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Impact of *Rhodopseudomonas palustris* on Fruit Yield and Quality of 'Keitt' Mango

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ABSTRACT

Using microorganisms can develop into a promising strategy for increasing photosynthetic activity and greater accumulation of assimilates in crops. This study aimed to evaluate the effects of *Rhodopseudomonas palustris* on fruit yield and quality of 'Keitt' mangoes grown under semi-arid environmental conditions. The experiment was carried out simultaneously in two mango orchards in Petrolina, Pernambuco, Brazil. The study comprised a randomized block design with treatments distributed in 7 treatments, 4 replications, and 3 plants per plot. The treatments consisted of different strategies for applying *R. palustris*. These treatments were T1) control treatment; T2) 1.43×10^7 CFU/plant via fertigation; T3) 2.85×10^7 CFU/plant via fertigation; T4) 4.27×10^7 CFU/plant via fertigation; T5) 5.70×10^7 CFU/plant via fertigation; T6) 1.43×10^7 CFU/plant via fertigation + 1.43×10^7 CFU/plant via leaf spray; T7) 2.85×10^7 CFU/plant via fertigation + 1.43×10^7 CFU/plant via leaf spray. The treatments were a pplied monthly, totaling seven applications. We evaluated fruit yield (M g ha^{-1}), longitudinal diameter, ventral diameter and transverse diameter , fruit mass, pulp firmness, pulp percentage, soluble solids (SS), pH, titratable acidity (TA), SS/TA ratio, and dry pulp mass. *R. palustris* improved mango yield and fruit quality characteristics regarding longitudinal and transversal diameters and the SS/TA ratio. Mango plants treated with *R. palustris* at 1.43×10^7 CFU/plant via fertigation (T2) produced 10 Mg ha^{-1} more than the control group, without loss of fruit quality while considering the European market demand.

Abbreviations
Colony forming units (CFU), Use strategies of *R. palustris* (R), Transvers e diameter (TD), Longitudinal diameter (LD), Ventral diameter (VD), Fr uit mass (FM), Pulp percentage (PP), Firmness (PF), Soluble solids (SS), Titratable acidity (TA), Dry mass (DM)

Introduction

The cultivation of mango (*Mangifera indica* L.) has a significant socio-economic impact on the Brazilian market, especially in the Northeast region, the São Francisco Valley, the primary fruit-producing region in the country (Carvalho et al., 2019). Keitt mango, among the cultivars in the São Francisco Valley, is a plant with an upright growth habit and long branches. The plant bears large oval-shaped fruits weighing between 600 and 1500 g, with low fiber content, a pulp/fruit ratio of around 70%, soluble solids content

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between 18.9 and 22 ºBrix, and an average titratable acidity of 0.31 g citric acid per 100 g of pulp. Its shelf life can reach up to 20 days when stored at room temperature (Mouco and Lima Neto, 2017). It is primarily destined for the European market.

The post-harvest quality of mangoes and their shelf life can be influenced by several factors, such as the dry matter content, which seems to play a fundamental role. The portion of carbohydrates allocated by the plant to the fruit depends on the amount synthesized through photosynthesis, considering the source-sink relationship and the availability of reserves (Léchaudel and Joas, 2007; Sanches et al., 2023). Therefore, more photosynthetic activity can lead to more synthesis and mobilization of assimilates necessary for fruit development and growth (Taiz et al., 2017), including in the case of mango (Cunha et al., 2022).

The use of products containing microorganisms has proven to be a promising strategy for increasing photosynthetic activity and greater accumulation of assimilates in crops due to their biostimulant action (Lino et al., 2023). In a study conducted on Chinese cabbage (*Brassica chinensis* L.) sprayed with the bacterium *Rhodopseudomonas palustris*, an increase in photosynthetic activity and greater biomass accumulation occurred (Xu et al., 2016). Furthermore, the bacterium did not exhibit harmful potential to the environment but served as a plant growth promoter (Harwood, 2022). There is no evidence to suggest that this bacterium poses a risk to human health.

R. palustris is a purple, gram-negative, rod-shaped bacterium. It is known for its large capacity in photosynthetic activity and its photo-heterotrophic use of light as an energy source that allows this bacterium to produce ATP, NADH2, NADPH2, and other compounds (Phongjarus et al., 2018).

The application of this bacterium is a viable strategy to increase fruit yield and improve fruit quality in grapes (*Vitis vinifera* L.), cayenne pepper (*Capsicum annuum*), and tomato (*Solanum lycopersicum*) by reducing acidity and increasing the fruit ratio (Shi et al., 1995; Gu et al., 2002). There is citable evidence that this bacterium can increase yields in crops such as rice (*Oryza sativa* L.) (Harada et al., 2005; Kantachote et al., 2016), cucumber (*Cucumis sativus* L.) (Ge et al., 2017), and Chinese dwarf cherry (*Prunus humilis* Bunge) (Yin et al., 2012). A study by Lino et al. (2023) provided data on tropical species grown in semi-arid conditions while using this bacterium. The authors found that *R. palustris* affects stomatal conductance, gas exchange, and nitrate reductase enzyme activities in mango cv. 'Keitt' in Brazilian semi-arid conditions, but the effects depend on the phenological phase evaluated. There is a knowledge gap in the literature that should demonstrate conclusively how this bacterium affects tropical species, especially those of great economic importance for the Brazilian semi-arid region. Therefore, the objective of this study was to evaluate the effect of *R. palustris* on mango yield and fruit quality after 22 days of storage. In this research, 'Keitt' mangoes were grown under semi-arid environmental conditions.

Materials and Methods

Plant material and growth conditions

Seven-year-old mango (*Mangifera indica* L.) trees, cv. Keitt, with uniform size and vigor in the fifth production cycle, were used in this study. The experiments were conducted from 2019 to 2020, simultaneously in two experimental orchards located in the municipality of Petrolina (09° 18' S and 40º 25' W; at an altitude of 349 m above sea level), State of Pernambuco, Brazil. The climate of this region is classified as BSh (Köppen), which corresponds to a semi-arid region. During the experiment, mean air temperature and relative humidity ranged from 24.1 ºC to 34.6 ºC and from 54.4% to 79.1%, respectively, with an accumulated precipitation of 424 mm year^{-1}.

Treatments and experimental design

The treatments consisted of different doses of *R. palustris*: T1) control; T2) 1.43 × 10^7 CFU/plant via fertigation; T3) 2.85 × 10^7 CFU/plant via fertigation; T4) 4.27 × 10^7 CFU/plant via fertigation; T5) 5.70 × 10^7 CFU/plant via fertigation; T6) 1.43 × 10^7 CFU/plant via fertigation + 1.43 × 10^7 CFU/plant via leaf spray; T^7) 2.85 × 10^7 CFU/plant via fertigation + 1.43 × 10^7 CFU/plant via leaf spray, applied monthly, totaling seven applications, with four blocks and three plants per plot. For the leaf spray treatments, it was used a mechanized sprayer (Arbus 2000 model) with a regulated volume of 1.2 L per plant. The source of *R. palustris* used was Bioavance™ (Vinhedo, Brazil) in which this bacterium was maintained in a medium with lipases, proteases, sugars, and carbohydrates at 750.000 CFU mL^{-1} of *R. palustris*, with a density of 1.0g cm^{-3}. Treatments were applied every 30 days after pruning until the beginning of the fruit set, totaling seven applications. The treatment definitions were based on bacterium characteristics (Kantachote et al., 2016) and previous studies by Phongjarus et al. (2018) and Xu et al. (2016).

The experiments were carried out in two orchards simultaneously using similar plants and management characteristics. Each experimental unit was 12.0 m^2. The plants, spaced with 6.0 m between the rows and 2.0 m between the plants, were daily drip-irrigated with twelve emitters (drippers) per plant, with a flow of nearly 1.5 L h^{-1} each. All management practices such as pruning, control of weeds, pests and diseases, plant growth regulators for gibberellin inhibition (Cultar™), and break dormancy (calcium nitrate and potassium nitrate) were performed following the instructions of Genú and Pinto (2002). Nutrients were added to a fertigation system per plant demand (Genú and Pinto, 2002). Tip pruning allowed synchrony in vegetative flush events in the canopy.

Data gathering and statistical analysis

Fruit yield

The fruit yield, expressed as Mg ha^{-1}, was estimated as production per plant (kg per plant) and was measured using a balance with a precision of 0.5 g at fruit harvest.

Postharvest quality parameters

After the harvest, the fruits were stored for 22 days at 12 °C in a refrigerated incubator to simulate their transfer to the exterior market. Then, the temperature was adjusted to 25 °C for 72 h until the maturation cycle finished (Brazilian Program for Horticulture Modernization, 2004).

Postharvest analyses of the mango fruits were performed following the instructions of Zenebon et al. (2008) and included parameters such as transverse diameter (TD, region of fruit shoulder), and longitudinal diameter (LD, region between peduncle and apex) which were measured with a digital caliper (0.01 mm–300 mm) and expressed as mm. Fruit mass was measured using a precision balance (0.01 g precision) and expressed in grams. Fruit firmness (kgf cm^{-2}) was measured using a fruit firmness tester on both fruit sides. The pH was determined by potentiometry. Soluble solid concentration (SS) was expressed as percentages (%) and measured with an Abbe® refractometer (Bausch and Lomb, Rochester, NY, USA). Titratable acidity (TA) was expressed in grams of citric acid per 100 g of pulp. TA was determined by titration with sodium hydroxide (0.1 N) using 1% phenolphthalein as an indicator according to the methodology of Zenebon et al. (2008). The soluble solids/titratable acidity ratio (SS/TA) was also calculated. Dry mass (DM) were estimated by mixing 30 g of fresh pulp sample dried in an oven at 70 °C. TS was expressed as percentages.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA). Statistical analyses were performed with the software 'R' (R Core Team, 2019) using combined data from both experimental orchards. The mean values were compared by Scott-Knott's test at $p<0.05$ and $p<0.01$.

Results

According to the analysis of variance, the treatments affected fruit yield (Fig. 1), longitudinal diameter (Fig. 2A) and transversal diameter (Fig. 2B). Fruit yield values were higher in response to T2, T4, T5, and T6 treatments, with respective increments of 18%, 12%, 11%, and 8% compared to the control treatment (Fig. 1). Treatments T1, T3, and T7 presented similar results, with fruit yield ranging between 53 and 56 Mg ha^{-1}. The treatment T2 had the lowest dose of *R. palustris* and was applied only via fertigation. It resulted in the best average fruit yield, representing a difference of 10 Mg ha^{-1} compared to the control treatment (T1).

For the physical quality parameters of the fruits, there were differences in the longitudinal and transversal diameters of the fruit. In both, the statistically equal and superior treatments were T2, T4, and T5, which had applications only via fertigation, with significant values of fruit longitudinal diameter (137.84, 137.56, and 138.31 mm) (Fig. 2A) and fruit transversal diameter (101.12, 102.05, and 101.74 mm) (Fig. 2B), respectively.

The application methods of *R. palustris* did not affect the mango chemical characteristics (Table 1), except the soluble solids/titratable acidity ratio (SS/TA), with emphasis on treatments T2 and T3 (Fig. 3). It is noteworthy that all treatments showed uniformity in the other chemical characteristics of the fruits, with no compromise in fruit quality despite the sharp increase in fruit yield of treatments T2, T4, T5, and T6. SS/TA ratio values were significantly higher for T2 and T3 (Fig. 3), both with the *R. palustris* application via fertigation, with mean values 25% (T2) and 26.6% (T3) higher than the control group (T1), respectively.

Titratable acidity, soluble solids, and pH (Table 1) ranged from 0.33 to 0.43 g of citric acid per 100 g of pulp, 13.61 to 14.67 °Brix, and 4.00 to 4.11, respectively. Pulp firmness values (Table 1) were not affected by treatments, ranging from 1.13 kgf cm^{-2} (T2) to 1.58 kgf cm^{-2} (T7). DM values averaged 13%, with no differences among the evaluated treatments (Table 1).

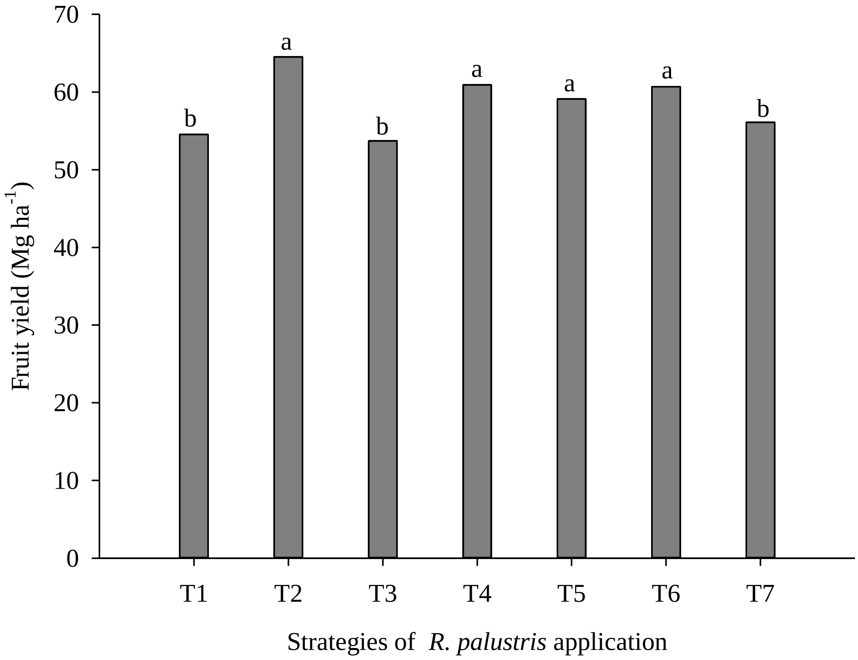

Fig. 1. Fruit yield of 'Keitt' mango as a function of different strategies of *R. palustris* application. Bars followed by the same lowercase letter do not differ according to the Scott-Knott's test (5%). T1) control treatment; T2) 1.43 × 10^7 CFU/plant via fertigation; T3) 2.85 × 10^7 CFU/plant via fertigation; T4) 4.27 × 10^7 CFU/plant via fertigation; T5) 5.70 × 10^7 CFU/plant via fertigation; T6) 1.43 × 10^7 CFU/plant via fertigation + 1.43 × 10^7 CFU/plant via leaf spray; T7) 2.85 × 10^7 CFU/plant via fertigation + 1.43 × 10^7 CFU/plant via leaf spray.

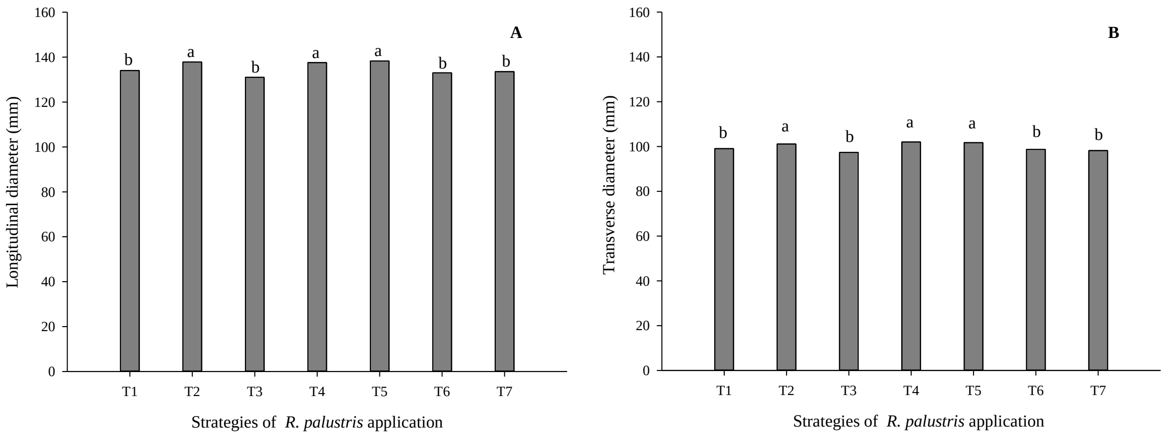

Fig. 2. Longitudinal (A) and transverse (B) diameter of 'Keitt' mango as a function of different strategies of *R. palustris* application. Bars followed by the same lowercase letter do not differ according to the Scott-Knott's test (5%). T1) control treatment; T2) 1.43 × 10^7 CFU/plant via fertigation; T3) 2.85 × 10^7 CFU/plant via fertigation; T4) 4.27 × 10^7 CFU/plant via fertigation; T5) 5.70 × 10^7 CFU/plant via fertigation; T6) 1.43 × 10^7 CFU/plant via fertigation + 1.43 × 10^7 CFU/plant via leaf spray; T7) 2.85 × 10^7 CFU/plant via fertigation + 1.43 × 10^7 CFU/plant via leaf spray.

Table 1. Analysis of variance for the variables in ventral diameter (VD), fruit mass (FM), pulp percentage (PP), pulp firmness (PF), titratable acidity (TA), soluble solids (SS), pH, and % of dry mass (DM) in 'Keitt' mango as a function of different methods for applying *Rhodopseudomonas palustris*.

SV	VD (mm)	FM (g)	PP (%)	PF (kgf cm^{-2})	TA (g $100g^{-1}$)	SS (Brix°)	pH	DM (%)
Value 'F'	1.878^{ns}	2.460^{ns}	0.598^{ns}	1.024^{ns}	2.123^{ns}	1.126^{ns}	0.630^{ns}	1.002^{ns}
T1	84.47	610.50	80.71	1.40	0.42	13.58	4.00	13.72
T2	86.07	632.40	79.08	1.13	0.33	13.61	4.11	13.82
T3	82.84	619.75	80.83	1.33	0.36	14.67	4.03	13.57
T4	86.09	650.38	81.81	1.26	0.38	14.01	4.07	12.75
T5	86.50	645.88	81.34	1.22	0.42	13.63	4.06	12.78
T6	83.76	593.38	81.24	1.50	0.40	13.92	4.07	13.07
T7	84.02	652.50	81.56	1.58	0.43	13.93	4.11	12.93
CV (%)	3.81	6.86	2.54	36.65	12.33	5.13	2.64	6.92

Means followed by common lowercase letters in the same column do not differ according to the Scott-Knott test at 5% probability level; **: significant ($p<0.01$); *: significant ($p<0.05$); ns: not significant; SV: Sources of variation; CV%: coefficient of variation. T1) control treatment; T2) 1.43 × 10^7 CFU/plant via fertigation; T3) 2.85 × 10^7 CFU/plant via fertigation; T4) 4.27 × 10^7 CFU/plant via fertigation; T5) 5.70 × 10^7 CFU/plant via fertigation; T6) 1.43 × 10^7 CFU/plant via fertigation + 1.43 × 10^7 CFU/plant via leaf spray; T7) 2.85 × 10^7 CFU/plant via fertigation + 1.43 × 10^7 CFU/plant leaf spray.

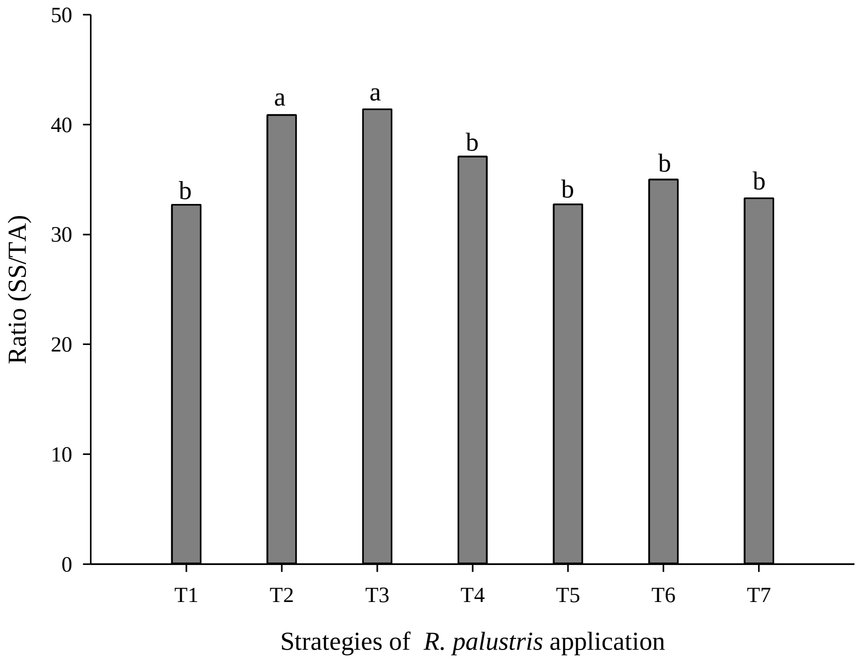

Fig. 3. Ratio (SS/TA) of 'Keitt' mango as a function of different strategies of *R. palustris* application. Bars followed by the same lowercase letter do not differ according to the Scott-Knott's test (5%). T1) control treatment; T2) 1.43 × 10^7 CFU/plant via fertigation; T3) 2.85 × 10^7 CFU/plant via fertigation; T4) 4.27 × 10^7 CFU/plant via fertigation; T5) 5.70 × 10^7 CFU/plant via fertigation; T6) 1.43 × 10^7 CFU/plant via fertigation + 1.43 × 10^7 CFU/plant via leaf spray; T7) 2.85 × 10^7 CFU/plant via fertigation + 1.43 × 10^7 CFU/plant via leaf spray.

Discussion

The significantly positive results regarding fruit yield may have occurred due to the effect of *R. palustris* on plant metabolism. Lino et al. (2023) evaluated the effects of such bacteria on crop physiological parameters of mango cv. 'Keitt' grown under tropical semiarid environmental conditions and concluded that *R. palustris* affects stomatal conductance, gaseous exchange, and nitrate reductase enzyme activities in mango cv. 'Keitt', promoting a peak of 35% more net photosynthesis than the control group. Indeed, the positive effects of *R. palustris* on fruit yield could be attributed to the increase in microbial activity and other key bacteria involved in C and nutrient cycling, both of which can potentially contribute to improved plant growth and development (Xu et al. 2016).

Lobo et al. (2019) verified a 100% increase in the fruit yield of mango cv. 'Kent' in the São Francisco Valley using a biostimulant containing water-soluble nutrients and L-α-amino acids, obtaining approximately 53 Mg ha^{-1}, a value similar to that obtained by the control treatment and lower than the best averages found in this study. Gu et al. (2002) used *R. palustris* and noted increases of 24% and 18% in fruit yield of cayenne pepper and tomato fruits, respectively, in addition to an improvement in fruit quality and a longer shelf life.

The average fruit yield of mangoes grown in the São Francisco Valley region is 27 Mg ha^{-1}, which is above the national average of 19.8 Mg ha^{-1} (EMBRAPA SEMIÁRIDO, 2022). The fruit yield values obtained in this work are numerically superior to those verified in the mango orchards of Petrolina County, with an average difference of 16.2 Mg ha^{-1} among the treatments that achieved the best fruit yield in this study (T2, T4, T5, and T6) and the average Petrolina fruit yield. It shows the potential that the bacterium *R. palustris* can benefit mango cultivation in the region.

Regarding the fruit yield, it is important to infer that the official statistical data presented by IBGE (2023) is general, i.e., it does not consider the differences among the mango cultivars cultivated and, consequently, the fruit production capacity of each cultivar.

The higher longitudinal and transversal fruit diameters promoted by T2, T4, and T5 are in response to the beneficial effects of *R. palustris* previously discussed and reported by Lino et al. (2023) and Xu et al. (2016). In addition, *R. palustris* specifically converts the sunlight and CO_2 into biomass with a direct effect on chlorophyll contents (Ge et al., 2017), which may have indirectly affected the fruit size.

Mango fruits cv. 'Keitt' treated with gibberellin (40 ppm) spray showed a longitudinal diameter of 138.7 and 153.9 mm and a transversal diameter of 100.3 and 133.3 mm in consecutive production cycles (Gattass et al., 2018), Souza et al. (2018) evaluating different mango cultivars under subtropical conditions also reached similar values for longitudinal diameter of 131.74 mm and transversal diameter of 111.15 mm for cv. Keitt, values close to those found in this study. Schnell et al. (2006) reported that 'Keitt' mango fruits have an oval shape and rounded base, generally with a 90 to 110 mm transversal diameter and a longitudinal diameter ranging from 85 to 100 mm. However, in this study, the fruits of all treatments reached longitudinal diameters above 100 mm (Fig. 2A), showing that the experimental conditions were adequate for irrigation and fertilizer management.

Regarding SS/TA, the best results occurred in response to T2 and T3 (Fig. 3), which included *R. palustris* application via fertigation. The photosynthetic fixed carbon could be used to synthesize starch in the chloroplasts and supply carbon and energy to other metabolic processes depending on plant demand. At the fruit development stage, the sucrose demand promotes synthesis in the leaves and translocation to developing fruits (Cunha et al., 2022). This demand may have happened in the present study since the SS/TA ratio expresses the fruit sweetness due to the balance of acids and sugars, being more representative than the measurement of these parameters individually. Thus, if the SS/TA ratio is high, the fruit has a good taste and adequate maturation stage, as this ratio increases when there is a decrease in acidity and high content of soluble solids derived maturity (Lobo et al., 2019b).

The SS/TA ratio values of T2 and T3 (Fig. 3) in this study meet the standards established by the Ministry of Agriculture, Livestock, and Supply (MAPA) in normative instruction Nº 37, of October 8, 2018 (BRASIL, 2018), in which the minimum value for ratio is 38.33. Indeed, the higher the fruit SS/TA, the higher the levels of soluble sugars against organic acids, directly affecting the flavor and constituting an important parameter for fruit selection (Rezende et al., 2023). For the Kent cultivar in the semiarid region as a function of the use of biostimulants, Lobo et al. (2019) reported SS/TA ratio values between 20.3 and 25.1 for their worst and best treatments, respectively. In a study with grapes in China, a decrease in acidity was verified concomitantly with an increase in the SS/TA ratio of fruits with the use of *R. palustris* (Shi et al., 1995).

Despite a lack of significant effects of *R. palustris* on some physical and chemical fruit characteristics of mangoes (Table 1), it is important to note the significant effects.

The average firmness values in this study fit the scale proposed by Brecht et al. (2017) as "soft ripe fruit", which is the best stage for consumption. This result is similar to that

reported by Lobo et al. (2019), who obtained average values between 1.21 and 1.97 kgf cm^{-2} in mango fruits cv. Kent as a function of biostimulants. Similar results by Gattass et al. (2018) portrayed the quality of mango fruits cv. Keitt in response to the application of plant regulators. They obtained mean firmness values between 1.27 and 2.18 kgf cm^{-2}.
The values related to soluble solids (Table 1) are similar to those found by Barbosa et al. (2023), who evaluated the effects of a biostimulant on the physical and physicochemical characteristics of Kent mango fruits. Although no differences were observed among treatments, the values found for these variables comply with the standards determined by the Ministry of Agriculture, Livestock and Supply (MAPA) in normative instruction Nº 37 (BRASIL, 2018). It states that the chemical composition of mango pulp should meet quality standards. They must fall within the following minimum thresholds: 0.30 g of citric acid per 100 g pulp for titratable acidity, 11 °Brix for soluble solids, and 3.5 for pH.
Mango is a fleshy fruit composed of nearly 80% water and 20% dry matter (DM). Pulp DM is determined by photoassimilates and nutrients accumulated during the growth and development of fruits, which can be affected by genetic factors and cultivation conditions that contribute to the chemical composition of the fruit and indirectly affect fruit DM (Léchaudel and Joas, 2007).
DM content correlates with the amount of reserve carbohydrates accumulated during fruit growth and development, such as starch, which is converted into sugars during the fruit ripening process, thus affecting the fruit flavor and quality (Subedi et al., 2007). Therefore, the higher the DM, the greater the sweetness of the ripe fruits. Fruits with high DM contents have a higher consumption quality (Nassur et al., 2015).
Fruit DM is a fruit quality variable adopted nowadays for mango. In this sense, Walsh et al. (2020) reported that fruit DM has been used to access the storage reserves (sum of starch) and soluble sugar content, and future soluble sugar content in climacteric fruit storing starch, i.e., after ripening with the conversion of starch to sugars, as seen in ripened apple or mango fruit. Thus, the dry matter content of fruit at harvest is related to future eating quality.

Conclusions

Rhodopseudomonas palustris improved fruit yield and fruit quality characteristics such as fruit longitudinal and transversal diameters and SS/TA ratio in mango cv. 'Keitt'. Mango plants treated with *R. palustris* at 1.43 × 10^7 CFU/plant via fertigation (T2) every 30 days after the production pruning stage until the beginning of the fruit set (totaling seven applications) had an increased yield by 10 Mg ha^{-1} compared to the control group, without loss of fruit quality while considering the European market demand.

Conflict of Interest

The authors indicate no conflict of interest for this work.

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