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EXPLORING THE SYNERGISTIC EFFECTS OF CALCIUM NITRATE AND BIOSTIMULANTS ON SEED YIELD IN *CAPSICUM ANNUUM* L. VAR. *GROSSUM*

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Abstract

This work aims to study the potential for increasing seed yield in round pepper (Capsicum annuum L. var. grossum) by applying calcium nitrate in combination with various biostimulants. The biological material used in the experiment was the semi-early cultivar "Asteroid 204", obtained from ICDLF Vidra. Treatments included calcium nitrate alone and in combination with different biostimulants, specifically two seaweed extracts - one containing Ascophyllum nodosum and the other containing Ecklonia maxima - as well as two inocula based on mycorrhizal fungi, one enriched with Trichoderma spp. and the other with Bacillus spp. The results indicate that the combination of calcium nitrate and biostimulants - either seaweed-based or mycorrhizal fungi-based - can lead to an increase in seed yield and improvements in seed quality.

Key words*: biostimulants, mycorrhizal fungi, seed quality.*

INTRODUCTION

The vegetable sector faces difficulties in increasing vegetable yields, and reducing harmful effects on the environment and human health is a significant challenge. In conventional agriculture, modern practices have become increasingly limited due to environmental damage and the depletion of natural resources. Nowadays, agriculture relies heavily on the use of less harmful products, aiming to minimize chemical inputs (Almășan and Datcu, 2022).

Biostimulants are increasingly used to improve crop yield and quality. These products have diverse chemical compositions, often containing a mixture of organic and inorganic compounds, including essential macro- and micronutrients. The main categories of biostimulants include humic and fulvic acids, protein hydrolysates, seaweed extracts, chitosan, and inorganic compounds. They significantly contribute to enhancing plant growth, resistance, and productivity by stimulating natural physiological processes (Du Jardin, 2015; Singh et al., 2025; Apostol et al., 2022).

Seaweed extracts, rich in auxins and cytokinins, promote plant growth, flowering, and shelf life (Yao et al., 2020). Studies have shown that these extracts can significantly increase the height and number of branches in peppers (Sridhar & Rengasamy, 2012; Ozbay and Demirkiran, 2019; Vijayakumar et al., 2019; Ashour et al., 2021; Azzam et al., 2022).

Brown algae biostimulants, such as *Ecklonia maxima* and *Ascophyllum nodosum*, contribute to increased eggplant (Constantin et al., 2023a) and seed production. *Ecklonia maxima* treatments can lead to higher seed production in eggplant, although the response may vary among cultivars. *Ecklonia maxima*, *Ascophyllum nodosum*, and *Laminaria digitata* can also improve the seed vigor index in eggplant (Dascălu et al., 2025). Seaweed treatments can enhance the seed vigor index when used as seed priming treatments (Constantin et al., 2023b). Additionally, *Ascophyllum nodosum* and calcium nitrate can increase fruit number per plant, seed production in peppers, and improve the seed vigor index in peppers (Constantin et al., 2025). The use of foliar treatments with *Ascophyllum nodosum* and calcium nitrate increased pepper and seed production when applied more frequently (at 7 or 10-day intervals), with treatments applied every 7 days also positively affecting the seed vigor index (Buzatu et al., 2024).

Biostimulant treatments in peppers have shown a significant increase in the number of seeds per fruit, as well as a significant increase in seed weight. This is mainly due to improved nutrient uptake and accessibility to the plants (Deori et al., 2023).

Calcium ions are essential nutrients for healthy plant growth and development, contributing to fruit quality and yield (Ragab et al., 2021). They play a crucial role in the formation of the plant cell wall and function as signaling molecules, influencing various physiological and pathological processes (Malinovsky et al., 2014). The application of calcium sources, such as calcium sulfate and calcium nitrate, can improve soil properties and increase nutrient availability, proving effective in saline soils (Khaled et al., 2019). Calcium stimulates root development, accelerates crop maturity, supports flowering and seed production, confers cold resistance, and promotes cell division (Sandhya, 2014). A low calcium concentration in plant tissues can cause physiological disorders such as blossom end rot in peppers, tomatoes, and eggplants, especially during periods of maximum fruit growth. The absorption of calcium ions is genetically influenced and depends on soil conditions, as well as biological and climatic factors (Shakoor & Bhat, 2014).

The aim of this work was to study the possibility of improving seed yield in round pepper by using calcium nitrate combined with biostimulants of different origins.

MATERIALS AND METHODS

In this study, the germination process of bell pepper seeds of the Asteroid 204 cultivar, produced at the Research and Development Institute for Vegetable and Flower Growing Vidra, was monitored under conditions of fertilization with various biostimulant products. Specifically, calcium nitrate was applied alone and in combination with four different biostimulants.

The chemical composition of these products and their respective concentrations used in the treatments are presented in Table 1. The treatments were applied every 10 days, beginning with the formation of the first fruits.

Table 1. Treatment applications and their compositions

Product	Chemical composition	Concentration used
Calcinit	Total nitrogen - 15.5; calcium oxide CaO 26.5%	5 g/l
E-Dalgin	Pure extract of *Ascophyllum nodosum*	2 ml/l
Kelpak	Pure extract of *Ecklonia maxima*	2 ml/l
Triptolemus HV	Mycorrhiza content: 0.1%; *Rhizosphere* bacteria content: 1 x 10^5 CFU/g; *Trichoderma* spp. content: 1.2 x 10^8 CFU/g.	3 ml/l
Albit	Poly-beta-hydroxybutyric acid; natural biopolymer synthesized from soil bacteria: *Bacillus magaterium* and *Pseudomonas aureofaciens*, min. 0.62%; Nitrogen min. 7.5%; Phosphorus min. 6%; Potassium min. 4.5%; Magnesium min. 0.6%; Sulfur min. 2.7%	0.1 ml/l

The experimental variants were: V1 - untreated; V2 - treated with calcium nitrate; V3 - treated with calcium nitrate and E-Dalgin; V4 - treated with calcium nitrate and Kelpak; V5 - treated with calcium nitrate and Triptolemus HV; V6 - treated with calcium nitrate and Albit.

The experimental variants were arranged in the field according to the experimental technique, using randomized block design, with three repetitions. The surface area of each experimental plot was 13 m². Planting was performed on May 16 using 60-day-old seedlings.

Fruits were harvested at physiological maturity, indicated by their red color, and the number of fruits per plant reaching physiological maturity was recorded. Three harvests were conducted (September 1, 15, and 30). Seed extraction was performed after cutting the fruits, removing the receptacles, and detaching the seeds. Drying was carried out by exposing the seeds to the sun for 4 hours, followed by drying in a dry, ventilated room.

After conditioning the seeds in the selector, seed yield, seeds per fruit, and 1000-seed weight were calculated for each sample. The study was conducted under controlled laboratory conditions. Seeds were germinated in an incubator at alternating temperatures of 20°C for 16 hours and 30°C for 8 hours. For each treatment, six replicates of 100 seeds each were germinated (STAS **SR 1634/1999. Seeds for sowing. Germination test).

Seeds were evenly distributed on filter paper inside Petri dishes. The percentage of germinated seeds was counted daily. Germination was evaluated 14 days after sowing. The germination speed index (GSI) was calculated using the formula: $GSI = (G_1/N_1) + (G_2/N_2) + ... + (G_n/N_n)$, where G_n is the number of seeds germinated on day n and N_n is the number of days (Maguire, 1962).

Statistical analysis was performed using IBM SPSS Statistics version 26 (SPSS Inc., Chicago, IL, USA). The results were analyzed via one-way ANOVA. To determine differences among means, Duncan's Multiple Range Test was employed. A significance level of $p \leq 0.05$ was used to assess statistical significance.

RESULTS AND DISCUSSIONS

The results of this study highlighted the effects of the fertilizers tested on certain quality indices of the round pepper variety 'Asteroid 204' (see Table 2). Significant fluctuations were observed in 1000-seeds weight, fruits per plant, seed yield, seeds per fruit, germination percentage, and germination speed index under the influence of interactions with fertilization methods ($p = 0.000$). The fertilization technique significantly affected all evaluated components, with estimated effect sizes of 67.2% for 1000-seeds weight, 74.2% for fruits per plant, 51.3% for seed yield, 54.4% for seeds per fruit, 81.7% for germination percentage, and 60.7% for germination speed index, according to the ANOVA results.

Table 2. Main effects of fertilization variant and germination days on germination percentage, mean germination time, germination rate index of seeds of the "Asteroid 204" pepper cv.

Evaluated component		1000 seeds weight	Fruits/ plant	Seed yield	Seeds/ Fruit	Germination percentage	Mean germination time	Germination speed index
Fertilization technique	Sig.	*** (p=0.000)	*** (p=0.000)	*** (p=0.000)	*** (p=0.000)	*** (p=0.000)	n.s. (p=0.114)	*** (p=0.000)
	Effect size	67.2	74.2	51.3	54.4	81.7	24.6	60.7

*The symbols and letters of significance refer to the fertilization variant and germination days: n.s. = non significant and *** = very significant (at $p \leq 0.001$, respectively).

The weight of 1000 seeds ranged from 4.06 to 8.11 g, with an average of 5.23 g. The number of fruits per plant had an average of 4.83, with a minimum of 4.23 and a maximum of 5.31. Seed yield varied between 107.69 and 334.31 kg/ha, with an average of 231.15 kg/ha. The seeds per fruit averaged 791.11 mg, with fluctuations between 552.26 and 1223.90 mg.

The germination percentage was 70.47 ± 3.15%, with minimum and maximum values of 65.00% and 77.00%, respectively. The average germination time was 5.72 ± 0.27 days, ranging from 5.00 to 6.30 days. The germination speed index was 13.20 ± 0.94 seeds germinated/day, varying between 11.66 and 15.43 seeds germinated/day.

Seed germination was monitored from the beginning of the process until its completion. It started on the fourth day after sowing the seeds in Petri dishes and concluded on the eighth day for all treatment types applied. According to Figure 1, the evolution of seed germination over the five days analyzed was variable. The number of germinated seeds decreased on days 5-8 compared to day 4 for the fertilization variants V1 (untreated control), V2 (calcium nitrate), V3 (calcium nitrate and E-Dalgin), and V6 (calcium nitrate and Albit). In contrast, variant V5 (calcium nitrate and Triptolemus HV) showed maximum germination on day 5, while variant V4 (calcium nitrate and Kelpak) reached its peak germination on day 7.

Fluctuations in seed germination behavior were observed, influenced by the six fertilization variants. Variant V1 (untreated control) exhibited the lowest number of germinated seeds on day 5, while variant V4 (calcium nitrate and Kelpak) recorded the fewest germinations on day 6. In variant V6 (calcium nitrate and Albit), the number of germinated seeds decreased continuously starting from day 4, although a slight increase was noted on day 6.

Table 3. Statistical descriptors (mean, median, standard deviation, absolute minimum and absolute maximum) for germination percentage, mean germination time, germination speed index for the pepper cv. "Asteroid 204"

Statistical descriptors	1000 seeds weight	Fruits/ plant	Seed yield	Seeds/ fruit	Germination percentage	Mean germination time	Germination speed index
Mean	5.23	4.83	231.15	791.11	70.47	5.72	13.20
Median	4.99	4.87	230.26	778.21	70.00	5.78	13.14
Mode	4.33a	4.88	107.69[a]	552.26[a]	68.00[a]	5.32[a]	12.39[a]
Std. deviation	0.86	0.27	48.95	130.68	3.15	0.27	0.94
Range	4.05	1.08	226.62	671.64	12.00	1.30	3.77
Minimum	4.06	4.23	107.69	552.26	65.00	5.00	11.66
Maximum	8.11	5.31	334.31	1223.90	77.00	6.30	15.43

[a] Multiple modes exist. The smallest value is shown

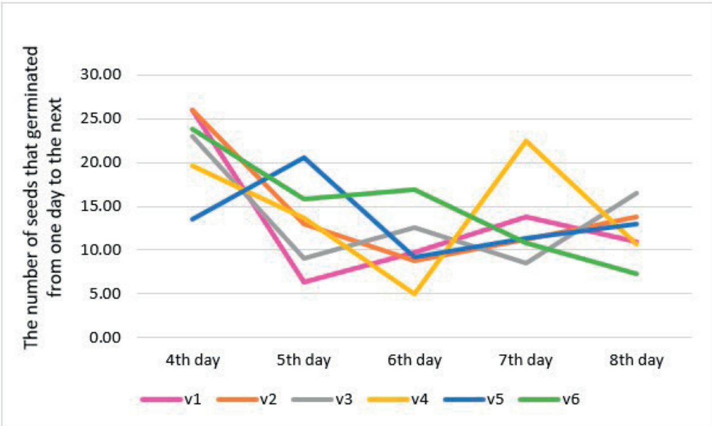

Figure 1. Seed germination evolution (days 4-8) according to fertilization variant

Among the experimental variants studied, the treatments used in variants V4 and V6 led to a significant increase in the weight of 1000 seeds, with the highest values observed in the case of variant V6 (an increase of 32.44%), which involved combined treatments with calcium nitrate and Albit (Table 4). This was followed by variant V4, where treatments with calcium nitrate and Kelpak were applied, resulting in an increase of 21.56%. Previously, increases in 1000 seed weight were obtained in round pepper using calcium nitrate treatments combined with a biostimulant based on *Ascophyllum nodosum* (Buzatu et al., 2025).

The number of fruits per plant increased significantly in all treatment variants compared to the untreated control. The increases ranged from 5.16% (in variant V6, treated with calcium nitrate and Albit) to 15.92% (in variant V4, treated with calcium nitrate and Kelpak). Previous studies have indicated that different biostimulants positively influence the number of fruits in peppers, with significant results observed when biostimulants based on *Trichoderma inoculum* (Constantin et al., 2024; Kumari et al., 2019), *Ascophyllum nodosum* (Buzatu et al., 2025), and *Ecklonia maxima* (Constantin et al., 2024) were used.

Seed yield and the amount of seeds per fruit increased significantly in all treated variants.

The highest seed yield value was obtained in the case of the variant treated with calcium nitrate in combination with the biostimulator Albit. This led to an increase of 62.92%. Similar differences were also observed in the case of the variant treated with calcium nitrate and the biostimulator Kelpak (V4), in which the increase was 60.94%.

The growth, in the case of the other treatment variants, ranged between 32.55% and 43.36%. Increases in seed yield in round peppers have also been obtained through treatments with calcium nitrate combined with a biostimulant based on *Ascophyllum nodosum* (Buzatu et al., 2025; Constantin et al., 2025).

Regarding the quantity of seeds/fruit, the highest values were also obtained in the case of variants V6 and V4, but significant increases were obtained in the case of all treated variants. The V6 variant led to a difference of 50.16%, and the V4 variant, to a different of 40.71%, compared to the untreated variant V1.

The positive effects of the treatments are due to both calcium nitrate and the biostimulants used. Nitrogen (Aminifard et al., 2012) and calcium (Buczkowska et al., 2016) are essential nutrients for optimal pepper yield. These have a great influence on the round pepper fruit quantity and quality (Constantin et al., 2024). The beneficial effect of seaweed-based biostimulants is due to the high content of phytohormones, amino acids and enzymes (Battacharyya et al., 2015).

Table 4. The influence of foliar treatments on fruits maturated and seeds quantity

Variant	1000 seeds weight (g)	Number of fruits/plant	Seed yield (kg/ha)	Seeds/fruit (mg)
V1	4.87±0.53 b	4.46±0.20 d	165.05±37.30 c	613.31±63.10 c
V2	4.63±0.23 b	4.78±0.12 c	218.78±56.48 b	768.90±69.13 b
V3	4.86±0.25 b	4.87±0.17 bc	231.94±11.51 ab	799.72±49.23 b
V4	5.92±0.38 a	5.17±0.13 a	265.63±21.59 ab	862.99±62.65 ab
V5	4.65±0.42 b	5.02±0.12 ab	236.62±26.49 ab	781.10±62.35 b
V6	6.45±0.99 a	4.69±0.11 c	268.90±47.90 a	920.67±188.32 a

*Duncan test: Significant differences ($p \leq 0.05$) are indicated by mean values in a column that do not share the same letter (a, b, c).

The germination percentage (Table 5) ranged from a minimum of 66.83% (V3, calcium nitrate and E-Dalgin) to a maximum of 74.67% (V6, calcium nitrate and Albit). According to Duncan's test, calcium nitrate and Albit treatment (V6) significantly improved the germination percentage by 5.17% compared to the untreated control (V1), followed by calcium nitrate treatments (V2), which increased germination by 3.33%, and calcium nitrate and Kelpak (V4), which resulted in a 2.00% increase.

A significant decrease in the percentage of germinated seeds was observed after applying calcium nitrate and E-Dalgin or calcium nitrate and Triptolemus HV (V5), with germination rates dropping from 69.50% (V1) to 66.83% (V3) and 67.50% (V5), respectively. The Asteroid 204 pepper variety exhibits superior germination performance, with a percentage of 85% (Șovărel et al., 2023). This performance is influenced by pedoclimatic conditions, the applied technology, the quality of the growing environment, and the level of infestation by pathogens (Hogea et al., 2023).

Previous studies suggest that seed germination performance is significantly influenced by the stage of maturation. Seeds that have reached physiological maturity contain fully developed embryos, giving them an advantage in the germination process compared to seeds at the green maturity stage (Brondo-Ricárdez et al., 2020; Dzib-Ek et al., 2025; Hernández et al., 2020; Sripathy & Groot, 2023) or at the overripe stage, where germination may begin to decline due to increased risks of seed damage (Dos Santos et al., 2016). The seed germination period is favorably influenced by certain substances with a biostimulating role (Dinu et al., 2019).

Environmental conditions such as temperature, humidity, and light also play a crucial role in seed germination. Seeds typically require warm temperatures (between 18-24°C) and adequate humidity to germinate effectively (Bewley & Black, 2013). The average germination time of seeds varies depending on environmental conditions and seed quality (Bewley & Black, 2013; Yazdanpanah et al., 2017). In general, under favorable conditions, germination time tends to decrease.

In the present study, a minimum germination time of 5.49 days was observed for the variant fertilized with calcium nitrate and Albit (V6), closely followed by calcium nitrate alone (V3) at 5.64 days, and calcium nitrate combined with E-Dalgin (V4) at 5.66 days. In contrast, the control variant exhibited a significantly higher average germination time of 5.81 days.

Table 5. Seed germination behavior

Fertilization option	Germination percentage	Mean germination time	Germination speed index
V1	69.50±0.84c	5.81±0.18a	12.91±0.50b
V2	72.83±1.17b	5.64±0.27ab	13.90±0.67a
V3	66.83±1.60d	5.66±0.32ab	12.73±0.74b
V4	71.50±2.26b	5.87±0.25a	13.03±0.49b
V5	67.50±1.05d	5.85±0.11a	12.25±0.35b
V6	74.67±1.37a	5.49±0.34b	14.39±0.91a

*Duncan test: Significant differences ($p \leq 0.05$) are indicated by mean values in a column that do not share the same letter (a, b, c).

Germination speed index is an indicator of the potential of a seed to germinate. The treatments applied to the seeds also led to significant improvements in the germination speed index (GSI), its value was 1.48 seeds germinated/day higher in the calcium nitrate and Albit-treated variant (V6) than in the untreated control variant (V1). Our results are in agreement with those obtained by Buzatu et al. (2025). Seed sources with a higher germination rate produce stronger seedlings, making them relevant in the selection process for breeding and improving the species (Wani & Singh, 2016).

The intensity of the correlations among the evaluated characteristics, presented in Table 6, indicates that the treatment variant resulted in a significant increase in 1000 seeds weight, fruits per plant, seed yield, and seeds per fruit (r = 0.516**, r = 0.406*, r = 0.613**, and r = 0.620**).

This result suggests that the treatment not only increased the total seed production but also improved the efficiency of each fruit in terms of the number of seeds produced.

Table 6. Correlation matrix between evaluated component

		Variant	1000 seeds weight	Fruits/ plant	Seed yield	Seeds/ fruit	Germination percentage	Mean germination time	Germination speed index
Variant	Pearson Correlation	1	0.516**	0.406*	0.613**	0.620**	0.228	-0.134	0.145
	Sig. (2-tailed)		0.001	0.014	0.000	0.000	0.181	0.437	0.398
1000 seeds weight	Pearson Correlation		1	0.168	0.435**	0.649**	0.502**	-0.156	0.369*
	Sig. (2-tailed)			0.328	0.008	0.000	0.002	0.363	0.027
Fruits/plant	Pearson Correlation			1	0.422*	0.368*	-0.142	0.92	-0.171
	Sig. (2-tailed)				0.010	0.027	0.410	0.595	0.319
Seed yield	Pearson Correlation				1	0.722**	0.250	-0.034	0.141
	Sig. (2-tailed)					0.000	0.141	0.844	0.411
Seeds/fruit	Pearson Correlation					1	0.267	-0.138	0.203
	Sig. (2-tailed)						0.116	0.422	0.235
Germination percentage	Pearson Correlation						1	-0.277	0.789**
	Sig. (2-tailed)							0.102	0.000
Mean germination time	Pearson Correlation							1	-0.794**
	Sig. (2-tailed)								0.000
Germination speed index	Pearson Correlation								1
	Sig. (2-tailed)								

**Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

A positive correlation between 1000 seeds weight and seed yield, seeds per fruit, and germination percentage was also highlighted (r = 0.435**, r = 0.649**, and r = 0.502**). This suggests that seeds with a larger mass may indicate better quality, which can contribute to a higher crop yield.

The relationship between seed yield and seeds per fruit is also positively significant and of very high intensity (r = 0.722**). The result indicates that, in order to improve seed yield, it is important to consider strategies that increase the number of seeds produced per fruit. Germination percentage correlates positively and significantly with the germination speed index (r = 0.789**).

The beneficial interaction between biostimulants, germination rate, and germination speed could enhance crop yields. A very high-intensity negative interdependence was found between mean germination time and the germination speed index (r = -0.794**). This interdependence offers insights into how plants adapt to various cultivation environments, aiding in the understanding of their diversity and survival strategies.

CONCLUSIONS

This study led to the following conclusions:

- treatments with calcium nitrate and Albit led to the lowest mean germination time, meaning a faster germination;
- the highest germination percentages were obtained in the case of variants in which calcium nitrate was combined with Albit, followed by the variants when it was combined with E-Dalgin and Kelpak;
- germination speed index was significantly positively influenced by treatments with calcium nitrate alone or combined with Albit;
- the weight of 1000 seeds was significantly positively influenced by treatments with calcium nitrate combined with Albit or Kelpak;
- the number of seeds per plant, seed yield and seeds/fruit were significantly influenced by all treatments used;
- the use of a biostimulator, in addition to calcium nitrate treatments, leads to better results in seed production and quality.

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