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Effect of Microbial Consortiums on Germination and Initial Growth Attributes of Mangosteen

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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

An experiment was conducted to study the influence of microbial consortiums / biofertilizers on germination and initial growth attributes of mangosteen grown in the College Orchard, Department of Fruit Science, College of Agriculture, Kerala Agricutural University, Vellanikkara, Thrissur, Kerala, India during 2023. The research work was conducted under rain shelter with five treatments, which include microbial consortiums / biofertilizers such as PGPR MIX-1 (T_1), *Piriformospora indica* (T_2), PGPR MIX-1 + *Piriformospora indica* (T_3), Arka Microbial Consortia (AMC) (T_4) and control (T_5). The various parameters of germination and initial growth attributes of mangosteen seedlings were recorded *viz*., time taken for germination, germination percentage, height of seedlings, girth of seedlings, number of leaves, length of leaves, breadth of leaves, length of roots, girth of roots, chlorophyll content, carotenoid content, seedling fresh weight and dry weight. Among the treatments, PGPR MIX-1 + *Piriformospora indica* (T_3) recorded significantly higher values for majority of the parameters under study over control (T_5), and *Piriformospora indica* (T_2), but it (T_3) was found to be on par with Arka Microbial Consortia (AMC) (T_4) and PGPR MIX-1 (T_1). The recorded values were lowest under control (T_5). The seeds of mangosteen treated with PGPR MIX-1 + *Piriformospora indica* or Arka Microbial Consortia alone or PGPR MIX-1 alone @ 100 g per kg of seeds were found to be the best in overall performance of the seedlings for various growth parameters under study.

Keywords: Mangosteen; microbial consortium; biofertilizer; PGPR MIX-1; Piriformospora indica; Arka microbial consortia.

1. INTRODUCTION

The mangosteen (*Garcinia mangostana* L.) is a tropical fruit native to Southeast Asia, known for its exquisite taste and remarkable health benefits. Often hailed as "queen of fruits," mangosteen has captured the attention of fruit enthusiasts worldwide. Here's a comprehensive overview of mangosteen fruit, its characteristics, nutritional profile, health benefits, and culinary uses. Mangosteen is not only a culinary delight, but also a nutritional powerhouse. Despite its relatively low-calorie content, fruit is rich in essential nutrients, vitamins, and antioxidants. A typical serving of mangosteen (100 g) provides calories: 76, carbohydrates: 18.4 g, fibre: 1.7 g, calcium: 9 mg, phosphorus: 14 mg, iron: 0.5 mg, copper: 0.11 g, Vitamin C: 2 mg, Vitamin B1: 0.09 mg and Vitamin B2: 0.06 mg [1]. Some potential health benefits of mangosteen are the presence of antioxidants, immune support system, anti-inflammatory properties, and improvement of skin health. Mangosteen is rich in xanthones, which are potent antioxidants that help to combat oxidative stress and inflammation in the body, thereby reducing the risk of chronic diseases such as heart disease and cancer. Immunity supported by vitamin C content of mangosteen helps to boost immune function, supporting body's defensive mechanism against infections and illnesses. Anti-inflammatory property compounds found in mangosteen have found to help to alleviate symptoms of inflammatory conditions such as arthritis and inflammatory bowel disease.

Mangosteen propagation remains primarily reliant on seed propagation, which offers most reliable method for cultivating this prized tropical fruit tree. Successful propagation depends on the use of good quality seeds, environmental conditions, and cultural practices. Though, grafting methods were explored, they are not commonly used due to their lower success rates. By understanding the characteristics of mangosteen seeds and providing optimal germination conditions, growers can successfully use mangosteen seedlings for orchard establishment or home cultivation. Despite its challenges, seed propagation remains the primary method for expanding mangosteen cultivation and ensuring the availability of this exquisite fruit to consumers worldwide. Continued research into seed handling innovative techniques can improve the success rates of seed germination of mangosteen and also further growth and development of seedlings which will result in ensuring the sustainable production.

As mangosteen is handicapped with low percentage of germination and slow growth of seedlings it hinders propagation and overall production of this fruit crop. Hence the use of microbial consortiums/biofertilizers containing microbes, which can mobilise nutrients through

biological processes from an unusable form to a useful form is found to be beneficial [2]. One of the most significant scientific developments in the coming ten years will be improving our understanding and management of rhizosphere processes so as to address the global concerns of climate change and population expansion [3]. Gaining further knowledge about these biofertilizers has become essential to preserve plant health, provide food for soil-dwelling creatures, extend soil productivity, and preserving environmental biodiversity [4]. By inoculating Azotobacter, nitrogenous fertiliser input can be reduced by 10-20% [5]. To increase soil organic carbon and also to preserve sustainability biofertilizers should be combined with chemical fertilisers and organic manures [6].

An *Azospirillum* sp. may fix up to 20-40 kg N ha^{-1}, and so when inoculated with it, yield will definitely increase and the species *Azospirillum lipoferum*is found to be capable of producing gibberellic acid (GA_3), which favourst he growth and development of crop plants [2]. Seed treatment with microbial consortiums in mangosteen could address the above said problems, and in this context research work on effect of microbial consortiums on germination and initial seedling growth attributes of mangosteen was taken up in the College Orchard, Department of Fruit Science, College of Agriculture, Vellanikkara, Thrissur.

2. MATERIALS AND METHODS

Research work was conducted during 2023 under rain shelter located at college orchard, Department of Fruit Science, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India with a longitude of 76.282351ºand latitude 10.55046º. The experiment was conducted in mangosteen seeds using Completely Randomized Design (CRD) with five treatments *viz*., T_1- PGPR MIX-1, T_2- *Piriformospora indica*, T3- PGPR MIX-1 + *Piriformospora indica,* T_4 - AMC- Arka Microbial Consortia and T_5- Control with four replications. The plumpy seeds of mangosteen were extracted and collected from fully ripened purple coloured fruits by removing snow-white pulp and seed meat, then dried under shade for two days. Further, seeds were treated with microbial consortiums PGPR mix-1 and *Piriformospora indica* received from department of Agricultural Microbiology, College of Agriculture, Vellayani. The PGPR mix-I contains *Azospirillumlipoferum*, *Azotobacter chroococcum*, *Bacillus megaterium* and *Bacillus sporothermodurans*. Whereas, Arka Microbial Consortia (AMC) is a mixture of *Azotobacter tropicalis* strain PAN MC1, *Bacillus aryabhattai* strain Bel 6 and *Pseudomonas taiwanensis* Mpf2 [7] from Indian Institute of Horticulture Research (IIHR), Hesaraghatta, Bangalore. The seeds were treated with these microbial consortiums at the rate 100 g per kg of seeds *i.e,.* 10 g per 100 g of mangosteen seeds.

In each replication, forty seeds were sown in poly bags of size 6x9". Observations were recorded during germination and seedling growth stage at 15 days intervals up to 45 days. Time taken for germination (days), germination percentage (%), seedling height at 15, 30 & 45days (cm). girth of seedling at 15, 30 & 45 days (mm), number of leaves at 15, 30 & 45 days, length of leaves at 15, 30 & 45 days (cm) and breadth of leaves at 15, 30 & 45 days (cm) were recorded. By destructive sampling method at the end of experiment *i.e*., after 45 days, various parameters recorded were chlorophyll-a (mg g^{-1}), chlorophyll-b (mg g^{-1}), total chlorophyll (mg g^{-1}), carotenoid (mg g^{-1}), length of taproot (cm), girth of taproot (mm) (0.5 cm below collar region), no. of secondary roots on taproot, seedling fresh weight and seedling dry weight. The time taken for germination was counted as number of days taken from first day of sowing to initiation of germination of seed and germination percentage was calculated by number of seeds germinated divided by total number of seeds sown multiplied by hundred. Whereas, chlorophyll-a, chlorophyll-b, and carotenoid were estimated by extraction using 80% acetone then centrifuged at 3000 rpm for 10 minutes followed by measuring the optical density (OD) values at 480, 510, 645 and 663nm by spectrophotometer. The calculation of chlorophyll-a, chlorophyll-b, total chlorophyll, ratio of chlorophyll-a/b and carotenoids were done using following formulae.

$$Chlorophyll - a = (12.7 \times OD\ at\ 663) - (2.69 \times OD\ at\ 645) \times \frac{v}{1000 \times xw}$$

$$Chlorophyll - b = (22.9 \times OD\ at\ 645) - (4.68 \times OD\ at\ 663) = \frac{V}{1000 \times W}$$

$$Total\ chlorophyll = (8.02 \times OD\ at\ 663) - (20.2 \times OD\ at\ 645) \times \frac{V}{1000 \times W}$$

$$Carotenoids = (7.6 \times OD\ at\ 480) - (1.49 \times OD\ at\ 510) \times \frac{V}{1000 \times W}$$

$$Chlorophyll-\ a\ and\ b\ ratio\ = \frac{Chlorophyll-a}{Chlorophyll-b}$$

Girth of seedlings was recorded at 15 days interval, using digital vernier caliper, which was also used for measuring the girth of taproot at the end of 45 days. The data of various parameters collected at regular intervals during seedling stage were compiled, and statistically analysed by using one factor CRD design of OPSTAT.

3. RESULTS AND DISCUSSION

The induction of early germination and fastening initial growth attributes of mangosteen seedlings are very challenging for the farming community engaged in mangosteen cultivation. As there existed a possibility of managing this by the use of microbial consortium / biofertilizers to a greater extent, this possibility was tried in the abovementioned experiment on mangosteen, wherein various microbial consortiums and their combinations were tested. These consortiums includePGPR MIX-1, *Piriformospora indica*, PGPR MIX-1 + *Piriformospora indica*, AMC-Arka Microbial consortia, and control (seeds that were not subjected to any seed treatment).

When the first set of observations like time taken for germination and germination percentage were recorded during germination period (Table 1), no significant difference could be observed among the treatments.

Height of seedlings was not found to be influenced by any of the treatments under study at 15th day after germination. At 30th and 45th day, seeds treated with PGPR MIX-1 + *Piriformospora indica* (T_3) recorded significantly higher of height of mangosteen seedlings over control (T_5) and *Piriformospora indica* (T_2), and was found to be on par with Arka Microbial Consortia (T_4) and PGPR MIX-1 (T_1). The control treatment recorded the least value for seedling height throughout the period of study. Girth of mangosteen seedlings did not show any significant difference throughout study period. However, seeds treated with a combination of PGPR MIX-1 + *Piriformospora indica* recorded higher values and lower values were noted in control at 15th day after germination and the same trend was observed at 30th and 45th day (Table 2).

Table 1. Effect of microbial consortiums / biofertilizers on germination attributes of mangosteen

Treatments	Time taken for germination (days)	Germination percentage (%)
T_1-PGPR MIX-1	24.11	87.50
T_2-*Piriformospora indica*	26.63	82.50
T_3-PGPR MIX-1 + *Piriformospora indica*	24.16	90.00
T_4-AMC- Arka Microbial Consortia	23.99	90.00
T_5-Control	27.11	75.00
SE(m)±	0.92	11.57
CD (p=0.05)	NA	NA

Table 2. Influence of microbial consortiums / biofertilizers on height and girth of mangosteen seedlings

Treatments	Height of seedlings (cm)			Girth of seedlings (mm)		
	15 days	30 days	45 days	15 days	30 days	45 days
T_1-PGPR MIX-1	4.66	5.01	5.76	2.23	2.53	2.89
T_2-*Piriformospora indica*	4.11	4.51	5.08	2.14	2.50	2.61
T_3-PGPR MIX-1 + *Piriformospora indica*	4.81	5.64	6.34	2.32	2.64	2.99
T_4-AMC- Arka Microbial Consortia	4.45	5.09	5.80	2.28	2.55	2.86
T_5-Control	3.63	3.91	4.63	2.08	2.31	2.44
SE(m)±	0.40	0.32	0.25	0.14	0.10	0.14
CD (p=0.05)	NA	0.97	0.77	NA	NA	NA

Table 3. Effect of microbial consortium / biofertilizers on number of leaves, length of leaves and breadth of leaves of mangosteen

Treatments	Number of leaves			Length of leaves (cm)			Breadth of leaves (cm)		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
T_1-PGPR MIX-1	2.22	2.82	3.32	2.93	3.91	5.04	1.91	2.43	2.57
T_2-*Piriformospora indica*	2.00	2.38	3.06	2.87	3.65	4.49	1.78	2.23	2.37
T_3-PGPR MIX-1 + *Piriformospora indica*	2.25	3.13	3.71	3.21	4.07	5.62	2.01	2.63	2.79
T_4-AMC- Arka Microbial Consortia	2.33	2.87	3.49	3.35	4.02	5.16	1.96	2.41	2.56
T_5-Control	2.00	2.13	2.62	2.68	3.08	4.18	1.58	2.08	2.16
SE(m)±	0.12	0.18	0.18	0.20	0.21	0.32	0.08	0.10	0.13
CD (p=0.05)	NA	0.55	0.56	NA	0.65	0.98	0.24	0.31	0.38

Table 4. Effect of biofertilizers on root growth and dry weight of mangosteen seedlings

Treatments	Length of taproot at 45th day (cm)	Girth of taproot at 45th day (mm) (0.5 cm below collar region)	No. of secondary roots on taproot at 45th day	Seedling fresh weight at 45th day (g)	Seedling dry weight at 45th day (mg)
T_1-PGPR MIX-1	5.53	1.65	8.50	2.17	422.50
T_2-*Piriformospora indica*	5.03	1.42	7.25	1.83	400.50
T_3-PGPR MIX-1 + *Piriformospora indica*	6.19	1.90	12.00	3.33	644.75
T_4-AMC- Arka Microbial Consortia	5.76	1.81	10.50	2.74	546.00
T_5-Control	4.10	1.32	5.75	1.62	357.50
SE(m)±	0.18	0.05	0.77	0.25	40.50
CD (p=0.05)	0.55	0.14	2.34	0.75	123.18

Table 5. Effect of biofertilizers on biochemical parameters of mangosteen leaf at 45th day

Treatments	Chlorophyll-a (mg g^{-1})	Chlorophyll-b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Ratio of chlorophyll-a/b	Carotenoid (mg g^{-1})
T_1-PGPR MIX-1	0.67	0.65	1.32	1.03	0.23
T_2-*Piriformospora indica*	0.57	0.51	1.08	1.13	0.23
T_3-PGPR MIX-1 + *Piriformospora indica*	0.75	0.71	1.46	1.05	0.25
T_4-AMC- Arka Microbial Consortia	0.71	0.71	1.42	1.01	0.24
T_5-Control	0.53	0.52	1.05	1.02	0.22
SE(m)±	0.02	0.01	0.03	0.04	0.01
CD (p=0.05)	0.07	0.04	0.09	NA	0.02

The maximum number of leaves of mangosteen seedling were significantly influenced by the treatments and values recorded were 3.13 and 3.71 at 30 and 45 days respectively in PGPR MIX-1 + *Piriformospora indica* (T_3) over control (T_5) and *Piriformospora indica* (T_2). The T_3 (PGPR MIX-1 + *Piriformospora indica*) was found to be on par with Arka Microbial Consortia (T_4) and PGPR MIX-1 (T_1) at 30th day and 45th day. No significant difference could be observed with regard to number of leaves among the treatments at 15th day of seedlings growth. Nevertheless, when higher number of leaves were recorded in T_3 (*Piriformospora indica*) and the least number of leaves were found in T_5 (control) (Table 3). With regard to length of leaves a similar trend was observed at 15th, 30th and 45th day as in the case of number of leaves, as no notable variations was noted among the treatments. During 30th day of seed germination, the treatments PGPR MIX-1 + *Piriformospora indica* (T_3), Arka Microbial Consortia (T_4) and PGPR MIX-1 (T_1) recorded significant difference with regard to length of leaves with values of 4.07 cm, 4.02 cm, and 3.91 cm respectively. While 45th day of seed germination also the treatments recorded significantly higher values for length of leaves in PGPR MIX-1 + *Piriformospora indica* (T_3) (5.62 cm), Arka Microbial Consortia (T_4) (5.16 cm) and PGPR MIX-1 (T_1) (5.04 cm). The minimum length of leaves was recorded *i.e.,* 4.18 cm in control (T_5) and 4.49 cm in *Piriformospora indica* (T_2) (Table 3).

The influence of microbial consortium / biofertilizers on breadth of leaves of mangosteen at 15 days interval is presented in Table 3. Statistically remarkable difference with regard to breadth of leaves was observed under T_3 (PGPR MIX-1 + *Piriformospora indica*),T_1(PGPR MIX-1) and T_4(Arka Microbial Consortia) over T_5 (control) and T_2 (*Piriformospora indica*)during 15th, 30th and 45th day. The control, T_5 recorded the least value for breath of leaves among the treatments, which were recorded at in 15 days interval up to 45 days.

The length of taproot at 45thday of germination varied significantly among the treatments. The treatment T_3 (PGPR MIX-1 + *Piriformospora indica*) recorded the longest taproot 6.19 cm, which was on par with T_4 (Arka Microbial Consortia) (5.76 cm). Treatments T_1 (PGPR MIX-1), T_2 (*Piriformospora indica*), and T_5 (control) recorded remarkably lower values, when compared to T_3. However, the shortest taproot was recorded in T_5 *i.e.,* control (4.10 cm). At 45th day, treatment, T_3 and T_4 were found to be significantly superior with regard to girth of taproot and values recorded were 1.90 mm and1.81 mm respectively. The control (T_5) recorded remarkably lower value. The treatments T_2 (1.42 mm) and T_1 (1.65 mm) recorded similar trend as observed for length of taproot. Number of secondary roots on taproot notably differed among the treatments, however T_3 (12) and T_4 (10.5) were found to be significantly superior over other treatments and were statistically on par. The lowest number was control (5.75) (Table 3).

Seedling fresh weight was found to be significantly higher in treatments T_3 (3.33 g) and T_4 (2.74 g) than in T_1 (2.17 g), T_2 (1.83 g), and T_5 (1.62 g). However, T_3 (PGPR MIX-1 + *Piriformospora indica*) and T_4 (Arka Microbial Consortia) remained statistically on par with each other, and the control recorded the lowest value for fresh weight of seedlings (Table 4). Seedling dry weight varied from 357.50 mg to 644.75 mg and the treatments exhibited significant difference. The treatment T_3 (644.75 mg) and T_4 (546.00 mg) were found to be on par with each other with significantly superior values. The control (T_5) was recorded significantly minimum value of dry weight of seedlings (357.50 mg) which was statistically on par with T_1 and T_2.

Thebiochemical parameters of mangosteen leaves such as chlorophyll-a, chlorophyll-b and carotenoidwere also estimated after 45th day and is represented in Table 5. The level of chlorophyll-a was found to be significantly higher in seeds treated with PGPR MIX-1 + *Piriformospora indica* (T_3) and Arka Microbial Consortia (T_4) with values of 0.75 mg g^{-1} and 0.71 mg g^{-1} respectively. Other treatments scored 0.67 mg g^{-1}, 0. 57 mg g^{-1} and 0.53 mg g^{-1} in T_1, T_2, and T_5 respectively. All the treatments recorded similar trends for chlorophyll-b, total chlorophyll and carotenoid as recorded in chlorophyll-a. Significantly higher chlorophyll-b amount of was recorded in T_3 (0.71 mg g^{-1}) and T_4 (0.71 mg g^{-1}), when compared to T_3 and T_4 remarkably lower amount of chlorophyll-b was analysed in T_1 (0.65 mg g^{-1}), T_2 (0.51 mg g^{-1}), and T_5 (0.52 mg g^{-1}). However, PGPR MIX-1 (T_1) was found to be significantly inferior to T_3 and T_4, but significantly superior over T_2(*Piriformospora indica*) and T_5 (control). Total chlorophyll content was notably significant in treatments T_3 andT_4, with values 1.46 mg g^{-1} and 1.42 mg g^{-1}

respectively. The least value of total chlorophyll was observed in T_5 (1.05 mg g^{-1}), which was found to be on par with T_2 (1.08 mg g^{-1}). No considerable variation was noticed among treatments with respect to ratio of chlorophyll-a/b. Carotenoid content of leaves was found to be statistically significant in T_3 (0.25 mg g^{-1}) and T_4 (0.24 mg g^{-1}) and superior over other treatments. No remarkable variation could be observed among the treatments like T_1 (0.23 mg g^{-1}), T_2 (0.23 mg g^{-1}), and T_5 (0.22 mg g^{-1}).

The aforementioned outcomes were consistent with conclusion of Barman et al. [7] in jamun, Sindhu et al. [8] in horticultural crops, Pathak et.al. [9] in guava, Abdelaal et al. [10] in Washington navel orange, Ramakrishnan and Selvakumar [11] in tomato, Singh and Banik, [12] in mango cv. Himsagar and Umar *et* al. (2009) in strawberry cv. Chandler.

4. CONCLUSION

From the experiment, it was observed that significantly no difference existed among the treatments with regard to initial time taken for germination, germination percentage and seedling growth attributes at 15thday of seed germination. But as the experiment progressed significant difference could be observed among the treatments during 30thand 45thday.Seed treatment of mangosteen with PGPR MIX-1 + *Piriformospora indica* (T_3) recoded numerically higher values for majority of the parameters under study and was on par with Arka Microbial Consortia (T_4).For some parameters T_3 and T_4 were found to be on par with PGPR MIX-1 (T_1). The performance of mangosteen seedlings grown from the seeds treated with different microbial consortiums werefound to be greatly influenced by there microbial consortiums of which the combination of PGPR MIX-1 + *Piriformospora indica* (T_3), Arka Microbial Consortia (T_4), and PGPR MIX-1 (T_1) were found have significantly positive influence on the growth of seedlings of mangosteen. From the results, it can be concluded that based on the availability of these microbial consortiums in their area, farming community can opt for PGPR MIX-1 + *Piriformospora indica* or Arka Microbial Consortia or PGPR MIX-1 they can use this at the rate of 100 g per kg of seeds for treating seeds for speeding up the growth of mangosteen seedlings.

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 manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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