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# Evaluating the Impact of Bioinoculants along with Organic Manures on Seed Germination of Fresh and Aged Seeds of Guava (*Psidium guajava* L.)

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The present investigation was conducted to evaluate the effect of bioinoculants along with organic amendments on seed germination of fresh and aged seeds of guava (*Psidium guajava* L.) under open field conditions. The experiment aimed to assess the influence of selected bioinoculants along with organic manures on germination behaviour and subsequent vegetative growth of seedlings. Treatments comprised 15 different combinations of bioinoculants along with organic manures with control as untreated seeds, arranged in RBD design under open field conditions. The

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study concluded that among all the treatments, T15 (Sand + Vermicompost + PSB + *Azotobacter* + VAM) consistently performed best across all parameters. Fresh and aged seeds treated with this combination exhibited substantial improvements in germination percentage, vigour index-I, vigour index-II, shoot length, seedling girth and root length, showing increases of 217.52%, 477.50%, 883.78%, 56.29%, 127.06% and 54.87%, respectively, for fresh seeds, and 215.35%, 802.34%, 888.24%, 83.67%, 153.42% and 87.06%, respectively, for aged seeds, compared to the untreated control which recorded the lowest values across all parameters. Results indicated that bioinoculant along with organic amendments application significantly enhanced seedling establishment and growth, likely through improved nutrient uptake and increased rhizospheric activity.

*Keywords: Guava; bioinoculants; sand; vermicompost.*

## 1. INTRODUCTION

Guava (*Psidium guajava* L.) is an important tropical fruit crop valued for its nutritional richness, hardiness and adaptability. Successful nursery production requires vigorous and uniform seedlings; however, guava seeds often face poor germination due to seed dormancy and hard seed coat. Bio-inoculants such as PSB, *Azotobacter* and VAM are known to improve nutrient availability, produce growth-promoting hormones and stimulate early seedling establishment. Organic manures such as FYM and vermicompost further enhance soil structure, microbial activity and nutrient cycling. Fresh seeds respond quickly to improved conditions and offer an ideal system to study the direct influence of microbial and organic amendments. The present study was conducted to evaluate the impact of bio-inoculants in combination with organic manures on germination and seedling vigour of fresh guava seeds.

Guava (*Psidium guajava* L.), popularly known as the “Apple of the Tropics” or “Poor man’s fruit” is an important fruit crop cultivated widely across tropical and subtropical regions. It belongs to the family Myrtaceae and is valued for its exceptional nutritional quality, hardiness and adaptability to diverse agro-climatic conditions. In India, guava holds a prominent position, ranking fifth in area and production among fruit crops. The main cultivation of guava in India is in northern agro-ecological regions. In India, it was grown in an area of 345.48 thousand hectares and gave yield of 5449.47 thousand MT with average productivity of 15.77 MTha$^{-1}$ during 2023-24, while in Haryana, it was grown in an area of 15.86 thousand hectares and gave yield of 189.71 thousand MT with average productivity of 11.96 MTha$^{-1}$ during 2023-24 (Anonymous, 2023). It is recognized for its high vitamin C content, pectin concentration and processing potential. Its ability to withstand a wide range of soil types, pH, drought and salinity makes it a highly dependable fruit crop for both commercial and small-scale growers (Sharma *et al*., 2020).

In recent years, the expansion of guava cultivation, along with the adoption of intensive production technologies such as high-density planting and meadow orcharding, has increased the demand for high-quality planting material. However, one of the major constraints in guava nursery production is poor and inconsistent seed germination. Guava seeds possess a relatively hard seed coat and physiological dormancy, resulting in delayed and low germination percentages. Seed ageing further exacerbates this problem, leading to deterioration of cellular components, reduced enzymatic activity, membrane leakage and decreased vigour and viability. Such deterioration hampers uniform seedling emergence, affecting the availability of vigorous rootstocks essential for budding and grafting.

To overcome these limitations, integration of bioinoculants and organic manures has emerged as a promising strategy. Bioinoculants such as *Azotobacter*, Phosphate Solubilizing Bacteria (PSB) and Vesicular Arbuscular Mycorrhiza (VAM) play crucial roles in enhancing nutrient mobilization, producing phytohormones ($GA_3$, auxins, cytokinins), improving soil microbial activity, and strengthening seedling establishment. Organic manures including farmyard manure (FYM) and vermicompost complement these effects by improving soil structure, aeration, moisture retention and supplying essential macro- and micronutrients

(Mohammed *et al.*, 2014). Vermicompost is also known to contain growth-promoting substances and enzymes that accelerate germination and enhance vigour, while FYM improves soil fertility, biological activity and nutrient cycling (Khare *et al.*, 2018).

Several studies have highlighted the synergistic benefits of combining bioinoculants with organic manures in various crops, reporting improvements in germination percentage, seedling vigour, shoot and root growth and nutrient uptake (Järvan *et al.*, 2017). However, comparison research on their influence specifically on fresh and aged guava seeds remains limited. The prime objective of the investigation was to evaluate the effect of bioinoculants combined with organic manures on germination and seedling growth of fresh and aged guava seeds. The study aims to provide a scientific basis for developing efficient, eco-friendly nursery practices for guava, thereby supporting sustainable fruit production systems.

## 2. MATERIALS AND METHODS

The experiment was carried out at the orchard of Department of Horticulture, CCS Haryana Agricultural University, Hisar, which is located at 215.2m above the mean sea level, with coordinates of 29° 10\` North latitude and 75° 46\` East longitudes during the year 2021-22. Fresh guava seeds of cultivar L-49 were collected from ripe fruits harvested of mrig season crop from guava orchard of Department of Horticulture, CCS HAU, Hisar. The seeds were extracted in July, 2021. After extraction, the seeds were properly cleaned, dried under shade and then stored at room temperature. For aged seeds, the artificial ageing treatment was given to freshly harvested seeds in ageing chamber having $42^0$ C with 100% relative humidity for 72 hours. The experiment consists of various treatments shown in Table 1. The experiment was laid out in RBD design having three replications. Different pre-sowing treatment were given to fresh and aged seed extracted along with control. Treated and untreated seeds were sown simultaneously in nursery beds in the month of August, 2021.

### 2.1 Accelerated Ageing Test

Guava seed sample of required seed weight were placed in plastic box over a wire mesh tray above 30 ml water in bottom. Uniform spread of seeds over wire mesh tray and transferred to an accelerated ageing chamber maintained at 42° C and relative humidity (90-100 percent) for 72 hours. The decline in accelerated ageing is related to initial degree of deterioration of seed lots.

Observations of seed germination parameters were recorded by using standard method. The data of germination and growth parameters was tabulated and subjected to statically analysis using method of analysis of variance (ANOVA) for Randomized Block Design (RBD) by Fisher and Yates (1963). Whenever 'F' test was found significant for comparing the means of two treatment, critical difference (C.D. at 5%) were worked out.

**Table 1. The treatments applied to the seeds**

| Sr. No. | Treatment Details |
|---|---|
| $T_1$ | Control |
| $T_2$ | Sand + FYM |
| $T_3$ | Sand + FYM + PSB |
| $T_4$ | Sand + FYM + *Azotobacter* |
| $T_5$ | Sand + FYM + VAM |
| $T_6$ | Sand + FYM + PSB + *Azotobacter* |
| $T_7$ | Sand + FYM + PSB + VAM |
| $T_8$ | Sand + FYM + PSB + Azotobacter + VAM |
| $T_9$ | Sand + Vermicompost |
| $T_{10}$ | Sand + Vermicompost + PSB |
| $T_{11}$ | Sand + Vermicompost + *Azotobacter* |
| $T_{12}$ | Sand + Vermicompost + VAM |
| $T_{13}$ | Sand + Vermicompost + PSB + *Azotobacter* |
| $T_{14}$ | Sand + Vermicompost + PSB + VAM |
| $T_{15}$ | Sand + Vermicompost + PSB + *Azotobacter* + VAM |

# 3. RESULTS AND DISCUSSION

## 3.1 Germination Percentage (%)

Germination percentage of fresh and aged seeds were increased significantly with different bio-inoculants in combination with organic manures treatments as compared to control (Table 2) at 60 DAS. Maximum germination percentage of fresh and aged seed of guava (29.53% and 15.20 %) was recorded with the treatment T15 (Sand + Vermicompost + PSB + *Azotobacter* + VAM) which was significantly higher as compared to the other treatments, followed by T8 (Sand + FYM + PSB + *Azotobacter* + VAM) with germination per cent of 29.00 % and 14.24%; T13 (Sand + Vermicompost + PSB + *Azotobacter*) with germination percent of 28.96 % and 13.23%, respectively, whereas, minimum germination percentage of fresh and aged seed was observed in (9.30% and 4.82%) was observed in control. It is amply clear from the results that fresh and aged seeds treated with Sand + Vermicompost + PSB + *Azotobacter* + VAM had a significant effect on seed germination percentage. The results clearly demonstrate that the use of bio-inoculants in combination with organic manures significantly enhanced the germination percentage of both fresh and aged guava seeds compared to the control. The highest germination was recorded in treatment T15 (Sand + Vermicompost + PSB + *Azotobacter* + VAM), followed by T8 and T13, indicating a strong synergistic effect of multiple beneficial microbes with nutrient-rich organic substrates. Vermicompost and FYM improve soil aeration, moisture availability, and nutrient release, while PSB enhances phosphorus solubilization essential for early root development. *Azotobacter* contributes nitrogen fixation and phytohormone production (IAA, $GA_3$), promoting seed metabolic activity, whereas, VAM improves nutrient uptake and root–soil interactions. $GA_3$ acts as growth regulator for breaking seed dormancy, plays an important role in germination of seed by leaching out retardants and activates the cytological enzymes which stimulate α-amylase enzyme that converts insoluble sugar into soluble sugar Babu *et al.* (2010), Hartmann and Kester (1979). These combined effects accelerate enzymatic activation and radicle emergence, resulting in superior germination percentages. VAM increase the permeability of air and water through seed coat, Devi *et al.* (2019). These findings are in consent with Pathak *et al.* (2013) reported that germination of guava seeds was significantly influenced by bio-inoculants with manures having synergistic effect. Results are in accordance with the findings of Vasantha *et al.* (2014) in tamarind and Surakshitha and Kumar (2015) in jamun crop.

In contrast, the control showed the lowest germination due to the absence of organic nutrients and beneficial microbial activity, leading to poor seed vigor and delayed metabolic processes. Aged seeds consistently recorded lower germination than fresh seeds because of natural deterioration during storage, including membrane damage, reduced enzymatic activity, oxidative stress accumulation, and weakened embryo viability. Although bio-inoculants improved germination of aged seeds, their inherent loss of vigor limited overall performance compared to fresh seeds.

## 3.2 Number of Days Taken for Germination

Data pertaining to number of days taken for germination is presented in Table 2. Different bio-inoculants in combination with organic manures treatments had a significant effect on number of days taken for germination. Among the various treatments, seeds treated with T15 (Sand + Vermicompost + PSB + *Azotobacter* + VAM) took minimum number of days taken for germination (32.43 and 48.30) in fresh and aged seeds, respectively, while maximum number of days in fresh and aged seeds (43.00 and 59.06) were taken to initiate germination was recorded in the control.

Pre sowing seed treatments significantly decreased number of days required for seed germination in freshly harvested and aged guava as compared to control. Seeds treated with PSB, *Azotobacter* and sown in sand mixed with vermicompost in 1:1 ratio and VAM culture required minimum number of days to initiate germination (32.43 and 48.30 respectively). Maximum number of days (43.00 and 59.06 respectively) for initiation of germination was observed in control. In aged seeds, days taken for germination are relatively more due to fungal attack, irradiation, degradation of genetic material and accumulation of toxic compounds. Bio-inoculants repair the damaged proteins, genetic material. Time required for seed germination was reduced with bio-inoculants and organic manures application improve the physical properties and conserve nutrients against leaching cause improved early germination Devi *et al.* (2019) Similar results were observed by Vasantha *et al.* (2014) in guava. They observed that bio-fertilizers produce GA3 and enzymes which increase the permeability of seed coat and reduced days to initiation of germination as compared to control.

## 3.3 Vigour Indexes

- **Vigour index -I**

It is apparent from the data presented in Table 2 that Vigour index –I was significantly affected by the different bio-inoculants in combination with organic manures treatments. Maximum Vigour index –I of fresh and aged seed (295.10 and 269.8) was recorded in T15 (Sand + Vermicompost + PSB + Azotobacter + VAM) that was followed by (288.01 and 238.9) in T8 (Sand + FYM + PSB + Azotobacter + VAM) while minimum Vigour index –I of fresh and aged seed (51.06 and 29.9) was observed in control.

- **Vigour index –II**

The data pertaining in Table 2 that Vigour index-II of fresh and aged seed was significantly affected by the different bio-inoculants in combination with organic manures treatments. Vigour index –II of fresh and aged seed was observed maximum (0.728 and 0.168) with T15 (Sand + Vermicompost + PSB + Azotobacter + VAM) while minimum Vigour index-II (0.074 and 0.017) was observed in control.

Vigour indexes of fresh and aged seeds were influenced by germination percentage but Vigour index-I depends on the seedling length (cm) which is maximum (295.10 and 269.82 respectively) in Sand + Vermicompost + PSB + Azotobacter + VAM treatment because the application of bio fertilizers increase the availability and mobilization of nutrients and vermicompost provide the favourable environment to the plant which enhances the length of fresh seedling. In aged seeds, they consistently showed lower vigour values than fresh seeds because ageing causes deterioration of membranes, reduced enzymatic activity, and weakened metabolic function, limiting the ability of seedlings to grow vigorously even under improved treatment conditions. The superior vigour in T15 can be attributed to the synergistic effect of multiple beneficial microorganisms along with nutrient-rich vermicompost.

In contrast, the low vigour observed in the control is due to the absence of microbial stimulation and poor nutrient availability, leading to weak, slow-growing seedlings with minimal dry matter accumulation.

Vigour index –II of fresh and aged seeds was mainly affected by dry weight of seedlings which is maximum (0.728 and 0.168 respectively) in Sand + Vermicompost + PSB + Azotobacter + VAM. It was due to higher dry matter accumulation and photosynthates production in plants by increase in plant growth hormones that directly participates to improve it. Whereas the minimum values of fresh and aged seeded plants (0.074 and 0.017 respectively).

## 3.4 Shoot Length (cm)

The data related to shoot length (cm) has been provided in Table 3. The shoot length varied significantly under different bio-inoculants in combination with organic manures treatments at 60, 90 and 120 DAS. Maximum average shoot length of fresh and aged seed (13.66 and 6.30 cm) was recorded in the treatment $T_{15}$ (Sand + Vermicompost + PSB + *Azotobacter* + VAM), respectively. Minimum average shoot length of fresh and aged seed (8.74 and 3.43 cm) was recorded in control.

Maximum shoot length (5.51cm, 8.86 cm and 11.86 cm) in fresh seeds and (4.91cm, 6.33 cm and 7.65 cm) in aged seed was recorded at 60, 90 and 120 DAS, respectively, in Sand + Vermicompost + PSB + Azotobacter + VAM as compared to control. Improvement in shoot length could be attributed to easily availability of required quantity of nutrient and improved soil conditions due to organic manures addition and bio-inoculants releases different micro and macro nutrient at proper stage. This resulted in production of more quantum of carbohydrates and subsequently their translocation (Singh *et al.* (2014). These results are in conformity with the findings of Vasantha *et al.* (2014) in tamarind. Bio-inoculants such as Azotobacter produce growth-promoting hormones (IAA, $GA_3$ and cytokinins), which stimulate cell division and elongation in the shoot meristem. Vermicompost provides readily available nutrients and humic substances that improve chlorophyll content and metabolic activity, resulting in faster shoot elongation. PSB improves phosphorus availability, which supports ATP synthesis, promoting shoot growth. VAM enhances nutrient uptake and water absorption, further supporting shoot development. In aged seeds, shoot lengths is relatively less due to fungal attack, irradiation, degradation of genetic material and accumulation of toxic compounds. Bio-inoculants repair the damaged proteins, genetic material because they synthesize vitamins, amino acids, auxin, gibberellins and other plant growth regulators which increase the cell growth, elongation and number that produce health and long seedling (Pathak *et al.*, 2013).

### 3.5 Stem Girth (mm)

It is apparent from the data that different bio-inoculants in combination with organic manures significantly affected the girth of seedling at 60, 90 and 120 DAS (Table 4). Maximum average girth of seedling of fresh and aged seed (17.62 and 12.95 mm) was recorded in seeds, treated with $T_{15}$ (Sand + Vermicompost + PSB + *Azotobacter* + VAM), respectively, while minimum average seedling girth of fresh and aged seed (7.76 and 5.11 mm) was found in control.

Results of the investigation revealed that maximum girth of seedling (9.53 mm, 18.02 mm and 25.30 mm) in fresh seeds and (5.91 mm, 13.70 mm and 19.23 mm) in aged seeds was observed in Sand + Vermicompost + PSB + Azotobacter + VAM and minimum girth of seedlings was recorded (5.13 mm, 7.16 mm and 10.99 mm) in fresh seeds and (2.81 mm, 4.95 mm and 7.58 mm) in aged seeds under control treatment at 60 DAS, 90 DAS and 120 DAS, respectively.

A thicker stem girth in bio-inoculant-treated seedlings is due to better nutrient supply and enhanced root activity. Nitrogen fixation by Azotobacter promotes vigorous vegetative growth, while vermicompost increases microbial biomass and improves soil structure, supporting stronger vascular tissue development. Phosphorus solubilized by PSB supports lignification and cambial activity, resulting in thicker stems (Veermachaneni and Ramachandrudu, 2020; Barani and Anburani, 2004). The results of present study accordance with Ram and Pathak (2007). Bacteria produce enzymes that can hydrolyse cellulose glucoside bonds and cellobiose dimers into simpler molecules (sugars) to provide nutrition, Widyastuti *et al.* (2021). The results are also in accordance with the findings of Vasantha *et al.* (2014) in tamarind and Shukla *et al.* (2014) in guava.

In aged seeds, stem girth is relatively less due to reduced seed vigour, lower enzyme activity, accumulation of reactive oxygen species, impaired hormonal response. Bio-inoculants repair the damaged proteins, genetic material. Results are in conformity with findings of Sourabh *et al.* (2018) in guava.

### 3.6 Root Length (cm)

It is perusal from the data presented in Table 5 that the root length showed significant difference at 60, 90 and 120 DAS. Maximum average root length of fresh and aged seed (8.75 and 3.18 cm) was recorded with T15 (Sand + Vermicompost + PSB + Azotobacter + VAM), respectively. The minimum average root length of fresh and aged seed (5.65 and 1.70 cm) was recorded in control.

Maximum root length of seedling (6.40 cm, 9.55 cm, 10.31 cm) in fresh seeds and (2.36 cm, 3.52 cm and 3.66 cm) in aged seed was recorded at 60, 90 and 120 DAS respectively in Sand + Vermicompost + PSB + *Azotobacter* + VAM whereas, minimum values found in (3.22 cm, 6.10 cm and 7.54cm) in fresh seeds of guava and (0.80 cm, 1.83 cm and 2.26 cm) in aged seeds of guava compared to control. VAM plays a crucial role in promoting extensive root systems by increasing the absorptive surface area and improving uptake of phosphorus and micronutrients. Vermicompost and FYM improve soil porosity and moisture retention, creating a favourable environment for root elongation. Azotobacter and PSB stimulate root growth through hormone production and improved nutrient cycling, leading to longer and healthier roots. Improvement in root length could be attributed to easily availability of required quantity of nutrient and improved soil conditions due to organic manures addition and bio-inoculants releases different micro and macro nutrient at proper stage. This resulted in production of more quantum of carbohydrates and subsequently their translocation, Singh *et al.* (2014). These results are in conformity with the findings of Vasantha *et al.* (2014) in tamarind.

In aged seeds, root length is relatively less due to fungal attack, reduced vigour, degradation of genetic material and accumulation of ROS. Bio-inoculants repair the damaged proteins, genetic material. Bio-inoculants synthesize vitamins, aminoacids, auxin, giberellins and other plant growth regulators which increase the cell growth, elongation and number that produce health and long seedling, Pathak *et al.* (2013).

**Table 2. Effect of bio-inoculants in combination with organic manures on germination percentage, number of days taken for germination and vigour indexes of fresh and aged seeds of guava**

| Treatment detail | Germination (%) 60 DAS | | Days to taken for germination (days) | | Vigour index-I (60 DAS) | | Vigour index-II (60 DAS) | |
|---|---|---|---|---|---|---|---|---|
| | Fresh Seeds | Aged Seeds | Fresh Seeds | Aged Seeds | Fresh Seeds | Aged Seeds | Fresh Seeds | Aged Seeds |
| $T_1$ (Control) | 09.30 | 4.82 | 43.00 | 59.06 | 51.1 | 29.9 | 0.074 | 0.017 |
| $T_2$ (Sand + FYM) | 13.90 | 7.23 | 38.30 | 58.22 | 104.2 | 66.8 | 0.174 | 0.034 |
| $T_3$ (Sand + FYM + PSB) | 17.43 | 8.16 | 36.97 | 56.22 | 145.6 | 83.2 | 0.262 | 0.045 |
| $T_4$ (Sand + FYM + *Azotobacter* ) | 25.03 | 11.39 | 37.83 | 51.30 | 210.8 | 156.3 | 0.438 | 0.084 |
| $T_5$ (Sand + FYM + VAM) | 19.33 | 8.92 | 36.33 | 54.62 | 170.8 | 99.6 | 0.314 | 0.051 |
| $T_6$ (Sand + FYM + PSB+ *Azotobacter*) | 26.86 | 13.17 | 34.13 | 49.74 | 239.2 | 205.5 | 0.516 | 0.081 |
| $T_7$ (Sand + FYM + PSB + VAM) | 22.43 | 9.38 | 36.13 | 52.88 | 204.5 | 108.5 | 0.391 | 0.064 |
| $T_8$ (Sand + FYM + PSB+ *Azotobacter* + VAM) | 29.00 | 14.24 | 33.23 | 49.04 | 288.0 | 238.9 | 0.636 | 0.136 |
| $T_9$ (Sand + Vermicompost | 17.00 | 7.60 | 36.53 | 56.66 | 139.2 | 72.9 | 0.241 | 0.038 |
| $T_{10}$ (Sand + Vermicompost + PSB) | 18.33 | 8.23 | 35.10 | 55.80 | 154.5 | 84.4 | 0.283 | 0.046 |
| $T_{11}$ (Sand + Vermicompost + *Azotobacter*) | 26.60 | 12.82 | 34.90 | 50.72 | 224.4 | 194.2 | 0.491 | 0.089 |
| $T_{12}$ (Sand + Vermicompost + VAM) | 21.13 | 9.18 | 34.10 | 54.11 | 190.6 | 104.5 | 0.347 | 0.062 |
| $T_{13}$ (Sand + Vermicompost + PSB+ *Azotobacter*) | 28.96 | 13.23 | 33.33 | 49.69 | 262.3 | 207.1 | 0.594 | 0.114 |
| $T_{14}$ (Sand + Vermicompost + PSB + VAM) | 23.70 | 11.26 | 35.13 | 51.98 | 226.0 | 152.1 | 0.438 | 0.080 |
| $T_{15}$ (Sand + Vermicompost + PSB + *Azotobacter* + VAM) | **29.53** | **15.20** | **32.43** | **48.30** | **295.1** | **269.8** | **0.728** | **0.168** |
| **C.D. (p=0.05)** | 0.80 | 0.77 | 1.94 | 1.75 | 21.5 | 20.6 | 0.042 | 0.016 |

**Table 3. Effect of bio-inoculants in combination with organic manures on shoot length of fresh and aged seeds of guava**

| Treatment detail | Shoot Length (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Fresh seed | | | | Aged seed | | | |
| | **60 DAS** | **90 DAS** | **120 DAS** | **Pooled Mean** | **60 DAS** | **90 DAS** | **120 DAS** | **Pooled Mean** |
| $T_1$ (Control) | 5.51 | 8.86 | 11.86 | 8.74 | 2.36 | 3.33 | 4.6 | 3.43 |
| $T_2$ (Sand + FYM) | 7.49 | 10.13 | 12.51 | 10.04 | 3.34 | 3.845 | 5.02 | 4.07 |
| $T_3$ (Sand + FYM + PSB) | 8.36 | 10.71 | 13.09 | 10.72 | 3.74 | 4.357 | 5.44 | 4.51 |
| $T_4$ (Sand + FYM + *Azotobacter*) | 8.42 | 10.83 | 13.9 | 11.05 | 3.9 | 4.977 | 5.98 | 4.95 |
| $T_5$ (Sand + FYM + VAM) | 8.83 | 11.35 | 13.69 | 11.29 | 4.17 | 4.51 | 5.97 | 4.88 |
| $T_6$ (Sand + FYM + PSB+ *Azotobacter*) | 8.9 | 11.58 | 14.26 | 11.58 | 4.24 | 5.093 | 6.64 | 5.32 |
| $T_7$ (Sand + FYM + PSB + VAM) | 9.11 | 11.75 | 14.67 | 11.84 | 4.26 | 4.765 | 6.36 | 5.13 |
| $T_8$ (Sand + FYM + PSB+ *Azotobacter* + VAM) | 9.92 | 12.71 | 15.68 | 12.77 | 4.73 | 5.769 | 7.35 | 5.95 |
| $T_9$ (Sand + Vermicompost | 8.19 | 10.3 | 12.8 | 10.43 | 3.55 | 4.049 | 5.12 | 4.24 |
| $T_{10}$ (Sand + Vermicompost + PSB) | 8.43 | 10.93 | 13.58 | 10.98 | 3.74 | 4.443 | 5.72 | 4.63 |
| $T_{11}$ (Sand + Vermicompost + *Azotobacter*) | 8.43 | 10.96 | 14.06 | 11.15 | 3.92 | 5.044 | 6.08 | 5.01 |
| $T_{12}$ (Sand + Vermicompost + VAM) | 9.01 | 11.6 | 13.98 | 11.53 | 4.17 | 4.571 | 6.1 | 4.95 |
| $T_{13}$ (Sand + Vermicompost + PSB+ *Azotobacter*) | 9.06 | 11.8 | 14.71 | 11.86 | 4.3 | 5.117 | 6.66 | 5.36 |
| $T_{14}$ (Sand + Vermicompost + PSB + VAM) | 9.54 | 11.83 | 14.45 | 11.94 | 4.36 | 4.856 | 6.43 | 5.22 |
| $T_{15}$ (Sand + Vermicompost + PSB + *Azotobacter* + VAM) | **9.99** | **14.15** | **16.84** | **13.66** | 4.91 | 6.326 | 7.65 | **6.30** |
| **C.D. (p=0.05)** | 0.84 | 1.98 | 1.62 | | 0.38 | 0.62 | 0.82 | |

**Table 4. Effect of bio-inoculants in combination with organic manures on stem girth of fresh and aged seeds of guava**

| Treatment detail | Stem Girth (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Fresh seed | | | | Aged seed | | | |
| | 60 DAS | 90 DAS | 120 DAS | Pooled Mean | 60 DAS | 90 DAS | 120 DAS | Pooled Mean |
| $T_1$ (Control) | 5.13 | 7.16 | 10.99 | 7.76 | 2.81 | 4.95 | 7.58 | 5.11 |
| $T_2$ (Sand + FYM) | 8.15 | 10.61 | 16.46 | 11.74 | 4.65 | 7.43 | 11.53 | 7.87 |
| $T_3$ (Sand + FYM + PSB) | 8.37 | 11.8 | 17.68 | 12.62 | 4.77 | 8.38 | 12.55 | 8.57 |
| $T_4$ (Sand + FYM + *Azotobacter*) | 9.21 | 16.86 | 23.71 | 16.59 | 5.54 | 12.48 | 17.55 | 11.86 |
| $T_5$ (Sand + FYM + VAM) | 8.81 | 14.13 | 21.9 | 14.95 | 5.1 | 10.18 | 15.76 | 10.35 |
| $T_6$ (Sand + FYM + PSB+ *Azotobacter*) | 9.32 | 17.17 | 24.1 | 16.86 | 5.69 | 12.71 | 18.08 | 12.16 |
| $T_7$ (Sand + FYM + PSB + VAM) | 8.91 | 16.25 | 23.17 | 16.11 | 5.26 | 11.87 | 17.03 | 11.39 |
| $T_8$ (Sand + FYM + PSB+ *Azotobacter* + VAM) | 9.51 | 17.67 | 24.77 | 17.32 | 5.8 | 13.43 | 18.83 | 12.69 |
| $T_9$ (Sand + Vermicompost | 8.3 | 11.48 | 17.11 | 12.30 | 4.65 | 8.04 | 11.98 | 8.22 |
| $T_{10}$ (Sand + Vermicompost + PSB) | 8.62 | 12.87 | 18.31 | 13.27 | 4.92 | 9.14 | 13 | 9.02 |
| $T_{11}$ (Sand + Vermicompost + *Azotobacter*) | 9.24 | 17.04 | 24.05 | 16.78 | 5.54 | 12.61 | 17.79 | 11.98 |
| $T_{12}$ (Sand + Vermicompost + VAM) | 8.88 | 15.72 | 22.92 | 15.84 | 5.06 | 11.32 | 16.44 | 10.94 |
| $T_{13}$ (Sand + Vermicompost + PSB+ *Azotobacter*) | 9.33 | 17.58 | 24.69 | 17.20 | 5.69 | 13.19 | 18.52 | 12.47 |
| $T_{14}$ (Sand + Vermicompost + PSB + VAM) | 8.96 | 16.53 | 23.49 | 16.33 | 5.2 | 12.07 | 17.15 | 11.47 |
| $T_{15}$ (Sand + Vermicompost + PSB + *Azotobacter* + VAM) | **9.53** | **18.02** | **25.3** | 17.62 | 5.91 | 13.7 | 19.23 | 12.95 |
| **C.D. (p=0.05)** | 0.28 | 0.69 | 0.68 | | 0.17 | 0.49 | 0.53 | |

**Table 5. Effect of bio-inoculants in combination with organic manures on root length of fresh and aged seeds of guava**

| Treatment detail | Root Length (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Fresh seed | | | | Aged seed | | | |
| | **60 DAS** | **90 DAS** | **120 DAS** | **Pooled Mean** | **60 DAS** | **90 DAS** | **120 DAS** | **Pooled Mean** |
| $T_1$ (Control) | 3.32 | 6.1 | 7.54 | 5.65 | 1 | 1.83 | 2.26 | 1.70 |
| $T_2$ (Sand + FYM) | 4.34 | 6.57 | 7.77 | 6.23 | 1.35 | 1.98 | 2.41 | 1.91 |
| $T_3$ (Sand + FYM + PSB) | 5.2 | 7.15 | 8.23 | 6.86 | 1.72 | 2.36 | 2.72 | 2.27 |
| $T_4$ (Sand + FYM + *Azotobacter*) | 5.06 | 6.93 | 8.2 | 6.73 | 1.61 | 2.22 | 2.61 | 2.15 |
| $T_5$ (Sand + FYM + VAM) | 5.59 | 7.75 | 8.36 | 7.23 | 1.96 | 2.71 | 2.93 | 2.53 |
| $T_6$ (Sand + FYM + PSB+ *Azotobacter*) | 5.43 | 7.58 | 8.32 | 7.11 | 1.85 | 2.58 | 2.83 | 2.42 |
| $T_7$ (Sand + FYM + PSB + VAM) | 5.84 | 8.05 | 8.67 | 7.52 | 2.07 | 2.9 | 3.12 | 2.70 |
| $T_8$ (Sand + FYM + PSB+ *Azotobacter* + VAM) | 6.38 | 8.3 | 9.24 | 7.97 | 2.24 | 3.07 | 3.42 | 2.91 |
| $T_9$ (Sand + Vermicompost | 5.04 | 6.73 | 8 | 6.59 | 1.56 | 2.09 | 2.48 | 2.04 |
| $T_{10}$ (Sand + Vermicompost + PSB) | 5.22 | 7.37 | 8.27 | 6.95 | 1.72 | 2.43 | 2.73 | 2.29 |
| $T_{11}$ (Sand + Vermicompost + *Azotobacter*) | 5.06 | 7.03 | 8.21 | 6.77 | 1.62 | 2.25 | 2.63 | 2.17 |
| $T_{12}$ (Sand + Vermicompost + VAM) | 5.75 | 7.98 | 8.54 | 7.42 | 2.01 | 2.79 | 2.99 | 2.60 |
| $T_{13}$ (Sand + Vermicompost + PSB+ *Azotobacter*) | 5.58 | 7.65 | 8.33 | 7.19 | 1.9 | 2.6 | 2.84 | 2.45 |
| $T_{14}$ (Sand + Vermicompost + PSB + VAM) | 6.23 | 8.08 | 8.81 | 7.71 | 2.1 | 2.9 | 3.17 | 2.72 |
| $T_{15}$ (Sand + Vermicompost + PSB + *Azotobacter* + VAM) | **6.4** | **9.55** | **10.31** | 8.75 | **2.36** | **3.52** | **3.66** | 3.18 |
| **C.D. (p=0.05)** | 0.6 | 1.63 | 1.26 | | 0.2 | 0.55 | 0.45 | |

## 4. CONCLUSION

The present investigation clearly demonstrates that the integrated application of bio-inoculants in combination with organic manures markedly enhances germination behaviour and early seedling growth of fresh as well as aged guava seeds. Among all the treatments, T15 (Sand + Vermicompost + PSB + Azotobacter + VAM) consistently performed best across all parameters. Fresh and aged seeds treated with this combination recorded the highest germination percentage, vigour index-I, vigour index-II, shoot length, root length and seedling girth, while the untreated control showed the least values in each parameter.

The beneficial effects of T15 may be attributed to the synergistic action of bio-inoculants that produce growth-promoting substances such as $GA_3$, auxins, cytokinins, improve nutrient mobilization, enhance soil physical properties and support repair of degraded proteins and genetic material, especially in aged seeds. Vermicompost further contributes by enriching the nutrient environment and improving aeration and moisture conditions around the seeds. As a result, seeds germinated earlier, produced longer shoots and roots, accumulated more dry matter, and ultimately expressed stronger seedling vigour.

Aged seeds generally performed poorer than fresh seeds due to deterioration factors such as fungal attack, reduced seed vigour, lower enzyme activity, accumulation of reactive oxygen species, impaired hormonal response. However, even in aged seeds, the T15 treatment substantially improved germination and seedling characteristics compared to control, highlighting the restorative potential of bio-inoculants.

This integrated approach may be recommended for raising healthy nursery plants and improving establishment success in guava cultivation.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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