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Original Article

# *Lithothamnion calcareum* Nanoparticles Increase Growth of Melon Plants

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**Abstract**

The application of alternative fertilizers to the soil in a sustainable way in order to supply nutrients to plants is important for growers and for the environment. Calcareous algae, *Lithothamnion calcareum* (Lit), is considered an alternative fertilizer because it is rich in nutrients, particularly magnesium and calcium, that are essential for plants. The objective of this study was to investigate the effect of different formulations, doses and fertilization intervals *of L. calcareum* on growth of melon plants. Two experiments were performed. The first experiment aimed to evaluate the effect of various formulations and doses of Lit on the growth of melon plants. The second experiment was carried out to evaluate the use of formulations and fertilization intervals of Lit. Formulations and doses of Lit had a significant effect on the efficiency in the development of melon plants. No impact was observed when using a concentrated suspension of Lit. However, four applications of Lit nanoparticle formulations every 7 days increased the growth of melon plants at doses close to 1 kg ha$^{-1}$. We provide the first data related to beneficial effects on melon growth of *L. calcareum,* in different formulations, application intervals and doses, applied to red-yellow soil.

***Keywords:*** application intervals; calcareous algae; *Cucumis melo*; fertilizer; formulations; powder-micronized

## Introduction

Calcareous algae have been used over time as corrective material on the French, English and Irish coasts to correct the acidic and/or calcium deficient soils. In these regions, the product is known as "Calcified Seaweed" or "Mäerl". Ancient studies report its use since 1186 (Le Bleu, 1983). France is the world's largest producer of bioclastic and lithoclast granulates for industrial use (Dias, 2000).

In Brazil a large continental shelf of North-Northeast limestone algae was discovered in the 1960's by researchers from the Oceanographic Institute of the Federal University of Pelotas. According to Kempf (1974), the potential exploration of the platform is comparable to the French "Mäerl".

Subsequent studies showed that the Brazilian continental zone represents an extensive coverage of carbonate sediments, extending from the Pará River (Lat. 00º 30 'S) to Rio Grande do Sul (Cavalcanti, 2011). This shelf presents deposits of algae with higher potential for economic exploitation than the French deposits. Among the different bioclastic materials found, sediment deposits formed by fragments of coral algae predominate, mainly of the genus *Lithothamnion* (Dias, 2000).

*Lithothamnion calcareum* (Pallas) Areschoug is a kind of seaweed of the Rhodophyta phylum, belonging to the order Corallinales of the family Lithothamniaceae. It has a red coloration and in its cell walls precipitate Calcium Carbonate ($CaCO_3$) and Magnesium (Mg) in the form of crystals of calcite, being a simple organic product (Woelkerling, 1985; Graham *et al.*, 2008; Guiry and Guiry, 2017).

*Lithothamnion calcareum* has been used in different ways as animal nutrition (Carlos *et al.*, 2011; Ucrós *et al.*, 2012; Hagg *et al.*, 2013; Lopes *et al.*, 2013; Schlegel and Gutzwiller, 2017), herbal medicines (Goetz, 2008), biopolymers (Thangavelu and Subramani, 2016) and cosmetics (Dias, 2000). In agriculture, *L. calcareum* products have been applied as fertilizers and soil acidity

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brokers (Melo and Furtini Neto, 2003; Goetz, 2008; Costa Neto *et al*., 2010). The use of *L. calcareum* in agriculture is characterized by the high proportion of Calcium (Ca) and Magnesium (Mg), essential elements for plants (Souza *et al*., 2007; Evangelista *et al*., 2013).

Studies reported by Filgueira (2008) showed that even in the face of cultural practices such as liming, the demand for calcium is not always sufficient to adequately supply the needs of plants. Therefore, the use of this alga may be a technology applied by the producers in planting or coverage to minimize symptoms of deficiencies of Ca and Mg in the field.

Although *L. calcareum* is widely used in different sectors of the world economy, its use in agriculture has been used increasingly in the last 20 years in Brazil. According to Kempf (1974), *L. calcareum* contains Ca=32%, Mg=2%, S=0.2%, Cl=0.2%, Mo=0.0005% and Fe=0.1%, which makes it a viable option in regions close to the coast, as a source of nutrition and soil correction.

Studies have shown that the use of this alga was efficient in the formation of seedlings in yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deneger) (Mendonça *et al*., 2006); in papaya (*Carica papaya* L.) (Hafle *et al*., 2009; Teixeira *et al*., 2009); in the development of 'Swingle' citrus (*Citrus paradisi* Mac × *Poncirus trifoliata* L. Raf.) (Araújo *et al*., 2007) and 'Cleopatra' mandarin (*Citrus reticulata* Blanco var. 'Cleópatra') (Cruz *et al*., 2008); in the increase of coffee productivity (*Coffea arabica* L.) (Evangelista *et al*., 2013) and red pitaya [*Hylocereus undatus* (Haw.) Britton & Rose], as well as increasing the quality of the fruits produced (Costa *et al*., 2015).

Although some studies demonstrate the importance of the use of this alga, there are still gaps regarding its use in soils used for melon cultivation. Its study is important as a tool to provide organic inputs for the management of this group of crops. Heretofore, there is no report of the use of this seaweed in melon culture (*Cucumis melo* L.), which makes this a pioneering work in this context. Due to the scarcity of information on the efficiency of *L. calcareum* in the development of melon plants, the objective of the present study was to evaluate the effect of different formulations, doses and application intervals of *L. calcareum* on the growth of melon plants.

## Materials and Methods

### *Algae sources*

*Lithothamnion calcareum* algae (Lit) were obtained as a commercial product Primaz® (PrimaSea, Brazil) and showed the following chemical properties: Calcium (CaO) = 46%; Magnesium (MgO) = 4.2% and Silica and insoluble = 16.25%.

### *Formulations of Lithothamnion calcareum*

Different formulations of Lit were used: concentrated suspension (CS), powder-micronized (PM) and nanoparticles (nano). The nano was obtained by milling the PM formulation to obtain sizes between 100 and 240 nm in a planetary type ball mill (Fritsch, pulverisette 7").

The high energy milling process was performed at room temperature in a 45 mL tungsten carbide container containing four spheres, also of the same material, each with a 15 mm radius and a mass of 25 g. The weight ratio of the ball to the powder was maintained at 10:1. The grinding was carried out at a rotation rate of 300 RPM, for a period of 20 h, 25 g of the product being processed by milling.

### *Soil and seed preparation*

The experiment was conducted in red-yellow, eutrophic, abrupt and sand-free sandstone, collected in a semiarid region in Rio Grande do Norte State, Brazil, showing the following chemical characteristics: pH ($H_2O$) = 7.0; MW = 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$^{-3}$; Al = 0.00 cmol dm$^{-3}$ (Santos *et al*., 2013). Soil was sterilized by autoclaving for 50 min at 121 °C and 1.2 ATM pressure in order to eliminate possible phytopathogenic agents.

The commercial substrate Tropstrato HT® from 'Vida Verde' was also used, with the following characteristics: humidity = 60% w / w, water retention capacity = 130% w / w, dry base density = 200 kg m$^{-3}$, wet basis density = 500 kg m$^{-3}$ and pH = 5.8.

Plastic pots containing two previously disinfected melon seeds of yellow melon cv. 'Gladial', from Rijk Zwaan, were used in both experiments. All irrigations were carried out with a manual irrigator, with a daily irrigation shift, aiming to keep the substrate always close to the field capacity.

Two experiments were carried out in a greenhouse (5°11'15" S and 37°20'39" W, 18 m altitude), with a BSh (hot semi-arid climate), according to Köppen's classification (Alvares *et al*., 2013).

### *Experiment I: Growth of melon in different formulations and doses of L. calcareum*

This experiment was conducted in a completely randomized manner, consisting of eight treatments and five replicates, which were: CONT-control; Lit CS (doses 10, 20 and 30 L ha$^{-1}$); Lit nano (doses 1.0, 5.0 and 10 kg ha$^{-1}$) and Lit PM at the dose of 50 kg ha$^{-1}$, respectively. All doses were calculated with 12,500 plants per hectare as the reference value.

Plastic pots with 500 mL capacity were filled with the Tropstrate HT® substrate. Melon seeds were applied two per pot at distances equidistant from the edges. Seven days after sowing, one plant per pot (replicate) was left.

At 15 days after sowing, the following variables were evaluated: plant height (cm), root length (cm), fresh and dry weight of shoot (g), fresh and dry weight of root (g). The plant height (H) was determined by measuring the height from plant crown to the apical meristem; root length (RL) was obtained by measuring the plant crown to the end of the primary root. A 'BL-3200-H' electronic scale of the 'Shimadzu' brand was used to measure the variables: shoot fresh matter (SFM) and root fresh matter (RFM). After the shoot, fresh matter (SFM) and root dry matter (RDM) were obtained by individually placing samples in previously weighed paper bags and drying in an oven with forced air circulation at 65 °C for a period of 72 hours, until reaching constant dry matter. The samples were then weighed on an analytical balance to obtain the dry weight of the root.

The data was submitted to analysis of variance. For the qualitative levels, orthogonal contrasts were constructed and the Scheffé test with 5% of significance was applied, aiming at the comparison between the treatments and those with the witnesses by ASSISTAT (Silva and Azevedo, 2016). For the quantitative levels the adjustment of regression modelling was performed using the curve adjustment program "Table Curve".

*Experiment II: Melon growth in different formulations and application intervals of L. calcareum*

The experimental design was completely randomized and distributed using a 3 × 3 + 1 factorial scheme. It included three formulations of *L. calcareum* (CS, PM and Nano); and three application intervals (7+7+7+7, 10+10+10 and 14+14 days of application) plus the control, with four replications. All experiments were repeated.

Plastic pots (1 kg capacity) were filled with a mixture of autoclaved soil, quartz sand, and the commercial substrate Tropstrato HT®, in a ratio of 1: 1: 1.

Melon seeds were sown chopped. Subsequently, the same applications were carried out as in the treatments of experiment I.

The data was submitted to analysis of variance (ANOVA) by ASSISTAT (Silva and Azevedo, 2016). The Tukey test ($P \leq 0.05$) was applied in cases where the treatment data presented significant differences according to the F test at $P \leq 0.01$ and $P \leq 0.05$ probability levels.

## Results and Discussion

*Experiment I: Growth of melon in different formulations and doses of L. calcareum*

The application of different formulations and doses of *L. calcareum* was efficient ($P < 0.05$) in the development of melon plants (Figs. 1 and 2). The analysis of the orthogonal contrasts indicates a significant effect at $P < 0.05$ among the different Lit formulations (CS, PM and nano) for most of the variables analyzed in the different contrasts (Table 1). In the case of contrast $\hat{y}1$ (control vs other treatments), the treatments in which Lit was applied showed more efficiency in relation to the control for H, RL and RFM of melon plants. The same effect was found for contrast $\hat{y}2$ (Lit CS vs Lit nano), in which all variables were significant ($P < 0.05$), except for RL (Table 1).

The SFM, RL, RFM and RDM of melon plants showed significant effects in the case of contrast $\hat{y}3$ (Lit CS vs Lit PM). However, the H of melon showed no difference between the formulations tested. Contrast $\hat{y}4$ (Lit nano vs Lit PM) presented a significant difference in four variables H, RL, RFM and RDM, which displayed increases of 15.3, 23.9, 31.3 and 125%, respectively (Table 1).

There was no impact ($P < 0.05$) of Lit CS application on the soil when comparing H and SFM because the maximum value was observed at 0 dose (Fig. 1). However, doses of 16, 6 and 20 L $ha^{-1}$ give maximum values to the variables RL, RFM and RDM, of 40.67 cm, 2.33 g, and 0.078 g respectively. The increases observed in these variables were probably due to the chemical and biological improvement behaviour with the application of *L. calcareum* in the substrate. According to Dias (2010), calcareous algae contribute to a more permeable soil and condition the effectiveness of the clay-humic complex, causing direct effects on the soil by increasing the nutrient absorption and improving soil structure, which allows greater root penetration and distribution. These factors contribute to production, productivity and quality of various crops. Here, plants that received Lit showed different results when applied in different formulations.

Melon plants treated with the Lit nano formulation increased H, RL, SFM, RFM and RDM (Fig. 2). Lit nano applied at doses of 1.2; 5.2; 1.2; 3.12 and 1.0 kg $ha^{-1}$ showed maximum RDM values of 17.88 cm of H; 44.76 cm of RL; 3.72 g of SFM; 3.12 g of RFM and 0.1 g, respectively (Fig. 2). These results suggest that *L. calcareum* in the nanoparticle formulation exerts a corrective action on the acidity of the substrate. Corroborating with the present study, Melo and Furtini Neto (2003) showed that the application of Lit improved nutrient assimilation, supporting the growth of the root system of melon plants, rather than that of the aerial part, when plants were treated in the CS formulation.

Increasing the dose of Lit in the nano formulation followed the same behaviour as Lit CS, which showed a reduction in the growth of melon plants with increasing dose (Fig. 2). Similar results were observed by Teixeira *et al.* (2009) when using doses of *L. calcareum* superior to 2 kg $m^{-3}$ in the production of papaya seedlings. This can be explained by the fact that high doses of the product may cause an imbalance in the cation ratios, resulting in possible phytotoxicity.

In all treatments with Lit nano application, the values of H, SFM, RFM and RDM were higher than those of the control. The highest H value for melons was recorded with Lit nano at 1.2 kg $ha^{-1}$. This dose was the maximum SFM point with 3.72 g (Fig. 2C). Melon plants developed better when Lit nano was applied at low doses, close to 1 kg $ha^{-1}$, for almost all variables, except for RL that obtained the maximum point at 5.2 kg $ha^{-1}$.

Studies reported by Mendonça *et al.* (2006) evaluating the quality of yellow passion fruit seedlings produced in different substrates and fertilized with *L. calcareum* showed that the use of this seaweed at doses up to 4.5 kg $m^{-3}$ was efficient in the development of yellow passion fruit seedlings. However, the authors observed that high doses of *L. calcareum* resulted in decaying effects on seedlings. Here, the high doses of Lit CS resulted in decaying effect on the initial growth of melon in shoot variables (Figs. 1A and 1C), but it was efficient in root variables (Figs. 1B, 1D, 1E). Such results can be explained as due to the short time of this experiment, and it is possible that a high dose of the product may have caused a phytotoxicity to the seedling, reflecting directly in its growth.

There is an ideal relationship between soil nutrients, as well as K, Ca and Mg cations. When the proportion of these nutrients is greater than indicated, it the manifestation of a nutrient deficiency can occur, as well as the reduction of its absorption by the plant (Zambolim *et al.*, 2012).

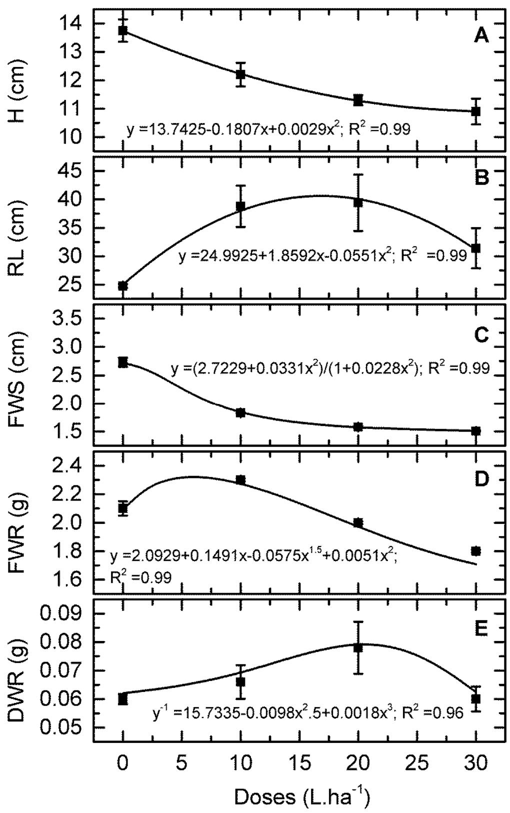


Fig. 1. Plant height (H), shoot fresh matter (SFM), root length (RL), root fresh matter (RFM) and root dry matter (RDM) of melon plants treated with different doses of *Lithothamnium calcareum* as a concentrated suspension

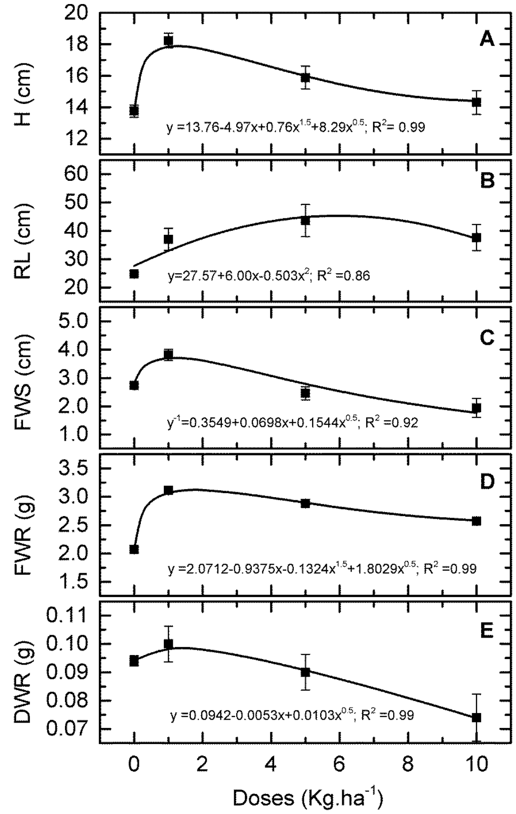


Fig. 2. Plant height (H), shoot fresh matter (SFM), root length (RL), root fresh matter (RFM) and root dry matter (RDM) of melon plants treated with different doses of *Lithothamnium calcareum* in a nanoparticle formulation

Table 1. Estimation of treatment contrasts to plant height (H), shoot fresh matter (SFM), root length (RL), root fresh matter (RFM) and root dry matter (RDM) of melon plants, submitted to different formulations of *Lithothamnion calcareum* (Lit)

| Variables | F values | | | | Group means | | | |
|---|---|---|---|---|---|---|---|---|
| | $\hat{y}_1$[1] | $\hat{y}_2$ | $\hat{y}_3$ | $\hat{y}_4$ | Control | CS | Nano | PM |
| H (cm) | 11.13* | 134.52* | 0.014ns | 69.19* | 11.70 | 11.47 | 16.14 | 14.00 |
| SFM (g) | 0.66ns | 30.55* | 19.26* | 0.23ns | 2.11 | 1.64 | 2.83 | 3.27 |
| RL (cm) | 4.39* | 0.81ns | 14.65* | 19.94* | 27.00 | 36.53 | 39.40 | 31.80 |
| RFM (g) | 105.19* | 1731.30* | 10.72* | 683.67* | 2.18 | 2.09 | 2.85 | 2.17 |
| RDM (g) | 0.29ns | 121.04* | 136.17* | 378.28* | 0.07 | 0.06 | 0.09 | 0.04 |

[1]$\hat{y}_1$ = control vs other treatments; $\hat{y}_2$ = *Lit*. concentrated suspension (CS) vs *Lit*. nanoparticles (Nano). $\hat{y}_3$= *Lit*. CS vs *Lit*. powder-micronized (PM). $\hat{y}_4$= *Lit*. Nano vs *Lit*. PM.

*Significant at 5% probability; ns = no significant.

*Experiment II: Growth of melon in different formulations and application intervals of L. calcareum*

The application of different formulations and forms of application of *L. calcareum* did not present significant effects on the initial growth of melon, using the Tukey test ($P < 0.05$). However, in unfolding, a difference was observed for the RFM variable, which, for an application interval at seven days (42.58 g), was superior in relation to the other treatments (Table 2).

The different formulations of the applied of *L. calcareum* followed the same pattern as the previous experiment, because the Lit nano was the most efficient treatment on melon growth compared to Lit CS and Lit PM (Table 2). Although there is no information in the literature on the effect of Lit nanoparticles on the growth of melon, it is suggested that the fractionation of this in nanoparticles increased the contact surface of the product with the root system in relation to the other formulation. This probably provided a higher solubilization and absorption of the nutrients by the roots of the seedlings, thus resulting in a greater development of these.

Melon plants that received Lit nano formulation showed superior results when compared to the PM and CS formulations, with application intervals of seven (7) days, for both SFM and RDM variables. However, the application of fourteen (14) days presented better SFM with the CS formulation (8.0 g). Regarding the growth of the root system, it was seen that Lit nano with application an interval of ten (10) days was superior (Table 3).

Studies conducted by Melo and Furtini Neto (2003) with the use of *L. calcareum* fertilizer as a corrective of soil acidity and source of nutrients for the common bean (*Phaseolus vulgaris* L.), concluded that the use of Lit promotes an increase in growth and production of beans plants. These results suggest that *L. calcareum* interacts, exerting a corrective action of the acidity of the substrate, improving the assimilation of the fertilizing elements, as well as favoring the development of the roots and aerial parts of the plants. Here, the application of Lit nano with an application interval of seven (7) days was the most efficient for increasing melon growth.

Table 2. Plant height (H), shoot fresh matter (SFM), root length (RL), root fresh matter (RFM) of melon plants, submitted to different formulations (FL) and forms of application (FA) of *Lithothamnion calcareum* (Lit)

| FA (days) | H (cm) | RL (cm) | SFW (g) | RFW (g) |
|---|---|---|---|---|
| 7+7+7+7 | 56.73 a | 12.93 a | 54.16 a | 42.58 a |
| 10+10+10 | 57.44 a | 13.41 a | 59.58 a | 30.83 b |
| 14+14 | 60.14 a | 12.62 a | 56.50 a | 33.08 b |
| FL[1] | H (cm) | RL (cm) | SFW (g) | RFW (g) |
| Nano | 65.91 a | 15.83 a | 62.91 a | 34.75 a |
| PM | 47.69 b | 9.96 b | 49.41 b | 36.25 a |
| CS | 60.71 a | 13.16 ab | 57.91 a | 35.50 a |

[1]Nano= Nanoparticles; PM= Powder-micronized; CS= Concentrated suspension. Different letters indicate significant differences (P < 0.05) by ANOVA followed by Tukey test.

Table 3. Shoot dry matter (SDM) and root dry matter (RDM) of melon plants, submitted to different formulations (FL) and forms of application (FA) of *Lithothamnion calcareum* (Lit)

| | SDM (g) | | | RDM (g) | | |
|---|---|---|---|---|---|---|
| FA<br>FL[1] | 7+7+7+7 | 10+10+10 | 14+14 | 7+7+7+7 | 10+10+10 | 14+14 |
| Nano | 7.10 aA | 6.91bA | 6.63cB | 0.82aA | 0.82aA | 0.77aB |
| PM | 5.77 aB | 5.44bB | 5.44bC | 0.29aC | 0.31aC | 0.28aC |
| CS | 5.65 cC | 6.97bA | 8.02aA | 0.57bB | 0.51cB | 1.10aA |

[1]Nano= Nanoparticles; PM= Powder-micronized; CS= Concentrated suspension.
*Different lowercase letter in horizontal and uppercase in vertical indicate significant differences (P < 0.05) by ANOVA followed by Tukey test.

## Conclusions

Results presented here are important in understanding how the use of natural algae influences melon growth and highlighting the benefits of *L. calcareum* as an alternative source of fertilizer. The application of *L. calcareum* clearly demonstrates the increase of initial growth of melon plants. In addition, application of *L. calcareum* in nanoparticle formulation in four applications every 7 days were more efficient in the plant growth development. We provide the first report about the use of of *L. calcareum* as an alternative source for fertilization of melon grown in red-yellow soil, and show the beneficial effect of different formulations, application intervals, and doses of *L. calcareum*. These data may be used as reference for future studies with different plants, as well as in the field and for longer periods.

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