Expansion of Growth and Yield of *Arachis Hypogaea* using different Biofertilizers

T. Mahakavi [1*], L. Baskaran[2], S. Prabakaran[1], R. Bakiyaraj[1],

[1]*Department of Botany, Government Arts College (Autonomous), Karur, Tamilnadu, India.*
[2]*Department of Botany, Government Arts College, Salem, Tamilnadu, India.*

ABSTRACT

The development of ecofriendly microorganisms, as alternatives to chemical fertilizers in agricultural applications are essential for the proper development of plants, vegetables, flowers and fruits, as they suggest rapid growth with better quality to all species. Pot culture experiment had been done in split design with 3 replicates in Botanical garden, Department of Botany, Government Arts College (Autonomous), Karur. Tamilnadu. To study the effect of single and combined inoculation of Azospirillum (T1), Basillus megaterium (T2), Rhizobium leguminosarum (T3) and Rhizobium leguminosarum + Basillus megaterium + Azospirillum (T4) on growth, photosynthetic pigments, biochemical and yield parameters of Arachis hypogaea L. at various stages. The results of this pot culture experiment revealed that single and combined inoculation with fertilizers significantly increased growth, photosynthetic pigments, biochemical and yield parameters of the test crop. Among various combinations, single inoculation performed better than uninoculated (control) while combined inoculation found better over single inoculation treatments. In general, it could be concluded that, biofertilizer is a good tool to improve the crop yield productivity and quantity and allow for batter environment.

Key Words: - *Arachis hypogaea, Basillus megaterium, Biofertilizer, Inoculation, Environment.*

1. INTRODUCTION

India is the third largest producer of oil seeds in the world. It accounts for 19% of world's area and 9% of the global production [1]. Mustard (*Brassica campestris*), sunflower and Groundnut (*Arachis hypogaea*) is an important oil seeded crop. Application of chemical fertilizers has contributed significantly to the huge increase in the world food production. But the adverse impacts of excessive inputs of chemical fertilizers in conventional agricultural practices are being well documented [2].

The broad apply of chemical fertilizers has concerned the delicate ecological balance of the soil, contaminated groundwater, developed resistant races of pathogens and increased human health risks [3]. Chemical pollutants are extremely dispersed in the environment and cause several problems to human health, soil as well as the environment. In agroecosystems, the use of synthetic toxic chemical pesticides affects the soil fertility and growth of cultivated crops [4]. Therefore, the development of ecofriendly microorganisms, as alternatives to chemical fertilizers in agricultural applications are essential for the proper development of plants, vegetables, flowers and fruits, as they suggest rapid growth with better quality to all species. They have the nutrients necessary for better development. In addition, the organic matter serves as nutrients and energy sources for soil microorganisms [5].

Biofertilizers are known to play an important role in increasing availability of nitrogen and phosphorus also improving biological fixation of atmospheric nitrogen and enhance phosphorus availability to legume plants [6]. Hence, introduction of *Rhizobium* in soil with low nitrogen may help augment nitrogen fixation and thereby boost production of crops. Phosphorus is known to play an important role in growth and development of the leguminous plant and have direct relation with root proliferations, straw strength, grain formation and crop maturation.

Numerous publications have indicated that the well known N_2-fixing microorganism interaction is the legume–rhizobia symbiosis, which is considered the most efficient and important process in crop production, so as to improve soil fertility and farming system flexibility [7]. The growth-stimulating bacteria are *Azospirillum*, *Azotobacter* and *Pseudomonas* which, additionally to biological fixation of nitrogen and solubilization of soil phosphate, considerably affect plant growth regulators especially auxin, gibberellin and cytokinin, hence develop the plant performance. *Azotobacter* is able to produce antifungal compounds that fight plant diseases and improve viability and germination of the plantlets and, as a result, improve the overall plant growth [8].

So it was of interest to investigate whether a synergistic effect would occur if both *Rhizobium leguminosarum*, *Basillus megaterium* and *Azospirillum* were used as an inoculants for legumes. Biofertilizer used in the current study, tested the effect of single and combined inoculation of on growth, biochemical and yield of *Arachis hypogaea* L. at various stages.

2. MATERIALS AND METHODS

During the pot culture experiment was carried out at Botanical garden, Department of Botany, Government Arts College (Autonomous), Karur, Tamilnadu. The treatments contained three fertilizer types, i.e. biofertilizer (*Azospirillum*, *Basillus megaterium* and *Rhizobium leguminosarum*), whereas untreated soil (control). The treatments are given below:

1. Control
2. T1 - *Azospirillum*
3. T2 - *Bacillus megaterium*
4. T3 - *Rhizobium leguminosarum*
5. T4 - *Rhizobium leguminosarum + Bacillus megaterium + Azospirillum*

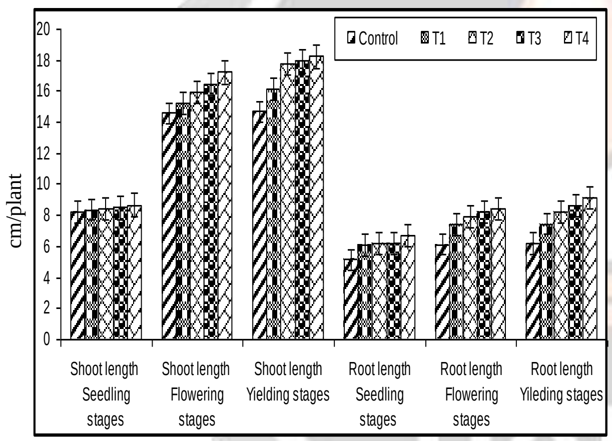
All the experiments were carried out in triplicates. For each set, the totally 16 numbers of biofertilizers inoculated pots were planted and three pots of uninoculated control were also planted. 3kg soil was taken in 3.5kg capacity earthen pot, where first 3kg of soil was filled. Then the soil was moistened with tap water and mixed with different biofertilizers. In each pot, 10 healthy seeds of *Arachis hypogaea* (groundnut) were sowed on the moistened soil. All the pots were watered regularly. Plants sampling was done on the seedling, flowering and yielding stages for the analysis. All the plant samples were immediately packed in labeled polythene bags and brought to the laboratory for analysis.

The following parameters were determined: shoot length (cm), root length (cm) and number of lateral roots were measured; fresh weight (g/plant) and dry weights (g/plant) were recorded after oven drying at 70˚C until reaching a constant weight. Leaf area was measured with an electronic leaf area meter (LAM 101, Disha Online Pvt. Ltd., Raipur, Chattisgarh, India). Number of pods per plant, seeds per pod and weight of 100 seeds was calculated at physiological maturity of plant.

Chlorophyll content (mg g^{-1} fresh weight of leaves) was determined through organic solvent (80% acetone) extraction method as described by [9]. Proteins were estimated according to the method described [10].Total free amino–acids were determined by the method described by [11]. Starch estimation was followed by Anthrone method [12]. The significance of treatments was analysed using one way ANOVA. Significant differences between treatments were determined using Tukey’s multiple range tests (P16.05).

3. RESULTS AND DISCUSSION

The comparison of the different bio-fertilization treatments on growth of groundnut showed Fig. 1. Shoot length and root length of groundnut was increased significantly due to the single application of bio-fertilizers (*Azospirillum* (T1), *Basillus megaterium* (T2), *Rhizobium leguminosarum* (T3) compared with control. While *Azospirillum*(T1) inoculation caused the least increase of these parameters. The highest shoot length and root length was obtained with the combined application of bio-fertilizers (*Rhizobium leguminosarum* + *Basillus megaterium* + *Azospirillum* (T4) in seedling, flowering and yielding stages. In the reason about PSB secrete some organic acids which can solubilize phosphate from insoluble and fixed forms to plant available forms, whereas PSB can convert atmospheric N_2 into plant available form of nitrogen in the soil. In this respect, combined inoculation of *Thiobacillus* with *Rhizobium* increased the shoot, root length and plant biomass on *Arachis hypogaea* [13]. Also, [14] found that the increase in the growth of the biofertilized plants might be due to the ability of *B. megaterium* to produce some growth promoting substances such as IAA, gibberellins and abscisic acid, it is also well-known that *B. megaterium* produces organic, inorganic acids and CO_2 which lead to increase in soil acidity and consequently convert the insoluble forms of phosphorus into soluble ones [15]. The highest value of stem diameter was obtained by using the combined treatment between *Brady*+ *Azoto* + PDB+ AM as it recorded 15.2 cm on *Prosopis chilensis* [16]. Seedlings inoculated with *Azotobacter chroococcum*, *Azospirillum brazilense* and *B. circulans* as soil inoculants gave significant increases in shoot length, root length and shoot dry weight when compared from control with the results obtained by [17] on *Moringa oleifera*.



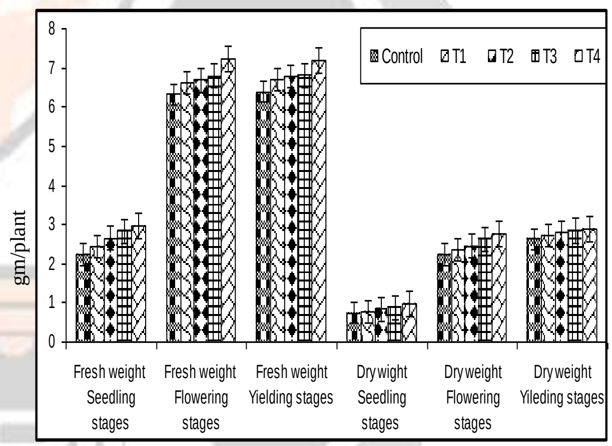


Fig. 1 & 2 Effect of different biofertilizers on morphological parameters (shoot length, root length, fresh weight and dry weight) of groundnut at various stages.

Interaction effect of bio-fertilizer showed significant differences the fresh weight and dry weight of groundnut in seedling, flowering and yielding stages Fig. 2. Compared to different biofertilizers, the lowest fresh weight and dry weight was recorded in control, whereas the combined application bio-fertilizers (*Rhizobium leguminosarum* + *Basillus megaterium* + *Azospirillum* (T4) gave the highest biomass in leguminous plant of groundnut, while *Azospirillum* (T1) inoculation caused the least increases of these parameters. Application of different bio-fertilizers significantly enhanced plant biomass in both seedlings, flowering and yielding stages. The increased shoot and root dry matter weight of plant could increase the chance for nutrients uptake through maximum exploitation of soils. Interaction effect of bio-fertilizer showed significant differences the fresh weight and dry weight of groundnut was recorded in control, whereas the combined application bio-fertilizers gave the highest biomass in groundnut of leguminous plant, while *Azospirillum* (T1) inoculation caused the least increases of these parameters. Application of different bio-fertilizers significantly enhanced plant biomass in both seedlings, flowering and yielding stages.

Accordingly, similar profile clearly illustrated that the application of biological fertilizers in *Calendula officinallis* L. and *Matricaria recutita* increase in plant height and dry and wet weights of the shoots in the medicinal plants [18].

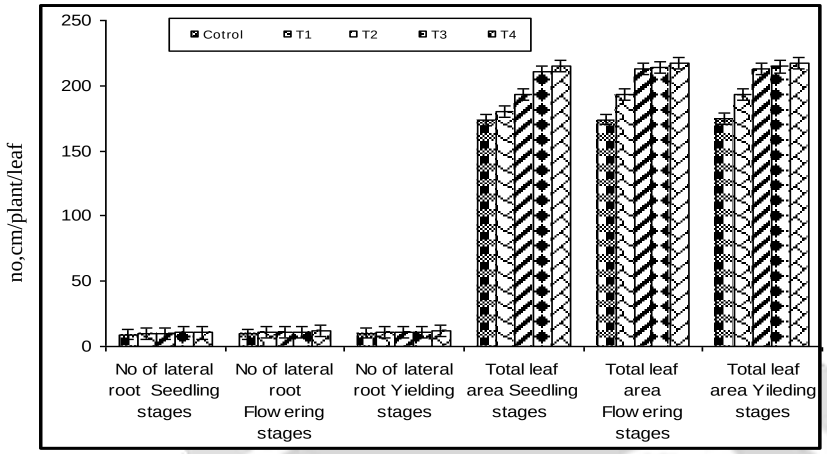


Fig. 3 Effect of different biofertilizers on morphological parameters (no. of lateral root and total leaf area) of groundnut at various stages

Data in Fig. 3 shows that, all tested biofertilizer (*Azospirillum* (T1), *Basillus megaterium* (T2), *Rhizobium leguminosarum* (T3) treatments increased the number of lateral root and total leaf area of *Arachis hypogaea* plant as compared with untreated (control) on seedling, flowering and yielding stages. Combined inoculation (*Rhizobium leguminosarum* + *Basillus megaterium* + *Azospirillum* (T4) produced higher in the lateral root and leaf area at different stages while *Azospirillum* (T1) inoculation caused the least increase of number of lateral root and total leaf area. The reason, improved leaf area in plants by nitrogen content of leaves is rapidly converted to protein and increased the leaf area. It has already been reported by [19,20].

The obtained results revealed that photosynthetic pigments chl ‘a’, ‘b’ and carotenoid was significantly increased by applied different biofertilizers on *Arachis hypogaea* shown in Fig. 4. However, the highest content of photosynthetic pigments (chl ‘a’, ‘b’ and carotenoid) was obtained by using the combined treatment of (*Rhizobium leguminosarum* + *Basillus megaterium* + *Azospirillum* (T4) as it recorded, while the inoculation with *Azospirillum* (T1) showed its superiority in this concern at seedlings, flowering and yielding stages. In this respect, [21] the combination treatments between PGPR and Mycorrhizae have the highest values in the presence of Putrescine at 2.5 mM compared with the individual application and the control. Similar results were reported by [16,22]. In some previous works ([23,24]).

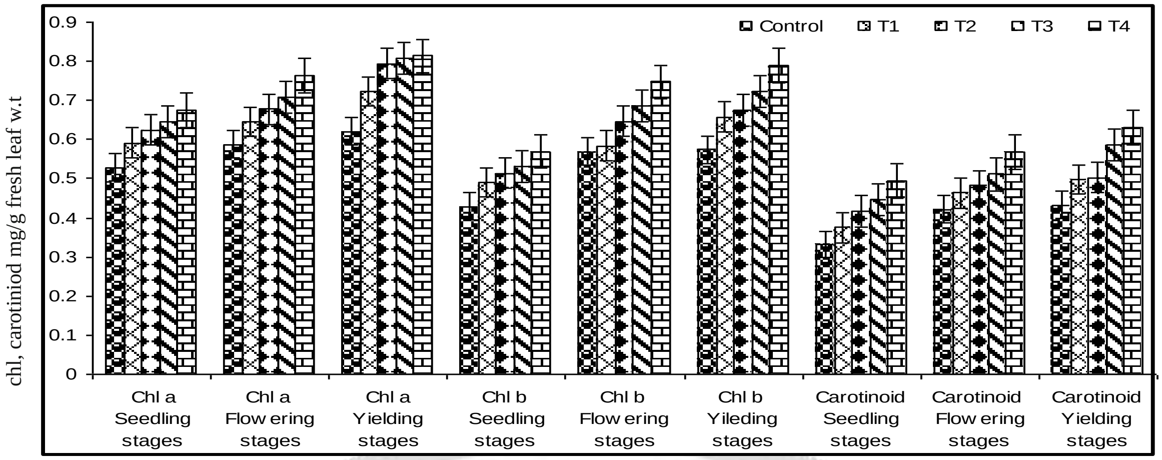
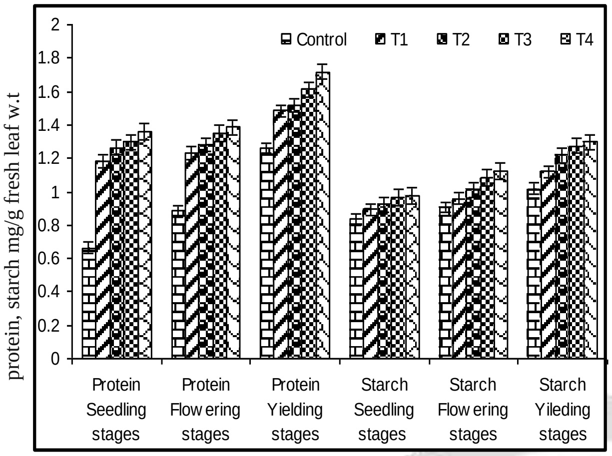


Fig. 4 Effect of different biofertilizers on biochemical parameters (chl a, b and carotenoid) content of groundnut at various stages.

From this Fig. 5 it could be concluded that all treatments of biofertilizer (*Azospirillum* (T1), *Basillus megaterium* (T2), *Rhizobium leguminosarum* (T3) gave an increase in protein and starch content when compared to control at seedlings, flowering and yielding stages. The treatments which gave the utmost increase in protein and starch (g/plant) were (*Rhizobium leguminosarum* + *Basillus megaterium* + *Azospirillum* (T4) used as combined inoculants while *Azospirillum* (T1) single inoculation caused the least increase of total protein and starch content of *Arachis hypogaea*. It may be described to increased nitrogen uptake of leaves due to the combined inoculation of biofertilizers. Because biofertilizer supports phytohormones production, which stimulate nutrients absorption as well as photosynthesis process, as a result of this protein content increases [25]. In addition, in a study, 1 % biofertilizer (*Ulva lactuca*) along with 50 % recommended rate of chemical fertilizers improved the content of protein and carbohydrate in *Tagetus erecta* [26].

The total sugar and amino acid content of the *Arachis hypogaea* plants as affected by inoculation with different biofertilizer at seedlings, flowering and yielding stages are presented in Fig. 6. Total sugar and amino acid content of groundnut was increased significantly due to the single application of bio-fertilizers (*Azospirillum* (T1), *Basillus megaterium* (T2), *Rhizobium leguminosarum* (T3) compared with control. While single inoculation of *Azospirillum* (T1) caused the least increase of this biochemical content. The highest total sugar and amino acid was obtained with the combined application of bio-fertilizers (*Rhizobium leguminosarum* + *Basillus megaterium* + *Azospirillum* (T4) in seedling, flowering and yielding stages. Our findings were similar with the earlier findings on *Sideritis Montana* ([27,28]) in mulberry leaves and on Iris [29]. However, it has been reported that seaweed liquid fertilizer at 10 % extracted from brown alga (*Sargassum wightii*) increased the content of protein and total sugars in *Vigna radiata* [30].



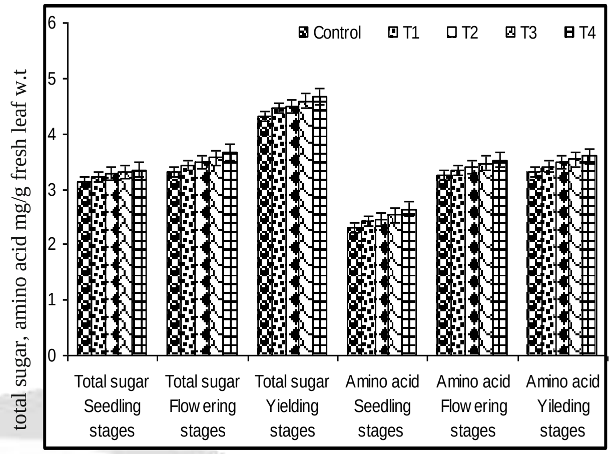
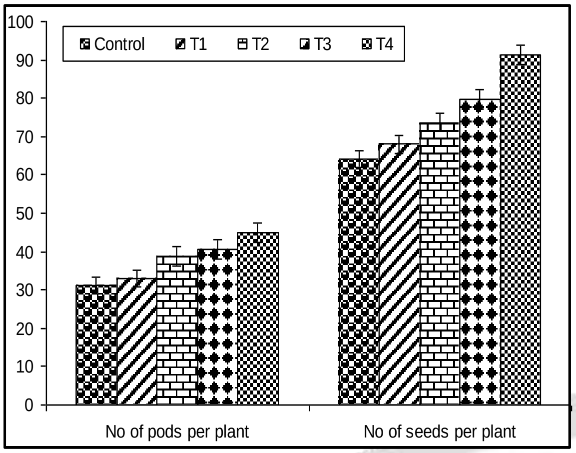


Fig. 5 and 6 Effect of different biofertilizers on biochemical parameters (protein, starch, total sugar and aminoacid) content of groundnut at various stages.

Biofertlizers application which consisted of (*Azospirillum* (T1), *Basillus megaterium* (T2), *Rhizobium leguminosarum* (T3) have a considerable effect on yield (number of pods per plant and number of seeds per plant) compared with untreated control Fig. 7. It could be noticed that all treatments single biofertilizers increased yield of *Arachis hypogaea*. Concerning to single inoculation of *Rhizobium leguminosarum* (T3) treatment gave the highest yield (number of pods per plant and number of seeds per plant) followed by *Basillus megaterium* (T2), and finally *Azospirillum* (T1) treatment. The best result was obtained in case of combined inoculation; a similar finding was also reported by [31]. The inoculation of effective microorganisms at the rate of 90 ml/palm/year combined with potassium sulfate at 1.5 kg/palm/year as a soil inoculation has enhanced fruit set percentage, retained fruit percentage, yield and fruit quality of ‘‘Hayany’’ date palm cv. [32].

The best result was obtained in case of combined inoculation, *Rhizobium leguminosarum* + *Basillus megaterium* + *Azospirillum* (T4). Yield in terms of weight of seeds per plant and weight of seeds per 100 seeds is presented in Fig. 8. weight of seeds per plant and weight of seeds per 100 seeds of groundnut *Arachis hypogaea* was increased significantly due to the single application of bio-fertilizers (*Azospirillum* (T1), *Basillus megaterium* (T2), *Rhizobium leguminosarum* (T3) compared with control, whereas the combined application of biofertilizers (*Rhizobium leguminosarum* + *Basillus megaterium* + *Azospirillum* (T4) gave the highest yield was observed, while *Azospirillum* (T1) inoculation caused the least increases of these parameters. Similar results agreement with [33] on guar plants. *Rhizobium* has been used as a proficient nitrogen fixer for many years. It plays a main role in increasing yield by converting atmospheric nitrogen into usable forms [34].



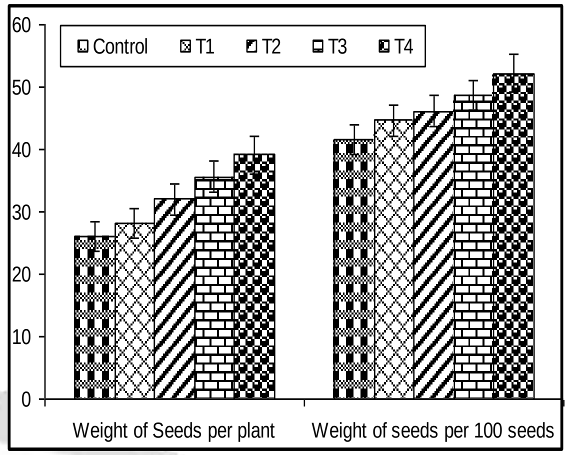


Fig. 7 and 8 Effect of different biofertilizers on yield parameters of groundnut at harvesting stage.

4. CONCLUSION

From the result of the study presented here, it can be concluded that groundnut (*Arachis hypogaea*) growth, biochemical and yield could be promoted by the different biofertlizers application. The combined application of different biofertilizers (*Rhizobium leguminosarum* + *Basillus megaterium* + *Azospirillum* (T4) had much more favorable results than other single application on the growth, biochemical and yield. Based on these findings, it is recommended that the combined application of bio-fertilizers (*Rhizobium leguminosarum* + *Basillus megaterium* + *Azospirillum* (T4) is economical and suitable for the growth of groundnut (*Arachis hypogaea*).

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