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Effects of Biofertilizers on Primary Growth and Biochemical Parameters of Green Gram

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ABSTRACT

Biofertilizers are microscopic living things that can improve soil quality. The fact that biofertilizers are eco-friendly is one of their many wonderful features. Because of this, they are a suitable fit for sustainable agricultural methods. To address this issue, the current investigation was conducted as a pot experiment to assess how different biofertilizers affected the development and biochemical composition of green gram (Vigna radiata). The findings of current research work explored that when compared to untreated control plants, highest plant height (37.18 cm), number of leaves (9.2), fresh weight (1.430 g/plant), dry weight (0.506 g/plant), chlorophyll a (0.564 mg/g fresh weight), chlorophyll b (1.018 mg/g fresh weight), protein (0.216 mg/g fresh weight) and sugar content (0.018 mg/g fresh weight) were observed in the combined inoculation of Azospirillum brasilense + Glomus fasciculatum + Pseudomonas fluorescens. Additional research is required to assess how biofertilizers affect green gram production and other characteristics.

Keywords: Biofertilizers, Primary Plant Growth, Phytochemical Contents, Green Gram.

1. INTRODUCTION

The soil contains a variety of microorganisms that affect plant development, including actinomycetes, bacteria, algae, and fungi. Plant growth promoting rhizobacteria (PGPR), which live near roots, promote both plant development and agricultural productivity. Plant growth-promoting bacteria (PGPB) [1] are Free-living bacteria found in soil, rhizosphere, rhizoplane, and phyllosphere that, under certain situations, are beneficial to plants [2].

Azospirillum brasilense, the most studied PGPB, has a lot of potential for usage in industry. The findings of several research works revealed that A. brasilense enhanced the growth and yield of numerous plant species, many of which are significant for agronomy or ecology [3-5].

An arbuscular mycorrhizal fungus (AMF) called Glomus fasciculatum develops a symbiotic connection with plants, enhancing their ability to absorb water and nutrients and strengthening their immune systems. By transforming insoluble forms into soluble ones and growing the root system, this

G. fasciculatum biofertilizer improves the plant's access to vital minerals including phosphorus and nitrogen. Consequently, it improves plant growth, quality, and resistance to environmental stress and diseases [6].

Pseudomonas fluorescens, a drought-tolerant strain, has characteristics that promote plant development. The quantity of osmolytes, antioxidants, and chlorophyll rose when P. fluorescens was used. It is related to the ability of this strain of bacteria to manufacture ACC deaminase. It also enhanced nutrient and water absorption, which maintains the pace of photosynthesis [7].

With an annual global production of 79 million metric tons, grain legumes are one of the most significant food crops grown worldwide [8]. They are essential to healthy diets, sustainable food production, and food security since they have long been regarded as an excellent source of protein. Additionally, through the processes of nutrient cycling and biological nitrogen fixation (BNF), legumes have a number of beneficial effects on maintaining soil health. Green gram (Vigna radiata (L.) R. Wilczek) is one of the major grain legumes of India. It is cultivated well in a variety of agricultural systems and has a short growing season. It is grown on 4.25 million hectares area an average yield of 567 kg ha^{-1} and an output of 2.41 million tons [9].

The organic fertilization approach reduces environmental impact while taking into account the flexibility of native PGPR strains and their capacity to encourage plant development [10]. To gauge the impact of organic derivatives on productivity and quality, it is especially important to understand the simple or mixed effects of native microbiological strains with possible applications in agriculture [11,12]. Hence, the current research work was conducted to evaluate the single and combined effects of A. brasilense, G. fasciculatum and P. fluorescens on primary growth and biochemical contents of V. radiata.

2. MATERIALS AND METHODS

2.1. Seed source

Seeds of green gram var. PK10 were obtained from the Department of Plant Breeding in Tamil Nadu Agricultural College and Research Institute, Madurai. After being surface sterilized with a 0.1v/v mercuric chloride solution, the seeds were planted in mud pots and maintained in nursery conditions. An equal amount of garden soil was placed in each pot. One pot was kept as control (uninoculated) and four pots as testing (biofertilizer inoculated) pots with three replications. For thirty days, the pots were watered every day.

2.2. Biofertilizers

The lignite-based biofertilizers included Pseudomonas fluorescens and Azospirillum brasilense, with a population load of 108 cfu/g, was acquired from culture collection centre of the Department of Agricultural Microbiology, Tamil Nadu Agricultural College and Research Institute, Madurai. In accordance with Gerdemann and Nicolson's method [13], Glomus fasciculatum was isolated from the rhizosphere soil of Azadirachta indica, which was found in the forest nursery garden in Madurai. It was then multiplied in pot culture using a 1:1 v/v mixture of sterilized soil and sand, and it was kept in the roots of Sorghum vulgare as the host plant.’

2.3. The use of biofertilizer

In the root zone of each seedling, 10 g of lignite-based A. brasilense and P. fluorescens with a population load of 108 cfu/g and 10 grams of G. fasciculatum inoculum containing extra metrical hyphae, chlamydospores, and infected root segments were added individually and combined (totally in 10 g of A. brasilense + G. fasciculatum + P. fluorescens).

2.4. Growth data

15 plants (five plant samples from each replication) were chosen at random on the 30th day following planting in order to record growth metrics such as shoot length, root length, total length, and number of leaves. Using an electronic weighing balance, the fresh and dried weights of the shoot and root were calculated individually. Standard deviation analysis was used to determine how the estimated data responded to different treatments.

2.5. Biochemical determination

The Arnon method [14] was used to evaluate the concentration of chlorophyll, the Lowry et al. [15] technique was used to estimate the protein content and the Dubois et al. [16] method was used to estimate the soluble sugar.

3. RESULTS AND DISCUSSION

The seedlings of green gram (V. radiata) were inoculated with various biofertilizer alone and in combination, and 30-day-old plants were examined for growth parameters like plant length (shoot and root length), number of leaves, fresh weight (shoot and root), dry weight (shoot and root), and specific biochemical parameters like chlorophyll, protein, and soluble sugar.

3.1. Growth parameters

For the current research work, green gram seeds were germinated in five pots with three replicates and the germination percentage of all the pots were equal (100%). The biofertilizers were applied in every pot except the control after five days of germination. The results revealed that when compared to uninoculated plants, treated plants showed much higher growth. The plants that received mixed inoculation of biofertilizers exhibited the highest growth.

3.1.1. Shoot length

The plants treated with a combination of biofertilizers showed a significant increase in shoot length followed by single inoculation. Plants treated with a combination inoculation reached their maximum shoot length of 26.58 cm. In comparison to the control (15.64 cm), seedlings treated with A. brasilense showed the highest height (23.40 cm), followed by P. fluorescens (20.56 cm) and G. fasciculatum (19.42 cm) among single inoculation (Table 1; Figure 1).

3.1.2. Root length

The findings of current research work showed that in comparison to the control (7.48 cm), the combination inoculation had a longer root length about 11.46 cm. When A. brasilense was used as a single inoculant, the seedlings exhibited the longest roots compared to P. fluorescens and G. fasciculatum measuring 10.6, 9.58, and 8.66 cm, respectively (Table 1; Figure 1).

Table 1: Effect of biofertilizers on the growth of green gram

Growth parameters	Treatments				
	Control	A. brasilense	G. fasciculatum	P. fluorescens	Combined
Shoot length (cm)	15.64 ± 0.1816	23.40 ± 0.1581	19.42 ± 0.2588	20.56 ± 0.1516	26.58 ± 0.1643
Root length (cm)	7.48 ± 0.2588	10.60 ± 0.2408	8.66 ± 0.2073	9.58 ± 0.1643	11.46 ± 0.2121
Total length (cm)	23.12 ± 0.2863	34.90 ± 0.2828	28.08 ± 0.2949	30.14 ± 0.2792	37.18 ± 0.1788
No. of leaves	2.6 ± 0.5477	5.4 ± 0.4472	5.2 ± 0.8366	4.8 ± 0.4472	9.2 ± 0.4472

Values are Means of three replicates ± Standard Deviation

3.1.3. Total length

When compared to the control (23.12 cm), the combined inoculation had a greater overall length (37.18 cm). When given a single inoculation, seedlings treated with A. brasilense had maximum total length than P. fluorescens and G. fasciculatum and it was recorded as 34.9, 30.14, and 28.08 cm, respectively (Table 1; Figure 1).

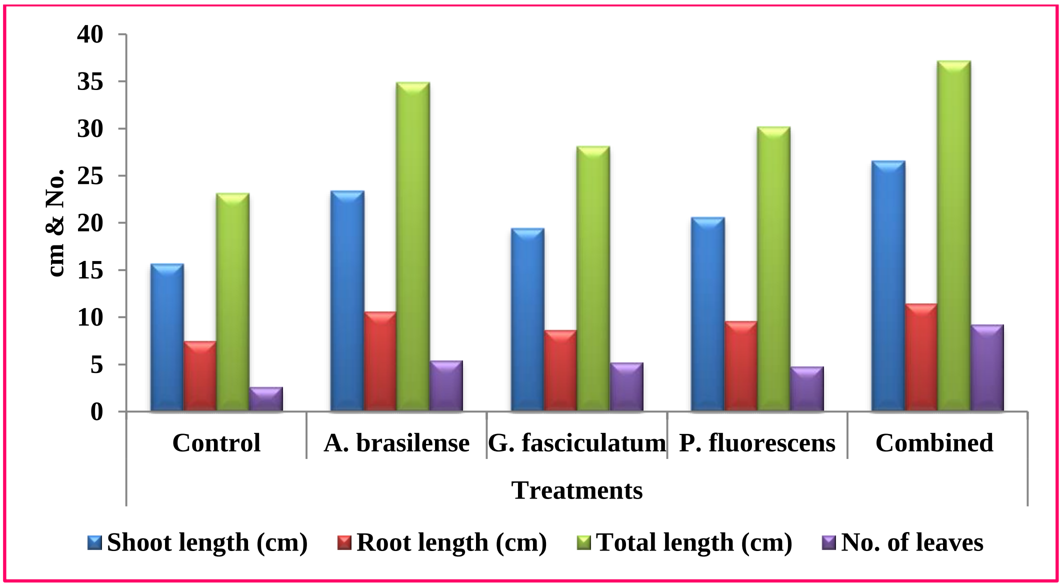

Figure 1: Effect of biofertilizers on the growth of green gram

3.1.4. Number of leaves

The seedlings that received a combination inoculation followed by individual inoculations of A. brasilense, G. fasciculatum and P. fluorescens had more leaves than the control seedlings. It was noted as as 9.2, 5.4, 5.2, 4.8 and 2.6 respectively (Table 1; Figure 1)

3.2. Biomass

The findings of present study also showed that in terms of fresh and dry weight, the green gram seedlings treated with biofertilizer had more biomass than the untreated control seedlings.

3.2.1. Total fresh weight

When compared to the control (0.914 g/plant), the triple inoculation of A. brasilense, G. fasciculatum and P. fluorescens produced the highest total fresh weight of both shoot and root (1.430 g/plant). In case of single inoculation, A. brasilense inoculated seedlings noted with maximum total fresh weight (1.390 g/plant) than P. fluorescens (1.234 g/plant) and G. fasciculatum (1.046 g/plant) (Table 2; Figure 2).

Table 2: Effect of biofertilizers on the fresh weight (g/plant) of green gram

Growth parameters	Treatments				
	Control	A. brasilense	G. fasciculatum	P. fluorescens	Combined
Shoot	0.838 ± 0.0130	1.256 ± 0.0207	0.954 ± 0.0181	1.116 ± 0.0151	1.276 ± 0.0114
Root	0.076 ± 0.0089	0.134 ± 0.0167	0.092 ± 0.1090	0.118 ± 0.0083	0.154 ± 0.0181
Total	0.914 ± 0.0089	1.390 ± 0.0300	1.046 ± 0.0270	1.234 ± 0.0114	1.430 ± 0.0122

Values are Means of three replicates ± Standard Deviation

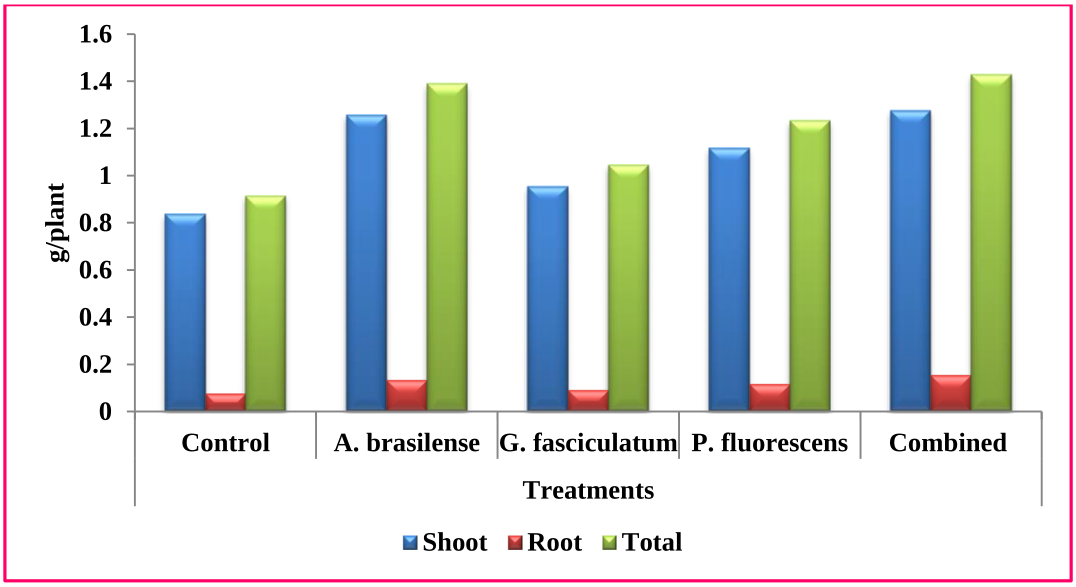

Figure 2: Effect of biofertilizers on the fresh weight (g/plant) of green gram

3.2.2. Total dry weight

In case of total dry weight, when compared to the control (0.144 g/plant), the combined inoculation of A. brasilense + G. fasciculatum + P. fluorescens produced the highest total dry weight of both shoot and root (0.506 g/plant). In case of single inoculation, P. fluorescens inoculated seedlings ahowed maximum total fresh weight (0.384 g/plant) than G. fasciculatum (0.316 g/plant) and A. brasilense (0.295 g/plant) g/plant (Table 3; Figure 3).

Table 3: Effect of biofertilizers on the dry weight (g/plant) of green gram

Growth parameters	Treatments				
	Control	A. brasilense	G. fasciculatum	P. fluorescens	Combined
Shoot	0.118 ± 0.0083	0.228 ± 0.0083	0.272 ± 0.0080	0.324 ± 0.0054	0.426 ± 0.1100
Root	0.026 ± 0.0089	0.036 ± 0.0089	0.044 ± 0.0114	0.660 ± 0.0070	0.074 ± 0.0089

Total	0.144 ± 0.0167	0.295 ± 0.0151	0.316 ± 0.0080	0.384 ± 0.0089	0.506 ± 0.0207

Values are Means of three replicates ± Standard Deviation

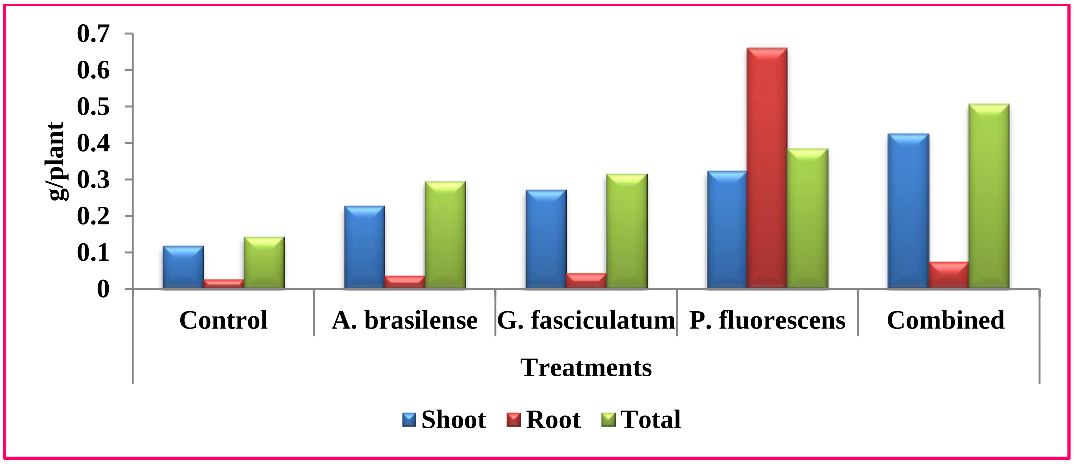

Figure 3: Effect of biofertilizers on the dry weight (g/plant) of green gram

3.3. Biochemical parameters

3.3.1. Chlorophyll

As demonstrated by increases in both chlorophyll a and chlorophyll b concentrations, green gram seedlings treated with different biofertilizers had higher total chlorophyll content than the control. The seedlings treated with a combination inoculation of A. brasilense + G. fasciculatum + P. fluorescens had the highest levels of chlorophyll a and b about 0.564 and 1.018 respectively (mg/g fresh weight) (Table 4; Figure 4).

3.3.2. Soluble protein

Soluble protein concentration increased marginally in green gram seedlings treated with various biofertilizers. The protein content of the plants treated with mixed inoculation, G. fasciculatum, P. fluorescens A. brasilense was determined as 0.216, 0.106, 0.080, and 0.053 mg/g fresh weight respectively. The uninoculated seedlings showed least protein content (0.026 mg/g fresh weight) (Table 4; Figure 4).

Table 4: Effect of biofertilizers on the biochemicals (mg/g fresh weight) of green gram

Biochemical parameters	**Treatments**				
	Control	**A. brasilense**	**G. fasciculatum**	**P. fluorescens**	**Combined**
Chlorophyll a	0.196	0.309	0.462	0.230	0.564
Chlorophyll b	0.354	0.558	0.833	0.415	1.018
Total chlorophyll	0.652	0.992	1.352	0.809	1.583
Soluble protein	0.026	0.053	0.106	0.080	0.216
Soluble sugar	0.010	0.008	0.007	0.012	0.018

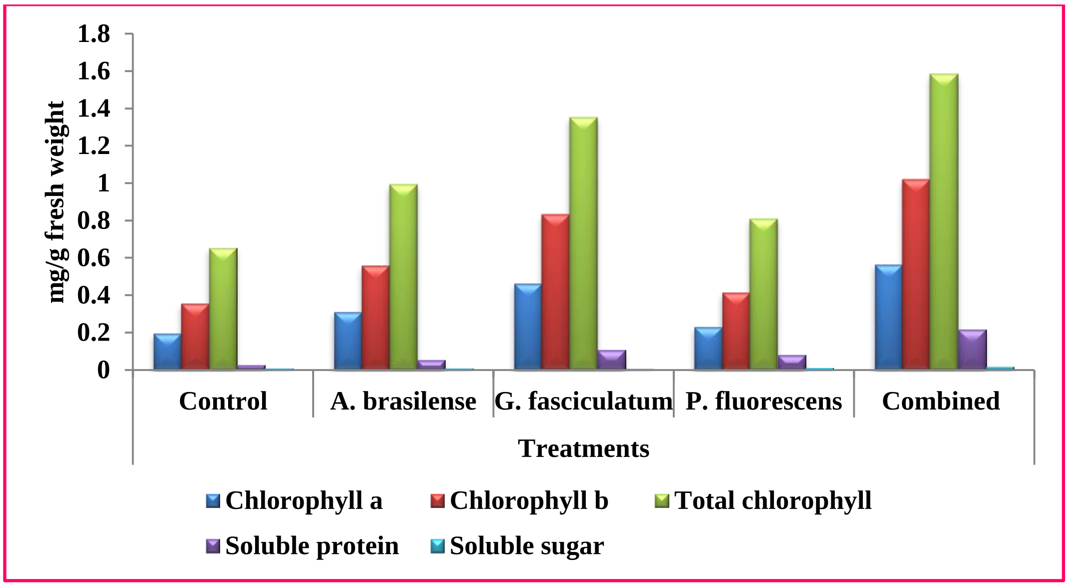

Figure 4: Effect of biofertilizers on the biochemicals (mg/g fresh weight) of green gram

3.3.3 Soluble sugar

The amount of soluble sugar in the green gram seedlings treated with different biofertilizers increased significantly. The plants treated with combination inoculation, P. fluorescens, A. brasilense and G. fasciculatum had sugar contents of 0.018, 0.012, 0.008, and 0.007 (mg/g fresh weight), respectively. The control seedlings were noted with least sugar content (0.010 mg/g fresh weight) (Table 4; Figure 4).

The current study estimated a number of characteristics of green gram plants, such as plant height, fresh and dried weights of the roots and shoots, and the amounts of protein, sugar, and chlorophyll. The seedlings treated with a combination inoculation of A. brasilense + G. fasciculatum + P. fluorescens showed the highest levels of all the parameters examined. According to earlier reports, A. brasilense promotes nutrient absorption and root development [17], which together optimize nutrient availability and minimize stress, hence bolstering the resilience and growth of green gram plants [18, 19].

It was also reported one of the most important impacts of mycorrhizal inoculation, such as that of G. fasciculatum, on the host plant is the increase in phosphorus uptake [20], which is caused by the increased ability of the plants to absorb more phosphorus from the soil. Through the provision of phosphorus and other immobile nutrients that are necessary for nitrogen fixation, AM fungus assisted nitrogen fixation in legumes [21]. Tavasolee et al. [22] reported that rhizobial infection performance can be improved by effective AM fungus, and vice versa, resulting in increased growth and other biochemical contents. This scientific evidence supports the findings of the current study.

Numerous studies have demonstrated that the adaptable bacteria Pseudomonas sp. detoxifies heavy metals and stimulates plant growth. To lessen the harmful effects of heavy metals on plants and other species, these bacteria release enzymes and compounds known as metal-chelating agents. Additionally, these bacteria promote plant development and nutrient uptake by producing phytohormones [23-25]. The results of our current investigation are consistent with several studies on the enhanced growth and other characteristics in Samanea saman [26], Feronia elephantum [27], Aegle marmelos [28], and other plants resulting from the combination inoculation of Azospirillum, AM fungus, and Pseudomonas.

4. CONCLUSION

Collectively, the findings of current research work highlighted that the combined application of Azospirillum brasilense, Glomus fasciculatum and Pseudomonas fluorescens showed encouraging favorable effects on the development and biochemical contents of green gram (Vigna radiata) cultivated in pots under natural conditions. According to this, it can be concluded that the application of combination inoculation of biofertilizer features may be a more innovative and successful strategy for improving development in natural conditions. More research is required to estimate the effect of various biofertilizers on yield and other attributes of green gram.

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