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EFFECT OF BRASSINOLIDE AND MORINGA LEAF EXTRACT FOLIAR APPLICATION ON GROWTH AND MINERAL CONTENT OF LOCAL LEMON TRANSPLANTS

L.H.A. AL-KARBOLI* and A.M.I. AL-JANABI

Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Ramadi, Iraq
*Corresponding author's email: lou21g5005@uoanbar.edu.iq
Email address of co-author: ag.atheer.mohammed@uoanbar.edu.iq

SUMMARY

The prevailing study aimed to assess the brassinolide, and moringa leaf extracts' foliar application influence on the growth and mineral content of local lemon transplants, commencing in 2022 in a wooden lath house affiliated with the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Ramadi, Iraq. The experiment's study factors comprised foliar application of growth regulator brassinolide (B0, B1.0, B1.5, and B2.0 mg L^{-1}) and moringa leaf extracts (M0%, M2%, and M4%) on local lemon. Study elements influenced all characteristics, especially foliar spraying of brassinolide (B1.5 mg L^{-1}), significantly enhancing the number of secondary shoots increment and leaves, leaf area, and leaves' nitrogen, phosphorus, potassium, and total chlorophyll content. The growth regulator brassinolide (B1.0 mg L^{-1}) revealed considerable secondary shoot length increment superiority. On spraying of moringa leaf extracts, moringa extract (M4%) achieved a substantial superiority in all the studied traits.

Keywords: Lemon (*Citrus limon* Burmann), brassinosteroids, moringa leaf extracts, foliar application, growth traits, mineral content

Key findings: The lemon vegetative growth and biochemical content traits bore improvement and significant increases, especially by foliar application made with the brassinolide and moringa leaf extracts at the concentrations of 1.5 mg L^{-1} and 4%, sequentially.

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INTRODUCTION

Lemon (*Citrus limon* Burmann) belongs to the Rutaceae family and has the notion to originate from the northeastern regions of India and southwestern regions of China. A belief also states it has evolved through bud mutation from citron (*Citrus medica* Linnaeus). Its occurrence has not resulted in growing wild in any region of the world. Lemon thrives well in

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semi-tropical areas but is more sensitive to cold temperatures (Saunt, 2000). With its lower acidity, many in Iraq favored the local cultivar of lemon compared with other global cultivars. It is also juicier, with oval-shaped fruits and a medium-thick, smooth peel.

At maturity, it looks light yellow, and an early-maturing cultivar, harvest starts in September and October, with the harvesting season extending until the spring months (Al-Khafaji *et al*., 1990). Lemon fruits are an essential nutritional source of various vitamins, especially vitamins C, A, B1, B2, and B3, as well as specific amino acids, organic and phenolic acids, minerals, carbohydrates, essential oils, and antioxidants like flavonoids (Ledesma-Escobar *et al*., 2015; Klimek-Szczykutowicz *et a*l., 2020).

Citrus cultivation in Iraq faces various challenges in general, including neglect of present orchards and lower productivity of the lemon trees compared with their adjoining countries. Additionally, ensuring suitable and high-quality lemon transplants for cultivation and slow growth in nurseries and post-transplantation establishments are the critical obstacles hindering the citrus cultivation expansion. However, various technical methods to enhance growth, such as foliar application of plant growth regulators, are crucial and fundamental in plant growth and development (Davies, 2004). Brassinosteroids (BRs) rank in the sixth group of plant hormones due to substantial evidence of their physiological effects, and in most cases, they have demonstrated effects similar to auxins, gibberellins, and cytokinins (Davies, 2004). The term 'Brassinosteroids' came from the rape plant *Brassica napus* L., where this hormones' extraction from their pollen grains (Rao *et al.,* 2002).

Past studies have enunciated that physiological effectiveness emerged from stimulating cell elongation and division, biosynthesis of nucleic acids, proteins, and cell wall components, and vascular system differentiation, enhancing photosynthetic efficiency, transport, distribution, and assimilation of manufactured nutrients and minerals uptake, postponing aging, regulating gene expression, and other functions (Haubrick and Assmann, 2006; Hayat and Ahmed, 2011; Li and He, 2020). Numerous studies have confirmed the positive effects of brassinolide spraying on boosting growth traits of various fruit saplings, including olive (Al-Khattab, 2017; Al-Dulaimi and Al-Janabi, 2021), Washington navel orange and blood orange (Al-Ahbaby and Al-Ani, 2021), and mandarin transplants (Marak *et al*., 2021).

In the present era, the extensive use of plant extracts has existed as an alternative or supplement to chemical fertilizers and growth regulators. These plant extracts can improve and enhance the growth and productivity of various plant species. In these extracts, the moringa (*Moringa oleifera* Lam.) leaf extract has considerable environment-friendly biostimulants that sizably contribute to numerous aspects of growth and development and is also a rich source of vitamins. Moringa leaf extracts also contain high levels of carbohydrates, proteins, and minerals, along with amino acids, organic acids, steroids, and natural antioxidants, such as flavonoids, ascorbates, and phenols, and some plant hormones, such as zeatin (El-Sohaimy *et al*., 2015; Udofia *et al*., 2020; Zulfiqar *et al*., 2020). Numerous studies have highlighted the importance of moringa leaf extract foliar spraying to enhance many fruit trees' vegetative growth and chemical content. Past studies revealed improvement in growth traits and biochemical composition of citrus rootstock species (Mohamed *et al.*, 2017), Washington navel orange and tangor transplants (Abd-Al-Rhman *et al*., 2018; Al-Sabbagh *et al.*, 2020), and date palm trees (Aubied *et al*., 2023).

MATERIALS AND METHODS

The presented study happened in a wooden lath house at the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Ramadi, Iraq. The study ran from April 2022 to December 2022 on two-and-a-half-year-old transplants of a local lemon cultivar, budded onto sour orange rootstock (*Citrus aurantium* L.) and planted in plastic containers with a capacity of eight kilograms. A selection totaled 108

Table 1. The experimental soil's chemical and physical properties.

Physical properties (g kg^{-1} soil)				
Sand	Silt	Clay	Texture	
637.3	158.5	204.4	Sandy loam	
Chemical properties				
pH	EC (1:1) ds m^{-1}	N (mg kg^{-1} soil)	P (mg kg^{-1} soil)	K (mg kg^{-1} soil)
7.1	1.85	69.7	17.1	187.8

Table 2. Chemical composition of moringa leaf extracts.

Seq.	Vitamins (mg 100 g^{-1} leaf powder)		Seq.		
1	Vit. A (*B*-carotene)	16.3	6	Phenylalanine	27.14
2	Vit. B1 (thiamine)	2.64	7	Tyrosine	18.88
3	Vit. B2 (riboflavin)	20.5	8	Valine	27.58
4	Vit. B3 (nicotinic acid)	8.20	9	Histidine	13.57
5	Vit. C (ascorbic acid)	17.3	10	Threonine	21.97
6	Vit. E (tochopherol acetate)	113	11	Serine	20.79
Seq.	Minerals (mg 100 g^{-1} leaf powder)		12	Glutamic acid	50.58
1	Phosphorus (P)	204	13	Aspartic acid	46.11
2	Potassium (K)	1.324	14	Proline	25.75
3	Magnesium (Mg)	368	15	Glycine	26.62
4	Calcium (Ca)	2.003	16	Alanine	28.67
5	Iron (Fe)	28.2	17	Arginine	30.28
6	Zinc (Zn)	2.5	18	Tryptophan	9.26
7	Copper (Cu)	0.57	Seq.	Other compounds	
8	Sulfur (S)	870	1	Protein (g 100g^{-1} leaf powder)	27.1
Seq.	Amino acids (g kg^{-1} leaf powder)		2	Carbohydrates (g 100g^{-1} L. P.)	38.2
1	Lysine	28.77	3	Fats (g 100g^{-1} leaf powder)	2.3
2	Leucine	42.89	4	Fiber (g 100g^{-1} leaf powder)	19.2
3	Isoleucine	22.53	5	Calories (Cal)	205
4	Methionine	8.96	6	Moisture (%)	7.5
5	Cystine	5.18	7	Phenols (g 100g^{-1} leaf powder)	154.3
			8	Zeatin (*m*cg g^{-1} F. W.)	213.6

[a] Amino acids (Makkar and Becker, 1996)
[b] Zeatin **+** Phenols (Fuglie, 2000)
[c] Vitamins **+** Minerals **+** Other compounds **(**Uphadek *et al.*, 2018)

uniform transplants that were as homogeneous in their growth as possible, and all maintenance operations proceeded as necessary. Plastic shading nets during May, June, July, August, and September covered the saplings to protect them from expected heat and extreme sunlight. Soil samples were collected for chemical and physical analyses before experimenting transpired (Table 1).

Study factors

The experiment included the foliar application of growth regulator 'Brassinolide,' a product from New Sunshine "Xiangtan" Agrochemical Co., Ltd., China, at the concentrations of B0, B1.0, B1.5, and B2.0 mg L^{-1} and moringa leaf extracts at the concentrations of M0%, M2%, and M4%. Moringa leaf extract preparation followed the formula according to the method described by Makkar and Becker (1996). Preparing the moringa concentrations of 2% and 4% comprised the following: taking 20 and 40 ml of the extract sequentially and diluting with one liter of distilled water for each concentration. Some components of the moringa leaf extracts are available in Table 2. The lemon transplants' spraying with brassinolide and moringa leaf extracts had the following timings, i.e., every first day of April, May, September, and October 2022.

Studied traits

The number and length of secondary shoots of lemon transplants were the traits measured before the treatments' implementation (25/3), and measured them again at the end of the experiment (25/11) to calculate the Increment in the number and lengths of secondary shoots**.** As for the rest of the studied traits, their measurements occurred at the end of the experiment, including leaves number and leaf area (Chou, 1966), percentage of nitrogen, phosphorus, and potassium content in the leaves **(**Bahargava and Raghupathi, 1999), and the total chlorophyll content in leaves (Gogoi and Basumatary, 2018).

Statistical analysis

A factorial experiment with two factors proceeded with a randomized complete block design (RCBD) (Al-Mohammedi and Al-Mohammedi, 2012). The experiment included three replications per treatment and three saplings per experimental unit. Data analysis used the statistical software GenStat, with means compared and separated using the Least Significant Difference (LSD) test at the 5% probability level.

RESULTS AND DISCUSSION

Growth traits

The results indicated that foliar application of the brassinolide significantly affected all the vegetative growth traits (Table 3). In particular, the brassinolide treatment of B1.5 mg L^{-1} notably outperformed the other treatments, showing the highest mean values for the average increase in the number of

Table 3. Effect of spraying of brassinolide and moringa leaf extracts and their interaction on number and length of secondary shoots, leaves number, and leaf area.

Treatments	Increment in secondary shoots number (shoot. transplant^{-1})	Increment in secondary shoots lengths (cm)	Leaves number (leaf. transplant^{-1})	Leaves area (dm^2)
Brassinolide (B) (mg L^{-1})				
B_0	4.33	16.34	152.3	38.28
$B_{1.0}$	5.66	21.84	180.6	46.85
$B_{1.5}$	7.72	20.95	229.7	62.83
$B_{2.0}$	5.72	20.87	193.2	50.82
$LSD_{0.05}$	0.93	1.95	9.77	1.81
Moringa leaves extract (M) (%)				
M_0	4.29	16.55	158.9	40.41
M_2	5.87	20.72	193.5	50.91
M_4	7.41	22.73	214.4	57.76
$LSD_{0.05}$	0.80	1.69	8.46	1.57
Brassinolide x Moringa leaves extract				
B_0M_0	2.66	9.41	116.7	28.28
B_0M_2	4.33	18.34	167.0	41.99
B_0M_4	6.00	21.27	173.2	44.56
$B_{1.0}M_0$	4.00	19.73	153.2	38.50
$B_{1.0}M_2$	5.66	22.61	182.0	47.19
$B_{1.0}M_4$	7.33	23.18	206.7	54.85
$B_{1.5}M_0$	6.17	18.22	197.3	52.29
$B_{1.5}M_2$	7.66	21.12	234.5	63.99
$B_{1.5}M_4$	9.33	23.52	257.2	72.22
$B_{2.0}M_0$	4.33	18.85	168.5	42.58
$B_{2.0}M_2$	5.83	20.82	190.7	50.47
$B_{2.0}M_4$	7.00	22.93	220.5	59.42
$LSD_{0.05}$	1.61	3.38	16.92	3.15

secondary shoots (7.72 shoots transplant^{-1}), leaf number (229.7 leaves transplant^{-1}), and leaf area (62.83 dm²). However, the treatment of brassinolide (B0 mg L^{-1}) gave the lowest values for these traits, i.e., 4.33 shoots transplant $^{-1}$, 152.3 leaves transplant $^{-1}$, and 38.28 dm², sequentially. For the increase in secondary shoot length, brassinolide treatment of B1.0 mg L^{-1} did not significantly differ from the other two treatments, i.e., B1.5 and B2.0 mg L^{-1}, exhibiting the highest value (21.84 cm), and was considerably superior to the treatment B0 mg L^{-1}, which recorded with the lowest value (16.34 cm).

Foliar application of moringa leaf extracts also demonstrated a significant rise in the number and length of secondary shoots, the number of leaves, and the leaf area of the lemon transplants (Fatonah *et al*., 2018; Tahir *et al*., 2023). The rise in growth traits was evident with a higher concentration of moringa (M4%), which achieved the highest values, i.e., 7.41 shoots transplant $^{-1}$, 22.73 cm, 214.4 leaves transplant $^{-1}$, and 57.76 dm² leaf area. Conversely, the lowest values for said traits were visible in the control treatment of moringa (M0%), i.e., 4.29 shoots transplant $^{-1}$, 16.55 cm, 158.9 leaves transplant $^{-1}$, and 40.41 dm².

The interaction between both study factors had a significant effect on all growth traits of transplants, especially the combination treatment of B1.5 mg L^{-1} + M4%, which achieved the highest values of 9.33 shoots transplant $^{-1}$, 23.52 cm, 257.2 leaves transplant $^{-1}$, and 72.22 dm² for the number and length of secondary shoots increment, the number of leaves per transplant, and leaf area, respectively. However, the interaction of both control treatments (B0 mg L^{-1} + M0%) exhibited these characteristics' lowest values, i.e., 2.66 shoots transplant^{-1}, 9.41 cm, 116.7 leaves transplant^{-1}, and 28.28 dm² leaf area.

The rise in the number and length of secondary shoots of lemon transplants as a consequence of foliar spray of brassinolide may refer to its role in stimulating the various processes responsible for cell elongation and division, including enhanced efficiency and activity of several enzymes (DNA, RNA polymerase, and ATPase) responsible for nucleic acids and protein synthesis (Haubrick and Assmann, 2006). Additionally, brassinolide contributes to the cell wall components formation and regulation of gene expression for various genes, some of which are vital to plant growth (Kauschmann *et al.,* 1996). Furthermore, the increase in the number and lengths of secondary shoots could also be due to the interaction of brassinolide with other endogenous hormones, including synergistic responses with auxins and complementary effects with gibberellins and cytokinins (Vert *et al.,* 2008). These results were consistent with the findings of Hussein (2018), who also observed a notable rise in the number and length of shoots in olive saplings due to foliar application of brassinolide. Marak *et al*. (2021) showed a marked rise in the number and length of shoots in mandarin saplings with brassinolide foliar spray.

The increase in leaf number and leaf area results from the treatment of brassinolide could depend on its role in stimulating the growth of leaf primordia (Sasse, 2003). Moreover, it could be because of its role in regulating the transfer and assimilation of products of photosynthesis in plant organs and the stimulation of mineral elements absorption and utilization in building overall green mass (Hayat and Ahmed, 2011). These results agreed with the findings of Al-Khattab (2017), who observed a significant rise in leaves area in olive transplants by foliar spray with brassinolide. Al-Swaidawi (2019) reported that foliar application of brassinolide on local sweet orange transplants remarkably increased the leaf number and there area.

Moringa extracts enhanced growth traits due to growth-promoting substances (Table 2), such as vitamins crucial in carbohydrate metabolism, amino acids, and other biochemical processes. The moringa extract also contains substantial amounts of proteins and carbohydrates that provide the necessary energy for the growth and development of plants (Taiz and Zeiger, 2010). Moreover, the said extract contains essential nutrients for vital plant activities like photosynthesis, respiration, carbohydrate metabolism, enzymatic activation, and various metabolic processes, which significantly contribute to cell division and elongation, with these processes positively correlating to the characteristics of vegetative growth (Marschner, 2012). Similarly, the moringa extract contains amino acids that provide a ready-made formula for the plant, from the building blocks of proteins to its role in building nucleic acids (Hildebrandt *et al*., 2015).

Furthermore, amino acids contribute to enhancing the efficiency of photosynthesis and chlorophyll synthesis (D'Mello, 2015). In addition, amino acids, especially tryptophan, phenylalanine, and arginine, are vital in the biosynthesis of various plant hormones, such as auxins, cytokinins, and polyamines. These hormones have broad and essential roles in the plant's growth and development (Singh, 1999; Davies, 2004). Considering these multifaceted functions and physiological effects of the components found in moringa leaf extracts, an expected increase of the lemon transplants by the biological activity is evident, reflected in improved growth traits, particularly the number and length of secondary shoots, number of leaves and there area (Table 3). These results also aligned with the findings of Al-Sabbagh *et al.* (2020), who observed a notable increase in the shoots number, leaf number, and leaves area of Washington navel orange by foliar spray with moringa extracts. Al-Abed-Allah (2022) also reported a weighty increase in the shoots and leaves number, and leaves area in Mexican lime transplants treated with moringa extracts.

Biochemical traits

The results showed that the content of nitrogen, phosphorus, potassium, and total chlorophyll in the lemon transplant leaves has significantly enhanced with the foliar application of the brassinolide, especially with the concentration B1.5 mg L^{-1} achieving a significant supremacy with the highest values of nitrogen, phosphorus, potassium, and total chlorophyll, i.e., 2.53%, 0.28%, 1.69%, and 1.37 mg g^{-1} fresh weight compared with the treatment of brassinolide B0 mg L^{-1}, recording with the lowest values of 2.41%, 0.21%, 1.40%, and 1.22 mg g^{-1} fresh weight in the leaves of lemon transplants (Table 4).

The outcome further revealed notable differences in the mineral content (NPK) and total chlorophyll in the leaves of lemon saplings with the spraying of moringa extracts. The moringa treatment (M4%) significantly outperformed the other concentrations, with the highest percentages of N, P, and K at 2.51%, 0.28%, and 1.68%, and total chlorophyll content (1.35 mg g^{-1} fresh weight), with the treatment (M0%) emerging with the lowest scores of 2.41%, 0.21%, 1.40%, and 1.23 mg g^{-1} fresh weight for these mineral characteristics and total chlorophyll content.

The results also indicated the significance of the interaction between brassinolide and moringa leaf extracts. The combination of B1.5 mg L^{-1} + M4% achieved the maximum values of 2.59%, 1.88%, and 1.45 mg g^{-1} fresh weight for nitrogen, potassium, and total chlorophyll in the lemon transplant leaves sequentially. However, control treatments of both factors (B0 mg L-1 + M0%) showed the lowest values in the above traits, i.e., 2.35%, 1.26%, and 1.15 mg g^{-1} fresh weight, sequentially. The interaction amongst both study factors had no significant effect on leaves content of phosphorus. The enhancement of nitrogen, phosphorus, and potassium possibly due to the stimulative influences of brassinolide in raising the uptake of minerals (Hayat and Ahmed, 2011). The increase in the percentage of mineral elements due to foliar spraying of moringa leaf extracts could be because of the nutritional elements in the moringa directly absorbed by the host plant (Table 2).

The increase in the leaf's total chlorophyll content due to the brassinolide treatment may refer to its role in inhibiting the activity of the enzyme chlorophyllase, which is responsible for the degradation of this pigment (Fariduddin *et al.,* 2003). Regarding the effect of moringa extracts in increasing the chlorophyll content, it can depend on the nutritional elements it contains, including magnesium involved in the structure of the chlorophyll molecule, iron, which aids in its formation, and copper, which stabilizes and prevents its degradation (Marschner, 2012). Moreover, it contains glutamic acid and glycine, also essential compounds contributing to the biosynthesis of this green pigment (D'Mello, 2015). These results were analogous to past findings reporting a sizable increase in the leaf's nitrogen, phosphorus, and potassium with the foliar application of brassinolide in citrus transplants (Al-Ahbaby and Al-Ani, 2021). Mohamed *et al.* (2017) also concluded a noteworthy increase in the leaf content of phosphorus, potassium, and total chlorophyll in four species of citrus rootstocks by the foliar application of moringa leaf extracts.

Table 4. Effect of spraying of brassinolide and moringa leaf extracts and their interaction on percentage of nitrogen, phosphorus, and potassium and total chlorophyll content in leaves.

Treatments	Leaves nitrogen content (%)	Leaves phosphorus content (%)	Leaves potassium content (%)	Total chlorophyll content in leaves (mg g^{-1} F.W.)
Brassinolide (B) (mg L^{-1})				
B_0	2.41	0.21	1.40	1.22
$B_{1.0}$	2.45	0.23	1.50	1.27
$B_{1.5}$	2.53	0.28	1.69	1.37
$B_{2.0}$	2.46	0.25	1.57	1.29
$LSD_{0.05}$	0.04	0.04	0.06	0.08
Moringa leaves extract (M) (%)				
M_0	2.41	0.21	1.40	1.23
M_2	2.46	0.24	1.54	1.29
M_4	2.51	0.28	1.68	1.35
$LSD_{0.05}$	0.03	0.03	0.05	0.07
Brassinolide x Moringa leaves extract				
B_0M_0	2.35	0.18	1.26	1.15
B_0M_2	2.42	0.22	1.39	1.23
B_0M_4	2.47	0.25	1.56	1.29
$B_{1.0}M_0$	2.39	0.20	1.37	1.21
$B_{1.0}M_2$	2.45	0.23	1.49	1.27
$B_{1.0}M_4$	2.51	0.27	1.65	1.34
$B_{1.5}M_0$	2.48	0.24	1.51	1.30
$B_{1.5}M_2$	2.53	0.28	1.68	1.37
$B_{1.5}M_4$	2.59	0.32	1.88	1.45
$B_{2.0}M_0$	2.43	0.23	1.46	1.26
$B_{2.0}M_2$	2.46	0.26	1.61	1.30
$B_{2.0}M_4$	2.50	0.28	1.66	1.33
$LSD_{0.05}$	0.06	N.S.	0.10	0.14

CONCLUSIONS

The promising research findings demonstrated that the interaction of foliar application with brassinolide (B1.5 mg L^{-1}) and moringa leaf extracts (M4%) positively affected local lemon saplings' growth and biochemical traits significantly. These results also provide valuable insights into potential strategies for enhancing lemon saplings' growth and nutrient content, which could impact citrus cultivation and production practices.

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