



## Amelioration of drought stress reduced effects by exogenous application of L- Phenylalanine on *Moringa oleifera*

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### Abstract

A field trial was conducted at 2018/2019 & 2019/2020 at National Research Centre, Experimental Station, Nubaria district, Beheira Governorate, Egypt, to investigate L-phenylalanine (Ph) (50, 100 and 150 mg/l) external treatments role on *Moringa oleifera* L plant development, productivity and nutritional value (carbohydrates%, protein% and some macro element contents), antioxidant compounds (flavonoids and phenol), antioxidant activity (DPPH%) and amino acid composition under water deficiency (drought stress). Drought stress decreased markedly morphological characters of *Moringa oleifera* plant (shoot length, leaves number, plant fresh and dry weights), photosynthetic pigments, yield components and carbohydrates%, protein%, N, P, K, Ca and Mg contents with marked increases in phenol and Flavonoid contents as well as, DPPH%. Meanwhile, exogenous application with Ph were effective in improving plant growth criteria and various studied physiological aspects at normal irrigation or drought stress conditions. Moreover, Ph external treatment increased markedly and significantly yield and its components, carbohydrates, protein, nitrogen, phosphorous, potassium, calcium and magnesium contents. Also Ph treatments caused more increases in phenol, flavonoids contents and antioxidant activity under normal irrigation or drought stress. 100 mg/l was the most effective concentration on alleviating drought stress adverse effect on *Moringa oleifera* plants.

**Key words:** Amino acid, Antioxidant activity, Drought, Flavonoids, *Moringa oleifera* Phenolics, Phenylalanine

### 1. Introduction

*Moringa oleifera* L. Moringaceae, is a medicinal and vegetable plant cultivated at tropical & subtropical countries [1]. *Moringa oleifera* plant is commonly known as 'magic tree' because of strong healing properties to different maladies and many chronic illnesses. Many researches could isolate different active chemicals using different plant parts [2]. One of the alternative techniques in medicinal fields is using herbal plants which are known as phytomedicine and this technique is widely used due to its affordable low cost [3]. Different uses of *Moringa oleifera* as a medicinal plant as anti-inflammatory, anti-spasmodic, antihypertensive, chemotherapy, antioxidant, anti-pyretic, anti-pyretic, anti-epileptic, anti-diabetic as well as, diuretic, anti-

lipidemic [2 & 3] and hepatoprotective activities [4]. In addition, *Moringa oleifera* has been used as great cosmetic compound, as well as, various health care compounds. Moreover, moringa seed oil could be used as vegetable oil also in soap manufacture. Moreover, its oil has oxidative degradation resistance and fuel characters [1].

One of the most important abiotic stress is water deficiency or drought stress. All over the world, water scarcely, limiting and decreased plant growth and yield [5]. Climatic variations & habitat use of rapidly increased population in the world made the agricultural land shrinking. Thus, it is necessary to exploit water deficit soil to overcome increased food demand. Stress plant response via maintaining various biochemical metabolism as photosynthesis, water

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Receive Date: 02 December 2021, Revise Date: 09 January 2022, Accept Date: 13 January 2022

DOI: 10.21608/EJCHEM.2022.109253.4978

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relations, etc, in addition to adjustments of the membrane system [6]. Photosynthetic process adversely affected by drought stress in all its phases via the decline in CO<sub>2</sub> transport to chloroplast [7]. Moreover, to overcome the oxidative stress resulted via reactive oxygen species, plant possess an effective antioxidant (non-enzymatic & enzymatic) defense system [8]. Antioxidant enzymes like superoxide dismutase and peroxidase might inhibit the stress injury. In addition, crops respond to stress by production of organic solutes (compatible solutes) or osmoprotectants which have the ability to decrease osmotic potential and absorb water molecules to maintain cell turgor. [5 & 9]. Various strategies are achieved which connect with one another, producing complicated network that causes alterations of specific proteins associated with cellular responses [10].

To increase plant tolerance to various abiotic stresses different strategies are used. One of these strategies is using natural exogenous plant growth regulators, antioxidants, vitamins, amino acids. Amino acids are organic compounds have N, C, H<sub>2</sub>, and O<sub>2</sub> with organic side-chain, a property which differentiates various amino acids [11]. They are important compounds for biosynthesis of different cell components used for plant development. Moreover, they have a function in signaling pathways via serving as signal molecules or via facilitating the conjugation of amino acids and phytohormones to alter hormone levels [12]. Moreover, amino acids have important effects; as stress relievers, nitrogen sources and hormone precursors [13&14]. Several studies stated the efficiency of amino acids uptake by plants [15&16]. One of these amino acids is L-phenylalanine, an aromatic amino acid and substrate of phenylalanine ammonia lyase (PAL) which catalyzes L-phenylalanine into Trans-cinnamic acid as the first step of the biosynthesis of different phenolic compounds [16]. PAL used in phenylpropanoid formation [17].

Therefore, this investigation was carried out to show L-phenylalanine exogenous treatment role on alleviation of reduced effect of drought stress on growth, yield and some biochemical aspects of *Moringa oleifera* L grown in sandy soil.

## Materials and Methods

*Moringa oleifera* L. plants were transplanted in the Experimental Station of National Research Centre, Nubaria district, Beheira Governorate, Egypt, at 2 winter seasons of 2018/2019 and 2019/2020. The experimental soil was sandy soil. Analysis characteristics are shown in Table (1) according to Carter & Gregorich [18].

Experiment was designed as split – plot with 4 replications. Main plots were water applications, L-phenyl alanine (Ph) application were random in sub – plots. The recommended agricultural practices were applied, for each plant, 40 g ca-superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) and 20 g K-sulphate (48.0 % K<sub>2</sub>O) and 40 g urea (46.5% N) mixed with 500 g green manures (compost). Plants were sprayed with L-phenyl alanine (50, 100 and 150 mg/l, as Ph 1, Ph 2 and Ph 3) while control plants were sprayed with water (Ph 0) at 45 and 60 days after cutting. Watering applications including normal (D0) and skipping two irrigation times (D1) were done after 2<sup>nd</sup> Ph application at 75 and 82 days. Plant samples were taken after second skipping irrigation by week. Some growth characters were analyzed (plant height cm, leaves No/plant, stem circumference cm, plant fresh and dry weights g), photosynthetic pigments, indole acetic acid (IAA), and phenolic contents. Foliage yield and its indices (shoot length cm, stem circumference (cm), plant fresh and dry weight (g). Some antioxidant compounds such as, flavonoids and DPPH activity in addition to nutritive contents of leaves as protein%, carbohydrates% and some element contents as nitrogen, phosphorus, potassium, calcium and magnesium.

### Biochemical analysis:

Photosynthetic pigments contents were carried out [19]. IAA acid were analysed according Larsen [20]. Phenolic contents were estimated [21]. Flavonoid levels were estimated as Ordoñez [22]. Free radical scavenging activity was estimated as Brand-Williams [23]. Protein levels were estimated as microkjeldahl [24]. Total carbohydrates were estimated as Dubois [25]. Some element levels were analyzed [18].

Table (1): Characteristics of experimental soil.

Sand %	Silt %	Clay %	pH	Organic matter %	CaCO <sub>3</sub> %	E.C. dS/m	Soluble N, ppm	Available P, ppm	Exchangeable K, ppm
91.2	3.7	5.1	7.3	0.3	1.4	0.3	8.1	3.2	20

### Statistical analysis

Analysis of variance method of split-plot design as [26], means were compared [27] at 5% LSD. Combined analysis of the two growing seasons was done.

### Results

**Growth parameters:** Subjecting *Moringa oleifera* to water deficiency significantly reduced growth parameters comparing to unstressed control (Table 2). Data clearly show that, plant height was reduced by 10.03%, stem circumference was reduced by 20.87% plant fresh and dry weight were reduced by 11.03% and 14.86 under drought stress. Meanwhile, exogenous application of L phenyl alanine (with 50, 100 & 150 mg/l) increased gradually and significantly all growth characters under two water levels (Table 2). The increases in various studied growth characters in response to Ph treatments were gradually increases till

100 mg/l then the increases were lower. Data show that, 100 mg/l caused the greatest increments in different growth criteria (Plant height 119.00 & 109.00, Leaves no/plant 17.00 & 13.00, Stem circumference 5.95 & 4.62, Plant fresh wt 85.65 & 63.50 and plant dry wt 22.65 & 17.73 under D0 and D1 respectively) (Table 2).

**Yield and its indices:** Table (3) represents the L-phenylalanine application impact on yield (shoot length, leaves No /plant, plant fresh and dry weight) of *Moringa oleifera* plant at water deficit. Water deficit by escaping two irrigation times significantly decreased the tested yield attributes While, treatment of *Moringa oleifera* by phenylalanine (50, 100 and 150 mg/l) improved yield attributes comparing with controls at normal or drought conditions. L-Phenylalanine 100 mg/l gave the highest yield attributes increases of *Moringa oleifera* (Table 3) except plant height under D0 the 150 mg/l Ph gave the highest increase (200.00 cm).

Table (2): L- phenylalanine (0, 50, 100 and 150 mg/l) role on growth parameters of *Moringa oleifera* L. under water deficit in sandy soil

Drought	Ph	Plant height (cm)	Leaves no/plant	Stem circum	Plant fresh wt (g)	Plant dry wt (g)
D0	Ph0	96.33	12.00	4.12	57.44	13.32
	Ph1	108.33	12.67	5.32	73.46	19.43
	Ph2	119.00	17.00	5.95	85.02	22.65
	Ph3	113.67	12.67	4.92	63.80	22.41
D1	Ph0	86.67	9.33	3.26	57.21	11.34
	P1	93.67	11.67	3.95	60.71	15.74
	Ph2	109.00	13.00	4.62	63.50	17.73
	Ph3	89.33	12.67	4.16	59.71	15.46
LSD at 5%		6.524	1.523	0.324	6.524	1.254

Table (3): L- phenyl alanine (0, 100, 150 and 200 mg/l) role on yield of *Moringa oleifera* L. at water deficit in sandy soil

Drought	Phenyl alanine	Shoot length (cm)	Leaves no/plant	Plant fresh wt (g)	Plant dry wt (g)
D0	Ph0	173.00	7.67	299.04	86.25
	Ph1	183.67	9.67	420.91	108.42
	Ph2	193.33	11.00	471.71	116.81
	Ph3	200.00	10.33	397.91	107.80
D1	Ph0	150.33	7.33	199.48	57.91
	Ph1	156.33	8.00	412.90	97.21
	Ph2	166.67	10.00	431.83	103.31
	Ph3	159.00	7.33	326.83	96.58
LSD at 5%		13.685	0.985	23.684	11.624

### Photosynthetic pigments:

Different *Moringa oleifera* photosynthetic pigments constituents (Chlo a, Chlo b, carotenoids and total pigments) significantly decreased by drought stress comparing with control plant leaves (Table 4). The decrease in chlorophyll a was 8.33%, chlorophyll b was 11.48, carotenoids was 10.26 and total pigments was 9.78%. While, different used phenylalanine levels increased significantly photosynthetic pigments contents not only under normal plants but also under water stressed conditions comparing by their corresponding controls. Increasing concentrations of Ph foliar treatments caused gradual significant increases in different constituents of pigments (Table 4). Phenylalanine with 100 mg/l caused the highest significant increases under normal irrigation and water stress.

**IAA contents:** Subjecting *Moringa oleifera* plants to water deficit significantly reduced endogenous IAA levels, the percentage of decreases was by 38.81% than that of control (Table 4). On contrast, L phenylalanine treatments (50, 100 and 150 mg/l) induced significant increments of IAA in both unstressed and stressed plants compared with untreated plants. The increases in response to foliar treatment were gradually increases with increasing Ph concentrations till 100 mg/l (Table 4)..100 mg/l phenylalanine gave the highest increases in IAA contents under normal conditions (42.65 µg/100 g fresh wt) and water stress (30.41 µg/100 g fresh wt).

### Nutritional value of the foliage yield:

Table (5) stated, water deficit significant reductions in carbohydrates and protein % of *Moringa oleifera* yield comparing with control. Table (5) stated significant increments in yielded leaves carbohydrates and protein

percentages by exogenous applications of phenylalanine comparing with their untreated controls under normal or stressed conditions. Increasing phenylalanine foliar treatment concentrations increased gradually the above mentioned nutritional values (carbohydrates% and Protein%).

The variations in element levels of *Moringa oleifera* by exogenous treatments with phenylalanine (50, 100 & 150 mg/l) under both unstressed and stressed conditions (Table 5). N, P, K, Ca and Mg levels of *Moringa oleifera* leaves were decreased significantly by escaping two irrigations times compared with those grown normally. Meanwhile, different concentrations of phenylalanine exogenous application increased various element levels of *Moringa oleifera* at both normal and water deficit irrigation conditions (Table 5). L-phenylalanine 100 mg/l induced the greatest increments of studied elements comparing with controls.

### Antioxidant compounds (Phenol and flavonoids) and antioxidant activity:

Table (6) states exogenous application of phenylalanine (50, 100 and 150 mg/l) on antioxidant contents such as phenols and flavonoid levels & antioxidant activity DPPH% of foliage yield of *Moringa oleifera* under normal conditions or water stress. Skipping two irrigation times increased significantly phenols and flavonoid contents and antioxidant activity as DPPH% of *Moringa oleifera* yield compared with normal irrigated control. Meanwhile, various levels of phenylalanine caused more significant increments in different studied parameters in comparison with untreated control at normal or drought stress.

Table (4): L- phenylalanine (0, 100, and150 mg/l) role on Chl a, Chlo b, carotenoids, total pigments and IAA contents (µg/100g fresh wt) of *Moringa oleifera* L. under water deficit in sandy soil .

Drought	Phenyl alanine	Chlo a	Chlo b	carot	Total pigments	IAA
D0	Ph0	853.49	640.03	319.07	1812.59	29.68
	Ph1	1016.44	715.82	327.06	2059.32	35.65
	Ph2	1227.13	937.75	396.65	2561.53	42.65
	Ph3	1045.61	809.31	359.08	2214.00	40.85
D1	Ph0	786.92	559.62	287.00	1633.54	21.52
	Ph1	960.35	572.89	259.44	1792.69	25.68
	Ph2	1024.91	526.92	228.02	1779.86	30.41
	ph3	1074.00	613.58	261.70	1949.28	28.65
LSD at 5%		106.351	95.653	58.975	196.845	3.15

Table (5): L- phenylalanine (0, 100, and 150 mg/l) role on nutritional value (carbohydrate%, protein% and some macro elements contents) of foliage yields of *Moringa oleifera* L. under water deficit in sandy soil .

Drought	Phenylalanine (mg/l)	Carbohydrates %	Protein%	N	P	K	Ca	Mg
D0	Ph0	33.41	20.06	3.21	1.37	0.83	1.68	0.33
	Ph1	34.01	20.56	3.29	1.45	0.97	1.87	0.38
	Ph2	34.75	20.88	3.34	2.12	1.12	2.01	0.43
	Ph3	34.12	20.50	3.28	2.08	1.26	1.93	0.37
D1	Ph0	33.01	19.06	3.05	1.25	0.78	1.23	0.23
	Ph1	33.85	19.50	3.12	1.48	0.94	1.25	0.27
	Ph2	33.95	19.94	3.19	1.75	1.03	1.43	0.37
	Ph3	33.15	20.00	3.20	1.36	1.14	1.52	0.33
LSD at 5%		2.68	1.15	0.674	0.678	0.314	0.458	0.131

Table (6): L- phenylalanine (0, 100, and 150 mg/l) role on phenol, flavonoids & antioxidant activity of *Moringa oleifera* L. yielded leaves under water deficit in sandy soil

Drought	Phenylalanine (mg/l)	Phenol	Flavonoids	DPPH %
		mg/g dry wt		
D0	Ph0	43.65	16.35	42.15
	Ph1	52.48	20.14	49.52
	Ph2	61.52	26.25	51.45
	Ph3	58.65	26.52	53.84
D1	Ph0	53.62	23.65	51.32
	Ph1	62.65	31.52	61.52
	Ph2	71.01	39.62	69.45
	Ph3	65.62	32.45	54.62
LSD at 5%		5.62	4.15	3.84

**Amino acid composition:** The patterns of variations in the amino acid constituents of foliage yield of *Moringa oleifera* treated with phenylalanine (0.0 and 100 mg/l) and grown under normal irrigation or drought stress are shown in (Table 7). Results revealed that, amino acid composed of 18 amino acids and proline were the largest levels of amino acids (major predominant). Proline levels ranged among 52.52: 57.96 followed by threonine (34.39: 37.65), glutamic acid (15.87: 20.97) and alanine (12.95: 15.05), these are known the predominant amino acids. Meanwhile, the rest are known as minor amino acids. Subjecting *Moringa oleifera* to drought stress increased markedly aspartic acid, serine, glutamic acid, proline, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine and histidine, total essential amino acids (threonine, valine, methionine, leucine, isoleucine, phenylalanine, histidine, lysine and arginine) and total amino acids

comparing by control. Meanwhile, decreased markedly threonine, glycine, cysteine, methionine and lysine and the ratio between essential AA /non-essential AA. Foliar treatment of phenylalanine with 100 mg/l increased markedly all amino constituents, essential amino acids (threonine, valine, methionine, leucine, isoleucine, phenylalanine, histidine, lysine and arginine) in addition to ratio between essential AA /non-essential AA comparing by control.

### Discussion

Drought stress emerged as an environmental problem affecting plant productivity. Water deficit decreased different growth and yield parameters comparing with those of control plants. While foliar treatment with L-phenylalanine (50, 100 & 150 mg/l) improved growth and yield of *Moringa oleifera* plant grown at both irrigation conditions (Table 2&3).

Table (7): L- phenylalanine (0 and 100 mg/l) role on amino acid constitutes of *Moringa oleifera* L. yielded leaves at water deficit in sandy soil

Treatment	Ph0	Ph2	Ph0	Ph2
Amino acid	Normal	Drought	Normal	Drought
Aspartic acid	6.32	6.52	7.10	7.51
Threonine*	34.39	37.65	33.62	35.95
Serine	6.66	7.62	8.12	8.19
Glutamic	15.87	18.65	19.35	20.97
Proline	52.52	57.62	56.35	57.96
Glycine	5.62	6.12	5.15	5.95
Cystine	2.99	3.62	2.86	2.87
Alanine	12.95	15.05	13.62	13.85
Valine*	5.76	6.95	6.85	6.97
Methionine*	3.59	3.75	2.65	4.85
Leucine*	5.69	7.95	7.12	7.35
Isoleucine*	6.68	8.82	7.16	7.15
Phenylalanine*	5.70	7.15	6.15	7.68
Tyrosine	2.65	4.06	3.12	3.42
Histidine*	2.53	4.65	4.12	4.95
Lysine*	4.69	5.62	4.32	4.85
NH <sub>4</sub>	0.02	0.09	0.00	0.00
Arginine*	2.79	3.24	3.52	4.62
Essential A A	71.81	85.78	75.51	84.37
Non-essential A A	105.60	119.35	115.67	120.72
Total amino acids	177.41	205.13	191.18	205.09
Ess AA /non ess AA	0.68	0.72	0.65	0.70

These obtained data of drought stress are similar to the earlier data on *Moringa oleifera* [28], *Salvia nemorosa* L [29], sunflower [30] and *Moringa oleifera* [31]. Kar [32] stated that water deficit induced ROS accumulation. Low level of ROS enhanced antioxidant protection, meanwhile increased ROS production triggered lipid peroxidation.

Water deficit seriously affects different biochemical processes of plants. Moreover, plant height reductions as a result of water deficit (Table 2 & 3) resulted by reductions in lengthening, turgidity, volume and finally cell growth [33]. These reductions of *Moringa* productivity caused by reductions in growth attributes (Table 2) & photosynthetic pigments (Table 4). These reductions are in accordance with the

earlier results of Sadiq [34] on mung bean plant, Dawood [35] on sunflower plant. Chlorophyll reductions of water deficit of *Moringa oleifera* that inhibits photosynthetic enzyme activities lead to reduced carbohydrate formation [36]. On the other hand, phenylalanine treatments improved different growth parameters and yield components of *Moringa oleifera* plant at unstressed or drought stress. These results are in accordance with Reham [37] and Bakry [38] using different concentrations of phenylalanine on genoveser basil and flax plant. External treatment of L-phenylalanine alleviated reduced water deficit results of *Moringa oleifera* growth and yield via improving photosynthetic pigments & IAA (Table 4). In addition, phenylalanine as organic nitrogenous materials are the building blocks of protein synthesis also components of enzyme [39]. Zhang [40] observed that L-phenylalanine treatments enhanced carbon and nitrogen assimilation via the stabilizing membrane components under stress thus increased growth.

Photosynthetic pigments of *Moringa oleifera* grown at water deficit in sandy soil and treated externally with different concentrations of L-phenylalanine, data are represented in (Table 4). Skipping of irrigation two times decreased chlorophylls pigments. These reduction effects of might be connected with the disorders of various metabolic processes caused by chloroplast oxidation so changes proteins and pigments forms [41]. Ezzo [28] on *Moringa oleifera* and Dawood [35] on sunflower plant confirmed these results. The promotive effect of L-phenylalanine application was in agreement with Abd El-Samad [42] on maize and Bakry [38] on flax cultivars. These promotive effects on enhancing photosynthetic pigments were resulted by succinyl COA (Kreb's cycle) and they initiate synthetic method causing chlorophyll formation [43].

Drought significantly reduced IAA level in *Moringa oleifera* leaves (Table 4). These reductions could be resulted by IAA oxidase activity increments that increase its breakdown [44]. Meanwhile, L-phenylalanine application improved IAA level in both unstressed and stressed plants. These results were in accordance with earlier studies which reported that, phenylalanine treatments increased total indoles [45]. Those IAA increments indicating bioregulator effect on promotion of cell division and/or enlargement causing finally growth. Different treatments of phenylalanine markedly and significantly increased total carbohydrate and protein percentage of yielded foliage plant. The same results were found in response

to amino acids treatment Bakry [38], Abd El-Samad [42] and El Awadi [46].

Regarding macronutrient contents in the foliage yield, phenylalanine improved macro and micronutrients levels of *Moringa oleifera* (Table 5). It could be concluded that the promotive role of amino acids is via improving biosynthesis of free amino acids and their incorporation into protein (Table 5). These data were confirmed with others stated by Abd Allah [47]. The respective increase in (K<sup>+</sup>) with phenylalanine might be reflected by nitrogenous materials role on protein formation [26]. Moreover, multiple membrane proteins may be used for cations absorption from soil [48].

Precursor external treatments, L-phenylalanine, was used to improve formation of secondary metabolites such as phenolics and flavonoids in plant cell [49]. Treatments of phenylalanine increased phenolics & flavonoids of *Moringa oleifera*. Phenolics has an important effect as protective compounds of cells from oxidative stress, improve cell membrane stability [50]. Those increases could resulted via phenols effect in regulation of plant metabolic processes. Similar to our results Govindaraju, &Arulselvi [51] and Shekari and Javanmardi [52] found that the amino acids improved phenolics of snap bean, medicinal herb – *Coleus aromaticus* Benth (L) and Broccoli. Heldt and Piechulla [53] stated, phenolics are resulted from amino acids and flavonoids increments levels were formed by various compounds able for inducing flavonoid synthesis by phenylalanine.

The amino acids constituent's data are in accordance with those of Kovács [54] and Abd Elhamid [55] on various plants. The increased total amino acids might be used in osmosis stabilization, ROS scavenging and stabilizing protein and membrane [56]. Proline known as main osmoprotectant, and its overproduction is used for decreasing cellular water potential and avoid toxic effect of increased ionic content [57]. Regarding amino acid constituents of the foliage yield of *Moringa oleifera* as affected by foliar treatment of phenylalanine application improved those characters, those data are in accordance with Abd Allah [47].

### Concolusion

L-Phenylalanine improved drought tolerance of *Moringa oleifera* plants and increased growth and photosynthetic pigments, IAA & foliage yield components, and some nutritional values and

antioxidant compounds in addition to antioxidant activities of yielded plant.

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