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Biochemical responses of white termis to pyridoxine and mycorrhizae treatment under salinity stress

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Abstract

Applying vitamins like pyridoxine to decrease the impact of various stressors on the majority of plants has an important efficiency. Thus, this research tries to study the physiological impact of pyridoxine and mycorrhizae exogenous treatments on improving white lupine tolerance to salinity stress. Mycorrhizae was added to soil (0.0 and 7 g/pot).White lupine seedlings were foliar applied by various levels of pyridoxine (0.0, 100 and 200 mg/l) and watered by two levels of salinity (0.0 or 5000 mg/l). Irrigation of white lupine plants with saltwater resulted in significant reductions in morphological aspects, photosynthetic pigments and productivity in comparison to irrigation with tap water, meanwhile, gradually increase osmolytes, comparing to control (plants irrigated by tap water). Adding mycorrhizae to soil with the recommended dose boosted white lupine growth, certain physiological and productivity of plants irrigated with saltwater. Furthermore, exogenous pyridoxine treatment with 100 & 200 mg/l enhanced growth and seeds productivity of white lupine plants under normal irrigation and improved salinity tolerance by increasing white lupine growth and productivity via inducing photosynthetic pigments, osmolytes levels comparing to their corresponding controls. In conclusion, 200 mg/l pyridoxine showed superiority on inducing beneficial role in improving lupine plant salinity tolerance.

***Key words*:** Lupine, Mycorrhyzae, Pyridoxine, growth, yield, Osmolytes, Nutritional value.

Introduction

White Lupine (*Lupinus termis* L.) is a useful traditional crop which is cultivated in a variety of environments. Their seeds have nutritional components which are similar to that of soybean seeds and greater than the seeds of other legumes. White lupine seeds are influential protein and oil supplier [1]. The lupine seeds are a significant source of oil (5-13%) and protein (33-40%) with valuable amino acids pattern [2]. Furthermore, husked seeds flour have high content of protein, oil, total ash, crude fiber & carbohydrate [3]. Bitter lupine seeds oil have high concentrations of antioxidants therefore, it is suitable for the use in various food processing [4 & 5]. Furthermore, lupine plant as other legume plants can fix atmospheric nitrogen which improves soil fertility, permeation and water storage [6]. Accordingly, plants have potentiality of growing better in low fertility soils and are used as supplement of green manure to improve its fertility.

Salinity is among most severe problems in the world's arid and semi-arid climate zones. The key reason for rising salinity stress in agricultural soils is saline water irrigation, insufficient drainage, irrigation techniques, increased transpiration and decreased rainfall. High salinity affects nearly 20% among agricultural area & 33 percent among watered agricultural area. By 2050, it is predicted that, salinity will affect about fifty percent of the arable area. Elevated salt levels decreased growth and yield of different crops in various ways. Salinity stress caused two stressors, ionic resulted via solute imbalance inside the cytosol and an osmotic stress resulted via decreasing water availability in soil [7]. Increased salinity contents limits plant ability to absorb water and minerals as potassium K^+ and calcium Ca^{2+} while, increased Na^+ and Cl^- absorption, affect directly cells via toxic effects on cell membranes [8]. Salinity initial effects cause other impacts as cell expansion reduction, formation of assimilate and reduced cyotosolic metabolism and increased free radicals production (ROS) [9].

To mitigate salt adverse impacts and enhance plant tolerance, various strategies were conducted. One of those strategies is exogenous treatments with different substances as plant growth regulators, antioxidants, vitamins, different nutrient elements etc. [10]. In this aspect, the use of *Arbuscular mycorrhiza* (AM) in addition to exogenous treatment of vitamins such as vitamins B (Pyridoxine) is a potential approach for improving plant tolerance and increase crop

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productivity. Arbuscular mycorrhizal fungi (AM) facilitate host plants to grow vigorously under stressful conditions via mediating a series of complex communication events between the plant and the fungus leading to enhanced photosynthetic rate and other gas exchange-related traits [11], as well as increased water uptake. Numerous reports describe improved resistance to a variety of stresses including drought, salinity, herbivory, temperature, metals, and diseases due to fungal symbiosis [12]. AM synergy are characterized as a more effective nutrient absorption and transport system than roots alone. The physiological feature of AM synergy is not only minerals absorption but also, transport to the host. Furthermore, AM and plant relationship has been shown to increase host plant more tolerant towards environmental challenges in most cases [13]. AM fungus is valuable in sustainable agriculture as it enhances plant water relations improving stress tolerance of plants, increase disease resistance and enhance nutrient absorption via increasing accumulation of low mobile minerals that decrease fertilizers needs. AM has the ability to degrade specific complicated nutrients and biomolecules in soil and make accessible to its hosts [14].

Vitamins are chemical substances necessary in tiny quantities by all organisms for sustaining optimal growth and performance; their functions as coenzymes therefore play an important role of metabolic control. They´re, known as development´s restricting impact [15]. Different vitamin treatments caused an effective role through plant bioregulators which then influence various biological processes also protects plant from stress negative influences [16]. Pyridoxine (Vitamin B6) is an essential coenzyme which is incorporated in a wide range of physiological processes, among them glycogen metabolism and biosynthesis of amino acids. Pyridoxine was demonstrated as a powerful antioxidant also may work as co-enzyme [17] and potent antioxidant [18]**.** It improved the efficiency of photosynthetic carbon reactions and increased dry matter formation. Moreover, pyridoxine application improved cell division, growth and differentiation and increased nutrient uptake [19]**.** In seedling growth, exogenous treatments with optimal pyridoxine concentrations were beneficial via increasing availability of water and nutrient. Pyridoxine is highly recommended for use in various plants.

Therefore, this investigation aimed to study pyridoxine effect on lupine plants grown in sandy soil amended with or without *Arbuscular mycorrhiza*.

Materials and Methods

A pot trial was done in greenhouse of the National Research Centre Dokki, Egypt at 2017/2018 and 2018/2019 winter seasons. Bitter lupine (*Lupinus termis* L.) cv., Giza 2 seeds were gets from Agriculture Research Centre, Egypt. They were inoculated using nitrogen fixing bacteria (*Rhizobia*). During November 2017 and November 2018, the seeds were planted in pots of 50 cm^3. At the pots, the planting media is clay and sand soils in the ratio of 2:1. *Arbuscular mycorrhiza* was added to the soil of each pot with the rate of 1Kg/fed. At 15 days old, plants were thinned leave 5 plants at each pot. The fertilization was conducted using superphosphate (5 g/pot), potassium sulfate (2.5 g/pot), and urea (6 g/pot). The experimental design was factorial complete randomized design. Mycorrhizae was added to half pots with the recommended dose (7g/pot). Pyridoxine treatment (0.0, 100 & 200 mg/l) were done at 30 and 45 age Pots were divided into two groups, every one watered by one of these salt levels (0.0 or 5000 mg/l). Plants irrigated three times with equal amounts (liter/pot) of the salt solution followed by one with tap water. Salt water prepared as Stroganov [20] Table 1. Pots were watered by different saline concentrations with same amounts.

Table (1): Salt constituents as % of total salt content.

$MgSO_4$	$CaSO_4$	NaCl	$MgCl_2$	$CaCO_3$
10	1	78	2	9

Certain anions and cation constituents in salt mixture as percentage of total mill equivalents.

Na^+	Mg^{+2}	Ca^{+2}	SO^{-2}	Cl^-	CO^{-2}
38	6	6	5	40	5

Measurements

At 60 days old, plants were taken to determine morphological characters (shoot length (cm), leaves No /plant, shoot fresh & dry weights (g/ plant) and some chemical analysis. Fresh samples were taken to analyze photosynthetic pigments, IAA and phenol, hydrogen peroxide and some antioxidant enzymes. Air dried samples of lupine plants were taken to analyze osmolytes. Three plants / pot have been left for yield determination. At harvest time, yield criteria, pods & seeds No/plant, seeds number/pod, pods & seeds dry weight/plant also 100 seed weight (g). Furthermore carbohydrate%, protein%, total oil% and flavonoids as well as DPPH activities of the seeds were done.

Total chlorophyll a, b & carotenoids were analyzed by Lichtenthaler and Buschmann [21]. IAA was determined according to Larsen [22]. Phenolics was analyzed according Danil and George [23]. Total soluble carbohydrates (TSS) were extracted [24] and analyzed [25]. Free amino acids and proline were extracted by Vartainan [26] method. Free amino acid were estimated as Yemm and Cocking [27]. Proline was estimated as Bates [28].

Nutritional values of yielded seeds: Total carbohydrate estimation was estimated as Herbert [29]. Flavonoid was analyzed as Chang [30]. Extraction of oil were done by Kates and Eberhardt [31]. Free radical scavenging activity was done by Gyamfi [32].

Data were statistically analyzed as Snedecor and Cochran [33] method. Combined analysis of two growing seasons was performed. Means were compared by using least significant difference (LSD) at 5% levels of probability [34].

Results

Results presented in Table (2) show impact of pyridoxine foliar treatment without or with the addition of *Arbuscular mycorrhiza* to soil on growth indices of lupine under salinity. Results showed that salinity stress (5000 mg/l) significantly reduced growth parameters of lupine plant (shoot length, no of leaves/plant, fresh and dry weights/plant, fresh and dry weight of root/plant) comparing by the control plant (Table 2).

Meanwhile, soil addition of mycorrhizae improved growth parameters of lupine plants (shoot length, no of leaves/plant, fresh & dry weights/plant, fresh and dry weight of root/plant) either irrigated with tap or saline water compared by plants without mycorrhizae (Table 2). Moreover, data also showed that, treating lupine plant with 100 and 200 mg/l of pyridoxine vitamin resulted in increments of lupine growth characters at tap water or saline water. Different pyridoxine concentrations significantly increased plant growth parameters grown without mycorrhizae and also caused more significant increases in plants grown with mycorrhizae. Moreover, within the above mentioned treatments, the obtained data indicate that, using higher level of pyridoxine (200 mg/l) resulted in the better growth than using lower level (100 mg/l).

Table (2): Impact of salinity (S0 0 & S1 5000 mg/l), mycorrhyzae and pyridoxine (0.0, 100 & 200 mg/l) on lupine plant growth indices (Data are means of two seasons).

Salinity (mg/l)	Mycorrhizae	Pyridoxine (mg/l)	Shoot length(cm)	leaves no/plant	shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
		0	32.33	11.00	20.72	3.71	3.70	1.58
	Without	100	35.00	20.33	23.02	5.86	3.95	1.69
		200	39.33	24.67	28.56	6.04	4.78	1.93
		0	37.67	16.00	23.42	4.83	4.17	1.65
		100	43.00	25.67	30.64	6.02	4.26	1.84
S0	With	200	46.00	29.33	33.03	7.19	4.37	2.12
		0	29.00	8.00	13.30	2.06	2.36	1.44
	Without	100	33.33	10.33	15.89	2.84	2.44	1.87
		200	35.33	14.67	18.03	3.13	3.66	1.95
		0	33.67	13.33	15.51	2.76	3.22	1.83
		100	40.33	16.00	19.14	3.81	1.45	0.68
S1	With	200	41.67	16.67	20.49	3.95	1.42	0.75
	LSD at 5%		1.352	0.942	0.869	0.214	0.152	0.065

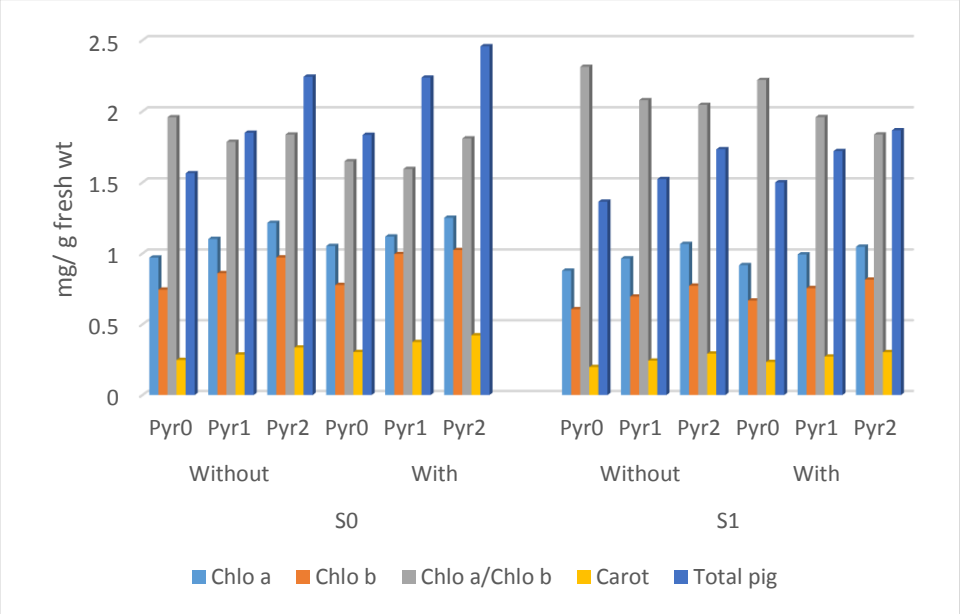
Salinity stress reduced *Chlo* a, *Chlo* b, *Chlo* a/*Chlo* b, carotenoids and total pigments of lupine plant leaves compared by control those irrigated with tap water (Fig 1). The percentages of decreases were 9.47%, 18.52%, 20.16% and 12.79% of *Chlo* a, *Chlo* b, carotenoids and total pigments, respectively.

With respect to mycorrhyzae, addition to soil. Data revealed that, addition of mycorrhizae significantly improved *Chlo* a, *Chlo* b, carotenoids and total pigments of lupine plants in comparison by those grown without this addition either of plants watered by tap or saline water. Meanwhile, decreasing the ratio of *Chlo* a/ *Chlo* b either at normal irrigation or saline irrigated plants (Fig 1).

Foliar spraying of lupine plants with pyridoxine vitamin with different concentrations (100 and 200 mg/l) significantly enhanced photosynthetic pigments in plants under salt either of plants grown without or with mycorrohyzae comparing by those control plants. Meanwhile, decreased the ratio of *Chlo* a/ *Chlo* b comparing by control under tap or salinity conditions (Fig 1)

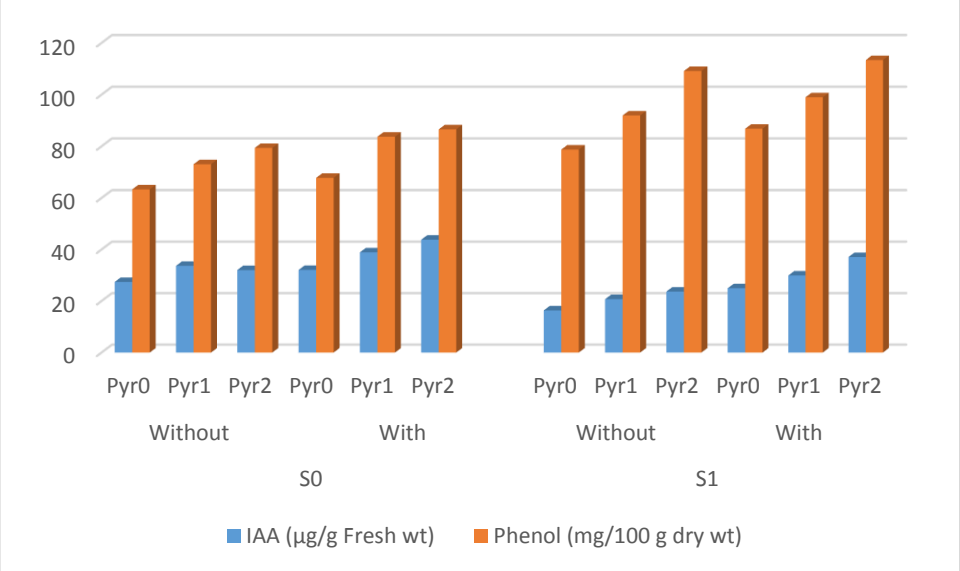
Figure (2) showed the effect of pyridoxine exogenous application without or with mycorrhizae on lupine plants grown at normal and saline conditions. Salinity reduced endogenous IAA levels of lupine in comparison to control, while increased phenolic contents as compared with normal irrigated plants. Addition of mycorrhizae to the soil increased significantly endogenous IAA and phenolics contents in lupine comparing by control without mycorrhyzae either in normal irrigated and salt stressed irrigated plants (Fig. 2).

Exogenous treatment as foliar spraying of lupine plants with pyridoxine vitamin levels (100 & 200 mg/l) significantly increased endogenous IAA & phenolic levels in comparison by corresponding untreated plants improved IAA & phenolic comparing by those untreated plants. It is obvious that greater pyridoxine was higher effective (100 mg/l) in increasing lupine plant IAA and phenols at normal and stressed (0.0 and 5000 mg/l) in soil without or with mycorrohyzae.



LSD at 5% for *Chlo* a: 0.035, *Chlo* b: 0.034, *Chlo* a/ *Chlo* b: 0.075, Carotenoids: 0.024 and Total pigments: 0.086

Fig (1): Impact of salinity (S0 0.0, S1 5000 mg/l), mycorrhyzae and pyridoxine (Pyr0 0, Pyr1 100 & Pyr2 200 mg/l) on chlorophyll a, b, a/b, carotenoid and total pigments (mg/g FWt) of lupine.



LSD at 5% for IAA : 3.542 and Phenolics : 10.526

Fig (2): Impact of salinity stress (S0 0.0, S1 5000 mg/l), mycorrhyzae and pyridoxine (Pyr0 0, Pyr1 100 & Pyr2 200 mg/l) on IAA (µg/g Fresh wt) and Phenolics, (mg/100 g dry wt) of lupine plant.

.Salinity stress effect and different treatments of pyridoxine in absence and presence of mycorrhizae on osmolytes (total soluble sugars TSS, proline and free amino acids) are presented in Table (3). Watering lupine with saline water significantly increased the compatible solutes. Moreover adding mycorrhizae to

soil and foliar treatment of pyridoxine impacted greater significant increments of TSS, proline and free amino acids comparing by those plants without addition of mycorrhizae (Table 3).

Yield and yield attributes:

Results of pyridoxine effect without or with the *Arbuscular mycorrhiza* addition to soil on yield and characters (Table 4**).** Data showed that saline water (5000 mg/l) caused marked reduction in all yield attributes of lupine plant (Number and weight of pods/plant, Number of seeds/pod, weight of seeds/plant and weight of 100 seeds) as comparing by the control plant.

On the other hand, addition of mycorrhizae to soil with the recommended dose increased yield and its characters in lupine plants either grown under normal conditions or salinity stress conditions.

Moreover, the data also showed that, foliar application of lupine plant with different concentrations of pyridoxine vitamin resulted in increases in the above mentioned yield and yield attributes in plants watered by normal water or saline water both without and with addition of mycorrhyzae.

Table (3): Impact of salinity stress (S0 0.0, S1 5000 mg/l), mycorrhyzae and pyridoxine (0, 100 & 200 mg/l) on TSS proline and free amino acids (mg/100 g dry wt) of lupine plant.

Salinity (mg/l)	Mycorrhizae	Pyridoxine conc	TSS	Proline	FAA
		0	2602.33	32.52	235.85
		100	2991.67	35.62	249.85
	Without	200	3137.67	46.35	259.68
		0	2848.00	42.62	287.85
		100	3157.67	49.85	324.85
0.0	With	200	3297.00	49.65	345.28
		0	2839.33	57.85	265.85
		100	3131.33	63.65	276.68
	Without	200	3266.00	72.62	286.95
		0	3077.00	68.65	312.52
		100	3225.00	81.65	324.65
5000	With	200	3376.33	89.85	330.84
	LSD at 5%		124.52	3.354	4.452

Table (4): Effect of salinity stress (S0 0.0, S1 5000 mg/l), mycorrhyzae and pyridoxine (0, 100 & 200 mg/l) on yield & its indices of lupine plant (Data are means of two seasons)

Salinity (mg/l)	MA	Pyridoxine (mg/l)	No of pods/plant	No of seeds/pod	Weight of seeds/plant (g)	Weight of 100 seeds (g)
		0	3.33	4.00	2.33	30.24
		100	4.00	4.33	3.67	31.38
	Without	200	4.33	5.00	4.33	42.42
		0	4.67	4.67	3.67	36.35
		100	4.67	5.00	4.67	42.42
0	With	200	5.00	5.67	5.67	43.49
		0	3.00	3.00	1.42	20.92
		100	3.33	3.33	2.33	25.12
	Without	200	3.67	4.00	3.33	31.27
		0	3.67	3.67	2.33	26.14
		100	4.00	4.00	3.67	31.26
5000	With	200	4.33	4.33	4.67	37.31
	LSD at 5%		0.031	0.034	0.028	0.95

Nutritional values of lupine seeds:

Table (5) show that, subjecting lupine plants to salinity stress caused marked decreases in carbohydrate percentage meanwhile increased markedly protein%, flavonoids content, oil % and DPPH% activities.

Treating *lupines termis* with pyridoxine (100 and 200 mg/l) markedly increased different nutritional components and antioxidant activities percentages of lupine seeds comparing by controls without or with mycorrhizae treatment either under normal or salinity stressed conditions.

Table (5): Impact of salinity stress (S0 0.0, S1 5000 mg/l), mycorrhyzae and pyridoxine (0, 100 & 200 mg/l) on nutritional value of lupine plant.

Salinity (mg/l)	MA	Pyridoxine (mg/l)	Carbo%	Protein%	Flavonoid (mg/100 g dry weight)	Oil%	DPPH%
S0	Without	0	37.54	23.52	25.65	6.65	45.65
		100	38.65	24.52	31.52	6.95	52.34
		200	38.95	24.95	33.65	7.12	55.74
	With	0	37.85	24.10	27.85	9.95	47.65
		100	38.51	25.35	35.54	7.67	55.5
		200	39.95	25.85	39.84	8.65	57.68
S1	Without	0	35.75	24.51	35.85	8.74	50.74
		100	36.51	25.68	36.75	9.68	55.65
		200	36.92	26.85	38.65	9.82	59.54
	With	0	36.95	27.85	37.52	9.12	57.85
		100	37.41	28.65	40.41	9.62	62.35
		200	37.95	28.75	42.65	9.85	67.54
LSD at 5%			1.023	0.054	1.134	0.085	2.652

Discussion

Growth criteria:

Results showed, salinity stress (5000 mg/l) impacted significant reductions of growth parameters of lupine plant. The results obtained earlier [35, 36, 37, 38 & 39] are in concurrent with these obtained data. These reductions in growth criteria could be resulted by reduced water uptake, disorders in various physiological activities, nutrients deficiency and the reduced role of increased Na^+ and Cl^- aggregation around root [40]. Moreover, salt stress cause osmotic stress lead to water balance disturbances and in turn lead to stomatal closure, decreases of photosynthesis, toxic ions aggregation thus growth reductions. Moreover, it was suggested that, salinity inhibitory impact on growth might be due to the reduction of cell division or the inhibition of both cell elongation and activity of meristematic tissues [7].

Data presented in Table (2) show that, increased effect of AM Fungi on lupine plants compared with plants grown without mycorrhizae. Those data of mycorrhizae are in agreement with Hafez [41] and Bakry [42] on olive and flax plants, respectively. These increases might be reflected via AM role on various nutrient uptake (N, Ca, K, Cu, Zn, S, P) [43]. Applying AM enhanced growth and influenced nutrient allocation and transport among stem and root, resulting in improved dry weight of shoot **[12]**.

Different pyridoxine levels improved different morphological indices grown without mycorrhizae and also caused more significant increases in plants grown with mycorrhizae. Earlier studies reported that treatment of vitamins caused an enhancement in plant growth on different plant species [44 & 45]. Pyridoxine treatment to wheat plants enhanced cell division, increased the root growth and nutrient uptake that enhanced efficiency of photosynthetic surface and increased dry matter production [19]**.**

Photosynthetic pigments:

Salinity stress reduced *Chlo* a, *Chlo* b, *Chlo* a/*Chlo* b, carotenoids and total pigment of lupine plant leaves comparing with those irrigated with tap water (Fig 1). These data are similar to those in wheat plant [46 & 47]. These reductions caused by salinity stress on photosynthetic pigments components could be ascribed to salt deleterious impact on biosynthesis, improving their destruction [48] and/or causing serious injury to chloroplast thylakoids [49]. Salinity improved chlorophyllase activity and inhibited protein *de-novo* formation, that link chlorophyll [50]. Meanwhile, K^+ is known as stimulator to several enzymes which are necessary to photosynthesis. So, decreases in K^+ content caused an inhibition of photosynthesis and, ultimately, decreased growth [51]. Plants subjected to salinity had lower Chlo levels, while the Chlo *a*/*b* improved, owing to chlorophyll *b* breakdown being faster than chlorophyll *a* (Fig 1). These is supported by the fact that transformation to Chlo a is the initial step in destruction of chlorophyll *b* [52]. Chlo *a*/*b* increment were related to variations in Chlo a & b constituents that have decreased contents of light harvesting proteins [53].

Data presented in Fig. (1) revealed that, adding mycorrhizae increased significantly *Chlo* a, *Chlo* b, carotenoids and total pigments lupine plants comparing to those grow without AM. Meanwhile decreasing the ratio of *Chlo* a/ *Chlo* b under tap irrigation or saline irrigated (Fig. 1). Many scientists assumed the AM, symbiosis role on improving *Chlo* a, *Chlo* b, carotenoids and total pigments [54].

Foliar spraying of lupine plants with pyridoxine vitamin with different concentrations significantly improved photosynthetic pigments, in plants subjected to salt stress either of plants grown without or with mycorrohyzae comparing to those of control plants. Meanwhile, decreased the ratio of *Chlo* a/ *Chlo* b as compared with untreated control (Fig 1). Pyrodoxine results are in good agreement with Hamada and Khulaef [55], they stated that seed and foliar treatments of bean by pyridoxine improved biosynthesis of photosynthetic pigments fractions. Furthermore, Hendawy and Ezz El- Din [56] confirmed the increased contents of photosynthetic pigments of *Foeniculum vulgare* var. *azoricum* and, Nassar [45] on sesame plant.

Indole acetic acid and phenolics contents:

Indole acetic acid as an endogenous bioregulator preserves the defensive function in plant cells towards stress through antagonist or complimentary actions by other hormones, gibberellic acid, cytokinins and abscicic acid which termed, signalling crosstalk. Salt reduced endogenous IAA, while increased phenolic levels of lupine (Fig. 1). This reduction might be explained by salt role on boosting IAA degradation or lowering its formation [57]. Jasim [58] and Sadak [47] confirmed these results. Meanwhile, the increment of phenolic compounds of lupine plant can alleviate the adverse salt effect. The increased reactivity of phenolics as H_2 or electron donor reflecting in antioxidant protective strategies [59]. That mechanism causes unpaired electron to be stabillised also delocalized [60].

Addition of mycorrhizae to the soil increased significantly endogenous IAA and phenolics contents in lupine without mycorrhyzae either in normal irrigated and salt stressed irrigated plants (Fig 2). It was hypothesised that the altered IAA balance with AM fungus aided plant development and helped in improving growth and yield [61].

Exogenous treatment as foliar spraying of lupine plants with pyridoxine vitamin significantly increased endogenous IAA and phenolic levels. It is obvious that pyridoxine greater conc. was effective (100 mg/l) in increasing lupine plant IAA and phenolic at normal and stressed conditions (0.0 and 5000 mg/l) in soil without or with mycorrohyzae. These increases might be due the role of pyridoxine in IAA biosynthesis and retarding its degradation [62].

Osmolytes

Salinity stress increased the studied compatible solutes of lupine. Moreover adding mycorrhizae to soil and foliar treatment of pyridoxine increased TSS, proline and free amino acids in comparison by those plants without addition of mycorrhizae (Table 3). Plants accumulate higher amounts of compatible solutes under the effect of salinity stress [63], these compounds shield plant from stress via membrane stabilization, tertiary structures of proteins and enzymes. Osmoprotectants (TSS, proline and free amino acids) have a major effect on cell acclimation to varying unfavorable environmental stress by enhancing osmosis in cytoplasm, balancing proteins and membranes, sustaining greater water level required to plant growth and cell activities [64]. Increment of TSS improve turgor up keeping and maintain cell membrane [65]. Proline buildup is thought to be a signal of stress in several plants, serving as an osmotic protective and aiding in cell turgor stability [66]. Moreover, the higher proline level might resulted via proline oxidase activity decrease. Also, it is proposed as C & N supplier to quick recovery and stabilization. Proline is a scavenging osmolyte that neutralize dangerous ROS [67]. Quench of singlet oxygen ($^1O_2$) and chemical interaction with OH radicals are two techniques that proline lowers ROS harm [68]. Free amino acid buildup correlated by stress can be a component of the adapting method that helps with osmotic balance.

Yield and yield attributes:

Data showed that, salt (5000 mg/l) caused marked reduction in all yield attributes of lupine plant as compared with the control plants. Regarding reducing salt role (5000 mg/l) on yield indices of lupine, these decreases are reflected from growth decreases (Table 2), photosynthetic pigments (Fig 1) reductions. Moreover, the reductions in chlorophylls content which caused decreases in photosynthesis activity, causing lower carbohydrates buildup thus reduce transportation from leaves to the new seeds [69].

On the other hand, addition of mycorrhizae to soil with the recommended dose increased lupine yield either at normal or salinity stress conditions. Those increments resulted via AM role on nutriments uptake like N, Ca, K, Cu. Zn, S, & P [43]. Applying AM enhance plant development and influences nutrient allocation and transport along stem and root resulting in higher dry weight of shoot [12].

Moreover, the data also showed that, treating lupine by pyridoxine vitamin concentrations resulted in increases in the above mentioned yield and yield attributes. Boghdady [44] and Nassar [45] confirmed these results. The positive role of pyridoxine was stated on plants development under stress. In addition, Vitamin B could act as an antioxidant in improving plant tolerance [70]. Vitamins have lately been stated to be powerful antioxidants with a special ability to quench ROS [71].

Nutritional values of lupine seeds:

Table (5) show that, subjecting lupine plants to salinity stress caused marked decreases in carbohydrate percentage meanwhile increased markedly protein%, flavonoids content, oil % and DPPH% activities. Sadak [72] confirmed these results. Carbohydrates

reduction is mostly resulted by decreases in Chlo a, b, carotenoids (Table 3). Seeds carbohydrate variations is useful due to relation with variable processes as photosynthesis, transfer, and respiration [72]. Salt reduced chlorophyll levels causing decreases in photosynthetic activity. Thus, lower carbohydrates build up in mature leaves and thus decrease carbohydrate transport from leaves to the seeds**.**

Treating *lupines termis* with pyridoxine increased markedly different nutritional components and antioxidant activities percentages of lupine seeds. The stimulating effect of mycorrhizae soil addition on seeds carbohydrate may be growth increases and photosynthetic pigments (Tables 2 & 3). Moreover, enhanced photosynthetic production boosted carbohydrates production of leaves and consequently improved carbohydrate transfer from leaves to seeds. The results of the variations of flavonoids and antioxidant capacity of lupine plant as affected by pyridoxine treatments (Table 5) salinity stress increased protein, flavonoids contents and antioxidant activities of the yielded seeds. Flavonoids, including flavones, flavanols and condensed tannins, are secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH [73]. The higher levels of flavonoid could indicate some types of defence towards salt stress, since salinity stress was accompanied with higher ROS levels [73]**.** Pyridoxine addition to lupine caused increases in flavonoids. These findings suggest that pyridoxine as a bioactive chemical can be considered as an activator for synthesis of secondary metabolites (flavonoids).

Conclusions

In conclusion, salinity reduced growth and biochemical parameters. Mycorrihyzae treatment significantly improved growth and productivity by improving photosynthetic pigments, IAA, phenolic compounds, total soluble sugars, proline and free amino acids contents. Moreover, lupine plant treated by mycorrhizae and pyridoxine have higher nutritional constituents of yielded lupine seeds as carbohydrate%, protein%, flavonoids and DPPH percentages.

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