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Research Article

Impact of various organic biofertilizers on the growth and biochemical constituents of Maize (*Zea mays* L.)

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ABSTRACT

The current study emphases on assessing the efficiency of different biofertilizers, namely *Phosphobacteria, Azospirillum*, Vesicular Arbuscular Mycorrhiza (VAM) and Potash Mobilizing Bacteria (PMB), concerning their impact on seeds germination, growth parameters and biochemical constituents of Maize (*Zea mays* L). The experimentation with seed inoculations of different biofertilizers to assess their influence on the germination, various growth parameters and biochemical constituents of maize. A germination study was conducted using maize seeds treated with a variety of biofertilizers. The investigation focused on multiple aspects, including germination percentage, vigor index, seedling growth, fresh and dry weights of the seedlings. The growth parameters and pigments of the maize plants at different intervals (15, 30, and 45 Days after sowing, or DAS), with comparisons made to a control group. The findings indicated that all the examined growth parameters and pigments exhibited gradual improvements with the application of various organic biofertilizers. Based on the data, among these biofertilizers, *Azospirillum* stood out as the most effective, leading to significant enhancements in maize seedling growth, morphological parameters, and pigment levels when compared to both other biofertilizers and the untreated control group.

INTRODUCTION

For numerous decades, maize (*Zea mays* L.) has held a dual role as a crucial global crop and a primary model organism for genetic research [1]. Maize is utilized for both food and animal feed purposes across the globe. Maize cultivation spans subtropical, tropical and temperate regions worldwide, showcasing its remarkable adaptability on a global scale [2].

Maize is listed as the third most extensively cultivated food crop worldwide and its susceptibility to climate change underscore the vital need for the development of adaptation strategies. This is crucial not only for agricultural production but also for ensuring food security, as emphasized [3,4]. In the current era of rapid economic development, challenges like population growth, shrinking land resources, resource scarcity and environmental degradation are prevalent. Maize cultivation science is presented with new, historic opportunities and challenges. At this pivotal juncture in history, it holds great significance to revisit the progress in scientific research and technology in the field of maize cultivation [5].

The ongoing depletion of Earth's natural resources and the escalating use of harmful chemical fertilizers raise significant apprehensions regarding the future of agriculture [6,7]. Biofertilizers offer a promising solution to these hazardous chemical inputs and are becoming increasingly important for achieving sustainable agriculture. They play an essential role in elevating crop yields and preserving the enduring fertility of the soil, which is critical for meeting the world's ever-growing food requirements. Microorganisms can establish interactions with crop plants, thereby bolstering their immunity, growth and overall development [8].

Besides the traditional biofertilizers, another interesting biofertilizer includes *Piriformospora indica*, which is a cultivable endophytic fungus, colonizes plants' roots and helps in promoting plant growth and biomass production [9]. Bacteria that can fix nitrogen and fall within genera such as *Azospirillum*, *Rhizobium*, *Azotobacter*, *Klebsiella* and others play a significant role in meeting plants' nitrogen needs through the process of biological nitrogen fixation. This process is a critical aspect of sustainable agriculture. Among these, *Azospirillum*, in addition to its nitrogen-fixing capability, is widely recognized for its positive impact on plant growth, as evidenced by numerous studies across various crop plants [10,11]. The use of plant growth promoting rhizobacteria with enhanced properties can improve the socio-economic status of poor farmers [12]. In order to increase the growth and yield of maize, this research investigation was undertaken with the aim of bringing out findings with the following objectives.

To find out the efficiency of various biofertilizers on the germination propensity, the growth tendency of maize and to find out the variation in biochemical contents in maize (*Zea mays* L.) treated with the various biofertilizers in the field experiment at various growth stages.

MATERIALS AND METHODS

Biological materials

The dry and dormant seeds of the Maize (*Zea mays* L.), variety Kaveri 25k55, were obtained from Kaveri Seed Company Ltd, Secunderabad, Telangana, India.

List of biofertilizers used for study

a) Phosphobacteria - obtained from Tari Bio – Tech Arulanandha Nagar, Thanjavur, India.

b) *Azospirillum* – obtained from Tari Bio – Tech Arulanandha Nagar, Thanjavur, India.

c) Vesicular Arbuscular Mycorrhiza (VAM) – Mani Dharma Biotech Pvt. Ltd. Porur, Chennai, India.

d) Potash Mobilizing Bacteria (PMB) – Tari Bio – Tech, Arulanandha Nagar, Thanjavur, India.

Seed treatments

After surface sterilizing the maize seeds with 80% ethanol and 0.1 percent mercuric chloride, they were repeatedly cleaned with distilled water. The seeds were combined with a specific organism biofertilizer and allowed to dry in the shade for half an hour. The seeds were sowed after drying in the shade.

T0 - Seeds without treatments (Control), T1 – Phosphobacteria, T2 – *Azospirillum,* T3 - Vesicular Arbuscular Mycorrhiza (VAM), T4 - Potash Mobilizing Bacteria (PMB). Non-treated dry seeds are pre-soaked in distilled water for 6 hours.

Experimental plots

The experiments were conducted in Botanical Garden, Department of Botany, A.V.C. College (Autonomous), Mannampandal, Mayiladuthurai District, Tamil Nadu, India. The experiments were conducted during April 2021 to June 2022 (Fig. 1). The soil mixture containing red soil; sand and farm yard manure at 1:1:1 ratio and one row was kept as control and remaining four rows were used for various biofertilizers treatment. Five seeds were sown in the ground and the seedlings were thinned to 3 on 10th day after sowing (DAS).

Fig. 1. Field view.

Irrigation schedule

To guarantee consistent germination, irrigation was used prior to sowing. With caution, irrigation was applied at 3 DAS to prevent excessive water flooding. Twice a week, uniform watering was carried out.

Germination percentage

On the seventh day following seeding, the number of seeds that germinated in each treatment was counted. Every treatment was kept in three duplicates. The following formula was used to get the overall germination percentage.

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Seedling growth (cm/seedlings)

From each treatment, ten seedlings were chosen at random to track the seedlings' progress. Using a centimeter scale, the growth of the maize seedlings that were seven days old was measured, and the results were recorded.

Vigour index

Seedling height and germination percentage were recorded and vigour index was calculated as per the procedure.

Vigour index = Germination percentage x Total length of seedling (cm)

Seedling fresh weight

Seedlings fresh weight was measured by using digital electronic balance and expressed in grams per plant.

Seedling dry weight

Using an electronic balance, the fresh weight of the seedling was measured after it had been cleaned with tap water. The results were given in grams. The seedlings were dried for 24 hours at 60 degrees Celsius in a hot air oven after a fresh weight was taken. Following drying, the weight was calculated and the results were given in grams.

Growth parameters

Root and shoot length

On multiple DAS, root and shoot lengths were measured. Total root length was measured below the point of root-stem transition to the tap root and the length of lateral roots. Stem length was defined as the distance between the tip of the stem and the point of the root stem transition region. Each plant's stem and root lengths were given in centimeters.

Total leaf area

With a LICOR Photo Electric Area Meter (Model LI-3100, Lincoln, USA), the total leaf area was calculated and expressed in centimeters per plant.

Whole plant fresh weight and dry weight

Using an electronic balance, the fresh weight of the plants was measured after they had been cleaned with tap water. The results were given in grams. The plants were dried for 24 hours at 60°C in a hot air oven after a fresh weight was taken. Following drying, the weight was calculated and the results were given in grams.

Weed management

Hand weeding was done two times at 25 and 45 days after sowing in order to remove the weeds from the field.

Photosynthetic pigments

Chlorophylls content

Chlorophyll contents were extracted from the leaves and estimated according to the method of Arnon [13].

Statistical analysis

With SPSS 16.0, all of the characters that were the subject of the data were statistically analyzed. Three replicates of each treatment and the control were used to calculate the values. The computed information presented as Mean ±SD.

RESULTS AND DISCUSSION

The goal of the current study is to evaluate how biofertilizers affect the growth parameters and pigmentation of maize plants. Maize seeds germination percentage, seedling length, vigour index, seedlings fresh and dry weight modified by the treatments of various Biofertilizers are presented in Table 1. The maximum germination percentage, seedling length, vigour index, fresh weight and dry weights were recorded in Maize seedlings grown with *Azospirillum*. Among various biofertilizers the PMB showed lowest germination percentage, seedling length, vigour index, seedlings fresh and dry weight. The lowest germination percentage seedling length, vigour index (seedling length x germination percentage), fresh weight and dry weight were recorded in Maize seedlings grown without treatment of biofertilizers.

Table 1. Effect of different biofertilizers on seed germination, seedling growth, vigour index, fresh and dry weight of Maize (*Zea mays* L.) seedlings.

Treatments	Germination percentage	Seedling length (cm/seedling)	Vigour index (seedling length × germination percentage)	Seedling fresh weight (g/seedling)	Seedling dry weight (g/seedling)
Control (T0)	90 ± 1.43	10.3 ± 1.05	773.2 ± 11.25	1.27 ± 0.83	0.47 ± 0.037
Phosphobacteria (T1)	92 ± 1.72	12.43 ± 0.85	975.2 ± 10.58	2.10 ± 0.13	0.58 ± 0.095
Azospirillum (T2)	94 ± 1.88	13.25 ± 1.33	998.7 ± 14.99	1.78 ± 0.17	0.74 ± 0.033
VAM (T3)	93 ± 1.29	11.57 ± 1.52	943.6 ± 16.92	1.53 ± 0.42	0.65 ± 0.082
PMB (T4)	92 ± 1.25	12.21 ± 0.26	934.4 ± 14.23	1.64 ± 0.71	0.56 ± 0.036

Data are average values of three replicates ± SD.

Similar results were observed in *Raphanus sativus* and *Cynara scolymus*. In three radish cultivars using bacteria strains significantly improved the percentage of seed germination. PGPR could be useful to obtain higher seed germination percentages in radish [14]. Seed inoculation of PGPR strains significantly increased germination rate of wheat compared with the control [15].

Shoot length (cm/plant)

The results on the effect of different biofertilizers on the shoot length (cm/plant) of maize at various stages of its growth (15, 30 and 45 DAS) are shown in Table 2. The shoot lengths gradually increased and the highest were recorded in maize grown in *Azospirillum*. The lowest shoot length was recorded in control in the crop grown without treatment of biofertilizers, followed by PMB, VAM and Phosphobacteria at various stages of its growth. Among various biofertilizers *Azospirillum* enhanced maximum shoot length (Fig. 2-4).

Table 2. Effect of different biofertilizers on shoot length of Maize (*Zea mays* L.) at different growth stages.

Treatments	Age of the plant in days (DAS)		
	15	30	45
Control (T0)	10.4 ± 1.34	25.1 ± 1.78	54.7 ± 1.28
Phosphobacteria (T1)	21.2 ± 1.77	47.8 ± 0.99	65.7 ± 1.29
Azospirillum (T2)	23.1 ± 1.98	70.37 ± 1.09	77.4 ± 1.42
VAM (T3)	22.2 ± 1.52	53.4 ± 1.10	73.8 ± 1.42
PMB (T4)	22.7 ± 1.96	51.2 ± 1.95	70.4 ± 1.58

Values are mean ± SD of three replicates

Fig. 2. Maize 15 DAS treated with different biofertilizers.

Root length (cm/plant)

The results on the effect of different biofertilizers on root length of Maize at various stages of its growth (15, 30 and 45 DAS) are shown in Table 3. The highest root length was recorded in maize grown in VAM. The lowest root length was recorded at various stages of its growth (15, 30 and 45 DAS) in the crops grown without biofertilizers (Fig. 2-4).

Fig. 3. Maize 30 DAS treated with different biofertilizers.

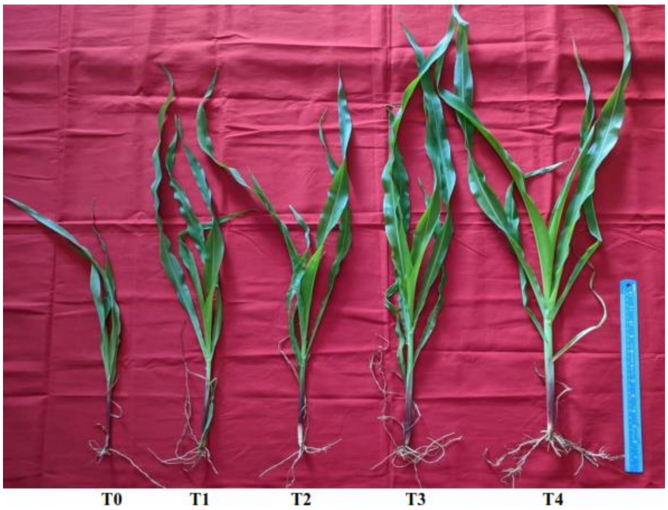

Fig. 4. Maize 45 DAS treated with different biofertilizers.

The same results of shoot and root length were observed in the following studies. According to Nagargade et al. [16], the PGPR are naturally occurring soil bacteria that actively colonize plant roots and improve the growth environment for plants. When PGPR strains were injected into wheat seeds, the result was a significant increase in root length as compared to the control [16]. Kapri and Tewari [17] observed that the inoculation of chickpea with Trichoderma sp. that produced phosphatase and phosphate solubilizing agents resulted in increased length of shoots and roots. When Rhizobium, Phosphobacteria, and *Azospirillum* were inoculated into cowpeas, the growth parameters increased [18].

Total leaf area (cm²/plant)

The results on the effect of different biofertilizers on total leaf area (cm^2/plant) of maize at various stages of its growth (15, 30 and 45 DAS) are shown in Fig. 5. The maximum total leaf area was recorded in maize crop grown in *Azospirillum* treatment. The lowest total leaf area was recorded at various stages of its growth (15, 30 and 45 DAS) in the crops grown without biofertilizers. Senthilkumar and Sivagurunathan [18] found that when cowpea plants were inoculated with Rhizobium, Phosphobacteria and *Azospirillum* at the same time, the leaf area (length and breadth) increased significantly. When compared to the control, inoculating the seeds with PGPR improved the sunflower's qualitative and quantitative characteristics, particularly the leaf area index [19].

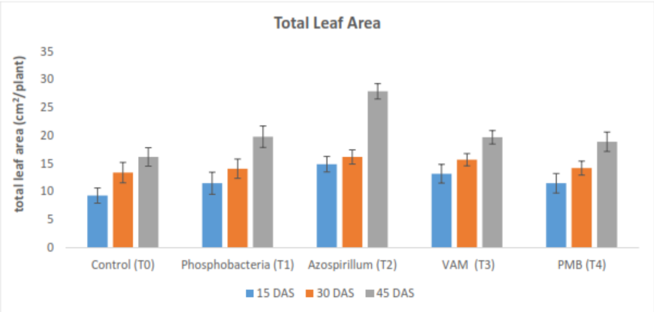

Fig. 5. Effect of different biofertilizers on total leaf area of Maize (*Zea mays* L.) at different growth stages.

Table 3. Effect of different biofertilizers on root length (cm/plant) of Maize (*Zea mays* L.) at different growth stages.

Treatments	Age of the plant in days (DAS)		
	15	30	45
Control (T0)	07.4 ± 1.02	14.7 ± 1.88	16.2 ± 1.17
Phosphobacteria (T1)	09.7 ± 1.34	17.3 ± 1.79	19.8 ± 1.90
Azospirillum (T2)	11.9 ± 1.28	19.77 ± 1.22	17.4 ± 1.24
VAM (T3)	14.7 ± 1.33	21.4 ± 1.56	23.5 ± 1.79
PMB (T4)	11.3 ± 1.05	20.2 ± 1.25	19.4 ± 1.32

Values are mean ± SD of three replicates

Whole plant fresh weight

Fig. 6 displays the findings regarding the impact of distinct biofertilizers on the fresh weight (g/plant) of maize at different growth stages (15, 30 and 45 DAS). A maize crop treated with *Azospirillum* reached its maximum fresh weight. The crops grown without biofertilizers had the lowest fresh weight at different growth stages (15, 30 and 45 DAS).

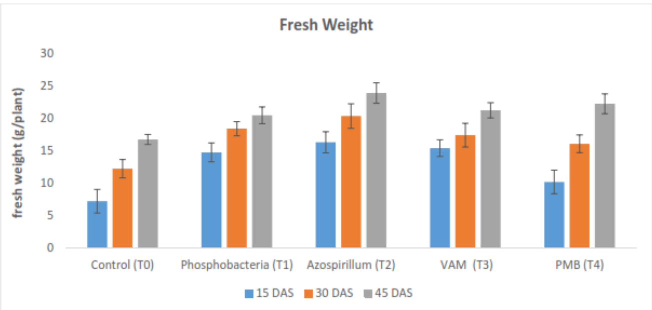

Fig. 6. Effect of different biofertilizers on fresh weight (g/plant) of Maize (*Zea mays* L.) at different growth stages.

Whole plant dry weight

The results on the effect of different biofertilizers on the dry weight (g/plant) of Maize at various stages of its growth (15, 30 and 45 DAS) are shown in Fig. 7. The dry weight gradually increased and the highest were recorded in maize grown in *Azospirillum*. The lowest shoot length was recorded in control in the crop grown without treatment of biofertilizers, followed by PMB, VAM and Phosphobacteria at various stages of its growth. Among various biofertilizers *Azospirillum* enhanced maximum shoot length.

The similar results were observed in *Sesbania grandiflora* treated with Bacillus subtilis and PGPR mixture showed highest total plant fresh weight and highest total plant dry weight [20]. The fresh and dry weight of chickpea significantly increased by biofertilizers [9]. According to Gholami et al. [21], under field conditions, various PGPR strains increased the shoot fresh and dry weight of maize. The consortium application of PGPR increased the fresh and dry weight of root and shoot portion in *Cajanus cajan* [22]. The shoot dry weight of plant increased due to application of Rhizobacteria [23,24].

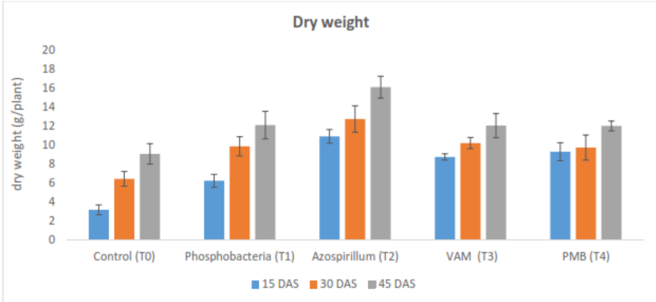

Fig. 7. Effect of different biofertilizers on dry weight (g/plant) of Maize (*Zea mays* L.) at different growth stages.

Photosynthetic pigments

The results on the effect of different biofertilizers on chlorophyll (g/plant) of Maize at various stages of its growth (15, 30 and 45 DAS) are shown in Fig. 8-10. The maximum fresh weight was recorded in Maize crop grown in *Azospirillum* treatment. The lowest fresh weight was recorded at various stages of its growth in the crops grown without biofertilizers. The similar results were noted in in tomato plants [25]. Chlorophyll "a," Chlorophyll "b," and total chlorophyll were all raised by the application of *Azospirillum*, *Azotobacter*, and *Pseudomonas* [26]. According to Lenin and Jayanthi [27], *Catharanthus roseus*'s chlorophyll content increased as a result of the PGPR. Strawberry plants' chlorophyll content was considerably raised by biofertilizer inoculations [28].

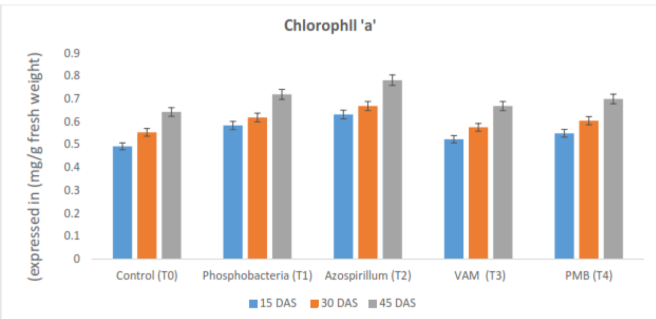

Fig. 8. Effect of different biofertilizers on Chlorophyll 'a' of Maize (*Zea mays* L.) at different growth stages (expressed in (mg/g fresh weight).

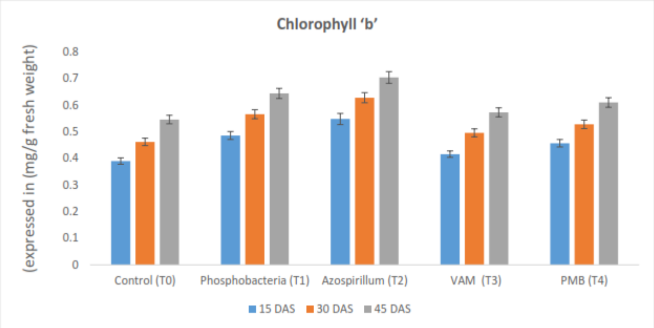

Fig. 9. Effect of different biofertilizers on Chlorophyll 'b' of Maize (*Zea mays* L.) at different growth stages (expressed in mg/g fresh weight).

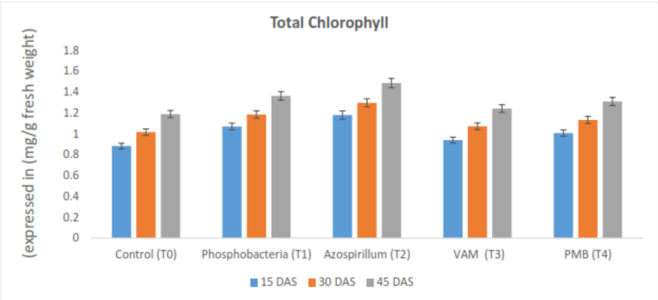

Fig. 10. Effect of different biofertilizers on total chlorophyll of Maize (*Zea mays* L.) at different growth stages (expressed in (mg/g fresh weight).

CONCLUSION

The current investigation highlights the efficacy of a range of biofertilizers, including Phosphobacteria, *Azospirillum*, Vesicular Arbuscular Mycorrhiza (VAM) and Potash Mobilizing Bacteria (PMB), on various growth parameters and biochemical constituents of maize (*Zea mays* L). This study encompassed an assessment of germination percentage, vigor index, seedling growth, fresh and dry weights of seedlings, as well as growth parameters such as shoot length, root length, total leaf area, fresh and dry weights, and pigments content in maize plants. When compared to the control group, all these growth parameters and pigment levels exhibited gradual increases with the application of various biofertilizers. Among the biofertilizers, *Azospirillum* displayed the most significant improvements in assessment to others and the control. In comparison, treatments with organic biofertilizers, such as *Azospirillum*, Phosphobacteria, VAM, and PMB, demonstrated enhanced plant growth and higher pigment content when compared to the untreated control.

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