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Morphophysiological and Productive Responses of Garlic (*Allium sativum* L.) 'Criollo' Cultivar under the Effects of Biostimulants

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ABSTRACT

At present, food production is of considerable importance, particularly vegetables used as condiments and in the pharmaceutical industry. The application of biostimulants to enhance yield presents a promising alternative. This research aimed to evaluate the morphophysiological and productive responses of garlic to different biostimulants. The effectiveness of the biostimulants VIUSID Agro®, FitoMas-E, Effective Microorganisms, and a formulation consisting of a mixture of all these was evaluated based on their differing compositions. Morphophysiological parameters such as bulb diameter, pseudostem diameter, number of cloves per bulb, growth rates, and yield, among others, were evaluated. The results revealed a significant increase in all morphological indicators with the application of the biostimulants. VIUSID Agro® had the greatest effect on bulb diameter, with a rise of 14.59% compared with the control group. In addition, the absolute growth rate showed an average increase of 0.032 g d-1 with this biostimulant. With regards to the net assimilation rate, the best results were obtained with VIUSID Agro®, Effective Microorganisms, and the combination of all biostimulants. Overall, VIUSID Agro® had the best performance in terms of morphophysiological and production parameters. These results suggest that the use of these biostimulants may be beneficial for increasing garlic production, which could be crucial in the current global food supply situation.

Abbreviation: Leaf area (LA), Leaf area index (LAI), Leaf area ratio (LAR), Net assimilation rate (NAR), Relative growth rate (RGR), Absolute growth rate (AGR), Relative water content (RWC), Membrane stability index (MSI), Saturation deficit (SD), Days after sprouting (DAS), Nitrogen (N), Effective Microorganisms (E.M.)

Introduction

Garlic (*Allium sativum* L.) is one of the world's oldest vegetable crops, native to Central Asia, and is a member of the *Alliaceae* family. It is the second most widely cultivated species in the *Allium* genus after onion. It is a widely cultivated vegetable worldwide, with an estimated production of 28 million t in 2020 (Sunanta et al., 2023), with China alone accounting for 70% of the global production of this crop (Han et al., 2024). In Cuba, garlic production in 2020 totalled 11,313 t, whereas in the province and municipality of Sancti Spíritus, it amounted to 1,284.4 and 1,212.6 t, respectively (Atlasbig, 2020; ONEI, 2020). These production volumes still do not meet the demand; hence, there is a need to look for alternatives to increase production, as it is currently affected by intensive agricultural models and the use of high levels of inputs, especially for pest control and fertilisation (Haney et al., 2015; Riahi et al., 2020). For this reason, the application of plant growth stimulators has increased in recent years (Carmona and Melendrez, 2019; Chandran et al., 2021; Kumar et al., 2021).

Garlic requires the rigorous management of agricultural practices and phytotechnologies, especially irrigation and nutrition since a deficit or excess of these resources in the soil causes significant environmental and productive losses (Bayan et al., 2014; Li et al., 2021). In this sense, the use and study of biostimulants is a viable alternative that contributes to the development of sustainable agricultural production systems (Yakhin et al., 2017; Caradonia et al., 2019; Keswani et al., 2020; Riahi et al., 2020). These are defined as any substance or microorganism capable of increasing nutrient efficiency, abiotic stress tolerance, and crop quality and yield (Yakhin et al., 2017; Chandran et al., 2021; Kumar et al., 2021). It is also one of the current high priorities of modern agricultural science, which focuses on transforming the production system in geographical and climatic regions affected by low agricultural productivity (Liane et al., 2021; Martínez-Balmori et al., 2021; Mitter et al., 2021).

There have been several studies on the use of agricultural bioproducts in the cultivation of *A. sativum*. Researchers such as Martínez-Balmori et al. (2021), who studied the physical, mechanical, and chemical properties of garlic grown with different concentrations of QuitoMax®, stand out. These authors reported changes in quality indicators (size, pungency, % organic acids, reducing carbohydrate and protein content) that contributed to an increase in internal quality. They also reported that these increases were dependent on the concentration of the biostimulant and the variety of garlic used. On the other hand, Carmona and Melendrez (2019) evaluated the effects of VIUSID Agro® doses on this crop and reported improvements in morphoagronomic indicators (plant height, number of leaves, stem diameter, pseudostem diameter, bulb mass and yield) at a dose of 0.25 L ha^{-1}. On the other hand, Hassan and co-workers (2024) recently evaluated the foliar application of potassium citrate and humic acids as soil fertilisers. The authors observed that high concentrations of humic acids, along with potassium citrate, exhibit effective fertiliser functionality. Additionally, they reported an increase in productivity and essential oil production (Hassan et al., 2024).

However, studies on the use of biostimulants in garlic cultivation are still insufficient, and the variables evaluated do not provide clear information on the physiology of crops treated with biostimulants. Therefore, this study aimed to assess the morphophysiological response of the Criollo garlic cultivar to the biostimulants VIUSID Agro®, FitoMas-E, effective microorganisms, and a combination of the three.

Material and methods

Generalities of the research

The experiment was carried out on a farm belonging to the CCS Julio Calviño, located in the town of Banao (21°49'26.0"N 79°34'06.2"W), in the municipality and province of Sancti Spiritus, Cuba. The garlic clone used was 'Criollo'. Cloves were subjected to a vernalisation process (10 °C for 10 d and 70% relative humidity) to stimulate homogeneous germination. The climatic variables were recorded by the Provincial Meteorological Station of Sancti Spiritus (Fig. 1).

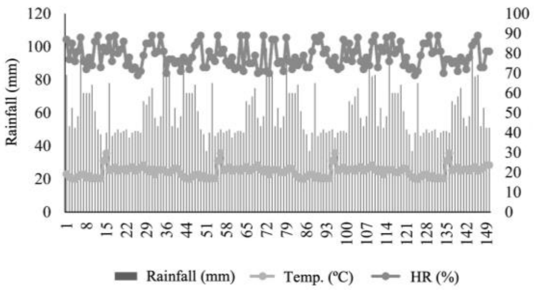

Fig. 1. Meteorological variables recorded during the experimental phase. Relative humidity RH (%), mean temperature (°C), and rainfall (mm).

The type of soil on which the experiment was carried out was ferrallitic red leached (Hernández-Jiménez et al., 2019). Irrigation was carried out using gravity-fed water runoff with an interval of 3 d between each irrigation. Fertilisation was carried out with 300 kg ha^{-1} NPK complete formula (9-13-17) from 15 d after sprouting (DAS), with a fortnightly frequency and a final application of 150 kg ha^{-1} nitrogen (N) at the end of the vegetative cycle.

Characteristics of the biostimulants

The following bioproducts were used: Effective Microorganisms (ME-50, hereafter referred to as E.M.) consisting of *Bacillus subtilis* B/23-45-10 Nato (5.4x10^4 CFU mL^{-1}), *Lactobacillus bulgaricum* B/103-4-1 (3.6x10^4 CFU mL^{-1}) and *Saccharomyces cerevisiae* L-25-7-12 (22.3x10^5 CFU mL^{-1}), with a quality certificate issued by the Cuban Research Institute of Sugar Cane Derivatives (ICIDCA) (R-ID-B-Prot-01-01-01-01). FitoMas-E is a phytostimulant derived from the sugar industry and was obtained and developed by the ICIDCA. FitoMas-E is a natural mixture of high-energy complex organic intermediates, including amino acids, low-molecular-weight peptides, nitrogenous bases, and bioactive carbohydrates (Gómez-Kosky et al., 2019). The composition of the VIUSID Agro® biostimulant is amino acids, aspartic acid (1.6%), arginine (2.5%), glycine (2.4%), tryptophan (0.5%), and organic nitrogen (1.8%), with pH 6.80 and net mass of 1.14 kg (Peña et al., 2018a; Gómez-Kosky et al., 2019).

Experimental design, application methods, and treatments

The experimental design was a randomised block design with five treatments and three replicates. The plots were 6 m^2 in size, with 2 m between plots and a total of 15 plots. The distance between rows was 0.30 m and the spacing between plants within a row was of 0.05 m. The planting frame was 0.04 × 0.35 m. Ten plants per treatment were randomly selected. The dose used and the time of application for each biostimulant was: FitoMas-E (1.5 L ha^{-1}), with an application frequency of every 15 d after bud break; VIUSID Agro® was applied at a rate of 0.25 L ha^{-1} every 15 d; Effective Microorganisms (100 mL L^{-1}) at a weekly frequency; and an untreated control where distilled water was sprayed every 15 d. Doses were determined according to the manufacturer's recommendations and previous studies.

The first application was performed at 12 DAS with a 16 L calibrated manual backpack sprayer (MATABI).

The combined treatment involved applying the three biostimulants evaluated (FitoMas-E, E.M., and VIUSID Agro®) to the same plant at the same concentrations and application times specified for each individual treatment.

Indicators assessed at 45 and 60 DAS

Plant height was measured from the base of the pseudostem to the apical bud via a 1,500 × 30 mm steel ruler. The leaf area (LA) was determined using the ImageJ free software (Schneider et al., 2012). Calculations of the leaf area index (LAI), leaf area ratio (LAR), net assimilation rate (NAR), relative growth rate (RGR), and absolute growth rate (AGR) were performed according to the methodology adapted from Gardner et al. (2017). The fresh mass of the plant organ was determined via a digital balance (Sartorius, model BS 124S) with an accuracy of ± 0.01 g. For the dry mass, an oven (MJW WS 100) was used at 75 °C until the mass was constant, and then the same mass was determined via a digital balance (Sartorius) with an accuracy of ± 0.01 g. Chlorophylls *a* and *b*: Discs were cut from leaves with a fresh mass of 0.10 g and then crushed in a mortar together with glass powder. First, 5 mL of 90% (v/v) methyl alcohol was added. This mixture was placed in a 10 mL plastic tube, rinsed in a mortar with the other 5 mL of alcohol, added to the plastic tube, covered with aluminium foil, and placed in the dark for 2 min. Then, the samples were centrifuged at 3,000 × g for 15 min. The absorbance (Abs) was then analysed at the appropriate wavelengths for each element (663 nm and 646 nm) via a spectrophotometer. The Abs values were then taken and substituted into the following equations (Porra et al., 1989):

$$Chl\ a\ (mg\ mL^{-1}) = 12.25 \times Abs_{663} - 2.79 \times Abs_{646}$$

$$Chl\ b\ (mg\ mL^{-1}) = 21.50 \times Abs_{646} - 5.10 \times Abs_{663}$$

Carotene content: Calculated via the following formula:

$$Carotenes = 1000 \times Abs_{470} - 1.82 \times Chl\ a - 58.02 \times Chl\ b$$

Indicators at 60 DAS

Relative water content (RWC): The relative water content is an indicator of the water status of a leaf and was determined via the following formula (Weatherly, 1950):

$$Relative\ water\ content\ (\%) = \frac{fresh\ mass - dry\ mass}{saturation\ mass - dry\ mass} \times 100$$

Saturation deficit (SD): The saturation deficit was calculated via the following formula (Turner, 1981):

$$Saturation\ deficit\ (\%) = \frac{saturation\ mass - fresh\ mass}{saturation\ mass - dry\ mass} \times 100$$

Membrane stability index (MSI): Membrane stability was estimated by sampling fresh leaves in two sets of test tubes containing deionised water. The first set was heated in a hot bath at 40 °C for 30 min, and the electrical conductivity was measured and recorded at the end of the indicated time (C1). The second set was autoclaved at 100 °C for 10 min, and the electrical conductivity was measured at the end of this time (C2). The stability of the membrane was calculated via the following formula:

$$IEM = \left[1 - \left(\frac{C1}{C2}\right)\right] 100$$

Pseudostem and bulb diameter: Both indicators were measured with a digital calliper (Digite, model ACC115-006-11) with an accuracy of ±0.03 mm, and the values were recorded for all randomly taken plants and expressed in millimetres (mm).

Number of cloves per bulb: The number of cloves per bulb was counted manually at the end of the experiment.

Agricultural yield (t ha^{-1}): It was quantified by multiplying the average number of bulbs by the fresh mass of the bulbs and by the area and expressed in t ha^{-1}.

Statistical analysis

The data were processed via the statistical package SPSS version 20.0 (2018). The Kolmogorov–Smirnov test for normality and Levene's t-test for homogeneity were used. When normality and homogeneity were present, simple rank analysis of variance (ANOVA) and Tukey's multiple range test were performed when $P \leq 0.05$.

Results

Plant height

When the plant height at 45 DAS was evaluated, the treatments with VIUSID Agro® and the combination of biostimulants (FitoMas-E + E.M. + VIUSID Agro®) had the best response (Fig. 2a). The highest value was obtained when the plants were treated with VIUSID Agro®, which was significantly different ($P < 0.05$) from that of the control, Phytomas-E and E.M. treatments. This treatment (VIUSID Agro®) exceeded the control by 2.57 cm (SE: 0.25, CV: 16.50), an increase of 30.77%.

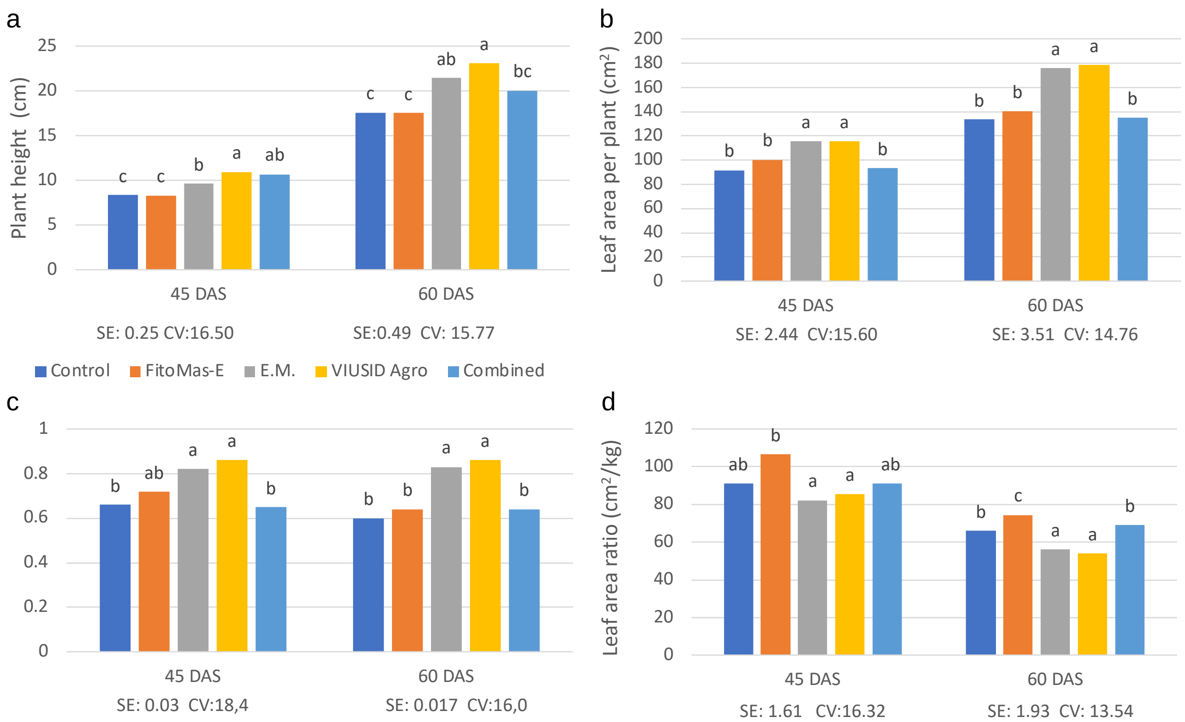

Fig. 2. Effects of treatments on (**a**) Plant height; (**b**) Total leaf area per plant; (**c**) Leaf area index (LAI); (**d**) and leaf area ratio (LAR) at 45 and 60 DAS in the absence of bioproducts (control), FitoMas-E (1.5 L ha^{-1}), Effective Microorganisms (E.M., 100 mL L^{-1}), VIUSID Agro® (0.25 L ha^{-1}) and the biostimulant mixture treatment (FitoMas-E + E.M. + VIUSID Agro®).

Different letters indicate significant differences within each independent time (45 and 60 DAS) according to Tukey's multiple range test ($P \leq 0.05$).

When analysing plant height at 60 DAS, E.M. and VIUSID Agro® had the best responses, and although the highest numerical value was obtained by VIUSID

Agro®, no difference was observed between them. This treatment significantly differed ($P \leq 0.05$) from the control and differed from the other treatments studied. The increase over the control was 31.17%, exceeding the control by 5.48 cm (SE: 0.49, CV: 15.77) (Fig. 2a).

Pseudostem diameter

When the pseudostem was evaluated, no difference ($P > 0.05$) was observed between the control and those treated with FitoMas-E, E.M. and the combined biostimulant (Table 1). The biostimulant VIUSID Agro® had the greatest significant difference ($P \leq 0.05$) with respect to the control, with an increase of 1.4 mm (SE: 0.17, CV: 15.95), representing a 31.1% increase.

Table 1. Effect of biostimulant treatments on morphological indicators.

Treatments	Pseudostem diameter (mm)	Bulb diameter (mm)	Number of cloves per bulb
Control	4.47^{b}	29.25^{b}	20.25
FitoMas-E	4.87^{b}	29.87^{b}	20.00
E.M.	5.05^{ab}	31.42^{ab}	20.75
VIUSID Agro®	5.87^{a}	33.52^{a}	21.00
Combined*	4.17^{b}	29.82^{b}	19.50
SE	0.17	0.44	0.66
CV (%)	15.95	6.51	14.53

Means with different letters in the same column are different ($P \leq 0.05$) according to Tukey's multiple range test (mean ± standard error). * FitoMas-E + E.M. + VIUSID Agro®.

Bulb diameter

As shown in Table 1, the treatments with the best results were VIUSID Agro® and E.M., with no differences ($P > 0.05$) between them, but VIUSID Agro® had the greatest significant difference ($P \leq 0.05$) with respect to the control and the other treatments studied. Compared with the control treatment, the VIUSID Agro® treatment exceeded the control by 4.27 mm (SE: 0.44, CV: 6.51), an increase of 14.59%. The other biostimulants did not significantly ($P > 0.05$) differ from the control.

Number of cloves per bulb

No significant differences ($P > 0.05$) were detected between the treatments studied when the number of cloves per bulb was analysed (Table 1).

Leaf area

The analysis of the leaf area at 45 DAS revealed no significant differences ($P > 0.05$) between the treatments with E.M. and VIUSID Agro®, but the other treatments did. These biostimulants exceeded the control by 23.71 cm^2 (SE: 2.44, CV: 15.60), an increase of 25.86% over the control (Fig. 2b). There were no differences between the FitoMas-E, combined (FitoMas-E + E.M. + VIUSID Agro®) and control treatments. When the leaf area was analysed at 60 DAS, similar differences were observed to those at 45 DAS (Fig. 2b).

Leaf area index

The leaf area index (LAI) is an allometric variable closely related to light capture capacity and plant growth. Figure 2c shows that at both 45 DAS and 60 DAS, the treatments with the highest leaf area indices were VIUSID Agro® and E.M. [$P < 0.05$, (45 DAS, SE: 0.03, CV: 18.4), (60 DAS, SE: 0.017, CV: 16.00)] with no differences between them ($P > 0.05$), but with the other treatments.

Leaf area ratio

Leaf area ratio analysis at 45 DAS revealed that the treatment with the greatest response ($P < 0.05$) was FitoMas-E (SE: 1.61, CV: 16.32), whereas VIUSID Agro® and E.M. had not difference ($P > 0.05$) with the control and combined treatments (Fig. 2d). Similarly, at 60 DAS, this biostimulant (FitoMas-E) still had the highest values, which differed ($P < 0.05$) from those of the other biostimulants studied and the control. The combined treatment (FitoMas-E + E.M. + VIUSID Agro®) showed no difference ($P > 0.05$) with the control but a difference ($P < 0.05$) with the other treatments was observed. The treatments with E.M. and VIUSID Agro® showed no difference ($P > 0.05$) between them, but showed a difference ($P < 0.05$) with the other treatments, being the ones with the lowest indices.

Growth rates

The analysis of the absolute growth rate (AGR) at 45 DAS revealed that the VIUSID Agro® and E.M. treatments presented the highest values, without differences between them, but with differences ($P \leq 0.05$) among the other treatments (Table 2). VIUSID Agro® outperformed the control by 0.041 g plant.d^{-1} (SE: 0.0023, CV: 31.90). As with the previous variables, FitoMas-E remains the biostimulant with the lowest result in terms of the parameters analysed.

At 60 DAS, VIUSID Agro® was significantly superior to all the other treatments studied ($P \leq 0.05$). In this case, the daily gain of this biostimulant with respect to the control was 0.0323 g plant d^{-1} (SE: 0.00208, CV: 29.10), an increase of 90%.

Table 2. Effects of treatments on growth rates at 45 and 60 DAS.

Treatments	AGR (g plant d^{-1})	NAR (g cm^{-2} d^{-1})	RGR (g g d^{-1})
	45 DAS		
Control	0.033^{c}	0.0006^{b}	0.049^{c}
FitoMas-E	0.038^{c}	0.0007^{b}	0.064^{b}
E.M.	0.065^{a}	0.0010^{a}	0.069^{ab}
VIUSID Agro®	0.074^{a}	0.0009^{a}	0.070^{a}
Combined*	0.058^{b}	0.0011^{a}	0.071^{a}
SE	0.0023	0.00008	0.0018
CV (%)	31.90	29.60	18.30
	60 DAS		
Control	0.0333^{c}	0.006^{bc}	0.074^{bc}
FitoMas-E	0.0334^{c}	0.004^{c}	0.063^{d}
E.M.	0.0536^{b}	0.007^{ab}	0.080^{ab}
VIUSID Agro®	0.0653^{a}	0.009^{a}	0.085^{a}
Combined*	0.0569^{b}	0.006^{bc}	0.066^{cd}
SE	0.00208	0.0004	0.002
CV (%)	29.10	40.00	17.00

Means with different letters in the same column and for each 45 and 60 DAS differ significantly ($P \leq 0.05$) according to Tukey's multiple range test (mean ± standard error). * FitoMas-E + E.M. + VIUSID Agro®.

When the net assimilation rate (NAR) was analysed at 45 DAS, no significant differences ($P > 0.05$) were detected between the treatments with the combined biostimulants (FitoMas-E + E.M. + VIUSID Agro®), VIUSID Agro® and E.M., whereas a significant difference ($P < 0.05$) was detected between FitoMas-E and the control (SE: 0.00008, CV: 29.60) (Table 2). In terms of the NAR at 60 DAS, the best response was obtained with the E.M. and VIUSID Agro® treatments, with no differences between them, but with the other treatments (SE: 0.0004, CV: 40.00).

When the relative growth rate (RGR) at 45 DAS was analysed, no significant differences ($P > 0.05$) were observed between the combined treatments (FitoMas-E + E.M. + VIUSID Agro®), VIUSID Agro® and E.M., but there were significant differences ($P < 0.05$) between them and the other treatments, with the control having the lowest value (SE: 0.0018, CV: 18.30) (Table 2). At 60 DAS, the bioproduct VIUSID Agro® had the best result, although with no difference from E.M. VIUSID Agro® exceeded the control by 0.011 g $g.d^{-1}$ (SE: 0.002, CV: 17.00), representing an increase of 14.86% with respect to the control (Table 2).

Relative water content and saturation deficit

Compared with the control, all the biostimulant treatments had a positive effect on the relative water content (RWC), except for the treatment with FitoMas-E, which did not differ from the control. The highest percentages of RWC were obtained when the plants were treated with the bioproducts VIUSID Agro® and E.M. (SE: 1.52, CV: 8.53), which exceeded those of the control by 12.20 and 14.45%, respectively (Fig. 3a).

When the saturation deficit (SD) was examined, the highest values were observed in the plants treated with the control and FitoMas-E, with no differences between them, but with the other treatments studied (SE: 1.85, CV: 11.60) (Fig. 3a).

Membrane stability

In the evaluation of membrane stability, the treatment with the best results was VIUSID Agro®, which was significantly different ($P < 0.05$) from the other treatments and the control, with an increase of 23.37% (SE: 1.07, CV: 12.60) (Fig. 3b).

Chlorophyll a and b contents and carotene

When the chlorophyll *a* content was evaluated at 45 and 60 DAS, the VIUSID Agro® treatment had the greatest significant difference ($P \leq 0.05$) with respect to the control and the other treatments, with values that exceeded those of the control by 1.20 and 2.96 times [(45 DAS, E: 0.108, CV: 9.79), (60 DAS, SE: 0.26, CV: 17.80)], respectively (Table 3). When chlorophyll *b* was evaluated at 45 DAS, no difference ($P > 0.05$) was detected between any of the treatments. At 60 DAS, the treatments that presented the highest values (SE: 0.113, CV: 22.40) and significant differences ($P \leq 0.05$) from the other treatments and the control were E.M. and VIUSID Agro®, with no differences between them (Table 3).

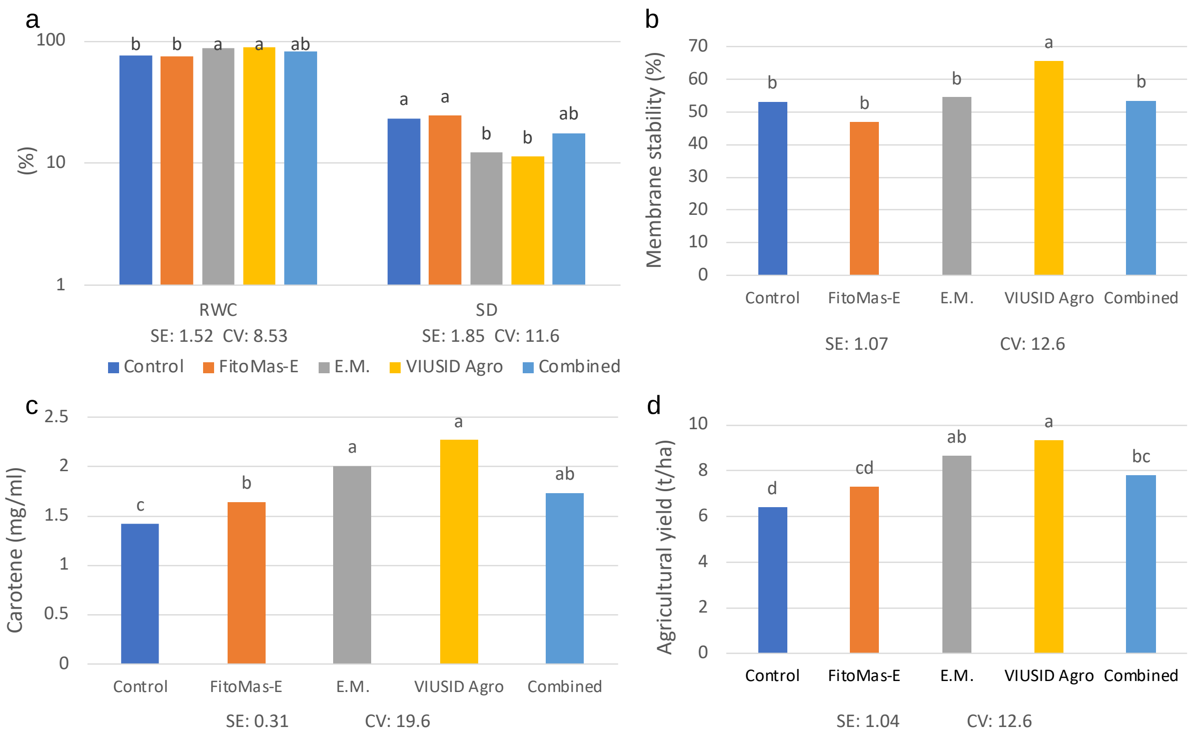

Fig. 3. (**a**) Plant response to relative water content (RWC) and saturation deficit (SD) at 60 DAS; (**b**) Membrane stability at 60 DAS; (**c**) carotene content at 60 DAS; (**d**) and Agricultural yield in the absence of bioproducts (control), FitoMas-E (1.5 L ha^{-1}), Effective Microorganisms (E.M., 100 mL L^{-1}), VIUSID Agro® (0.25 L ha^{-1}) and the combined treatment (FitoMas-E + E.M. + VIUSID Agro®). Different letters indicate significant differences according to Tukey's multiple range test ($P \leq 0.05$).

Table 3. Response of plants to the different treatments in terms of chlorophyll *a* and *b* contents at 45 and 60 DAS.

Treatments	**Chlorophyll *a* (mg mL^{-1})**	
	45 (DAS)	**60 (DAS)**
Control	4.5655^{c}	5.6631^{c}
FitoMas-E	4.9574^{b}	5.9137^{c}
E.M.	4.6619^{c}	8.5608^{a}
VIUSID Agro®	5.7540^{a}	8.6281^{a}
Combined*	4.5814^{c}	7.0463^{b}
SE	0.108	0.26
CV (%)	9.79	17.80
	Chlorophyll *b* (mg mL^{-1})	
Control	1.6232	1.7456^{c}
FitoMas-E	1.5596	2.0117^{bc}
E.M.	1.6724	3.1107^{a}
VIUSID Agro®	1.6471	3.0949^{a}
Combined*	1.3286	2.2149^{b}
SE	0.169	0.113
CV (%)	22.22	22.40

Means with different letters in the same column and for each chlorophyll differ significantly ($P \leq 0.05$) according to Tukey's multiple range test (mean ± standard error). * FitoMas-E + E.M. + VIUSID Agro®.

When analyzing the carotene content at 60 DAS, the treatments with the best response were VIUSD agro®, E.M. and the combination of all the biostimulants (SE: 0.31, CV: 19.60), with no significant difference ($P > 0.05$) between them, although the highest value was obtained with VIUSD agro® (Fig. 3c). The control had the lowest carotene content, so it can be concluded that the biostimulants favor the synthesis of these compounds to a greater or lesser extent.

Agricultural yield

All the biostimulants had a positive effect on the agricultural yield (Fig. 3d). The best value for this indicator was obtained with the application of VIUSID Agro®, although it did not significantly differ ($P > 0.05$) from the E.M. but did differ from the other treatments ($P < 0.05$). VIUSID Agro® outperformed the control by 2.95 t ha^{-1} (SE: 1.04, CV: 12.60), which represents a significant increase in yield (Fig. 3d).

Discussion

Garlic cultivation holds significant global importance due to its widespread use in culinary and pharmaceutical applications (Bayan et al., 2014; Verma et al., 2023). Like all organisms, garlic is influenced by various environmental factors including climate, soil quality, and water availability. Among these factors, soil nutrient levels play a crucial role in determining crop productivity. To boost yields, an increasing reliance on chemical fertilizers has been observed, despite their detrimental effects on both human health and the environment. These fertilizers contribute to irreversible soil salinity and chemical alterations, impacting nearby water reservoirs and streams surrounding cultivation areas.

In recent years, substantial strides have been made in the research and development of biological products aimed at enhancing plant growth and protection. Biostimulants offer alternatives that mitigate environmental harm while promoting agricultural productivity. Various biostimulants with specific applications tailored to different crops are now available on the market. In this study, we evaluated the efficacy of plant growth biostimulants VIUSID Agro®, FitoMas-E, E.M., and their combinations (FitoMas-E + E.M. + VIUSID Agro®) on several morphophysiological parameters of garlic.

Regarding garlic plant height, our findings align with previous studies indicating E.M. as an effective growth promoter (Sharma et al., 2017; Huaman, 2019; Gabriela de Melo et al., 2022). Our results surpassed those reported by Carmona and Melendrez (2019), who evaluated VIUSID Agro® effects on garlic and noted a maximum increase in plant height of 22.40 and 17.85% with doses of 0.25 and 0.50 L ha^{-1}, respectively. Analysis of pseudostem diameter revealed that FitoMas-E yielded lower results, consistent with Carmona and Melendrez (2019)'s findings on VIUSID Agro®, which reported a notable 52.38% increase in pseudostem diameter compared to the control.

At 60 d after the budding of the stem, the rate of growth decreases, the plant begins to tuberize, the rate of growth of the aerial part decreases, and the leaves senesce as a result of the transfer of photosynthates to the bulb. The positive effects of biostimulants on plant stimulation and development through improvements in nutrition and nutrient and water uptake are well known (Chandran et al., 2021; Kumar et al., 2021). In terms of bulb diameter, similar results were reported by Pupo-Feria et al. (2016), where treatment with Phytomas-E and its combination with QuitoMax yielded the best results, although the largest diameter obtained was 3.9 cm, which was far from those presented in this research. These results were also similar to those reported by Huaman (2019), who applied different doses of E.M. in the cultivation of *A. sativum*. E.M. had a significant effect on the bulb diameter, which reached values of 6.01 cm, representing a 63.44% increase over the control. Similarly, when analysing the number of cloves per bulb, Abreu et al. (2021) reported that when different biostimulants were applied, including E.M., there were no significant differences in the number of cloves per bulb. However, Pupo-Feria et al. (2016) reported an increase in the number of cloves per bulb by applying FitoMas-E and the combination of FitoMas-E + EcoMic.

At 60 DAS, the total leaf area exhibited a similar response to that observed at 45 DAS, with the E.M. and VIUSID Agro® treatments achieving the highest significant values. There was no significant difference between these two treatments, and they both outperformed the other treatments and the control, which showed no significant differences among themselves. According to Murchie et al. (2009), a higher leaf area enhances laminar photosynthetic activity, as the response of dry matter accumulation to increased planting density is primarily dependent on leaf area. Plants with a larger leaf area are more Effective in nutrient utilization and solar energy capture, leading to more effective photosynthesis. Keswani et al. (2020) reported that E.M. treatment has the ability to produce indole-3-acetic acid, which leads to increased plant leaf growth. These results are somewhat similar to those reported by Peña et al. (2018a), who evaluated the effects of the biostimulant VIUSID Agro® on tobacco crops and obtained similar results.

The leaf area index (LAI) is a fundamental variable for studying the development and growth of crops. It is the basis for estimating water and nutrient requirements, bioenergetic efficiency, and the potential for phytosanitary damage. There is a close relationship between the LAI and solar radiation interception, which is related to photosynthesis and transpiration processes, aspects that are strongly linked to biomass accumulation and productivity (Wadas and Dziugieł, 2020). Therefore, the LAI is a crucial variable for quantifying agronomic crop growth and yield (Elings, 2000; Murchie et al., 2009). The LAI values obtained in our study confirm those reported by Osman (2018) for sunflower (*Helianthus annuus*) crops, where no significant differences were detected between the FitoMas-E treatments and the control. As has been observed with other variables already described, FitoMas-E did not yield different results from the control, which is a very important aspect to consider when designing a biostimulant application scheme for this crop. Regarding the leaf area ratio (LAR), our findings suggest that treatments with VIUSID Agro® and E.M. enhance the physiological processes in plants. The LAR, directly associated with chlorophyll content, indicates that plants with lower LAR values are more effective (Gardner et al., 2017). This implies that these products positively impact the efficiency of chlorophyll-containing parenchyma tissue. Specifically, VIUSID Agro®'s effectiveness could be attributed to its composition, which includes amino acids known to be precursors and components of proteins vital for stimulating cell growth (Rai, 2002). Among the growth indices evaluated, the absolute growth rate (AGR) showed significant differences, consistent with the findings of Peña et al. (2018b), who observed similar results with different doses of VIUSID Agro® applied to radish (*Raphanus sativus*) crops, reporting an increase of 0.0689 g d^{-1} compared to the control. Analysis of the net assimilation rate (NAR) at 45 DAS revealed no significant differences between treatments; however, a significant difference was noted at 60 DAS. These results indicate that VIUSID Agro® and E.M. treatments enhance the production of photoassimilates, essential for achieving high yields. This is supported by the findings of Rodriguez and Peña (2021), who reported a 26% increase in NAR with VIUSID Agro® treatments in sugar beet (*Beta vulgaris* L.), with all treated groups showing statistical differences from the control (0.0013 g cm^{-2} d^{-1}). NAR measures the efficiency of foliage in producing photoassimilates for dry matter production, recording the net photosynthesis rate over time (Konings, 1989). The relative growth rate (RGR), defined as biomass gain over time, is another crucial variable for plant growth analysis (Wandera et al., 1992; Osone et al., 2008). Our results indicate a significant increase in RGR at 60 DAS, aligning with the findings of Abu-Qaoud et al. (2021), who reported increased leaf biomass growth rates in wheat (*Triticum aestivum* L.) with the application of various effective microorganisms.

Compared to the control, all biostimulant treatments improved the relative water content (RWC) except for FitoMas-E, which showed no difference. The biostimulants enhanced growth and root formation, enabling plants to absorb more water and improving their water status. Conversely, the saturation deficit (SD), indicating the water deficit needed for tissue saturation, was highest in the mock and FitoMas-E treatments, with no differences between them, but differed from the other treatments. VIUSID Agro® treatment potentially enhanced tissue hydration, resulting in a lower deficit. Chlorophyll content, crucial for photosynthetic capacity and yield (Kumar et al., 2021; Zhang et al., 2023). Leal-Almanza et al. (2018) suggested that this increase could be due to adequate nitrogen fertilization stimulated by biostimulants. Chen et al. (2019) highlighted carotene as antenna pigments transferring energy to chlorophyll, observing an 11.27% increase in carotene content in ginger (*Zingiber officinale*) with microorganism treatment [0.5 mL kg^{-1} (soil)]. Costa et al. (2020) indicated that carotene help plants capture more light energy, enhancing growth.

Interestingly, when all biostimulants (FitoMas-E + E.M. + VIUSID Agro®) were applied in combination, an increase in plant height, AGR, RGR, and NAR was observed at 45 DAS. Moreover, a significant increase in carotene content, AGR, and productivity was observed at 60 DAS. For the other parameters measured, no significant differences were observed when the plants were treated with the combination of biostimulants. This lack of effect could be due to antagonism caused by the interaction of different components present in each formulation (Rouphael et al., 2018).

Conclusions

Garlic is a crop that is very sensitive to environmental conditions, and its yield can be significantly affected. The results obtained in the present research were superior to those obtained by Carmona and Melendrez (2019). The biostimulants used had better effects on the morphophysiological parameters evaluated, with VIUSID Agro® and E.M. resulting in the highest values. All the biostimulants had a positive effect on agricultural yield, with VIUSID Agro® outperforming the control by 2.95 t ha^{-1}, an increase of more than 38%, and E.M. by 2.25 t ha^{-1}. VIUSID Agro® and E.M. also proved to be the most effective treatments when analyzing other commercially relevant parameters, such as pseudostem diameter, bulb diameter, and absolute growth rate.

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Author Contributions
Conceived and designed the experiments, MTGG, LIRC, KPC, ZGU, MMRJ, YRC, FD, and GD; Performed the experiments, MTGG, LIRC, KPC, MMRJ, and YRC; Analysed the data, MTGG, LIRC, KPC, ZGU, and GD; Contributed reagents/materials/analysis tools, MTGG and KPC; Wrote the paper, MTGG, LIRC, KPC, ZGU, and GD. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest
The authors indicate no conflict of interest in this work.

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