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The impact of calcite-based biostimulants applied during the growing season on sweet pepper seed germination

Utjecaj primjene biostimulatora na bazi kalcita tijekom vegetacije na klijavost sjemena paprike

Monika VIDAK[1,2] (✉), Dijana ČEH[1], Zlatko ŠATOVIĆ[1,2], Ivica BUDOR[3], Klaudija CAROVIĆ-STANKO[1,2]

[1] University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, Zagreb, Croatia
[2] Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Svetošimunska cesta 25, Zagreb, Croatia
[3] Agroledina j.d.o.o., Prigorska 32, Moravče, Croatia

✉ Corresponding author: mvidak@agr.hr

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ABSTRACT

Sweet pepper (*Capsicum annuum* L.) is an annual herbaceous plant of the *Solanaceae* family, which is one of the most important agricultural crops due to its exceptional economic importance, the nutritional value of its fruits and its confirmed antioxidant activity. The aim of this work was to determine the influence of the application of calcite-based biostimulants during the growing season on the germination of sweet pepper seeds. In the 2019 growing season, the calcite-based biostimulants Eco Green and Zeogreen+P were foliar applied on two sweet pepper cultivars (bell fruit type – 'Šorokšari' and capia fruit type – 'Kurtovska kapija'). At the end of the growing season, 300 seeds from each cultivar were collected (100 seeds from plants treated with each of the two biostimulants and 100 seeds from plants that had not been treated during the growing season - control) and placed for germination under controlled conditions at a constant temperature of 25 °C and a light regime of 16/8 h day/night. At the end of the experimental period, the seed germination parameters were calculated. It was found that the application of the calcite-based biostimulant Eco Green had a statistically significant positive effect on the germinability (G, %), mean germination rate (MR), the uncertainty of the germination process (U) and germination index (GI) in both cultivars and on mean germination time (MGT, day) in the cultivar 'Kurtovska kapija'. The calcite-based biostimulant Zeogreen had no statistically significant positive effect on the germination parameters, except for the germinability (G, %) in the cultivar 'Kurtovska kapija'.

Keywords: *Capsicum annuum* L., cultivar, Eco Green, germination parameters, Zeogreen+P

SAŽETAK

Paprika (*Capsicum annuum* L.) je jednogodišnja zeljasta biljka iz porodice *Solanaceae*, koja se zbog svoje iznimne gospodarske važnosti, nutritivne vrijednosti plodova i antioksidativnog djelovanja ubraja u najznačajnije poljoprivredne kulture. Cilj ovog rada bio je utvrditi utjecaj primjene biostimulatora na bazi kalcita tijekom vegetacije na klijavost sjemena paprike. Tijekom vegetacije 2019. dvije sorte paprike (babura - Šorokšari i rog - Kurtovska kapija) tretirane su biostimulatorima na bazi kalcita Eco Green i Zeogreen+P. Na kraju vegetacije sakupljeno je po 300 sjemenki od svake sorte (100 sjemenki s biljaka tretiranih svakim od dva biostimulatora i 100 sjemenki s biljaka koje nisu bile tretirane tijekom vegetacije – kontrola) i stavljeno na klijanje u kontrolirane uvjete pri konstantnoj temperaturi od 25 °C i svjetlosnom režimu od 16/8 h dan/noć. Na kraju pokusnog razdoblja izračunati su parametri klijavosti sjemena. Utvrđeno je da primjena biostimulatora na bazi kalcita Eco Green ima statistički značajan pozitivan učinak na klijavost (G, %), srednju stopu klijavosti (MR), pouzdanost procesa klijanja (U) i indeks klijavosti (GI) kod obje sorte te na prosječno vrijeme klijanja (MGT, dan) kod sorte 'Kurtovska kapija'. Biostimulator na bazi kalcita Zeogreen+P nije imao statistički značajan pozitivan učinak na parametre klijavosti, osim na klijavost (G, %) kod sorte 'Kurtovska kapija'.

Ključne riječi: *Capsicum annuum* L., Eco green, parametri klijavosti, sorta, Zeogren+P

INTRODUCTION

Three major current global challenges are food security, environmental degradation and climate change, which can be solved by more efficient use of nutrients in agricultural production and the stabilisation of yields through sustainable agriculture (Searchinger et al., 2018). Accordingly, organic agriculture has become increasingly important in recent years. One way to improve the sustainability of modern agriculture and overcome the challenges mentioned above is to introduce new technologies that improve grain yield and germination rates while maintaining crop quality (Rifna et al., 2019). Biostimulants are considered innovative agronomic tools, as evidenced by the increase in scientific publications and the constant expansion of their market (Bulgari et al., 2019). Although the most important effect of biostimulants from an agronomic point of view is to increase plant production, rapid seed germination is crucial for plant establishment and is the most important stage in the plant's life cycle (Wozniak et al., 2020). A plant biostimulant is a substance or microorganism that is applied to plants to improve germination, flowering, seedling and plant growth, fruit set, plant productivity and nutrient utilisation efficiency, to increase tolerance to a variety of abiotic stress factors and/or to improve the quality characteristics of plants, regardless of their nutrient content (Du Jardin, 2015; Rouphael and Colla, 2020).

Plants need a correct supply of minerals for optimal productivity, i.e. to organise their growth and development as well as their size and yield quality (White and Brown, 2010; Buczkowska et al., 2016). Calcium (Ca) is an essential macronutrient that is important for plants and plays an important role in increasing growth, seed quality and post-harvest storage duration, and to fulfill the structural role, moreover, Ca must be available for the plant in sufficient amounts (Anany et al., 2019; Thor, 2019). In general, calcium deficiency because of low soil availability is not very common but its low mobility through the phloem makes it difficult to translocate developing tissues, including fruits (Thor, 2019; Navarro-León et al., 2022). Since the plant takes up Ca mainly from the soil and the process of uptake, transport and distribution in a plant is influenced by many soil, biological and climatic factors, foliar fertilisation can improve nutrient uptake and efficiency in plants with a large leaf area and is particularly useful for poorly mobile nutrients, i.e. it is more economical and effective (Fageria et al., 2009; Buczkowska et al., 2016).

Sweet pepper (*Capsicum annuum* L.) is a very important vegetable plant worldwide due to its high nutritional value, a wide range of fruit colours, and its uses (Kim et al., 2019). It is consumed raw or cooked, as jarred food in powder form, as a spice or as a colouring agent in the agricultural, pharmaceutical and food industry (Guevara et al., 2021). Pepper is worm season vegetable, and the optimum germination temperature is about 25 - 30 °C (Demir et al., 2008; Abdel at al., 2016). Rapid germination and vigorous growth of early seedlings are essential for the establishment of peppers, a warm-season plant that germinates poorly in cold conditions (Samarah et al., 2016). In sweet pepper production, one of the most common plant nutritional disorders is the occurrence of blossom-end rot (BER) on the fruit, and it has been shown that the occurrence of BER on the fruits can be prevented by foliar application of Ca (Buczkowska et al., 2015). The fastest and most effective method of limiting Ca deficiency is foliar application of a Ca preparation to the parts of the plant where Ca translocation is limited, i.e. leaves and fruit (Buczkowska et al., 2015; Pimentel et al., 2023).

Various biostimulants have been used in sweet peppers to improve the physical and chemical properties of the fruit (Mahmood et al., 2017; Maraei et al., 2019; Majkowska-Gadomska et al., 2021), germination and germination parameters (Baroud et al., 2019; Fagioli et al., 2019). Foliar application of calcium during the growing season in sweet pepper (Cardoso et al., 2022) and common bean (*Phaseolus vulgaris* L.) (Costa et al., 2014; Anany et al., 2019) has been applied and increased seed germination, but to our knowledge there has been no study in which sweet pepper plants were treated with biostimulants during the growing season and then the effect on seed germination was tested.

Based on the aforementioned studies, it is believed that the application of calcite-based biostimulants during the growing season has a positive effect on sweet pepper seed germination and that said treatment could be used to achieve faster and more uniform germination and sprouting, especially at lower temperatures and direct sowing, as well as to increase the quantity and quality of production. The aim of this study was therefore to determine the influence of the foliar application of calcite-based biostimulants during the growing season on the germination of sweet pepper seeds.

MATERIALS AND METHODS

Plant Material and Experimental Design

The research material included seeds collected from plants of two cultivars of sweet pepper (bell fruit type – 'Šorokšari' (Figure 1A) and capia fruit type – 'Kurtovska kapija' (Figure 1B)) cultivated at the Maksimir experimental station (45° 49' N; 16° 20' E) of the Department of Seed Science and Technology (now Department of Plant Biodiversity) at the University of Zagreb Faculty of Agriculture, Croatia. Seedlings and plants were grown as described in Vidak et al. (2021).

The commercial calcite-based biostimulants Eco Green (Agroledina j.d.o.o.) and Zeogreen+P (Velebit Agro d.o.o.) were applied, according to producers' guidelines, three times during the growing season, and water represented control. Eco Green is a natural mineral powder obtained from calcite mineral by tribomechanical activation for foliar fertilisation of plant crops (Agroledina j.d.o.o.). Zeogreen+P is an organic, specially activated combination of calcite, zeolite and phosphorus for foliar fertilisation of plant crops (Velebit Agro d.o.o.). The first application of calcite-based biostimulants took place at the beginning of fruit formation (2 July 2019) and the second and third at 7-day intervals (9 and 16 July 2019). The biostimulants and water were sprayed until complete wetness of the plants. The treatments with the treatment abbreviation and concentration applied are given in Table 1.

Table 1. Treatments used in the experiment

Abbreviation	Treatment	Applied Recommended Concentration (g/L)
T1	Control (water)	-
T2	Eco Green	5
T3	Zeogreen+P	5

The harvest of fruits for this research was performed on 16 September 2019 and 300 seeds from each cultivar were collected (100 seeds from plants treated with each of the two biostimulants and 100 seeds representing the control). The experimental design was two-factor factorial arranged in a randomized complete design with four repetitions (4 Petri dishes) with 25 seeds each. A filter paper (Munktell 21/N, 580 x 580 mm, 80 g/qm) moistened with distilled water was placed on the bottom of the Petri dishes (Steriplan®, DURAN®, DWK

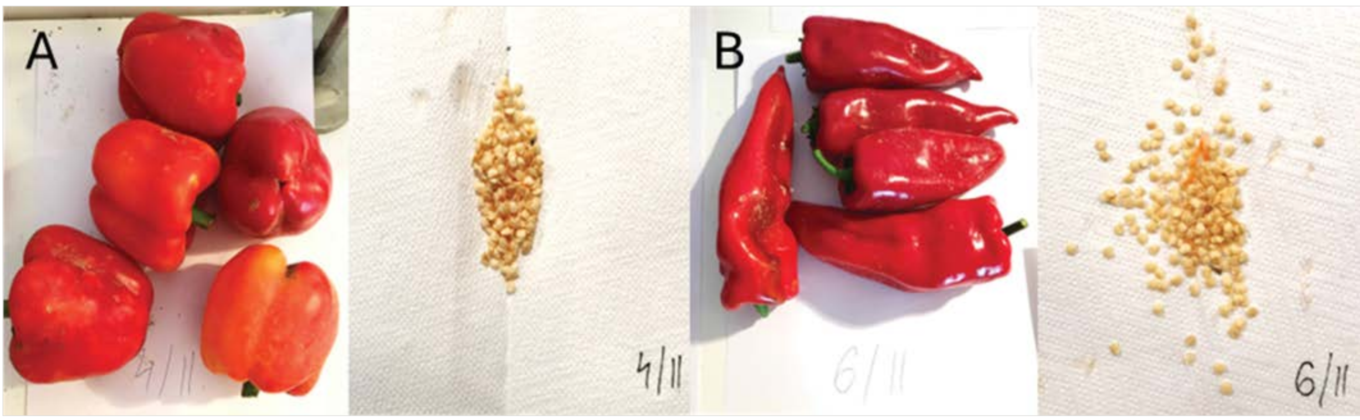

Figure 1. Fruits and seed of bell peper – 'Šorokšari' (A) and horn pepper – 'Kurtovska kapija' (B)

Life Sciences GmbH, Mainz, Germany) and the seeds were placed on top. The Petri dishes were placed in a germination test chamber under controlled conditions according to the instructions of the International Seed Testing Association (ISTA, 1993), at a temperature of 25 °C, 70% relative air humidity and a 16 h day and 8 h night regime. The number of germinated seeds (seeds with root size ≥ 2 mm) was determined every 48 h for 14 days.

Data analysis

The germination parameters [Germinability (G, %), Mean germination time (MGT, day), Coefficient of variation of germination time (CV_t, %), Mean germination rate (MR), Uncertainty of the germination process (U), Synchrony of the germination process (Z), Germination index (GI)] were calculated at the end of the experiment. The same equations presented in the work of Vidak et al. (2022) were used to calculate the germination parameters.

Using SAS software PROC GLM (SAS Institute, 2004), a two-way analysis of variance (ANOVA) was conducted to determine if there were significant differences in measured germination parameters among the treatments. Tukey's test ($P \leq 0.05$) was used to compare the mean differences between the values of the quantitative variables of the treatments. The original variables of G and CV_t expressed as percentages, were transformed before the analysis using formula y = arcsin (x / 100) (SAS Institute, 2004).

RESULTS AND DISSCUSION

In recent years, seed priming has emerged as an alternative and cost-effective technique that allows seeds to germinate quickly and evenly, overcoming environmental stress and reducing the time needed to absorb water and essential nutrients (Tian et al., 2023). In addition, some novel technologies such as high pressure and ozone treatment, pulsed electric field, ultrasound, ultraviolet, magnetic field, microwave radiation, non-thermal plasma, electrolyzed oxidising water and plasma-activated water have been proven to improve the germination and growth properties of various types of seeds (Rifna et al., 2019). Foliar application of biostimulants or some nutrients during the growing season not only promotes growth, yield, fruit quality and stress tolerance but also has a very important influence on the germination of the next generation of plants (Andreasen et al., 2014; Wozniak et al., 2020; da Silva Domingos et al., 2021; Boscaro et al., 2023). Also, the effect of biostimulants often depends on the type of biostimulant, the concentration and the plant species (Makhaye et al., 2021). For plants, Ca is one of the essential elements taken up directly from the soil and has essential functions in the formation, development and quality of fruit (Thor, 2019). Although it is available in soil and it is the third most-used plant nutrient, it is considered as a low-mobile nutrient in the plant (Prasad and Shivay, 2020). Young reproductive structures have low vascular differentiation, which restricts the flow of calcium to flowers and fruits during their development (Nakada-Freitas et al., 2021). Since the distribution of Ca in the plant is preferentially via the xylem, the redistribution rate for fruits and seeds is very low, making foliar application essential for the production and quality of seeds (Nakada-Freitas et al., 2021; Cardoso et al., 2022).

In this research, the influence of the application of calcite-based biostimulants Eco Green and Zeogreen+P during the growing season on the germination of seeds of two sweet pepper cultivars (bell fruit type – 'Šorokšari' and capia fruit type – 'Kurtovska kapija') was studied.

Analysis of variance (ANOVA) (Table 2) revealed a significant difference between the cultivars in the parameters germinability (G, %) and germination index (GI), while for other parameters the difference between the cultivars was not statistically significant. A highly significant difference between treatments was found for the parameters germination index (GI). A significant difference between treatments was found for the parameters mean germination time (MGT, day) and mean germination rate (MR). A weakly significant difference between treatments was found for the parameters germinability (G, %) and uncertainty of the germination process (U).

Table 2. Analysis of variance (ANOVA) for pepper seed germination parameters

Source of Variability	G (%)	MGT (day)	CV_t	MR	U	Z	GI
Cultivar (C)	**	ns	ns	ns	ns	ns	**
Treatment (T)	*	**	ns	**	*	ns	***
Interaction Cultivar x Treatment	ns	ns	ns	ns	ns	ns	ns

G (Germinability), MGT (Mean germination time), CV_t (Coefficient of variation of the germination time), MR (Mean germination rate), U (Uncertainty of the germination process), Z (Synchrony of the germination process), GI (Germination index)

P (F) – the significance of the F-test: $^{ns}P > 0.05$, $^{*}0.05 > P > 0.01$, $^{**}0.01 > P > 0.001$, $^{***}P < 0.001$

The coefficient of variation of the germination time (CV_t) and the synchrony of the germination process (Z) showed no statistically significant differences between the treatments. No statistically significant difference was found in the interaction between cultivar and treatment for any of the parameters.

The results of this study showed that foliar application of calcite-based biostimulant Eco Green (T2) during the growing season had significant impact on germinability (G, %), mean germination time (MGT, day), mean germination rate (MR), uncertainty of the germination process (U) in regard to control (T1) and germination index (GI) in regard to control (T1) and Zeogreen+P (T3) (Table 3).

Table 3 shows that the seeds of the plants treated with the calcite-based biostimulant Eco Green (T2) during the growing season achieved statistically significantly higher germination (G = 95.50%) compared to the control (G = 84.00%). There are no statistically significant differences in the germination of seeds from plants treated with the calcite-based biostimulant Zeogreen (T3) during the growing season (G = 86.50%) compared to the germination of seeds from plants treated with the Eco Green (T2) and the control (T1).

Table 3 shows that the seeds of plants treated with the calcite-based biostimulant Eco Green (T2) during the growing season achieved a statistically significant shorter mean germination time (MGT = 5.15 days) compared to the control (T1; MGT = 5.9 days). There are no statistically significant differences in the mean germination time of the plants treated with the calcite-based biostimulant Zeogreen (T3) during the growing season (MGT = 5.54 days) compared to the mean germination time of the plants treated with the Eco Green biostimulant (T2) and the control (T1). The calcite-based biostimulant Eco Green (T2) accelerated the germination process of the pepper seeds, especially at the beginning. The germination of the seeds started on the second day, when two germinated seeds were recorded in the control (T1). On the fourth day, twice as fast germination was observed in the seeds of plants previously treated with calcite-based biostimulant Eco Green (T2; 47.5% of germinated seeds) compared to the control (T1; 22.5% of germinated seeds). In addition, at the end of the germination test (day 14th), a significantly lower percentage of ungerminated seeds was observed in plants previously treated with calcite-based biostimulant Eco Green (T2; 4.5%) compared to the control (T1; 16%). According to Demir et al. (2008), the shorter mean germination time (MGT) of pepper seed lots can be used to predict the size and uniformity of seedlings in germination tests. The shorter mean germination time of the seeds after treatment with the calcite-based biostimulant Eco Green (T2) could influence the appearance of larger and more uniform seedlings (with longer roots and hypocotyls) and shorten the mean germination time.

The seeds of the plants treated with the calcite-based biostimulant Eco Green (T2) during the growing season achieved a statistically significant higher germination index (GI = 4.89) compared to the germination index of the seeds of the plants treated with the calcite-based biostimulant Zeogreen (T3) during the growing season (GI = 4.17) and those of the control (T1; GI = 3.87). There are no statistically significant differences in the germination index of seeds from plants treated with the calcite-based biostimulant Zeogreen (T3) during the growing season and those in the control (T1; Table 3).

In addition, the seeds of the plants treated with the calcite-based biostimulant Eco Green (T2) during the growing season achieved a statistically significant higher uncertainty of the germination process (U = 1.28) compared to the control (T1; U = 1.63), considering that a lower value indicates a better uniformity of germination. There are no statistically significant differences in the uncertainty of the germination process of plants treated with the calcite-based biostimulant Zeogreen (T3) during the growing season (U = 1.48 days) compared to the uncertainty of the germination process of plants treated with the calcite-based biostimulant Eco Green (T2) and those of the control (T1; Table 3).

The seeds of the plants treated with the calcite-based biostimulant Eco Green (T2) during the growing season also achieved a statistically significantly higher mean germination rate (MR = 0.19) compared to the control (T1; MR = 0.17). There were no statistically significant differences in the mean germination rate (MR = 0.18) of plants treated with the calcite-based biostimulant Zeogreen (T3) during the growing season compared to the mean germination rate of plants treated with the calcite-based biostimulant Eco Green (T2) and the control (T1; Table 3).

There are also no statistically significant differences between the treatments (T1, T2 and T3) in the synchrony of the germination process (Z) and the coefficient of variation of the germination time (CV_t) (Table 3).

Table 4 shows the interaction effects of the germination parameters of the experiment between two pepper cultivars for the germination parameters. It was found that foliar application of the calcite-based biostimulant Eco Green (T2) during the growing season had a statistically significant positive effect on the germinability (G; C1 = 96.00%, C2 = 95.00%), the mean germination rate (MR; C2 = 0.20), the uncertainty of the germination process (U) and the germination index (GI; C1 = 4.73, C2 = 5.07) in both cultivars and on mean germination time (MGT; day; C2 = 4.95) in the cultivar 'Kurtovska kapija' compared to the calcite-based biostimulant Zeogreen (T2) and the control (T1). The calcite-based biostimulant Zeogreen (T3) had no statistically significant positive effect on the germination parameters, except for the germinability (G) in the cultivar 'Kurtovska kapija' (C2).

Similar to our study, foliar application of Ca was observed in common bean, where it improved the physiological potential of seeds (Costa et al., 2014) and significantly increased the percentage of seed germination (Anany et al., 2019). In a similar study by Mohammadi et al. (2016), it was found that the seeds of four okra (*Abelmoschus esculentus* L.) cultivars derived from the micronutrient-treated plants had higher seed quality, i.e. seed germination. In addition, the study by Gupta et al. (2022) found that foliar application of nano-ZnO and nano-Fe_3O_4 improved the percentage of seed germination, germination rate and seedling vigour in cucumber (*Cucumis sativus* L.). Cardoso et al. (2022) found that foliar application of calcium to peppers increased pollen grain number, seed production and seed albumin

Table 3. Differences between treatments for pepper seed germination parameters

	G (%)	MGT (day)	CV_t	MR	U	Z	GI
T1	84.00^{B}	5.91^{A}	28.29^{A}	0.17^{B}	1.63^{A}	0.36^{A}	3.87^{B}
T2	95.50^{A}	5.16^{B}	24.61^{A}	0.19^{A}	1.28^{B}	0.45^{A}	4.90^{A}
T3	86.50^{AB}	5.54^{AB}	28.60^{A}	0.18^{AB}	1.49^{AB}	0.38^{A}	4.18^{B}

T1 (Control), T2 (Eco Green), T3 (Zeogreen+P).

G (Germinability), MGT (Mean germination time), CV_t (Coefficient of variation of the germination time), MR (Mean germination rate), U (Uncertainty of the germination process), Z (Synchrony of the germination process), GI (Germination index).

Values followed by the same letter in each column are not significantly different based on the Tukey test at 0.05 probability.

Table 4. Interaction effect of germination parameters of the experiment between two pepper cultivars for the germination parameters

	G (%)	MGT (day)	CV_t	MR	U	Z	GI
C1*T1	76.00^{B}	6.11^{A}	35.30^{A}	0.16^{B}	1.70^{A}	0.35^{A}	3.49^{C}
C1*T2	96.00^{A}	5.36^{AB}	24.32^{A}	0.19^{AB}	1.25^{A}	0.44^{A}	4.73^{A}
C1*T3	78.00^{AB}	5.49^{AB}	29.28^{A}	0.18^{AB}	1.25^{A}	0.46^{A}	3.79^{BC}
C2*T1	92.00^{AB}	5.70^{AB}	21.27^{A}	0.18^{AB}	1.58^{A}	0.37^{A}	4.25^{ABC}
C2*T2	95.00^{A}	4.95^{B}	24.91^{A}	0.20^{A}	1.31^{A}	0.45^{A}	5.07^{A}
C2*T3	95.00^{A}	5.60^{AB}	27.91^{A}	0.18^{AB}	1.72^{A}	0.31^{A}	4.56^{AB}

C1 ('Šorokšari'), C2 ('Kurtovska kapija'). T1 (Control), T2 (Eco Green), T3 (Zeogreen+P).

G (Germinability), MGT (Mean germination time), CV_t (Coefficient of variation of the germination time), MR (Mean germination rate), U (Uncertainty of the germination process), Z (Synchrony of the germination process), GI (Germination index).

Values followed by the same letter in each column are not significantly different based on the Tukey test at 0.05 probability.

content, and they concluded that calcium foliar application is recommended in pepper seed production, although it did not affect seed germination. Additionally, the study by Boscaro et al. (2023) showed that male-sterile inbred lines of maize (*Zea mays* L.) can benefit from a foliar application of mixed minerals and organic biostimulants during the growing season at the five-leaf stage, with the treatments improving root growth, producing a greater number of commercial seeds and increasing the number of grains per ear.

Although foliar application of calcite-based biostimulants at fruit formation of peppers had a positive effect on seed germination in our study, future research should focus on the application of calcite-based biostimulants first at flower formation to achieve better results. This is due to some studies that found that Ca application leads to high fertility, which increases pollen germination and pollen tube growth, and also increases seed yield and seed quality when applied during flowering (Costa et al., 2014; Anany et al., 2019; Nakada-Freitas et al., 2021).

CONCLUSIONS

Based on the results of this study, it can be concluded that the foliar application of calcite-based biostimulants during the growing season had a positive effect on the germination of the sweet pepper seeds. The calcite-based biostimulant Eco Green had a statistically significant positive effect on the germinability (G), mean germination rate (MR) and germination index (GI) in both cultivars and on mean germination time (MGT; day) in the cultivar 'Kurtovska kapija'. The calcite-based biostimulant Zeogreen had no statistically significant positive effect on the germination parameters, except for the germinability (G) in the cultivar 'Kurtovska kapija'.

However, foliar application of calcite-based biostimulants during the growing season in sweet pepper could be used to achieve faster and more uniform germination and sprouting of pepper seeds in the next growing season, especially under conditions of unfavorable temperatures and direct sowing, and to increase the quantity and quality of production, which is particularly important in seed production aimed at ensuring satisfactory quality and high germination of agricultural crop seeds.

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