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Foliar Application Influence of Pyridoxine and Thiamine on Growth, Qualitative and Quantitative Traits of Faba Bean Grown in Sandy Soil

[1]A.S.M.Younis, [2]Mervat S.H. Sadak, [1]B.A. Bakry and [2]Amany A. Ramadan

[1]Field Crops Research Department, Agriculture and Biology Research Division,
[2]Department of Botany, Agricultural and Biological Research Division,
National Research Centre, 33 El-Bohouth Street, P.O. Box: 12622, Dokki, Giza, Egypt

Abstract: The effect of foliar applications on physiological traits of faba bean with pyridoxine (vitamin B6) at 50, 100 and 150mg/L and thiamine (vitamin B1) at 100, 200 and 300mg/L was investigated during of two winter seasons 2017/2018 and 2018/2019 at the Research and Production Station, National Research Centre (NRC), Nubaria district, Behaira Governorate, Egypt. Results revealed that, foliar treatments with pyridoxine or thiamine at all tested concentrations induced marked increases in growth traits (plant height, branches and leaves number/plant, fresh & dry root weight), photosynthetic pigments, indole acetic acid (IAA), phenolics, carbohydrate components, free amino acids and proline contents. Seed yield and its related characters (pods number /plant, seeds number/pod, seed yield/plant and 100 seeds weight) as well as carbohydrate percentages in seeds were also increased. It is worthy to mention that; 200 mg/L of thiamine followed by 100 mg/L pyridoxine are the most effective treatment in increasing seed yield/fed by 86 and 61%, respectively. It may be concluded that pyridoxine and thiamine have a beneficial effect in improving growth and seed yield of faba bean under sandy soil conditions.

Key words: Pyridoxine · Thiamine · Faba bean · Protein · Carbohydrates · IAA · Proline · Sandy soil

INTRODUCTION

Faba bean (*Vicia faba* L.) plant is one of the most important leguminous crops, it considered one of the major sources of protein and carbohydrates as it provides about 28-30% and 51-60% of dietary human protein and carbohydrates, respectively. It is regarded as a source of natural antioxidants, with high contents of starch, cellulose, minerals and vitamin C [1]. Moreover, it is among the important plants used in farm animals' feed, since the vegetative parts of plants and broken seeds are mixed with animal diets [2]. Because seeds are good source of lysine and arginine, its use can augment the poor contents in cereals diets and also improves manure content of soil so it used in plant rotation [3].

Due to the rapid increases in human population and the increased depletion of agricultural lands due to desertification and erosion problems, Egypt suffers from decreasing productivity of different crops. Thus, it is important to improve the yield of faba bean under sandy soil which is mostly affected by the combination between abiotic adverse conditions such as nutrient scarcity, poor available water, temperature fluctuations and increased solar radiations. Therefore, improving plant tolerance to these adverse environmental conditions can be achieved through selection of tolerant genotypes, using the optimum cultural strategies from soaking seeds before planting or seedlings treatment at various growth stages with different natural and safety compounds, e.g. plant growth promotors, vitamins and antioxidant compounds. These compounds play significant role for improving the tolerant of plants to fulfilled partially the adverse conditions and mitigate their damaging impacts on seed yield and quality [4]. When plants subjected to adverse abiotic stress conditions at different growth stages, various effects physiological and biochemical functions disrupted [5]. This disturbance induces a decrease in plant growth and productivity.

Corresponding Author: A.S.M.Younis, Field Crops Research Department, Agriculture and Biology Research Division, National Research Centre, Dokki, 33 El-Bohouth Street, P.O. Box: 12622, Dokki, Giza, Egypt.

Vitamins are one of the most important plant growth promotor's precursors which could enhance plant growth and yield of different crops under different environmental stress. Vitamins are organic nutrients needed for development of living organisms. However, vitamins are endogenously synthesized in autotrophic organisms [6], still, external treatment of vitamins affect positively plant growth, CO_2 –absorption and protein biosynthesis [7]. Different treatment of vitamins caused an effective role via growth regulating factors which affect different biochemical processes and protect plant from adverse effects of abiotic stress [8].

Pyridoxine (Vitamin B6) is a significant coenzyme which incorporated in many physiological processes, among them glycogen metabolism and biosynthesis of amino acids [9]. Pyridoxine act as a co-enzyme for lot of metabolic enzymes [9], also, it is regarded as an efficient antioxidant [10]. It improved the efficiency of photosynthetic carbon reactions and increased dry matter production. In the same time, treatment of different plants with pyridoxine enhanced cell division, improved growth and differentiation and increased nutrient uptake [11].

Thiamine (vitamin B1) is water soluble compound, is one of vitamin B group and is produced from plants as well as microbes. Thiamine found in plants as free or in phosphorylated states [12]. Thiamine may be used as coenzyme in decarboxylation of α-keto acids, such as pyruvic and keto-glutamic acids which gain its importance in carbohydrates and fats metabolism [13]. The pyrophosphate works as a coenzyme for enzymes related to different metabolic pathways citric acid and Calvin cycles [14-16]. Moreover, thiamine is a significant cofactor for trans-ketolation reactions in nucleotide biosynthesis [17]. In addition, thiamine treatment improves plant tolerance to environmental adverse conditions by preventing DNA damage [14]. This empowering influence of thiamine was confirmed earlier on growth, photosynthetic pigments, different biochemical aspects and yield in many plants [18-21].

Thus the aim of this work was to investigate the physiological effect of pyridoxine and thiamine foliar treatment on growth, some biochemical traits, yield as well as its components of faba bean plants grown under sandy soil.

MATERIALS AND METHODS

Two field experiments were conducted at the Research and Production Station, National Research Centre, Nubaria Province, Behaira Governorate, Egypt during the two growing seasons of 2017/2018 and 2018/2019 to study the physiological response of Egyptian faba bean (var. Nubria 1) to foliar application with different concentrations of Pyridoxine (50, 100, 150 ppm) and Thiamine (100, 200, 300 ppm). Seeds of faba bean were obtained from Legume Research Department, Field Crop Institute, Agricultural Research Center, Giza, Egypt. Both tested materials (Pyridoxine and Thiamine) bought from Sigma Chemical Company, St. Louis, MO, USA.

Experimental Procedure: The seeds of faba bean were sown on 15th October in both seasons. The experiment was designed in a randomized complete block (RCBD) with three replicates, in experimental plot area of 17.5 meter (5meters long and 3.5 width and 70 cm between rows). Soil of the experimental site was sandy soil. Mechanical, chemical and nutritional analysis of the experimental soil is reported in Table (1) according to Chapmanand Bratt [22].

Seeds were sown in hills on each side of a ridge at 20 cm apart. The plants were thinned after (15 days of sown) to two plants/hill. Precipitation was nil in all months of faba bean growing season for both seasons. All other agricultural practices were followed according to the recommendations of Agricultural Research Centre (ARC), Egypt.

The plants were sprayed twice at 45 and 60 days after sowing with freshly prepared solutions of Pyridoxine and Thiamine with different levels. Meanwhile, untreated plants were sprayed by distilled water to serve as control.

Data Recorded: Plant samples were collected at 75 days of age for determination of some growth parameters (plant height (cm), number of branches per plant, number of leaver per plant, plant fresh weight (g), plant dry weight (g), root fresh weight (g) and root dry weight (g)). At harvest, the following characters were recorded at random of ten guarded plants from each plot: plant weight (g), number of pods per plant, number of seeds per pod (measured for ten plants per plots), seed yield per plant (g). 100-seeds weight (100-SW, g), seed yield per feddan (SYPF, kg) by adjusting seed yield/plot to seed yield per feddan.

Chemical Analysis: Photosynthetic pigments (Chl. a, Chl. b and carotenoids) were determined using the methods described by Lichtenthaler and Buschmann [23]. Indole acetic acid contents were extracted and analyzed by the method of Larsen *et al.* [24]. Phenols were analyzed

Table 1: Mechanical, chemical and nutritional analysis of the experimental soil. Mechanical analysis

Items	Component and concentrations				
	Sand				
	Course 2000-200 µ%	Fine 200-20µ %	Silt 20-0µ %	Clay < 2µ %	Soil texture
Mechanical analysis	47.46	36.19	12.86	4.28	Sandy

					Soluble cations (meq/l)				Soluble anions (meq/l)			
Chemical analysis	pH 1:2.5	EC dSm^{-1}	$CaCO_{3\%}$	OM %	Na^{+}	K^{+}	Mg^{+}	Ca^{++}	CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{--}
	8.25	0.11	0.9	0.9	0.7	0.02	0.1	0.3	0.0	0.2	0.8	0.12

	Available nutrients						
	Macro element (ppm)			Micro element (ppm)			
Nutritional analysis	N	P	K	Zn	Fe	Mn	Cu
	12.9	3.6	52.9	0.12	1.98	0.46	0.06

by using the method of Folin-Ciocalteau reported by Tavarini *et al*. [25]. Total carbohydrate was determined according to Dubois *et al.* [26]. Total soluble sugars were extracted by the method of Prud'homme *et al.* [27] and analyzed according to Yemm and Willis [28]. Polysaccharides were calculated by the subtracting total carbohydrates and total soluble sugars. Free amino acids and proline were extracted according to the method described by Vartainan *et al.* [29]. Free amino acid was determined with the ninhydrin reagent method of Yemm and Cocking [30]. Proline was assayed according to the method described by Bates *et al.* [31]. Crudeprotein percentage was extracted and determined by Micro-Kjeldahl using methods outlined by A.O.A.C. [32] (1990).

Statistical Analyses: Combined analysis of variance of RCBD for substances and their concentrations across the two seasons were performed using SAS ® [33]. Least significant differences (LSD) were calculated according to Steel *et al.* [34].

RESULTS

Changes in Growth Traits: The effect of pyridoxine and thiamin vitamins treatments on growth traits of faba bean plants grown in sandy soil are presented in Table (2). Different treatments increased markedly growth parameters of faba bean plant (plant height, cm; number of branches and leaves/plant, plant and root fresh & dry weight, g) as compared with untreated control. The most pronounced treatments were150 mg/Lpyridoxine and 200 mg/Lthiamine which showed the highest significant ($P<0.05$) increments in most of the studied growth traits, except for plant height(cm) with both of tested materials which was non-significant.

Photosynthetic Pigments Performance: Data in Fig. (1) shows the effect of different concentrations of pyridoxine at (50, 100 & 150 mg/L) and thiamine at (100, 200 and 300 mg/L) on photosynthetic pigments of faba bean plants grown in sandy soil. Data clearly shows that foliar treatments of pyridoxine or thiamine with different concentrations significantly increased photosynthetic pigments constituents (Chl. a, Chl. b, carotenoids and consequently total pigments) of the tested plants. The treatment of pyridoxine at 100 mg/L recorded the maximum increases of photosynthetic pigments constituents, followed by the treatment with 200 mg/L thiamine as compared with other tested concentration treatments.

Changes in Indole Acetic Acid and Phenolic Contents: The data in Fig. (2) shows the effect of pyridoxine different concentrations (50, 100 and 150 mg/L) and thiamine (100, 200 and 300 mg/L) on endogenous phytohormone indole acetic acid (IAA) and phenolic levels of faba bean plants grown in sandy soil. Data clearly shows that foliar applications of pyridoxine or thiamine with different concentrations caused significantly increased IAA as well as phenolic levels of faba bean plants compared with control plants. Pyridoxine concentrations 100 mg/Land thiamine 300 mg/L recorded the highest values of IAA constituents.

Meanwhile, foliar application of 300 mg/L thiamin treatment recorded the highest value of phenolic contents followed by 200 mg/L treatment compared with the other concentrations used from both tested substances.

Changes in Carbohydrates Constituents, Free Amino Acids and Proline Contents: Data in Table (3) stated that carbohydrates, free amino acids and proline contents of

Table 2: Effect of different concentrations of pyridoxine and thiamine on growth traits of faba bean plants grown in sandy soil. (Combined analysis of two seasons)

Treatments (mg/L)		Plant height (cm)	Branches no./plant	Leaves no./plant	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control		43.33	3.33	56.33	112.20	16.18	8.74	1.16
Pyridoxine	50	50.67	5.67	74.33	153.77	22.89	16.24	1.63
	100	54.67	5.67	78.33	192.07	27.14	15.91	2.05
	150	55.67	6.00	67.33	289.50	34.53	20.51	2.01
Thiamine	100	55.00	5.67	61.33	153.34	26.60	21.48	1.75
	200	50.00	7.33	84.33	265.58	28.84	26.90	3.33
	300	48.67	5.33	86.00	145.72	21.27	12.27	1.76
$LSD_{0.05}$		NS	1.46	14.54	40.92	8.05	4.49	0.47
$LSD_{0.01}$		NS	2.04	20.38	57.36	11.29	6.30	0.66

NS: Not significant

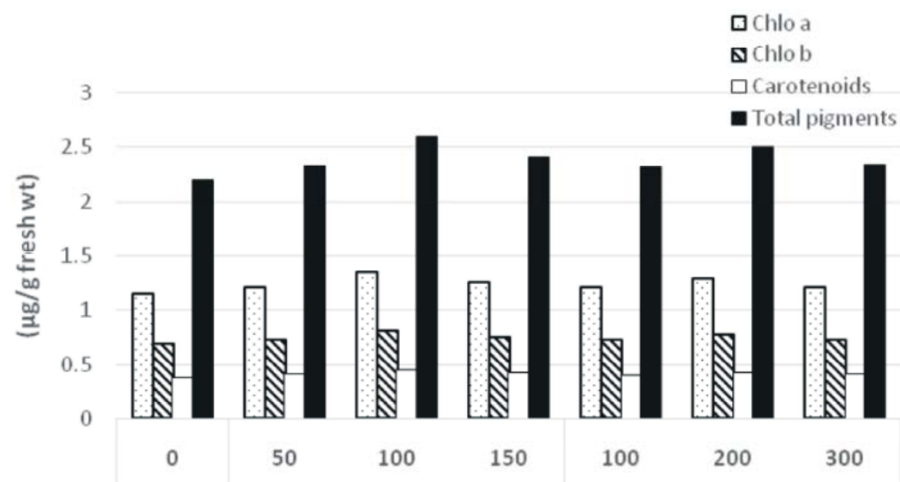
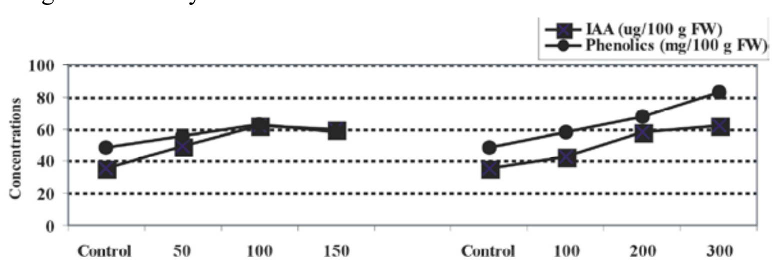


Fig. 1: Effect of different concentrations (mg/L) of pyridoxine and thiamine on photosynthetic pigments (µg/g fresh wt.) of faba bean plants grown in sandy soil



Pyridoxine treatments
LSD (0.05): 2.53; (0.01): 3.54

Thiamine treatments
LSD (0.05): 1.02; (0.01): 1.43

Fig. 2: Effect of different concentrations of pyridoxine and thiamine (mg/L) on IAA (µg/100 g fresh weight) and phenolic (mg/100 g fresh weight) of faba bean plants grown in sandy soil.

faba bean plants were positively responded to different concentrations of either pyridoxine or thiamine vitamins. For carbohydrates constituents, each concentration of both tested materials caused significant (P<0.05) increase in total carbohydrates and polysaccharides content. Meanwhile, total soluble sugars of faba bean plants were decreased significantly (P<0.05) as compared with untreated ones (control). The treatment with 100 mg/L pyridoxine followed by treatment with 200 mg/Lthiamine were the most effective concentrations which gave the highest increases in the studied parameters. Moreover, foliar applications of pyridoxine and thiamine with different concentrations caused significant (P<0.05) increasing in the free amino acids and proline contents of

Table 3: Effect of different concentrations of pyridoxine and thiamine (mg/L) on TSS%, polysaccharides, free amino acids, proline (mg/100 g) and total carbohydrates %of faba bean plants grown in sandy soil

Treatments (mg/L)		TSS %	Polysaccharides	Free amino acids	Proline	Total Carbohydrate %
Control		2.37	17.49	233.42	38.78	19.86
Pyridoxine	50	2.11	20.45	244.60	42.99	22.55
	100	2.01	21.93	254.28	52.12	23.94
	150	1.85	19.74	244.85	45.82	21.59
Thiamine	100	2.47	20.47	252.34	46.75	22.94
	200	2.53	20.94	262.72	57.89	23.47
	300	2.40	19.75	259.22	52.37	22.15
$LSD_{0.05}$	0.06	0.96	3.54	0.44	0.97	
$LSD_{0.01}$	0.08	1.34	4.97	0.61	1.37	

Table 4: Effect of different concentrations of pyridoxine and thiamine (mg/L) on yield, yield components, carbohydratesand protein % of faba bean produced seeds. (Combined analysis of two seasons)

Treatment (mg/L)		Plant height (g)	Number of pods /plant	Seeds no./pod	Pods yield/ plant (g)	Seed yield/ plant (g)	100 seeds weight (g)	Seed yield/fed (kg)	Carbo. %	Protein %
Control		58.35	9.33	4.00	52.46	36.74	87.38	404.06	46.85	20.52
Pyridoxine	50	61.52	11.33	4.67	66.27	50.80	103.19	613.99	48.86	21.50
	100	64.35	15.00	5.33	68.91	54.09	108.21	653.76	50.42	22.35
	150	61.52	14.33	5.67	63.67	53.42	101.06	524.79	49.80	21.52
Thiamine	100	62.35	15.00	5.67	64.40	45.09	84.47	544.98	48.55	22.18
	200	65.65	13.67	5.00	73.87	62.20	101.13	751.78	49.94	23.10
	300	64.08	13.67	4.67	67.37	52.19	100.00	509.93	48.94	22.79
$LSD_{0.05}$		8.68	2.50	0.75	10.71	17.66	1.74	56.65	0.25	0.31
$LSD_{0.01}$		11.68	3.51	1.06	13.71	24.77	2.44	79.85	0.35	0.44

faba bean plants grown in sandy soil. Treating plants with 200 mg/L thiamine was the most effective treatment as it caused the highest increases in plants free amino acids content (262 mg/100 g) and proline content (57.89 mg/100 g) followed by 100 mg/L thiamine treatment which recorded 254.28 and 52.12 mg/100 g for both parameters, respectively.

Changes in Yield and its Components: Data in Table (4) shows the effect of different concentrations of pyridoxine (50, 100 and 150 mg/L) and thiamine(100, 200 and 300 mg/L) on the yield and its components number of pods/plant, number of seeds/pod, pods and seed yield/plant, 100-seed weight, seed yield/feddan, carbohydrate and protein%) of faba bean plants grown in sandy soil. Data clearly shows that foliar application of pyridoxine or thiamine with different concentrations increased the entire above mentioned seed yield and its components traits of faba bean under the conditions of this trail. Treatment with thiamine at 200 mg/L followed by pyridoxine with 100 mg/L recorded the highest increases in seed yield/plant and seed yield/feddan. The percentages of increases in seed yield/plant were 69.30% and 47.22% and in seed yield/fed. were 86.06 & 61.80%, respectively in response to 200 mg/L thiamine and 100 mg/L pyridoxine treatments.

DISCUSSION

Data presented in Table (2) shows that different concentrations of pyridoxine and thiamine increased growth traits of faba bean grown in sandy soil. In agreement with the obtained results, Barakat [11] who found that foliar application of pyridoxine on wheat plant strengthened cell differentiation, enhanced root growth and nutrient uptake thus improved photosynthetic surface performance which resulted in improved dry matter production. Similarly, the efficiency of pyridoxine on growth, of *Lupinus termis* and sesame plant were investigated by Boghdady [35] and Nassar *et al.* [36]. With respect to thiamine effect, thiamine is a good antioxidant and have a significant effect on various biochemical metabolic processes affecting plant growth and development. El-Awadi *et al*. [20] and El-Metwally and Sadak [37] stated that thiamine treatment enhanced growth characters of Lupine and faba bean plants, respectively. Thiamine as a vitamin and its major role as a functional coenzyme (thiamine pyrophosphate) has a significant effect on carbon metabolism maintenance. Moreover, these results might be resulted via thiamine effect on improving cell differentiation and endogenous phytohormones [37].

External treatments of various concentrations of either pyridoxine or thiamine caused significant increases in photosynthetic pigments of faba bean plants (Fig. 1). The enhancing role of the used vitamins on photosynthetic pigments could be due to its enhancing role on chlorophyll biosynthesis or decreasing its degradation and thus reflect directly on photosynthetic process. The obtained results of pyridoxine are in good harmony with those obtained by Hamada and Khulaef [38] who noticed that exogenous treatments of bean seedlings with pyridoxine enhanced biosynthesis of photosynthetic pigments fractions and net photosynthetic rate. Moreover, Hendawy and Ezz El-Din [39] confirmed the increased contents of photosynthetic pigments of *Foeniculum vulgare* var. *azoricum* by treatments of vitamins, they referred these increases to the role of vitamin B as co-enzymes in the enzymatic reactions in carbohydrates, fats and protein metabolism and used in respiration and photosynthesis. Regarding to the role of thiamine treatments, the positive role of thiamine on photosynthetic pigments, chlorophyll was stated in many plants because thiamine enhances or assist resynthesize chlorophyll [38, 36] on faba bean and sesame plant respectively. Thiamine treatment is principally concerned with adequate regulation of photosynthesis and energy-providing reactions in treated plants [20]. Thiamine serves as a precursor of thiamine diphosphate, that serves as a Qingming prospective coenzyme in large main metabolic pathways, including plant pigment biosynthesis and carbohydrate metabolism [40].

Using pyridoxine and thiamine as foliar applications enhanced indole acetic acid and phenolic contents of faba bean plant (Fig. 2). In agreement with these obtained data, Aminifard *et al*. [41] who stated that pyridoxine increased phenolic contents of fenugreek plants. Also, Poutaraud *et al.* [42] found that treatment of grapevine plant with thiamine increased the total phenol contents to constitute 97% of total antioxidants, which considered the main class of natural antioxidants present in plants as protective agents against abiotic stress [43].

With respect to the improving role of different treatments of pyridoxine and thiamine on carbohydrates constituents, proline and free amino acids of faba bean plants (Table 3), these obtained data were in agreement with those obtained earlier using pyridoxine [44, 20] and thiamine [45, 46, 41] on different plant species. Using thiamine, Proline synthesis in this system depends upon excessive transcription of the D1-pyrroline-5-carboxylate formation and inhibition of proline dehydrogenase enzyme needed to prevent its breakdown [47]. The valuable function of pyridoxine comes through its role as an important cofactor of various metabolic enzymes as amino acid metabolism [48]. Furthermore, the overproduction of different osmoregulators (soluble sugars, proline and free amino acids) by thiamine application may rise the turgor pressure required for cell differentiation and hence increase plant growth via altering water potential of plant [49]. Thiamine has a significant effect on carbohydrate catabolism also NADPH and ATP biosynthesis [50]. Furthermore, thiamine derivative functions as a cofactor in enzymatic reactions of many metabolic pathways such as glycolysis, Krebs cycle, the pentose phosphate pathway, Calvin cycle, amino acid metabolism, nitrogen assimilation and synthesis of branched chain amino acid, isoprenoid and nucleic acids [51].

Data in (Table 4) clearly shows that foliar application of pyridoxine or thiamine increased the entire yield and its components in addition to carbohydrates and proteins% of seed yield of faba bean plants grown in sandy soil. The effectiveness of pyridoxine on growth, yield, seed quality and anatomy of *Lupinus termis* was investigated by El-Awadi *et al.* [20] and Nassar *et al.* [36]. As mentioned previously, pyridoxine treatment encourages root growth which improves nutrient absorption. Thus enhances plants' vegetative and reproductive growth as well as increased active ingredients quantities leading to higher economic yield [52]. Khoshlahjeh *et al.* [53] confirmed this empowerment role of pyridoxine on *Physali salkekeng* yield. In response to thiamine treatment El-Awadi *et al*. [20] and Aminifard *et al*. [41] found that thiamine treatment enhanced seed yield, oil and protein contents in Lupine and fenugreek plants. Thiamine is necessary for dividing meristematic stem cells and organ initial cells [51].

Pyridoxine and thiamine stimulating effects on carbohydrate contents of the seed yield of faba bean resulted via the increased growth traits and photosynthetic pigments (Table 2 & Fig. 1). Moreover, these increases might be due to the increased photosynthetic output, so increased carbohydrates formation in leaves and thus increased its consequent translocation to seeds. With respect to the present work data, El-Metwally and Sadak [37] found that foliar application of faba bean plants with thiamine increased the content of carbohydrates and proteins in produced seeds.

CONCLUSION

Finally, it could be concluded that pyridoxine or thiamine exogenous treatments had an enhancement effect on growth, some biochemical traits and yield quantity and quality of faba bean plants grown in sandy soil.

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