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Pivotal Impact of Some Bio-stimulants on Morphophysiological and Yield Characters of *Triticum aestivum* L.

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ABSTRACT

Two field trials were carried out during 2023/24 and 2024/25 growing seasons to evaluate the impact of some bio-stimulants as a foliar application on the physiological, morphological and quality traits of wheat cultivar Misr 3. These bio-stimulant treatments were (control, Cina green plus, Lithovit with boron, and with nitrogen). Foliar application showed significant changes in the studied characteristics of wheat. The Cina green plus recorded the maximum significant values of all studied characteristics and didn't significantly differ from Lithovit with boron in the characters (spike length and grain yield). The application of Lithovit with nitrogen didn't differ substantially from the Cina green plus treatment, especially in terms of shoot length, number of spikes m^{-2}, and 1000-kernel weight (g). The control plants (unfertilized) recorded the minimum values at all studied traits. Plants that grew in the second season achieved the best values of all characteristics, except the plants that grew in the first season recorded the maximum significant values of shoot length (cm). Based on this investigation, it is recommended that the application of Cina green and Lithovit with nitrogen can improve the growth and productivity of the Misr 3 wheat cultivar.

Keywords: wheat, biostimulants, Cina green, agronomic characters, yield production.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most vital crops, and it is widely grown throughout Egypt (Alhusnain et al., 2025; Alomran et al., 2025; Elsherbini et al., 2025). The grain has also become a staple food in more than 40 countries worldwide. As a source of calories and proteins, wheat provides 82% of the world's population (Sharma et al., 2019). Wheat is a staple food for about one-third of the world's population due to its great nutritional content and usefulness (Giraldo et al., 2019). In Egypt, the need for wheat production is increasing due to the steady population increase. Many studies have been conducted to increase wheat grain yield using growth-promoting compounds and biostimulants under both normal and stressful conditions (Mosalem et al., 2021, 2023; Galal et al., 2023; Khedr et al., 2023; Abdelaal et al., 2024; Alafari et al., 2024; Al-Shammari et al., 2025).

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In wheat improvement efforts, a key goal for breeders is the development of more resilient varieties that can deliver higher yields while requiring fewer inputs, such as water and fertilizers, and exhibiting greater resistance to various stress factors (Giraldo et al., 2019; Hafez et al., 2022; Omara et al., 2023). In 2016, the Wheat Research Department, FCRI, ARC, Egypt, introduced Misr 3, a promising bread wheat cultivar that enables wheat growers a new resource for a high-yielding, rust-resistant genotype (Abdel-Majeed, 2018). In addition, define the optimum package of cultural practices for wheat; especially, fertilization is important to obtain the highest yield potential. Biostimulants, natural compounds capable of promoting plant growth and development even in minimal quantities, have been explored extensively for their potential advantages across various crops (El Sabagh et al., 2017; Elwaziri et al., 2023; Alharbi et al., 2025). Further research should be conducted to evaluate the efficacy of bio-stimulants in cereal crop production. Lithovit is a type of bio stimulant, which is a finely milled limestone composed mainly of (Ca, Mg)-CO_3 as well as various micronutrients that are essential for plant physiology (Patent DE202006011165 U1). The use of Lithovit led to improve growth characters and yield, furthermore, Lithovit has been found to increase CO_2 levels in leaf intercellular spaces, thereby improving photosynthesis (Gharib et al., 2025). Moreover, it has been demonstrated to enhance chlorophyll concentration, and dry matter of tomato plants under salinity conditions (Sajyan et al., 2019). This study aimed to evaluate the impact of Lithovit and Cina green plus as biostimulants on agronomic, physiological, and quality characters in the spring bread wheat cultivar Misr 3.

MATERIAL AND METHODS

Tow field trials were conducted at Agricultural Faculty Farm, Kafrelsheikh University (2023/24 and 2024/25 seasons) to evaluate the impact of some bio-stimulants as a foliar application on physiological, morphological, and quality traits of wheat cultivar Misr 3. These bio-stimulant treatments were (control, Cina green plus, Lithovit with boron, and Lithovit with nitrogen). The location is at 31°07' N latitude, 30°57' E longitude, and around 6 m above mean sea level. The experiment was performed by using RCBD with three replications. The plot area was 4.2 m^2; including six rows 3.5 m long and 20 cm apart. Sowing was carried out on 25th and 27th November in 2023/24 and 2024/25, respectively. The bread wheat cultivar Misr 3 was used, in addition, four foliar spray treatments were applied (control, Cina green plus, Lithovit with boron, and Lithovit with nitrogen) with a concentration of 1.5 g liter^{-1} at 40, 50, and 60 days after sowing. All cultural practices were conducted according to the recommended doses, the previous crop was maize in the first season and rice in the second season, respectively. The following data were recorded:

Plant height (cm), no. of spikes m^{-2}, spike length (cm), no. of spikelets spike^{-1}, 1000-kernel weight (g), biological and grain yield m^{-2} (g), flag leaf area (cm^{-2}), grain protein content % (AOAC, 1990), wet gluten content %: (AACC, 10-38, Anonymous 1983), shoot and root length (cm) and germination %. The analysis of variance was performed according to the RCBD design. Combined analysis across the two seasons was performed when the assumption of error homogeneity cannot be rejected (Levene, 1960). Statistical analyses were done using the statistical routines available in Microsoft EXCEL (2016) and GenStat 18 (Payne et al., 2017).

RESULTS AND DISCUSSION

Data presented in Tables 1 and 2 showed that the mean values of all characters under the foliar spray treatments were similar across both seasons. Regarding the impact of the two growing seasons, there were significant differences for all studied characters. While the impact of foliar treatments had a highly significant impact on all characters except for germination %, shoot length (cm), root length (cm), spikes number m^{-2}, and 1000-kernel weight (g). Data showed that plants grown in the second season achieved the best values for flag leaf area (cm^2), grain protein content (%), wet gluten content, germination %, root length (cm), plant height (cm), No. of spikes m^{-2}, spike length (cm), No. of spikelets/spike, 1000-kernel weight (g), biological yield, and grain yield. The results in the first season recorded the highest significant values of shoot length (cm). The significant effect of seasons indicated that these sources of variation had sufficient variability. The increased values of most studied characters in the 2023/24 season were due to the decreased temperature and higher relative humidity than in the second season. The foliar application had a significant impact on grain protein (%), wet gluten, germination %, shoot length (cm), root length (cm), spikes number m^{-2}, spike length (cm), No. of spikelets/spike, 1000-kernel weight (g), biological and grain yield.

Plants treated with Cina green plus recorded the highest values for most parameters and did not differ significantly from Lithovit combined with boron in terms of spike length and grain yield. Similarly, Lithovit combined with nitrogen did not differ significantly from Cina green plus in plant height, number of spikes m^{-2}, spike length, and 1000-kernel weight.

The untreated control consistently showed the lowest performance across all evaluated traits, confirming the positive effect of foliar biostimulant application.

Overall, nanostructured fertilizers such as nano-carriers, nano-capsules, and nano-nutrients showed a significantly positive effect on wheat growth and productivity. These “smart fertilizers” improve nutrient use efficiency, regulate nutrient release, and contribute to reduced environmental pollution. In particular, Lithovit (a nano-calcium carbonate formulation) improved growth, physiological traits, and yield components, especially when applied as a foliar spray under both normal and stress conditions.

Among treatments, nano-nitrogen and Lithovit-based formulations enhanced key yield attributes, including spike length, number of spikelets per spike, spike weight, 1000-kernel weight, and grain yield, as confirmed by the results presented in Tables 1 and 2.

Table 1. Mean squares and the mean performance of flag leaf area (cm^{-2}), grain protein (%), wet gluten content, germination %, shoot length (cm), and root length (cm) as affected by bio stimulants treatments as a foliar spray application in 2023/24 and 2024/25 seasons

Treatments	Flag leaf area (cm^{-2})	Grain protein content (%)	Wet gluten content	Germination %	Shoot length (cm)	Root length (cm)
Season	0.21	0.12*	0.12	0.02	0.21**	0.21
2023/24	32.43	11.88	24.09	23.89	11.92	9.42
2024/25	32.62	12.02	24.23	23.94	11.82	9.61
Foliar spray (F)	1.00**	0.31**	1.63**	0.20**	0.38**	0.30**
Control	32.07d	11.68c	23.47c	23.73c	11.48c	9.23c
Lithovit with boron	32.35c	11.92b	24.19b	23.83bc	11.78b	9.49b
Lithovit with nitrogen	32.65b	11.96b	24.24b	23.95b	11.98ab	9.55b
Cina green plus	33.03a	12.24a	24.73a	24.15a	12.05a	9.78a
S x F	0.09*	0.04*	0.073*	0.003	0.01	0.004
CV	0.44	0.76	0.59	0.45	1.31	1.62

**, * highly significant and significant at 0.01 and 0.05 levels, respectively, NS: Not Significant. Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

Table 2. Mean squares and the mean performance of plant height (cm), no. of spikes m^{-2}, spike length (cm), no. of spikelets $spike^{-1}$, 1000-kernel weight (g), biological and grain yield m^{-2} (g) as affected by bio stimulants treatments as a foliar spray application in 2023/24 and 2024/25 seasons

Treatments	Plant height (cm)	No. of spikes m^{-2}	Spike length (cm)	No. of spikelets/spike	1000-kernel weight (g)	Biological yield	Grain yield
Season (S)	12.04	150	0.23**	0.65**	0.81	79350**	177.62*
2023/24	106.67	364.17	10.93	22.28	34.79	2690.83	914.51
2024/25	108.08	369.17	11.12	22.61	35.16	2805.83	919.95
Foliar spray (F)	27.49**	2091.67**	0.17**	1.15**	1.17**	12133.33**	892.39**
Control	104.5 c	348.33 b	10.78b	21.89 c	34.36 c	2691.67 c	900.33 c
Lithovit with boron	107.83 b	354.17 b	11.1a	22.47 b	34.98 b	2748.33 b	923.34 ab
Lithovit with nitrogen	107.5 b	375.83 a	11.07a	22.44 b	35.15 ab	2751.67 b	916.91 b
Cina green plus	109.67 a	388.33 a	11.15a	22.97 a	35.4 a	2801.67 a	928.33 a
S x F	1.71*	2.78	0.02*	0.10*	0.06	5927.78*	272.68**
CV	0.51	3.12	0.58	0.68	0.89	1.39	0.71

**, * highly significant and significant at 0.01 and 0.05 levels, respectively. NS: Not Significant. Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

Interaction effect

No. of spikes m^{-2}

As shown in Figure 1a, the foliar application of Cina green plus and Lithovit with nitrogen resulted in the highest values of spikes number m^{-2} in both seasons. In contrast, the control treatment gave the lowest values in both seasons.

1000-kernel weight (g)

The weight of 1000 kernels in Figure 1b showed a higher value by the foliar application of the used bio-stimulants compared to the control plants in both seasons.

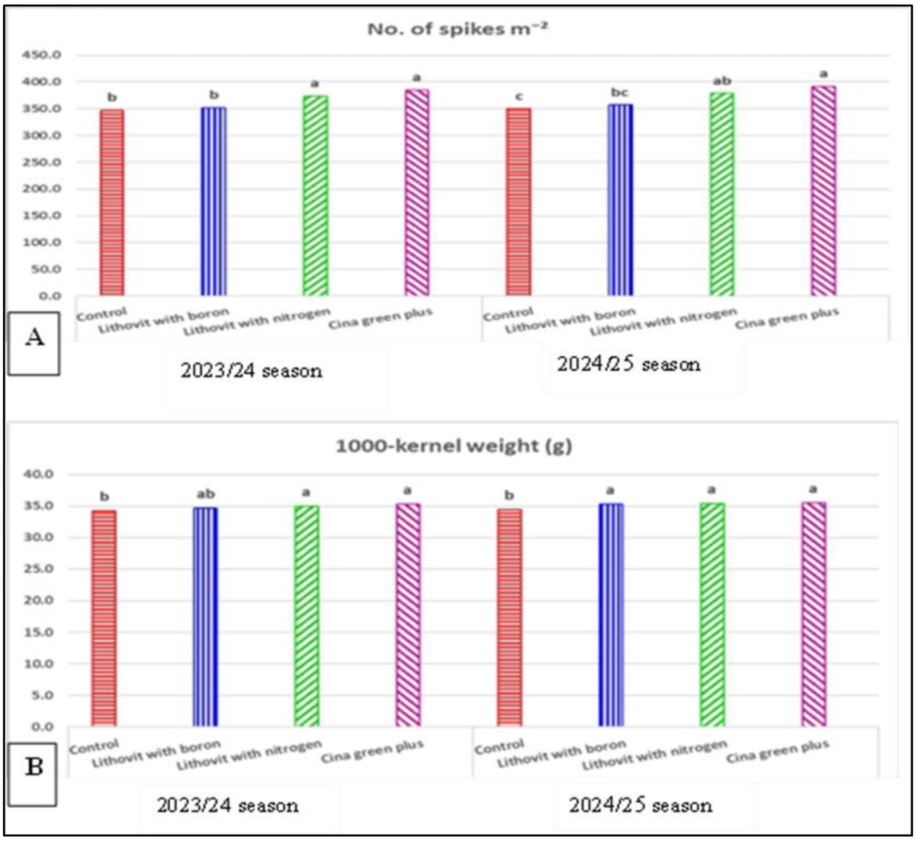

Figure 1. Impact of the interaction between seasons and foliar spray treatments on no. of spikes m^{-2} (a) and 1000-kernel weight (b)

Biological yield (kg m^{-2}) and Grain yield (kg m^{-2})

The presented results in Figure 2a showed that applications of Cina green plus gave the maximum values of biological yield in the first season, while the spray treatments did not differ significantly from the control treatment in the second season.

As shown in Figure 2b, foliar applications of Cina green plus and Litovit with boron resulted in the highest values of grain yield in the first season, while all spray treatments did not differ significantly and gave the highest values compared with the control plants in the second season.

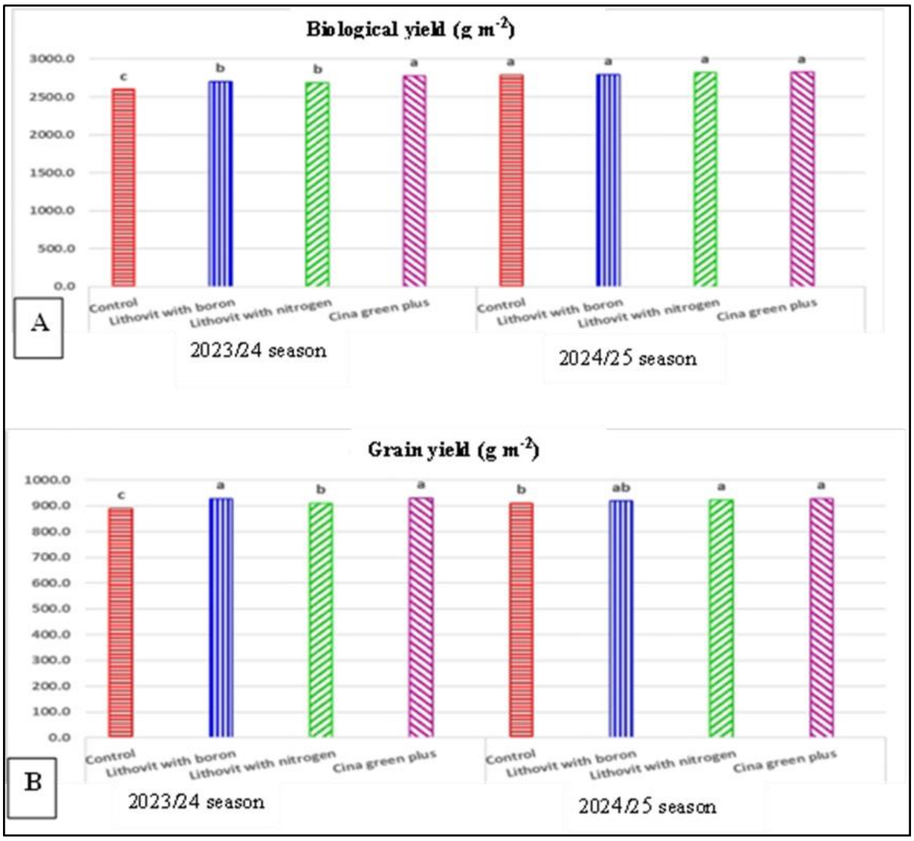

Figure 2. Impact of the interaction between seasons and foliar treatments on biological yield (a) and grain yield (b)

Flag leaf area (cm^{-2})

The flag leaf area in Figure 3a had the highest values using the application of Cina green plus in the first season and using Cina green plus and Lithovit with nitrogen in the second season, while the control treatment gave the minimum value.

Grain protein content (%)

The foliar application of Cina green plus gave highly significant values of grain protein content compared with the other spray treatments, while the control treatment showed the lowest values in both seasons (Figure 3b).

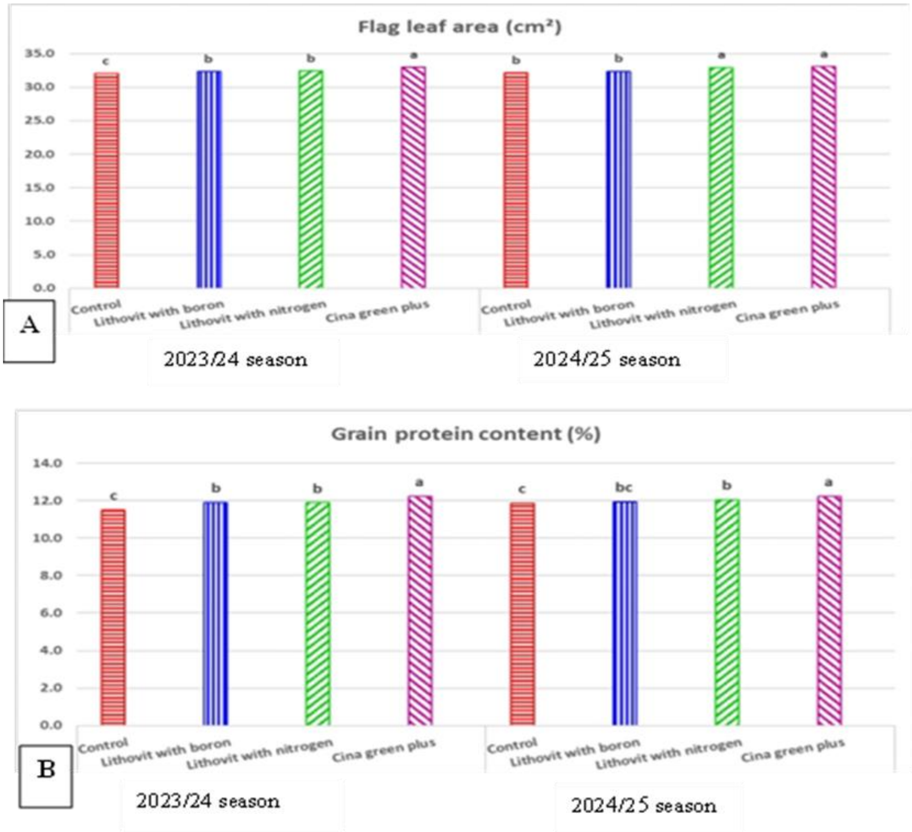

Figure 7. Impact of the interaction between seasons and foliar treatments on flag leaf area (a) and grain protein (b)

The impact of foliar spray treatments had a highly significant impact on the studied characteristics except for shoot length (cm), root length (cm), and 1000-kernel weight (g). The best results for grain protein and wet gluten content, germination %, plant height (cm), spikes number m^{-2}, spike length (cm), 1000-kernel weight (g), and grain yield. The significant effect of seasons indicated that these sources of variation had sufficient variability. The increased values of most studied characters in the 2023/24 season were due to the decreased temperature and higher relative humidity than in the second season. These results were in agreement with the results of Shehab-Eldeen and Farhat (2020), Farhat et al. (2021), Morsy et al. (2021), and Mu et al. (2021) who found that the rising temperatures during grain filling resulted in reduced grain growth and shortened the period for normal grain development. The foliar spray led to a significant impact on flag leaf area (cm^2), grain protein content (%), wet gluten content, germination %, plant height (cm), 1000-kernel weight (g), and grain yield.

The plants that received Cina green plus recorded the highest significant values of all studied traits and didn't significantly differ from Lithovit with boron. The control treatment registered the lowest values at all studied traits. The useful effect of nanomaterials as smart fertilizer may improve the efficacy of plant nutrient use, control the nutrient release, and decrease the ecological contamination. Also, Lithovit (a Nano-$CaCO_3$), is a natural fertilizer that releases CO_2, enhances the net photosynthetic rates, and promotes the growth characteristics (Zakaria et al., 2018). The promoting effect of Lithovit in enhancing plant growth has been previously reported (Ghatas and Mohamed, 2018).

Lithovit plays a crucial role in enhancing CO_2 levels within leaf intercellular spaces, thereby boosting photosynthesis efficiency, as noted by Gharib et al. (2025). Furthermore, its composition, which includes Mg, Ca, Fe, and Si, contributes to elevated levels of chlorophyll and carotenoid pigments. This compound has demonstrated the ability to increase the weight and water content of plant parts, facilitating improved water movement in plants experiencing salinity stress (Dessoky et al., 2025). In previous results, Nano-calcium exhibited a growth-promoting impact by enhancing leaf area and dry weight in green bean plants, while Ca and Si proved effective in alleviating salinity adverse effects (Gomaa et al., 2017). Sajyan et al. (2019) reported that Lithovit increased chlorophyll content, leaf area, and dry matter in tomato cultivated in saline soil. Additionally, it significantly boosted key growth metrics in potatoes - such as plant height, branch count per plant, shoot fresh and dry weights, leaf area per plant, tuber number, and overall tuber yield per plant (Farouk, 2015). The positive impact of Lithovit on tomato chlorophyll content further underscores its potential in improving crop performance under challenging growth conditions.

The increase in spike length, spike weight, 1000-grain weight, and grain yields with the application of Nano nitrogen was recorded with Saad et al. (2022). Also, Saad et al. (2022) reported that Misr 1 showed the maximum spike length, and grain yield using 120 kg of nitrogen with 14 L Nano nitrogen per hectare. Increasing the nitrogen concentration is an important method to increase nutrient efficacy, improve plant nutrition, and increase yield traits (Saad et al., 2022). The significance of Cina green plus as an NPK fertilizer lies in its ability to provide essential nutrients required for plant growth while improving nutrient uptake and utilization (Al-Juthery and Al-Shami, 2019). The plants treated with Nano NPK, micro-element fertilizers, and yeast extract showed notable enhancement in both macro- and micro-nutrient content, alongside a significant rise in protein levels. Macro- and micronutrients play critical roles in plant processes such as growth, metabolism, photosynthesis, chlorophyll synthesis, root development, cellular respiration, and enzyme effectiveness in primary and secondary metabolic pathways (Khanday et al., 2017). Additionally, the foliar application of bio-stimulants, particularly yeast extract, significantly improved the vegetative growth, yield, quality, and overall development of wheat plants. Nano fertilizers stand out due to their high surface area-to-volume ratio, making them more effective than many modern polymer-based fertilizers. Their unique properties enable a gradual release of nutrients and enhanced absorption efficiency (Elemike et al., 2019; Al-Juthery and Al-Shami, 2019).

The application of Lithovit has been shown to enhance the process of photosynthesis, likely due to its high carbonate content, which serves as a source of CO_2 and calcium. Research indicates that elevated CO_2 levels and the external application of calcium positively influence photosynthesis. Additionally, Lithovit functions as a long-term reservoir, supplying plants with CO_2, which promotes growth by boosting photosynthesis, reducing photorespiration, and increasing the availability of carbon assimilates essential for plant growth and development. Higher CO_2 concentrations typically stimulate plant growth by enhancing carbon assimilation, biomass accumulation, and leaf area expansion. The foliar application of Lithovit on cluster bean (guar) plants increased plant height and the number of branches per plant compared to the untreated control (Nassar et al., 2018). Lithovit B (LB), which is enriched with boron, also exhibits notable benefits. Boron foliar application was found to significantly improve root weight and diameter in sugar beet (Kandil et al., 2020). The use of LB effectively boosted root and top yields (t/ha). Similarly, Sajyan et al. (2020) highlighted the positive effects of LB on root and shoot dry weights, as well as on pepper yield. Moreover, application of micronized marine calcite ($CaCO_3$), a key compound in Lithovit led to increase both leaf and root yields in sugar beet (Artyszak et al., 2014).

CONCLUSIONS

The results revealed significant differences among treatments for most of the studied traits.Cina green plus had the best values, similar to Lithovit with boron for spike length and grain yield. Lithovit with nitrogen also performed comparably; however, the control treatment had the lowest values. Plants in the second season had better overall traits, while the first season had the highest shoot length. Based on the obtained results, Cina green plus and Lithovit combined with nitrogen can be recommended to enhance growth performance and yield of wheat cultivar Misr 3.

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