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Optimization of Phenological Stages and Growth of Guava cv. Taiwan Pink through Organic Amendments and Bioinoculants

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ABSTRACT: Guava (*Psidium guajava* L.) is a valuable tropical fruit crop known for its nutritional benefits and economic significance, especially in India. Nevertheless, guava farming encounters difficulties, including early flower and fruit drop, which are frequently linked to inadequate nutrient management and excessive reliance on chemical inputs. This research sought to evaluate the effects of organic fertilizers, seaweed extract, and Pink Pigmented Facultative Methylotrophs (PPFM) on the growth stages, physiological characteristics, and yield factors of the guava variety Taiwan Pink. A field experiment was carried out in Rajampalayam village, Tamil Nadu, from February 2024 to May 2025 utilizing a Randomized Block Design (RBD) with 12 unique treatment combinations, each replicated three times. The treatments included various combinations of goat manure, poultry manure, seaweed extract (2–3%), and PPFM (1–2%). Observations were made on phenological parameters (such as bud emergence and fruit set), vegetative parameters (including shoot and leaf count, and leaf area), and physiological parameters (chlorophyll index). The data were analyzed using standard ANOVA methods at a 5% significance level. Treatment T_3 (Goat manure 2.5 kg combined with 3% seaweed extract) achieved the earliest bud emergence at 12.42 days. Meanwhile, T_{10} (Poultry manure 2.5 kg with 2% PPFM) resulted in the quickest fruit set at 27.96 days, alongside the highest number of flower buds (144.69) and the most fruit set (139.67). T_5 (Goat manure with 2% PPFM) exhibited the greatest leaf area at 61.16 cm². Additionally, T_{10} recorded the highest chlorophyll index during the flowering stage at 43.96, while T_2 (Goat manure with 2.5% seaweed extract) excelled during fruit set with an index of 38.46. Combining organic fertilizers with bio-inputs such as seaweed extract and PPFM greatly improved vegetative growth, chlorophyll levels, and fruiting characteristics in guava cv. Taiwan Pink. This environmentally friendly method not only boosts yield and fruit quality but also promotes sustainable agriculture by decreasing reliance on chemical fertilizers.

Keywords: Guava, Seaweed extract, PPFM, Organic manure, Poultry manure, Goat manure.

INTRODUCTION

Guava (*Psidium guajava* L.) is a prominent tropical fruit crop, originating from Tropical America and belonging to the Myrtaceae family. This fruit is widely appreciated for its multitude of nutritional benefits, including high levels of protein and fiber, which make it a favored choice among health-conscious consumers (Kareem and Kadhim 2024).

According to Lim (2012); Singh *et al*. (2023), Guava has more vitamin C than oranges, boosting immune health. In addition to that, it is rich in dietary fiber, antioxidants, and essential minerals like potassium and magnesium. Its pink flesh contains lycopene, an antioxidant associated with a reduced risk of chronic diseases such as cancer and heart conditions. Guava has several varieties, including Arka Kiran, Lucknow-49, Taiwan Pink, and Lalit. Among these varieties, Taiwan Pink is particularly noted for its consumer acceptance, economic value and agricultural adaptability. Taiwan Pink Guava is a high-yield, profitable crop for farmers in tropical and subtropical areas. Its sweetness, few seeds, and appealing look have boosted its global popularity, with exports including fresh fruit, juices, jams, and purées (Morton, 1987; Singh *et al.*, 2023a). This guava variety adapts well to various climates and soils, showing drought resilience, making it ideal for sustainable farming in water-limited areas.

In 2024, India led the world in guava production, accounting for 45% of the total, followed by China and Thailand. The total area under guava cultivation is approximately 357.64 Mha, yielding 5262.73 MT of fruit with a productivity of 14.72 MT/ha. Madhya Pradesh is the top-producing state, followed by Uttar Pradesh and Andhra Pradesh. Notably, Allahabad in Uttar Pradesh is known for its high-quality guavas. In Tamil Nadu, the area is 14.56 Million Hectares, producing 367.71 million tonnes with a productivity of 25.26 tonnes per hectare (Indiastat, 2024).

Soil fertility is essential for growing guava (*Psidium guajava* L.), influencing plant health, fruit yield, and quality. While guava tolerates various soil types, optimal fertility increases productivity. Applying goat and poultry manure improves soil structure, boosts microbial activity, and enhances nutrient retention in guava (Afreen *et al.*, 2024). Organic fertilizers enhance soil health by providing essential nutrients and improving its structure and fertility. Their importance in sustainable agriculture has risen due to environmental and health concerns. They increase organic matter, improving soil aeration, water retention, and microbial activity, crucial for long-term fertility. By gradually releasing nutrients, they match plant absorption and minimize leaching. Unlike synthetic fertilizers, they are less likely to contaminate soil and water, reduce greenhouse gas emissions, and support biodiversity. Organic fertilizers boost productivity while being safe for humans, animals, and beneficial insects (Reganold & Wachter 2016).

Beneficial microbes like PPFM (Pink Pigmented Facultative Methylotrophs) also aid in nitrogen fixation and nutrient absorption in plants (Bairwa *et al*., 2009). These are beneficial bacteria mainly*Methylobacterium* spp. that promote plant growth by producing phytohormones and enhancing stress tolerance. Seaweed extract is a natural biostimulant rich in plant hormones, trace minerals, and amino acids that enhance growth, yield, and stress resistance.

A key challenge in guava cultivation is premature flower and fruit drop, causing yield losses of 45-65% and also deterioration in fruit quality (Gomasta *et al*., 2024). Inorganic chemicals in agriculture can harm the environment. A sustainable alternative is using seaweed extract and Pink Pigmented Facultative Methylotrophs (PPFM) alongside organic manure, which boosts nutrient absorption, productivity, and fruit quality (Du Jardin, 2015). Recent studies show that this combined approach can enhance yield and yield-related traits in guava, including fruit weight and quality (Al-Saif *et al*., 2023; Babu *et al*., 2023).

METHODOLOGY

A. Experimental Site

The field experiment was conducted on a farmer's field in Rajampalayam village, Maduranthanakam Taluk, Tamil Nadu, from February 2024 to May 2025. The location is positioned at a latitude of 12°23'N and a longitude of 79°42'E. The relative humidity in the area is 65 to 70 percent, and the average annual rainfall is 1200 mm. Temperatures in the region typically range from 25°C to 32°C. The soil in the experimental field is sandy loam, exhibiting low to medium levels of nitrogen (N) at 0.014 kg/ha, phosphorus (P) at 0.32 kg/ha, potassium (K) at 220 kg/ha, and organic carbon at 0.4%. The soil pH is 7.01, with an electrical conductivity (EC) of 0.04 ds/m.

B. Experimental Design

The field experiment aimed to investigate the effects of seaweed extractand Pink Pigmented Facultative Methylotrophs (PPFM) on the floral and yield performance of four-year-old guava trees spaced 2.5 × 2.5 meters apart. The selected guava variety for this study was Taiwan Pink, and the experimental design employed was a Randomized Block Design (RBD) with 12 different treatments, each replicated thrice. The treatments combinations comprises are T_1 – Goat Manure 2.5 kg + Seaweed extract 2%,T_2 - Goat Manure 2.5 kg + Seaweed extract 2.5%,T_3- Goat Manure 2.5 kg + Seaweed extract 3%,T_4 - Goat Manure 2.5 kg + PPFM 1%,T_5 - Goat Manure 2.5 kg + PPFM 2%,T_6 - Poultry Manure 2.5 kg + Seaweed extract 2%,T_7 - Poultry Manure 2.5 kg + Seaweed extract 2.5%,T_8 - Poultry Manure 2.5 kg + Seaweed extract 3%,T_9 - Poultry Manure 2.5 kg + PPFM 1%,T_{10} - Poultry Manure 2.5 kg + PPFM 2%,T_{11} - Control (Water spray) and T_{12} -Absolute control. The application of manures and bioinoculants was given immediately after pruning, at the time of bud emergence, and at the time of fruit set.

The observations were recorded and analyzed statistically to assess the impact of different treatment combinations. The duration for bud emergence is determined by counting the number of days from the application date of the treatment to the day when the first bud is visibly noticed on the plant. The days taken for fruit set are determined by counting the days from when the flower buds appear until the moment visible fruit begins to form and are expressed in days. The total number of new shoots for each plant was determined by counting all the newly developed shoots that emerged from the main stem and branches throughout the observation period. The total number of newly grown leaves on each plant was counted throughout the study, and the number for each plant. To determine the number of flower buds, you can manually count all the visible buds on each plant during a specific growth stage and then calculate the average total across all the sampled plants or replications. The total number of fruits that develop from the flowers on each plant during the fruit-setting stage is determined by counting the successful fruit formations.

The chlorophyll index during the flowering stage and at the time of fruit set is determined using a chlorophyll meter, such as the SPAD device, which gauges the relative greenness of the leaf by measuring light absorption at specific wavelengths associated with chlorophyll content. With the help of a leaf area meter (LI-COR LI-3100C Area Meter), the leaf area is determined by placing the leaf on the scanning surface. The device measures the actual area by examining the leaf's length and width with optical or digital sensors, and it directly displays the result in square centimeters (cm^2).

The data was analyzed statistically by using SPSS (version 30). A Randomized Block Design was employed to evaluate the impact of treatments on the guava trees. Following the computation of analysis of variance (ANOVA), standard error (SE(d)), and coefficient of variation, mean comparisons were performed, with a significance level of five percent established for the critical difference.

RESULTS AND DISCUSSION

A. Effect of biofertilizer application on phenological and physiological parameters and growth parameters of guava

The research revealed notable differences across treatments in every measured parameter, such as vegetative growth, flowering, fruiting, chlorophyll levels, and leaf area. The use of goat and poultry manure together with seaweed extract or PPFM positively affected the growth and development of guava cv. Taiwan Pink.

B. Days taken for bud emergence

The duration for bud emergence varied from 12.42 days in T_3 (which included Goat manure and 3% seaweed extract) to 27.41 days in T_9 (which contained Poultry manure and 1% PPFM). The quicker bud emergence observed in T_3 highlights the effectiveness of seaweed extract in enhancing rapid vegetative growth. The critical difference (CD) was 0.86, the standard error (SEd) was 0.42, and the coefficient of variation (CV) was 2.62% (Table 1).

The application of biofertilizers, particularly goat manure and seaweed extract, results in faster bud emergence. Notably, seaweed extract contributes to increased shoot length and diameter due to its rich content of growth regulators. This enhancement is linked to the presence of cytokinins and auxins in the extract. Cytokinins promote the growth of lateral shoots and floral development, while auxins play a role in preventing the formation of the abscission layer, which in turn improves flower retention. These results are consistent with studies conducted on guava (Patier *et al*., 1993).

C. Days taken for Fruit Set

In the experimental results, treatment combination T_{10}, which consisted of poultry manure (2.5 kg) combined with 2% PPFM, exhibited the fastest fruit set, occurring in an impressive average of just 27.96 days. This is in stark contrast to treatment T_9, where the fruit set experienced a significant delay, taking as long as 42.20 days. These observations strongly suggest that the synergistic effect of combining 2.5 kg of poultry manure with 2% PPFM plays a crucial role in promoting quicker reproductive initiation in the studied plants. Such rapid fruit set is beneficial for overall yield and can be particularly advantageous in agricultural practices aimed at enhancing productivity. Furthermore, the statistical analysis yielded a critical difference of 1.05, indicating a relatively low level of variation in the data, with a standard error difference (SEd) of 0.50. The overall coefficient of variation across the treatments stood at 1.76%, affirming the reliability and consistency of the results obtained from this study (Table 1).

The treatment comprised 2.5kg of poultry manure along with 2% of PPFM (T_{10}), which exhibited superior nutrient absorption capabilities, significantly enhancing plant growth and development. This accelerated transition from flower to fruit can be attributed to not only improved nutrient uptake but also the hormonal effects of crucial plant hormones such as auxins and cytokinins. These hormones play a vital role in regulating various growth processes, including the stimulation of cell division and elongation, thereby facilitating a more rapid transformation from flowers into fruits. Overall, the combination of effective nutrient delivery and hormonal regulation creates an optimal environment for fruitful development.

D. Number of new shoots

The number of new shoots varied from 40.84 (T_{12}) to 68.92 (T_3), demonstrating a significant vegetative enhancement due to seaweed extract. This implies that the bioactive compounds found in seaweed have promoted shoot proliferation. The critical difference (CD) was 1.83, the standard error of difference (SEd) was 0.87, and the coefficient of variation (CV) was 1.98%. A strong positive correlation ($r = 0.62$) was observed between the number of new shoots and the number of new leaves, indicating that plants with increased shoot growth tend to produce a greater number of leaves. This underscores the importance of healthy and vigorous vegetative growth for reproductive success (Table 1).

Seaweed extract plays a significant role in promoting shoot growth, primarily through the action of growth regulators found within the extract. These compounds contribute to enhanced cell division, largely driven by auxins along with various other plant hormones that facilitate the activation of vegetative buds. This mechanism of promoting healthy growth has been highlighted by Marhoon and Abbas (2015), who observed that algae extract is an effective bio-stimulant for plant growth. The rich composition of auxins and cytokinins in seaweed extract makes it particularly valuable, as these are key plant growth regulators that not only support initial shoot development but also influence overall plant vitality and productivity. By leveraging these natural compounds, gardeners and farmers can cultivate stronger and more vigorous plants, ultimately resulting in healthier crops.

E. Number of new leaves

The treatment combination of 2.5 kg of poultry manure in combination with 3% of seaweed extract (T_8) showed the highest number of new leaves at 436.27, whereas T_{12}, the absolute control, recorded the lowest at 248.52. This suggests that the combination of poultry manure and seaweed has a significant positive effect on leaf growth. The critical difference (CD) was 9.26, with a standard error of difference (SEd) of 4.44, and the coefficient of variation (CV) was 1.56%. Additionally, there was a moderate positive correlation (r = 0.53) between the number of new leaves and fruit set, indicating that an increase in leaf growth contributes to improved fruit development by enhancing photosynthetic efficiency and nutrient distribution (Table 2).

The highest number of leaves was recorded in the T8 treatment, which consisted of 2.5 Kg of poultry manure paired with a 3% seaweed extract. This significant increase in leaf count can be attributed to the beneficial effects of the seaweed treatments on vegetative growth. Additionally, the reduction of mineral fertilization to half the recommended dosage plays a crucial role in this enhancement. By modifying the soil's alkalinity, this approach not only supports healthier plant development but also enhances the rate of nutrient absorption, as highlighted by the research conducted by Farag *et al*. (2023). The results highlight the potential of combining organic and natural inputs to optimize plant growth.

F. Number of flower buds

The results revealed a variation in the number of flower buds, ranging from an average of 108.53 buds in treatment T_2 to 144.69 buds in treatment T_{10}. Notably, treatments that incorporated PPFM were associated with a significant enhancement in flower bud initiation, indicating the positive impact of these microorganisms on plant growth. The analysis also identified a critical difference of 5.26 between the treatments, with a standard error of difference calculated at 2.52. Additionally, the coefficient of variation was determined to be 2.46%, reflecting a low level of variability in the data, which lends further credibility to the results observed in the study (Table 2).

G. Number of fruits set

The highest fruit set was recorded in T_{10} (139.67), followed by T_5 and T_8, while T_{12} showed the lowest at 119.95. The use of poultry manure combined with PPFM at a 2% concentration (T10) significantly enhanced fruit retention. The critical difference (CD) was 3.63, with a standard error of the difference (SEd) of 1.74 and a coefficient of variation (CV) of 1.71%. A very strong positive correlation (r = 0.89) was identified between the number of flower buds and the number of fruits set, confirming the expected biological relationship that an increase in flowers leads to more fruit. Furthermore, a moderate positive correlation (r = 0.53) was observed between the number of new leaves and fruit set, indicating that robust vegetative growth contributes to improved reproductive success (Table 2).

H. Chlorophyll index at Flowering stage

The chlorophyll index during flowering varied between 38.25 (T_1) and 43.96 (T_{10}), indicating that treatments high in organic inputs contribute to sustaining elevated pigment levels that are crucial for photosynthesis (Fig. 1).

When comparing various treatments, the combination of T_{10} (2.5kg poultry manure and 2% PPFM) stands out as the most effective option. This effectiveness can be attributed to several factors, primarily the range of plant pigments found in seaweed extracts that play a crucial role in the development of pigments within plants. Research indicates that reducing the use of chemical fertilizers allows for improved nitrogen absorption, which in turn leads to an increase in the formation of essential plant pigments in the leaves. This optimal condition is vital for plant health and productivity. A notable example of this is the treatment with chlorophyll algae extract, which has been shown to significantly enhance the pigment content in snap beans, leading to improved quality and growth (Seif *et al*., 2016). Therefore, the synergistic effects of poultry manure and PPFM not only optimize nutrient availability but also promote pigment synthesis, making them highly effective for plant growth.

I. Chlorophyll index at Fruit set

During the fruit set stage, the chlorophyll index was highest in treatment T_2 at 38.46 and lowest in treatment T_{10} at 31.03. The decrease in chlorophyll levels in high-yielding treatments might be due to nutrients being redirected to developing fruits. The CD was 1.00, SEd was 0.48, and the CV was 1.71%. A moderate positive correlation (r = 0.37) was found between leaf area and chlorophyll index at fruit set, suggesting that larger leaf areas are linked to higher chlorophyll content, which in turn improves photosynthetic efficiency during fruit development (Fig. 1).

The Chlorophyll index experiences a decline during the fruit set stage when compared to the flowering stage. Among the various treatments investigated, T_2, which consists of 2.5 kg of goat manure combined with 2.5% seaweed extract, proved to be the most effective in promoting chlorophyll levels. Research conducted by Seif *et al*. (2016) indicated that the application of chlorophyll algae extract significantly increased the pigment content in snap beans. This enhancement may be attributed to the presence of antioxidants and growth-promoting properties found in the algal extract, which likely contribute to improved plant health and vitality. These findings suggest a beneficial relationship between the use of organic amendments and the development of plant pigments during critical growth stages.

J. Leaf area (cm²)

Leaf area ranged from 54.31 cm² (T_6) to 61.16 cm² (T_5). The application of goat manure and PPFM resulted in the largest leaf areas, which are essential for maximizing light capture and photosynthetic productivity. CD: 1.51, SEd: 0.72, CV: 1.54% (Fig. 1).

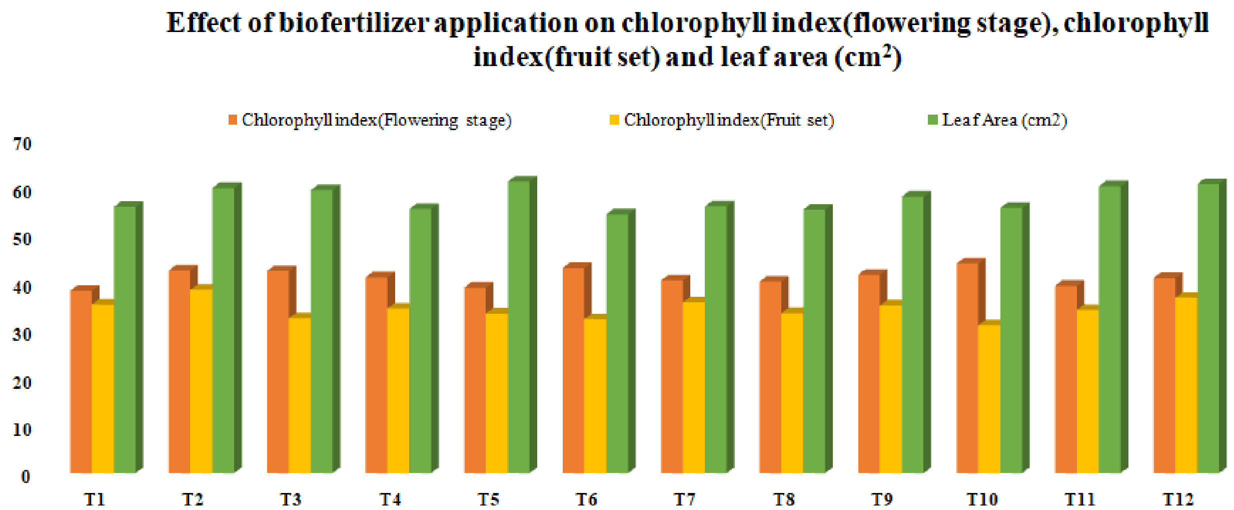
The leaf area hasa high range in T_5 (2.5 kg of Goat manure + PPFM 2%).By this, the leaf length and breadth have been calculated. The leaf area of the guava genotypes G.12 and G.11 was significantly higher than that of G.6 (32.27), which was considerably lower than that of G.12 (65.75), and the other guava genotypes were in between. These findings align with those reported by El-Sharkawy and Othman (2009).

Table 1: Effect of biofertilizer application on days taken for bud emergence, days taken for fruit set and number of new shoots.

Treatment	Days taken for Bud emergence	Days taken for fruit set	No. of new shoots
T_1 : 2.5 Kg Goat manure + Seaweed extract 2%	19.86	39.14	53.63
T_2 : 2.5 Kg Goat manure + Seaweed extract 2.5%	20.02	33.67	52.26
T_3 : 2.5 Kg Goat manure + Seaweed extract 3%	**12.42**	31.79	**68.92**
T_4 : 2.5 Kg Goat manure + PPFM 1%	24.79	37.78	45.76
T_5 : 2.5 Kg Goat manure + PPFM 2%	13.98	32.32	62.88
T_6 : 2.5 Kg Poultry manure + Seaweed extract 2%	22.44	35.69	57.02
T_7 : 2.5 Kg Poultry manure + Seaweed extract 2.5%	19.79	32.94	52.11
T_8 : 2.5 Kg Poultry manure + Seaweed extract 3%	17.53	30.89	59.30
T_9 : 2.5 Kg Poultry manure + PPFM 1%	**27.41**	**42.20**	53.40
T_{10}: 2.5 Kg Poultry manure + PPFM 2%	15.31	**27.96**	57.99
T_{11}: Control (H_2O)	16.70	41.11	45.35
T_{12}: Absolute control	22.93	34.34	**40.84**
C.D.	**0.866**	**1.055**	**1.834**
SE(d)	**0.415**	**0.505**	**0.879**
C.V.	**2.618**	**1.768**	**1.981**

Table 2: Effect of biofertilizer application on the number of new leaves, number of flower buds, and number of fruit set.

Treatment	No. of new leaves	No.of flower buds	No. of fruit set
T_1 : 2.5 Kg Goat manure + Seaweed extract 2%	307.17	111.58	108.42
T_2 : 2.5 Kg Goat manure + Seaweed extract 2.5%	357.61	**108.53**	112.25
T_3 : 2.5 Kg Goat manure + Seaweed extract 3%	359.98	127.58	125.95
T_4 : 2.5 Kg Goat manure + PPFM 1%	319.24	127.35	122.93
T_5 : 2.5 Kg Goat manure + PPFM 2%	348.49	138.01	135.92
T_6 : 2.5 Kg Poultry manure + Seaweed extract 2%	353.88	113.13	125.10
T_7 : 2.5 Kg Poultry manure + Seaweed extract 2.5%	354.39	125.38	126.07
T_8 : 2.5 Kg Poultry manure + Seaweed extract 3%	**436.27**	130.36	131.91
T_9 : 2.5 Kg Poultry manure + PPFM 1%	377.09	132.85	126.12
T_{10}: 2.5 Kg Poultry manure + PPFM 2%	386.76	**144.69**	**139.67**
T_{11}: Control (H_2O)	333.18	124.40	124.46
T_{12}: Absolute control	**248.52**	120.93	**119.95**
C.D.	**9.269**	**5.263**	**3.637**
SE(d)	**4.441**	**2.521**	**1.742**
C.V.	**1.560**	**2.463**	**1.713**



T_1 : 2.5 Kg Goat manure + Seaweed extract 2%,T_2 : 2.5 Kg Goat manure + Seaweed extract 2.5%,T_3 : 2.5 Kg Goat manure + Seaweed extract 3%,T_4 : 2.5 Kg Goat manure + PPFM 1%,T_5 : 2.5 Kg Goat manure + PPFM 2%,T_6 : 2.5 Kg Poultry manure + Seaweed extract 2%,T_7 : 2.5 Kg Poultry manure + Seaweed extract 2.5%,T_8 : 2.5 Kg Poultry manure + Seaweed extract 3%,T_9 : 2.5 Kg Poultry manure + PPFM 1%,T_{10}: 2.5 Kg Poultry manure + PPFM 2%,T_{11}: Control (H_2O),T_{12}: Absolute control

Fig. 1. Effect of biofertilizer application on chlorophyll index (flowering stage), chlorophyll index(fruit set), and leaf area (cm^2).

CONCLUSIONS

The study highlights the significance of sustainable practices in guava cultivation, especially for the Taiwan Pink variety, which provides important nutritional and economic benefits. Utilizing organic fertilizers like goat and poultry manure, along with Pink Pigmented Facultative Methylotrophs (PPFMs) and seaweed extract, can enhance soil fertility and support plant growth. This approach reduces reliance on chemical fertilizers and addresses issues such as premature flower and fruit drop, ultimately improving yield and fruit quality. The findings from the study suggest that these integrated strategies can boost productivity and sustainability in guava farming, essential for India's leading role in global guava production. Further research could lead to even better practices in cultivation.

FUTURE SCOPE

Future research should examine the lasting impacts of organic amendments and bioinoculants on soil health and nutrient dynamics. Studying guava plants' molecular responses to seaweed extract and PPFM may uncover important physiological insights. Applying these findings in various agro-climatic zones can demonstrate their wider relevance. Combining precision farming techniques with organic inputs could optimize guava yields and resource use.

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Conflict of interest. None.

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