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GAS AND BIOCHEMICAL EXCHANGE OF THE 'TOMMY ATKINS' MANGO TREE: APPLICATION OF BIOSTIMULANTS IN THE BRAZILIAN SEMI-ARID REGION

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ABSTRACT
In the cultivation of mango trees in the climatic conditions of the Brazilian semi-arid region, the plants undergo metabolic variations due to abiotic stresses. The present study aimed to evaluate the gas exchange and biochemical responses of the 'Tommy Atkins' mango tree, on the application of humic substance-based biostimulants during the phenological fruiting phase in the Brazilian semi-arid region. The studies were carried out at the Surubim farm, located in the municipality of Petrolina - PE, and two distinct experiments were conducted

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simultaneously, with applications of two biostimulants via fertigation. The experimental design was in randomized blocks, in a 2 x 5 factorial scheme, with two production cycles (2021 and 2022), and five doses of the products: Sagersolo® (BST1): (0.0, 7.0, 14.0, 21.0 and 28.0 L $^{ha-1}$) and Fulvumin® (BST2): (0.0, 10.0, 20.0, 30.0 and 40.0 L $^{ha-1}$). The treatments were applied in the periods of 60, 75, 90 and 105 days after full flowering. Gas exchange and biochemical responses in leaf tissue were evaluated. BST1 doses ranging from 17.5 to 23.0 L $^{ha-1}$ provided maximum increase in stomatal conductance and internal CO2 concentration compared to the control treatment. For BST2, the doses between 24.2 and 25.0 L $^{ha-1}$ favored the maximum increase in leaf transpiration and CO2 assimilation. For both BSTs, the increase in doses during the fruiting phase promoted a linear reduction in starch contents and an increase in total free amino acids, proteins and total soluble carbohydrates in the leaf tissues of the 'Tommy Atkins' mango tree.

Keywords: Soluble carbohydrates. Photosynthesis. Fruiting. *Mangifera indica* L. Humic substances.

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INTRODUCTION

The production of mango fruits (*Mangifera indica* L.) has a great economic expression for Brazilian agriculture (SIMÕES et al., 2021). The micro-region of the Submédio do Vale do São Francisco accounts for more than 90% of Brazil's mango exports, concentrating its production mainly in the states of Bahia 60% and Pernambuco 40% (DE OLIVEIRA et al., 2023).

Among the various mango cultivars produced and marketed in the Brazilian semi-arid region, 'Tommy Atkins' stands out for its high versatility and can be marketed *in natura*, or industrially processed in the form of pulp, juices, jam, among other products (Veras, 2017). For the São Francisco Valley, according to Kist et al. (2019), the cultivar 'Tommy Atkins' occupies about 30% of the total cultivated area in the region.

The semiarid region presents variations in climatic elements, such as high temperatures and low air humidity, which makes it a challenge for mango management during the fruiting phase, mainly due to possible metabolic losses in relation to abiotic stresses. These factors can negatively influence photosynthesis and carbohydrate production in plants (Taiz et al., 2017), thus contributing to yield loss, since developing fruits represent the main drains in photoassimilate translocation (Singh et al., 2017).

As integral agents of production systems and mitigating the effects of abiotic stress, the use of humic substance-based biostimulants (HSs) can regulate physiological events in plants and increase productivity (El-Hoseiny et al., 2020). However, the use of HSs in fruit trees has been widely studied, and its effects have been well elucidated (Andreotti et al., 2022). According to the work of El-Hoseiny et al. (2020), results showed that the application of humic acids promoted improvements in bud fertility and increased fruit weight in mango trees.

Thus, the present study aimed to evaluate the gas exchange and biochemical responses in the leaf tissue of the 'Tommy Atkins' mango tree, as a function of the application of biostimulants based on humic substances during the phenological phase of fruiting in the Brazilian semi-arid region.

METHODOLOGY

The work was carried out in a commercial 'Tommy Atkins' mango orchard of the Surubim farm, located in the Senador Nilo Coelho Irrigated Perimeter, city of Petrolina –

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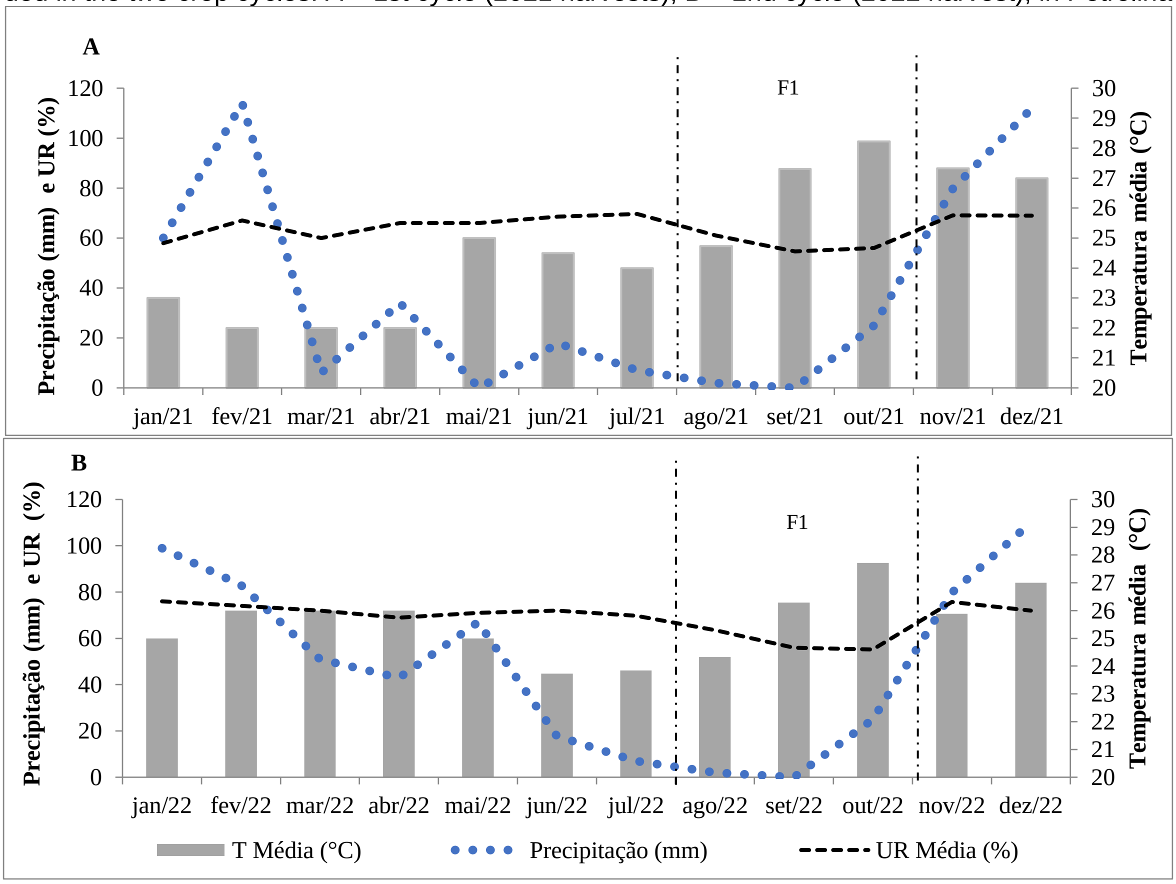
PE, with the geographic coordinates of 9° 14' 42" S latitude, 40° 27' 40" W longitude, at an altitude of 402 m above sea level.

The climate of the region, according to Koopen's classification is BSh, characterized as semi-arid, with an average annual temperature of 26.0 °C and average annual precipitation of 500 mm, concentrated between the months of January and April, and relative humidity of approximately 66% (Leão et al., 2020). Meteorological data for the two production cycles of this work (2021 and 2022) were recorded at an automatic weather station installed near the farm (Figure 1).

Figure 1. Average monthly data on accumulated precipitation, relative humidity – RH, average air temperature, recorded in the two crop cycles: A – 1st cycle (2021 harvests); B – 2nd cycle (2022 harvest), in Petrolina-PE.



Source: Prepared by the authors. F1: Fruit growth phase and application of treatments.

For experimental conduction, an orchard with 20 years of planting, with spacing 10 x 5 m, was used. Irrigation was carried out by the localized method, with one microsprinkler per plant (flow rate of 100 L $^{h-1}$). In each cycle, after the floral induction period, the fertility of the experimental area was characterized, collecting soil samples in the layers from 0 to 20, and from 20 to 40 cm. The samples were submitted to the Soil Laboratory of the Brazilian

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Agricultural Research Corporation EMBRAPA Semi-arid for chemical analysis, the results of which are described in Table 1.

Table 1. Chemical analysis of the soil in the experimental area with the 'Tommy Atkins' mango tree, before the production cycles, 2021 and 2022

Layer	pH 1:2.5	C.E. (25°C)	Macronutrients						Micronutrients				
			K+	Na+	Ca2+	Mg2+	SO42-	P	Cu2+	Fe2+	Mn2+	Zn2+	B
Cm	(H2O)	dS.m-1	--------- Cmol C.DM-3 ----------				------------------------- mg.dm-3 ---------------------------						
1st cycle (2021 Harvest)													
0 - 20	5,80	0,52	0,34	0,04	3,94	0,51	19,18	48,1	12,4	37,3	26,5	22,8	1,45
20 - 40	6,00	0,46	0,37	0,03	3,38	0,44	16,12	43,7	11,9	41,2	23,6	19,5	1,52
2nd cycle (2022 Harvest)													
0 - 20	6,10	0,59	0,28	0,01	3,84	0,41	16,58	51,1	9,4	38,0	29,3	22,2	1,29
20 - 40	6,25	0,44	0,33	0,01	2,18	0,34	15,18	46,8	4,9	39,4	26,7	18,3	1,30

pH = hydrogen potential; EC= electrical conductivity of the saturation extract; K= exchangeable potassium; Na= exchangeable sodium; Ca= exchangeable calcium; Mg= exchangeable magnesium; S = exchangeable sulfur P = available phosphorus extracted by Mehlich; Cu = exchangeable copper; Fe= exchangeable iron; Mn= exchangeable manganese; Zn= exchangeable zinc; and B = boron.

Irrigation management was carried out based on climatic data obtained from a meteorological station installed near the experimental area. For the application of the irrigation depths, daily calculations of reference evapotranspiration (ETo) were performed using the Penman-Monteith method, parameterized by the Food and Agriculture Organization (FAO), (Allen et al., 1998). ETc was obtained through ETo product and culture coefficient (Kc). The Kc used for the study period was equal to 1, as determined by Teixeira et al. (2008) for 'Tommy Atkins'.

The other managements performed during the experiment were the same as those used for all production areas of the farm: pruning after harvest, mineral fertilization, application of PBZ, floral induction, shade control pruning, as recommended by Mouco (2015), and control of spontaneous plants, pests, pathogens and diseases (level of economic damage), following the technical standards of Integrated Mango Production defined by Lopes et al., 2003.

The studies were carried out from two experiments, conducted in different and simultaneous ways, with the application of biostimulants (BSTs) in the fruiting phase. The experimental designs were in randomized blocks, in a factorial scheme (2 x 5), with two crop cycles (2021 and 2022), and five doses of the products: Sagersolo® (BST1): (0.0, 7.0, 14.0, 21.0 and 28.0 L $^{ha-1}$) and Fulvumin® (BST2): (0.0, 10.0, 20.0, 30.0 and 40.0 L $^{ha-1}$).

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Four blocks were used. The products tested were composed of stabilized organic matter, whose active ingredients described by the manufacturers are shown in Table 2.

Table 2. Descriptions of the active components of the commercial products Sagersolo® (BST1) and Fulvumin® (BST2)

Commercial Product	Active ingredient of biostimulants	Additional elements (g $^{L-1}$)
BST1	Total Humic Acids 150 (g $^{L-1}$); Fulvic Acids 75 (g $^{L-1}$); 10% Polyflavonoids; 5% Salicylic Compounds and 10% Amino Acid.	K2O 120.0 (g $^{L-1}$); Ca 180.0 (g $^{L-1}$); S 90.0 (g $^{L-1}$); B 15.0 (g $^{L-1}$); Mo 1.5 (g $^{L-1}$); Zn 30.0 (g $^{L-1}$)
BST2	Total soluble carbon 180.2 (g $^{L-1}$), Amino Acid, Ligno-Sulfonated, Leonardite, Peat	N 36.7 (g $^{L-1}$) Water-soluble nitrogen; N 36.7 (g $^{L-1}$) Nitrogen in organic form.

Source: Data provided on the labels of commercial products.

The biostimulants were applied via an irrigation system, using a system assembled with PVC pipes (lungs), in which the diluted solution entered the system by pressure difference (Oliveira et al., 2022). The doses were divided into four periods of 60, 75, 90 and 105 days after full flowering of the 'Tommy Atkins' mango tree in both production cycles.

The determinations of the gas exchange of the 'Tommy Atkins' hose were carried out 5 days after the last application of the biostimulants, using a portable IRGA analyzer (Infra Red Gas Analyser – Model Li 6400 Liquor®), with photon irradiation [1500 µmol $^{m-2}$ $^{s-1}$], and the readings were carried out between 9 and 11 am, in two completely expanded and mature leaves, with good health and exposed to the sun, located in the middle third of the plants, (Fernandes et al., 2021). The following parameters were determined: CO2 assimilation rate (*A*; µmol CO2 $^{m-2}$ $^{s-1}$), internal CO2 concentration (*Ci*; mmol CO2 $^{m-2}$ $^{s-1}$), stomatal conductance (*gs*$^{;\ mol\ of\ H2O}$ m-2 $^{s-1}$), leaf transpiration (E ; mmol of H2O $^{m-2}$ s-1$^{).}$

Samples for biochemical analysis in leaf tissue were collected 5 days after the last application of the biostimulant, collecting 4 leaves per sampling unit, originating from the last vegetative flow, at the median height of the mango canopy. Then, the samples were wrapped in aluminum foil, dipped in liquid nitrogen and packed in plastic bags and stored in a cooler containing ice. Later they were taken to the Cytology and Physiology laboratory of UNIVASF - Campus Juazeiro - BA.

The following contents were quantified: total free amino acids (*AAT in* µmol $^{g-1}$), according to the methodology described by Yemm et al. (1955); total soluble proteins (*PST in* mg mL-1), according to Bradford. (1976); total soluble carbohydrate (*CST in* mg $^{g-1}$) and Starch (mg $^{g-1}$), following the methodology described by Dubois et al. (1956). All variables were determined in relation to fresh leaf mass (MF), after obtaining the standard curve for

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each substance, considering their respective wavelengths (nm), using a spectrophotometer.

The analysis of variance of the data was performed separately for each experiment, with the measurements of Sagersolo® and Fulvumin®, with the application of the F test at $p<0.05$. In the variables with significant results for cycles, the means were submitted to Tukey's test at 5%. The results, when significant for doses, were submitted to regression analyses. The analyses were carried out using the Sisvar program - version 5.6 (Ferreira, 2017).

RESULTS DISCUSSION

Based on the results of the analysis of variance obtained for the experiment as a function of the biostimulant Sagersolo® (BST1), (Table 3), no significant interaction (F<0.05 probability) was observed between the doses and productive cycles for gas exchange. However, significant effects were found between the doses applied in all variables studied, except leaf transpiration (*E*). In addition, there was a significant difference between the production cycles for the variables CO_2 assimilation rate (*A*), internal CO_2 concentration (*Ci*), (Table 3).

Table 3. Summary of the analysis of variance for CO_2 assimilation rate (*A*), stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), leaf transpiration (*E*) in the leaf tissues of the 'Tommy Atkins' mango tree submitted to doses of the biostimulants Sagersols® (BST1) and Fulvumin® (BST2). Petrolina – PE, 2021 and 2022

FV	GL	Test F			
		The (µmol CO_2 m^{-2} s^{-1})	*Gs* (mol H_2O m^{-2} s^{-1})	*Ci* (mmol CO_2 m^{-2} s^{-1})	*And* (mmol H_2O m^{-2} s^{-1})
BST1					
Blocks	3	1,04	0,003	152,55	0,09
Servings (D)	4	11,26**	0,023**	2554,72*	0.08ns
Cycles (C)	1	29,07**	0.002ns	3385,94**	0.05ns
(D)*(C)	4	0.20ns	0.002ns	484.07ns	0.20ns
Error	27	2,00	0,001	348,78	0,06
CV – (%)		**13,35**	**12,74**	**8,62**	**11,92**
BST2					
Blocks	3	4,93	0,0005	79,47	0,11
Servings (D)	4	9,00*	0.0017ns	527.65ns	0,98**
Cycles (C)	1	21,66**	0.0006ns	268.63ns	0,72*
(D)*(C)	4	1.46ns	0.0002ns	151.10ns	0.14ns
Error	27	2,03	0,0004	199,04	0,05
CV – (%)		**14,64**	**13,77**	**6,89**	**8,82**

Source: Prepared by the authors. FV: source of variance; LG: degrees of freedom; CV %: coefficient of variance; consecutive values (**): significant (p<0.01); (*): significant (p<0.05); (n>s): not significant (p0.05) F-test.

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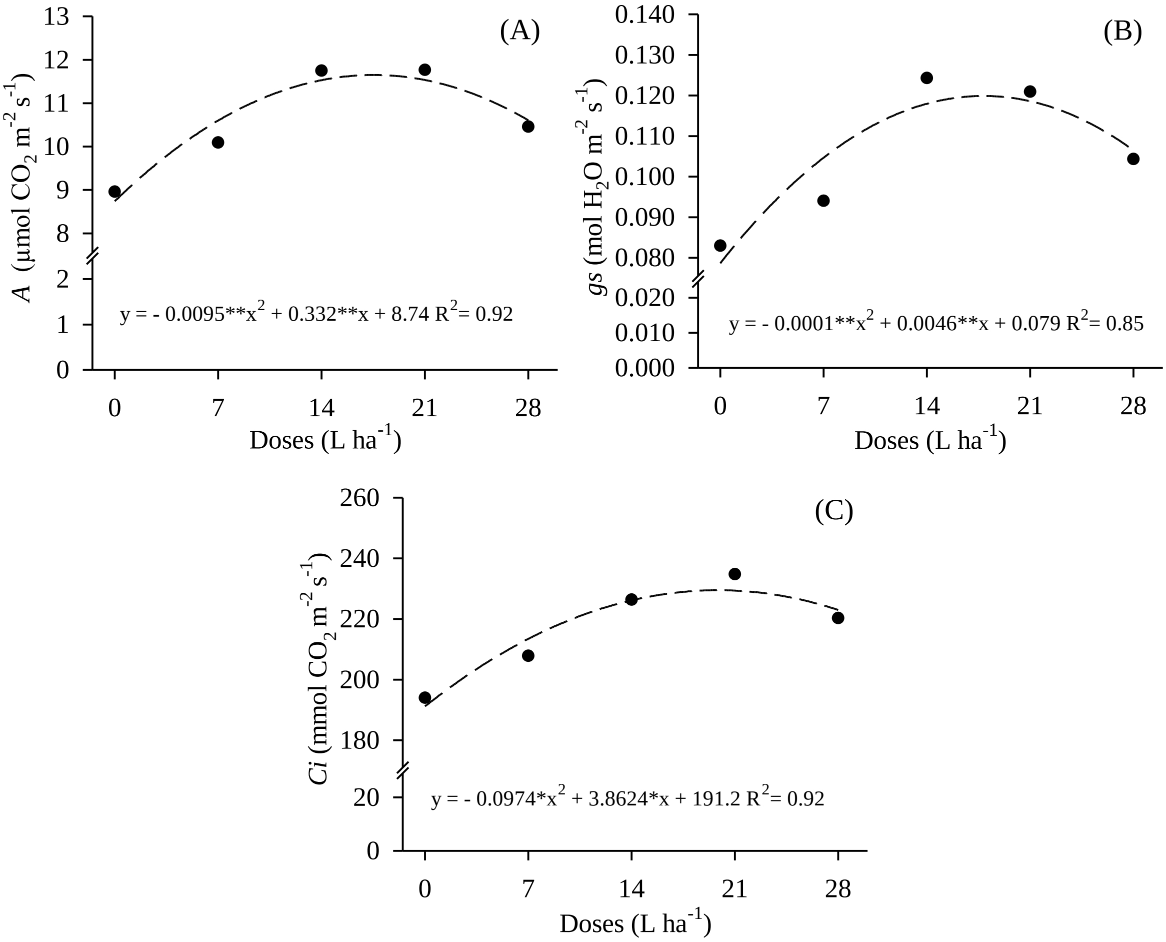
For the experiment with the application of the biostimulant Fulvumin® (BST2), there was no interaction between doses and production cycles. However, a significant effect was found of the isolated doses for *A* and *E*. In addition, a distinct response was found in relation to the cycles for *A* as shown in Table 3.

When analyzing the assimilation of CO2 and stomatal conductance in the leaves of the 'Tommy Atkins' hose (Figures 2A and 2B), significant effects of the doses applied were observed as a function of the treatments with BST1 product. The highest values observed were 11.6 µmol CO2 $^{m-2\ s-1}$ for *A* and 0.131 mol H2O m-2 s-1 for *gs*, corresponding to the doses of 17.5 and 23.0 L $^{ha-1}$, respectively.

Figure 2. Assimilation rate CO2 - *A* (A), stomatal conductance - *gs* (B) and internal concentration of CO2 -*Ci* (C) in 'Tommy Atkins' hose, submitted to doses of the biostimulant Sagersolo® (BST1).



Source: Prepared by the authors. Equations followed: (**) significant at p < ŷ 0.01 and (*) significant at p < ŷ 0.05 at test F.

In this context, the increase in BST1 doses up to the limit of 17.5 L $^{ha-1}$ promoted an increase in photosynthesis in the first experiment (Figure 2A), with a positive correlation for the increments in stomatal conductance (Figure 2B) and internal concentration (Figure 2C).

Thus, it was possible to verify that the trend of maximization of the three variables studied occurred with the estimated concentration of humic substances at the dose of 17.5 L $^{ha-1}$ for *A*.

In this sense, for the same region of the Brazilian semi-arid region, Simões et al. (2021), when evaluating the gas exchange of the 'Tommy Atkins' hose under different arrangements of the irrigation system, observed that the treatment with a higher transpiration rate (3.50 mmol H2O $^{m-2\ s-1}$) conditioned a significant increase in photosynthesis due to the diffusive flow of water vapor and CO2 through the stomatal pore. Trends similar to these were observed in this study (Figure 2, A and B).

According to the model proposed by Shah et al. (2018), (SHs) can upregulate genes involved in the Calvin cycle, such as those encoding for ribuloso-1,5-bisphosphate carboxylase (RUBPCs); glyceraldehyde-3-phosphate dehydrogenase (G3PdHs); glucose-1-phosphate adenyl transferase (GPATs), thus increasing photosynthetic activity, and consequently promoting increased levels and starch contents in leaves.

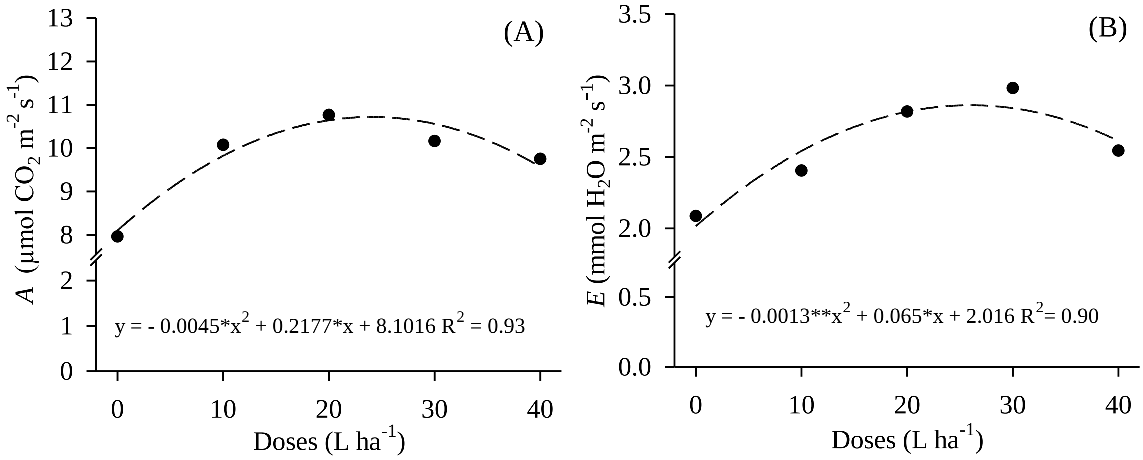
For the internal CO2 concentration in the leaves of the 'Tommy Atkins' hose, significant effects of the doses applied were observed as a function of the BST1 treatments. The highest value observed was 229.5 mmol CO2 $^{m-2\ s-1}$ for Ci*, corresponding to the dose of 20 L* ha-1 (Figure 2.C). Possible explanations for such responses would be the improvement of a series of physiological events of plants that influence photosynthetic activity, such as better rubisco activity, improved water availability for electron transport, and NADPH and ATP production, among other factors (Taiz et al., 2017).

Another possible explanation for the increase in the internal CO2 concentration in the leaves of the 'Tommy Atkins' hose may be associated with the presence of amino acids present in BST1. As described by Mudo et al. (2020), when evaluating the gas exchange of the 'Tommy Atkins' mango tree in conditions of the Brazilian semi-arid region in response to different doses of Biostimulant Bulk (Alltech®), consisting of amino acids, they observed that treatments with a higher concentration of amino acids resulted in higher stomatal conductance and also had higher CO2 assimilation rates.

In view of the results obtained, it is possible to highlight that the high internal concentration of CO2 in the leaves tends to favor the photosynthesis process (Taiz et al., 2017), which corroborates the findings regarding the effects of the biostimulant Sagersolo® (BST1) on the physiological parameters of the 'Tommy Atkins' mango tree when applied in the phenological fruiting phase.

For the BST2 experiment, when the influence of the isolated doses in relation to the gas exchange of 'Tommy Atkins' was evaluated (Figures 3A and 3B), there was an increase in the rate of CO2 assimilation and leaf transpiration as a function of the increase in the dose of Fulvumin® when compared to the control treatment. The highest values observed were 10.7 µmol CO2 $^{m-2}$ s-1 *for* A $^{and\ 2.8\ mmol\ H2O}$ m-2 s-1 for E , corresponding to the doses of 24.2 and 25.0 L $^{ha-1}$, respectively. There were increases of 25.3% in photosynthesis and 28% in leaf transpiration when compared to the control treatments.

Figure 3. Assimilation rate CO2 - *A* (A), leaf transpiration - *E* (B), in 'Tommy Atkins' hose, subjected to doses of the biostimulant Fulvumin® (BST2).



Source: Prepared by the authors. Equations followed: (**) significant at p < ŷ 0.01 and (*) significant at p < ŷ 0.05 at test F.

The 28% increase in the leaf transpiration rate in the BST2 experiment in relation to the control treatment (Figure 3B) indicates greater efficiency in terms of water flow through the stomatal pores due to a greater supply and availability of the substances contained in the humic biostimulant based on humic compounds from the Fulvumin® ^{source}.

This phenomenon probably acted as a mitigating agent of a possible stress caused in the mango plants due to the high temperatures and low air humidity incident in the region during the study period (Figure 1). These conditions tend to increase the speed of water transport from the root to the shoot and result in a greater loss of water from the leaves to the environment due to the increase in water deficit and reduction of the borderline layer in the leaves according to (Taiz et al., 2017).

Olk et al. (2018) found that the accumulation of humic substances in the soil can become a primary response mechanism in plants under conditions of abiotic stress. According to Jannin et al. (2012), receptors in the plant cell membrane can detect HSs,

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increasing intracellular concentrations of NO^-, H2O2 and H2S, activating genetic mechanisms such as post-translational and post-transcriptional modifications determined by the regulation of gene expression. This can trigger several homeostatic mechanisms in the plant cell, including the acceleration of the rate of assimilation of CO2 (Orsi, 2014).

However, it is possible to infer that the superiority of photosynthesis as well as the stomatal conductance (Figure 3A and 3B), for 'Tommy Atkins' mango with the increment (4.3 and 4.5 kg total organic carbon) present in the estimated doses (24.2 and 25.0 L $^{ha-1}$) of BST2, may have potentiated the physiological processes of mango plants.

Based on the results of the analysis of variance obtained for the biochemical contents of the experiment with the application of BST1 (Table 4), significant effects were found (1% probability of the F test) as a function of the doses applied and the cycles studied for all the variables analyzed: total free amino acids (*AAT*), total soluble proteins (*PST*), total soluble carbohydrates (*TSF*)) and Starch, in the 'Tommy Atkins' mango leaf tissue.

Considering the experiment with the application of BST2, there was no interaction between doses and production cycles. However, a significant effect was found ($p < 0.01$) for the doses applied as a function of the variables: total free amino acids (*TAA*), total soluble proteins (*TSP*), total soluble carbohydrates (*TSC*) and Starch. In addition, different responses were found in relation to the cycles for all biochemical variables (Table 4).

Table 4. Summary of the analysis of variance for total free amino acids (*AAT*), total soluble proteins (*PST*), total soluble carbohydrates (*TSF*) and Starch, in the fresh mass (MF) of leaf tissue in 'Tommy Atkins' mango tree submitted to doses of the biostimulants Sagersolo® (BST1) and Fulvumin® (BST2). Petrolina – PE, 2021 and 2022

FV	GL	Test F			
		AAT (µmol $^{g-1}$ MF)	*PST* (mL-1 mg MF)	*CST* (mg $^{g-1}$ MF)	Starch (mg $^{g-1}$ MF)
BST1					
Blocks	3	0,06	0,001	1,61	0,01
Servings (D)	4	5,34**	0,116**	46,65**	2,47**
Cycles (C)	1	1,62**	0,075**	22,65**	2,49**
(D)*(C)	4	0.09ns	0.001ns	0,52	0.02ns
Error	27	0,06	0,001	0,95	0,05
CV – (%)		**7,87**	**6,42**	**9,61**	**8,12**
BST2					
Blocks	3	0,16	0,008	0,48	0,09
Servings (D)	4	4,43**	0,056**	39,59**	4,39**
Cycles (C)	1	3,58**	0,045**	22,17**	2,30**
(D)*(C)	4	0.08ns	0.005ns	0.05ns	0.07ns
Error	27	0,06	0,009	0,96	0,09
CV – (%)		**10,02**	**8,34**	**10,51**	**13,62**

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Source: Prepared by the authors. FV: source of variance; LG: degrees of freedom; CV %: coefficient of variance; consecutive values (**): significant ($p<0.01$); (*) : significant ($p<0.05$); (ns): not significant ($p>0.05$) F. MF test: fresh pasta;

When evaluating the levels of total free amino acids (*TAA*) and total soluble proteins (*TSPs*) in the leaf tissue of the 'Tommy Atkins' mango tree in the BST1 experiment (Figure 4), a positive correlation was found between the increase in Sagersolo® doses and these variables. 3.9 μmol $^{g-1}$ MF of *AAT* were recorded for the estimated dose of 20.1 L $^{ha-1}$ and 0.75 mg mL-1 of *PST* for the dose of 28 L $^{ha-1}$, providing an increase in the levels of amino acids and soluble proteins, in relation to the control treatment.

During the fruiting phase of 'Tommy Atkins', the increase in total amino acid contents in relation to the control may have been influenced by the applications of BST1, considering that in its composition containing 10% of amino acids. It is important to highlight that, in this phase, with recommended crop treatment, nitrogen (in the form of nitrate) should be applied, a common practice during crop management in the study region, and can thus be considered a sum factor of the increase in amino acid biosynthesis by the supply of nitrogen associated with the availability of amino acids through the application of the product.

The process of assimilation of nitrate activates the formation of the enzyme nitrate reductase (Anusuya et al., 2018), which sometimes acts in the production of total free amino acids (Sudha et al., 2012; Sanches et al., 2023), which play a central role in protein synthesis and in the regulation of plant metabolism in general. Therefore, it is possible that the increase in *AAT* levels in leaf tissues (Figure 4A) exerted a positive influence on the ability of plants to produce *PST* (Figure 4B) in response to conditions of higher demand in the transport of photoassimilates during the fruiting phase of the 'Tommy Atkins' mango.

In the study by Silva et al. (2020b), carried out in the Brazilian semi-arid region, the application of Alltech®'s Bulk biostimulant on the cultivar 'Tommy Atkins' was evaluated. This biostimulant contains 12% KCl, 9.87% humic substances (SHs) and 20% amino acids. The results showed concentrations of total free amino acids between 130 and 150 μmol $^{g-1}$ of fresh mass (MF) in leaf tissues in the vegetative phase. These values are higher than those found in the present study, which showed a leaf concentration of 3.9 μmol $^{g-1}$ of MF. This suggests that, in the phase evaluated 115 days after full flowering, amino acids were used in the physiological process of fruit nutrition.

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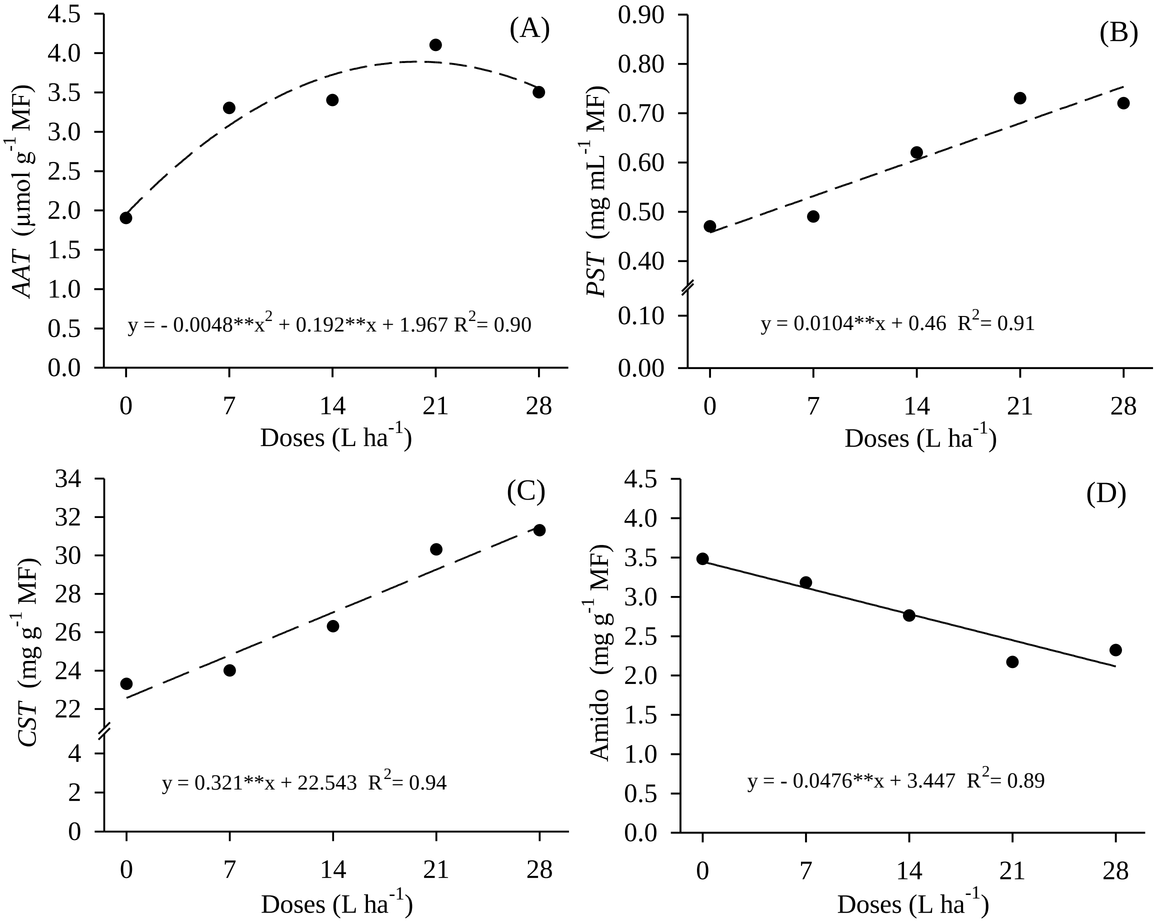
As for the carbohydrate contents in the leaf tissue with BST1 application, it was found as the greatest representativeness of the results when adjusting the increasing linear equation for total soluble carbohydrates (*TSC*) in relation to the Sargesolo® doses. The highest *CST* content of 31.6 mg $^{g-1}$ MF observed for the dose 28.0 L $^{ha-1}$, representing an increase of 15% in relation to the control. However, in relation to starch (Figure 4D), there was a linear decrease in leaf contents, with the lowest value recorded being 2.11 mg $^{g-1}$ MF for a dose of 28.0 L $^{ha-1}$. This represents a decrease of how many 40% and relation to the witness.

Figure 4. Total free amino acids - *AAT* (A), total soluble proteins - *PST*, (B), total soluble carbohydrates - *CST* (C), and Starch (D), in a 'Tommy Atkins' hose, submitted to doses of the biostimulant Sagersolo® (BST1).



Source: Prepared by the authors. Equations followed: (**) significant at p **<** ŷ 0.01 and (*) significant at p < ŷ 0.05 at test F.

Although the relationship between humic substances, amino acids in soil and carbohydrate levels in plants is a complex process, the fact that BST1 contains: 150 g $^{L-1}$ of total humic acids, 75 g $^{L-1}$ of fulvic acids, 10% of polyflavonoids, 5% of salicylic compounds and 10% of amino acids, may have contributed to the efficiency in carbohydrate transport.

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influencing the reduction of starch and the increase of total soluble carbohydrates in the mango leaves during the fruiting phase of 'Tommy Atkins'. In this perspective, Tanou et al. (2017) state that the increase in growth and yield of fruit trees with the use of biostimulants has been associated with modulation in different fruit substances and species.

Convergent responses with the reduction of starch content, as observed in this study, were also reported by Silva et al. (2020b), when determining the biochemical patterns in the leaf tissue of 'Tommy Atkins' with the application of a biostimulant (Alltech® Bulk), which contains 12% KCl, 9.87% (SH) and 20% amino acids, at different periods of the vegetative phase, in the conditions of the Semi-arid region of Brazil. The aforementioned authors observed that the leaf starch contents decreased over time, regardless of the treatment, and that in the penultimate collection there was a significant reduction in the starch contents and an increase in the average values in the total soluble carbohydrate contents in the leaf tissues of the mango tree in the flowering phase.

In this context, the observation that the applied dose of 28 L $^{ha-1}$ of BST1 resulted in a higher *CST* content at a level of 31.1 mg $^{g-1}$ MF and a lower starch content of 2.11 mg $^{g-1}$ MF (Figure 4C), signals a possible improvement in the metabolism of mango plants in relation to the efficiency in the allocation of carbohydrates for fruit development, when compared to control treatment.

Starch, produced in the chloroplasts in the leaves, is mobilized and converted into soluble sugars, such as glucose, fructose, sucrose, among others, through enzymatic hydrolysis processes (Vieira et al., 2010). The authors emphasize that the reduction in starch content in leaf tissues during the fruiting phase is directly related to the need to provide energy and biosynthesis of other organic compounds for fruit development due to the source-drain relationship. Fruits developing in the plant production phase are the main drains of plants, although they are not the only ones, and therefore efficient translocation of photoassimilates is crucial (Singh et al., 2017).

For the experiment with BST2, in relation to the AAT and *PST* levels present in the leaf tissue (Figures 5A and 5B). According to the results, significant effects ($F< 0.01$) were observed for doses applied in both variables, with the maximum point being 3.2 µmol $^{g-1}$ MF of total free amino acids, observed with the estimated dose of 23.3 L $^{ha-1}$, while the maximum point of 0.61 mg mL-1 MF total soluble proteins was observed with an estimated dose of 23.5 L $^{ha-1}$.

However, this increase in *AAT* in the leaf tissues of the 'Tommy' mango tree was probably influenced by the amount of nitrogen present at the estimated dose of 23.3 L $^{ha-1}$ in BST2. It is important to note that in this fruiting phase, the application of Nitrogen is common in the management of the crop for the northeastern semi-arid region. This conditioning may have provided an increase in amino acid biosynthesis due to stress attenuation in mango plants, a phenomenon also verified by Helaly et al. (2017) when studying four mango varieties and ratified by Silva et al. (2020b) for 'Tommy Atkins' in the vegetative phase.

Establishing a correlation between the levels of amino acids and proteins in leaf tissues, associated with the substances found in the compost based on humic substances in the soil, is highly complex. However, the increase of 0.15 mg mL-1 in the *PST contents* in the leaves of 'Tommy Atkins' in the fruiting phase in relation to the control (Figure 5B), is possibly related to the increase in the biosynthesis of total free amino acids (Figure 5A) due to the application of BST2.

Following the model proposed by Shah et al. (2018), receptors in the plant cell membrane detect (SH) in the rhizosphere, which induces the production of indolyl-3-acetic acid (IAA), raising the concentration of $NO3^-$ in root cells. The same authors emphasized that this biochemical pathway activates the generation of alkaloids, which upregulate the nitrate transporter ($NO3^-$) maximizing nitrogen assimilation. This may have favored the improvement of the relationship between the increase in *AAT* and *PST contents* in the fruiting phase of the mango tree.

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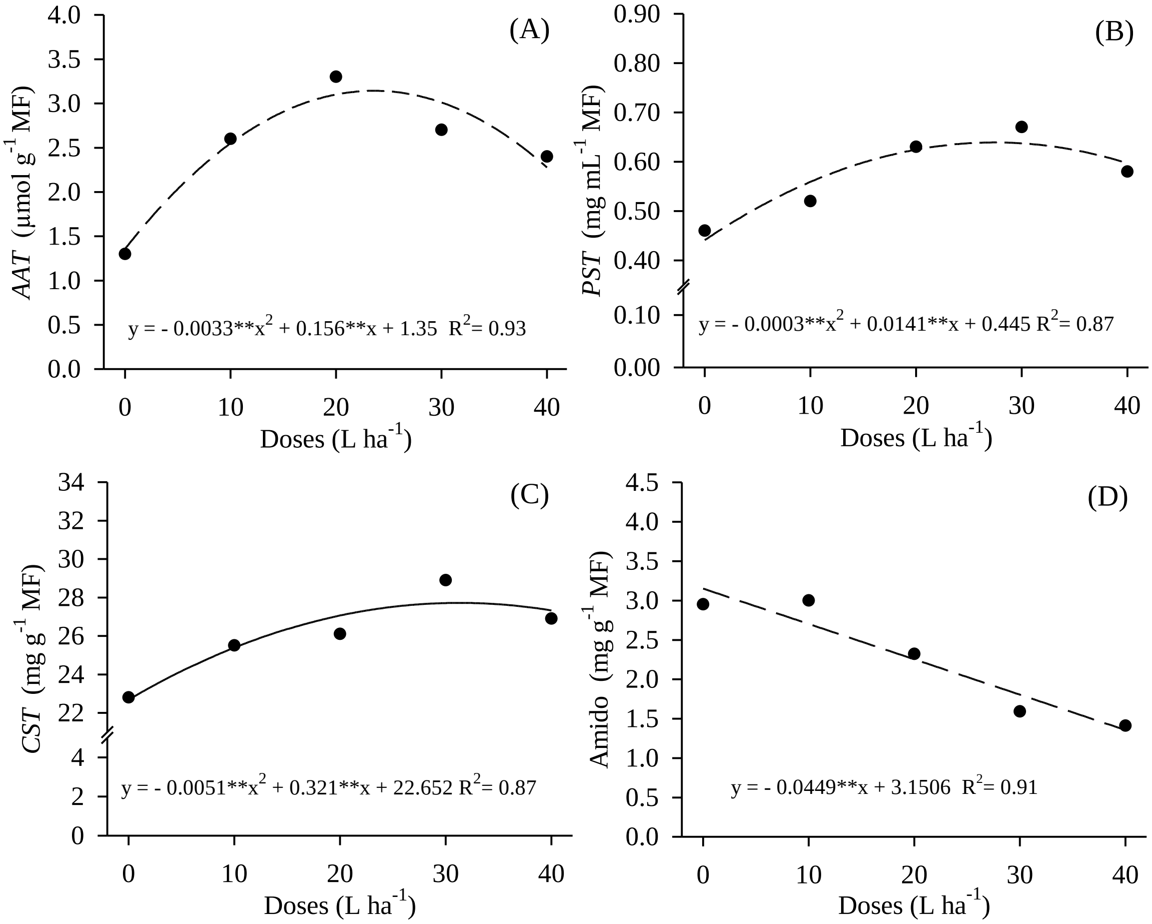
Figure 5. Total free amino acids - *AAT* (A), total soluble proteins - *PST* (B), total soluble carbohydrates - *CST* (C), and Starch (D), in 'Tommy Atkins' hose submitted to doses of the biostimulant Fulvumin® (BST2).



Source: Prepared by the authors. Consecutive equations: (**) significant at p < ŷ 0.01 and (*) significant at p < ŷ 0.05 at test F.

With the application of the BST2 product, better responses were identified in the production and partitioning of carbohydrates in the leaf tissue of the "Tommy Atkins' mango tree as a function of the increase in the dose, with the highest content of total soluble carbohydrates of 27.7 mg $^{g-1}$ MF, observed for the dose 31.5 L $^{ha-1}$, estimated by the quadratic equation with R2= 0.87, (Figure 5C).

However, there was a linear reduction in starch content as a function of the increase in BST2 doses, when compared to the control treatment, with the lowest observed value of 1.3 mg $^{g-1}$ MF for the highest dose studied of 40.0 L $^{ha-1}$ (Figure 5D). This answer may be linked to the fact that, during the fruiting phase studied, 105 days after the full flowering of the mango tree, the plant's energy demand increases significantly. According to Vieira et al. (2010), the reduction of starch in the leaf tissues of vegetables during the fruiting phase is directly related to the need to provide energy for fruit development by the source and drain relationship.

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When evaluating the application of the product BST1 in the fruiting phase for the 2021 and 2022 production cycles, it was found that the second cycle recorded average values: *A* 11.45 µmol CO2 $^{m-2\ s-1}$; *Ci* 231.12 mmol CO2 $^{m-2\ s-1}$. These results were significantly higher compared to the first cycle. In relation to the biochemical variables, all parameters presented higher averages with 5% probability, according to the Tukey test, for the first cycle compared to the second cycle (Table 5).

Table 5. Leaf concentrations: assimilation rate CO_2 (*A*); stomatal conductance (*gs*); internal CO2 concentration (*Ci*); transpiration (*E*); total free amino acids (*AAT*); total soluble proteins (*TSPs*), total soluble carbohydrates (*TSCs*) and Starch, in a 'Tommy Atkins' hose submitted to doses of Sagersolso® (BST1) and Fulvumin® (BST2). Petrolina – PE, 2021 and 2022

FV	*The* (µmol CO2 $^{m-2\ s-1}$)	*Ci* (mmol CO2 $^{m-2\ s-1}$)	*And* (mmol H2O $^{m-2\ s-1}$)	*AAT* (µmol $^{g-1}$ MF)	*PST* (mL-1 mg MF)	*CST* (mg $^{g-1}$ MF)	Starch (mg $^{g-1}$ MF)
BST1							
1st cycle	9.75b	202.16b	2.03a	3.45a	0.64a	27.79a	3.02a
2nd cycle	11.45a	231.12a	2.10a	3.04b	0.56b	26.28b	2.53b
DMS	0,91	12,11	0,16	0,16	0,02	0,63	0,14
BST2							
1st cycle	9.00b	202.15a	2.43b	2.77a	0.60a	26.75a	2.49a
2nd cycle	10.47a	207.34a	2.70a	2.17b	0.53b	25.26b	2.01b
DMS	0,92	9,15	0,14	0,17	0,02	0,65	0,19

Source: Prepared by the authors. Cycles with averages followed by equal letters in the column do not differ from each other, at the level of 5% probability by Tukey's test.

In the second experiment with the application of BST2, in two production cycles in the 'Tommy Atkins' fruiting phase, it was identified that in the second cycle it obtained higher average values in relation to the first cycle for: *A* 10.47 µmol CO2 $^{m-2\ s-1}$; and *E* 2.70 mmol H2O $^{m-2\ s-1}$. However, the first cycle obtained higher mean values for all biochemical variables at 5% probability, according to the Tukey test (Table 5).

The variations observed in gas exchange in the fruiting phase of 'Tommy Atkins' throughout the production cycles were relatively small. The inter-cycle difference in mean CO2 assimilation values was 1.65 µmol CO2 $^{m-2\ s-1}$ for Sagersolo® (BST1) treatment and 1.47 µmol CO2 $^{m-2\ s-1}$ for Fulvumin® (BST2) treatment.

These variations can be attributed to several factors, such as fluctuations in climatic conditions at the time of data collection and water availability during the fruiting phase at the time of readings. Since relative humidity, air temperature, and radiation flux are factors that influence photosynthesis (Taiz et al., 2017).

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Regarding the contents of total free amino acids, total soluble proteins, total soluble carbohydrates, and Starch, present in the leaf tissues of the 'Tommy Atkins' mango tree during the fruiting phase, it was observed that they are superior in the first cycle in both experiments. These answers may be related to the seasonality of the cultivar, which presents alternation of productivity between cycles, as observed by (Modesto. 2013; Simões et al., 2021).

Cycles with higher productivity demand greater amounts of carbohydrates, amino acids and proteins, requiring more intense photosynthetic activity and greater production of these compounds. Since developing fruits act as the main photoassimilate drains in plants (Singh et al., 2017).

CONCLUSION

The doses of the biostimulants Sagersolo® (BST1) between 17.5 and 23.0 L $^{ha-1}$, applied during the fruiting phase of the mango variety 'Tommy Atkins', resulted in increases in CO2 assimilation under the conditions of the Brazilian semiarid region.

For the biostimulant Fulvumin® (BST2), the doses of 24.2 and 25.0 L $^{ha-1}$, applied during the fruiting phase, improved the efficiency in CO2 assimilation of the 'Tommy Atkins' variety for the Brazilian semi-arid region.

The use of both biostimulants (BSTs) favored the efficiency in the production and translocation of carbohydrates in the leaf tissues of the variety 'Tommy Atkins' during the fruiting phase under the conditions of the Brazilian semi-arid.

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