fertilizer increased from 150 to 300 or 450 mg kg-1, additionally, increasing the N level to 300 mg kg-1 did not cause additional increase in the number of leaves per plant and even decrease number of leaves in comparison with 150 mg kg-1 level.

Table 2 Effect of species, watering periods and organic fertilizer on morphological seedling growth characteristics.

Factors	Leaf Number increment	Stem diameter increment(cm^2)	Seedling height increment(cm)	Number of branches increment
Species				

Q. aegilops	4.33 ± 0.67 a	1.1 ± 0.1 a	1.91 ± 0.46 a	1.22 ± 0.1 a
Q. libani	3.19 ± 0.65 b	0.1 ± 0.01 b	1.03 ± 0.17 b	1.33 ± 0.12 a
P- value	*0.0083 ***	0.001 **	*0.0026***	*0.4109 Ns*
Watering period				
W4	5.22 ± 0.73 a	0.52 ± 0.12 b	2.2 ± 0.46 a	1.44 ± 0.17 b
W8	5.17 ± 0.85 a	0.86 ± 0.2 a	1.86 ± 0.48 a	1.33 ± 0.14 ab
W12	0.89 ± 0.25 b	0.41 ± 0.09 b	0.34 ± 0.13 b	1.06 ± 0.06 b
P- value	*0.0001***	0.0001 **	*0.0001***	*0.06 Ns*
Organic fertilizer				
0 (control)	4.72 ± 0.77 a	0.56 ± 0.15 a	2.39 ± 0.58 a	1.39 ± 0.14 a
25 gm pot^{-1}	2.5 ± 0.64 b	0.69 ± 0.17 a	0.76 ± 0.2 b	1.33 ± 0.14 a
50 gm pot^{-1}	4.06 ± 0.94 a	0.54 ± 0.13 a	1.25 ± 0.35 b	1.11 ± 0.11 a
P- value	*0.0003 ***	0.1636 NS	*0.001 ***	*0.21 Ns*

Means with ± standard error) have the same letters in columns are not significantly different by Duncan at p ≤ 0.05 (*) or (**).

3.2 Effects of species, different watering periods and organic fertilizer from chlorophyll content, leaf area (cm^{-2}) and moisture% of seedlings.

The result obtained in table 3 show significant effects of the different watering periods and two species seedling oak trees from chlorophyll content, leaf area (cm^{-2}) and Moisture seedling growth oak tree. The highest values of chlorophyll content, leaf area and moisture% (51.54, 12.27cm^2 and 51.12%, respectively) were recorded from the *Q. aegilops* under greenhouse condition. However, the lowermost values of Chlorophyll content, Leaf area and moisture% (44.68, 7.81cm^2 and 42.41%, respectively) were recorded from the *Q. libani* has given in Table 3. The highest values of moisture% was (53.48%) were recorded from the (8 days watering plant^{-1}). However, the lowest values of moisture (41.76%) were recorded from the (12 days watering plant^{-1}). Also, no significant different were founded in Chlorophyll content and leaf area on the different level watering period as shown in Table (3).

The highest values of leaf area (10.95cm^2) were recorded from the (zero or control organic fertilizer plant^{-1}). However, the lowest values of leaf area (9.32cm^2) were recorded from the (50gm organic fertilizer plant^{-1}) different levels of organic fertilizer. However, no significant effect of the different levels of organic fertilizer on studied chlorophyll content and moisture% in seedlings were found as shown in Table (3). This result is agreed with study of Petropoulos *et al*., (2008), when stated that there was no significant increase in plant growth when the level of applied fertilizer increased from 150 to 300 or 450 mg kg-1, additionally, increasing the N level to 300 mg kg-1 did not cause additional increase in the number of leaves per plant and even decrease number of leaves in comparison with 150 mg kg-1 level. This result found in the leaf area is contrary to the result of Gopal *et al.*, (2010), which stated the largest leaves area was recorded on treatments with vermicomposting. While the least leaf area was recorded from control plants; he also stated the leaves area is large when nutrients content in organic fertilizer was increased. This is due to availability of dry matter in organic fertilizer that contain rich nutrients and higher light interception resulted in increased leaf area and high photosynthetic activity leads to an increase in the plant growth. (Balraj *et al*., 2014).

Table 3 Effect of species, watering periods and organic fertilizer from chlorophyll content (SPAD), leaf area (cm^{-2}) and moisture% from seedling growth.

Factors	Chlorophyll content	Leaf area (cm^2)	Moisture%
Species			
Q. aegilops	51.54 ± 0.91 a	12.27 ± 0.8 a	51.12 ± 2.63 a

Q. libani	44.68 ± 0.96 b	7.81 ± 0.5 b	42.41 ± 1.74 b
P- value	0.0001**	0.0001**	0.0001 **
Watering period			
W4	48.03 ± 1.23 a	9.28 ± 0.99 a	45.05 ± 3.09 b
W8	47.72 ± 1.43 a	10.9 ± 1.27 a	53.48 ± 3.16 a
W12	48.59 ± 1.59 a	9.93 ± 0.47 a	41.76 ± 1.52 b
P- value	0.8353 NS	0.2933 NS	0.0001 **
Organic fertilizer			
0 (control)	46.67 ± 1.15 a	10.95 ± 1.17 a	43.58 ± 2.24 b
25 gm pot^{-1}	49.35 ± 1.33 a	9.85 ± 1 a	49.42 ± 3.77 a
50 gm pot^{-1}	48.31 ± 1.67 a	9.32 ± 0.66 a	47.29 ± 2.42 ab
P- value	0.1993 NS	0.0001**	0.0703 NS

Means with ± standard error) have the same letters in a columns are not significantly different by Duncan at $p \leq 0.05$ (*) or (**).

3.3 Effect of species, watering periods and level organic fertilizer on the nitrogen(N)%, phosphorus(p)%, and potassium(k)% characteristics.

The highest values of N, P and K (1.47%, 0.80%and0.19%, respectively) were recorded from the (8 days watering plant^{-1}, 8 days watering plant^{-1} and 4-12 days watering plant^{-1}, respectively) as presented in table 4. However, the lowest values of N, P and K (1.29%, 0.71% and0.18%, respectively) were recorded from the (12 days watering plant^{-1}, 12 days watering plant^{-1} and 8 days watering plant^{-1}respectively). In regarding of the effectiveness of organic fertilizer. The maximum values of N, P and K (1.45%, 0.79% and 0.20%, respectively), were recorded from the (50 gm organic fertilizer plant^{-1}, 25gm organic fertilizer plant^{-}1 and 50gm organic fertilizer plant^{-1}). But, the minimum values of N, P and K (1.27%,0.70% and 0.17%, respectively), were recorded from the (25g organic fertilizer plant^{-1} 50gm organic fertilizer plant^{-1}and 25gm organic fertilizer per plant) Effects of different levels of organic fertilizer as shown in Table (4). In General the result showed that seedlings responses to effects of both species of oak tree, watering period and levels of organic fertilizer. Dried leaf samples and organic matter were analyzed for percent concentrations of the macronutrients N, P, K and Mg, using an auto analyzer, after digestion with H2SO4 (Faithful, 1971). Previous work by Malinovszky (1994) showed that these were the nutrients most likely to be limiting to tree growth in overburden. Amended and un-amended overburden samples were analyzed for extractable levels of the macronutrients P, K, and Mg. Sodium bicarbonate was used as an extract ant for P and 1 mole NH4NO3 for K and Mg (Rowell, 1994). Effects of organic fertilizer and other fertilizers and manures in the agricultural farming has been studied by several authors (Sultana *et al.*, 2015; Siddique, 2015). Many studies throughout the world have demonstrated that fertilization of young trees increase the growth performance. Many previous studies showed that nutrient availability; especially N and P are important factor responsible for seedling growth (Lin and Stemberg, 2007). This indicates that water stress reduced N, P and K content, compared to well-watered treatment (control) as Hussain *et al.*, (2019) declared the plant without fertilizer had the lowest N, P and K content than other treatments. Similarly, Dania *et al.*, (2014) reported that N and K content significantly decreased in seedling without fertilizer treatments. Our results are in line with those findings by Al-Mansor *et al*., (2015) and Xiukang and Yingying (2016) reported that the N, P and K uptake of eggplant seedlings increased with increasing application rates of N, P and K fertilizers. Our result also in line with a pervious study on growth and nutrient uptake in oak seedlings (Lunt *et al.*, 2003)

Table 4 Effect of species, watering periods and organic fertilizer on the nitrogen (N), phosphorus (p) and potassium (k) characteristics.

Factors	N%	P%	k%
Species			
Q. aegilops	1.36 ± 0.06 b	0.7 ± 0.04 b	0.19 ± 0.01 a
Q. libani	1.38 ± 0.02 a	0.79 ± 0.07 b	0.18 ± 0.01 b
P- value	0.0001 **	0.0001 **	0.0001 **
Watering period			
W4	1.35 ± 0.05 b	0.73 ± 0.04 b	0.19 ± 0 a
W8	1.47 ± 0.07 a	0.8 ± 0.11 a	0.18 ± 0.02 b
W12	1.29 ± 0.04 c	0.71 ± 0.05 c	0.19 ± 0.02 a
P- value	0.0001**	0.0001 **	0.0003 **
Organic fertilizer			
0 (control)	1.39 ± 0.07 b	0.75 ± 0.08 b	0.2 ± 0.02 a
25 gm pot^{-1}	1.27 ± 0.04 c	0.79 ± 0.06 a	0.17 ± 0.01 b
50 gm pot^{-1}	1.45 ± 0.04 a	0.7 ± 0.09 c	0.2 ± 0.01 a
P- value	0.0001 **	0.0001 **	0.0001 **

Means with ± standard error) have the same letters in a columns are not significantly different by Duncan at $p \leq 0.05$ (*) or (**).

4. Conclusion

The results obtained in this study showed that oak tree differ significantly in their response to drought and hence its tolerance. The extent of tolerance depended on the interactions between irrigation periods and organic fertilizer. Water stress and organic fertilizer were obviously shown in the character of the plants, and significantly affected the morphological and chemical characteristics of the seedlings. Also, irrigation interval (especially once in 12 days) had presented significant detrimental effects on most parts of seedlings oak tree. This indicates that the (*Quercus spp*) sections differed in their ability to withstand different levels organic fertilizer and water stress. This will help to discover more physiological and developmental parameters that may be associated with water stress sensitivity. It can be argued that reduced water availability could reduce plant growth, although fertilizers significantly affected plants.

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