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Effect of Zinc and Boron foliar application on leaf chemical composition of *Moringa oleifera* and on yield and characters of its seed oil

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Abstract

Extracted oils from plants make up an important part of human life wellbeing all over the world, especially oils containing polyunsaturated acids, such as oleic, linoleic and palmitoleic acids. In Egypt, vegetable oils are produced from multiple sources such as: cotton, soybeans and sunflower. Unfortunately, all these sources do not fulfill consumer requirements. So it is important to find alternate sources of edible oils. Therefore, this study aimed to evaluate the chemical composition of three-year-old *Moringa oleifera* trees and their seed oil characterization as affected using foliar application with zinc (Z) and boron (B). Treatments included two doses of solo-foliar application of Zinc (Zn1 & Zn2) or Boron (B1 & B2) and four combinations between the two doses of Zn and B (Zn1B1, Zn1B2, Zn2B1 and Zn2B2) compared to untreated control. Chemical composition of Moringa oleifera leaves, i.e. N, P, K, Zn, B, Protein (%) and vitamin C including physical and chemical characters of *Moringa oleifera* oil, i.e. oil yield (%), specific gravity, refractive index, acid number, saponification number, ester number and free fatty acids were evaluated. The obtained results reflected that all foliar application treatments improved *Moringa oleifera* chemical composition in leaf samples, not to mention seed oil physical and chemical characters compared to the untreated control.

Keywords: Moringa, Zinc, Boron, Leaves chemical composition, Seeds oil, Oil physical and chemical characters

1. Introduction

Vegetable oils have many important uses in our daily life routines, leading to a predicted increase in the global demand by 33 million tons by 2030 [1]. Locally, Egypt has suffered for a long time from a chronic shortfall in domestic production of vegetable oils, where the producers are unable to fulfill consumers requirements which has led to low self-sufficiency in vegetable oils ratio of up to 14.4% in 2015. Also, self-sufficiency in vegetable oils is decreasing annually by 1.07%. Of course, this situation may be due to a decrease in the planted areas year after year, in addition to the increase in consumption resulting from the increase in population, and also increasing health and nutritional awareness nowadays. So, there is a need for alternate sources of edible oils to augment global production by using untraditional oil crops just like *Moringa oleifera* plants [2]. Moringa - the miracle tree earns its title from being a dietary supplement for both human and animals, and from the importance of its medical benefits in folk medicine [3, 4, 5, 6]. Furthermore, *Moringa oleifera* seeds oil contains from 28 - 42% of a brilliant yellow oil, high in oleic acid and crude oil has a pleasant, like peanut flavor. Moringa oil ranked the first in its quality when compared with other traditional vegetable oils such as: soybean oil, cotton oil, sunflower oil and olive oil [7]. The oil consists of 82% unsaturated fatty acids, 70% of which is oleic acid [8, 9]. Moreover, Moringa oil contains moisture content (0.60%) - ash (1.50%) - crude protein (2.19%) - crude fat (39.3%) and carbohydrate (56.42%). Moringa oil chemical characters include pH 5.96 - saponification value 164.09mg/g - iodine value 68.23g/mol - free fatty acid 8.27 mg KOH/g and specific gravity 0.86 [10]. In addition, Moringa oil contains fatty acids such as: Palmitic acid 6.8% - Oleic acid 70% - Stearic acid 6.5% - Behenic acid 5.8% - Palmitoleic acid 2.9% - Arachidic acid 4.2% and Linoleic acid 0.9% [11].

Zinc and boron are necessary for plant growth and development. Whereas, Zn is the main composition of the ribosome and is essential for their development. It also plays an important role in the production of biomass and physiologic metabolism, hormonal regulation, and membrane structure and function [12]. It is also a co-factor for a variety of enzymes with roles in protein synthesis, membrane stability, cell division and metabolism [13]. Boron

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plays an important physiological role in the formation and structural integrity of the cell wall and contributes to water retention in tissues. It also has a good impact on growth and differentiation of tissues, functioning of plasma membranes, regulation of enzyme activity, metabolism of carbohydrate, nucleic acid, phenol and indole acetic acid, and sugar transport and seed filling, membrane functions and integrity. Boron has a big role in translocation of soluble sugars. Zn in combination with other micronutrients enhanced plant productivity even under stress conditions [14, 15]. Zinc and boron deficiency during the reproductive phase lead to poor seed setting [16].

Accordingly, the aim of this research study was to investigate the effect of foliar application treatments of zink and boron fertilizers in different doses levels applied to Moringa plants, on some chemical ingredients in leaves and also their effect on seed oil yield including seeds oil physical and chemical properties.

2. Materials and Methods:

The field experiments were carried out at a farm in the newly reclaimed land of Belbeis desert, Sharkia governorate, North-east of Egypt on three-year-old Moringa trees during the two successive seasons of 2019 and 2020. *Moringa oleifera* plants were subjected to foliar application treatments with zink and boron fertilizers, as follows:

1- 1. Zn0+B0 (Control).
2. Zn0+B1 (Foliar applications of 0.2% B (2g/l)).
3. Zn0+B2 (Foliar applications of 0.4% B (4g/l)).
4. Zn1+B0 (Foliar applications of 0.2% Zn (2g/l)).
5. Zn2+B0 (Foliar applications of 0.4% Zn (4g/l)).
6. Zn1+B1 (Foliar applications of 0.2% Zn combined with 0.2% B).
7. Zn1+B2 (Foliar applications of 0.2% Zn combined with 0.4% B)
8. Zn2+B1 (Foliar applications of 0.4% Zn combined with 0.2% B)
9. Zn2+B2 (Foliar applications of 0.4% Zn combined with 0.4% B).

Moringa oleifera plants initially cultivated in the open field in sandy soil were treated with the above nine foliar applications (100 ml/ plant) three times in April, May and June during 2019 and 2020. Zinc sulphate (36% Zn) was used as a Zn source and Boric acid (17% B) was used as a B source for foliar application treatments. In addition, a basic fertilization regime was implemented with 200 kg/fed Ammonium nitrate, 200 kg/fed Superphosphate and 100 kg/fed Potassium sulfate. Each soil plot area contained 20 Moringa plants in the ridge (10 m long and 1 m wide) at 50 cm apart between each two plants on the ridge.

2.1 Chemical characters measured in leaves:

1. Total Nitrogen (%) was determined using Kjeldahl Gerhard vapodest 20s according to [17].
2. Phosphorus (%) was determined using UV/VIS Spectrophotometer according to [17].
3. Potassium (%) was determined by the flame Photometer pfp7 according to [17], and the results were calculated using a standard curve of potassium chloride and Calcium Chloride.
4. Zinc amount (ppm) and Boron amount (ppm) were determined using an Atomic Absorption Spectrophotometer according to [18], and the results were calculated using a calibration curve prepared from our data.
5. Protein (%) = Nitrogen amount × 6.25.
6. Vitamin C (mg/100 D.W) was determined as mg/kg dry weight of leaves using dye 2, 4, 6-di-chlorophenolindophenol method as described in [19].

2.3 Physical and chemical characters in seed oil:

1. Oil yield (%) was estimated using thirty grams of Moringa seeds extracted with petroleum ether (40-60°C) using a soxhlet for 6h and the petroleum ether was then evaporated using a rotary evaporator apparatus as stated in the [20].
2. Specific Gravity was determined according to [21].
3. Refractive Index was determined using ATAGO hand refractometer model N-1E Brix 0- 32% according to [21].
4. Acid Number was determined according to the procedure stated by [21].
5. Saponification Number was determined according to [21].
6. Ester Number = (Saponification number – Acid number).
7. Free Fatty Acids (FFA**)** = Acid number × 0.503.

2.4 Experimental design and statistical analysis:

The experiments were carried out in a randomized complete block design with three replicates and the results of treatments were subjected to analysis of variance. The mean values were compared using LSD test at $P < 5\%$ according to [22].

3. Results and Discussions

3.1 Effects on leaf chemical composition

The data pertaining to the effect of foliar application of Zn and B on the *Moringa oleifera* leaves chemical composition are presented in Table (1). From the result data, it is evident that both micronutrients (Zn and B) had significant positive effects on leaves chemical elements and substances studied. The foliar spraying treatment with highest concentration of Zn and B resulted in the maximum values of total Nitrogen (%), Phosphorus (%), Potassium (K %), Zinc content (ppm), Boron content (ppm), Protein (%) and vitamin C).during both seasons of the study when compared to the untreated control treatment.

Similarly, [23] reported synergistic zinc and boron interactions on growth and accumulation of other nutrients such as N, P, K, and Ca. The foliar application of corn with zinc and boron had an influence principally on Ca concentration in the leaves, while K levels were not affected by zinc and boron supplied. In addition, [24] recommended that application mixture of (Fe + Zn + Mn) at 500 ppm + H_3BO_3 at 500 ppm on olive trees and reported an increase in leaf N, Fe, Zn, Mn and B contents, compared to the control during the two growing seasons. In contrast, leaf P decreased in both experimental seasons while leaf K was not significantly affected by boric acid and chelated mixture alone treatments as compared with the control. Moreover, our results are in agreement with [25], who showed that spraying with zinc sulfate at 1750 ppm + boric acid at 348 ppm gave the highest leaves nutrient concentrations as N, P, K, B, and Zn in walnut trees. Furthermore, [26] found that the most effective treatment was T200 (olive trees sprayed with 200 mg/L Mn, Zn and B) to increase concentrations of (calcium, magnesium, phosphorus and nitrogen, as well as iron) in olive trees (cv. " Chondrolia Chalkidikis "). Chlorophyll concentrations were not influenced by foliar treatment.

It is well known that Zinc helps in starch synthesis and protein biosynthesis [27]**.** Furthermore, zinc and boron supplementation may modulate the uptake and accumulation of other nutrients. Also, the increment of leaves chemical composition might be due to an increase in length and width of leaves since Zn is responsible for cell division and increasing the growth hormone such as indole acetic acid [28].

3.2 Seeds oil yield

Result data in Table (2) showed that all different spraying treatments significantly increased seed oil yield (as a percentage) on dry weight basis than in the control during both seasons studied. Moringa trees sprayed with the highest concentrations (0.4%) of both Zn and B gave the maximum significant seed oil percentage in both seasons. On the other side, the control trees exhibited the minimal significant seed oil percentages in both seasons. Meanwhile, the other remaining treatments were in between in their values of seed oil percentage. (Table 2)

3.3 Seed oil physical and chemical properties

Result data in Table (2) indicated that the treatment with the highest concentration (0.4%) of both Zn and B resulted in the maximum values of specific gravity, acid number, saponification number, ester number and free fatty acids in both seasons when compared to almost all of the remaining treatments. Meanwhile, the refractive index slightly responded to foliar application of Zn and B. The differences among treatments were not enough to be significant in the first season. This due to the fact that the refractive index is consider of a seed oil physical character and these characters are not easily affected by agricultural treatments and environmental factors, unlike seed oil chemical characters.

In a similar manner, [29] found that cotton plants treated with zinc at 57.6 g/fed obtained an increase in seed oil content and oil yield per hectare (38.2 kg oil ha^{-1}). Also, they recorded a significant increase in the oil refractive index, and a significant decrease in unsaponifiable matter, compared with untreated control. While, oil properties (such as Acid number, Saponification number and Iodine values,) were not significantly affected. Whereas, they recorded an increase in total unsaturated fatty acids (by 3.49%) and the ratio between total unsaturated fatty acids (TU), total saturated fatty acids (TS) and TU/TS ratio (by 15.25%). In addition, [30] recorded that olive trees treated with zinc sulfate at 0.25% + boric acid at 0.25% had an increase in oil content from 11.7 to 19.4%. Also, it increased the ratio of unsaturated/saturated fatty acids compared to untreated plants. Also, it gave the highest level of oleic acid (57.2%) and the lowest level of palmitic acid (15.19%) in oil. In the same trend, [31] noticed that 4000 mg L^{-1} Zinc Sulphate + 4000 mg L^{-1} Boric + urea 7500 mg L^{-1} respectively showed the maximum seed oil (32.67 %) from olive fruits. Moreover, [32] on canola reported that the application of boron at 4 mg/l resulted in the highest values of refractive index, specific gravity, saponification number, ester number and iodine number including the lowest value of acid number. Furthermore, [33] presented that the highest canola seed oil was obtained from S + B + Zn treatments. While, minimum seed oil was obtained from control treatments. Also, the maximum of oleic acid (229.6 mg/g^{-1}) and linolenic acid (27.14 mg/g) were obtained from B + Zn + S treatment and maximum of linoleic acid (55.55 mg/g) were obtained from B + Zn treatment. Finally, [34] showed that the highest oil content in sunflower hybrids were realized with the combined application of Zn; 10 kg/ha and B; 2 kg/ha which also produced the higher oil content from stearic acid, palmitic acid and linoleic acid as compared to control.

The increment of oil yield and oil quality might be due to zinc and boron important role on pollination by inducing pollen tube growth resulted from its role on tryptophan synthesis as an auxin biosynthesis precursor. In addition, Zinc has a major role metabolism of RNA and also, in stimulation of carbohydrates. Furthermore, Boron has an important role in sugar transport and hormones development and boron may be required in stigma and styles to physiologically inactivate callus present in pollen tube walls that would otherwise elicit phytoalexin production to inhibit pollen tube growth [30]. In addition, [32] noticed that the slight changes in oil properties may be due to that the oil properties are related to genetics more than the environmental effects during plant growth in the field. Moreover, [35] reported that the genotype is considered as the most important determinant factor in fatty acid composition. However, the interaction of genotype with environmental conditions affected the quality of canola fatty acid compounds.

Table (1): Effect of foliar applications of Zn and B fertilization on leaf chemical composition of *Moringa oleifera* plants in the two seasons 2019 and 2020.

Treatments	N (%)	P (%)	K (%)	Zn (ppm)	B (ppm)	Protein (%)	Vitamin C (mg/100.D.W)
First season (2019)							
Foliar applications:							
control	2.20	0.16	1.51	19.83	28.67	13.77	31.67
B1	2.95	0.17	2.16	21.85	33.84	18.44	39.78
B2	4.00	0.19	2.35	23.23	36.22	25.02	48.89
Zn1	3.58	0.17	2.06	24.20	29.89	22.40	41.45
Zn2	4.29	0.19	2.34	27.39	34.22	26.79	47.11
Zn1B1	3.61	0.16	2.16	23.00	29.39	22.58	35.78
Zn1B2	4.05	0.19	2.37	26.80	40.22	25.31	65.11
Zn2B1	4.41	0.19	2.51	28.96	42.00	27.56	70.11
Zn2B2	4.56	0.21	2.99	30.12	45.33	28.52	76.66
LSD at 5%	*1.37*	*0.04*	*0.65*	*6.76*	*8.31*	*8.56*	*19.54*
Second season (2020)							
Treatments	**N (%)**	**P (%)**	**K (%)**	**Zn (ppm)**	**B (ppm)**	**Protein (%)**	**Vitamin C (mg/100.D.W)**
Foliar applications:							
control	3.84	0.18	1.93	27.17	32.00	24.00	42.27
B1	4.11	0.19	2.27	29.77	45.17	25.71	53.60
B2	5.22	0.23	2.36	31.03	51.11	32.63	65.95
Zn1	3.92	0.20	2.25	30.19	38.33	24.48	54.85
Zn2	5.00	0.25	2.38	31.65	41.89	31.25	70.56
Zn1B1	4.14	0.21	2.13	29.38	39.11	25.88	55.69
Zn1B2	4.73	0.22	2.35	31.77	47.78	29.56	72.11
Zn2B1	5.43	0.23	2.59	33.40	51.78	33.94	78.25
Zn2B2	5.74	0.28	3.03	35.56	53.78	35.85	86.35
LSD at 5%	*1.19*	*0.05*	*0.25*	*2.94*	*7.76*	*7.43*	*14.11*

Table (2): Effect of foliar applications of Zn and B fertilization on seeds oil yield (as percentage) and on physical and chemical characters of *Moringa oleifera* plants in the two seasons 2019 and 2020.

Treatments	Seed oil yield (%)	Specific Gravity	Refractive Index	Acid Number	Saponification Number	Ester Numbe r	Free Fatty Acids
First season (2019)							
Foliar applications:							
control	24.33	0.62	1.49	0.56	145.86	145.3	0.28
B1	27.73	0.66	1.53	0.59	147.30	146.71	0.30
B2	32.54	0.69	1.53	0.40	149.81	149.41	0.20
Zn1	27.04	0.66	1.54	0.84	133.23	132.39	0.42
Zn2	31.79	0.67	1.72	0.59	147.26	146.67	0.29
Zn1B1	28.06	0.66	1.84	0.45	149.18	148.73	0.23
Zn1B2	36.40	0.67	1.76	0.22	153.80	153.58	0.11
Zn2B1	38.85	0.67	1.76	0.22	155.21	154.99	0.11
Zn2B2	40.73	0.68	1.61	0.20	158.17	157.97	0.10
LSD at 5%	*10.7*	*0.002*	*Ns*	*0.20*	*9.64*	12.87	*0.10*
Second season (2020)							
Treatments	**Seed oil yield (%)**	**Specific Gravity**	**Refractive Index**	**Acid Number**	**Saponification Number**	**Ester Number**	**Free Fatty Acids**

Foliar applications							
control	**24.17**	**0.62**	**0.45**	**0.56**	**140.10**	**139.54**	**0.28**
B1	**27.85**	**0.69**	**1.53**	**0.47**	**147.31**	**146.84**	**0.17**
B2	**32.70**	**0.71**	**1.57**	**0.26**	**152.87**	**152.61**	**0.13**
Zn1	**30.62**	**0.66**	**1.26**	**0.37**	**144.29**	**143.92**	**0.16**
Zn2	**32.37**	**0.70**	**1.52**	**0.45**	**151.59**	**151.14**	**0.11**
Zn1B1	**30.12**	**0.69**	**1.50**	**0.46**	**148.26**	**147.8**	**0.21**
Zn1B2	**37.63**	**0.71**	**1.65**	**0.64**	**150.25**	**149.61**	**0.12**
Zn2B1	**40.30**	**0.69**	**1.80**	**0.18**	**158.63**	**158.45**	**0.16**
Zn2B2	**42.99**	**0.71**	**1.87**	**0.56**	**160.66**	**160.1**	**0.16**
LSD at 5%	***7.84***	***0.002***	***0.32***	***0.28***	***10.95***	***22.85***	***0.10***

4. Conclusions

The combined foliar application of both Zn and B at 0.4% concentration of each as $ZnSO_4$ + H_3BO_3 had a profound positive effect on leaves of *Moringa oleifera* chemical composition in addition to an increase in seed oil yield (as a percentage) and it also improved seed oil physical and chemical characters.

5. Conflicts of interest

There are no conflicts to declare.

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