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ORIGINAL RESEARCH PAPER

Effect of biostimulants and bioactivators on reproductive parameters of *Daucus carota* (L.)

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Abstract: *Daucus carota* L. (carrot) belongs to the Apiaceae family. Every year, tons of carrots are produced for national consumption and export, and Brazil is one of the largest producers of this vegetable. This study aimed to evaluate biostimulants and bioactivators inoculated in *D. carota* seeds and verify post-harvest morphological attributes for root length and diameter, fresh and dry mass of carrot root. The experiment was carried out in the field in full sun using pots. *Daucus carota* seeds were inoculated with 1.5 mL/kg seed^{-1} and grown in fertilized soil. After the reproduction period, carrots were collected during the commercial maturity period, and analyses were carried out in the laboratory. The results demonstrated that ST Pro and Stingray biostimulants applied had a positive effect on the root length parameter with average values of 20.7 and 21.1 cm, for root diameter Stingray demonstrated a difference between the other biological products with an average of 3.1 cm, for ST fresh mass Pro and Stingray presented average values of 91.0 and 91.8 g and for dry mass with an average of 10.1 g for ST Pro. The applied biostimulants demonstrated a positive effect, however, the Biozyme bioactivation did not demonstrate gains in any of the post-harvest parameters for the carrot cultivar ‘BRS Planalto’.

Keywords: *Daucus carota*; Carrots; Stingray; Biozyme; ST Pro; Seaweed extract.

1. Introduction

Daucus carota (L.) belongs to the Apiaceae family (formerly Umbelliferae) composed of different colors, varying lengths, and diameters. The cultivated carrot is one of the most important vegetable plants in the world because of its high yield potential and use as a fresh or processed product, presenting important vitamins, nutraceutical contents, and important biological activities such as antioxidant, antitumor, and photoprotective (Ahmad et al., 2019).

Annual global carrot production is more than 428 million tons and a total cultivation area of around 11.5 million hectares (FAO, 2019). According to Dawid et al. (2015), carrot cultivation is among the top 10 vegetable crops in the world. The consumption of fresh or processed carrots plays an important role in human nutrition, due to their high dietary value, significant content of carotenoids, vitamins, nutrients, and antioxidants (Leja et al., 2013; Umar et al., 2015).

Raees-ul & Prasad (2015) adds that cultivation of the crop is favored from September to November in tropical and subtropical regions while temperate conditions offer a wide option for cultivation throughout the year.

In Brazil, carrot cultivation is of great importance among the vegetables grown in different regions and types of Brazilian soil (Coelho et al., 2009). According to Marouelli et al. (2007), carrot cultivation ranks fifth among the most cultivated vegetable crops in order of economic importance for Brazil.

The average annual carrot harvest area in Brazil is 27 thousand hectares with an average production of 760 thousand tons, with an average wholesale value of 690 million reais. The cultivation is of great socioeconomic relevance for the regions of the States of Minas Gerais (MG), São Paulo (SP), Paraná (PR), Rio Grande do Sul (RS), Santa Catarina (SC), Distrito Federal (DF) and Bahia (BA) (Vilela et al., 1997; Zátare et al., 2006; Marouelli et al., 2007).

Observing the national and international carrot markets, we see that they are constantly expanding, and with this, the demand for greater production, quality, and good morphological aspects of this vegetable has been influenced by new technologies applied to different crops, including carrot cultivation.

In this sense, researchers aim to improve new fertilizer formulas and also the use of algae and fungal extracts in biostimulant and bioactivator formulas applied to obtain exponential germination rates, development in the vegetative and reproductive phases and mainly the morphological parameters of post-harvest (Luz et al., 2009; Ávila et al., 2016).

Biostimulants or bioactivators are natural or synthetic products composed of regulators and plant hormones, amino acids, nutrients, microorganisms, and extracts of red and brown algae such as *Ascophyllum nodosum* (Parrado et al., 2007; Sauvu-Jonasse et al., 2020; Soppelsa et al., 2020).

Biostimulants can be applied exogenously to plants, promoting passive changes in biological and biochemical processes, as well as obtaining higher productivity rates, improving the quality of marketable products, and obtaining better arrangements of plants and their products in the process post-harvest (Chiavegato et al., 2007; Zulfigar et al., 2020).

A. nodosum (Figure 1) is a brown marine alga cultivated in North American waters. The original name (basionym) was *Fucus nodosus* Linnaeus 1753. The species was transferred to the genus *Ascophyllum* (as Ascophylla) by Stackhouse (Papenfuss, 1950), under the name *Ascophyllum laevigata* (Guiry; Guiry 2020). The combination *Ascophyllum nodosum* was made by Le Jolis (1863) (Pereira et al., 2020).

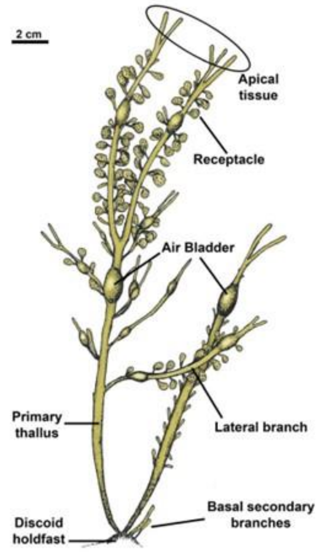

Figure 1. *Ascophyllum nodosum* with its main structures and branched system, which has a compressed primary thallus with apical growth. Source: Pereira et al. (2020).

A. nodosum has a chemical composition rich in bioactive compounds, poly- and oligosaccharides that are absent in plants, including laminaran, fucan, and alginate; betanas; sterols; vitamins; amino acids; macro and micronutrients; phytohormones, such as abscisic acid, cytokinins, and auxins; and unidentified compounds with hormone-like activities (Khan et al., 2009; Craigie, 2011; Quilty; Gado 2011; González et al., 2013; Calvo et al., 2014; Sharma et al., 2014; Yakhin et al., 2017; De Saeger et al., 2020).

Inoculation with plant regulator gibberellin (GA_3) was tested by Bevilaqua et al. (1998) with a dose of 100 mL L^{-1} in *D. carota* seeds, where they observed encouraging results on the acceleration of seed metabolism to a greater extent than vigor and better values in germination rates, emergence speed index and seed length aerial part. Promising results were also found by Vieira et al. (2021) where they evaluated the *Solieria filiformis* algae extract in inoculated *D. carota* seeds, the researchers demonstrated that the extract has effective biostimulatory activity on the development of carrots grown between 20-30 ºC.

In this sense, to improve new technologies to obtain better germination results, good vegetative and reproductive development, and superior morphological parameters with high quality in post-harvest, this study aimed to evaluate the inoculation of *Daucus carota* L. seeds with two biostimulants and a bioactivator regarding the morphological parameters of post-harvest carrots.

2. Material and Methods

2.1. Experimental location

The experiment was conducted in an open area in full sun in the research area Menezes Agricultural Research, Antônio Menezes & Filhos Farm, located in the municipality of Rio Verde, State of Goiás, Brazil, at geographic coordinates (17º43'06.7" S and 50º53'07.0" W) between August to November 2023. The soil in the area was classified as clayey Dystrophic Red Oxisol (Embrapa, 2013).

The experiment site is located at an altitude of 748 m above sea level, with an AW (tropical rainy) climate according to the Köppen-Geiger classification, with an average of 25.6 ºC and two well-defined seasons, one rainy (October to May) and dry (May to October. Rainfall between 750 and 2000 mm/year (Cardoso et al., 2014).

2.2. Equipments

Electric oven with air circulation (Thoth, Mod. Th-510-480, Brazil), digital caliper (Mitutoyo, Mod. 150 mm, Japan), and digital analytical balance (Shimadzu, Mod. AY220, Japan).

2.3. Carrot cultivar

The carrot cultivar 'BRS Planalto' (Sementes Isla, Brazil) was used. Rustic type, with excellent quality roots, intense orange color, uniform, smooth, and with a rounded tip, summer cycle 80 days, type of leaves cut with pubescent hair, orange skin color, green leaf color, white flower color, color orange pulp, resistance to leaf burn and nematodes, average length or height between 18-22 cm, diameter 3 cm and germination between 7-14 days. The germination rate by the producer is 90%. Study germination rate 93%.

2.5. Planting soil analysis

Soil parameters were determined in a layer between 0-20 cm deep with the following results: Ca = 2.13, Mg = 1.43, K = 0.30, P = 3.0, S = 9.0, Na = 1.0, organic matter (OM) = 61.1 and pH = 4.9. Clay = 30.3, Silt = 25.2 and sand = 44.4. The mineral supplement was added to the planting soil in the proportion of 2.5 g containing 04-14-08 (NPK + Fe, Mn, Mo, Cu, S, Ca, and Co) and doses containing 1.6 g of B and Zn. A top dressing was applied after 25 days and 65 days with 1.5 g of NPK.

2.6. Biostimulants and bioactivator

Biostimulant ST Pro (Grap)® mixed mineral fertilizers with Cobalt (Co) 1% (12.8 g/L), and Mn 7.0% (89.6 g/L), density 1.28 g mL-1, electrical conductivity (EC) = 51.53 mS cm^{-1}, saline index 19.56%, physical nature fluid in solution and *Ascophyllum nodosum* algae extract. Stingray (Koppert)® composed of K_2O 5.3% (61.46 g/L), Total organic carbon (C) 6% (69.60 g/L), pH = 8.0, density 1.16 g/mL^{-1} at 20 ºC, saline index 18% and extract of Ascophyllum nodosum algae 100%, and biostimulator Biozyme (Arysta LyfeScience)® composed of Nitrogen (N2) 1% (18 g/L), Potassium oxide (K_2O) 5% (60 g/L), B 0.08% (0.96 g/L), Fe 0.40% (4.8 g/L), Mn 1% (12 g/L), Zn 2% (24 g/L) and total C 3.50% (42 g/L).

2.7. Experimental design, planting, and cultural treatments

The experimental design adopted was a randomized block design (RBD) 3x8+1, where 3 were the number of inoculations (ST Pro, Stingray, and Biozyme), eight replications + one control.

The experiment was carried out in 5 L black high-density polyethylene pots. Each replication received 4.5 kg of soil with the addition of 2 g of limestone. In addition, the limestone was homogenized with the soil and allowed to react for 10 days with 2 daily waterings using natural lagoon water. After this period, *D. carota* seeds were inoculated using 1.5 mL/kg of $seed^{-1}$ of biostimulant and bioactivator.

After inoculation, the seeds remained resting for 15 min in a biostimulant and activator solution. After this time, 10 seeds were sown per repetition at a depth of 0.5 cm. 15 days after germination, thinning was carried out, leaving only 3 carrot seedlings per repetition. Daily watering was always carried out early in the morning and late in the afternoon with natural lagoon water.

20 days after germination, three applications were carried out at an interval of 15 days of the insecticide Fastac Duo® BASF via spraying using a pressurized backpack sprayer with CO_2 to control *Diabrotica speciosa* "little cow our *vaquinha*" and *Bemisia tabaci* "whitefly our *mosca-branca*".

2.8. Reproductive morphological analyzes

For post-harvest evaluation, 2 waxwings were randomly collected per pot, at commercial maturity (i.e., 100% of the surface with an orange color). Root length (RL) and root diameter (RD) were analyzed using a digital caliper with results expressed in centimeters (cm).

The fresh mass (FM) and dry mass (DM) of carrot roots were obtained using a digital analytical balance and the results are expressed in (g). For DM, the roots were placed in metal trays and transferred to an electric oven with forced air circulation with a temperature set at 75 ºC for 36 h.

2.7. Statistical analysis

Based on the average values obtained in the post-harvest parameters, an analysis of variance (ANOVA) was carried out and when a significant variation was found, the Tukey test was adopted with 5% significance using SISVAR software (Ferreira, 2019).

3. Results

3.1. Post-harvest morphological parameters

Positive effects were verified in the RL parameter for ST Pro and Stingray ($p < 0.05$) where they formed a statistical group (a) not differing from each other, but, different from the control and Biozyme bioactivator ($p > 0.05$). For the RD parameter, significant effects ($p < 0.05$) were obtained with an average value of 3 cm in plants with seeds inoculated by Stingray. The other biostimulants and bioactivators did not differ from each other or the control ($p > 0.05$). For FM again, the biostimulants ST Pro and Stingray demonstrate positive effects ($p < 0.05$), however, there was no significant difference between them, however different between the bioactivator and control. The control and bioactivator did not demonstrate a significant difference between them, forming a group (b). For DM, the average value was 10 g higher than the other treatments and control ($p < 0.05$) for the ST Pro biostimulant (Table 1).

Table 1. Post-harvest morphological parameters for *Daucus carota* L. investigating biostimulants and bioactivator.

Bio and Act	RL	RD	FM	DM
Control	14.95 b	2.48 b	62.40 b	6.06 c
ST Pro	20.74 a	2.71 b	91.00 a	10.10 a
Stingray	21.17 a	3.12 a	90.84 a	9.32 ab
Biozyme	17.02 b	2.58 b	64.25 b	7.07 bc
CV (%)	10.95	7.68	19.67	27.15

Note: Means followed by the same letters are statistically equal by the Tukey test at 5% significance. Source: Authors, 2024.

4. Discussion

Post-harvest morphological patterns are always a concern for producers, traders, and consumers who want high-standard agricultural products. As observed in this study, biostimulants demonstrated positive effects on morphological patterns in carrot crops for RL, RD, FM, and DM, where they demonstrated the high potential in increasing this new technology for the development of vegetables capable of providing producers and traders with quality to the final product.

Promising studies with other biostimulant compounds were also described by Vieira et al. (2021) evaluating the inoculation of carrot seed cultivars 'Brasília, Danvers, Splanada, and Planalto' with an extract based on S. filiformis algae and additional micronutrients 7.5% Mn and 13% S in variable doses 0, 2, 4 and 8 mL L^{-1}. In this study, the authors found averages higher than the control at temperatures ranging between 20-30 ºC, with germination rates and germination speed, length of the aerial part and roots, and aerial and root dry mass encouraging for the carrot cultivars analyzed *in vitro*. Encouraging results were also found by Ávila et al. (2016) evaluating carrot cultivar 'Brasília' where they found that the application of N associated with the biostimulant Stimulate composed of 0.009% kinetin, 0.005% GA and 0.005% AUX resulted in a greater average length of 12.8 cm and productivity with an average of 40,000 kg ha^{-1} of Extra A carrot, commercial carrot and waste carrot.

According to Shukla et al. (2016), brown and red algae (Rhodophyta) have carrageenans in their cellular structure that promote plant growth, activating synthesizing enzymes, participating in the process of cell division, in metabolic routes during the process of germination and seedling growth. In other vegetables, Torres et al. (2018) observed significant effects in the treatment of lettuce seedlings (*Lactuca sativa* L.) with extract of red algae *Gracilaria caudata* and *G. domingensis*, which promoted better root and shoot development when compared to the control. In pepper seedlings treated with extracts of *Kappachycys alvarezii* and *Gracilaria eduli* via seeds, Dutta et al. (2019) found encouraging results for the vigor index and fresh and dry mass of seedlings.

The different compounds found in marine algae of agricultural interest in the production of biostimulants have different effects at different concentrations, which must be evaluated when arriving at a specific concentration for a given group of vegetables (Rathore et al., 2009; Paulert et al., 2010), it must be tested for bioactivating compounds.

5. Conclusion

The biostimulants Stingray and ST Pro composed of Ascophyllum nodosum algae extract promoted good results for the morphological parameters of post-harvest carrots evaluated. The bioactivator Biozyme did not demonstrate effectiveness in promoting any of the parameters evaluated.

Possibly future studies could be analyzed by applying biostimulants and bioactivators via juice and foliar to verify new significant effects on the development of carrot crops in different cultivars.

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Author's Contributions

Thais de Oliveira Silva: study design, planting, soil analysis, cultural care, harvesting, parameter analysis, and article writing. *Carlos Henrique da Silva Santos*: study design, planting, soil analysis, cultural care, harvesting, parameter analysis, and article writing. *Antonio Carlos Pereira de Menezes Filho*: experimentation, data analysis, writing and translation. *Porshia Sharma*: translation, translation corrections, grammar, analysis of ideas, and final corrections. *Aurélio Ferreira Melo*: corrections, analysis of ideas, final evaluation. *Matheus Vinícius Abadia Ventura*: statistical analysis, final corrections, and publication.

Ethics

Not applicable.