

Revolutionizing watermelon growth: harnessing the power of *Lithothamnium calcareum* nanoparticles

Revolucionando o cultivo da melancia: aproveitando o poder das nanopartículas de *Lithothamnium calcareum*

Moisés B. Tavares¹ , Andréia M. P. Negreiros¹ , Edicleide M. da Silva¹ , Gilmar V. S. Moreira¹ , Erika V. Medeiros² ,
Naama J. de A. Melo^{1*} , José I. de Jesus C. Carlos¹ , Rui Sales Júnior¹ 

¹Department of Agronomic and Forest Sciences, Universidade Federal Rural do Semi-Árido, Mossoró, RN, Brazil. ²Universidade Federal do Agreste de Pernambuco, Garanhuns, PE, Brazil.

ABSTRACT - *Lithothamnium calcareum*, a marine alga, can be used as a low-cost and eco-friendly tool to improve soil conditions, thereby enhancing crop development and meeting calcium requirements of plants grown in acidic soils. In this sense, the present study evaluated effects of different application rates, formulations, and application intervals of *L. calcareum* on development of watermelon plants, *Citrullus lanatus* cv. 'Crimson Sweet.' Two experiments were conducted in a completely randomized design. The first experiment evaluated different formulations and application rates of *L. calcareum*: micronized powder (MP: 50 Kg ha⁻¹), concentrated suspension (CS: 10 L ha⁻¹, 20 L ha⁻¹, and 30 L ha⁻¹), and nanoparticles (1 Kg ha⁻¹, 5 Kg ha⁻¹, and 10 Kg ha⁻¹). In the second experiment, MP, CS, and nanoparticle formulations were assessed under three application intervals (7+7+7, 10+10+10, and 14+14 days). Results indicated that application of 5 L ha⁻¹ increased shoot length, which was 45% higher than control. Moreover, Nano formulation (1 kg ha⁻¹) promoted the best root system performance, showing root dry mass three times greater than control. In the second experiment, seven-day application intervals associated with Nano formulation provided the largest increase in root dry mass (0.42 g). In conclusion, the use of *L. calcareum* nanoparticles applied at 1 kg ha⁻¹ and 7-day intervals represents the most efficient strategy to optimize watermelon root development, constituting a viable tool for organic production systems.

RESUMO - *Lithothamnium calcareum*, uma alga marinha, pode ser usada como uma ferramenta de baixo custo e ecologicamente correta para ajustar o solo, melhorando o desenvolvimento das culturas e atendendo às necessidades de cálcio das plantas em solos ácidos. O objetivo deste estudo foi avaliar os efeitos de diferentes dosagens, formulações e intervalos de aplicação de *L. calcareum* no desenvolvimento de plantas de melancia. Dois experimentos foram conduzidos em delineamento inteiramente casualizado. No primeiro, diferentes formulações e dosagens de *L. calcareum* foram avaliadas: pó micronizado (MP: 50 Kg ha⁻¹), suspensão concentrada (CS: 10 L ha⁻¹, 20 L ha⁻¹ e 30 L ha⁻¹) e nanopartículas (1 Kg ha⁻¹, 5 Kg ha⁻¹ e 10 Kg ha⁻¹). No segundo experimento, as formulações de MP, CS e nanopartículas foram avaliadas em três intervalos de aplicação (7+7+7, 10+10+10 e 14+14 dias). Os resultados indicaram que a aplicação do nível 5 L ha⁻¹ resultou em maior comprimento da parte aérea, sendo 45% superior ao controle. A formulação Nano (1 kg ha⁻¹) promoveu o melhor desempenho do sistema radicular, apresentando uma massa seca de raiz três vezes maior que a do tratamento controle. No segundo experimento, o intervalo de aplicação de sete dias associado à formulação Nano proporcionou o maior incremento na massa seca da raiz (0,42 g). Conclui-se que o uso de *L. calcareum* em nanopartículas na dose de 1 kg ha⁻¹ com intervalos de 7 dias é a estratégia mais eficiente para otimizar o desenvolvimento radicular da melancia, configurando-se como uma ferramenta viável para a produção orgânica.

Keywords: Calcareous algae. *Citrullus lanatus* L. Concentrated suspension. Fertilizer. Micronized powder.

Palavras-chave: Alga calcária. *Citrullus lanatus* L. Fertilizante. Suspensão concentrada. Pó micronizado.

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***Corresponding author:**
<naama.melo@ufersa.edu.br>

INTRODUCTION

The growth of agriculture has been supported by various inputs, primarily chemical fertilizers, which increase production costs and may lead to soil and water pollution (ZHENG; YIN; YU, 2022). In recent years, agricultural systems have increasingly emphasized sustainability, particularly from an environmental perspective (COSTA et al., 2024), includes adoption of distinct strategies such as alternative inputs (NEGREIROS et al., 2023). In this context, marine algae species, such as *Lithothamnium calcareum* (Lit), can precipitate calcium carbonate (CaCO₃) and magnesium carbonate (MgCO₃) crystals in their cell walls (CHATTERJEE et al., 2017; SILVA et al., 2021). This characteristic makes limestones derived from these algae a biogenic and, therefore, renewable source of solid carbonates. After extraction and processing, limestone deposited in cell walls can be used as a calcium source in animal feed and applied to the soil as a pH adjuster and/or calcium source for plants (GOETZ, 2008; TAKIYA et al., 2023).

Calcium is a macronutrient essential for plant development because of its critical roles in cellular metabolism. It acts as a key component of cell wall structure, maintaining cell integrity by facilitating adhesion between cell walls and consequently contributing to plant tissue growth and development

(SHARMA; SINGH, 2023). In soil management, calcium sources, such as calcitic lime (calcium carbonate) and dolomitic lime (calcium magnesium carbonate) play a crucial role in ameliorating soil acidity by increasing soil pH. This adjustment is essential for nutrient availability, soil health, and crop productivity (NEGREIROS et al., 2023). However, even in regions where liming practices are essential because of acidic soils with high aluminum content, calcium ion (Ca^{2+}) supply may still be insufficient to meet metabolic requirements of plants (SILVA et al., 2021). Therefore, research on alternative calcium sources is important to provide producers with low-cost tools to improve watermelon plant development.

Several studies have highlighted positive effects of *L. calcareum* as a calcium source on plant development in crops such as coffee (RODRIGUEZ et al., 2017), red pitaya [*Hylocereus undatus* (Haw.) Britton & Rose] (COSTA et al., 2015), and melon (NEGREIROS et al., 2023). Different commercial *L. calcareum* formulations are currently available on the market and may result in distinct crop responses. For instance, Negreiros et al. (2019) identified diverse responses in melon cultivation depending on formulation and application interval. Negreiros et al. (2023) observed positive effects of micronized powder and concentrated suspension formulations on melon yield, with productivity increases of 29.8 t ha^{-1} . Cavalcante et al. (2025) verified that

Lithothamnium represents a viable alternative to synthetic calcium and magnesium inputs by improving postharvest quality of watermelon fruits.

Although positive effects of *L. calcareum* on plant development have been reported, responses of watermelon plants to different formulations and application rates still remain poorly understood. In this study, we hypothesized that different formulations and application rates would promote distinct responses in watermelon development. Thus, the present study aimed to evaluate effects of different application rates, formulations, and application intervals of *L. calcareum* on development of watermelon plants, *Citrullus lanatus* (Thumb.) Matsum. & Nakai cv. 'Crimson Sweet.'

MATERIALS AND METHODS

Experiments localization

Two experiments were conducted in a greenhouse in the municipality of Mossoró, Rio Grande do Norte State, Brazil ($5^{\circ}11'15'' \text{ S}$, $37^{\circ}20'39'' \text{ W}$, and 18 m above sea level). The local climate is classified as hot semi-arid (*BSh*) according to Köppen classification (DUBREUIL et al., 2018). Figure 1 displays temperatures and relative humidity recorded during the experiment. Average temperature and relative humidity were 28°C and 66%, respectively.

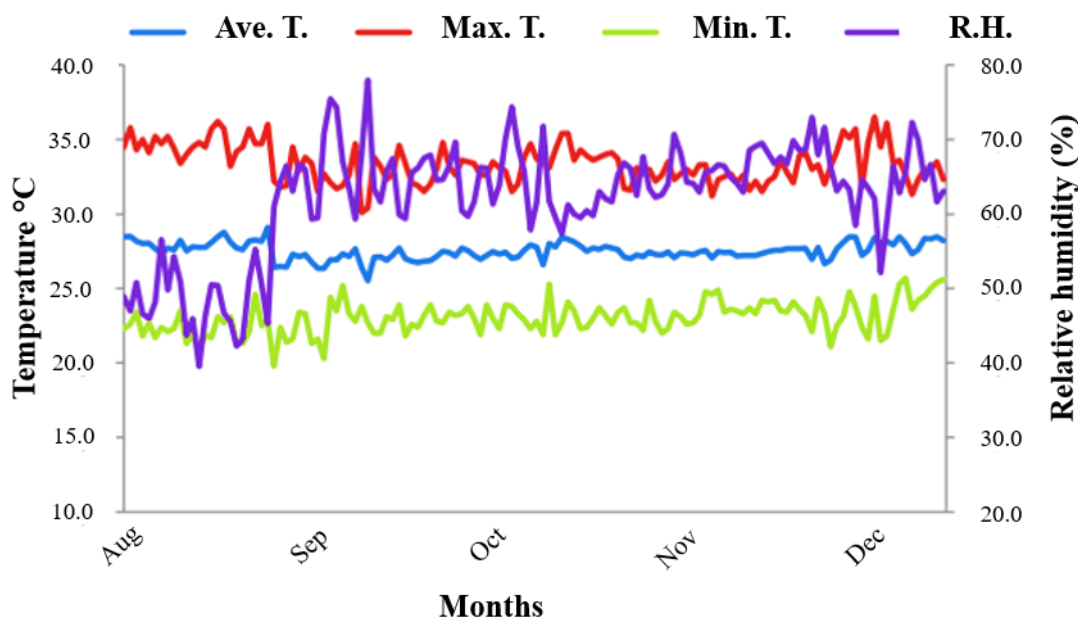


Figure 1. Minimum, average, and maximum temperatures and relative humidity recorded from August to December 2014.

Lithothamnium calcareum formulations

Concentrated suspension (CS) and micronized powder (MP) formulations of *L. calcareum* (Lit) were obtained from commercial product Primaz® (PrimaSea, Brazil), composed of 46% calcium oxide (CaO), 4.2% magnesium oxide (MgO), and 16.25% silica and insoluble materials. Nanoparticle formulation (Nano) was obtained by milling MP formulation

of Lit in a planetary ball mill (Fritsch, Pulverisette 7), producing particles ranging from 100 to 240 nm in size. Milling process was carried out at ambient temperature in a 45 mL tungsten carbide container containing four spheres with a radius of 15 mm and a mass of 25 g each. Ratio between sphere mass and powder mass was maintained at 10:1. Grinding was performed at 300 rpm for 20 h, processing 25 g of product per cycle.

Soil and seed preparation

Experiments were conducted in 1.0 L pots filled with a mixture of soil, sand, and commercial substrate at a 1:1:1 ratio. Soil was classified as eutrophic abrupt Red-Yellow Argisol with sandy clay loam texture and presented following chemical properties: pH (H₂O) = 7.0; organic matter (OM) = 0.26%; P = 210 mg dm⁻³; K = 0.43 cmol dm⁻³; Na = 0.15 cmol dm⁻³; Ca = 3.3 cmol dm⁻³; Mg = 1.8 cmol dm⁻³; and Al = 0.00 cmol dm⁻³.

Commercial substrate used was Tropstrato HT® (Vida Verde, Brazil), with dry density of 200 kg m⁻³, wet density of 500 kg m⁻³, pH of 5.8, moisture content of 60% (w/w), and water retention capacity of 130% (w/w).

Watermelon plants, *Citrullus lanatus* cv. 'Crimson Sweet,' received different application rates and formulations of Lit and commercial calcium-based products applied once at seven days after sowing (DAS). Treatments were added directly to mixture of soil, sand, and substrate. Plants were irrigated manually using a watering can. No additional fertilizers were applied during experimental period.

Experiment I - Watermelon growth under different *Lithothamnium calcareum* formulations and application rates

The first experiment was conducted in a completely randomized design (CRD) with 10 treatments and five replications, with one plant per experimental unit: 1. Control (without calcium source); 2. Lit concentrated suspension (CS) at 10 L ha⁻¹; 3. Lit CS at 20 L ha⁻¹; 4. Lit CS at 30 L ha⁻¹; 5. Calcium nitrate at 5 L ha⁻¹; 6. Lit nanoparticles (Nano) at 1 kg ha⁻¹; 7. Lit Nano at 5 kg ha⁻¹; 8. Lit Nano at 10 kg ha⁻¹; 9. Lit micronized powder (MP) at 50 kg ha⁻¹; and 10. Quantis at 10 L ha⁻¹.

At 15 days after sowing (DAS), shoot length (SL, cm), root length (RL, cm), shoot fresh weight (SFW, g), root fresh weight (RFW, g), and root dry weight (RDW, g) were evaluated for each plant. Shoot length was measured from crown to apical bud, whereas root length was measured from crown to tip of main root. Shoot and root fresh weights (SFW and RFW) were determined using a Shimadzu electronic scale (model BL-3200-H). Subsequently, samples were dried in pre-weighed paper bags in a forced-air oven at 65°C for 72 h until constant weight was achieved. Shoot and root dry weights (SDW and RDW) were then determined using an analytical balance.

Experiment II: Watermelon growth under different *Lithothamnium calcareum* formulations and application intervals

The second experiment was conducted in a completely randomized design consisting of 10 treatments with four replications. Treatments resulted from combination of three *L. calcareum* formulations [CS (10 L ha⁻¹), MP (50 kg ha⁻¹), and Nano (1 kg ha⁻¹)] and three application intervals (7+7+7+7, 10+10+10, and 14+14 days), in addition to control treatment.

Treatments comprised: 1. Control; 2. Lit Nano (7+7+7+7); 3. Lit Nano (10+10+10); 4. Lit Nano (14+14); 5. Lit MP (7+7+7+7); 6. Lit MP (10+10+10); 7. Lit MP (14+14); 8. Lit CS (7+7+7+7); 9. Lit CS (10+10+10); and 10. Lit CS (14+14).

Watermelon seeds were sown in a mixture of autoclaved soil, sand, and commercial substrate Tropstrato HT® at a 1:1:1 ratio in plastic pots with a capacity of 1.0 L.

At 35 DAS, shoot length (SL, cm), root length (RL, cm), shoot fresh weight (SFW, g), root fresh weight (RFW, g), and root dry weight (RDW, g) were evaluated for each plant.

Statistical analysis

In both experiments, data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test at 5% significance level. Quantitative variables were analyzed by regression using TableCurve curve-fitting software. Principal component analysis (PCA) was performed using PAST software (version 4.03; Paleontological Statistics software package for education and data analysis).

RESULTS AND DISCUSSION

Experiment I

Different application rates and formulations of *Lithothamnium calcareum* influenced initial growth of watermelon plants (Table 1). Plants treated with calcium nitrate (5 L ha⁻¹) showed greater shoot length, which was 45% higher than that of control treatment.

Interestingly, treatment with calcium nitrate [Ca Nit. (5 L ha⁻¹)] resulted in the highest values for shoot-related variables, whereas the best root system performance was observed in plants treated with Nano formulation (1 kg ha⁻¹). For instance, plants receiving this treatment exhibited root dry weight three times greater than that of control treatment. Calcium nitrate is a highly soluble source of nitrogen (N) and calcium (Ca). Nitrogen is primary nutrient responsible for vegetative growth because it is an essential component of chlorophyll and amino acids. High nitrate availability signals favorable resource conditions for leaf expansion, promoting cytokinin synthesis and translocation to shoots, which stimulates cell division and stem elongation (MARSCHNER, 2012). Negreiros et al. (2019) demonstrated that use of *Lithothamnium* nanoparticles significantly increases melon plant growth because of greater penetration of these elements into plant tissues and activation of basal metabolic enzymes. In addition, nanoparticles present extremely high surface-to-volume ratio, allowing minerals (Ca and Mg) and trace elements contained in algae to be released more efficiently into rhizosphere.

To better understand effects of different Nano application rates (kg ha⁻¹) of *L. calcareum* on shoot length (SL), shoot fresh weight (SFW), root length (RL), root fresh weight (RFW), and root dry weight (RDW) of watermelon plants, regression curves were developed to determine dose associated with maximum growth response (Figure 2). Shoot length increased with Nano application rates up to 3.18 kg ha⁻¹, which was estimated as rate associated with maximum growth. However, application rate of 1 kg ha⁻¹ resulted in shoot length of 8.74 cm, indicating that substantial benefits may be achieved even at low application rates. For remaining variables, an application rate of 1 kg ha⁻¹ promoted the greatest increases, followed by reductions in mean values beyond this threshold (Figure 2).

Table 1. Effects of different application rates and formulations of *Lithothamnium calcareum* on growth parameters of ‘Crimson Sweet’ watermelon plants.

Treatments ¹	SL (cm)	SFW (g)	RL (cm)	RFW (g)	RDW (g)
1. Control	7.05 d	0.73 b	26.40 ab	0.77 c	0.014 de
2. CS (10 L ha ⁻¹)	7.10 d	0.84 b	27.60 a	0.79 bc	0.022 cd
3. CS (20 L ha ⁻¹)	7.20 cd	0.85 b	25.00 bc	0.96 a	0.012 e
4. CS (30 L ha ⁻¹)	7.18 d	0.90 b	24.60 bcd	0.86 abc	0.010 e
5. Ca Nit (5 L ha ⁻¹)	12.70 a	2.93 a	24.20 cde	0.91 ab	0.014 de
6. Nano (1 Kg ha ⁻¹)	8.74 b	0.75 b	27.60 a	0.90 ab	0.042 a
7. Nano (5 Kg ha ⁻¹)	8.90 b	0.68 b	25.50 abc	0.61 d	0.032 b
8. Nano (10 Kg ha ⁻¹)	7.90 bcd	0.66 b	22.50 de	0.58 de	0.028 bc
9. MP (50 Kg ha ⁻¹)	5.90 e	0.68 b	20.20 f	0.88 ab	0.034 ab
10. Quantis	8.22 bc	0.67 b	22.10 ef	0.47 e	0.018 de
SMD	1.04	0.44	2.14	0.12	0.010
VC (%)	6.05	21.32	4.11	7.18	20.28

¹CS = concentrated suspension, Ca Nit = calcium nitrate, Nano = nanoparticles, MP = micronized powder, Quantis = plant biostimulant, SL = shoot length, SFW = shoot fresh weight, RL = root length, RFW = root fresh weight, RDW = root dry weight. Different letters indicate significant differences according to ANOVA followed by Tukey's test ($P < 0.05$).

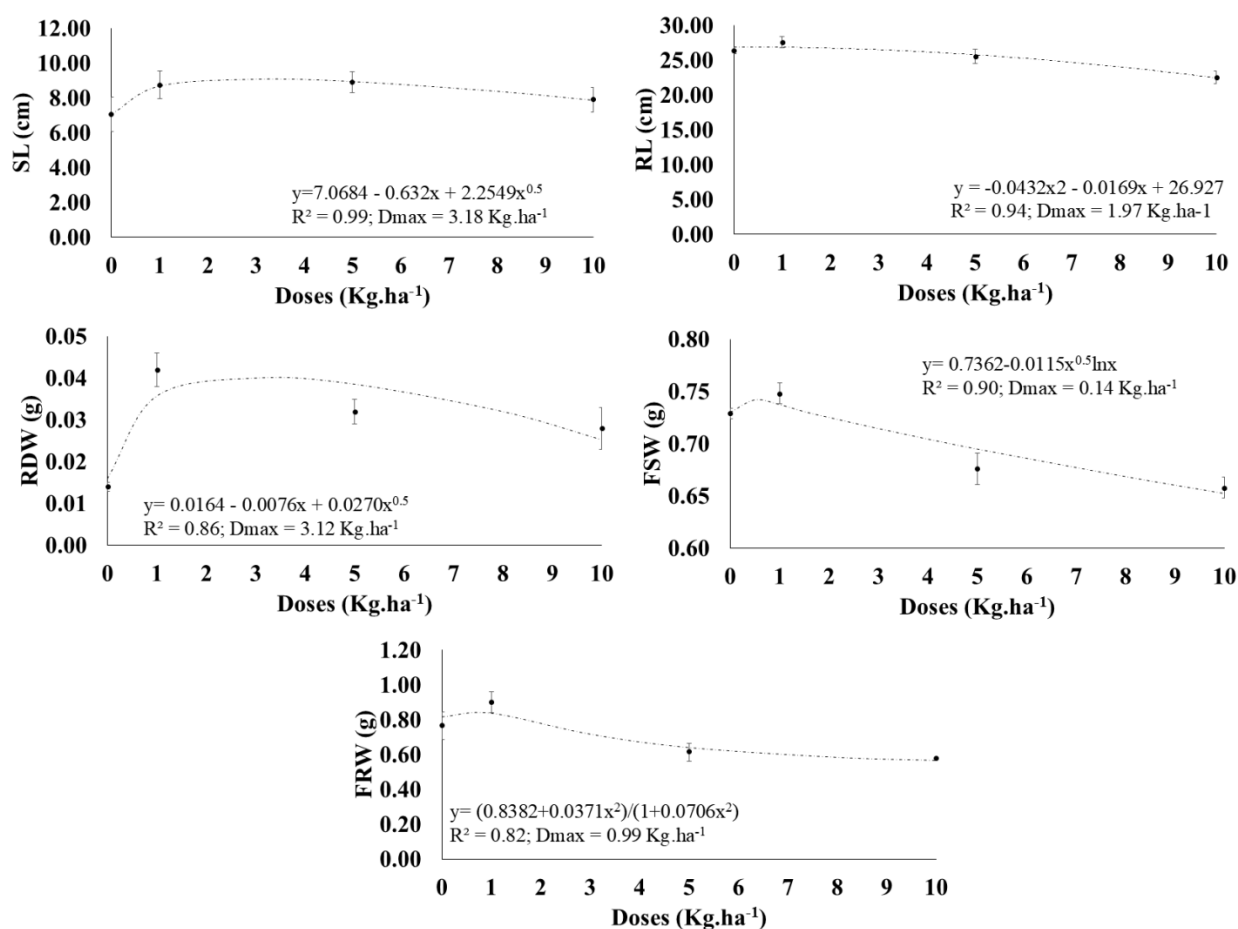


Figure 2. Effects of different dosages (Kg ha⁻¹) of *Lithothamnium calcareum* nanoparticles on shoot length (SL) (A), root length (RL) (B), root dry weight (RDW) (C), shoot fresh weight (SFW) (D), and root fresh weight (RFW) (E) of watermelon plants.

Results of present study confirmed hypothesis that *L. calcareum* nanoparticles promote watermelon growth. Nano formulation (1 kg ha^{-1}) promoted greater development of root system variables in watermelon plants. These findings suggest that *L. calcareum*, characterized as an ultraporous material with high specific surface area (RAMOS et al., 2023), may exhibit enhanced properties when transformed into nanoparticles. *L. calcareum* contains calcite, aragonite, magnesium, and humic substances (AMATUSSI et al., 2020; ALMEIDA et al., 2021; OLIVEIRA et al., 2021), which may contribute to nutrient absorption and root development in watermelon plants because these elements are essential for plant growth.

In addition to these compounds, *L. calcareum*-based products provide micronutrients important for plant development (NEGREIROS et al., 2023). These include iron (Fe), essential for chlorophyll synthesis and enzyme activation; manganese (Mn), involved in photosynthesis, nitrogen metabolism, and enzyme activation; boron (B), important for cell division, carbohydrate metabolism, and pollen germination; nickel (Ni), associated with nitrogen metabolism and enzyme activity; copper (Cu), required for photosynthesis, protein synthesis, and enzyme activation; zinc

(Zn), important for enzyme activity, hormone regulation, and chlorophyll production; and molybdenum (Mo), essential for nitrogen fixation and enzymatic activity in plants (SILVA et al., 2021). In addition, these nanoparticles may improve soil structure, increase nutrient availability, or stimulate hormones associated with root growth, thereby promoting early plant growth and development (NEGREIROS et al., 2023).

Principal component analysis (PCA) revealed distinct responses among Nano, CS, and calcium nitrate treatments (Figure 3). Principal component 1 (PC1) explained 43.25% of experimental variation, whereas principal component 2 (PC2) explained 23.68%, totaling 66.93% of total variation. Most important variables associated with PC1 were shoot length (SL) and shoot fresh weight (SFW), which were more strongly related to use of calcium nitrate as calcium source. In PC2, root length (RL) and root fresh weight (RFW) were more closely associated with *L. calcareum* application rates, particularly CS treatments at 10 and 20 L ha^{-1} and Nano formulation at 1 kg ha^{-1} . These results highlight greater effect of these formulations, especially at lower application rates, on root development of watermelon plants treated with *L. calcareum* (Figure 3).

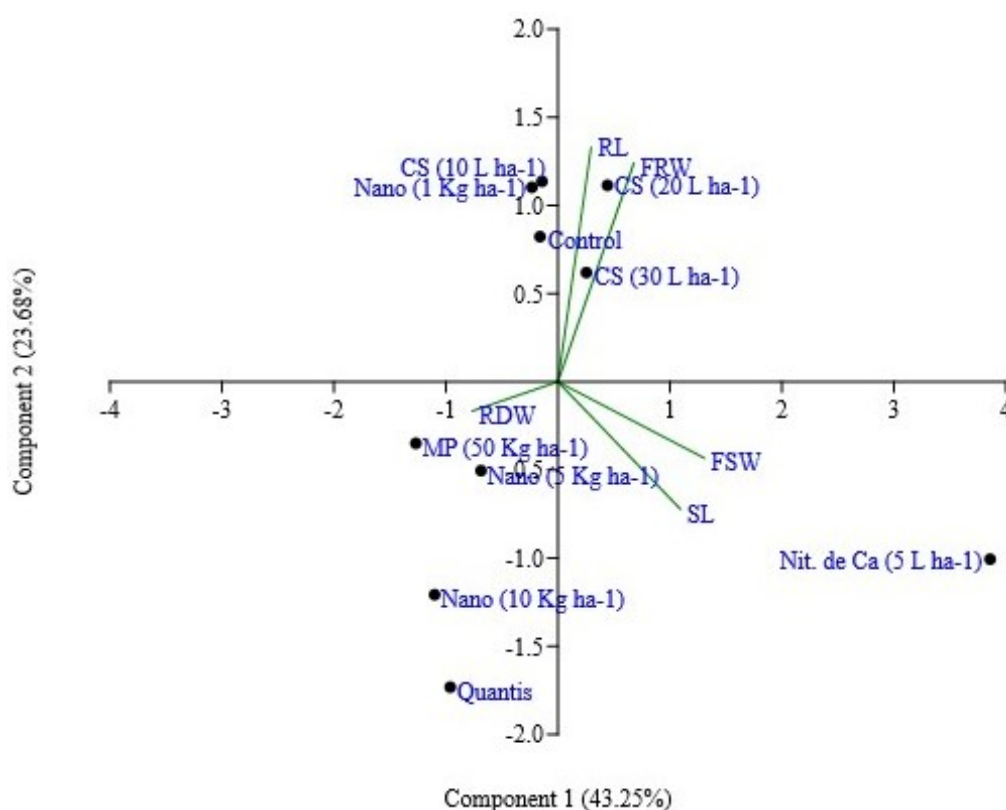


Figure 3. Principal component analysis (PCA) of shoot length (SL), shoot fresh weight (SFW), root length (RL), root fresh weight (RFW), and root dry weight (RDW) of watermelon plants subjected to different application rates and formulations of *Lithothamnium calcareum*: concentrated suspension (CS; 10, 20, and 30 L ha^{-1}), nanoparticles (Nano; 1, 5, and 10 kg ha^{-1}), and micronized powder (MP; 50 kg ha^{-1}), compared with control treatment and conventional calcium sources [Quantis (10 L ha^{-1}) and calcium nitrate (5 L ha^{-1})].

Observed reductions in growth variables at application rates above 1 kg ha^{-1} for root system variables and 3.18 kg ha^{-1} for shoot variables may be associated with

nutritional imbalance or excessive pH alteration in rhizosphere. Although *Lithothamnium* acts as a biogenic soil conditioner, excessive calcium carbonate input may increase

local pH to levels that restrict availability of essential micronutrients such as iron, zinc, and manganese, whose solubility decreases under more alkaline conditions (MARSCNER, 2012).

PCA revealed clear functional specialization among treatments. Strong association between calcium nitrate and shoot-related variables [shoot length (SL) and shoot fresh weight (SFW)] may be explained by immediate nitrogen supply in nitrate form, which is major driver of vegetative growth and leaf expansion. In contrast, association between *Lithothamnium* nanoparticles and root system variables [root length (RL) and root dry weight (RDW)] suggests biostimulant activity. Reduced particle size increases surface area available for release of calcium and trace elements, such as boron and zinc, which function as secondary messengers in signaling pathways associated with root hormones such as auxin (NEGREIROS et al., 2019; RAMOS et al., 2023).

Experiment II

Interaction between application interval and *L. calcareum* formulation affected growth of watermelon plants, except for SL, SFW, RL, and RFW. Ten-day application interval promoted the greatest SL (108.67 cm), not differing significantly from fourteen-day interval (95.75 cm). The largest SDW was observed under ten-day interval (7.13 g), which was superior to remaining treatments. In contrast, the highest RDW was obtained under seven-day interval (0.42 g), differing significantly from other treatments (Table 2).

Lithothamnium calcareum formulations evaluated individually did not significantly affect SL, SFW, or RL (Table 2). However, Nano formulation showed the highest SDW and RDW. Nano and MP formulations also resulted in greater RFW, differing significantly from CS formulation.

Table 2. Shoot length (SL), shoot fresh and dry weights (SFW and SDW, respectively), root length (RL), and root fresh and dry weights (RFW and RDW, respectively) of watermelon plants subjected to different application intervals (AI) and formulations (FL) of *Lithothamnium calcareum*.

AI	SL (cm)	SFW (g)	SDW (g)	RL (cm)	RFW (g)	RDW (g)
7+7+7+7	87.92 b	55.92 a	6.90 b	40.58 a	5.71 a	0.42 a
10+10+10	108.67 a	59.03 a	7.13 a	35.75 a	5.79 a	0.21 c
14+14	95.75 ab	53.80 a	6.18 c	40.67 a	5.34 a	0.34 b
LSD	20.13	12.22	0.02	7.47	0.65	0.02
FL*	SL (cm)	SFW (g)	SDW (g)	RL (cm)	RFW (g)	RDW (g)
Nano	102.08 a	58.57 a	6.88 a	39.17 a	6.10 a	0.43 a
PM	89.67 a	50.63 a	6.47 c	38.08 a	5.85 a	0.27 b
CS	100.58 a	59.55 a	6.85 b	39.75 a	4.90 b	0.28 b
LSD	20.13	12.22	0.02	7.47	0.65	0.02

*Nano= nanoparticles; PM= micronized powder; CS= concentrated suspension. SL= shoot length; SFW= shoot fresh weight; SDW= shoot dry weight; RL= root length; RFW= root fresh weight; RDW= root dry weight; LSD= least significant difference. Different letters indicate significant differences according to ANOVA followed by Tukey's test ($P < 0.05$).

Regarding interaction between application interval and formulation, Nano formulation promoted the highest RFW under seven-day application interval. The best application interval for MP formulation was ten days, whereas Nano formulation showed best performance under seven-day interval. Across different application intervals, Nano formulation resulted in the highest RDW values. Overall, seven-day application interval promoted the greatest growth increments in watermelon plants for most formulations evaluated. However, under MP treatment, fourteen-day interval did not differ significantly from seven-day interval (Table 3). Significant interactions between formulations and application intervals (Table 3) demonstrate that biological efficiency of *L. calcareum* in watermelon cultivation depends on both formulation granulometry and frequency of root exposure. Nano formulation, particularly under seven-day application interval, promoted expressive increases in RFW and RDW, reaching 7.36 g and 0.56 g, respectively. This response may be associated with high specific surface area of

nanoparticles, which enhances chemical reactivity and release of Ca^{2+} and Mg^{2+} ions into rhizosphere. According to Negreiros et al. (2019), reduced particle size of calcified seaweed facilitates penetration into plant tissues and activation of basal metabolic pathways, which may explain greater root vigor observed under Nano-7+7+7+7 treatment.

PCA of second experiment showed that principal component 1 (PC1) explained 43.39% of experimental variation, whereas principal component 2 (PC2) accounted for an additional 21.37%, totaling 64.76% of total variation. The main variables associated with PC1 were SFW, SDW, and RFW, which were strongly influenced by Nano formulation under seven-day application interval (Nano-7+7+7+7), grouped in the second quadrant. These results corroborate previous analyses, in which combination of the lowest Nano application rate and the shortest application interval promoted superior performance compared with other treatments. SL was also associated with PC1 and was more strongly influenced by Nano-10+10+10 and CS-10+10+10 treatments (Figure 4).

Table 3. Root fresh weight (RFW), shoot dry weight (SDW), and root dry weight (RDW) of watermelon plants subjected to different formulations and application intervals of *Lithothamnium calcareum*.

AI ²		RFW (g)	
FL ¹	7+7+7+7	10+10+10	14+14
Nano	7.36 aA	4.98 bB	5.95 aB
MP	5.28 bB	6.82 aA	5.43 abB
CS	4.50 bA	5.55 bA	4.63 bA
VC(%)		11.48	
AI ²		SDW (g)	
FL ¹	7+7+7+7	10+10+10	14+14
Nano	7.77 aA	6.72 bB	6.14 bC
MP	6.47 bB	6.61 cA	6.33 aC
CS	6.45 bB	8.05 aA	6.05 cC
VC(%)		5.34	
AI ²		RDW (g)	
FL ¹	7+7+7+7	10+10+10	14+14
Nano	0.56 aA	0.38 aB	0.36 aB
MP	0.36 bA	0.26 cB	0.39 aA
CS	0.35 bA	0.19 bC	0.28 bB
VC (%)		5.32	

¹Nano= nanoparticles; MP= micronized powder; CS= concentrated suspension. ² Application interval. *Lowercase letters in columns and uppercase in rows. Different letters indicate significant differences according to ANOVA followed by Tukey's test ($P < 0.05$). VC (%) = variation coefficient.

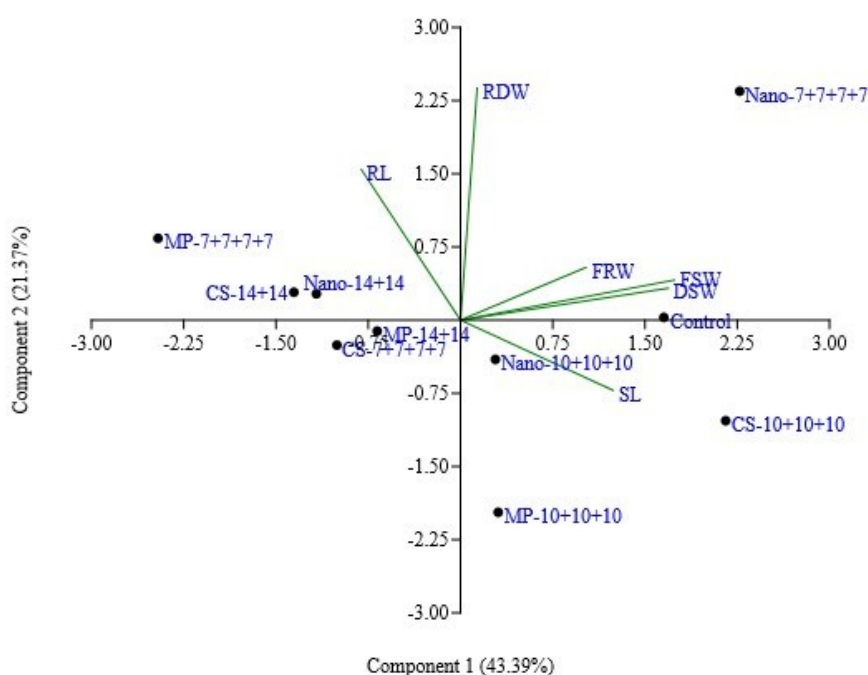


Figure 4. Principal component analysis (PCA) of shoot length (SL), shoot fresh weight (SFW), root length (RL), root fresh weight (RFW), and root dry weight (RDW) of watermelon plants subjected to different formulations and application intervals of *Lithothamnium calcareum*: concentrated suspension [CS; 10+10+10, 14+14, and 7+7+7+7], nanoparticles [Nano; 10+10+10, 14+14, and 7+7+7+7], and micronized powder [MP; 10+10+10, 14+14, and 7+7+7+7].

Lower application rates of Nano and CS formulations promoted optimal root development in watermelon plants treated with *L. calcareum*. These formulations likely stimulated root growth and proliferation by enhancing nutrient uptake and activating mechanisms associated with root development, as previously observed in other crops (RODRIGUEZ et al., 2017; NEGREIROS et al., 2019; RAMOS et al., 2023).

Overall, Nano and CS formulations, particularly at lower application rates, promoted greater root development in watermelon plants treated with *L. calcareum*. These findings highlight potential of *L. calcareum* as an effective strategy to improve watermelon cultivation. Even under conventional practices such as liming, calcium availability may remain insufficient to fully meet plant requirements. Similar responses were previously reported in melon plants treated with these formulations (NEGREIROS et al., 2019), suggesting that *L. calcareum* nanoparticles may represent a promising technology for use during planting or topdressing to alleviate calcium and magnesium deficiency symptoms under field conditions. In addition, this material may serve as an alternative organic fertilizer source (RAMOS et al., 2023).

CONCLUSION

Different formulations and application intervals of *Lithothamnium calcareum* exert distinct effects on vegetative development of watermelon cv. 'Crimson Sweet.'

Nano formulation at an application rate of 1 kg ha⁻¹ represented most efficient strategy for stimulating root system development, promoting root dry weight three times greater than that of control treatment. Maximum root vigor was obtained under seven-day application intervals.

Although calcium nitrate at 5 L ha⁻¹ promoted shoot length 45% greater than control treatment, use of nanostructured calcareous algae represented most effective technical strategy to optimize root architecture of watermelon plants. Therefore, for production systems focused on basal strengthening and sustainable watermelon cultivation, application of *L. calcareum* nanoparticles at 1 kg ha⁻¹ under weekly intervals is recommended.

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