Journal of Tropical Crop Science Vol. 12 No. 1, February 2025
www.j-tropical-crops.com

Received 27/05/2024; Revised 18/12/2024; Accepted 20/02/2025
https://doi.org/10.29244/jtcs.12.01.185-194

Effects of Pruning, Branch Bending, and Biofertilizer Application on Flowering and Fruiting of Guava "Crystal"

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Abstract

Guava "Crystal" is one of Indonesia's most popular varieties; its demand continues to increase, so it is important to examine methods to increase fruit production. This research aims to increase the flowering and fruiting of guava 'Crystal' through branch-bending and application of biofertilizer. The results showed that pruning + bending significantly increased the number of shoots by 15.8%, shoot length by 11.16%, and leaf number by 15.09%. This treatment also increased flower number by 88.84%, fruit number by 77.9%, and double the fruit weight. Biofertilizers significantly increased vegetative growth in the form of the number of shoots (5.12%), shoot length (9.21%), and number of leaves (10.29%). Pruning + bending and biofertilizer did not significantly affect weight per fruit, fruit diameter, fruit volume, fruit firmness, total soluble solids, total titratable acids, and vitamin C content.

Keywords: bending, biofertilizer, fruit quality, fruit set, generative shoots, vegetative shoots

Introduction

Production of red guava fruit in Indonesia has been increasing; it was 200.495 tons in 2017 and increased to 472,686 tons in 2022 (Badan Pusat Statistik, 2023). Guava consumption shows an increase as well; in 2022, it was 4.404 kg per capita per week, whereas in 2023, it was 4.31 kg per capita per week (Badan Pusat Statistik, 2024). One of Indonesia's most popular guava varieties is 'Crystal'; it is a mutation of the "Muangthai Pak", the guava cultivar introduced by Taiwan Engineering Mission in Mojokerto, Indonesia, in 2001. Guava 'Crystal' has seeds of <3% of the total fruit weight and a crunchy fruit flesh texture (Direktorat Perbenihan Hortikultura, 2007), high vitamin C (146.1.8 mg per 100 g of fruits (Romalasari et al., 2017). Guava "Crystal" has the advantage of producing fruits throughout the year.

Guava 'Crystal' has high economic prospects for agro-industrial business. Therefore, it is important to understand techniques to promote flowering and increase fruit production. One method to increase flowering and fruiting is to use plant growth regulators and modify the growing environment. Environment modification includes withholding irrigation, thinning the flower buds, pruning the shoots, and applying different fertilizers (Kumari and Choudary, 2019).

Increasing flowering and fruiting in guava 'Crystal' is generally done by pruning the branches (Susanto et al., 2019). Pruning is a technique to modify plant canopy (Fumey et al., 2011) so the plant's canopy is not too dense, allowing better capture of light and stimulating the growth of new, productive shoots (Susanto et al., 2019). Pruning can shorten the distance between source and sink so that photosynthate translocation becomes more efficient (Taiz and Zieger, 2010).

Pruning has been widely proven to enhance fruit crops' flowering and fruit production. In guava "Paluma," pruning at one-third of the branch length without fruit thinning, half-length with 10–20% fruit thinning, and two-thirds length with 10% fruit thinning increased photosynthetic accumulation in the first growing season (Santos et al., 2023). Pruning while leaving four pairs of leaves significantly improved fruit set to 64.5% compared to the control (49.8%) (Fitria, 2016). Similarly, pruning guava "Allahadad Safeda" branches to 10 cm promoted vegetative shoot growth, increased fruit diameter, average fruit weight, and total yield (Lakpathi and Rajkumar, 2018). In tangerine "Pulung", V-shaped and side pruning methods resulted in the highest number of flowers and fruits (Sugiyatno et al., 2019). These studies confirm that pruning is an effective technique for optimizing fruit plant productivity.

Received 27/05/2024; Revised 18/12/2024; Accepted 20/02/2025
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Branch bending is another technique to enhance flowering and fruiting. However, this technique has not yet been tested on guava "Crystal." Previous studies on tangerines "Borneo" showed that bending stimulated fruit production in non-fruiting plants (Azizu et al., 2016). In wax apples, bending angles 65° and 85° improved flowering, fruit growth, and quality (Khandaker et al., 2020). Similarly, research on bending has been conducted on guava "Sardar" (Tamang et al., 2021), but no studies have examined this technique for *Crystal* guava, particularly in Indonesia.

The success of pruning and bending can be further optimized with fertilizer application to ensure nutrient availability. While inorganic fertilizers are widely used for their efficiency and immediate effects on crops, long-term use can have negative environmental consequences, necessitating more sustainable alternatives. Biofertilizers, which contain beneficial microorganisms, improve nutrient availability from natural sources and reduce reliance on chemical fertilizers (Alalaf, 2020).

Biofertilizers facilitate nutrient uptake through increased nitrogen fixation using Rhizobacteria, phosphorus solubilization using phosphate-solubilizing fungi, and enhanced nutrient absorption using Arbuscular mycorrhizal fungi. Plant growth-promoting *Rhizobacteria* (PGPR) contribute to nitrogen fixation, hormone production, and disease suppression via the production of siderophores, glucanase, and chitinase (Simanungkalit et al., 2006). Studies have shown that liquid biofertilizers improve soil nitrogen availability (Perazzoli et al., 2020), for example, *Azotobacter* enhances phosphorus and potassium uptake (Ghaly et al., 2020), whereas *Bacillus cereus* increases soil potassium levels (Ali et al., 2021).

Biofertilizers are commonly used in annual crops, and rarely on perennial fruit trees. Research on *Tarroco* blood orange (*Citrus sinensis*) demonstrated that *Bacillus subtilis* improved internal fruit quality, juice yield, and root density (Qiu et al., 2021). *Sinorhizobium mexicanum* biofertilizer enhanced soil quality and plant growth in guava "Rio Grande" (Francisco et al., 2023), and *Pseudomonas aeruginosa* significantly boosted the growth of "Red Delicious" apples (Sharma and Sharma, 2022).

Combining physical techniques like pruning and bending with biofertilizers may maximize flowering and fruiting. Previous studies demonstrated that applying *Azotobacter* and mycorrhiza and pruning improved the growth and yield of orange "Kinnow" (*Citrus reticulata*) (Brar et al., 2024). Similarly, research on coffee found that pruning + bending combined with chicken manure enhanced shoot diameter, shoot length, chlorophyll content, and soil organic carbon (Rohani et al., 2024). This study aims to evaluate the effectiveness of these integrated techniques in promoting the flowering and fruiting of guava "Crystal."

Material and Methods

The research was carried out from July 2023 to January 2024 at the Cikabayan Experimental Station Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural Institute (-6.550780, 106.714531), which is in the lowlands (240 m above sea level). Fruit quality analysis was conducted at the Postharvest Laboratory, Department of Agronomy and Horticulture, IPB University. The materials used in this research were five-year-old 'Crystal' guava plants, chicken manure, NPK, and biofertilizer containing the bacteria *Rhizobium* sp., *Bacillus* sp., and *Pseudomonas* sp. LI-COR LI-3000C portable area meter was used to measure photosynthesis and transpiration rates.

The study was organized in a split-plot design. The main plots are with and without biofertilizer. The subplots are pruning and pruning + bending. The experiment was repeated 12 times, so there were 48 experimental units. Pruning and bending were conducted on four branches of each tree, totaling 192 branches.

Guava trees were selected with more than eight branches and a tertiary branch diameter of at least 1 cm. Four branches were selected to be pruned only, and four were to be pruned and bent. At the start of the study, biofertilizer was applied after pruning and bending, twice every three months. Biofertilizer is provided by mixing 1 kg of PGPR Rhizomax with 100 kilograms of chicken manures, which was applied around the trees at 20 kg per tree.

In the pruning + bending treatment, after the tertiary branches are pruned, leaving four pairs of leaves, the branch is bent at 90^0 from the primary or 135^0 from the tertiary stem. The branches are pulled with a rope towards the ground until they bend; the other end of the rope is tied to a stake stuck in the ground (Figure 1).

Vegetative growth measurements include the number of new shoots, vegetative shoots, generative shoots, shoot length, and number of leaves. Generative growth measurements include the number of flowers, fruit set, number of fruits, and total weight. Fruit

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quality was examined in terms of weight per fruit, fruit diameter, fruit volume, fruit softness, fruit skin color, total dissolved solids (TDS), total titrated acids (TTA), and vitamin C levels. Quantitative data were analyzed using analysis of variance at α=5%. If the treatment effects were significant, further comparison tests were conducted using the Honestly Significant Difference (HSD) test at α=5%.

Results and Discussion

Soil Chemical Analysis

The C-organic, total N, potential P and potential K were higher in the soil with biofertilizer (Table 1). According to Simanungkalit et al. (2006), biofertilizers facilitate the availability of nutrients in the soil. Soil treated with biofertilizer showed high potential and available phosphorus. It is possible that the biofertilizers dissolve insoluble phosphates to make them available for the plants.

The soil C-organic with biofertilizer was 1.74%, whereas without biofertilizer it was 1.52% (Table 1), indicating that the availability of organic matter in both soils was relatively low. According to Just et al. (2024), microorganisms in biofertilizers require organic matter as a substrate to decompose and increase the C-organic content. However, if the organic matter in the soil is low, these microorganisms cannot function effectively to increase the C-organic content.

The nitrogen content of the soil with biofertilizer was 0.26%, and that of the soil without biofertilizer was 0.21%, or in the moderate category. There was a slight increase in the N nutrient of the soil with biofertilizer. The biofertilizer used contained *Rhizobium*. Fahde et al. (2023) showed that the N content increased in the soil applied with *Rhizobium.*

The potential P of soil treated with biofertilizer and without biofertilizer are 100.60 and 74.92 mg P_2O_5/100g, whereas the available P is 31.51 and 16.32 ppm P_2O_5. Therefore, both indicate high status (Table 1). However, there was an increase in the P-potential and P-available content in soil treated with biofertilizer. Previous research by Yadav (2022) showed that *Bacillus* sp. and *Pseudomonas* sp. could dissolve phosphates into available phosphates. Similarly, potassium levels were higher with biofertilizer (14.92 vs 14.20 mg K_2O 100 g^{-1}), showing a low nutrient status. Based on the results of this study, the provision of biofertilizer can slightly increase the K nutrient content.

Photosynthesis and Transpiration Rates

Pruning + bending and pruning alone did not significantly result in different photosynthesis rates (Table 2). Although the photosynthesis rates were similar, the pruning + bending treatment had a photosynthesis rate of 48 µmol CO2$m^{-2}s^{-1}$, higher than that of those only pruned, 28.29 µmol $CO_2m^{-2}.s^{-1}$. According to Poerwanto and Susila (2014), bending

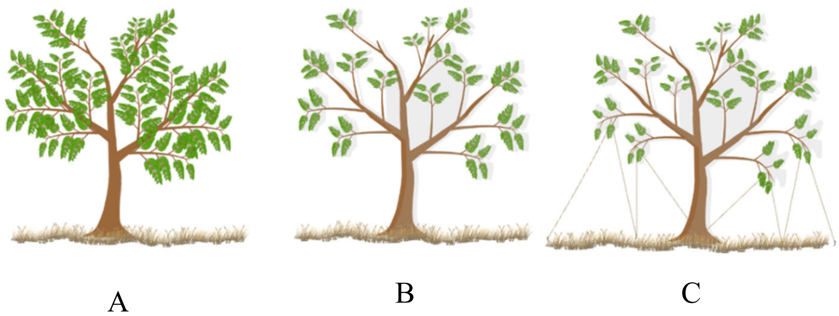

Figure 1. A tree before pruning (A); after pruning the branches, leaving four pairs of leaves per branch (B), and bending of the pruned branches (C)

Table 1. Soil chemical properties of the study site

Parameters	Biofertilizer		Without biofertilizer	
	Values	Status*	Values	Status*
C-organic (%)	1.74	Low	1.52	Low
Total N (%)	0.26	Moderate	0.21	Moderate
Potential P (mg P_2O_5.100 g^{-1})	100.60	High	74.92	High
Available P (ppm P_2O_5)	31.52	High	16.32	High
Potential K (mg K_2O.100 g^{-1})	14.92	Low	14.20	Low

Notes *: based on Soil and Fertilizer Instrument Standard Testing Center (2023).

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and pruning will cause the canopy to be more open and hence can receive more sunlight, increasing the photosynthesis rate.

The transpiration rates in the pruning + bending and pruning treatments showed significant differences (Table 2). The transpiration rate on branches that were pruned + bent was significantly higher than the ones pruned. This result is likely due to the increased exposure of the pruned and bent branches to sunlight. The higher the temperature, the higher the transpiration rate. This research aligns with research by Xiang et al. (2021) on Pear Korla Fragrant, whose branches were bent 90^0; higher leaf temperatures can accelerate water evaporation and increase transpiration compared to branches that were not bent.

There was no significant difference in photosynthesis and transpiration rates between plants with and without biofertilizers. Physical training did not interact with fertilizers in affecting guava photosynthesis and transpiration rates.

Vegetative Growth

Pruning + bending resulted in significantly more shoots, generative shoot length, and leaves than just pruning. Significant differences were also found in the application treatment of biofertilizers. Biofertilizer significantly increases the number of shoots, vegetative shoots, shoot length, and leaves (Table 3). Pruning and bending increased the number of vegetative shoots by 15.84% and generative shoots by 100% more than only pruned branches (Table 3). Bending the branches will break apical dominance, likely because the flow of the hormone auxin is hampered in the bent branch and stimulates the outgrowth of lateral shoots. The study aligns with research by Devy et al. (2023), which shows that bending can reduce auxin activity in shoot tissue, thereby encouraging increased cytokinin activation in the lateral shoots of Mandarin Citrus. In other research conducted by Azizu et al. (2016) on orange "Borneo Prima," and apple (Zhang et al., 2017) bending branches result in more shoots emerging than those without. Branch bending can increase the flow of carbohydrates to lateral shoots, which play an important role in supporting the growth and development of generative shoots that will bear flowers and fruits.

The application of biofertilizer significantly increased the number of shoots and vegetative shoots by 5.12% and 13.26% compared to without biofertilizer (Table 3). This study's results align with research by Karakurt et al. (2011) on sour cherry. The application of biofertilizers increased the number of sour cherry shoots due to the ability of biofertilizers to fix N and dissolved insoluble phosphates, so they can be utilized by plants to increase vegetative growth, especially in producing new shoots. Further test results showed no interaction between technical treatment and fertilizer application on the number of new, generative, and vegetative shoots.

The pruning + bending treatment significantly increased shoot length by 11.16% compared to only pruned branches (Table 3). Auxin promotes shoot elongation (Sosnowski et al., 2023), which is consistent with the results of this study where pruned + bent shoots can elongate faster than shoots that were pruned only.

Applying biofertilizer significantly increased guava shoot length by 9.21% compared to without biofertilizer (Table 3). In this study, the biofertilizers contained *Bacillus* sp. and *Pseudomonas* sp. This study is similar to previous research by Aslantaş et al. (2007), which used PGPR biofertilizers of *Pseudomonas* BA-8 and *Bacillus* OSU-142 bacterial strains proven to increase the number of shoots and shoot length in

Table 2. Effect of pruning + bending and biofertilizer on photosynthesis and transpiration rates of guava "Crystal"

Treatment	Photosynthesis rate ($\mu mol\ CO_2.m_{-2}.s_{-1}$)	Transpiration rate ($\mu mol.m_{-2}.s_{-1}$)
Physical training (P)		
Pruning + bending	28.46	0.0022 a
Pruning	28.29	0.0021 b
Fertilizer (F)		
Biofertilizer	28.47	0.0022
Without biofertilizer	28.29	0.0021
P x F	ns	ns

Notes: Values followed by the same letter in the same column show no significant difference according to the HSD test at α <0.05; ns = not significant.

Received 27/05/2024; Revised 18/12/2024; Accepted 20/02/2025
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Granny Smith and Stark Spur Golden apple cultivars. Pruning + bending branches significantly increased the number of leaves by 15.09% compared to only pruned branches (Table 3). The results of this study are similar to previous research by Azizu et al. (2016) on Borneo Tangerine. Pruning and bending make the branches and crowns open so that the leaves are not shaded by each other and can grow well compared to only pruned branches.

Biofertilizer application also significantly increased the number of leaves by 10.29% compared to without biofertilizer. The result is similar to reports by Shankarappa et al. (2018) on Alphonso mango. Biofertilizers improve soil health and increase nutrient absorption, such as nitrogen, an important element in forming Alphonso mango leaves (Shankkarappa et al., 2018).

Generative Growth

The pruning and bending treatment significantly improved generative growth, i.e., number of flowers, fruits, and total fruit weight compared to pruning alone. At the same time, the biofertilizer application had no significant effect on any parameter (Table 4).

Pruning + bending increased flower production by 88.84% compared to pruning alone (Table 4). According to Poerwanto and Susila (2014), this may be due to the more open canopy structure, allowing increased exposure to morning light, which induces flowering. Improved exposure to sunlight promotes flower induction by increasing carbohydrate accumulation and reducing nitrogen levels, leading to a higher C/N ratio in bent branches (Table 5). A high C/N ratio is essential for flowering, as seen in longan (Yulianto et al., 2008) and apple (*Fuji* and *Gala*) trees (Zhang et al., 2017).

Biofertilizer application did not significantly affect flower production, indicating its limited role in inducing flowering. The biofertilizers used in this study were plant growth-promoting rhizobacteria (PGPR), which primarily enhance vegetative growth through nitrogen fixation and phosphate solubilization (Aloo et al., 2022). PGPR can produce hormones such as auxins and cytokinins, which support root and leaf development rather than flowering. No interaction was recorded between pruning/bending treatments and biofertilizer application in affecting flower production.
Pruning + bending significantly increased fruit number and total fruit weight by 77.9% and 100%,

Table 3. Effect of pruning + bending and biofertilizer on vegetative growth of guava “Crystal”

Treatments	Number of shoots	Number of generative shoots	Number of vegetative shoots	Shoot length	Number of leaves
Physical training (P)					
Pruning + bending	27.94a	6.25a	21.28	19.13a	147.16a
Pruning	24.12b	3.03b	20.84	17.21b	127.87b
Fertilizer (F)					
Biofertilizer	26.68a	4.05	22.37a	18.97a	144.25a
Without biofertilizer	25.38b	5.22	19.75b	17.37b	130.79b
P x F	ns	ns	ns	ns	ns

Notes: Values followed by the same letter in the same column show no significant differences according to the HSD test at α <0.05; ns = not-significant.

Table 4. Effect of pruning + bending and biofertilizer on generative growth of guava “Crystal"

Treatment	Number of flowers	Fruit set (%)	Number of fruits	Total weight of fruits (g)
Technique				
Pruning + bending	11.84a	39.51	5.48a	1367.9a
Pruning	6.27b	39.78	3.08b	651.7b
Fertilizer				
Biofertilizer	7.89	38.80	3.82	861.8
Without biofertilizer	10.21	40.49	4.75	1157.8
Interaction	ns	ns	ns	Ns

Notes: Values followed by the same letter in the same column show no significant difference in the HSD test. ns = not significant. ** significant α >0.01. *significant at α <0.05.

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respectively (Table 4), likely due to increased carbohydrate accumulation in bent branches (Table 5). These findings align with previous studies, where branch bending in tangerine trees led to carbohydrate accumulation, supporting fruit development (Azizu et al., 2016). Similar results were reported for ‘Khaja’ guava (Nandi et al., 2017) and *Citrus mandarin* (Budiarto et al., 2018).

Biofertilizer application did not significantly affect fruit number (Table 4), which could be related to its inability to increase potassium (K) levels in the soil (Table 5). As Marschner (2012) noted, potassium is essential for carbohydrate transport, which is critical for fruit development. Additionally, no interaction was found between physical treatments and fertilizer application in fruit production.

Carbohydrates and C/N Ratio

Pruned + bent branches had lower N content than pruned-only branches (Table 5). Bending disrupts

Table 5. Effect of pruning + bending and biofertilizer on guava leaf NPK content, carbohydrates, and leaf C/N ratio

Treatment	N (%)	P (%)	K (%)	Carbohydrate (%)	C/N ratio
Technique					
Pruning + bending	2.00b	0.18	1.68	8.17	4.08a
Pruning	2.20a	0.19	1.66	7.32	3.32b
Fertilizer					
Biofertilizer	2.10	0.18	1.64	7.98	3.82
Without biofertilizer	2.10	0.18	1.70	7.51	3.58
Interaction	ns	ns	ns	ns	ns

Notes: Values followed by the same letter in the same column show no significant difference in the HSD test. ns = not significant. ** significant α >0.01. *significant at α <0.05.

Table 6. Effect of pruning + bending and biofertilizer on physical fruit quality

Treatment	Weight per fruit (g)	Volume (ml)	Fruit diameter (cm)	Fruit firmness (mm.100 gr^{-1} per 10 sec)	Peel Color
Technique					
Pruning + Bending	281.06	316.60	8.35	24.91	Yellowish Green
Pruning	297.23	330.66	8.55	22.91	Yellowish Green
Fertilizer					
Biofertilizer	299.53	335.93	8.55	23.17	Yellowish Green
Without Biofertilizer	278.76	311.33	8.35	24.65	Yellowish Green

Notes: Values followed by the same letter in the same column show no significant difference in the HSD test. ns = not significant. ** significant α >0.01. *significant at α <0.05.

Table 7. Effect of pruning + bending and biofertilizer on internal fruit quality

Treatment	TSS (^{0}Brix)	ATT (%)	TSS:ATT ratio	Vitamin C (mg.100 g^{-1})
Technique				
Pruning + Bending	9.14	0.31	29.86	139.71
Pruning	9.97	0.34	30.24	159.08
Fertilizer				
Biofertilizer	9.88	0.33	30.18	140.59
Without Biofertilizer	9.23	0.32	29.29	158.20
Interaction	ns	ns	ns	Ns

Notes: Values followed by the same letter in the same column show no significant difference in the HSD test. ns = not significant. ** significant α >0.01. *significant at α <0.05.

Received 27/05/2024; Revised 18/12/2024; Accepted 20/02/2025
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carbohydrate translocation to the roots, reducing root respiration and impairing nutrient and water absorption (Budiarto et al., 2018). In this study, the higher carbohydrate content in pruned + bent branches suggest an inhibition in the photosynthate translocation, while the reduced leaf N content indicates nutrient uptake disruption.

Although not statistically significant, pruning and bending tended to increase leaf carbohydrate content. Samant et al. (2016) demonstrated that bending slows carbohydrate movement, leading to accumulation in bent branches. The C/N ratio depends on carbohydrate and N levels—higher carbohydrates result in a higher C/N ratio.

Reduced N concentration limits vegetative growth, promoting flower bud formation (Zhang et al., 2017). A high C/N ratio is a key indicator of flowering induction. This study confirms that the elevated C/N ratio with the pruning + bending treatment (Table 5) led to increased flowering (Table 4). Similar results were reported in Mandarin citrus, where bent trees had a higher C/N ratio than pruned trees (Budiarto et al., 2018). Similarly, 85° branch bending in peach (*Prunus persica)* resulted in the highest C/N ratio (Zhang et al., 2023).

Physical and Internal Quality of Fruit

Fruit's physical quality directly influences consumer acceptance. In this study, pruning + bending and biofertilizer application did not significantly affect the fruit's physical quality (Table 6). The average fruit weight, volume, diameter, and softness ranged from 281.06–299.53 g, 311.33–335 ml, 8.35–8.55 cm, and 22.91–24.91 mm.100 g^{-1} per 110 sec, respectively, with a uniform yellowish-green skin color.

The fruit quality parameters, including total soluble solids (TSS), titratable acidity (ATT), TSS: ATT ratio, and vitamin C content, showed no significant differences across treatments (Table 7). The respective values ranged between 9.1–9.4 °Brix, 0.31–0.34%, 29.29–30.24, and 139.71–158.20 mg.100g^{-1} (Table 7). Previous studies reported no significant impact of pruning (Susanto et al., 2019) or strangulation (Widyastuti et al., 2020) on the physical and internal quality of guava "Crystal" and tangerine “Siompu” (Azizu and Peliyarni, 2022). Similarly, biofertilizers did not affect pear fruit firmness or titratable acidity (*Pyrus communis* L (Perazzoli et al., 2020).

These findings suggest that pruning and bending or biofertilizers do not influence the fruit quality of guava "Crystal". Instead, fruit ripeness is the dominant factor affecting TSS and ATT content, as ripening increases sugar levels while reducing acidity (Dolkar et al., 2017).

Conclusions

Pruning and branch bending enhanced vegetative and generative growth in guava "Crystal" by increasing the C/N ratio, promoting shoot formation, shoot length, and leaf production, and improving flowering, fruit set, fruit number, and total fruit weight. The biofertilizer application enhanced vegetative growth but did not affect the generative development, including flowering, fruit set, fruit count, and total fruit weight. There was no interaction between pruning, bending, and biofertilizer application, nor did these treatments affect the physical (fruit weight, diameter, volume, softness) or internal (total dissolved solids, titratable acids, vitamin C) fruit quality. The recommended practice for guava growers is to bend branches at a 90° angle from the main stem after pruning.

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