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Seed treatment with magnesium nanoparticles alters phenology and increases grain yield and mineral content in maize

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Abstract

Magnesium is an essential macronutrient for maize crop and its efficiency of absorption and utilization by plants can be improved when used in the form of nanoparticles. The objective of this work was to evaluate the efficiency of different sources of magnesium oxide via seed treatment in the agronomic performance of maize. The experiments were carried out under field conditions in three locations in the state of Santa Catarina, Brazil. In all experiments, the P4285HYR-hybrid maize seeds were used, with three sources of magnesium: MgO-NPs, MgO@C-NPs and $Mg(NO_3)_2$; and six concentrations: 0 (control); 37.5, 75, 150, 300 and 600 mg L^{-1}. The following variables were analyzed: SPAD reading, NDVI, plant height, ear insertion height, number of grains per ear, grain moisture, thousand grain weight, yield and quantification of Mg, Ca, P and K in the grains. The results showed that the seed treatment with magnesium oxide alters the V4 and V5 phenological stages (fourth and fifth developed leaves), shortening the plant life cycle by four to five days ($P<0.05$). All applied concentrations increased maize grain yield, with greater improvement in the order of 38% with 150 mg L^{-1} of MgO-NPs and 57% with 300 of MgO@C-NPs in comparison to control, respectively. The use of 75 and 150 mg L^{-1} of magnesium nitrate increased grain yield by only 2.3% and 6.6%, respectively, when compared to the control. The P>K>Mg>Ca levels in the harvested grains were increased by seeds treated with Mg. The results were significantly influenced by the nanoparticles, when comparing with conventional Mg nitrate or with the control. Therefore, it was concluded that the use of magnesium nanoparticles via seed treatment is a viable strategy to increase maize grain yield.

Keywords: Maize Cycle Acceleration, Magnesium Nanofertilizers, Maize Yield, Maize Treatment with Magnesium Nanoparticles.
Abbreviations: P_phosphorus; K_potassium; Ca_calcium; Mg_magnesium; MgO NP_magnesium oxide nanoparticles; MgO@C NPs_magnesium carbon oxide core-shell nanoparticles; Mg(NO3)2_magnesium nitrate; FAO_Food and Agriculture Organization of the United Nations; CQFS RS/SC_Comissão de Química e Fertilidade do Solo dos Estados do Rio Grande do Sul e Santa Catarina; NGE_ number of grains per ear; UNOCHAPECÓ_Universidade Comunitária da Região de Chapecó; SPAD_Soil Plant Analysis Development; NDVI_ Normalized Difference Vegetation Index; TGW_thousand grain weight; UPF_unidade de produção familiar/family farm.

Introduction

Despite the current knowledge and the scientific research studies available, the challenges of obtaining higher yields in maize crops are still imminent. The processes of making magnesium available to maize plants are based on the direct application of magnesium precursor compounds to the soil, the most common being dolomitic limestone or magnesian limestone (Cqfs RS/SC, 2016). Magnesium is an essential macronutrient for plant growth and development (Gransee and Führs, 2013). In plant cells, Mg^{2+} is essential in the synthesis of nucleic acids and proteins, and for the activity of more than 300 enzymes, including direct participation in photosynthesis (Taiz et al., 2017). According to Marschner (2012), magnesium is present in maize plants in more mobile states and, owing to its high mobility in the phloem, it is easily translocated to the growing active parts of maize, where it is necessary for chlorophyll synthesis, enzymatic activation, protein biosynthesis and export via photosynthetic phloem.

Table 1. Physical and Chemical soil attributes from three experimental areas [Caxambu do Sul, Lages (Tambo) and Lages (Fecav)].

SMP - index	pH H_2O	O.M.	Clay	P	K	Ca	Mg	H+Al	CEC pH7.0	Al
Caxambu do Sul (2017/18 season)										
	(1:1)	--%--	mg dm^{-3}	--------------cmolc dm^{-3}---------						
5.9	5.4	4.6	34.0	17.1	219	9.6	3.4	4.9	18.5	0.1
Lages (Tambo) (2017/18 season)										
6.0	6.0	5.0	44.0	17.1	127	11.8	4.4	3.9	19.3	0
Lages (Fecav) (2018/19 season)										
6.1	6.3	2.45	27.7	8.4	47	10.6	5.8	3.7	21.6	0

Such factors ensure the growth and development of maize plants.

In the last decade has it been systematically dedicated to the development of agricultural products (Balaure et al., 2017). Currently, nanotechnology procedures are applied in agriculture for supply of plant hormones, manufacture of nanofertilizers for seed treatment and foliar fertilization, manufacture of nanosensors and for products with controlled release of agrochemicals (Worrall et al. 2018). In the agricultural and food industry, advances in nanotechnology have been providing new tools and strategies for molecular management of diseases and for increasing the nutrient absorption capacity of plants, increasing the yield and the nutritional value of grains (Tarafdar et al., 2013).

However, the effects of nanoparticles (NPs) on plants are still poorly known, and they may be positive or negative, or dependent on factors such as plant species, pathways of incorporation (seed, root or leaf), chemical composition and concentration of NPs (Yang et al. 2017; Liu et al. 2016). The main characteristics of NPs are established to allow better absorption, movement and release of nutrients in plant tissues, which in turn, allow efficient and targeted delivery of essential nutrients to specific tissues at rates compatible with demand compared to particles with larger size shapes (Elmer and White, 2018 Jayarambabu et al., 2016).

Fadeel et al. (2018) reported that carbon-based NPs can have beneficial effects on plants. Segatto et al. (2020) found that the treatment of maize seeds with 75 mg L^{-1} of MgO-NPs and MgO@C-NPs, associated with 150-day storage time of treated seeds, promotes a slight improvement in the germinative performance of maize seeds. Nanotechnology is confirmed as a technology in progress, with numerous applications; however, it still requires advanced techniques to solve the current problems in major crops (Alshehddi and Bokhari 2020). Studies indicate that the treatment of maize seeds with ZnO-NPs and MgO-NPs provided synergistic gains in seed and plant protection from germination to the vegetative stage, including a potential to increase grain yield as a result of the antimicrobial activity of NPs (Wani and Shah 2012; Segatto et al. 2018).

In turn, few studies have reported the effects of seed treatment with MgO-NPs on maize grain yield, especially in field conditions (which reflects the reality of growers). Therefore, this work aims to analyze the effect of the treatment of maize seeds by different concentrations of magnesium oxide nanoparticles on productive performance under field conditions in a maize crop.

Results

Normalized Difference Vegetation Index - NDVI and Soil Plant Analysis Development - SPAD index at the V6 stage

The analysis of variance indicated that there was no significant effect ($p>0.05$) of the magnesium sources and their concentrations on the NDVI of V6-stage maize plants when using MgO-NPs, MgO@C-NPs and $Mg(NO_3)_2$ applied via seed treatment. Likewise, the analysis of variance indicated that there was no significant effect ($p>0.05$) of the magnesium sources and their concentrations on the SPAD index of maize plants at the phenological stages V8, V11, VT and R3, for the Caxambu and Tambo experiments, when using MgO-NPs, MgO@C-NPs or $Mg(NO_3)_2$ applied via seed treatment. The value of the SPAD index in leaves of V6-stage maize plants was significantly influenced ($p\leq0.05$) by the magnesium sources, in the Caxambu experiment, as shown in Table 2.

The results in Table 2 show a statistically significant difference in the SPAD values of V6-stage plants when the leaves were treated with MgO-NPs. The SPAD index of V6-stage plants under the influence of the MgO-NPs source were 48.9 SPAD units, while with MgO@C-NPs and with $Mg(NO_3)_2$, they showed lower SPAD values of 47.7 and of 47.6, respectively. Such values of the SPAD index in the treated maize plants, regardless of magnesium sources, can be considered as satisfactory.

Plant height and ear insertion height

The analysis of variance indicated no significant effect ($p>0.05$) of magnesium sources and concentrations on

Table 2. SPAD values at maize plant stage V6; plant height (PH) and ear insertion height (EIH) of maize plant at stage R6; number of grains per ear (NGE) of maize plant at stage R6; grain moisture contents of maize plants at stage R6 on the basis of single effects of magnesium sources, in three distinct places in the state of Santa Catarina state/BR.

Mg Sources	**SPAD values at stage V6 - Caxambu do Sul- SC/BR**
MgO-NPs	48.9 a
MgO@C-NPs	47.7 b
$Mg(NO_3)_2$	47.6 b
CV%	4.3

	Plant height (PH) and ear insertion height (EIH) x assayed locations					
	Caxambu		Tambo		Fecav	
	PH	EIH	PH	EIH	PH	EIH
MgO-NPs	221.1a	111.2a	201.6a	104.9a	218.9a	111.9a
MgO@C-NPs	220.3a	110.4a	199.9a	105.5a	221.2a	110.4a
$Mg(NO_3)_2$	217.1a	109.2a	201.0a	106.0a	219.9a	109.2a
CV%	4.0	6.4	9.2	8.4	5.1	4.9

	Number of grains per ear (NGE) x assayed locations		
	Caxambu	Tambo	Fecav
MgO-NPs	518.0a	405.0a	449.1b
MgO@C-NPs	526.5a	382.3a	478.2a
$Mg(NO_3)_2$	518.3a	383.0a	408.5c
CV%	7.2	11.1	13.1

	Grain moisture (%) x assayed locations		
	Caxambu	Tambo	Fecav
MgO-NPs	15.0a	26.8a	21.4a
MgO@C-NPs	14.7a	25.7a	22.1b
$Mg(NO_3)_2$	18.8b	25.5a	28.3c
CV%	8.0	7.8	5.2

Means followed by different lowercase letters differ by the Scott-Knott test ($P \leq 0.05$).

plant height and ear insertion height, in the three experiments carried out in three different environments (Table 2). Even without promoting improvements in these responses, it can be inferred that the treatment of seeds with nanoparticles is not detrimental to the growth and development of maize plants, as they did not alter the longitudinal growth of the plant stem.

Number of grains per ear

The number of grains per ear (NGE), according to the analysis of variance, has no statistically significant difference in relation to the magnesium sources, for the Caxambu and Tambo experiments (Table 2). However, it indicated that there was a significant effect ($p \leq 0.05$) only in the Fecav experiment, with a simple effect of sources (Table 2) and of interactions of source factors and magnesium concentrations (Figure 1). The fact that there are significant differences in the number of grains in only one of the three experiments reinforces the possibility of (weak) influence of the environment in the responses to NGE (Table 2).

In the Fecav experiment, the number of grains per ear was significantly influenced by the three sources and concentrations. NGE increased by 22% with the concentration of 600 mg L^{-1} of MgO-NPs while the increase was 52% with MgO@C-NPs at the same concentration. In turn, with magnesium nitrate, the increase was only 9% in NGE (Figure 1). This greater increase in NGE with MgO@C-NPs treatments may be due to biocompatibility, since NPs are coated with a carbon layer that, in turn, would be more biologically compatible than the other sources.

Thousand grain weight

The analysis of variance showed a significant effect ($p \leq 0.05$) of both magnesium sources and concentrations for TGW. The weight values related to the sources and the concentrations of MgO-NPS, MgO@C-NPs and $Mg(NO_3)_2$ are shown in Figure 2, considering the three experimental areas, Caxambu (Figure 2a), Tambo (Figure 2b) and Fecav (Figure 2c).

TGW is considered as an important yield component of maize crops. Based on the results shown in Figure 2a, it appears that TGW was positively influenced in all treatments with the MgO-NPs and MgO@C-NPs

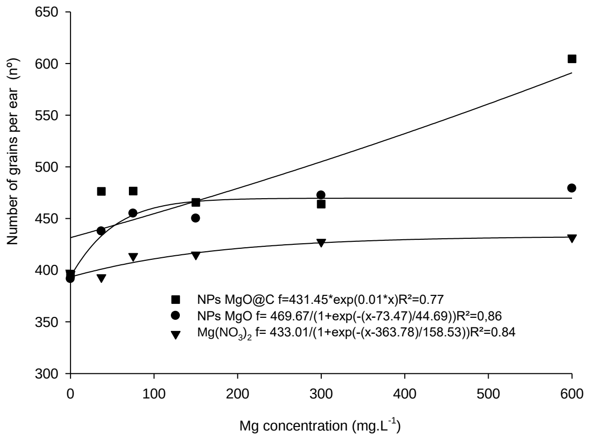

Fig 1. Number of grains per ear (NGE) of maize plants at stage R6 on the basis of magnesium concentrations and magnesium sources, in Lages (FECAV), SC/BR.

sources, providing increases in comparison to the control and in comparison to the treatment with $Mg(NO_3)_2$. It is noteworthy that TGW, for the magnesium nitrate concentrations, was lower than the rate obtained in the control in the Caxambu and Fecav experiments, as shown in Figures 2a and 2c.

In the Tambo experiment, the effect of MgO-NPs, MgO@C-NPs and $Mg(NO_3)_2$ on the TGW of the treated plants (Figure 2b) showed different results in comparison to Caxambu. For all concentrations of MgO-NPs, TGW was higher in comparison to the control, with emphasis on the concentrations of 37.5 and 75.0 mg L^{-1} which provided increases by 20.4% and 19.4%, respectively, when compared to the control. Plants treated with MgO@C-NPs and with a concentration of 150 mg L^{-1} increased by 17.7% for TGW in comparison to the control. For magnesium nitrate, the concentration of 75 mg L^{-1} provided an increase of 8.6% in comparison to the control (Figure 2b).

A similar response was found between the Fecav and Tambo experiments. It appears that TGW was positively influenced for all concentrations of MgO@C-NPs, providing increases of 12.6% and 9.1% in comparison to the control of each experiment (Figures 2b and 2c). However, under conditions of water deficiency during the development of the plants, in the case of Tambo (Figure 2b), the MgO@C-NPs source only responded positively for TGW up to the concentration of 150 mg L^{-1}, while the MgO-NPs source showed positive response stability for TGW in the concentration range from 37.5 to 600 mg L^{-1} (Figure 2c).

Grain moisture

The analysis of variance indicated a significant effect ($p \leq 0.05$) with interaction of magnesium sources and concentrations in grain moisture (U%) at the time of harvest in the Caxambu experiment. Figure 3 shows that grain moisture, for plants treated with MgO-NPs, showed a reduction from ~24% (control) to ~14% for both NPs sources (MgO and MgO@C). There was a similar response with grain moisture from seeds treated with magnesium nitrate; however, this reduction ranged from ~24% (control) to ~18% (Figure 3). In general, the use of magnesium favors lower moisture content for the grains at the time of harvest when compared to the control, and the pattern of response to concentration is constant in the range of 37.5 to 600 mg L^{-1}, regardless of source (Figure 3).

In the Tambo experiment, the analysis of variance indicated a non-significant effect ($p>0.05$) of magnesium sources on the moisture of the harvested grains. Table 2 shows a similar response to that of the Caxambu and Fecav experiments, in which the sources containing nanoparticles showed moisture values in the order of 3 to 5% lower in comparison to the conventional source of magnesium [$Mg(NO_3)_2$].

Further details on the effects of treatments on the reduction of final moisture of grains produced were explored in the Fecav experiment, via earlier assessments of this variable in plants in three different phenological stages. The analysis of variance indicated a significant interaction ($p \leq 0.05$) between the magnesium sources and the concentration values for the moisture of grains in the phenological stages R5, R6 and at harvest time. In the R5 phenological stage, grain moisture was reduced with increased concentrations, depending on magnesium nanoparticles sources. With concentrations higher than 75 mg L^{-1} of MgO-NPs or MgO@C-NPs, there was a reduction in moisture grain percentage, compared to the control, from ~42% to ~38.5% (Figure 4a), while for $Mg(NO_3)_2$, this value decreased in the control from ~42% to ~40.5% (Figure 4a). When moisture percentage was assessed at the R6 stage, there was the same downward trend found in the previous stages; however, it was close to 37% in the control, but when using the seeds treated with $Mg(NO_3)_2$, MgO@C-NPs and MgO-NPs, it stabilized at 36%, 35% and 34%, respectively (Figure 4 b). At the time of harvest, as expected, the moisture values were lower, but moisture percentage in the control was close to 31%; as a function of concentrations, it stabilized at 28%, 22% and 20% with $Mg(NO_3)_2$, MgO@C- NPs and MgO-NPs, respectively (Figure 4c). This experiment confirmed that all the sources of Mg used in the treatment of seeds reduced grain moisture percentage in comparison to the control. However, the NPs sources were more efficient at concentrations of 150 to 300 mg L^{-1}, consistently providing a reduction of approximately 10% in grain moisture percentage (Figure 4c).

Based on these differences, it can be inferred that the cycle of maize crop is probably accelerated, a fact previously observed in the phenological evaluation at V6, when the chlorophyll values were measured by the indicator SPAD. It was also found that under the

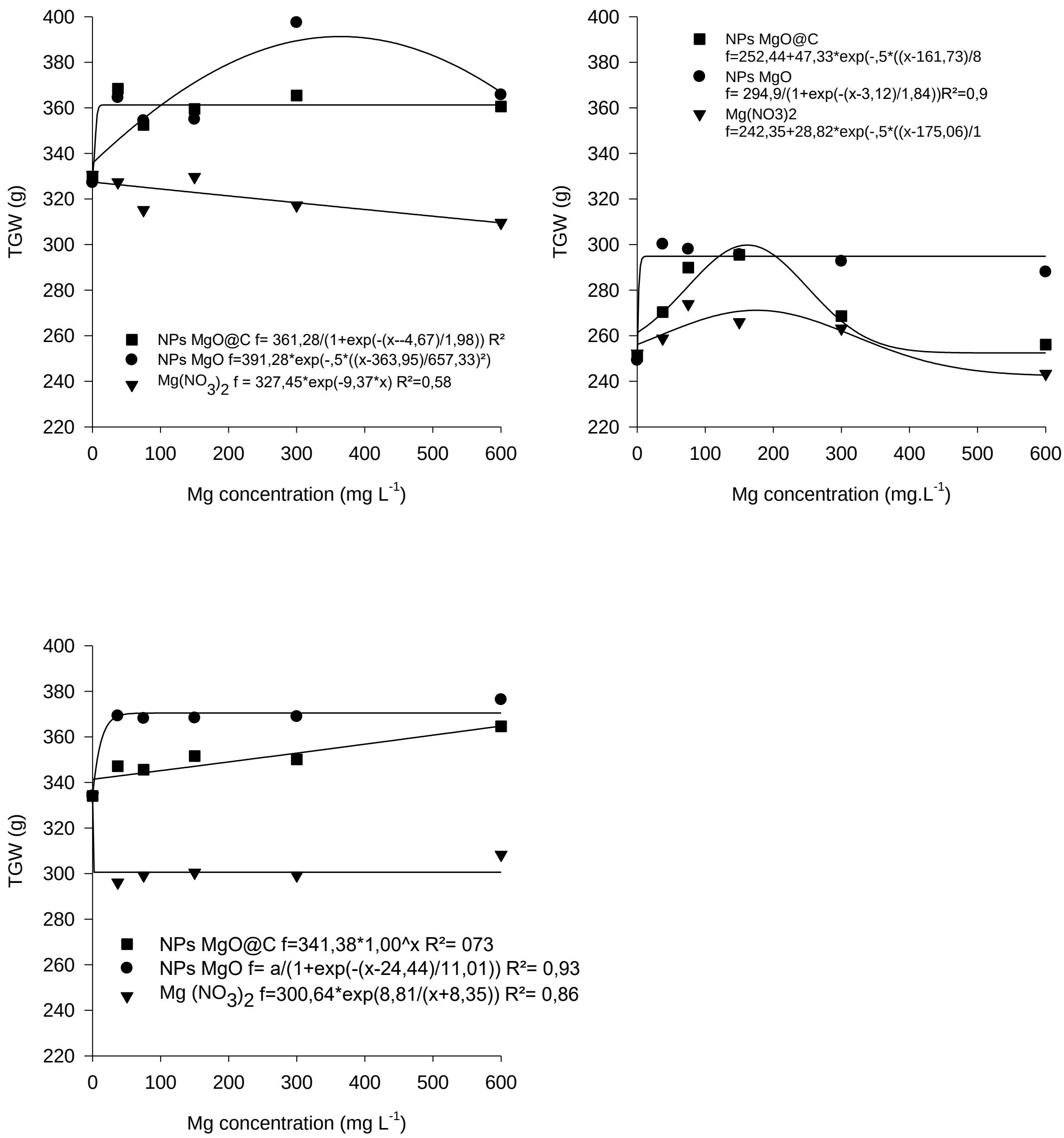

Fig 2. Thousand grain weight (TGW) of maize plants at stage R6 on the basis of magnesium concentrations and magnesium sources, in: a) Caxambu do Sul - SC/BR, b) Lages - SC/BR Tambo and c) Lages-SC/BR Fecav.

influence of NPs (MgO or MgO@C), the maize plants had a higher number of developed leaves in comparison to the control (dose 0) or the plants treated with all concentrations of conventional Mg-[$Mg(NO_3)_2$].

The finding concerning the lowest degree of grain moisture percentage, combined with the phenological differences found in the V4 and V5 phenological stages, suggest the hypothesis that the maize seed treatment with magnesium nanoparticles promotes acceleration of the cycle (precocity) by around four days. The results indicate that the development of the crop is accelerated in comparison to the control between the V4 and V5 stages. This difference in cycle was maintained until harvest time, thus allowing the harvest to be anticipated or to harvest grains with a lower moisture content, which can be a benefit to the production chain as well as generate savings in grain drying.

Grain yield

The analysis of variance showed a significant effect on the grain yield ($p \leq 0.05$) of the source and concentration of MgO-NPs, MgO@C-NPs and $Mg(NO_3)_2$ for all experiments. Figure 5a shows the maize grain yield in comparison to the sources and concentrations of MgO-NPs, MgO@C-NPs and $Mg(NO_3)_2$. As found in the weight values of TGW, there was an increase in yield with all concentrations; however, grain yield was higher, i.e., around 38.8% in comparison to the control plants, with the concentration of 300 mg L^{-1} of MgO-NPs.

Plants treated with MgO@C-NPs also showed an increase in yield compared to the control, with the highest yield found with the concentration of 150 mg L^{-1} with an increase of 33%. For the plants treated with $Mg(NO_3)_2$, the concentrations of 75 and 150 mg L^{-1} provided an increase of 6.6% and 9.9%, respectively.

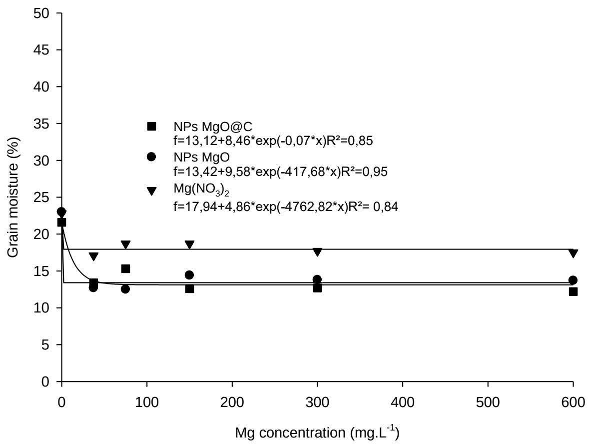

Fig 3. Maize grain moisture at harvest on the basis of magnesium sources and concentrations. Caxambu do Sul - SC/BR, 2017/18 growing season.

The smallest increase in productive performance with the use of conventional $Mg(NO_3)_2$ compared to treatments with nanoparticles is possibly associated with the phytotoxicity (saline effect) of these particles when in high concentrations (300 and 600 mg L^{-1}). This negative effect was also found in the germinative performance of seeds treated with conventional $Mg(NO_3)_2$, as shown in Figure 5a.

Figure 5b shows the values found for grain yield in the Tambo experiment, with a much lower grain yield than those obtained with the Caxambu and Fecav experiments. These lower grain yields can be assumed to be associated with climatic factors, especially water restriction (supplementary file), occurrence of hail, more compacted soil, occurrence of diseases associated with *Ustilago maydis* and *Sphacelothe careiliana*, and presence of physiological disorders in the ears. Based on the grain yield obtained under adverse conditions (mentioned) in the Tambo experiments, there were still positive effects of using nanoparticles and magnesium nitrate for maize crops.

There were significant increases in grain yield with all concentrations of MgO-NPs, especially the concentration of 300 mg L^{-1}, which provided an increase of 57.1% in comparison to the control. For plants treated with MgO@C-NPs, there was an increase of 58.1% with a concentration of 150 mg L^{-1}.

In a similar way to the results found for TGW using the concentration of 150 mg L^{-1} of conventional magnesium nitrate, there was an increase of 2.3% in grain yield. However, when using the highest concentrations of 300 and 600 mg L^{-1}, the negative effect was maintained, with a reduction in yield of 2.8% and 21.3%, respectively.

Figure 5c shows the response of grain yield in the Fecav experiment. It appears that there was a significant increase in grain yield, in comparison to the control, at all concentrations of magnesium oxide nanoparticles. Using concentrations of 150 and 300 mg L^{-1}, there was a higher increase than in the other concentrations, with 27.1% and 29.6%, respectively, in comparison to the control plants.

For the results of grain yield, treatments with MgO@C-NPs and MgO-NPs provided a similar response (Figure 5c). Likewise, the number of grains per ear (Figure 5c) provided an increase of 80.2% in comparison to the control with the treatments at the concentration of 600 mg L^{-1}. In turn, plants treated with conventional magnesium nitrate at a concentration of 300 mg L^{-1} showed an increase of 9.8%. On the other hand, with a concentration of 600 mg L^{-1}, yield was lower in comparison to controls.

Content of Ca, Mg, P and K in maize grain

The analysis of variance indicated a significant effect ($p \leq 0.05$) of the sources and concentrations of magnesium on the levels of calcium, potassium, magnesium and phosphorus in the grains. For calcium content in the maize grains, regardless of the source being used, there was a higher Ca content at all Mg concentrations tested when compared to those found in the control. Although the adjustment was quadratic, it is noteworthy that the response was quadratically increasing for the levels of Ca in the grains owing to the increase in the concentration of Mg applied via seed treatment. However, only the seeds treated with a concentration of 300 mg L^{-1} showed a difference in Ca content depending on the type of magnesium sources. With nanoparticles, there was an increase of 11.6% in Ca content in the grains, when compared to the same magnesium nitrate concentration (Figure 6a).

The treatment of seeds with MgO-NPs positively influenced K content in the grains with all treatment concentrations. There was a remarkable quadratic response with a point of maximum technical efficiency and K content in the grains of 6.09 g kg^{-1} after application of 211.98 mg L^{-1} of MgO-NPs via seed treatment. The application of $Mg(NO_3)_2$ also had a quadratic response with a point of maximum K content in the grains of 5.9 g kg^{-1} when applying 195.03 mg L^{-1} of $Mg(NO_3)_2$ via seed treatment. The concentration of 150 mg L^{-1} of Mg applied via seed treatment provided an increase of 32.8% in the potassium content in the grains in comparison to the control. However, when comparing the applied Mg sources, magnesium nitrate provided a K content in the grains that was similar to the treatments carried out with the two sources of nanoparticles (MgO-NPs and MgO@C-NPs) (Figure 6b).

Figure 6c shows that phosphorus content was higher in comparison to the control, regardless of magnesium source, for the treatments with all concentrations of Mg. The treatment with the concentration of 75 mg L^{-1} provided an increase of 62.9% in P content in the grains. Regardless of the source of Mg, the response

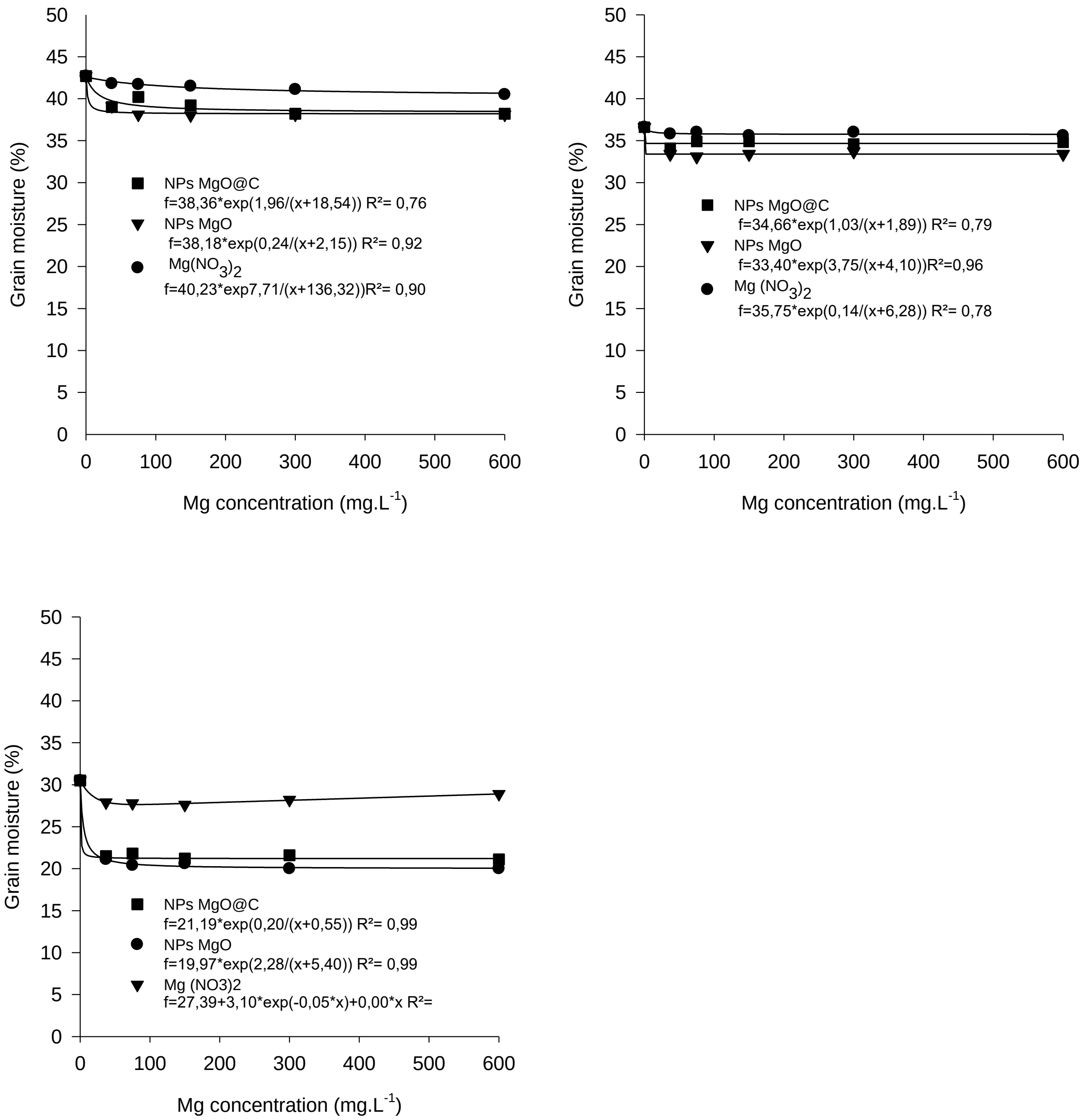

Fig 4. Maize grain moisture on the basis of magnesium sources and concentrations: a) harvested at plant stage R5; b) early R6; and c) late R6 c). Lages SC/BR – Fecav.

on P content in grains was quadratic with a point of maximum of 0.877 g kg^{-1} after the application of 187.7 mg L^{-1} of Mg via seed treatment, whether the source is MgO-NPs or $Mg(NO_3)_2$.

The grains from plants that received the seed treatment with MgO-NPs had a considerable increase of 0.81 g kg^{-1} of phosphorus content in relation to the grains treated with magnesium nitrate with a content of 0.51 g kg^{-1} (Table 3). The analysis of variance indicated a significant effect on the magnesium content in the grains ($p \leq 0.05$) depending on the type of magnesium source. Table 3 shows the Mg and P contents in the maize grains treated with the nanoparticles, which were higher when the seeds were treated with Mg sources, illustrating a 17.5% increase in magnesium content in the grains owing to the application of MgO-NPs in comparison to the application of the non-nano (conventional) Mg source [$Mg(NO_3)_2$]. These results corroborate the hypothesis that the addition of Mg via seed treatment by nanoparticles results in grains with higher magnesium content.

It can be argued that the treatment of seeds with nanoparticles increases Ca, Mg, K and P in maize grains, thus benefiting consumers of these grains, owing to a better nutritional quality. Another finding is that the use of MgO-NPs is more effective in increasing minerals in grains than $Mg(NO_3)_2$, except for the levels of K and P in grains in which the trend is similar but dependent on the concentration of Mg applied.

Table 3. Magnesium and phosphorus contents into maize grains on the basis of Mg sources [MgO-NPs (nanoparticles) and magnesium nitrate (conventional Mg)].

Mg sources	Mg into grains (g kg^{-1})	P into grains (g kg^{-1})
MgO-NPs	0.94 a	0.81 a
$Mg(NO_3)_2$	0.80 b	0.51 b
CV%	13.4	35.0

Means followed by different lowercase letters differ by the Scott Knott test (P≤0.05).

Discussions

Plant and agronomic traits

Magnesium content in the soils at pre-sowing was 4.5 $cmol_c\ dm^{-3}$. This value was considered as sufficient to supply the need for maize crops according to Cqfs-RS/SC (2016). In this way, the seed treatments with magnesium provided an additional amount of this element available to the plant, but this additional amount did not change the chlorophyll indexes between the different treatments (p>0.05). Photosynthesis is an important indicator of plant adaptability under biotic and abiotic stresses, and it was recently used to determine the phytotoxicity of metallic nanoparticles and metallic oxides, such as ZnO-NPs in plants of *Oryza sativa* (Chen et al. 2018). In *Chlamydomonas reinhardtii*, AgO-NPs strongly inhibited the photosynthetic net (Navarro et al. 2015).

According to Zuffo et al. (2012), the adequate SPAD index ranges between 45 and 48 for the V6 phenological stage. Wang et al. (2015) also reported that ZnO-NPs at concentrations between 200 and 300 mg L^{-1} significantly reduced the content of chlorophyll *a* and chlorophyll *b* in *Arabidopsis* leaves. Kanjana (2020) found an increase in the SPAD index with a value of 41.1 owing to foliar application of MgO-NPs compared to the value 38.4 of the (normal) MgO source in cotton plants. It should be noted that the SPAD index values of maize plants under the influence of MgO-NPs have not yet been published; thus, these results are the first to be published.

The number of grains per ear is established during the flowering period, after the extrusion of the tassel and stigmas of the ear and is greatly influenced by the flow of photoassimilates that occurs during this period (Fancelli and Dourado-Neto 2004). The positive results concerning the addition of grains per ear may be related to the physiological roles of magnesium in the development of pollen and in male fertility. Therefore, with sufficient magnesium for pollen mitosis, it caused an increase in the number of mature pollen grains and, consequently, it provided a greater number of grains per ear (Xu et al. 2015). However, such improvement in the supply of Mg provided increases of up to 52% in NGE with the use of 600 mg L^{-1} of the MgO@C-NPs source.

The increase in TGW may be associated with an increase in the photosynthetic activity of the plant because of the presence of MgO-NPs and MgO@C-NPs. The increase in this activity provides the conversion of synthesized carbohydrates into sucrose and starch, which can be used in grains that are considered as the main drains of the plant during the reproductive stages. The increase in TGW may be associated with the translocation of these nutrients to fill the grains. According to Cakmak and Yazici (2010), magnesium in equilibrium with potassium has known effects on plant nutrition and contributes to the translocation of photoassimilates and carbohydrates in plants. According to Taiz et al. (2017), the levels of nutrients in crops at certain growth stages influence the productivity of economically important tissues such as grains.

There are few reports in the scientific literature on acceleration, as caused by magnesium compounds, in the initial phenological stages of maize crops. According to Marschner (2012), magnesium is present in more mobile forms in plants, and it is easily translocated to their growing active parts owing to its high mobility in the phloem, where it is required for chlorophyll and enzymatic activation of the biosynthesis of proteins and the export, via phloem, of photosynthates to ensure plant growth and development. Because MgO-NPs have greater solubility in water, their penetration into the xylem through parenchymal cells can be improved, and they can be accumulated in the vacuole and adsorbed by the plant, thus enhancing plant growth (Al-Khazali and Alghanmi 2019).

It is very likely that nanoparticles transport a greater amount of Mg to chloroplasts because of their smaller dimensions in comparison to conventional magnesium compounds; for example, it was found that magnesium transporter molecules are a family of genes, such as ZmMGT12. This characteristic may favor the synthesis of chlorophyll and the mechanisms of photosynthesis, which will allow maximum accumulation of photoassimilates (Li et al. 2018). Through the translocation of magnesium, this accumulation of photoassimilates at V4 and V5 stages may be the factor responsible for the acceleration of the maize crop cycle.

It can be argued that plants treated with nanoparticles in the form of a magnesium-carbon oxide-core-shell may be more suitable under unfavorable environmental conditions, since the nanoparticles are coated with carbon, thus increasing biocompatibility, according to the results shown in Figure 5b. These

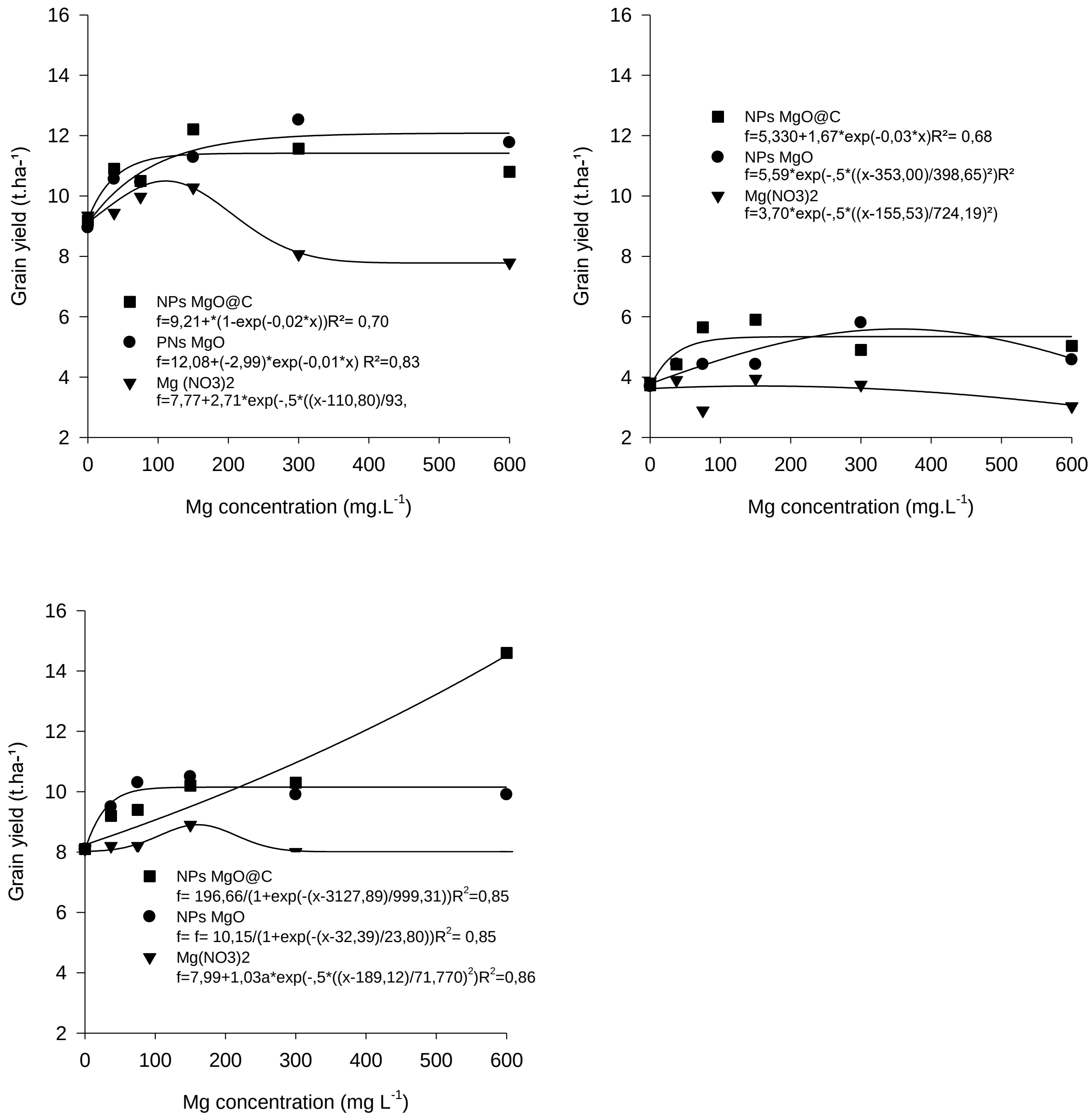

Fig 5. Maize grain yield on the basis of magnesium sources and concentrations. Assay carried out in (a) Caxambu do Sul-SC/BR, (b) Lages – SC/BR (Tambo) and (c) Lages – SC/BR (Fecav).

results corroborate the findings of Vijai-Anand et al. (2020), who reported that germination performance and seedling vigor improved with the seed treatment with 100 mg L^{-1} of MgO-NPs in *Vigna radiates*. There can be an increase in grain yield around 58% bigger than in the control, and similar to the GY value obtained with MgO-NPs but with half dose of the Mg concentration (150 x 300 mg L^{-1}). These results corroborate the findings of Dimkpa et al. (2020), who reported a significant increase between 39 to 51% of the wheat grain yield compared to the control in water stress conditions when they applied ZnO-NPs and urea coated with ZnO-NPs. Dimkpa et al. (2017) reported mitigation of water stress and an increase in grain yield between 22 to 183% in the sorghum crop with exposure to ZnO-NPs directly in the soil. Drought is one of the climatic events that most affects crop yield. The results found in this study are significant, regarding the importance of using nanoparticles as a strategy to mitigate the effects of drought on maize grain yield.

Recently, Debnath et al. (2020) reported that the treatment of rice seeds with TiO_2-NPs was very effective, since the applied concentration ranges between 20 mg L^{-1} and 50 mg L^{-1}, reflecting an increase in grain yield. The authors related the increase in grain yield to improvements in the number of tillers, the greater number of primary branches and the formation of longer panicles (Debnath et al. 2020). Although TiO is a non-nutritional chemical element, it is noteworthy that the application of NPs has brought benefits for the promotion of crop growth and development, improvements in phytosanitary aspects and, when the element is a nutrient, it brings nutritional improvements to plants and grains. In *Gossypium hirsutum*, Kanjana (2020) found an increase in plume and grain yield associated with improvements in the levels of N, P, K and Mg in the plant tissue. There probably is a synergistic effect between the application of Mg with increases of N, P and Mg in the dry weight of the plant, and that there could be an antagonistic effect with K, but this did not occur, probably because K is absorbed before Mg.

Content of Ca, Mg, P and K in maize grain

Coelho and Resende (2008) reported that the nutrients N, P and S absorbed by maize plants are exported in greater quantity to the grains, while much of the K, Ca, Mg and micronutrients are retained in the straw and return to the soil during crop straw decomposition. For cotton crops, Kanjana (2020) reported increases in the contents of N, P, K and Mg in the whole plant owing to the foliar fertilization of these crops with MgO-NPs (nano size 50 nm, up to 60 mg L^{-1}), as well as with the other sources of Mg that are not NPs (MgO and $MgSO_4$). In view of the results presented by this work, it can be argued that, as they are smaller in size, MgO-NPs or MgO@C-NPs are biologically more efficient and have positive effects, in agronomic and mineral terms, on maize ears.

Materials and Methods

Research sites

The evaluations of the agronomic performance of maize were carried out under field conditions in three different locations during the 2017/18 and 2018/19 spring/summer agricultural season: (i-Caxambu) in a family production unit (Upf) in the municipality of Caxambu do Sul - SC/Brazil Udesc at 27°10'37" south latitude, S; 52°53'19" longitude and 343 m of altitude, and in two locations in the municipality of Lages - SC / Brazil, (ii-Tambo) on the Tambo experimental farm – Udesc at 27°47'03" south latitude, S; 50°18'06" longitude and 904 m of altitude and (iii-Fecav) on the Fecav experimental farm Cav-Udesc (Centro de Ciências Agroveterinárias/ Santa Catarina State University) at 27°45'38" south latitude, S; 50°04'53" longitude and 872 m of altitude.

Definition of seed and nanoparticles

The experiments used P4285VHYR hybrid maize seeds, provided by the company Pioneer®, with Optimum® Intrasect® technology. The cultivar is characterized as a high-technology single-cross hybrid, recommended for planting in a population of 60,000 plants per hectare (Pioneer 2020).

The magnesium oxide nanoparticles and the magnesium oxide and carbon coated shell-core nanoparticles were provided by the Laboratório de Materiais Multifuncionais da Universidade Comunitária da Região de Chapecó – Unochapecó,

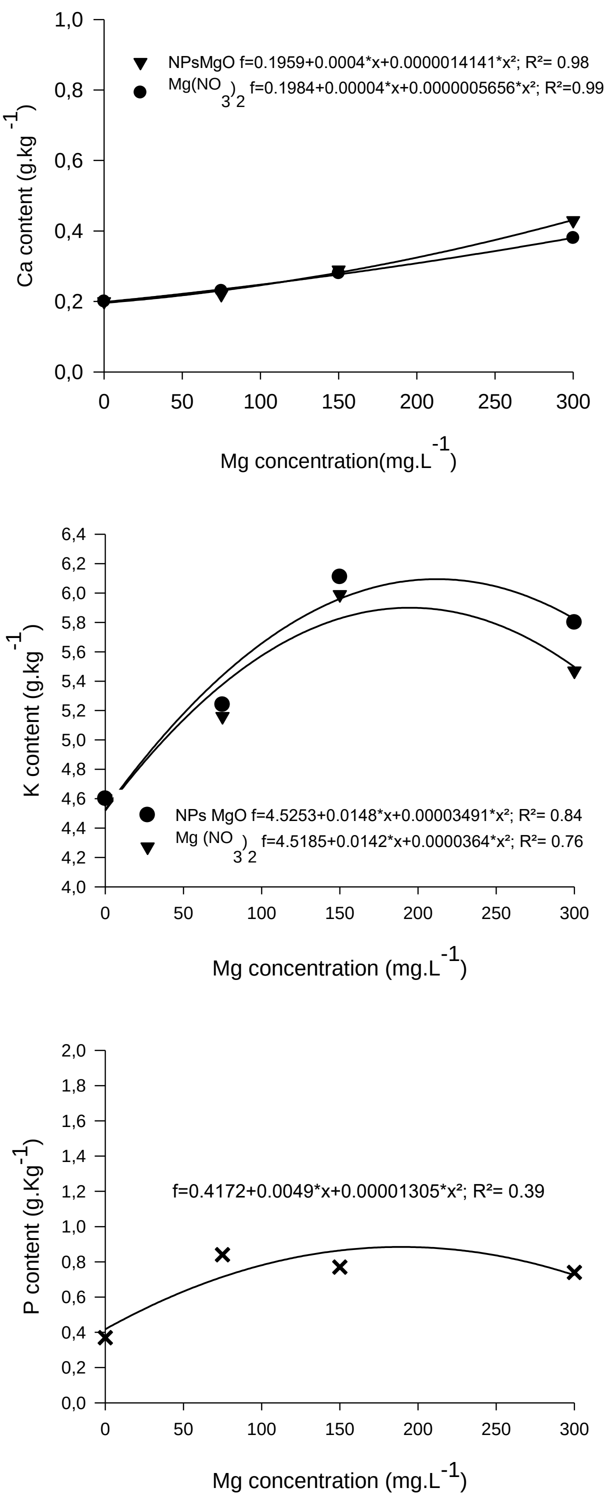

Fig 6. Calcium, potassium and phosphorus contents into maize grain on the basis of MgO-NPs and magnesium nitrate concentrations.

Brazil, with an average particle size of 25 nm and a purity of 99.5%. Magnesium nitrate [$(Mg(NO_3)_2$] (Vetec Brand, standard PA, with 98% purity) was used as non-nano (conventional) magnesium, as additional control for the control treatments (without magnesium application).

Maize seed treatment with nanoparticles and magnesium nitrate

The treatment of maize seeds was carried out the day before sowing at room temperature, in open glass reactors (Becker type), with magnesium sources and their respective concentrations, using ultrapure water as a vehicle and in sufficient quantity for rapid absorption by the seeds, without soaking them (3 mL kg^{-1} of seeds). After the treatment process, the seeds were packed in paper bags and labeled according to the respective treatments and them stored in a dry chamber with humidity of 50±5% and temperature of 8±4 ºC until the following day. All procedures of handling magnesium sources and preparing dilutions to the defined concentrations and seed treatment were carried out at the Laboratório de Plantas de Lavoura, da Universidade do Estado de Santa Catarina – Udesc SC/Brazil.

Soil characteristics and sampling

Soil samples were collected in July 2017 in areas of Caxambu and Tambo, and in September 2018 in the Fecav area. The soils were collected with a soil-press auger at five random points within each experimental area, and in each location, at a depth between 0 and 10 cm, according to instructions in the Manual de Adubação e de Calagem para os Estados do Rio Grande do Sul/BR e de Santa Catarina/BR (Cqfs-RS/SC 2016). After interpreting the results of the soil analysis (Table 1), the required amounts of fertilizer were applied, for a potential yield of 12 t ha^{-1} of grains, according to recommendations (Cqfs RS/SC 2016).

Experimental design

A complete randomized block design in a (3x6) factorial scheme was used to define the experimental conditions with different concentrations and types of magnesium sources. Three sources of magnesium and six concentrations with four replications were adopted as variables, totaling 72 plots for both Caxambu and Tambo experiments. For the Fecav experiment, a (3x6) factorial scheme with 24 repetitions was used, totaling 432 experimental plots. Such increase in the number of repetitions and area of the plots aimed to ensure greater assertiveness to commit the type I statistical error (or significance level of 5%) and, therefore, to reduce the experimental error.

Sowing procedure

The experimental area was allocated, and the fertilizer was distributed in the sowing row using a tractor-seeder set in all study sites. The perimeter of the plots was delimited using stakes and rope. In Caxambu, the sowing rows were previously marked and fertilized with the use of a seven-row seeder; each row was 0.46 m apart, and sowing was carried out on August 15, 2017. In Tambo, a five-row seeder was used, and rows were spaced 0.50 m apart; seeding was carried out on October 25, 2017. In these areas, sowing was carried out manually with the aid of a manual seeder to control seed density and distribution. In Fecav, sowing and fertilization were carried out on November 6, 2018, using a tractor fitted with a five-row seeder-fertilizer, with rows spaced 0.50 m apart, placing the seeds at a depth of 4 cm, in order to approach the reality of commercial crops.

Farming practices

In the Caxambu experiment, weed control was performed by using the herbicides glyphosate (Roundup WG 720 g acid equivalent – a.e. ha^{-1}, on September 19, 2017) and atrazine (Primoleo, 2400 g active ingredient – a.i. ha^{-1}, applied on October 10, 2017). In the Tambo experiment, the weeds were managed by applying the herbicides glyphosate (Roundup WG 720 g a.e. ha^{-1}, on December 4, 2017), atrazine (Primoleo, 2400 g a.i. ha^{-1}, on December 10, 2017) and mesotrione (Callisto, 168 g a.i. ha^{-1}, on December 18, 2017). In the Fecav experiment, the herbicides glyphosate (Roundup WG 720 g a.e. ha^{-1}, on December 3, 2018) and atrazine (Primoleo, 2400 g a.i. ha^{-1}, on December 13, 2017) were applied.

When the maize plants achieved the phenological stage V4, according to the phenological scale proposed by Ritchie; Hanway; Benson (2003), the excess plants were thinned to reach a population equivalent to 60,000 plants per hectare. After thinning, the crop was fertilized using 80 kg ha^{-1} of nitrogen (urea), distributed into two applications, and 25 kg ha^{-1} of equivalent K_2O by using potassium chloride. The other phytosanitary treatments were carried out according to the crop requirements after monitoring and constant observation of the experiments. There was a need to apply 0.8 L ha^{-1} of triflumuron insecticide (Certero, 48 g ai ha^{-1}) to control fall armyworm (*Spodoptera frugiperda*, J. E. Smith).

In the Tambo experiment, when the crop was at the V2 stage on November 10, 2017, hail occurred, causing damage to the plants. Most damage occurred on the leaves, stalk and apex of growth, and promoted the reduction of the leaf area, in addition to injuries that led to the incidence of diseases and the death of some plants. Immediately after hail, the insecticide thiamethoxam + lambda-cyhalothrin (Eforia, 21.2 + 28.2 g ai ha^{-1}) was applied on November 25, 2017, and top-dressing nitrogen fertilization was also applied at 100 kg ha^{-1} of N, using urea. These management practices aimed to assist plant recovery. In the three experiments, no fungicides were applied to manage fungal diseases in the shoot of maize plants.

Harvesting experiments

In the Caxambu experiment, maize was harvested on February 07, 2018, and the evaluations were carried out considering the usable area of the plot formed by three 5.0 m central rows, totaling a usable area of 6.9

m², where the plants of each experimental plot were collected to determine qualitative and quantitative agronomic traits.
Harvesting in the Tambo experiment was carried out on May 08, 2018, and the evaluations were made considering the usable area of the plot formed by two 7.0 m central rows, totaling a usable area of 7.0 m², where the plants of each experimental plot were collected to determine qualitative and quantitative agronomic traits.
Harvesting in the Fecav experiment was carried out on July 06 and 07, 2019, and the evaluations were made considering the usable area of the plot formed by a central row with 25.0 m in length, totaling a usable area of 12.5 m² where the plants of each experimental plot were collected to determine qualitative and quantitative agronomic traits.

Measured variables
a) SPAD index: the chlorophyll index on the maize leaves was assessed by measuring the SPAD value on the leaves with a portable chlorophyll meter (Minolta, model SPAD 502). It was carried out on plants in the six-leaf phenological stages V6, tasseling stage (VT) and R3, using four leaves per plot (from different plants). At the V6 and V10 stages, measurements were taken on the leaves (V6 and V10, respectively), and at the VT and R3 stages, measurements were taken on the index leaf. The measurements of the chlorophyll meter (one per leaf) were made in the middle third of the length of the sampled leaf blade, from the base, disregarding the space of the margin and the central rib of the leaf;
b) NDVI – the Normalized Difference Vegetation Index of the leaves of maize plants was assessed by using PlanPen 300U. Measurements were carried out on plants in the phenological stages of seven leaves (V7) and R5, using four leaves per plot (from different plants). At the vegetative stage, NDVI measurements were taken on the last fully expanded leaf of each plant. At the reproductive stage, they were performed on the index leaf (ear in the sheath). The readings were performed in the median part of the blade of each sampled leaf;
c) Plant height (PH): average plant height was determined in relation to the plant base (soil level) until the insertion of the flag leaf, when the plants were in the R1 phenological stage. This evaluation was carried out in five consecutive plants, on the central row of each experimental plot;
d) Ear insertion height (EIH): EIH was determined and defined as the distance between the base of the plant (soil level) and the base of ear insertion. This evaluation was carried out in five consecutive plants, on the central row of each experimental plot, when the plants were at the R1 phenological stage;
e) GPR - number of grains per row in the ear: GPR was determined by counting the number of grains per row, considering the average obtained from five ears harvested in the central row of each experimental plot;
f) NRG - number of rows of grains in the ear: NRG was determined by counting the number of rows, considering the average value in five ears harvested in the central row of each experimental plot;
g) NGE - number of grains per ear: NGE was determined by multiplying GPR by NRG, considering the average value in five ears harvested in the central row of each experimental plot;
h) U% - grain moisture: U% was measured in a sample of 100 g of grains free of foreign matter and impurities. This sample was weighed (wet weight - WW) and dried in a forced air circulation oven at 60 °C to constant weight (dry weight –DW). With both values, humidity on a wet basis was calculated according to the following equation (1):

$$U\% = 100 * \left[\frac{WW-DW}{WW}\right] \quad (1)$$

Where: WW = wet weight and DW = dry weight.

i) Thousand grain weight (TGW): the weight of 1,000 grains was determined using a sample of 1,000 grains from each experimental plot, in which an exact 1,000 grains were counted, using an electronic counter (Sanick, model ESC 2011). After counting, weighing was performed on a precision electronic scale (0.01 g), correcting the value obtained at the standard humidity of 13%.

j) GY – Grain Yield (t ha^{-1}): GY was determined by weighing the grains harvested in the usable area of each experimental plot for all experiments (WW). After obtaining the values of the plots, these data were corrected to the standard humidity of 13% (CW). Then calculations were made to estimate yield, according to equation 2.

$$GY = CW\left(\frac{t}{ha}\right) = WW * \left[\frac{(100-RH)}{(100-SH(13\%))}\right] \quad (2)$$

Where: CW = corrected weight, WW = wet weight, RH = real humidity and SH = standard humidity.

Acid digestion and quantification of magnesium, calcium, phosphorus and potassium
Samples were obtained from grains produced under seed treatment (ST) with the sources of magnesium oxide and magnesium nitrate nanoparticles in concentrations of 0, 75, 150 and 300 mg L^{-1}, including the control grains with three repetitions. The samples were ground in a knife-mill (Brand Solab, model SL-32).
An amount of 0.2 g of ground dry matter was transferred into an 80 mL digester tube, and 1 mL of 35% H_2O_2 (p.a.) was added. In the digestion chapel, 2 mL of 98% H_2SO_4 (p.a.) was added slowly, and then 0.7 g of the “digesting mixture” was added. This “digesting mixture” was composed of three pure reagents,

prepared as follows: 100 g of Na_2SO_4, 10 g of $CuSO_4$ and 1 g of Se; the three reagents were mixed in a mortar to form a fine powder. The tubes in the digester block were heated for 1 hour at 170 ºC and, subsequently, heated to a temperature of 360 ºC to form a greenish viscous liquid. The tubes were cooled under room conditions, and then 50 mL of distilled water was added. For each group of samples, a blank test (no vegetable sample) was prepared, adopting the same procedures and reagents.

Ca, Mg, K and P contents were quantified in the Laboratório de Solos – UNOESC, located in Campos Novos, SC, with the necessary dilution being performed for each mineral/analyte. Dilution was performed with a volume of 5 mL of each sample, in which an additional 5 mL of 0.3% strontium in 0.2 M HCl was added. The samples were diluted by adding 10 mL of deionized water; for potassium, 30 mL of deionized water was used for analysis by Atomic Absorption Spectrometry to determine the concentration of magnesium, calcium, phosphorus and potassium.

Statistical analysis

Data underwent analysis of variance using the F test ($p \leq 0.05$). When there was a statistically significant difference (qualitative factor - sources), means were compared with the Scott-Knott test ($P<0.05$) using the Sisvar software (Ferreira, 2019). For the quantitative factor (concentration), regression adjustment was performed using the Sigmaplot 10.0 software. Each experimental area was considered as an independent experiment.

Conclusions

Seeds treatment with magnesium oxide nanoparticles alters the phenology of maize plant, accelerating its cycle. The agronomic performance of the maize crop under field conditions cultivation is favored by the application of magnesium with increments in the range of 30% to 50% in yield associated with a reduction of up to four days in the crop cycle. The content of nutrients in the grains was positively influenced by the nanoparticles containing Mg in this order of magnitude P>K>Mg>Ca. The proposed method of introducing nanoparticles in seed treatment is shown to be a viable technology for agriculture.

Conflict of Interest: The authors declare no conflict of interest.

Ethics Approval: Not applicable

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