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Mitigating the Harmful Effects of Water Deficiency Stress on White Lupine (*Lupinus albus* L.) Plants by Using Algae Extract and Hydrogen Peroxide.

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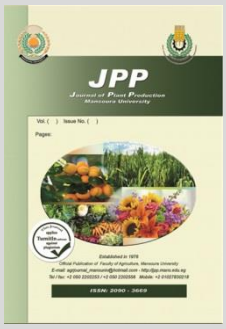

ABSTRACT

Water deficit is a major growth limitation factor affecting plant growth and productivity. To counteract the harmful effects of drought stress, lupine plants were sprayed with two concentrations of algae extract (1 g L^{-1} and 2 g L^{-1}) and hydrogen peroxide solution (0.5% and 1%) under low irrigation level. Morphological, physiological and anatomical studies were performed. It was found that all treatments enhanced plant height, root length, number of leaves, average leaf area, shoot and root fresh and dry weights. A significant increment in chlorophyll a and b, carotenoids and total pigments contents, as well as a significant decrease in electrolyte leakage was noted in treated plants compared with untreated under water deficit. Increment in levels of osmoprotectants such as proline, total free amino acids, glycine betaine and total soluble sugars contents was highly significant in case of 2 g L^{-1} algae extract treatment compared with untreated plants group. Anatomical studies of root, stem and leaf of treated and untreated plants revealed that 2 g L^{-1} algae extract treatment followed with 0.5% H_2O_2 induced significant increments in the thickness of the studied plant organs compared with the untreated plants under water deficit conditions. In general, the concentrations of 2 g L^{-1} algae extract and 0.5% H_2O_2 were the most effective doses regarding all the studied characteristics.

Keywords: Algae extract, anatomy, drought, hydrogen peroxide, lupine, osmoprotectants.

INTRODUCTION

By 2030, many countries will suffer water shortages in addition to poor water quality which is considered as a global problem for food markets (Pieters *et al.*, 2004). According to Kasim *et al.*, 2013, by 2050, drought is expected to affect more than 50% of arable lands. Shortage of water could be induced naturally in response to climate change or artificially due to anthropological activities (Al-Riffai *et al*, 2019). Water deficiency is an abiotic stress with a drastic effect on agricultural productivity. Plant responses to water stress include decreases in plant growth and development, plant pigments content, CO_2 uptake, in addition to accumulation of reactive oxygen species (ROS) (Winnicki *et al*., 2019).

Consequently, induction of crop tolerance to water shortage is a major concern of many recent agricultural studies (Cantale *et al*. 2018).

Lupine (*Lupinus albus* L.) is a leguminous plant that is rich in high good quality protein percentage (44%), and seed dry matters like oil and dietary fiber, lupine cultivation reached to 661 thousand hectares in 2013 with Australia, Russian Federation and Ukraine as the world's highest production areas (El-Harty *et al*. 2016). In Egypt, *Lupinus* is cultivated in different soil types because its agricultural requirements are very low. The annual production of lupine in Egypt is approximately 20488 kg/ha ranking the ninth on the world at 2018 (FAO 2020). Harmful effects of water shortage on lupine plants include flowers falling, decrease of pods number, and low productivity and seed chemical composition changes, e.g., increasing alkaloid content (Hane 2017).

Algae extract was reported as a useful biostimulant for plant growth, it is rich with auxins and cytokinins which are plant growth regulators (Marhoon and Abbas, 2015). *Chlorella* spp., *Spirulina* spp. and *Amphora* spp. microalgae are important commercially to produce algal products such as β-carotene (Pro-vitamin A), amino acids, lutein and phycocyanin (Badr *et al.* 2017). *Amphora coffeaeformis* is a marine and fresh water diatom which produces biochemical components like protein, carbohydrates and lipids (Rajaram *et al*., 2018). In addition, *Amphora coffeaeformis* extract is rich with high levels of plant pigments like chlorophyll, carotenoids as β-carotene, macro and micro-elements especially phosphorous, iron and zinc and antioxidants components (El-Sayed *et al.* 2018). Youssef and Ali (1998) stated that algal extracts (*Anabena oryzae*, *Nostoc calcicola* and *Spirulina* spp.) improved some biometric characteristics of cowpea plants (shoots fresh and dry weights and roots and length of shoots). According to Aung (2011), growth rate and leaf area increased by using *Spirulina* suspension treatments on mung bean.

Hydrogen peroxide (H_2O_2) is a stable Reactive Oxygen Species (ROS) which is naturally produced during cell metabolism especially under stress (Ismail *et al*. 2015).

It has great role in signaling several physiological processes like photosynthesis, respiration, transpiration and

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translocation (Kilic and Kahraman 2016). Furthermore, Wang and Song (2008) explained that abscisic acid increases under drought stress leading to stomatal closure, and according to the relationship between H_2O_2 and abscisic acid, the latter increases the former in plant cell inducing stomatal closure under stress. Spraying H_2O_2 on soybean plants helps in maintaining leaf water content and overcoming drought stress problems (Ishibashi *et al.* 2011), induces adaptation to osmotic stress in maize (Terzi *et al.* 2014), enhances barely seed germination and seedling growth under salinity (Kilic and Kahraman 2016).

The aim of this study is to introduce new approaches to reduce global loss in lupine plant production by enhancing plant performance under water shortage conditions, investigate the physiological effect of algae extract and H_2O_2 treatments on lupine plants under water deficit conditions and to study the morphological and anatomical changes induced by both treatments in lupine plants.

MATERIALS AND METHODS

Two pot experiments were conducted at Faculty of Education, Ain-shams University, Cairo, Egypt in two growing seasons 2017 /2018 and 2018/2019.

Plant material

Lupine seeds were obtained from Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Plastic pots (25 cm in diameter) were filled with clay soil mixed with sandy soil (4:1v:v). Homogenate seeds were selected, sterilized by 0.1% $HgCl_2$ (w/v) and sown at a rate of six seeds per pot on 23rd of October 2017 and 19th October 2018.The pots were kept in appropriate place receiving direct sun light, then seedlings were thinned to three per pot six days after emergence.

Experimental procedures

Seedlings were watered twice per week for two weeks. Drought stress was induced after 30 days from seed sowing at the emergence of first 2-3 true leaves.

The experiments were arranged in complete randomized block design with three replicates, three pots for each replicate (9 pots for each treatment). The treatments was divided as follows: control; normal irrigation (3-day interval), drought stressed plants; plants irrigated at 8-day interval, drought stressed treated plants; plants irrigated at 8-day interval and sprayed with: a- H_2O_2 at the concentrations of 0.5 and 1%, b- Algae extract (*Amphora coffeaeformis*) at the concentrations of 1 and 2 g L^{-1}. The aforementioned substances were first applied 55 days from sowing date and the foliar spray was repeated after two weeks from the first one.

Algae extract

Algae extract (*Amphora cofeaeformis*) was acquired from Algal Biotechnology Unit, National Research Centre (NRC), Giza, Egypt. It was isolated from the local irrigation water of Egypt. Preparation of Algae extracts was described by El-Sayed *et al.* (2018).

Data recorded

Morphological characters

After 85 days from sowing, random samples of 15 plants per treatment (5 plants per replicate) were taken at flowering stage (55 days from drought treatment) The following morphological parameters were recorded in both seasons: plant height, root length, leaf number/plant, average leaf area (cm2) was measured with an area meter (Planix Digital Planimeter, Modlel 2107). Also, shoot and root fresh and dry weights/plant (g).

Physiological characters

1- Determination of electrolyte leakage (EL)

Electrolyte leakage was determined according to Lutt *et al.* (1996) using five leaf discs from the youngest fully expanded leaf. Electrical conductivity was determined in two values; (EC_1) and (EC_2) at room temperature and 121°C, respectively and the (EL) value was calculated as EC_1/EC_2 and expressed in percentage.

b- Determination of photosynthetic pigments

Photosynthetic pigments were extracted using chilled acetone solution. Three wave lengths were used 645, 663, and 480 nm, the absorbance of the supernatant was recorded against 80% acetone as blank. The chlorophyll contents were estimated according to Arnon (1949), while the carotenoid content was determined according to the formula given by Kirk and Allen (1965).

c- Determination of osmolyte compounds

The proline content in fresh plants was estimated according to the method of Bates *et al.* (1973). Absorbance was determined at 520 nm.

Sugars were determined according to Dubois *et al.* (1956) and then absorbance was measured at 485 nm.

Total free amino acids were estimated following the method of Yemm and Cocking (1955). The absorbance was measured at 570 nm

Glycine betaine was extracted from air dried plants using deionized water according to Grieve and Grattan (1983). The absorbance was measured at 365 nm.

Anatomical studies

Anatomical specimens were taken for each of: control, drought stressed, drought stressed treated plants from the middle portions of root, stem and leaflet blade of compound leaf on the middle internode of the stem. The selected specimens were chosen from plants aged 55 days from drought stress treatment.

The specimens, were killed and fixed in F.A.A. solution (50 ml 95% ethyl alcohol + 10 ml formalin + 5 ml glacial acetic acid + 35 ml distilled water) for 48 hours.

Thereafter, samples were washed in 50% ethyl alcohol, dehydrated and cleared in tertiary butyl alcohol series, embedded in paraffin wax of 54-56 C m.p. Cross sections, 20 thick, were cut by a rotary microtome, adhesived by Haupt's adhesive and stained with the crystal violet-erythrosin combination (Nassar and El-Sahhar, 1998). Slides were photographed and read to determine histological aspects and measurements of different characters were expressed in microns (µ).

Statistical analysis

Data of each season were statistically analyzed according to Snedecor and Cochran (1994). New Least Significance Differences (New L.S.D.) at 5% level of probability were calculated to determine the mean comparisons among treatments.

RESULTS AND DISCUSSION

Morphological characters

Table (1) revealed that plants exposed to water deficit conditions showed a prominent significant decrease in all morphological characters estimated, except root length, which increased more than the control plants in both seasons.

Foliar application with algae extract (1 and 2 g L^{-1}) enhanced significantly all morphological characters in both seasons under drought conditions compared with the untreated plants, with exception of root length in the first and second seasons, and number of leaves/plant and root

fresh weight/plant in the first season which increased insignificantly at 1 g L^{-1} extract. Moreover, all vegetative traits in both seasons increased significantly at the concentration 0.5% of hydrogen peroxide, while 1% of hydrogen peroxide caused significant increase only in plant height and leaf area in both seasons in comparison with the untreated plants under the same stress conditions. Among all these treatments, algae extract at 2 g L^{-1} gave the highest significant values for all the morphological studied characters under drought stress conditions, however these values are still less than those of the control plants.

Water deficiency has adverse effects on plant growth and physiological processes; cell structure and enlargement is affected due to loss of turgor and inhibition of different metabolic processes which in turn affects plant growth rate (Zlatev and Lidon, 2012). Inhibition of shoot growth was recorded as an adaptive response helping plants to cope with drought stress by diverting essential solutes to face stressful conditions such as osmotic adjustment (Neumann, 2008).

The results of this study are in agreement with Emam *et al.* (2010) reported that Plant height, leaves number and area and dry weight of stem branches of legumes were reduced under soil water deficit. Ouzounidou *et al.* (2014) which recorded a significant decrease in plant height of broad bean under drought conditions. Moreover, Prabha and Negi (2014) on capsicum plants and Siddiqui *et al.* (2015) on faba bean, stated that drought stress affected plant height, root and shoot fresh and dry weights and leaf area.

On the other hand, an enhancement was detected in morphological characters in response to both treatments with algae extract and H_2O_2. Algae extracts -specifically *A. coffeaeformis*- are characterized by the presence of many useful phyto-constituents including photosynthetic pigments such as chlorophyll and carotenoids (β-carotene and fucoxanthin) which enhance different biological activities of the cell (El-Sayed *et al.*, 2018). Moreover, algae extract is rich with auxins and cytokinins which enhance cell division and enlargement leading to increase in plant vegetative characteristics (Marhoon and Abbas, 2015). The application of algae extract to tomato plants under drought stress enhanced plants fresh weights (Petrozza *et al.*, 2014). According to Kasim *et al.* (2015), seaweed extract enhanced root depth, shoot height and leaf area of wheat plants under drought stress. ,Likewise, Abu Seif *et al.* (2016) reported that foliar application of snap bean with fresh water algae (*Spirulina*) improved plant length, leaf area per plant and plant dry weight (g).

Application of *Spirulina* algae extract increased significantly vegetative growth and yield/fed in sugar beet plants (Enan, *et al.* 2016).

Recently, an increasing concern was given to the importance of H_2O_2 in counteracting the adverse effects of plant stress. Despite being an ROS, H_2O_2 plays an important role in many physiological and biochemical processes of plant such as: seed germination, root system development, flowering and senescence (Niu and Liao, 2016). Ishibashi *et al.* (2011) on soybean showed that exogenous application of H_2O_2 enhanced the up-regulation of stress responsive genes and improved drought stress tolerance., Sun *et al.*, (2016) on cucumber stated that the biomass of plants significantly increased by H_2O_2 application under drought stress. Orabi *et al.*, (2018) stated that H_2O_2 improved growth and yield characters of canola plant with specific emphasis on dry matter of shoots and root, seed yield/ pod and seed number/plant.

Table 1. Effect of algae extract and hydrogen peroxide on vegetative growth characters of lupine plants under water deficit conditions during two seasons of 2017/2018 and 2018/2019.

Treatments	Vegetative growth characters							
	1st season							
	Plant height (cm)	Root Length (cm)	No. of Leaves / plant	Average Leaf area (cm^2)	Shoot fresh weight/plant (g)	Root fresh weight/plant (g)	Shoot dry weight/ plant (g)	Root dry weight/plant (g)
Control	45.9	16.6	17.78	31.6	9.62	3.45	1.64	0.51
Drought	30.7	20.1	14.95	21.0	6.20	2.19	0.98	0.32
Drought+Algae1g L^{-1}	37.6	23.3	17.00	27.3	7.64	2.37	1.40	0.42
Drought+Algae 2 g L^{-1}	42.9	25.9	17.61	29.0	9.06	3.15	1.58	0.46
Drought+H_2O_2 0.5%	38.1	24.4	17.32	28.3	8.34	2.64	1.30	0.39
Drought+H_2O_2 1%	35.2	22.7	16.83	23.4	6.62	2.25	1.12	0.33
New L.S.D. (0.05)	4.21	3.64	1.75	2.23	0.98	0.23	0.172	0.068
	2nd season							
Control	41.5	14.5	16.82	28.4	8.20	3.90	1.38	0.56
Drought	26.8	18.6	14.24	20.5	6.44	2.28	0.92	0.29
Drought+Algae1g L^{-1}	34.7	21.3	16.23	26.0	7.42	2.46	1.34	0.38
Drought+Algae 2 g L^{-1}	40.5	22.8	17.35	27.5	7.90	2.87	1.46	0.44
Drought+H_2O_2 0.5%	39.2	22.7	16.71	25.6	7.24	2.56	1.24	0.36
Drought+H_2O_2 1%	33.3	20.6	15.92	21.8	6.82	2.43	1.02	0.32
New L.S.D. (0.05)	3.86	3.41	1.42	1.86	0.64	0.19	0.154	0.059

Physiological characters

Membrane stability

An increase in electrical conductivity indicates loss of membrane integrity which increases the leakage of ions; the damage of great range of plant tissues could be tested by determining the ability of plasma membranes to control the rate of ion movement in/out of cells (Masoumi *et al.*, 2010). Data in Table (2) showed threefold increments in the percentage of electrical conductivity (EL) in plants that experienced drought compared with control plants which received sufficient amount of water. While all treatments showed a significant decrease in the EL percentage compared with drought suffering untreated plants, both treatments algae extract (2 g L^{-1}) and hydrogen peroxide (0.5%) showed the least values compared with other treatments. Prabha and Negi (2014) showed that membrane stability was affected in drought stressed plants due to loss of membrane integrity which resulted in elevated leakiness of ions and in turn an increase in electrical conductivity. A noticeable electrolyte leakage was reported as a result of prolonged drought stress (Orabi and El-Noemani, 2015).

According to Sun *et al.* (2016), H_2O_2 treatment decreased electrolyte leakage and increased the capacity for osmotic adjustment regaining cell turgor in drought stressed plants.

Esmaielpour *et al.* (2020) indicated an increase in electrolyte leakage in basil plants in response to drought conditions followed by enhancement in growth and a noticeable decrease in electrolyte leakage after the application of seaweed extract.

Table 2. Effect of algae extract and H_2O_2 treatments on electrolytes leakage, chlorophyll a, b, carotenoids and total pigments contents in lupine plants under water deficit conditions.

Treatments	EL %	Chl a mg/g F. wt.	Chl b mg/g F. wt.	Carotenoids mg/g F. wt.	Total pigments mg/g F. wt.
Control	20.6	0. 783	0.548	0.490	1.821
Drought	65.9	0. 387	0.194	0.221	0.802
Drought +Algae 1 g L^{-1}	36.1	0. 622	0.372	0.316	1.31
Drought +Algae 2 g L^{-1}	25.6	0.743	0.511	0. 441	1.695
Drought +H_2O_2 0.5%	27.2	0. 686	0.498	0.385	1.564
Drought +H_2O_2 1%	39.8	0.574	0.305	0.250	1.129
New L.S.D. (0.05)	3.18	0.010	0.011	0.007	0.003

Photosynthetic pigments

Results in table (2) showed changes in pigments contents in lupine plant shoots under water deficit conditions in treated and untreated stressed plants compared with well irrigated control plants. Generally, a significant increment in chlorophyll a, b, carotenoids and total pigments contents was recorded in response to treatments compared with untreated plants under drought stress. Algae extract (2 g L^{-1}) treatment showed almost 2-fold increment in chlorophyll a, carotenoids and total pigments contents compared with untreated plants. Also, hydrogen peroxide 0.5% treatment showed a significant enhancement in the same contents. On the other hand, both treatments 1 g L^{-1} algae extract and 1% H_2O_2 recorded less effect on photosynthetic pigments contents.

Photosynthesis is a major plant metabolic process which is crucial for crop production, so it is directly affected by water deficit conditions (Farooq *et al.*, 2009).

Stomatal closure and reduction in activity of photosynthetic enzymes were recorded as plant response mechanisms under stressful environments which in turn result in a clear reduction in photosynthetic activity (Nyachiro *et al.*, 2001). The photosynthetic rate is adversely affected by the formation of reactive oxygen species (ROS) as well as increased activity of chlorophyllase and ethylene production (Farooq *et al.*, 2009, Escalante-Magaña *et al.*, 2019).

Plant pigments contents were significantly reduced in bean (Beinsan *et al.*, 2003), *Carthamus tinctorius* (Siddiqi *et al.*, 2009), wheat cultivars seedlings (Aly and Latif, 2011), and indian mustard (Mostafaei *et al.*, 2018) under water stress conditions. The aforementioned results supports the findings of this study as plant pigments content showed a significant decrease under water deficit conditions.

In accordance with the results of this study, algae extract treatment enhanced plant pigments content in snap bean (Abu Seif *et al.*, 2016), sugar beet plants (Enan *et al.*, 2016). This effect could be attributed to the activity of antioxidants (Lee *et al.*, 2008, El-Sayed *et al.*, 2018) and growth promoter components in algae extract (El-Eslamboly *et al.*, 2019). In addition, Sun *et al.*, (2016) and Guler and Pehlivan (2016) claimed that pigments contents increased due to H_2O_2 treatment which alleviated the drought stress in cucumber and soybean plants, respectively. Moreover, Habib *et al.*, (2020) recorded an increment in drought stressed wheat plant pigments contents in response to H_2O_2 treatment.

Osmolyte compounds

Plants tend to accumulate osmolytes such as soluble sugars, free amino acids, proline (Pro) and glycine betaine (GB) to adapt to stresses (Hoekstra *et al.*, 2001). The present results showed that algae extract at 2 g L^{-1} and H_2O_2 at 0.5% treatments induced an increment in studied osmolytes contents in plants subjected to drought in comparison with untreated ones under the same conditions.

Algae extract treatment 2 g L^{-1} induced a significant increment in glycine betaine content as shown in Table (3) compared with drought stressed untreated plants. At the same time, 0.5% H_2O_2 treatment showed noticeable increment in the same contents. Moreover, total soluble sugars, total free amino acids and proline contents increased in response to 1 g L^{-1} algae extract while, 1% H_2O_2 treatments gave the least value compared with untreated drought affected plants. Osmolytes present in the cytoplasm of plants play an important role in reducing cell osmotic potential and maintaining cell turgor (Pathan *et al.*, 2004) without decreasing actual water content (Serraj and Sinclair, 2002). Proline (Pro) accumulation could be a plant mechanism to avoid water stress as it is one of the most important osmolytes associated with plants drought stress, in addition, it contributes to stabilizing sub-cellular structures (e.g. proteins and membranes) and scavenging free radicals (Hayat *et al.*, 2012, Huang *et al.*, 2014).

Accumulation of Glycine betaine (GB) was recorded in water stressed plants which assumed a protective role in stressed tissues (Park *et al.*, 2006). Glycine betaine could maintain stable structures and activities of enzymes and protein complexes which reflect the integrity of membranes against the damaging effects of water stress (Sakamoto and Murata 2002). Moreover, under drought stress conditions, sugar accumulation was found to prevent the oxidation of cell membrane, maintain the turgidity of leaves and prevent dehydration of membranes and proteins, reduces the rate of photosynthesis and maintains the leaf water content and osmotic adjustment of plants (Sami *et al.*, 2016). The present study showed a significant increase in (Pro) and (GB) contents in response to water stress. Findings of Silvente *et al.* (2012) on soybean and Lum *et al.* (2014) on rice support results of this study.

Table 3. Effect of algae extract and H_2O_2 treatments on proline, total soluble sugars, total free amino acids and glycine betaine contents in lupine plants under water deficit conditions.

Treatments	Proline (mg/g FW)	Total s. sugars (mg/g FW)	Total free amino acids (mg/g FW)	Glycine betaine (mg/g DW)
Control	0.701	24.78	6.09	1.13
Drought	1.035	32.33	17.01	2.98
Drought+Algae 1 g L^{-1}	1.982	42.6	25.34	3.45
Drought+Algae 2 g L^{-1}	2.103	49.50	32.65	4.41
Drought+H_2O_2 0.5%	1.740	37.15	22.70	3.06
Drought+H_2O_2 1%	0.985	28.09	13.81	3.41
New L.S.D. (0.05)	0.031	0.648	0.653	0.226

Results of the current study noted further accumulation in osmolytes content in response to algae extract and H_2O_2 treatments. According to Nair *et al.*, (2012), algae extract increased proline and total soluble sugars contents contributing to abiotic stress tolerance in plants. In addition, application of algae extract increased sugar content in leaves and root of sugar beet plants (Enan *et al*., 2016). Sun *et al.*, (2016) on cucumber showed an increase in soluble sugar and proline contents in drought stressed plants treated with H_2O_2. In addition, a positive correlation between H_2O_2 treatment and biosynthesis of proline under water stress has been reported in wheat plant (Habib *et al*., 2020).

Anatomical studies:

For anatomical studies, plants treated with algae extract at 2 g L^{-1} and H_2O_2 at 0.5% concentrations under drought stress conditions (remarkable positive responses), in addition to untreated drought stressed and control plants (normal irrigation) were chosen.

1- The Root

The microscopical measurements (µ) and counts of root transverse sections of Lupine plants treated by 2 g L^{-1} algae extract and 0.5% H_2O_2 grown under drought stress, the untreated drought stressed as well as the control plants are presented in Table (4) and Fig. (1).

Concerning the drought effect, water deficit caused reduction in all anatomical characters compared with well irrigated control plants. On the other hand, plants which received 2 g L^{-1} of algae extract under drought stress conditions exhibited the most remarkable thickness of root overcoming the untreated plants under the same drought stress. This increment could be attributed to the increase in cortex, vascular cylinder diameter and pith diameter. The increment in vascular cylinder diameter is attributed to the increase of secondary xylem and phloem thickness. Moreover, plants treated with H_2O_2 under drought stress showed an increase in root diameter exceeding the untreated plants at the same conditions by 11.5%.

However, the root diameter was less than that exhibited by plants treated with algae extract at 2 g L^{-1}.

This increment is attributed to the increase in vascular cylinder, secondary xylem and phloem thickness, in addition to pith diameter. On the other hand, the cortex thickness showed a reduction of 14.3% less than the untreated stressed plants. The plants treated with algae extract at 2 g L^{-1} showed a prominent increase in root diameter more than those treated by H_2O_2 at 0.5%. This increment is related to the increase in cortex thickness, vascular cylinder diameter, xylem tissue and pith diameter (Table 4 and Fig. 1).

Table 4. Measurements in microns (µ) of some anatomical features of root transverse sections of lupine plants treated with algae extract and hydrogen peroxide under water deficit conditions.

Measurements (µ)	Control	Drought	Drought +Algae 2 g L^{-1}	±% to Drought	Drought + H_2O_2 0.5%	±% to Drought
Root diameter	2585	1685	2170	+28.7	1880	+11.5
Cortex thickness	490	420	425	+1.2	360	-14.2
Vascular cylinder diameter	1525	830	1325	+59.6	1120	+34.9
Xylem thickness	432	225	357	+58.6	325	+44.4
Phloem thickness	75	60	75	+25	70	+16.6
Vessel diameter	44	31	42	+35.4	40	+29
Pith diameter	520	260	451	+73.4	330	+26.9

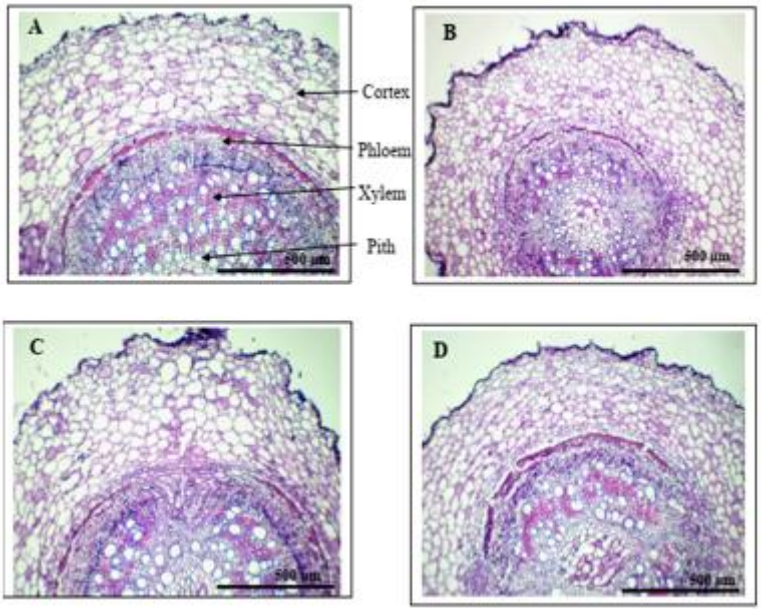

Fig. 1. Transverse sections through median portion of the tap root of lupine plants sprayed with algae extract, and hydrogen peroxide under water deficit conditions, and the well watered control plants.

A- Control Plants.
B- Stressed Plants
C- Stressed plants treated with 2 g L^{-1} algae extract.
D- Stressed plants treated with 0.5% of hydrogen peroxide.

2- The Stem:

Microscopical counts of histological characters in transverse sections through the median internode of lupine plants main stem are given in Table (5) and Fig. (2) for plants affected with drought stress, those treated with 2 g L^{-1} algae extract and 0.5% H_2O_2 at the same stress level, and the well watered control plants It is noted that drought stress severely affected the whole stem diameter which decreased

by 37.2% less than the well irrigated control plants. This deficiency is completely reflected on all histological characters of the stem. On the other hand, plants treated with algae extract at 2 g L^{-1} concentration under low water conditions gave the best results in which the whole stem diameter increased by 30.1% more than the drought stressed.

This increment in stem diameter was linked to the remarkable increase in thickness of both stem wall as well as hollow pith diameter more than the drought stressed. This increase in stem wall thickness could be attributed mainly to the increment in the thickness of cortex, phloem and xylem tissues, in addition to the parenchymatous pith thickness. Stressed plants treated by H_2O_2 at 0.5% showed an increase in stem diameter, however, this increment remains less than that of stressed plants treated by algae extract at 2 g L^{-1}.

Relative to the untreated drought stressed plants, the percentages resulted from this treatment, were higher by 12.6, 15.7, 5.0, 9.0, 13.3 and 20.5% for stem diameter, cortex, fiber cap thickness, phloem tissue, xylem tissue and the hollow pith diameter, respectively. Stem wall thickness was increased by 8.7% as a result of the increment in parenchymatous pith thickness which reached to 5.1% more than the untreated stressed plants.

It is worthy to mention that the plants treated by algae extract at 2 g L^{-1} were thicker compared to those treated with H_2O_2 at 0.5% concentration under same stress conditions. Stem diameter of the former plants increased by 15.5% than the latter ones, and this increment could be attributed mainly to the increase of stem wall thickness, cortex thickness, xylem tissue, parenchymatous pith thickness and hollow pith diameter by 10.9, 9.0, 21.0, 6.2 and 23.3%, respectively.

Table 5. Measurements in microns (µ) of some anatomical features of stem transverse sections of lupine plant treated with algae extract and hydrogen peroxide under water deficit conditions.

Measurements (µ)	Control	Drought	Drought + Algae 2 g L^{-1}	±% to Drought	Drought +H_2O_2 0.5%	±% to Drought
Stem diameter	4650	2920	3800	+30.1	3290	+12.6
Stem wall thickness	1368	970	1171	+20.7	1055	+8.7
Epidermis Thickness	30	30	30	0.0	30	0.0
Cortex thickness	284	190	240	+26.3	220	+15.7
Fiber cap thickness	66	60	63	+5.0	63	+5.0
Phloem thickness	68	55	63	+14.5	60	+9.0
Xylem thickness	354	210	288	+37.1	238	+13.3
Vessel diameter	47	32	38	+18.7	36	+12.5
Parenchymatous pith thickness	548	425	475	+ 11.7	447	+5.1
Hollow pith diameter	1914	975	1449	+48.6	1175	+20.5

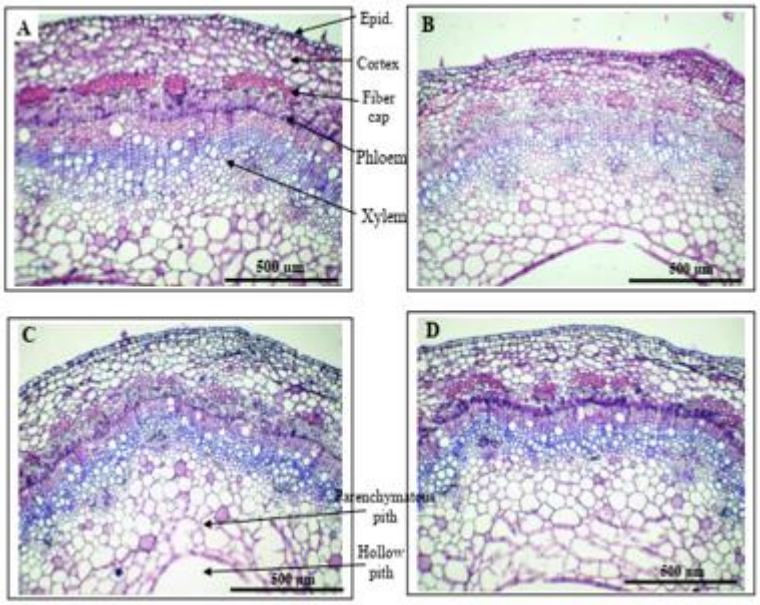

Fig. 2. Transverse sections through median portion of the stem of Lupine plants sprayed with algae extract, and hydrogen peroxide under water deficit conditions, and the well watered control plants.

A- Control Plants.
B- Stressed Plants
C- Stressed plants treated with 2 g L^{-1} algae extract.
D- Stressed plants treated with 0.5% of hydrogen peroxide.

1- The Leaf:

Microscopical characters in transverse sections of the middle leaflet blade of the compound leaf grown on the median internode of the main stem of lupine plants are presented in Table (6) and Fig. (3 and 4) for plants affected with drought stress, those treated with 2 g L^{-1} and 0.5% concentrations for both algae extract and H_2O_2, respectively grown under low level of water, as well the control plants.

As a result of drought stress, the midrib as well as the lamina thickness was decreased by 31.7 and 32.4% less than the well irrigated control plants. Subsequently, the palisade and spongy tissues, and the dimension of midvein bundle were negatively affected compared with control plants. The reduction in midrib and the lamina thickness in drought stressed plant leaves could be attributed to the malformation in palisade and spongy layers as a result of exposing plants drought (Abd Elbar *et al.*, 2019).

Moreover, Abd Elbar *et al.* (2019) explained that the rate of cell division of mesophyll decreases significantly by drought stress which indicates a decrease in leaf elongation.

Relative to drought stressed plants; it is obvious that spraying plants with algae extract at 2 g L^{-1} enhanced all the anatomical characters of the leaflet. Midrib and lamina thickness increased by 30.3 and 31.3%, respectively. This thicker leaflet could be attributed to the increase in palisade and spongy tissues, and the dimension of midvein bundle (length and width).

Plants treated with H_2O_2 at 0.5% still occupy the second rank after algae extract in the development of anatomical structure of leaf. Data also revealed that the plants received algae extract at 2 g L^{-1} showed 10.6 and 6.4% increments in midvein and lamina thickness over the plants treated by 0.5% H_2O_2 under the same drought conditions. This increment was reflected on all internal tissues; palisade, spongy, width of midvein bundle by 3.3, 4, and 10.7% higher than plants treated with 0.5% H_2O_2. The present results are almost in harmony with those obtained by (Boghdady *et al.,* 2016) on chickpea, where, using 2 g L^{-1} seaweed extract increased the stem diameter due to an increment in the thickness of cortex as well as phloem and xylem tissues. This concentration of seaweed also provided xylem vessels with wider cavities which in turn increased water conductive capacity due to increased area. In addition, the increased thickness of palisade and spongy tissues as well as vascular bundle of midvein lead to an increase in thickness of lamina and midvein of leaflet blades of chickpea. Moreover, Marhoon and Abbas (2015) stated that treatment of sweet pepper with seaweed extract at 6 g increased the thickness of cortex, and the diameter of vascular tissues significantly, in contrary, the thickness of pith area decreased compared with control. Salama *et al.* (2015) on basil plants and Salama *et al*. (2016) on stevia plants, stated that spraying seaweed extract at 1 and 1.5 g, respectively, caused an increase in stem diameter mainly resulting from the increment induced in thickness of cortex, phloem and xylem tissues and pith parenchym area compared to the control. Likewise, seaweed extract induced increase in thickness of midvein and lamina due to the increase in the thickness of palisade and spongy tissues as well as in the dimensions of the midvein bundle, being in harmony with the present investigation.

The effect of spraying H_2O_2 on anatomical structure of lupine root, stem and leaves are extensively discussed in literature. According to Gil *et al.* (2009) the xylem vessel diameter and xylem/phloem ratio of roots and shoots tended to be greater for trees grown in soil treated with H_2O_2 than for controls, which resulted in increase in water conductivity and better enhancement of the aerial parts growth. Jafariyan and Zarea (2016) found that, soaking seeds of wheat plants in H_2O_2 at various concentrations (0, 25, 50, and 80%), enhanced the stomata density and increased the length and the anatomical features of leaf.

Table 6. Measurements in microns (µ) of certain anatomical features of leaflet blade transverse sections of lupine plant treated with algae extract and hydrogen peroxide under water deficit conditions

Measurements (µ)	Control	Drought	Drought +Algae 2 g L^{-1}	±% to Drought	Drought +H_2O_2 0.5%	±% to Drought
Mid rip thick.	410	280	365	+30.3	330	+17.8
Lamina thick	222	150	197	+31.3	185	+23.3
Upper epidermis	10	10	10	0.0	10	0.0
Lower epidermis	10	10	10	0.0	10	0.0
Palisade tissue	110	70	93	+32.8	90	+28.5
Spongy tissue	90	60	78	+30.0	75	+25
Midvein bundle dimension						
Length	115	105	120	+14	115	+9.5
Width	160	130	155	+19.2	140	+7.6

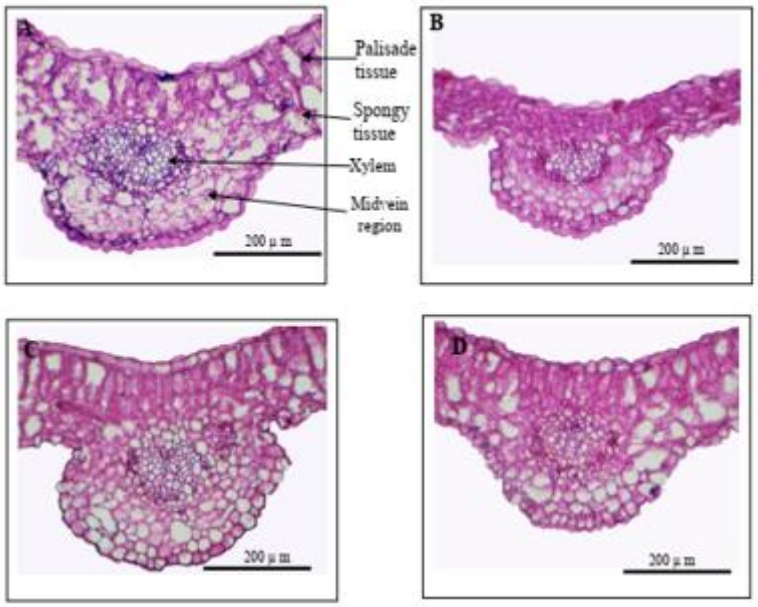

Fig. 3. Transverse sections through leaflet mid-rib of lupine plants sprayed with algae extract, and Hydrogen peroxide under water deficit conditions, and the well watered control plants.

A- Control Plants.
B- Stressed Plants
C- Stressed plants treated with 2 g L^{-1} algae extract.
D- Stressed plants treated with 0.5% of hydrogen peroxide.

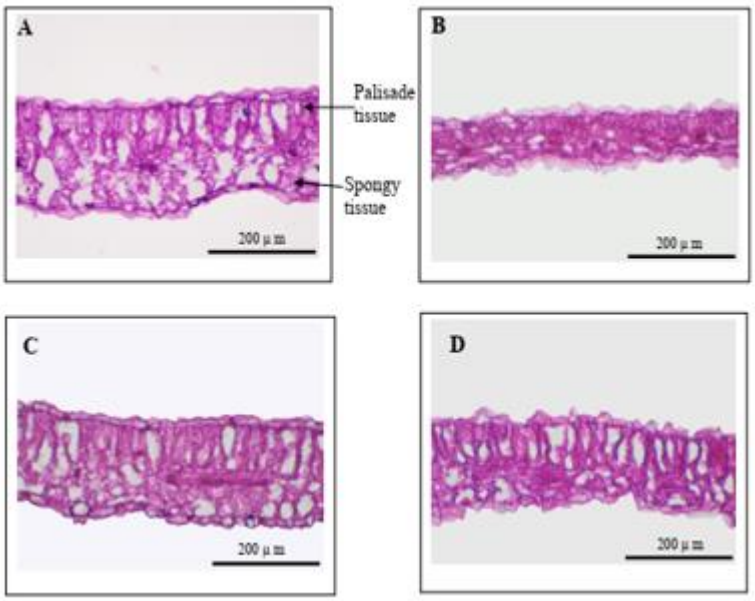

Fig. 4. Transverse sections through marginal portion of leaflet blade of lupine plant sprayed with algae extract, and hydrogen peroxide under water deficit conditions, and the well watered control plants.

A- Control Plants.
B- Stressed Plants
C- Stressed plants treated with 2 g L^{-1} algae extract.
D- Stressed plants treated with 0.5% of hydrogen peroxide.

CONCLUSION

In summary, lupine plants exposed to drought stress exhibited a severe reduction in plant growth. On the other hand, the exogenous application of *Amphora coffeaeformis* extract (rich in protein, carbohydrates, lipids, pigments and plant growth regulators) alleviated the harmful effect of drought stress on lupine plants. Additionally, foliar application of H_2O_2 solution improved lupine plants tolerance to drought stress due to its role in some physiological processes. However, the algal *Amphora coffeaeformis* extract was more effective than H_2O_2 solution in helping plants to tolerate drought stress.

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تخفيف الآثار الضارة الناتجة عن اجهاد نقص المياه على نباتات الترمس الأبيض باستخدام مستخلص الطحالب وفوق اكسيد الهيدروجين.

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اجريت تجربتي اصص بكلية التربية، جامعة عين شمس، في موسمين متتاليين 2017 /2018 و 2018/ 2019 وذلك بهدف تحسين النمو و الصفات الفسيولوجية لنبات الترمس (*Lupinus albus* L.) في ظل ظروف نقص المياه باستخدام مستخلص الطحالب "*Amphora coffeaeformis*" و مادة فوق اكسيد الهيدروجين. و أوضحت النتائج أن معاملة النباتات بالرش بمستخلص الطحالب بتركيزات (1 و 2 جم/لتر) و فوق اكسيد الهيدروجين بتركيزات (0.5 و 1 %) أدى الى تحسين جميع الصفات المورفولوجية (طول النبات، طول الجذر، عدد الأوراق، متوسط مساحة الورقة، الوزن الرطب و الجاف لكل من المجموع الخضري والجذري) وذلك مقارنة بالنباتات الغير معاملة تحت نفس ظروف نقص المياه واعطت الجرعة 2 جم/لتر من مستخلص الطحالب وتركيز 0.5% من فوق اكسيد الهيدروجين أعلى نسبة زيادة بالمقارنة بالتركيز الاخر. كما ادت معاملة نبات الترمس بالمواد السابق ذكرها الى حدوث زيادة معنوية في محتوى النبات من الصبغات (كلوروفيل a، كلوروفيل b، الكاروتينات بالاضافة الى المحتوى الكلي للنبات من الصبغات) ومحتوى النبات من البرولين، الاحماض الامينية الحرة الكلية، Glycine betaine بالاضافة الى المحتوى الكلي من السكريات الذائبة مقارنة بالكنترول تحت نفس ظروف نقص المياه. وعلى العكس حدث نقص معنوي في قدرة التوصيل الكهربي (Electrolyte Leckage 'EL') للنباتات المعاملة مقارنة بالنباتات الكنترول تحت نفس ظروف نقص المياه. اوضحت الدراسات التشريحية لكل من الجذر و الساق و الورقة ان تعرض النباتات لنقص المياه يؤدي لحدوث انخفاضا ملحوظا في جميع القياسات التشريحية، بينما ادت المعاملة بمستخلص الطحالب و مادة فوق اكسيد الهيدروجين الى زيادة معنوية في قطر الجذر والساق والعرق الوسطي للورقة تحت ظروف نقص الماء.