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Biostimulants increase the yield of greenhouse-grown tomato plants in summer under a tropical climate

Biosetimulantes aumentam a produtividade de tomateiro em casa de vegetação no verão sob clima tropical

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ABSTRACT - While tomatoes can be grown year-round in a greenhouse, the high temperatures of tropical climates are a limitation. As such, cooling the growing environment is key to cultivating tomatoes in controlled environments during summer, but effective cooling systems are expensive and involve high production costs. The use of biostimulants has been reported to increase yield and can mitigate the effects of high temperature on greenhouse-grown tomatoes. Our hypothesis is that biostimulants can improve tomato yield, particularly in the event of stress during cultivation. Our aim was to assess the effects of three biostimulants on greenhouse-grown tomato plants in the summer under a tropical climate. The experiment was conducted in a greenhouse on the Uberaba Campus of the Federal Institute of the Mineiro Triangle (IFTM). We used a completely randomized design consisting of a plot containing five plants, with 1.0 x 0.5 m spacing, an estimated density of 20,000 plants ha^{-1}, and six repetitions. The three biostimulants applied were Alquifishmel®, Booster® and Stimulate®, compared to a control (plants with no biostimulant). Production, fruit quality, yield and plant growth were assessed. All three biostimulants improved yield by more than three metric tons per hectare in relation to the control, primarily due to the larger number of ripe fruits. It is suggested that the biostimulants mitigated heat stress, promoting a larger number of fruits per truss and resulting in higher yield.

Keywords: Alquifishmel©. Booster©. Stimulate©. Heat stress.

RESUMO - Embora o tomate possa ser cultivado durante todo o ano em casa de vegetação, as altas temperaturas dos climas tropicais são uma limitação. Assim, resfriar o ambiente de cultivo é fundamental para cultivar tomates em ambientes controlados durante o verão, mas sistemas de resfriamento eficazes são caros e envolvem altos custos de produção. O uso de bioestimulantes tem sido relatado para aumentar a produtividade e pode mitigar os efeitos da alta temperatura em tomateiros cultivados em casa de vegetação. Nossa hipótese é que os bioestimulantes podem melhorar a produtividade do tomateiro, principalmente em caso de estresse durante o cultivo. Nosso objetivo foi avaliar os efeitos de três bioestimulantes em plantas de tomate cultivadas em casa de vegetação no verão sob clima tropical. O experimento foi conduzido em casa de vegetação no Campus Uberaba do Instituto Federal do Triângulo Mineiro (IFTM). Utilizou-se o delineamento inteiramente casualizado com seis repetições, parcela contendo cinco plantas, com espaçamento de 1,0 x 0,5 m e densidade estimada de 20,000 plantas ha^{-1}. Os três bioestimulantes aplicados foram Alquifishmel®, Booster® e Stimulate®, comparados a um controle (plantas sem bioestimulante). Foram avaliados a produção, a qualidade dos frutos, a produtividade e o crescimento das plantas. Todos os três bioestimulantes melhoraram a produtividade em mais de três toneladas por hectare em relação ao controle, principalmente devido ao maior número de frutos maduros. Sugere-se que os bioestimulantes atenuaram o estresse térmico, promovendo maior número de frutos por cacho, o que resultou em maior produtividade.

Palavras-chave: Alquifishmel®. Booster®. Stimulate®. Estresse por calor.

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INTRODUCTION

Tomato production during the hot wet season of tropical and subtropical regions is limited by unfavorable conditions such as high temperatures, flooding, strong winds and a high incidence of diseases. Although greenhouse systems enable tomatoes to be grown year-round, the main limitation is the high temperature inside these structures. Providing a cooling effect in the growing environment is therefore key to tomato cultivation in controlled environments during summer. There are several methods for cooling greenhouses, including evaporative cooling, shading and natural ventilation. However, high ambient relative humidity and temperature limit the efficiency of evaporative cooling systems and natural ventilation, respectively. Forced ventilation with humidity control and shading is the most effective method, but these greenhouses are expensive and involve high production costs (NICOLA; TIBALDI; FONTANA, 2009).

The use of plant stimulants (biostimulants, growth regulators, foliar biofertilizers, algal extracts etc.) has been reported to improve yield in different crops (DU JARDIM, 2015; YAKHIN et al., 2017) and may mitigate the effects of high temperatures in greenhouse-grown tomatoes (COLLA et al., 2017; CHEHADE et al., 2018). However, their use in plants remains highly controversial and despite promising and well-founded results indicating important yield improvements with their large-scale application, some of the manufacturers' claims have yet to be confirmed; as well as the effects are typically only observed in plants exposed to some form of stress (DU JARDIM, 2015; YAKHIN et al., 2017).

Our hypothesis is that biostimulants can improve tomato yield, particularly in the event of stress during cultivation. As such, the present study aimed to assess the effect of three biostimulants on the production, fruit yield and quality, and plant growth of greenhouse-grown tomatoes in summer under a tropical climate.

MATERIAL AND METHODS

The experiment was conducted from October 11, 2019, to January 10, 2020, in a 3.5-meter-high arched roof greenhouse, 51 meters long and 14 meters wide, covered in 150 micra transparent plastic sheeting with 50% shade cloth on the sides, located at the Uberaba Campus of the Federal Institute of the Mineiro Triangle (IFTM). Temperature in the greenhouse was not controlled. Climate in the region is classified as Aw (Köppen), characterized by a cold dry period from April to September and a hot wet season from October to March (BECK et al., 2018). The temperature (ºC) inside the greenhouse was measured daily between 11 a.m. and 12 p.m. after biostimulant application with a thermohygrometer (J Prolab®). Meteorological data (maximum and average temperatures; number of hours per day with a temperature above 30 ºC) were obtained from the IFTM weather station (Davis Vantage Pro 2®).

The tomato cultivar used was the Conquistador hybrid produced by SAKATA®, with the following characteristics: indeterminate salad variety, high vigor, excellent performance in greenhouse systems, average first harvest period of 110 days, short internodes, large fruits, high fruit set, and excellent fruit color and uniformity. It is also highly resistant to *Verticillium dahlia* race 1 (Vd 1), *Fusarium oxysporum* f.sp *lycopersici* races 1 and 2 (Fol 1-2), Tomato mosaic virus (ToMV), *Meloidogyne incognita* (Mi) races 1, 2, 3 and 4 and *Meloidogyne javanica* (nematode), and tomato spotted wilt virus (TSWV) (SAKATA, 2022).

Sowing was performed on September 2, 2019, in 64-cell expanded polystyrene trays filled with Bioplant Prata® substrate. The seedlings were transplanted on October 11, 2019 into 13-liter plastic pots filled with the same substrate.

A completely randomized design was adopted, with four treatments and six repetitions. The treatments were three commercial products characterized as biostimulants (Alquifishmel®, Booster® and Stimulate®) and a control (standard cultivation, without biostimulants). The experimental plot contained five plants (one per pot), spaced 0.5m apart and 1.0 m between rows, at an estimated density of 20,000 plants ha^{-1}.

Alquifishmel® (Ophicina orgânica) is registered as an organomineral foliar fertilizer certified for organic farming and contains raw materials such as fish, crustacean shells, sugarcane molasses and algal extract, with 1% nitrogen (10 g L^{-1}) and 6% organic carbon (61.8 g L^{-1}). Booster® (Agrichem) is composed of 239 g L^{-1} of algal extract (*Ecklonia maxima*), 24.4 g L^{-1} of molybdenum and 36.6 g L^{-1} of zinc. The algal extract used typically contains 10.7 mg L^{-1} of auxins and 0.03 mg L^{-1} of cytokinins (MEYER et al., 2021). Stimulate® (Stoller) is a blend of growth regulators consisting of 90 mg L^{-1} of N6-furfuryladenine (a cytokine compound), 50 mg L^{-1} of GA_3 (a gibberellin) and 50 mg L^{-1} of 4-(indol-3-yl) butyric acid (an auxin).

The dosages used were those recommended for the crop on the product labels, namely 0.6, 0.5 and 0.5 L ha^{-1} for Alquifishmel, Booster and Stimulate, respectively. Alquifishmel and Booster were applied weekly (totaling eight applications) and Stimulate every 14 days (totaling four applications), using a 1-liter manual sprayer. Spray application of the biostimulants began on November 11, 2019, when the first flower buds emerged on the racemes. A screen was used to isolate one pot from the others during spraying.

The plants were vertically trained to a height of 2.2 m using plastic twine and then pruned, leaving only one stem per plant. The lateral branches were thinned every 4 days and staked and/or tied as needed. Drip irrigation was carried out for 30 minutes three times a day, using two emitters per pot, each with a flow rate of 2 L hour^{-1}. Fertilization was performed via full fertigation, adapted from Furlani et al. (1999), with electrical conductivity of 2.0 dS m^{-1} and pH 6.5. The solution described by Furlani et al. (1999) was adapted by using ConMicros Standard® (Allplant) to supply micronutrients, applying 400 mL per pot, three times a week (Monday, Wednesday and Friday). The fertilizers used and amount of nutrients in the nutrient solution in grams m^{-3} of water were: calcium nitrate (Ca [152.0], N-NO_3^- [116.0], N-NH_4^+ [8.0]); potassium nitrate (K [179.2], N-NO_3^- [60.0]); monoammonium phosphate (P [92.8], N-NH_4^+ [42.0]); magnesium sulfate (Mg [10.0], S [13.0]). ConMicros (B [0.455], Cu [0.455], Fe [1.815], Mn [0.455], Mo [0.090], Ni [0.084], Zn [0.183]).

Beginning on December 10, 2019, the first, second and third trusses per plant were selected and the following assessed: total number of fruits (green and ripe) on all three trusses (TNF3T), number of ripe fruits on all three trusses (NRF3T), mass of ripe fruits on all three trusses (MRF3T, g) and mean mass per ripe fruit (MRF, g). Estimated yield was expressed in kilograms per hectare, calculated by multiplying MRF3T by 20,000 plants, in line with the spacing adopted in the experiment. Lost fruit was also evaluated on the three trusses and included fallen fruit that was still green, rotting or split fruit, or tomatoes attacked by insects. In addition to the variables cited above, the following were assessed in ten fruits per plot on the second truss: longitudinal diameter (LD, mm), transverse diameter (TD, mm), pulp thickness (PT, mm), locule diameter (LcD, mm) and total soluble solids (TSS), using a manual refractometer and expressed in ºBrix.

A final analysis was carried out on January 10, 2020 to determine the total number of fruits per plant (TNFP), which corresponded to the number of green fruits that were left on all the trusses of each plant added to the ripe tomatoes already assessed on the original three trusses. Shoot dry mass (SDM, g), root volume (RV, cm^3) and stem diameter (SD, mm) were measured on a randomly selected plant from each plot. Chlorophyll *a* fluorescence was also determined (OJIP test) on a fully expanded leaf at a height of around 1.40 m, using a portable chlorophyll meter (FluorPen 100, Photon Systems Instruments). Fluorescence measurements were taken after 11 p.m. in plants adapted to the dark, obtaining the following variables: dissipated energy flux per reaction center (DIo/RC), maximum quantum efficiency (Fv/Fm) of photosystem II (PSII), absorption flux per RC of PSII (ABS/RC), trapped energy flux per RC (TRo/RC) and electron transport rate per RC (ETo/RC).

The data were submitted to analysis of variance (ANOVA) and post-hoc comparisons between means were performed using Tukey's test ($p \leq 0.001$) in R® software. Descriptive statistical analysis was carried out with Jamovi® software, obtaining boxplots and alluvial diagrams. Heat map analysis, hierarchical clustering, principal component analysis (PCA) and variable intercorrelations were performed in accordance with Araújo et al. (2018) and Carvalho et al. (2018).

RESULTS AND DISCUSSION

The temperature inside the greenhouse generally varied from 30 to 35 °C between 11 a.m. and 12 p.m., occasionally reaching 38 °C or dipping below 30 °C. The maximum outside temperature throughout the day was similar to that recorded in the greenhouse, ranging between 30 and 35 °C, with peaks of 34 °C and temperatures below 28 °C at different times. The average outside temperature was usually between 23 and 28 °C.

On 12 days the maximum daily temperature and, consequently, the temperature in the greenhouse, remained higher than 30 °C, totaling 82.5 hours above this temperature from the onset of flower bud formation to the end of the experiment (Figure 1). In hot, tropical regions, high temperatures (≥ 35 ºC) can prevail for days (ABDUL-BAKI; STOMMEL, 1995). Greenhouse temperature for tomato cultivation under tropical climate conditions was measured throughout the day and began to rise at 8:00 a.m. (≥ 26 ºC), increasing exponentially until 10:00 a.m. (≥ 32 ºC) and reaching a plateau of 35 to 38 °C between 10:00 a.m. and 4 p.m. (SHAMSHIRI, 2017). These data are consistent with the temperatures measured between 11 a.m. and 12 p.m. in the greenhouse in the present study, under a tropical climate.

Temperatures ≥ 30 ºC negatively affect tomato fruit production due to factors such as a decline in flower production caused by abortion and blossom drop; reduced ovule and pollen viability; less exposure of stigmata and anthers; decreased pollen tube growth; and lower photosynthetic rates and photoassimilate translocation to the fruits (ABDUL-BAKI; STOMMEL, 1995; HAZRA et al., 2007; SHAMSHIRI et al., 2018). High temperatures also have a negative impact on Rubisco activity and therefore on photosynthesis For RuBisCo, temperatures between 30 and 38 ºC are considered moderate heat stress and ≥ 38 ºC severe stress. Both moderate and severe stress lower RuBisCo and catalytic site concentration, reduce RuBisCo and RuBisCo activase activity, and result in the presence of inhibitors (GALMÉS et al., 2013).

The effects of heat stress on tomato plants are well documented in the literature, indicating that the plants grown in the present study may have been exposed to high temperature stress, providing favorable conditions for the action of the biostimulants.

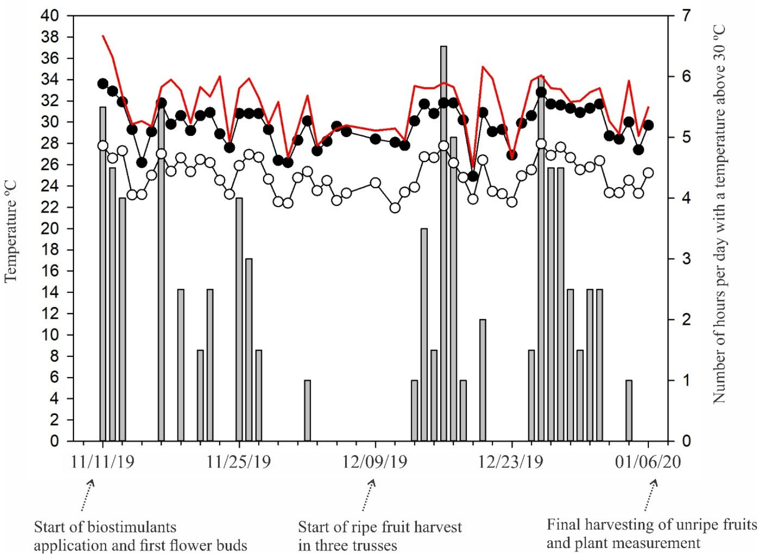

Figure 1. Greenhouse temperature between 11 a.m. and 12 p.m. (solid red line), measured with a thermohygrometer. Maximum daily temperature (solid black line with black dots), average outdoor temperature (solid black line with circles) and times of day with temperature above 30 ºC (bars) measured at a weather station, from the start of treatment application to the end of the experiment.

In general, the plants produced four to seven trusses, with an average of six per plant in all the treatments (data not shown). There was no significant difference in the total number of fruits (green and ripe) per plant; however, this variable increased in plants treated with Alquifishmel (+1.47) and Stimulate (+1.10) when compared to controls and there were fewer lost fruits in plants treated with Alquifishmel (-1.17) and Stimulate (-1.17) in relation to controls (Table 1).

Table 1. Fruit quality, production and yield of tomato plants treated with biostimulants and controls. Variables: total number of fruits (green and ripe) per plant (TNFP), total number of fruits (green and ripe) on three trusses (TNF3T), number of ripe fruits on three trusses (NRF3T), fresh mass of ripe fruits on three trusses (MRF3TB, g), mean fresh mass per ripe fruit (MRF, g), yield (Y, kg ha^{-1}), total soluble solids (TSS, ºBrix), and lost fruits per plant (LF).

	Biostimulants	TNFP	TNF3T	NRF3T	MRF3T	MRF	Y	TSS	LF
Mean	Alq	19.6a	14.9a	7.63a	967a	128a	19.3a	4.43a	0.50a
	Boo	18.7a	14.4a	7.60a	973a	128a	19.4a	4.56a	0.33a
	Sti	19.3a	14.6a	7.83a	961a	123a	19.2a	4.56a	0.83a
	Cont	18.2a	13.4a	6.43b	792b	123a	15.8b	4.39a	1.50a
p value	-	0.72	0.38	<0.001	<0.001	0.67	<0.001	0.27	0.40
CV(%)	-	12.34	10.68	7.24	6.30	8.24	6.29	4.07	157.6

Different lowercase letters indicate a significant difference according to Tukey's test (n=6). Coefficient of variation (CV). Control (Cont); Alquifishmel (Alq); Booster (Boo); Stimulate (Sti).

Plants sprayed with Booster, Alquifishmel and Stimulate showed an 18.2, 18.7 and 21.8% increase in the number of ripe fruits on three trusses in relation to controls. The alluvial diagram shows that most of the control plants had 6.4 ripe fruits on three trusses, the lowest among the classes in the diagram. On the other hand, for Stimulate this variable was concentrated in three classes, 8.3, 7.9 and 7.5 ripe fruits on three trusses, the highest in the diagram (Figure 2).

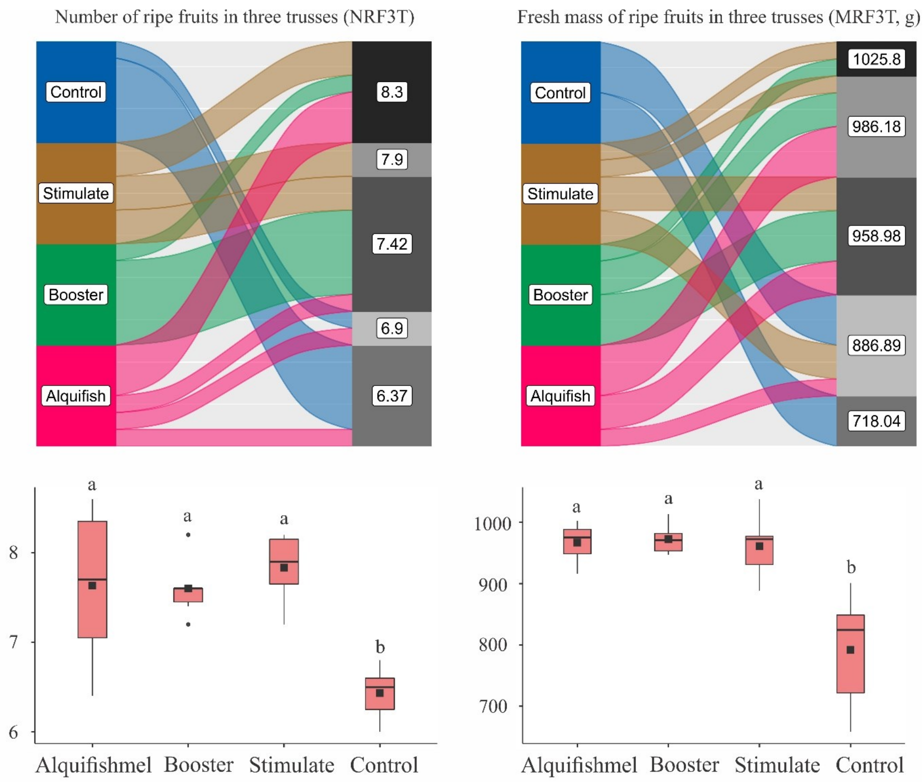

Figure 2. Fruit production of tomato plants grown with biostimulants and a control. The alluvial diagram at the top shows a series of images that explain the distribution of data for each treatment as a function of five classes for NRF3T and MRF3T. The values shown on the diagram are the mean for that class. The boxplot at the bottom depicts the mean (black square), median (black line), outliers (black dots) and percentiles (pink box (50%) and vertical bars (25%)). Different lowercase letters indicate a significant difference according to Tukey's test (p <0.001; n=6).

The fresh mass of ripe fruits on three trusses was 22.0, 22.8 and 21.3% grams higher in plants treated with Alquifishmel, Booster and Stimulate, respectively, than in controls. The alluvial diagram demonstrates that for most control plants, these data are concentrated in the lower classes of 718.0 and 887.0 g. On the other hand, the data for Booster are concentrated in the three highest classes for this variable, ranging from 959.0 to 1025.8 g (Figure 2).

Plants treated with Alquifishmel, Booster and Stimulate showed an increase in ripe fruits on three trusses of more than 3 metric tons per hectare in relation to controls (Table 1). There was no difference in mean fruit mass per plant (MFM), total soluble solids (TSS) and lost fruits per plant (LF) (Table 1). The variables related to fruit size (PT, LcD, LD and TD) and plant growth (SDM, SD, RV) did not differ (Table 2).

Table 2. Fruit quality and biometric traits of tomato plants treated with biostimulants and controls. Variables: pulp thickness (PT, mm), locule diameter (LcD, mm), longitudinal diameter (LD, mm), transverse diameter (TD, mm), shoot dry mass (SDM, g per plant), stem diameter (SD, mm per plant) and root volume (RV, cm^3 per plant).

	Biostimulants	PT	LcD	LD	TD	SDM	SD	RV
Mean	Alq	8.27a	49.2a	54.3a	64.9a	65.4a	9.25a	243a
	Boo	7.96a	48.0a	53.1a	63.7a	58.4a	8.28a	237a
	Sti	7.77a	47.7a	53.4a	62.5a	58.7a	8.78a	195a
	Cont	7.78a	46.9a	52.4a	63.5a	57.0a	8.92a	194a
p value	-	0.45	0.46	0.58	0.75	0.60	0.35	0.64
CV (%)	-	7.54	5.31	4.43	5.88	19.22	10.34	39.6

Different lowercase letters indicate a significant difference according to Tukey's test (n=6). Coefficient of variation (CV). Control (Cont); Alquifishmel (Alq); Booster (Boo); Stimulate (Sti).

In the physiological assessments, a difference was observed only for Dio/RC (Table 3). Fv/Fm is one of the main indicators of photoinhibition when plants are subjected to stress, including high temperatures (ARAÚJO; DEMINICIS, 2009). However, Fv/Fm is a robust variable and the lack of significant decreases in control plants indicates that heat stress was not sufficient to cause photoinhibitory damage, but the control plants adjusted their use and dissipation of light energy into heat, confirmed by the Dio/RC results. The increase in DIo/RC is related to greater light energy dissipation into heat (nonphotochemical quenching), largely associated with the xanthophyll cycle (STRASSER; TSIMILLI-MICHAEL; SRIVASTAVA, 2004; BUCHANAN; GRUISSEM; JONES, 2015), confirming that the control plants were more affected by high temperature.

Table 3. Quantum efficiency variables in tomato plants treated with biostimulants. Variables: dissipated energy flux per reaction center (DIo/RC), maximum quantum efficiency (Fv/Fm) of photosystem II (PSII), absorption flux per RC of PSII (ABS/RC), trapped energy flux per RC (TRo/RC) and electron transport rate per RC (ETo/RC).

	Biostimulants	Dio/RC	Fv/Fm	ABS/RC	TRo/RC	ETo/RC
Mean	Cont	0.37 a	0.820 a	2013.1 a	1655.0 a	1.01 a
	Boo	0.35 ab	0.821 a	2020.2 a	1653.7 a	1.02 a
	Sti	0.34 b	0.825 a	2033.1 a	1648.8 a	1.02 a
	Alq	0.33 b	0.821 a	2023.5 a	1665.3 a	1.03 a
p value	-	0.00	0.24	0.98	0.97	0.69
CV (%)	-	4.57	0.50	3.87	3.82	2.34

Different lowercase letters indicate a significant difference according to Tukey's test (n=6). Coefficient of variation (CV). Controle (Cont); Alquifishmel (Alq); Booster (Boo); Stimulate (Sti).

There was a clear difference in fruit production and yield between the biostimulants and control treatment, but not among the biostimulants themselves. However, the dendrogram shows the formation of three groups (1-Control; 2 -Stimulate; 3-Alquifishmel and Booster), whereby there was greater similarity between the Alquifishmel and Booster biostimulant treatments in relation to Stimulate, particularly for mean mass of ripe fruits and lost fruit (Figure 3). This similarity is evident in the data in Table 1 and the heat map for the magnitude of the variables in the dendrogram, which shows a difference in the orange and white rectangles for these variables between Stimulate, Alquifishmel and Booster (Figure 3).

The results of principal component analysis (PCA) indicated that 47.1% of total variance was explained by the first principal component (PC1), and 22.8% by the second (PC2), totaling 69.9% variance in the original data. The third principal component (PC3) explained 12.5% of total variance, with the sum of all three components explaining 82.5% of the variance in the original dataset (Table 4).

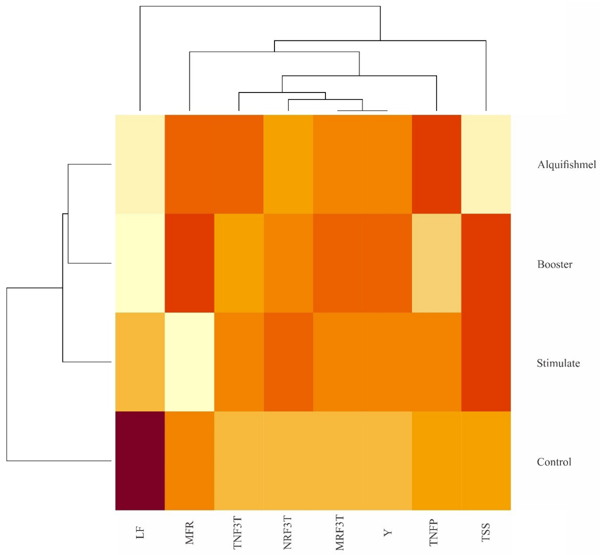

Figure 3. Heat map analysis and hierarchical clustering dendrogram considering all the characteristics related to tomato fruit production, quality and yield. The darker the color on the heat map, the greater the magnitude of the variable. Variables: total number of fruits (green and ripe) per plant (TNFP), total number of fruits (green and ripe) on three trusses (TNF3T), number of ripe fruits on three trusses (NRF3T), fresh mass of ripe fruits on three trusses (MRF3T), mean fresh mass per ripe fruit (MRF), yield (Y, Kg ha^{-1}), total soluble solids (TSS), lost fruits per plant (LF).

Table 4. Eigenvalues, percentage explained variance, percentage cumulative variance, correlation coefficient and eigenvectors of the correlation matrix between the fruit production, quality and yield attributes of tomato plants treated with biostimulants for the first three principal components.

Components	PC1	PC2	PC3
Eigenvalue	3.77	1.82	1.00
Explained variance (%)	47.1	22.8	12.5
Cumulative variance (%)	47.1	69.9	82.5
Correlation coefficients (eigenvectors)			
TNFP	0.50 (0.25)	-0.59 (-0.43)	-0.40 (-0.40)
TNF3T	0.80 (0.41)	-0.34 (-0.25)	-0.28 (-0.28)
NRF3T	0.76 (0.39)	-0.30 (-0.22)	0.56 (0.56)
MRF3T	0.92 (0.47)	0.29 (0.21)	0.16 (0.16)
MRF	0.27 (0.14)	0.75 (0.56)	-0.53 (-0.53)
Y	0.92 (0.47)	0.29 (0.21)	0.16 (0.16)
TSS	0.19 (0.09)	0.71 (0.52)	0.15 (0.15)
LF	-0.67 (-0.35)	0.11 (0.08)	0.26 (0.26)
Interpretation	Yield, fresh mass of ripe fruits on three trusses, total number of fruits (green and ripe) on three trusses, number of ripe fruits on three trusses and lost fruits.	Mean fresh mass per ripe fruit and total soluble solids.	Number of ripe fruits on three trusses and mean fresh mass per ripe fruit.

Yield, fresh mass of ripe fruits on three trusses, total number of fruits (green and ripe) on three trusses, number of ripe fruits on three trusses and lost fruits.
Variables: total number of fruits (green and ripe) per plant (TNFP), total number of fruits (green and ripe) on three trusses (TNF3T), number of ripe fruits on three trusses (NRF3T), fresh mass of ripe fruits on three trusses (MRF3T), mean fresh mass per ripe fruit (MRF), yield (Y, Kg ha^{-1}), total soluble solids (TSS), lost fruits per plant (LF).

In the biplot, the eigenvalues and eigenvectors of the correlation matrix clearly demonstrate the formation of only two groups (biostimulants vs control) (Figure 4), also observed in hierarchical clustering (Figure 3). The variables yield, fresh mass of ripe fruits on three trusses, total number of fruits (green and ripe) on three trusses, number of ripe fruits on three trusses and lost fruits showed greater discriminatory power to distinguish between the control and biostimulants for PC1. Mean fresh mass per ripe fruit and total soluble solids exhibited higher discriminatory power for the trend of separation between Stimulate (low MRF and high TSS) and Alquifishmel (high MRF and low TSS) for PC2. The discriminatory power of each variable within each principal component can be measured based on the correlation coefficients (loadings) of each variable for the respective principal component (Table 4).

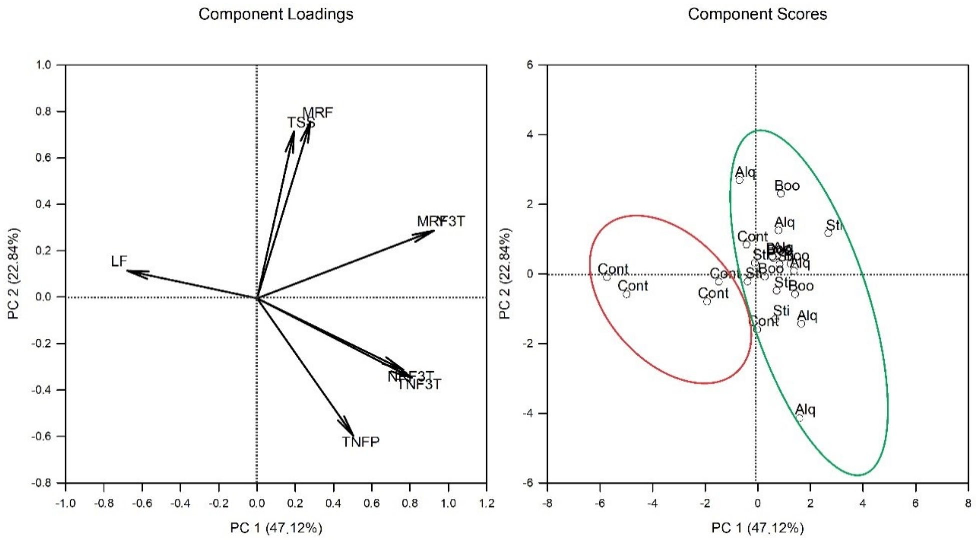

Figure 4. Biplot of the first and second principal components depicting tomato fruit production, quality and yield. Control (Cont); Alquifishmel (Alq); Booster (Boo); Stimulate (Sti). The red circle represents the control group and the green circle the biostimulant group. Variables: total number of fruits (green and ripe) per plant (TNFP), total number of fruits (green and ripe) on three trusses (TNF3T), number of ripe fruits on three trusses (NRF3T), fresh mass of ripe fruits on three trusses (MRF3T), mean fresh mass per ripe fruit (MRF), yield (Y, Kg ha^{-1}), total soluble solids (TSS), lost fruits per plant (LF).

Since there was no difference in mean fresh mass per ripe fruit, the higher production and yield of plants treated with the biostimulants can be attributed to the larger number of ripe fruits on three trusses (NRF3T). This is corroborated by Pearson's correlation analysis, with a positive correlation (0.72) between NRF3T and MRF3T and between NRF3T and yield (Figure 5).

Stimulate® is a blend of growth regulators (auxin + cytokinin + gibberellin) that promote a hormonal effect in plants, contributing to growth and development, in addition to improving yield and flower and fruit set. Auxins and gibberellins are general growth factors and play and important role in the onset of fruit development (BUCHANAN; GRUISSEM; JONES, 2015). Most of the putative tomato regulators identified to date are common signaling components of these hormones and their reduced expression often results in pleiotropic effects in plants, including parthenocarpy. In short, this combination of hormones controls the expression of genes involved in tomato fruit development (JONG; MARIANE; VRIEZEN, 2009). Cytokinins play a role in cell division, common in the early stages of reproductive structures, as well as in photoassimilate translocation and nutrient mobilization to sinks (BUCHANAN; GRUISSEM; JONES, 2015). Stimulate application increased the mean fresh weight of Micro-Tom tomatoes by 24% (CATO et al., 2013). Foliar spraying of 0.5L ha^{-1} (five applications) on field-grown Debora Max hybrid tomatoes (one stem per plant, with up to eight racemes and complete harvesting) increased yield by around five metric tons per hectare (LIMA JR, et al. 2009).

Booster® is composed of 239 g L-1 of algal extract (*Ecklonia maxima*), 24.4 g L-1 of molybdenum (Mo) and 36.6 g L-1 of zinc (Zn). The algal extract used typically contains 10.7 mg L-1 of auxins and 0.03 mg L-1 of cytokinins (MEYER et al., 2021). The hormones in Booster contribute to plant growth and development and promote the development of reproductive structures, improving flower and fruit set (BUCHANAN; GRUISSEM; JONES, 2015; MEYER et al., 2021). Mo acts as a cofactor of nitrate reductase and can increase nitrogen assimilation, especially in nonnodulating plants such as tomato, where most of the nitrogen occurs in the form of nitrate. Mo is also a cofactor of ABA3 (molybdenum cofactor sulfurase), which participates in ABA synthesis, an important hormone for seed development and stomatic control. Zn participates in the cytochrome c oxidase complex (complex IV) of the mitochondrial electron transport chain and is a cofactor of superoxide dismutase (SOD), which combats oxidative stress (BUCHANAN; GRUISSEM; JONES, 2015).

Figure 5. Pearson's correlation analysis of data on the production, quality and yield of tomato fruits. Variables: total number of fruits (green and ripe) per plant (TNFP), total number of fruits (green and ripe) on three trusses (TNF3T), number of ripe fruits on three trusses (NRF3T), fresh mass of ripe fruits on three trusses (MRF3T), mean fresh mass per ripe fruit (MRF), yield (Y, Kg ha^{-1}), total soluble solids (TSS), lost fruits per plant (LF).

Thus, we suggest that the action of Booster involves the hormonal effect of auxin and cytokinin from the algal extract and the modulating effect of Mo and Zn on important plant enzymes. Foliar application of "Kelpak" (*Ecklonia maxima*), a concentrated seaweed extract, increased the marketable yield of greenhouse-grown tomatoes by 18.3% and reduced nonmarketable yield by 41.3% (COZZOLINO et al., 2021). Foliar spraying of the same product on greenhouse-grown tomatoes during the summer in Italy improved yield and marketable yield by 14.1 and 6.6%, respectively, but with no difference in total soluble solids (COLLA et al., 2017).

Alquifishmel® contains 1% nitrogen (10 g L^{-1}) and 6% organic carbon (61.8 g L^{-1}), raw materials such as crustacean shells and fish (origin of chitosan and chitin polymers), sugarcane molasses and algal extract (not specified by the manufacturer) and is the only biostimulant in the present study that can be used in organic tomato farming. The amount of nitrogen in Alquifishmel is not sufficient to cause a nutritional effect in plants when applied to the leaves (FERNANDES; SOUZA; SANTOS, 2018). On the other hand, chitosan and chitin are naturally occurring compounds with potential for agricultural applications, particularly in disease control (ABDELBASSET et al., 2010; HADRAMI et al., 2010).

A biofertilizer based on cattle manure, milk, bone meal, sugarcane molasses and fish waste, that is, with some ingredients similar to those of Alquifishmel, promoted a 15% increase in the number of tomato fruits (TANAKA et al., 2003). Postharvest treatment of tomato fruits with chitin induced strong resistance to mold rot caused by *Botrytis cinerea* (SUN et al., 2018). The authors attributed the improved disease resistance of chitin-treated tomato to the accumulation of oxygen reactive species, callose deposition, and increased activity of defense-related enzymes such as superoxide dismutase, catalase, peroxidase, and chitinases. As such, we suggest that Alquifishmel® primarily exerted a protective effect, stemming from chitosan and chitin, to mitigate stress; this was likely associated with the effect of the algal extract.

Tomato yield generally varies between 40 and 100 metric tons ha^{-1} (HEUVELINK; DORAIS, 2005). The estimated yield of the tomato plants grown in the present study was lower than that reported in the literature; however, only fruits from three trusses were used for calculations. Nevertheless, yield increased by more than three metric tons per hectare, rising by 22.0, 22.8 and 21.,3% for Alquifishmel, Booster and Stimulate, respectively. The average cost of the biostimulants in December 2021 was BRL 38.00 (Alquifishmel 1L), BRL 155.00 (Booster 1L) and BRL 147.00 (Stimulate 1L). In the present study, there were eight applications of Alquifishmel and Booster and four of Stimulate, incurring costs of 195.43 BRL ha^{-1} (Alquifishmel), 664.29 BRL ha^{-1} (Booster) and 315.00 BRL ha^{-1} (Stimulate), excluding operating costs. The increase in metric tons per hectare was 3.50, 3.61 and 3.39 for Alquifishmel, Booster and Stimulate, respectively. As such, the cost of the products per metric ton of tomato greater than the yield of the control was 55.85 BRL ha^{-1} (Alquifishmel), 184.01 BRL ha^{-1} (Booster) and 93.06 BRL ha^{-1} (Stimulate). Considering that the average amount received by rural producers per metric ton of tomato in the previous 12 months in Brazil was BRL 3,242.50 (CONAB, 2021), there is an economic advantage to applying these biostimulants, Alquifishmel being the most cost effective.

Biostimulant application has shown positive effects in greenhouse-grown tomato plants by improving crop performance and fruit nutritional parameters, and despite increasing production costs, the net income of farmers has risen significantly (COLLA et al., 2017; PETROPOULOS, 2020). The present study clearly demonstrated that biostimulants produced higher yields than those recorded in the control and acted as mitigators of heat stress, promoting a larger number of fruits and higher fruit mass, possibly due to less fruit abortion and greater retention, resulting in fewer lost fruits per plant. The biostimulants tested in the present study may be a viable alternative for tomato production in the hot season of tropical climates, especially for open-field farmers or those without access to greenhouses equipped with cooling systems. Alquifishmel is also an alternative to obtain these effects in organic farming.

CONCLUSION

The biostimulants Alquifishmel®, Booster® (eight applications) and Stimulate® (four applications) in doses of 0.6 L ha^{-1}, 0.5 L ha^{-1} and 0.5 L ha^{-1}, respectively, applied at the onset until approximately 60 days after flowering, promoted an increase of more than three metric tons of ripe Conquistador (SAKATA®) hybrid tomatoes in relation to controls, when grown in a greenhouse under high summer temperatures. Yield increased, primarily due to the larger number and greater mass of ripe fruits per plant. Of the biostimulants tested, Alquifishmel was the most cost effective and can be used in organic farming.

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