

## **Short Research Article**

**Native liquid biofertilizer consortia of *Staphylococcus equorum*,  
*Bacillus velezensis* and *Staphylococcus epidermidis* enhanced the  
growth of nutmeg seedlings**

---

### **ABSTRACT**

**Aims:** The aim was to evaluate the three most distinct native liquid biofertilizer consortia on the growth of nutmeg seedlings under pot culture studies.

**Study design:** Completely Randomized Block Design

**Place and Duration of Study:** College of Agriculture, Vellanikkara, Thrissur, Kerala and Regional Agricultural Research Station, Ambalavayal, Wayanad, Kerala. The pot culture experiment was conducted from February 2023 to August 2023.

**Methodology:** The experiment was carried out in a completely randomized block design with three replications along with control. Each treatment was applied with a combination of NPK fertilizers (50% and 75% of recommended dose of fertilizer, based on soil test data).

**Results:** Application of *Staphylococcus equorum* + *Bacillus velezensis* + *Staphylococcus epidermidis* (microbial consortium – 2) along with 50% of the recommended dose of fertilizer, recorded the highest growth parameters. The biofertilizer inoculation had a positive impact on the population of soil microbes also.

**Conclusion:** The present study indicated that inoculation of native liquid biofertilizer consortia enhanced the growth of nutmeg seedlings under pot culture studies. Thus liquid biofertilizer consortia can be utilized for earlier establishment of nutmeg seedlings and thereby reduce the quantity of chemical fertilizers.

*Keywords:* nutmeg, plant growth promotion, liquid biofertilizer, consortium

## 1. INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt.), a native of Maluku islands (Indonesia), is a major export spice crop from India. Kerala, Karnataka, Maharashtra, and Tamil Nadu are the contributors to nutmeg production in India, with Kerala being the major producer. The perennial nutmeg spice tree is grown for: the aril and the kernel. Nutmeg possesses a strong aroma with a mildly sweet flavor. Nutmeg butter and essential oils are significant derivatives of the spice. These spices find application in enhancing food flavors and utilized in traditional medicinal practices. Nutmeg generally requires a large quantity of major nutrients (1), leading to soil health deterioration. Therefore, organic nutmeg cultivation needs to be encouraged, as it is a directly consumed spice product. As an alternative to chemical fertilizers, biofertilizers are widely used to reduce the quantity of chemical fertilizers in many crops like paddy, vegetables, spices etc (2).

Microorganisms help the host plants to receive an appropriate amount of nutrients, support healthy plant development (3), and maintain good physiological balance without any harmful effect on the environment. Biofertilizers are made up of efficient living microorganisms which help in root microbe partnership leading to plant growth and development. Soil sustainability can be enhanced by isolating microbial strains with increased plant growth-promoting characteristics from diverse agroecological zones and subsequently introducing them for commercial use (4). In terms of biofertilizers, liquid variants surpass carrier-based ones, exhibiting a greater number of viable microorganisms over an extended shelf life (5). In a solid state, the shelf life of *Azotobacter*, potassium mobilizing bacteria, and *Rhizobium* is around 6 months, while phosphorus solubilizing microorganisms can endure for up to 8 months. Conversely, the liquid formulations of *Azotobacter*, *Azospirillum*, phosphorus solubilizing microorganisms, and potassium mobilizing bacteria can last up to 2 years, with *Rhizobium* maintaining viability for a period of 14 months. Carrier based inoculants have some pitfalls like poor quality, higher contamination, clump formation upon drying leading to significant loss of viability and unpredictable field performance (6). Liquid biofertilizers also offer superior absorption and more convenient applications compared to the carrier-based inoculants. By plant and soil relationship, these beneficial bacteria boost nutrient accessibility, hinder detrimental organisms, and instigate various physiological processes in plants. Liquid *Rhizobia* with 2% polyvinylpyrrolidone, were observed to sustain a higher colony forming unit (cfu) count and proved highly successful in boosting soybean yield, in regions such as the United States (specifically Hawaii under NifTal), Brazil, Europe (including Spain and Argentina), and Asia (including China and Vietnam) (7).

Soil application of *Azospirillum* and *Azotobacter* were found beneficial for nutmeg seedlings (8). The effect of *Bacillus* sp. consortia on nutmeg

seedlings were also found beneficial (9). Similar results (10) were obtained for nutmeg seedlings, where inoculated nitrogen fixing bacteria and phosphorus solubilizing microbes enhanced growth of nutmeg seedlings. There are very few research work on the inoculation of nutmeg seedlings with liquid biofertilizers consortia, particularly in Kerala. At present, there are no liquid biofertilizer consortia for nutmeg in Kerala. Hence, a study was undertaken with a major objective of evaluating the three most promising liquid biofertilizer consortia for growth enhancement in nutmeg seedlings under pot culture studies.

## 2. MATERIAL AND METHODS

Nutmeg rhizosphere soil samples were collected from Wayanad district in Kerala and nitrogen fixers, phosphorus solubilizers, and potassium solubilizers were isolated using standard protocols. These bacterial strains were assessed quantitatively and qualitatively under *in vitro* for growth promoting attributes such as nitrogen fixation (11), phosphorus solubilization (12), potassium solubilization (13) and indole acetic acid (IAA) production (14). The nine most promising isolates with abiotic stress (moisture and temperature) tolerance were selected for pot culture studies. The liquid biofertilizer consortia were prepared in nutrient broth supplemented with 15mM trehalose and 2.5% polyvinylpyrrolidone (15).

The pot culture experiment was conducted using 6-month-old nutmeg seedlings collected from Model nursery, Department of Plantation and Spices crops, College of Agriculture, Vellanikkara, Thrissur. The experiment was conducted at Block 10, Regional Agricultural Research Station, Ambalavayal, Wayanad district, Kerala (GPS coordinates -11.615517°N latitude, 76.2133285°E longitude). The nutmeg seedlings were replanted in black polybags of 15 x 40 cm (width x height) with potting mixture of soil, sand, and farmyard manure in the ratio of 3:3:1 (16). The nutmeg seedlings were maintained under 50% shaded condition. Initial soil physicochemical properties such as soil pH, electrical conductivity, organic carbon, total nitrogen, available phosphorus and available potassium along with bacterial populations such as nitrogen fixers, phosphorus solubilizers and potassium solubilizers were recorded using standard protocols. The experiment was conducted in Completely Randomized Design with 12 treatments and replicated 3 times. Plot size was of 3 plants. The treatment details were as follows:

- T<sub>1</sub>: Microbial consortium - 1 (*Staphylococcus equorum* VTN2 + *Staphylococcus pasteurii* PPP + *Pseudomonas veroni* MHK) with 50% RDF of N, P, K
- T<sub>2</sub>: Microbial consortium - 2 (*Staphylococcus equorum* KPN3 + *Bacillus velezensis* KKP1 + *Staphylococcus epidermidis* NMK2) with 50% RDF of N, P, K

- T<sub>3</sub>: Microbial consortium - 3 (*Enterobacter* sp. KPN1 + *Pseudomonas aeruginosa* MKP2 + *Sphingobacterium anhuiense* MPK) with 50% RDF of N, P, K
- T<sub>4</sub>: PGPR Mix-1 (KAU) with 50% RDF of N, P, K
- T<sub>5</sub>: Microbial consortium - 1 (*Staphylococcus equorum* VTN2 + *Staphylococcus pasteurii* PPP + *Pseudomonas veroni* MHK) with 75% RDF of N, P, K
- T<sub>6</sub>: Microbial consortium - 2 (*Staphylococcus equorum* KPN3 + *Bacillus velezensis* KKP1 + *Staphylococcus epidermidis* NMK2) with 75% RDF of N, P, K
- T<sub>7</sub>: Microbial consortium - 3 (*Enterobacter* sp. KPN1 + *Pseudomonas aeruginosa* MKP2 + *Sphingobacterium anhuiense* MPK) with 75% RDF of N, P, K
- T<sub>8</sub>: PGPR Mix-1 (KAU) with 75% RDF of N, P, K
- T<sub>9</sub>: KAU Package of practices for organic farming crops (17)
- T<sub>10</sub>: RDF (Recommended dosage of fertilizer, based on soil test data)
- T<sub>11</sub>: KAU Package of practices (1)
- T<sub>12</sub>: Control (No inoculum, no fertilizers)

Commercial PGPR Mix - 1 (*Azospirillum lipoferum*, *Azotobacter chroococcum*, *Bacillus megaterium* and *Bacillus sporothermodurans*) from KAU was also used. Twenty ml of liquid consortium per plant was applied for each treatment. PGPR Mix - 1 was applied at the rate of 20 g l<sup>-1</sup>. The population of the bacterial strain in liquid biofertilizer consortia was 10<sup>10</sup> g cfu ml<sup>-1</sup>. All the liquid biofertilizer inoculants were mixed with farmyard manure and kept for 12 h before soil application. PGPR Mix - 1 was mixed with FYM and applied. Two applications of recommended fertilizer and liquid biofertilizers consortia were carried out at planting and then at 90 days after planting. Monthly observations on plant height, number of leaves, leaf length, leaf breadth, leaf area and diameter of the stem were recorded, and statistical analysis was performed using ANOVA through Web based Agricultural Statistics software Package (WASP 2.0). The bacterial strain population in each treatment was recorded at 3 and 6 months after planting. The soil pH in the potting mixture were recorded at 6-months after planting using standard protocol.

### 3. RESULTS AND DISCUSSION

A total of 55 beneficial bacterial isolates comprising of 21 nitrogen fixers, 18 phosphorus solubilizers and 16 potassium solubilizers were obtained from the nutmeg rhizosphere soils of Wayanad district in Kerala. Qualitative and quantitative screening for nitrogen fixation, phosphorus solubilization, potassium solubilization and IAA production were carried out to determine the most efficient bacterial strains. Potential 15 isolates (5 nitrogen fixers, 5 phosphorus solubilizers and 5 potassium solubilizers) were selected

and screened for abiotic stress tolerance of temperature and moisture. These 15 isolates were also assessed for compatibility among themselves. Three best isolates, each of nitrogen fixers, phosphorus solubilizers and potassium solubilizers were selected based on efficiency and compatibility to produce 3 distinct liquid biofertilizer consortia. The 3 distinct liquid biofertilizer consortia were designated as consortia -1 (*Staphylococcus equorum* VTN2, *Staphylococcus pasteurii* PPP, *Pseudomonas veroni* MHK), consortia -2 (*Staphylococcus equorum* KPN3, *Bacillus velezensis* KKP1, and *Staphylococcus epidermidis* NMK2) and consortia -3 (*Enterobacter* sp. KPN1, *Pseudomonas aeruginosa* MKP2, and *Sphingobacterium anhuiense* MPK).

### **Pot culture studies**

#### **1. Effect of different treatments on shoot and root growth**

The physiological growth parameters such as plant height, number of leaves, leaf area, leaf length, leaf breadth and diameter of the stem in nutmeg seedlings at monthly intervals are presented in Table 1. The liquid bio-inoculants had varying effects on the growth parameters. Plant height (22.12cm), leaf length (13.03cm), leaf breadth (4.87cm), leaf area (43.78cm<sup>2</sup>), and diameter of the stem (0.6cm) were highest in the case of Microbial consortium - 2 (*Staphylococcus equorum* KPN3 + *Bacillus velezensis* KKP1 + *Staphylococcus epidermidis* NMK2) with 50% RDF of N, P, K (T<sub>2</sub>). The number of leaves was highest (22.67) in Microbial consortium - 1 (*Staphylococcus equorum* VTN2 + *Staphylococcus pasteurii* PPP + *Pseudomonas veroni* MHK) with 50% RDF of N, P, K (T<sub>1</sub>). For many crops, leaf length and leaf breadth are good indicators of yield. The longer the leaves, it will receive more sunlight, which will result in increased rate of photosynthesis (18). The highest plant height was observed in the case of Microbial consortium - 2 (*Staphylococcus equorum* KPN3 + *Bacillus velezensis* KKP1 + *Staphylococcus epidermidis* NMK2) with 50% RDF of N, P, K (T<sub>2</sub>). The results are in accordance with the experiment (19) in walnut seedlings. The co-inoculation of *Pseudomonas chlororaphis* and *Acinetobacter pascens*, along with rock phosphate application, led to increased walnut seedling growth parameters such as height, shoot and root dry weight, as well as uptake of phosphorus and nitrogen. This approach also resulted in the highest levels of available phosphorus and nitrogen in the soil, observed under shade house conditions. In this study, the number of leaves were not significantly different. Leaf fall was noticed which resulted in decreased number of leaves. There were significant differences in the leaf length, leaf breadth and stem diameter. Study of oil palm seedlings under greenhouse conditions with 70% chemical fertilizers and 30% biofertilizer mixture (*Kocuria rhizophila*, *Arthrobacter methylotrophus*, *Bacillus* spp. such as *B. pumilus*, *B. subtilis* ssp. *Spizizenii*, *B. vallismortis*, *B. thurengiensis*, *B. mycoides*, *B. mucilaginosus*, *Brevibacillus reuszeri*, *Paenibacillus polymax*, *Paenibacillus azoreducens*, *Azospirillum brasilense*, *Aspergillus niger*,

*Aspergillus awamori* and *Saccharomyces cerevisiae* Hansen) revealed enhanced girth size, chlorophyll content, with improved nutrient uptake by the seedlings (20).

**Table 1: Effect of different treatments on the growth parameters of nutmeg at 6 months after planting under pot culture studies**

Treatments	Plant height (cm)	Number of leaves	Leaf length (cm)	Leaf breadth (cm)	Leaf area (cm <sup>2</sup> )	Stem diameter (cm)
T <sub>1</sub>	20.93	22.67	12.27 <sup>bcd</sup>	4.83 <sup>ab</sup>	39.58	0.60 <sup>a</sup>
T <sub>2</sub>	22.12	14.22	13.03 <sup>a</sup>	4.87 <sup>a</sup>	43.78	0.60 <sup>a</sup>
T <sub>3</sub>	20.93	13.50	11.97 <sup>bcd</sup>	4.67 <sup>abc</sup>	41.35	0.52 <sup>cd</sup>
T <sub>4</sub>	18.57	12.50	11.84 <sup>def</sup>	4.60 <sup>abcd</sup>	38.98	0.55 <sup>abc</sup>
T <sub>5</sub>	19.43	18.67	11.93 <sup>cde</sup>	4.37 <sup>cd</sup>	36.93	0.60 <sup>a</sup>
T <sub>6</sub>	15.43	09.67	11.22 <sup>f</sup>	4.38 <sup>cd</sup>	41.16	0.52 <sup>cd</sup>
T <sub>7</sub>	17.69	08.67	12.28 <sup>bcd</sup>	4.54 <sup>bcd</sup>	39.35	0.52 <sup>bcd</sup>
T <sub>8</sub>	18.97	14.67	11.83 <sup>def</sup>	4.30 <sup>d</sup>	38.08	0.57 <sup>abc</sup>
T <sub>9</sub>	18.05	20.17	12.57 <sup>abc</sup>	4.44 <sup>cd</sup>	40.91	0.58 <sup>ab</sup>
T <sub>10</sub>	19.13	07.67	12.63 <sup>ab</sup>	4.53 <sup>bcd</sup>	37.66	0.47 <sup>d</sup>
T <sub>11</sub>	13.37	05.67	11.70 <sup>def</sup>	4.47 <sup>cd</sup>	35.53	0.40 <sup>e</sup>
T <sub>12</sub>	18.11	15.56	11.29 <sup>ef</sup>	4.34 <sup>d</sup>	34.77	0.57 <sup>abc</sup>
<b>CD (0.05)</b>	NS	NS	0.675	0.310	NS	0.062

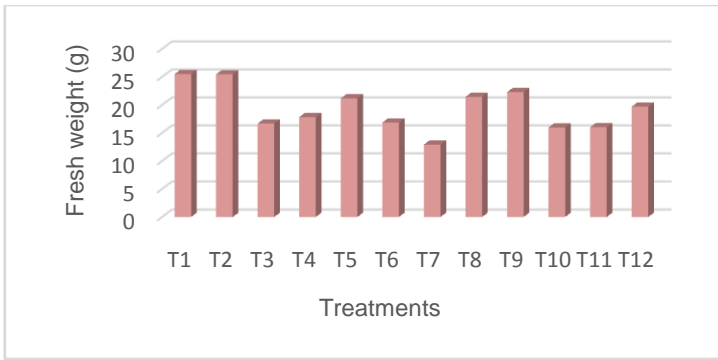
Each value represents a mean of three replications. Mean values in a column sharing similar alphabets do not differ significantly in ranking as determined by the DMRT test ( $p < 0.05$ ). NS - Non-Significant

The monthly observations revealed that 50% of recommended dose of chemical fertilizer application with consortium, C<sub>2</sub> was the best treatment. A study (21) revealed that application of vermicompost, 50% recommended dose of fertilizers and biofertilizers (*Azotobacter* and phosphate solubilizing bacteria) could result in higher growth, yield, and yield attributes with good quality cucumber. The present study confirmed that the chemical fertilizer dependency of the nutrient exhaustive crop, nutmeg could be reduced with biofertilizers.

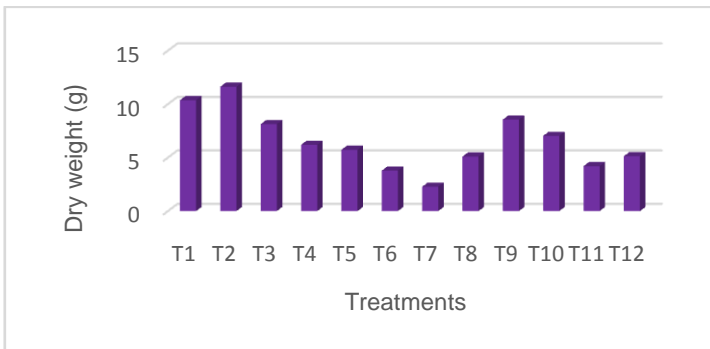
During the experimental period, nutmeg seedlings were diseased, the causative microorganism was identified as *Fusarium*. *Trichoderma* sp. (KAU) was applied to nutmeg seedlings, 2 months after planting. The second application of biofertilizers was carried out after *Trichoderma* sp. application, as the management of disease might have affected the biofertilizer strains.

After 6 months of planting, different physical attributes such as shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), root dry weight (g) and number of secondary roots were recorded (Fig.1 - 5). Shoot fresh weight was highest (25.5 g) in Microbial consortium -1 (*Staphylococcus equorum* VTN2 + *Staphylococcus pasteurii* PPP + *Pseudomonas veroni* MHK) with 50% RDF of N, P, K(T<sub>1</sub>), shoot dry weight (11.63 g) and maximum number of secondary roots (21.67) were recorded in the case of Microbial consortium - 2 (*Staphylococcus equorum* KPN3 + *Bacillus velezensis* KKP1 + *Staphylococcus epidermidis* NMK2) with 50% RDF of N, P, K (T<sub>2</sub>). Root fresh weight (17.97 g) and dry weight (10.09 g) was highest in the case of KAU Package of practices for organic farming crops (17), T<sub>9</sub>. There was a significant difference among the treatments with respect to root and shoot growth at 6 months after planting. In a study (22), the treatment with the highest dose of biofertilizers (*Azotobacter* and *Azospirillum*), 500 g, revealed the most significant effect on seedling development, increased growth, stimulated rooting, and the highest increase in leaf number in *Eucalyptus grandis*. Another study of field application of biofertilizer, *Bacillus thuringiensis* A5-BRSC, showed statistically significant increase in seed germination, shoot height, root length, leaf diameter, vigor index, fruit weight, seed weight, total fresh weight, dry weight of inoculated *Abelmoschus esculentus* plants in comparison to the control plants under field conditions (23).

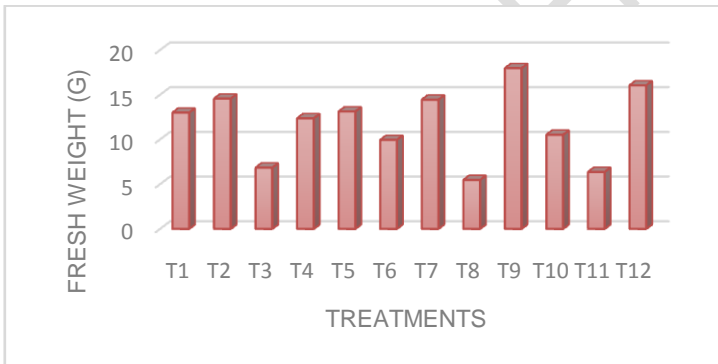
**Fig 1: Fresh weight of nutmeg shoot as influenced by different treatments**



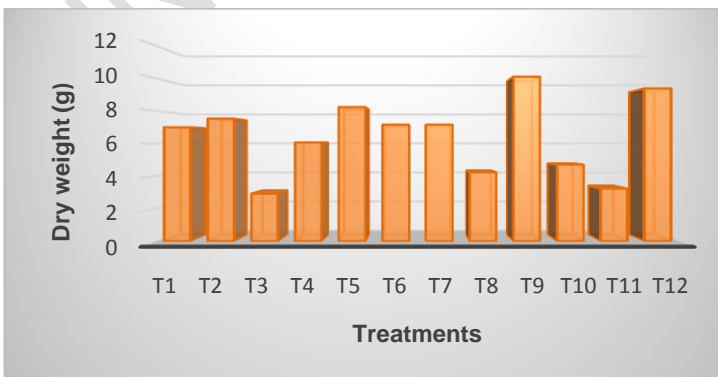
**Fig 2: Dry weight of nutmeg shoot as influenced by different treatments**



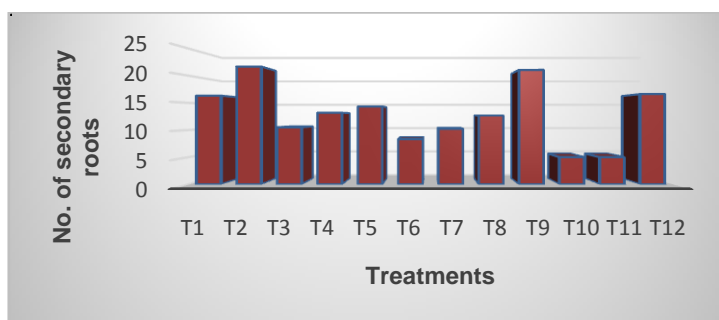
**Fig 3: Fresh weight of nutmeg root as influenced by different treatments**



**Fig 4: Dry weight of nutmeg root as influenced by different treatments**



**Fig 5: Number of secondary roots of nutmeg seedlings as influenced by different treatments.**



## 2. Effect of different treatments on root to shoot ratio.

The mean root to shoot ratio (R/S) recorded at 6 months after planting ranged from 0.26 to 1.12 (Table 2). Highest R/S ratio was observed in the case of Microbial consortium - 3 (*Enterobacter* sp. KPN1 + *Pseudomonas aeruginosa* MKP2 + *Sphingobacterium anhuiense* MPK) with 75% RDF of N, P, K (T<sub>7</sub>). The results are in accordance with the earlier studies in nutmeg seedlings (10), where it ranges from 0.38-0.45. Lower the R/S ratio, the higher proportion of shoots over roots.

**Table 2: Effect of different treatments on root to shoot ratio of nutmeg seedlings at 6 months after planting under pot culture studies.**

Treatments	Root to shoot ratio
T <sub>1</sub>	0.5098
T <sub>2</sub>	0.5723
T <sub>3</sub>	0.4107
T <sub>4</sub>	0.6942
T <sub>5</sub>	0.6202
T <sub>6</sub>	0.5905
T <sub>7</sub>	1.1184
T <sub>8</sub>	0.2553
T <sub>9</sub>	0.8066

T <sub>10</sub>	0.6594
T <sub>11</sub>	0.3973
T <sub>12</sub>	0.8151

Each value represents a mean of three replications.

### 3. Effect of different treatments on the population of inoculated biofertilizer strains

The final soil pH and beneficial bacterial population were recorded at 6 months after planting (Table 3). Initial soil pH was 5.68 and at 6 months after planting, different treatment soil pH increased to 7.71. In the study (10), the initial soil acidity was 4.7, which later increased with different treatments to 5.4, by the end of 24-week-old experiment in nutmeg seedlings.

**Table 3: Effect of different treatments on soil pH and population of nitrogen fixers, phosphorus solubilizing bacteria and potassium solubilizing bacteria at 6 months after planting under pot culture studies.**

Treatments	Soil pH	Nitrogen fixing bacteria (x 10 <sup>7</sup> cfu g <sup>-1</sup> )	Phosphorus solubilizing bacteria (x 10 <sup>7</sup> cfu g <sup>-1</sup> )	Potassium solubilizing bacteria (x 10 <sup>7</sup> cfu g <sup>-1</sup> )
T <sub>1</sub>	6.52	13.33 (8.123) <sup>e</sup>	115.33 (9.060) <sup>a</sup>	11.33 (8.050) <sup>f</sup>
T <sub>2</sub>	6.83	29.33 (8.470) <sup>bc</sup>	47.67 (8.68) <sup>c</sup>	45.33 (8.66) <sup>c</sup>
T <sub>3</sub>	6.89	24.67 (8.390) <sup>cd</sup>	31.67 (8.500) <sup>d</sup>	12.33 (8.090) <sup>f</sup>
T <sub>4</sub>	7.18	11.33 (8.050) <sup>e</sup>	31.67 (8.503) <sup>d</sup>	51.33 (8.710) <sup>bc</sup>
T <sub>5</sub>	6.98	12.67 (8.097) <sup>e</sup>	11.33 (8.050) <sup>g</sup>	71.33 (8.853) <sup>a</sup>

T <sub>6</sub>	7.56	72.67 (8.860) <sup>a</sup>	22.00 (8.340) <sup>e</sup>	31.33 (8.497) <sup>d</sup>
T <sub>7</sub>	6.92	32.00 (8.507) <sup>b</sup>	19.33 (8.283) <sup>f</sup>	54.00 (8.730) <sup>b</sup>
T <sub>8</sub>	7.20	11.67 (8.063) <sup>e</sup>	21.33 (8.327) <sup>ef</sup>	13.33 (8.123) <sup>f</sup>
T <sub>9</sub>	7.09	30.00 (8.477) <sup>b</sup>	33.00 (8.520) <sup>d</sup>	11.33 (8.050) <sup>f</sup>
T <sub>10</sub>	7.71	22.00 (8.340) <sup>d</sup>	61.67 (8.790) <sup>b</sup>	22.67 (8.353) <sup>e</sup>
T <sub>11</sub>	7.62	13.00 (8.110) <sup>e</sup>	21.33 (8.327) <sup>ef</sup>	13.33 (8.123) <sup>f</sup>
T <sub>12</sub>	6.74	31.67 (8.517) <sup>b</sup>	68.33 (8.83) <sup>b</sup>	12.67 (8.10) <sup>f</sup>

Bracket values are log transformation values. Each value represents a mean of three replications. Mean values in a column sharing similar alphabets do not differ significantly in ranking as determined by the DMRT test ( $p < 0.05$ ).

An initial population of nitrogen fixers ( $18.33 \times 10^4$ ), phosphorus solubilizers ( $5.33 \times 10^5$ ) and potassium solubilizers ( $20.3 \times 10^4$ ) were recorded, in the potting mixture. Soil application of liquid biofertilizers allowed the microbes to colonize the rhizosphere region more quickly. Bacterial population increased significantly in nutmeg seedling rhizosphere region. These results are in accordance with the studies (10). In the study, bacterial population of  $10^3$  cfu g<sup>-1</sup> of nitrogen fixing bacteria and  $10^6$  cfu g<sup>-1</sup> f phosphorus solubilizing bacteria increased to  $10^6$  nitrogen fixing bacteria and  $10^8$  phosphorus solubilizing bacteria at the end of the 24-week old experiment. In the present study, T<sub>1</sub> revealed the highest population of beneficial bacteria (NPK), followed by T<sub>2</sub>. Many researchers have concluded that inoculation of efficient native soil microbes had a significant role in plant establishment and robustness of seedling growth (8,24). The present results revealed that biofertilizers play a promising role in nutrient input of nutmeg seedlings under pot culture, by reducing the chemical fertilizers.

The promising results exhibited by liquid biofertilizer consortia with *Staphylococcus equorum* KPN3 + *Bacillus velezensis* KKP1

+*Staphylococcus epidermidis* NMK2 along with 50% RDF of NPK indicated that biofertilizers could help in improved seedling growth (Fig 6) with reduced soil degradation. *Staphylococcus equorum* has been identified as a halotolerant and seed endophyte that boosts the shoot and root length, as well as the root weight, of various plant species (25,26). Recent studies have reported that *Bacillus velezensis* Ag75 had the ability to solubilize phosphorus, leading to increased growth and yield in maize plants (27). *B. velezensis* when combined with organomineral fertilizer reduced the phosphate doses, improved the soil microbial chemical properties and sugarcane biomass (28). The present study of *B. velezensis* agrees with the previous reports that it is a promising phosphorus solubilizer. *Staphylococcus epidermidis* YJ101, from white clover rhizosphere soil is an efficient phosphate solubilizer and IAA producer, which improved the growth of white clover under pot studies (29). In the present study, it has been observed that *Staphylococcus equorum*, a nitrogen fixer; *Bacillus velezensis*, a phosphate solubilizer and *Staphylococcus epidermidis*, a potassium solubilizer as a liquid biofertilizer consortium makes a remarkable increase in the overall growth development of nutmeg seedlings.

Fig 6: Effect of the best treatment on the growth of nutmeg seedlings



#### 4. CONCLUSION

In this study, we successfully prepared three liquid biofertilizer consortia, which can impart properties like nitrogen fixation, phosphorus solubilization and potassium solubilization. Nutmeg seedlings treated with the liquid biofertilizer consortia exhibited enhanced growth with respect to various growth parameters of nutmeg. Notably, applying a 50% recommended dose of chemical fertilizer based on soil test along with the liquid consortium *Staphylococcus equorum* KPN3 + *Bacillus*

*velezensis*KKP1+ *Staphylococcus epidermidis* NMK2 resulted in the highest plant height, leaf length, leaf breadth, stem diameter, leaf area, shoot dry weight, and maximum number of secondary roots. The R/S ratio less than 1, indicated that the aerial growth of nutmeg seedlings surpassed that of the roots, in most of the treatments. Soil application of the liquid biofertilizer consortium 2, not only reduces 50 % dependence on chemical fertilizers but also enhances the robustness of nutmeg seedlings, facilitating their earlier establishment when planted in pots.

## REFERENCES

1. KAU (Kerala Agricultural University). Package of Practices Recommendations Crops. Directorate of Research, Vellanikkara, Thrissur. 134-136 p. 2016.
2. Ajmal M, Ali HI, Saeed R, Akthar A, Tahir M, Mehboob MZ, Ayub A. Biofertilizer as an alternative for chemical fertilizers. RRJAAS, 7:1-7. 2018.
3. Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil beneficial bacteria and their role in plant growth promotion: a review. *Ann Microbiol* **60**: 579–598. 2010. <https://doi.org/10.1007/s13213-010-0117-1>.
4. Singh D, Thapa S, Geat N, Mehriya ML, Rajawat MVS. Biofertilizers: Mechanisms and application, In: Biofertilizers (Rakshit, A.; Meena, V.S.; Parihar, M.; Singh, S.B. and Singh, A.K. (eds.)) Woodhead Publishing, 151-166p. 2021.
5. Bhaskara V. Comparative performance of liquid and lignite formulations of *Azotobacter chroococcum* and *Bacillus megaterium*

(PSB) on aerobic rice (*Oryza sativa* L.), MSc thesis, University of Agricultural Sciences, Bengaluru, India (2011).

6. Sahu PK, Brahmaprakash, GP. Formulations of biofertilizers – approaches and advances. In: Singh, D., Singh, H., Prabha, R. (eds) Microbial inoculants in sustainable agricultural productivity, Springer, New Delhi, 179–198. 2016. doi:10.1007/978-81-322-2644-4\_12.
7. Krishnaprabu S. Liquid microbial consortium: A potential tool for sustainable soil health. *J Pharmacogn Phytochem.* **9**(2):2191-2199. 2020.
8. Nair SK, Chandra N. Effect of biofertilizer application on growth of nutmeg (*Myristica fragrans* Houtt.) seedlings. *J. Trop. Agric.*, **39**:65-66. 2001.
9. Kalay M, Kesaulya H, Talahaturuson A, Rehatta H, Hindersah R. Application of biofertilizer consortium of strain *Bacillus* sp. with different concentrations and application methods on growth of nutmeg seeds (*Myristica fragrans* Houtt.) *Agrologia*, **9**:30-38. 2020.
10. Hindersah R, Kalay AM, Kesaulya H, Suherman C. The nutmeg seedlings growth under pot culture with biofertilizers inoculation. *De Gruyter*, **6**:1-10. 2021.
11. Jackson ML. Soil chemical analysis. Indian reprint, Prentice Hall in India, New Delhi, 498 p. 1973.

12. Olsen SR. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture, Washington. 19p. 1954.
13. Etesami H, Emami S, Alikhani HA. Potassium solubilizing bacteria (KSB): Mechanisms, promotion of plant growth, and future prospects - A review. *J. Soil Sci. Plant Nutr.*,**17**(4):897-911. 2017.
14. Gordon SA, Weber RP: Colorimetric estimation of indoleacetic acid. *Plant Physiol.*,**26**(1):192-195. 1951
15. Surendra-Gopal K, Baby A. Enhanced shelf-life of *Azospirillum* and PSB through addition of chemical additives in liquid formulations. *IJST*.**5**(4): 2023-2029. 2016.
16. Thangaselvabai T, Sudha KR, Selvakumar T, Balakumbahan R. Nutmeg (*Myristica fragrans* Houtt) - The Twin Spice - A review. *Agric. Rev.*,**32** (4): 283 – 293. 2011.
17. KAU (Kerala Agricultural University). Package of practices recommendations for organic farming crops. Directorate of Research, Vellanikkara, Thrissur. 124-126 p. 2017.
18. Chithra R, Janaki D, Jansirani P. Effect of bioinoculants on growth and yield of betel vine. *J. Plant. Crops.*, **48**(3): 232-236. 2020.
19. Yu X, Liu X, Zhu TH, Liu GH, Mao C. Co -inoculation with phosphate solubilizing and nitrogen fixing bacteria on solubilization of rock

phosphate and their effect on growth promotion and nutrient uptake by walnut. *Eur. J. Soil Biol.*, **50**:112-117. 2012.

20. Ajeng AA, Abdullah R, Malek MA, Chew KW, Ho YC, Ling TC, Lau BF, Show PL. The effects of biofertilizers on growth, soil fertility, and nutrients uptake of Oil Palm (*Elaeis Guineensis*) under greenhouse conditions. *Processes*, **8**:1681. 2020. <https://doi.org/10.3390/pr8121681>.
21. Bindiya Y, Prabhakar RI, Srihari D. Response of cucumber to combined application of organic manures, biofertilizers and chemical fertilizers. *Veg. Sci.*, **41**:12-15. 2014
22. Gonzalez-Diaz A, Ojeda-Morales ME, Hernandez-Rivera MA, Cordova-Bautista Y, Diaz-Flores LL, Lopez-Lazaro JS, Alvarez-Ramirez JG. Effect of biofertilizers application on the growth of *Eucalyptus grandis* seedlings under greenhouse conditions. *J. Plant Nutr.*, **42**: 2560-2576. 2019. DOI: [10.1080/01904167.2019.1655040](https://doi.org/10.1080/01904167.2019.1655040).
23. Bandopadhyay S. Application of plant growth promoting *Bacillus thuringiensis* as biofertilizer on *Abelmoschus esculentus* plants under field condition. *J. Pure Appl. Microbiol.*, **14**:1287-1294. 2020. <https://doi.org/10.22207/JPAM.14.2.24>.
24. Xiong Y, Zhang X, He X, Li M, Liao B. Species specific effects of P solubilizing and N<sub>2</sub> fixing bacteria on seedling growth of three salt tolerant trees. *Russ. J. Ecol.*, **47**: 259-265. 2016.

25. Vega C, Rodriguez M, Llamas I, Bejar V, Sampedro I. Silencing of phytopathogen communication by the halotolerant PGPR *Staphylococcus equorum* strain EN21. *Microorganisms*, **8**:42. 2020.
26. Mukherjee A, Singh BK, Verma JP. Harnessing chickpea (*Cicer arietinum* L.) seed endophytes for enhancing plant growth attributes and bio-controlling against *Fusarium* sp. *Microbiol. Res.*, **237**:126469. 2020.
27. Mosela M, Andrade G, Massucato LR, de Araujo Almeida SR, Nogueira AF, de Lima Filho RB, et al. *Bacillus velezensis* strain Ag75 as a new multifunctional agent for biocontrol, phosphate solubilization and growth promotion in maize and soybean crops. *Sci. Rep.*, **12**(1). 2022
28. Santos HL, Silva G Fd, Carnietto MRA, Oliveira LC, Nogueira C Hd C, Silva Md A. *Bacillus velezensis* associated with organomineral fertilizer and reduced phosphate doses improves soil microbial - chemical properties and biomass of sugarcane. *Agron.*, **12**(11):2701. 2022.
29. Cheng Y, Luo J, Wang Y, Ge J, Zhou S, Yang W, Kou J. *Staphylococcus hominis* YJLJH and *Staphylococcus epidermidis* YJ101 promote the growth of white clover (*Trifolium repens* L.) by increasing available phosphorus. *Symbiosis*, **83**:103–114. 2021.