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Research Article

Amino Acids Substance Biostimulants Application for Vegetable Development of Cauliflower (*Brassica oleracea* var. *botrytis* L.)

Marcelle Michelotti Bettoni[1], Rafael Kudlawiec[1], Vivyan Justi Conceição[2], Lucas Bernaski[1], Tefide Kizildeniz[3]

ABSTRACT

In Brazil, cauliflower crop (*Brassica oleracea* var. *botrytis*) is mainly a backyard gardening production, conducted in small areas of the country. However, its farming practice requires manpower as the production is mostly driven by family farming. Indeed, demand in labor and in sums, further coupled with environmental concerns and high production costs, seeks otherwise alternatives in providing greater efficiency, generating greater yield and quality, compared to other vegetables. Thus, the present study aimed to evaluate the biostimulant effect based on amino acids associated with a nutrient complex with high calcium and boron content in cauliflower cultivation. The experiment was carried out in the municipality of Campo Largo-PR, Brazil in a completely randomized design, with four treatments and three replications. The treatments consisted of 0.50; 0.75 and 1.0ml L^{-1} doses, plus the control treatment (producer standard), which follows all recommendations for culture, without the addition of the product under study in this article. The applications were performed by foliar spraying, at fortnightly intervals. At the end of the cycle, at 96 days after transplantation, the following parameters were evaluated: plant height, head height, head width and length, number of leaves, stem diameter, the fresh mass of the head, and average yield. The use of the biostimulant based on amino acids, calcium, and boron had a positive effect on the cauliflower crop, with the best results obtained at the dose of 1 ml L^{-1}.

Keywords: Amino acids, Brassica, Cauliflower, Mineral nutrition, Olericulture, Vegetables

Karnabaharın (*Brassica oleracea* var. botrytis L.) Sebze Gelişimi için Amino Asit Biyostimülan Uygulaması

ÖZ

Brezilya'da karnabahar mahsulü (*Brassica oleracea* var. botrytis) esas olarak ülkenin küçük bölgelerinde yürütülen bir arka bahçe bahçeciliği üretimidir. Bununla birlikte, üretim çoğunlukla aile çiftçiliği tarafından yönlendirildiğinden, çiftçilik uygulaması insan gücü gerektirir. Gerçekten de, çevresel kaygılar ve yüksek üretim maliyetleriyle daha da birleşen işgücü ve meblağlardaki talep, diğer sebzelere kıyasla daha fazla verimlilik sağlama, daha fazla verim ve kalite üretme konusunda başka alternatifler aramaktadır. Bu nedenle, bu çalışma karnabahar yetiştiriciliğinde kalsiyum ve bor içeriği yüksek bir besin kompleksi ile ilişkili amino asitlere dayalı biyostimülan etkiyi değerlendirmeyi amaçlamıştır. Deney, Brezilya'nın Campo Largo-PR belediyesinde, dört tedavi ve üç replikasyonla tamamen randomize bir tasarımda gerçekleştirilmiştir. Tedaviler 0.50; 0.75 ve 1.0ml L-1 dozları ve ayrıca bu makalede incelenen ürün eklenmeden kültür için tüm önerileri takip eden kontrol tedavisi (üretici standardı). Uygulamalar iki haftalık aralıklarla yapraktan ilaçlama ile gerçekleştirilmiştir. Döngünün sonunda, transplantasyondan 96 gün sonra, aşağıdaki parametreler değerlendirildi: bitki boyu, baş yüksekliği, baş genişliği ve uzunluğu, yaprak sayısı, gövde çapı, başın taze kütlesi ve ortalama verim. Amino asitler, kalsiyum ve bor bazlı biyostimülanın kullanımı karnabahar mahsulü üzerinde olumlu bir etkiye sahipti ve en iyi sonuçlar 1 ml L-1 dozunda elde edilmiştir.

Anahtar Kelimeler: Amino asitler, Brassica, Karnabahar, Mineral beslenme, Olerikültür, Sebzeler

ORCID ID (Yazar sırasına göre)
0000-0003-2493-5890, 0000-0003-0715-6860, 0000-0002-6582-732X, 0000-0003-4807-0756, 0000-0002-5627-1307

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[1] Universidade Tuiuti do Paraná, Rua Sydnei Antonio Rangel Santos, 238, CEP 82.010-330, Curitiba, PR, BRAZIL
[2] Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Departamento de Produção Vegetal - LPV Avenida Pádua Dias, 11 - Piracicaba/SP - CEP 13418-900. Tel.: (19) 3429-4190 – vivyan.conceicao@usp.br.
[3] Niğde Ömer Halisdemir University, Faculty of Agricultural Sciences and Technologies, Biosystem Engineering Department, 51240, Niğde, TÜRKİYE
[*] E-Posta: tizildeniz@ohu.edu.tr

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Introduction

Brazilian agriculture is the basis of the country's economic development, with the "green belts" being the main responsible for the supply of fruit and vegetables, which, for the most part, are supplied by small and medium-sized producers. The main vegetable-producing region in the state comprises the regional nucleus of Curitiba, which holds 40% of the state's production (DERAL/SEAB, 2021).

Vegetable crops are highly representative in the state of Paraná, according to the agricultural and vegetable production forecast of the Paraná rural economy department, which in its report includes tuberous, herbaceous, and fruit crops, with a cultivated area of 118,600.00 hectares and a production of 2,917,900 thousand tons generating a balance of 4,250,000,000 billion reais in the 2020 harvest (DERAL/SEAB, 2021).

Cauliflower (*Brassica oleracea* var. botrytis) can be produced throughout the year in much of Brazil (FILGUEIRA, 2008). Paraná has a cultivated area of 2,535 thousand hectares and an estimated production of 58,547.00 thousand tons, having generated a balance of 287,026,668 million reais in the 2020 harvest, corresponding to 7% of the total of other vegetable crops (DERAL/SEAB, 2021).

Crop yield and quality are affected by several factors, such as climatic factors, latitude, longitude, seasons of the year (Morais et al., 2012), soil type, water availability, and mineral nutrition, among others.

Aiming at achieving best performance of the culture, adequate management of fertilization must be carried out, it is recommended the administration of nitrogen, in the initial phase, followed by calcium and boron. Boron deficit creates serious issues in cauliflower, which is extremely susceptible to it. Boron has various roles in cauliflower, including flowering and nourishment, and has a direct influence on quality and final yield (Coutinho et al., 1993). Furthermore, boron is involved in various physiological processes targeting calcium metabolism, auxin synthesis, sugar metabolism, solute translocation, and protein synthesis (Thakur et al., 2019). Likewise, boron, calcium is of paramount importance in the plant, in addition to its structural role in the interconnection of cell wall components (Brett and Waldron, 1996), it participates in physiological processes that occur in the cytoplasm, acting as a protective agent of metabolism (Plieth, 2005). For cauliflower, studies show that this nutrient is important both for increasing yield and for decreasing physiological disorders (Tremblay et al., 2005).

Among the various techniques used to obtain qualitative and quantitative increases, there is the use of products with biostimulant effects, the biostimulants, which can be defined as mixtures of bioregulators or the mixture of these with other chemical compounds (Klahold et al., 2005). This definition, however, is neither formal nor accepted by regulatory bodies in Brazil, so far (Du Jardin, 2015).

The biostimulant has a systemic characteristic, having the ability to activate several sites in different locations in the plant (Pecha et al., 2012), acting in the cascade of signals and activating or deactivating several routes, optimizing the productive aspects according to the positive physiological effects that occur in the plant (Oliveira et al., 2017).

In Europe, in 2017, the biostimulant became part of plant nutrition products, being considered “a product that stimulates the nutrition processes of plants, regardless of the nutrient content of the product, with the sole objective of improving one or more of the following plant characteristics: (a) nutrient use efficiency, (b) tolerance to abiotic stresses, or (c) crop quality” (European Parlament, 2019).

Compounds such as amino acids belong to the list of substances of biostimulants that are naturally produced by plants, in addition to their structural function, they are related to the synthesis of vitamins, enzymes, hormones, and chlorophyll, as they have specific receptors inside cell membranes where interact to transmit signals, causing several morphophysiological and biochemical changes in plant structures (Ryan et al., 2002).

Some are also involved in the transport and storage of nutrients, such as glutamate, glutamine, and asparagine, which are involved in nitrogen metabolism (Campos, 2022). There are also amino acids that act in the reduction of free radicals by acting on the routes of the antioxidant system, which has the effect of reducing stress in the plant and consequently affecting its yield (Teixeira, 2017).

Based on the above reference, the objective of this work was to evaluate the application of an amino acid-based biostimulant associated with a nutrient complex with high calcium and boron content in cauliflower cultivation.

Materials and Methods

Experimental Design, Plant and Soil Materials

The course conclusion work was conducted at the Fazenda Fausto Bernaski, located in the municipality of Campo Largo- Paraná, Brazil (25°23ˈS - 49°28ˈW). Soil analysis in the experimental area showed the following pH values ($CaCl_2$) = 4.70; pH SMP = 5.79 (Due to its simplicity, effectiveness, and rapidity, The Shoemaker-McLean-Pratt (SMP) buffer created by Shoemaker et al. (1961) is one of the most extensively used methods to estimate soil potential acidity in Brazil, utilized for evaluating lime demand of soil includes chromium and p-nitrophenol).; Mo g dm^{-3} = 24.10; P Mehlich (mg dm^{-3}) = 27.07; K (cmol dm^{-3}) = 0.56; Ca (cmol dm^{-3}) = 4.00; Mg (cmol dm^{-3}) = 2.10; Al (cmol dm^{-3}) = 0.00; H+Al (cmol dm^{-3}) =5.80; Sum in Base (cmol dm^{-3}) = 6.66; CTC (continuous thermophilic composting) (pH 7.0) (cmol dm^{-3}) = 12.46; Bases V% = 53.4; Al (M%) = 0.0; Ca%= 32.1; Mg% = 16.8; K% = 4.5; S (mg dm^{-3}) = 14.87; B (mg dm^{-3}) = 0.17; Fe (mg dm^{-3}) = 36.27; Cu (mg dm^{-3}) = 0.80; Mn (mg dm^{-3}) = 17.50; Zn (mg dm^{-3}) = 2.15 (Agro TecSolo, agronomic analysis and consultancy, May 2022). The soil was prepared 15 days in advance and the transplant of seedlings of cauliflower (*Brassica oleracea* var. botrytis L.), cultivar "Barcelona", was carried out on March 25, 2022, using a spacing of 0 .75 m between rows and 0.60 m between plants. The experimental design was completely randomized, with 4 treatments and 3 replications, constituting 12 experimental plots, containing 24 plants per plot, totaling 288 plants, and being evaluated 4 central plants per plot.

The treatments consisted of the application, via foliar, of the biostimulant product, in the following doses: 0.50, 0.75 and 1.0 mL L^{-1}, in addition to the control, which was the producer standard, as recommended for the culture, without the addition of the biostimulant. The treatments were applied at regular intervals of 15 days, starting 15 days after transplantation (DAT).

Biostimulant Properties

The commercial product used was Calmax®(OMEX), being composed of (%): 14.60 of Nitrogen (N); 21.80 of Calcium (Ca); 2.90 of Magnesium (Mg); 0.15 of Manganese (Mn); 0.073 of Iron (Fe EDTA); 0.073 of Boron (B); 0.058 of Copper (Cu EDTA); 0.029 of Zinc (Zn EDTA) and 0.0015 of Molybdenum (Mo); pH (10% solution), 6.0-7.0, specific gravity 1.47-1.51 at 18°C.

Agricultural Practices

The producer standard consisted of the following applications as listed in Table 1: 28 DAT-Fylloton (foliar) composed of 6% water-soluble N (76.1 g L^{-1}), 6% N in the organic form (76.1 g L^{-1}), and 20.8% TOC (total organic carbon) (263.7 g L^{-1}); 30 DAT-urea; 42 DAT- K-bomber (leaf), composed of 5% phosphorus (P_2O_5) and 55% potassium (K_2O); Belgen Calcium and boron (leaf) composed of 1% nitrogen, 10% calcium and 2.00% boron.

At 45 DAT, the fertilizer formulated 15-00-14 was applied, as a top dressing, coinciding with the first application of the treatments with the biostimulant. At 60 DAT, together with the second application on the plants that received the treatments, the control plants received K-bomber (leaf) composed of 5% phosphorus (P_2O_5) and 55% potassium (K_2O).

Table 1. Data of the treatment standard producer

Commercial name	Application mode	DAT
Phylloton	Foliar	28
Urea	Roof	30, 60
K-bomber	Foliar	42
Belgen	Foliar	42
N.P.K	Roof	45

Phytosanitary control was carried out on all plants (Table 2), applying Wetcit, orange oil (surfactant), together with fungicides, herbicides, and insecticides, at 28 DAT. These pesticides were: Dithane (fungicide) composed of 800g kg^{-1} of Mancozeb and 200g kg^{-1} of other ingredients; Ridomil (fungicide) composed of metalaxyl-m 40g kg^{-1}, mancozeb 640g kg^{-1} and other ingredients 320g kg^{-1}; and Avatar (insecticide) composed of indoxacarb 150g L^{-1} and other ingredients 797g L^{-1}. At 42 DAT, the following were applied: Ampligo (insecticide) composed of 50 g L^{-1} Lambda-cyhalothrin and 100 g L^{-1} Chlorantraniliprole; Dithane (fungicide) composed of 80% mancozeb and 20 % other ingredients; and Wetcit orange oil. At 60 DAT, Red copper (fungicide) composed of 25 % Cu was applied; Engeo plena S composed of 141 g L^{-1} thiamethoxam and 106 g L^{-1} lambda-cyhalothrin; and Wetcit orange oil.

Table 2. Data on phytosanitary control applied to all plants.

Commercial name	Chemical group	DAT
Wetcit	Surfactant	28, 42, 60
Dithane	Fungicide	28, 42
Ridomil	Fungicide	28
Avatar	Insecticide	28
Ampligo	Insecticide	42
Red Copper	Fungicide	60
Full engine S	Fungicide	60

Measurements

At the end of the cycle (96 DAT), 4 central plants per plot were evaluated for the following characteristics: plant height (cm), head height (cm), head length (cm), head width (cm), number of leaves, stem diameter (cm), head fresh mass (g) and average yield (t ha^{-1}).

Climatic Data and Irrigation Regime

The experiment was conducted in the open field of the Fazenda Fausto Bernaski, located in the municipality of Campo Largo on March 25th until June, 25th 2022 in Curitiba, Paraná, Brazil (25°23ˈS - 49°28ˈW). Curitiba is in the southern region of Brazil and summer begins in the end of January and finishes in December here. Its climate has two climates as warm, and temperature and it gets a great deal of rainfall throughout the year. Despite being driest month there is still significant amount of rainfall. Cfb is it's the Köppen-Geiger climatic categorization. Curitiba's mean temperature is 17.2 ˚C. The annual rainfall totals 1630 mm (Figure 1). Upon transplanting the seedlings to the open field, the irrigation was applied and during the whole period of the experiment, the humidity level was enough to cultivate the cauliflower crop without irrigation (Figure 2).

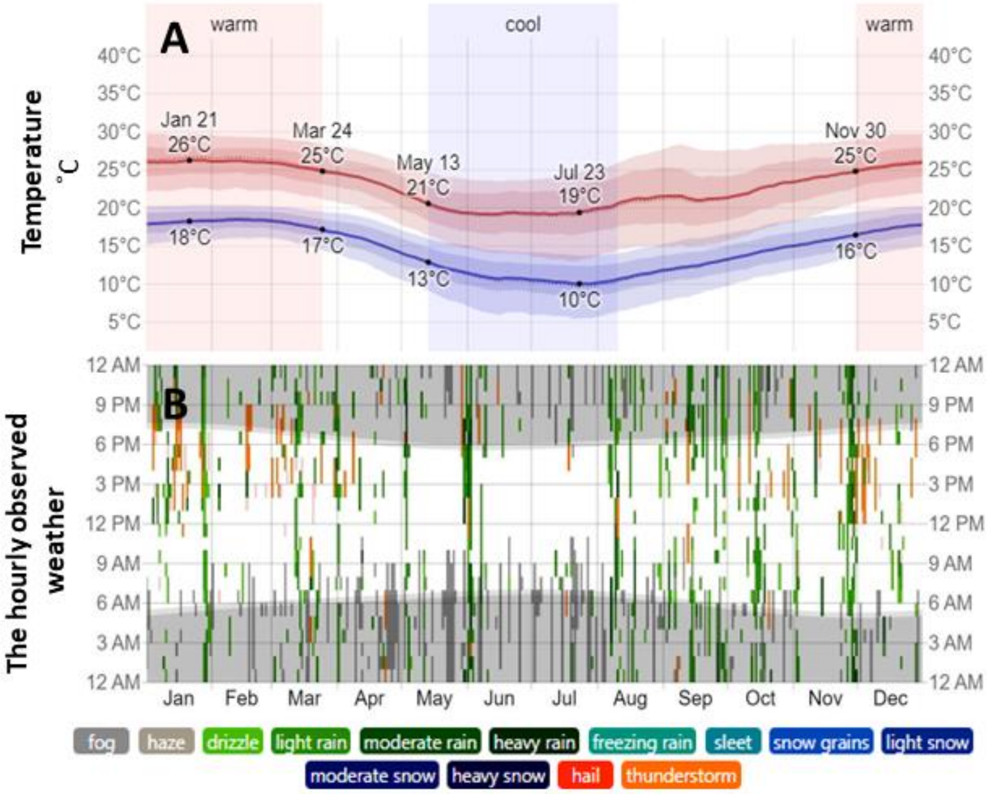

Figure 1. The daily temperature variance (gray bars) and 24-hour highs (red ticks) and lows (blue ticks), ranked the daily average high (faint red line) and low (faint blue line) temperature, with 25th to 75th and 10th to 90th percentile bands (A) and the hourly observed weather, color coded by category (in order of severity and the multiple colors are indicated, the most severe code) (B) in Curitiba, Paraná, Brazil (WeatherSpark, 2022)

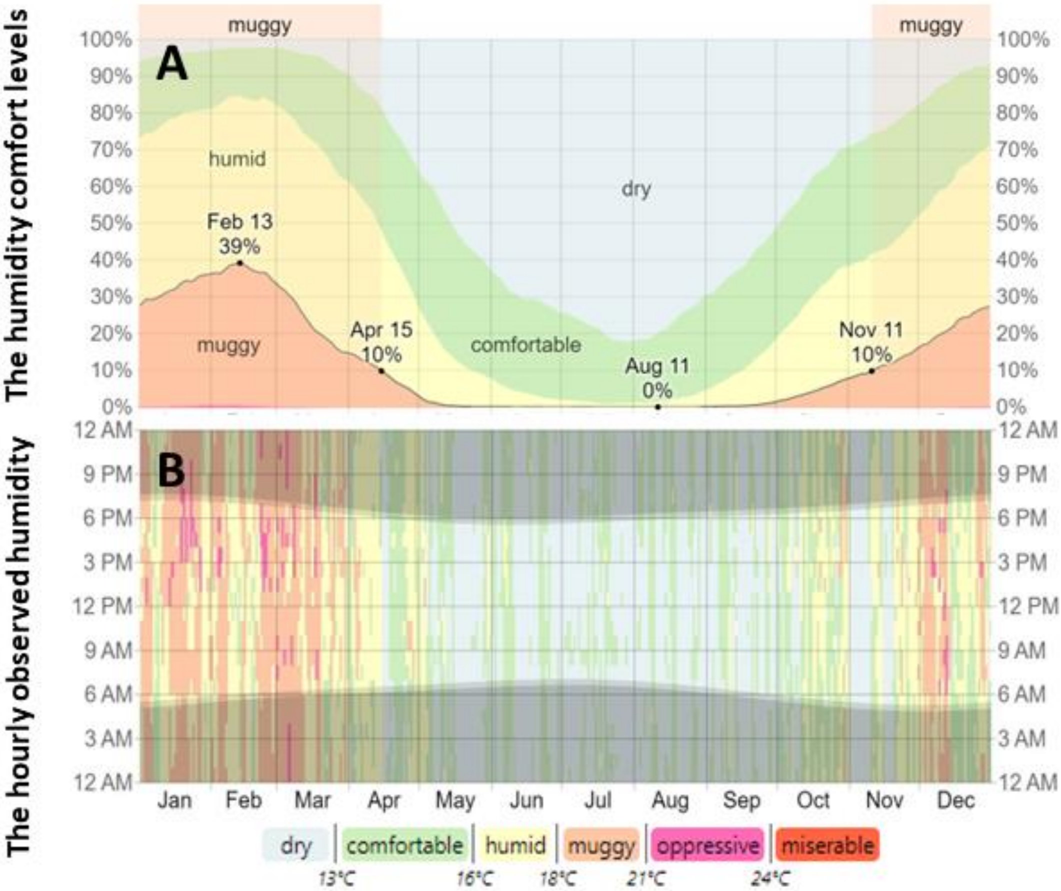

Figure 2. Classified by dew point, both the percentage of spending time at different degrees of humidity comfort (A) and the recorded humidity level of comfort hourly (Night and civil twilight are shown by the dark coverings)(B) in Curitiba, Paraná, Brazil (WeatherSpark, 2022)

Data Analyses

The values obtained were submitted to normality analysis using the Shapiro-Wilk test and the F test, and when significant, the polynomial regression analysis was carried out. The data were processed in the statistical software Sisvar ® and the graphs were generated in Excel®.

Results and Discussion

The analysis of variance showed a significant effect of the doses of the product used for all the variables analyzed, except for head height. The regression analysis (Figure 3) for the biometric variables: plant height, head length, and head width, as a function of the applied doses, indicated a linear behavior, in which the variables respond to the progressive increase in doses, according to the test F.

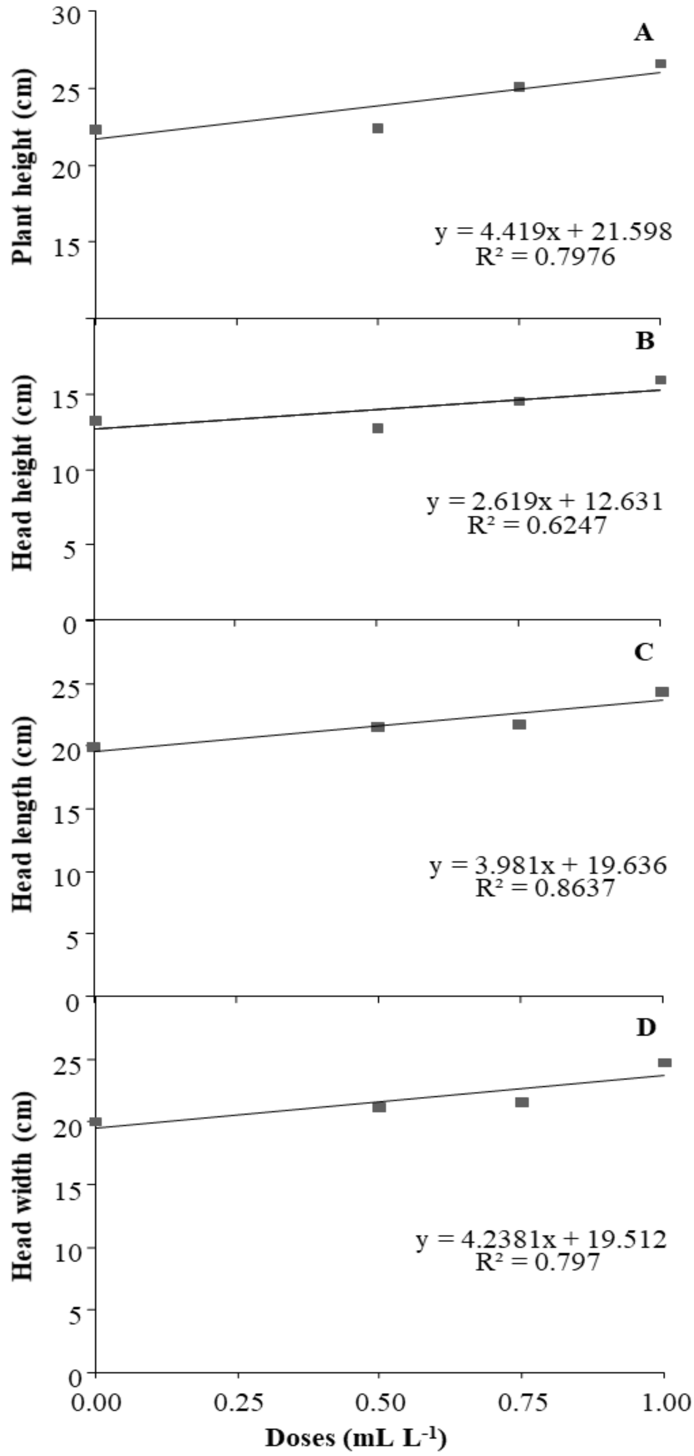

Figure 3. Plant height (A), head height (B), head length (C) and head width (D) of cauliflower

Such results demonstrate the positive effect of the product on the variables mentioned, in which the regression was significant, the maximum dose, 1 mL L^{-1}, was the one that presented the highest results, being 26.6 cm for plant height (19 % greater than the control), 24.3 and 24.8 cm for head length and width, respectively (Figure 3A). Following the linear trend of the results, this dose could be even higher (22 and 24 % increment; Figure 3A). Plant height of *Brassica oleracea* var. botrytis L. cauliflower can have been significantly influenced by environmental factors. 0, 1 and 2 kg ha^{-1} doses of boron application of the same variety demonstrated 48.53, 51.41 and 54.25 cm of plant height in India (Kumar et al., 2023). These results are exceeding even our control treatment result (24.3 cm) and these can lead to conclude as it is highly related to genetic and environmental factors influence.

The mean height of head (Figure 3 B) was 14.1 cm, ranging between 12.8 and 15.9 cm, not being affected by the application of the product, therefore, regression analysis was not performed. This factor can be explained by the plant's genetics. According the study conducted by Kumar et al., (2023) in India, the same variety of cauliflower seedlings (*Brassica oleracea* var. botrytis L.) were targeted to different doses of boron treatments. The control plants mean height of head was reached 12.44 cm that is confirmed our study. In 1 and 2 kg ha^{-1} boron application was resulted in 13.15 and 16.45 cm increased, respectively (Kumar et al., 2023). These results also agreed to our study that boron application does not result in a significant impact.

The regression analysis (Figure 4) for the biometric variables: number of leaves, stem diameter, fresh head mass, and average yield, as a function of the applied doses, indicated a linear behavior, according to the F test, with the highest dose being 1 ml L^{-1}, which presented the highest values.

The number of leaves (Figure 4A) reached an average of 15.8 leaves, at the dose of 1 ml L^{-1}, 23 % higher than the control. This result is in agreement with that observed in lettuce *(Lactuca sativa* L.) treated with an amino acid-based biostimulant (Limberger and Gheller, 2012), with the highest number of leaves obtained at higher doses than the highest applied dose of 1 mL. The stem diameter (Figure 4B) showed an increase of 17 % at the highest dose, concerning the control, with 5.3 cm, following the linear trend. For the fresh mass of the head (g) (Figure 4C), a linear behavior was also observed, with an increase of 26 % at the highest dose, concerning the control, with 987 g. For the variable average yield (Figure 4D) the same trend was verified as for the fresh mass, with an increase of 26 % in yield about the control, reaching 21.9 t ha^{-1}.

For the variables related to yields (fresh head mass and yield), a result similar to that obtained in broccoli cultivars (*Brassica oleracea* var. Itálica) treated with an amino acid-based biostimulant was observed (Bettoni et al., 2013). To best of our knowledge, there is not any reported study on amino acid-based biostimulant effect on cauliflower however a study conducted in Iraq on seaweed based biostimulant impact on cauliflower indicates that any significant impact on crop development and yield (Youssif and Tawfeeq, 2022). This can lead us to conclude that the significant improvement of plant development and yield parameters in cauliflower could cause by applied biostimulant including amino acid and high content of boron. Additionally, the result obtained can be related to the amino acid alanine, which participates in the pyruvate pathway, the precursor of proteins that act in the development and growth of plants (Kerbauy, 2008).

The observed increases in fresh mass can be explained by the increase in the largest diameter of the stem, which is a parameter that influences yield (Monteiro et al., 2010). This is due to the increase in water translocation and photoassimilates vessels. Moreover, the results obtained for most variables agree with the literature, being observed that the plant is more responsive, in general, to increased doses of amino acid-based biostimulant products.

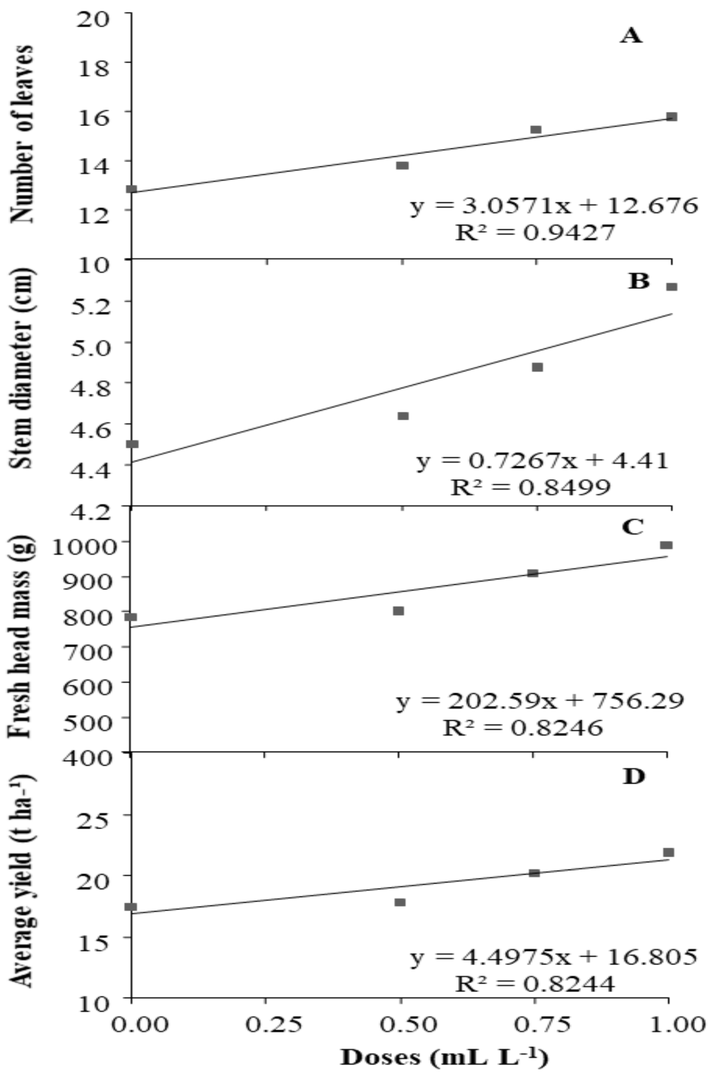

Figure 4. Number of leaves (A), stem diameter (B), fresh head mass (C) and average yield (D) of cauliflower

Conclusions

Cauliflower is a valuable cool season vegetable crop developed for its rich nourishment, great taste, and susceptibility to environmental conditions. The genetic factors have individually and interacting with environmental factors has also significant impact on cauliflower's quality and yield. *Brassica oleracea* var. botrytis L. cauliflower variety resulted in significant influenced by environmental factors. Many times, the environmental factors are not easy to control however the other factors such as fertilizers application can allow producers to diminish negative impacts caused by soil and climatic factors. In our study, leaf of biostimulant, Calmax® (OMEX) demonstrated the positive effect on the development of cauliflower plants. All variables evaluated have showed a linear behavior according to the F test, with the maximum efficiency found for the biometric variables at the dose of 1.00 mL L^{-1}.

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Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest.

Authors' Contribution

R.K: investigation, formal analysis, and writing. M.M.B.: conceptualization, methodology, validation, resources, writing, reviewing and editing, visualization, supervision. Conceptualization, methodology, validation, formal analysis, writing, reviewing and editing, visualization. T.K.: conceptualization, methodology, validation, writing, reviewing and editing. All the authors approved the paper for publication.

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