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Micronutrients do not improve agronomic efficiency under field conditions without nutritional stress to maize plants

Os micronutrientes não melhoram a eficiência agronômica em condições de campo sem stress nutricional para as plantas de milho

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ABSTRACT

Maize plants (*Zea mays* L.) are a cultivated crop of significant importance in global food security, because they have a high nutritional value and are used in the composition of chicken, pig, cattle, and goat feed. However, the physiological potential of maize plants has not been fully explored. Micronutrients are mineral elements required by enzyme activators in the processes of obtaining energy and in the accumulation of dry matter. We examined the hypothesis that maize plants treated with a micronutrient mixture (MNM), via foliar application, will not improve plant growth and, consequently, agronomic efficiency, under field conditions without nutritional stress. The experiment was carried out using the commercial hybrid *3646YHR* (Pioneer®) with the application of boron (B), zinc (Zn), copper (Cu) and molybdenum (Mo) available in the 'Mover' commercial product (Stoller-do-Brasil®) to the leaves of either 0.0 $L.ha^{-1}$, 1.25 $L.ha^{-1}$, 2.5 $L.ha^{-1}$, 5.0 $L.ha^{-1}$, and 7.5 $L.ha^{-1}$ distributed in a randomized block experimental design. We measured plant growth parameters and also calculated physiological indexes and agronomic efficiency. The data were subjected to an analysis of variance (ANOVA) and their means were compared using Tukey's test at 5% significance (α=0.05). In summary, our findings did not show improvements in plant growth and agronomic efficiency data. Therefore, we accepted our original hypothesis ('H0'), which suggests that mineral elements did not enhance the biological productivity and agronomic efficiency performance of our maize plants when they were not under nutritional stress. Investigation on maize leaf micronutrient mixture application is limited and so an excellent opportunity for future study.

Keywords: agronomic efficiency, micronutrient mixtures, plant growth, physiological indexes.

RESUMO

O milho (*Zea mays* L.) é uma espécie vegetal de significativa importância na segurança alimentar mundial, pois, possui relevante valor nutricional e é utilizada na composição de rações para frangos, suínos, bovinos e caprinos. Todavia, o potencial fisiológico e agronômico dessa espécie não é totalmente explorado. Os micronutrientes são elementos minerais considerados ativadores enzimáticos nos processos de obtenção de energia e no acúmulo de matéria seca. Examinamos a hipótese se plantas de milho tratadas, via foliar, com mistura de micronutrientes (MNM) não incrementarão o crescimento das plantas e, consequentemente, a eficiência agronômica sem estresse nutricional. O experimento utilizou o híbrido comercial 3646YHR (Pioneer®) com aplicação foliar de boro (B), zinco (Zn), cobre (Cu) e molibdênio (Mo) disponíveis no produto 'Mover' (Stoller-do -Brasil®) com as doses 0,0, 1,25, 2,5, 5,0 e 7,5 $L.ha^{-1}$, em que as plantas foram distribuídas em delineamento experimental em blocos casualizados. Avaliamos parâmetros do crescimento linear vegetais, assim como calculamos os índices fisiológicos e a eficiência agronômica. Os dados foram submetidos à análise de variância (ANOVA) e suas médias comparadas pelo teste de Tukey a 5% de significância (α=0,05). De maneira geral, relatamos que não houve incrementos no crescimento linear, índices fisiológicos e na eficiência agronômica. Diante disso, aceitamos nossa hipótese inicial ('H0') de que os micronutrientes não otimizaram a produtividade biológica e a eficiência agronômica de plantas de milho sem estresse nutricional. A investigação científica sobre a aplicação foliar de micronutrientes no milho é limitada ou inexistente, apresentando-se como excelente oportunidade para estudos futuros.

Palavras-chave: crescimento vegetal, eficiência agronômica, índices fisiológicos de crescimento, micronutrientes.

INTRODUCTION

Maize plants (*Zea mays* L.) contribute significantly to the Brazilian Gross Domestic Product. In the 2023/2024 agricultural year, maize grain production reached 316.7 million tons which was 1.5% or 4.7 million tons below that obtained in 2022/23 (Brasil, 2023). According to Caldarelli and Bacchi (2012), the maize production chain is one of the most important in Brazilian agribusinesses, responsible for around 37% of national grain production. Together with soybeans [*Glycine max* (L.) Merrill], these two crop plants are considered basic inputs for swine and poultry farming, and require the conversion of available natural resources (CO_2, water, essential mineral elements) into biomass (Branco et al., 2021).

Plant nutrition with micronutrients via foliar application is intended to complement, rather than replace, basic fertilization for supplying mineral elements. According to Eichert and Fernandéz (2023), plants have the capacity to take up nutrients through their leaves, although the majority of uptake sites are located in the tissues of the plant's roots. However, there are few studies that aim to investigate the agronomic efficiency of receiving supplementary doses of nutrients via the leaves of the plant.

The foliar application of supplemental mineral micronutrients aims to achieve nutritional balance and promote enzymatic interactions in biochemical and metabolic processes (Nciizah et al., 2020). The chemical elements called "micronutrients" are essential for plant development, as they participate in several biochemical and physiological processes such as enzymatic activation mechanisms, membrane and cell wall structural composition, redox potential, electron transport or cellular osmoregulation in their ionic form (Tavanti et al., 2021; Ahmed et al., 2024; Hussein and Amany, 2024).

Root tissues are the main mineral element uptake site in angiosperm plants (Hodge, 2023). The literature reports that certain mineral elements have restrictions regarding their mobility/translocation in individual plants, especially micronutrients with a 'low mobility', such as molybdenum (Mo), copper (Cu) and iron (Fe), as well as the 'intermediate mobility' element boron (B). On the other hand, the use of mineral elements (via foliar application) is a valid agronomic strategy to provide specific nutrients (Ishfaq et al., 2022; Eichert and Fernández, 2023; Zhao et al., 2024). Although micronutrients are well documented, leaf morphoanatomical mechanisms necessary for their uptake are poorly elucidated. Thus, it is assumed that plants under stress will tend to use mineral elements inefficiently, just as non-stressed plants will tend to be efficient in their use of mineral elements in their agronomic efficiency.

In general, plant growth parameters (biological productivity) and economic productivity (agronomic efficiency) with the use of natural resources are well documented in crop plants, such as yerba mate (Tang et al., 2023), wheat (Ren et al., 2023) and beet (Shrivastava et al., 2024). On the other hand, it is not clear whether crop plants under cultivation conditions with minimal or no stress will enhance economic productivity with the micronutrient supply. Considering this under investigated issue, we examined the hypothesis that maize plants treated with a micronutrient mixture (MNM), via foliar application, will not improve plant growth and, consequently, agronomic efficiency, under field conditions without nutritional stress.

MATERIAL AND METHODS

Experimental conditions and soil fertilization

The study was conducted in an experimental area belonging to the Universidade Federal of São Carlos

(UFSCar), Lagoa do Sino *campus*, located in the Buri municipality of São Paulo State, Brazil (average altitude of 596 m, geographical coordinates 23°47'57" S latitude and 48°35'15" W longitude and an average slope of 3%). According to the Köppen-Geiser climate classification, the region has a climate classified as Cwa, characterized as a tropical high-altitude climate with a rainy season in the summer and a dry season in the winter with an average temperature in the hottest month above 22°C. Furthermore, the region experiences a wide annual temperature range as the coldest month averages around 10.1ºC, while the hottest temperatures reach around 29.8ºC. The average annual precipitation is 1253 mm and the soil is labeled as Red Latosol Eutroferric as indicated in Table 1.

Fertilization was performed according to Van Raij et al. (1985) using 290 kg.ha^{-1} of the formulated granulated compost NPK 08-20-10 + micronutrients (B, Mn, Zn), and top dressed with 206 kg.ha^{-1} of the formulated granulated NPK 36-00-12. The water was applied by using center-pivot (CP) irrigation, and low-pressure spray sprinkler systems, with necessary and sufficient water volumes according to Pereira-Filho (2002). The authors of this study emphasize that the conditions of field cultivation were rigorous, especially giving great attention to the protection of the plants (phytosanitary management) and irrigation performance.

Plant materials and treatments

Our study investigated maize plants [*Zea mays* L. hybrid 3646YHR (Pioneer®)] as a 'crop-model' with productivity potential close to 120 bags of 60kg.ha^{-1} (7200 kg.ha^{-1}). The seeds were previously treated with *Azospirilum strain* Sp7 bacteria ('organic biostimulants') and, subsequently, sowed under straw in the season described as "second harvest cultivation" (February) with a plant population of around 75 thousand plants/hectare directly using a seeder equipped with 9 rows and a tractor with a 120 HP assisted traction system carried at the time. During the experimental investigation, we carried out phytosanitary management of maize seedlings and young plants to ensure that they produced photosynthetically active expanded leaves (known as phenological stage 'V8'). Foliar applied plant growth regulators, such as kinetin (CK), gibberellic acid (GA_3), and indole-3-acetic acid (IAA) ('inorganic biostimulants') were applied through the commercial product 'Stimulate' (Stoller-do-Brasil®). In addition, a sprayed micronutrient mixture (MNM) containing boron (B) (4.0% or 52.4 g.L^{-1}), copper (Cu) (0.17% or 2.23 g.L^{-1}), molybdenum (0.015% or 0.19 g.L^{-1}) zinc (Zn) (4.5% or 59.0 g.L^{-1}) and macronutrient nitrogen (N) (5.0% or 65.5 g.L^{-1}) was suppled using the commercial product 'Mover' (density product 1.31 g.ml^{-1}) (Stoller-do-Brasil®). The MNM was sprayed onto the plants using a backpack sprayer equipped with a manometer and a flat-fan nozzle (Teejet® 110 02 XR), while the solubilization of the syrup used deionized water (100 L.ha^{-1}). The pH value was corrected to 4.5 using an acidic phosphate solution and a 0.01 M NaOH solution was applied with wind speeds below 10km.h^{-1}, a relative humidity above 50% and temperatures between 20 to 26°C with the aid of a knapsack sprayer with a capacity of 20 L composed of a full conical nozzle. The treatments were applied at the 'V8' phenological stage (8 pairs of fully expanded and photosynthetically active leaves) with 2.5 L.ha^{-1} (dosage recommendation average or treatment 3) according to the manufacturer's recommendations; and dosages of 0.0 L.ha^{-1} (control treatment without foliar feeding or treatment 1),

Table 1. Chemical attributes of the Red Latosol Eutroferric soil of the experimental area in the 0-20 cm depth layer belonging to the Universidade Federal of São Carlos (UFSCar) Lagoa do Sino *campus* (Buri-SP, Brazil).

$P_{Mehlich}$	M.O	pH	K	Ca	Mg	H+Al	AL	SB	CTC	V	m
mg.dm^{-3}	g.dm^{-3}	H_2O	cmol$_c$.dm^{-3}							%	%
18	34.2	6.2	0.56	7.2	3.3	2.7	0.0	11	13.8	81	0.0
Micronutrients											
B	Cu	Fe	Mn	Zn	S						
mg.dm^{-3}											
0.12	1.90	123.1	48.3	9.90	8.7						

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1.25 L.ha^{-1} (treatment 2), 5.0 L.ha^{-1} (treatment 4) and 7.5 L.ha^{-1} (treatment 5), according to personal notes and communications with the corn farm producers.

Plant growth measurements and agronomic efficiency

The evaluations were performed with five replicates collected for each treatment. Plant growth parameters, such as stem diameter (collar diameter, mm), leaf area (dm^2), and plant height (cm) were performed according to Lopes et al. 2009. In addition, the total dry mass matter (g) was collected from maize plants and the individual specimens were divided into their aerial (leaf blade + sheath) and root parts (aerial and underground fasciculate adventitious roots). The different plant tissues were immediately washed with distilled water and packaged in paper bags before being kept in a forced air circulation oven for 72 hours at 65ºC and weighed on a precision scale accurate to 0.001 g. The maize grains were harvested from the moment they reached physiological maturity with a humidity around 18% (150 days after sowing). After reaching physiological maturity, we manually harvested and threshed the grains using an electric thresher (Trapp®) and, finally, we estimated grain productivity in maize bags of 60kg.ha^{-1}.

Physiological indexes measurements

From the leaf area (dm^2), shoot, and total dry matter mass (g) data, we calculated physiological indexes, such as leaf area ratio (LAR, dm^{-2}.dia^{-1}), specific leaf area (SLA, dm^{-2}.dia^{-1}), net assimilation rate (NAR, g.dm^{-2}.dia^{-1}), leaf weight ratio (LWR, g.g^{-1}), and relative growth rate (RGR, g.g^{-1}.dia^{-1}) (Radford, 1967).

Experimental design and statistical analysis

We used a random block design for our study with five experimental plot blocks. Each experimental plot was composed of four sowing rows of 5.0 m in length with a spacing of 0.5 m between rows, resulting in a population of 50,000 plants per hectare. The data were subjected to Levene's test to ensure homogeneity with the statistical assumptions (homoscedasticity among the variances) analyzed statistically by analysis of variance (ANOVA). Finally, the averages were compared by Tukey's test using a 5% probability threshold using SAS 9.0 statistical analysis software.

RESULTS

The climatic conditions are crucial for maximizing maize plant yields by supporting plant growth and reproductive development (Table 2). In the present study, we investigated whether micronutrients improve maize plant growth and agronomic efficiency cultivated in field conditions without nutritional stress. It is worth highlighting that the local climatic conditions were favorable for plant growth, which leads us to affirm that the planted crop was not affected by moderate or severe stresses.

The Levene's test showed homogeneity of variances between treatments. Our data collections show no statistical differences in plant growth parameters, physiological indexes, and agronomic efficiency (Tables 3 and 4, Figure 1).

Table 2. Minimum and maximum temperatures and rainfall during the experimental period between February and July 2017 in the experimental area belonging to the Universidade Federal of São Carlos, Lagoa do Sino *campus* (Buri-SP, Brazil).

Month	Minimum temperature average (°C)	Maximum temperature average (°C)	Rainfall (mm)
February	18°C	26°C	171
March	18°C	26°C	131
April	16°C	24°C	77
May	13°C	21°C	91
June	12°C	21°C	67
July	12°C	21°C	64

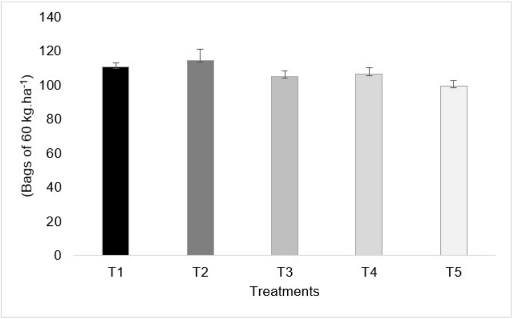

Figure 1. Agronomic efficiency (bags of 60 kg.ha^{-1}) of *Zea mays* L. Pioneer® 3646YH measured during grain harvest at 153 DAS. Boron (B), zinc (Zn), copper (Cu) and molybdenum (Mo) micronutrients were supplied in the commercial product 'Mover' (Stoller-do-Brasil®) and applied in dosages of 0.0 L.ha^{-1} (T1), 1.25 L.ha^{-1} (T2), 2.5 L.ha^{-1} (T3), 5.0 L.ha^{-1} (T4) and 7.5 L.ha^{-1} (T5). Means do not differ by Tukey's test at 5% probability (*n* = 5, ± standard error).

Table 3. Analysis of variance (ANOVA) performed on plant growth parameters (stem diameter, leaf area, plant height, and total dry matter mass) in *Zea mays* L. hybrid 3646YHR (Pioneer®).

Tratamento	Stem diameter (mm)	Leaf area (dm^2)	Plant height (cm)	Total dry matter mass (g)
0.0 $L.ha^{-1}$ (T1)	29.4 ± 1.2	699.84 ± 79	100.0 ± 20	61.7 ± 8.7
1.25 $L.ha^{-1}$ (T2)	30.8 ± 1.2	726.37 ± 54	120.0 ± 10	69.6 ± 5.8
2.5 $L.ha^{-1}$ (T3)	26.1 ± 2.1	561.28 ± 77	110.0 ± 10	66.0 ± 6.8
5.0 $L.ha^{-1}$ (T4)	29.2 ± 1.3	639.70 a ± 57	113.0 ± 20	67.2 ± 9.6
7.5 $L.ha^{-1}$ (T5)	29.0 ± 1.2	685.05 a ± 74	112.0 ± 10	69.2 ± 11.0
C.V. (%)	10.89	9.88	12.69	20.92
F	1.7^{ns}	1.618^{ns}	0.277^{ns}	0.817^{ns}

Means do not differ by Tukey's test at 5% probability (*n* = 5, ± standard error). ns = no significance.

Table 4. Analysis of variance (ANOVA) performed on physiological indexes, such as leaf area ratio (LAR), specific leaf area (SLA), net assimilation rate (NAR), and relative growth rate (RGR) in *Zea mays* L. hybrid 3646YHR (Pioneer®).

Treatment	LAR (dm^{-2} day^{-1})	SLA (dm^{-2} day^{-1})	NAR (g dm^{-2} day^{-1})	RGR (g g^{-1} day^{-1})
0.0 $L.ha^{-1}$ (T1)	0.24 ± 0.04	0.38 ± 0.05	0.72 ± 0.29	0.015 ± 0.006
1.25 $L.ha^{-1}$ (T2)	0.23 ± 0.05	0.35 ± 0.04	0.71 ± 0.27	0.017 ± 0.005
2.5 $L.ha^{-1}$ (T3)	0.22 ± 0.05	0.33 ± 0.06	0.69 ± 0.27	0.014 ± 0.007
5.0 $L.ha^{-1}$ (T4)	0.21 ± 0.04	0.32 ± 0.06	0.68 ± 0.25	0.015 ± 0.016
7.5 $L.ha^{-1}$ (T5)	0.22 ± 0.04	0.32 ± 0.07	0.69 ± 0.28	0.015 ± 0.014
F	0.87^{ns}	0.69^{ns}	0.44^{ns}	0.71^{ns}
C.V. (%)	21.36	17.98	14.85	16.66

Means do not differ by Tukey's test at 5% probability (*n* = 5, ± standard error). ns = no significance.

The values of agronomic efficiency (economic productivity) are presented in Figure 1. We observed that these values are similar to the productivity values expected for plant material cultivated at the time of year in which the experiment was implemented in the crop field of Buri/SP.

DISCUSSION

In our study, B, Zn, Cu, and Mo (MNM mixture) application to maize (*Zea mays* L.) occurred in the vegetative phenological growth stage ('V8'). The effects of micronutrients on plant growth performance in early phenological growth stages have not been investigated in the literature. Abiotic and biotic stresses can trigger losses in crop production (economic losses); however, our experiment does not reach the economic injury level (EIL) established in regard to insect pests, fungal/bacterial pathogens, or weed competition over time according to technical manuals for maize production (Cruz et al., 2011). Additionally, abiotic factors, such as carbon dioxide (CO_2), water (H_2O), and temperature are also described as inherent to the cultivation of plant species as they directly contribute to the synthesis of organic matter through processes like photosynthesis and cellular respiration. According to environmental data collected during our experiment, we report that our plants were not exposed to thermal and light stresses.

In the present study, MNM mixture application did not increase plant growth and, additionally, the maize plants did not show any visual symptoms of mineral imbalance compared to the control without any application. Foliar application of mineral elements is thought to benefit the plant as long as the plant is under recommended cultivation conditions, where the photosynthetic apparatus remains functional with satisfactory gas exchange in the leaves. Based on this premise, the different types of plant cells, such as stomatal complexes, remain turgid most of the time, which will allow for the efficient entrance of water (H_2O) and carbon dioxide (CO_2) into the leaf, ensuring favorable conditions for mineral ions to penetrate the ostiole and to be absorbed in the substomatal chamber formed by parenchymal tissue (mesophyll) (Lopes et al.

2009; Baron et al. 2018; Dos Santos et al. 2019). In this scenario, the entry of mineral elements that are part of redox reactions (Mo, Cu, Zn) improve the transport of electrons in the thylakoid membrane, in order to favor the production of photosynthates and, thus, serve as a cellular building block combined with the presence of B to ensure cellular structural integrity (Haensch and Mendel, 2009; Zhao et al., 2024). According to Cakmak et al. (2023), around 98% of the entire demand for mineral elements by higher plants is acquired by enzyme systems (membrane carriers) located in their root cells. While micronutrient leaf uptake may have occurred, the plant's requirements must be have been met via its roots supply, since the control performed satisfactorily in the absence of elements delivered directly to the leaves. Although some authors recommend the application of mineral elements to the leaf (Eichert and Fernández, 2023), the 'real' biochemical leaf uptake mechanisms are not currently experimentally described, except for the mineral element sulfur (S), macronutrient for higher plants (Maathuis, 2009), but this was not investigated in our study.

It is important to consider the possibility that mineral elements applied to the leaves may have trickled down to the roots after heavy rainfall or irrigation where they are mainly taken up via the roots (Lopes et al., 2024). This speculation could partly explain the reason why our treatments involving micronutrient dosages did not show any statistical differences. On the other hand, in the case of our control treatment, even though micronutrients were not directly applied to them (and thus not susceptible to runoff after heavy rain or irrigation), we must consider that the soil used for cultivation has a long history of grain production and, therefore, is a likely partial store of micronutrients needed by crop plants.

Dos Santos et al. (2019) reports that the micronutrient silicon (Si) is taken up at root sites, even when supplied via foliar application. Their study shows that the Si concentration in the xylem sap of monocot species is much higher than in eudicot species. This is due to the differences in Si transport from the cortical cells to the xylem vessels which is passive (without energy expenditure) in eudicots while monocots use membrane-specific ionic carriers for xylem loading (with energy expenditure). Plant physiologists and/or botanists (plant researchers) use the growth analysis data obtained at different plant developmental stages to monitor the increase in organic matter by photosynthetic activity and provide a detailed study of the physiological activities affected by the soil and climate conditions on crop plants. The relative growth rate (RGR) is determined by the accumulation of plant biomass over time, which is influenced by the improved photosynthetic activity described by the net assimilation rate (NAR), leaf biomass (SLA), or both. Philip J. Radford, in his pioneering study in the 1960s (Radford, 1967), reported that physiological index calculations reflect the edaphoclimatic effects that crop plants are subjected to. In this way, we can speculate that the application of mineral elements (micronutrients) to the leaves did not result in increased plant growth given the non-stressed cultivation condition. Such speculation is corroborated by our report of primary linear growth data that did not differentiate during plant development.

CONCLUSION

We rejected our initial hypothesis and accepted the original hypothesis ('H0'), that the micronutrients (mineral elements) applied to the leaves did not optimize biological productivity and agronomic efficiency of our maize plants without nutritional stress. Investigations into maize leaf micronutrient mixture application are limited and/or non-existent creating an excellent, worthwhile opportunity for future study.

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