Beneficial Effect Of Biofertilizers And Chemical Fertilizer On The Growth And Biochemical Characters Of Green Gram (Vigna Radiata (L.) R. Wilczek)

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Abstract— **Nitrogen and phosphorus are essential for the normal functioning of plants, as it participates in the composition several biomolecules. The continuous application of these elements through inorganic fertilizers to agricultural field affects the soil environment. It is directly affected the sustainable growth crop plants. The use of plant growth-promoting bacteria (PGPB) may be an alternative to reduce the usage of chemical fertilizer or inorganic fertilizers. Thus, using beneficial microorganisms that promote efficient growth from seedling to plant is relevant in the crop production system, especially in regard to the organic system, which is dependent on alternative inputs. Under nursery conditions, the applications of Azospirillum and PSB have the beneficial effect on growth, development and biochemical characters of green gram. The beneficial effect was higher in dual inoculation of biofertilizers rather than single application. Further, the effect was comparable to urea as inorganic fertilizer. Hence, the response of dual application of biofertilizers (Azospirillum and PSB) as organic fertilizer is comparable the urea as inorganic fertilizers.**

Index Terms— **organic fertilizer, inorganic fertilizers, Azospirillum, PSB, crop response.**

I. INTRODUCTION

Modern agricultural practices are emphasizing the widespread use of fertilizer and this approach has certainly increased grain yields in many countries in the last three decades. However, long term use of chemical fertilizers also led to a decline in crop yields and soil fertility in the intensive cropping system. There is evidence that over fertilization has increased the concentration of many plant nutrients in both surface and ground water, which has created a potential health hazard. This has in turn paved the way for integrated plant nutrition involving judicious and integrated use of chemical/synthetic sources of nutrients along with biofertilizers in addition to nutrient recycling through use of organic manures, green manuring and biodegradable wastes, etc. Biofertilizers offer a cheaper low capital intensive and eco-friendly route to boosting farm productivity depending upon their activity of mobilizing different nutrients.

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Integrated Nutrient Management (INM) holds great promise in meeting the growing nutrient demands of intensive agriculture and maintaining the crop productivity at higher levels with overall improvement in the quality of resource base [1].

Biofertilizers are carrier based preparations containing beneficial microorganisms in a viable state. They improve soil fertility and promote plant growth. Biofertilizers are broadly classified into nitrogen fixers, phosphate solubilizers, phosphorus mobilizers and organic matter decomposers. They enhance certain biological processes by which the nutritionally important elements make available to the plants. Biofertilizers alone cannot produce yield similar to chemical fertilizers [2]. Integration of biofertilizers with inorganic fertilizers at rates below those recommended for optimum plant production may result in saving of inorganic fertilizers and sustain production [3].

Azospirillum is a rhizosphere bacterium colonizing the roots of crop plants making use of root exudates and fixes substantial amount of atmospheric nitrogen. They exert beneficial effects on growth and yield of many economically important crops. There are extensively used in rice and other cereal crops as biofertilizers [4]. Biofertilizers accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants. They help in restoring soil health and thus provide a cost effective way to manage crop yield along with balancing the environment [5]. Phosphate Solubilizing Bacteria (PSB) plays an important role in converting insoluble chemically fixed soil phosphorus into available form. The mechanism of solubilization of insoluble phosphate is ability to secrete organic acids and phosphatase enzyme [6]. PSB are able produce phytohormones and inhibition of deleterious pathogens in the soil. The plant growth benefits due to the addition of PSB include increases in germination rate, root growth, yield, leaf area, chlorophyll content, tolerance to drought, shoot and root weight. Such group of bacterial inoculation as biofertilizers enhances P accumulation and biomass production of plants [7].

Biofertilizers improve growth and yield of ginger by increasing availability and uptake of nutrients, other micronutrients and production of growth promoting substance. Organic manures and biofertilizers offer as an alternative to chemical inputs and are being increasingly used in spice production today. On the other hand, biofertilizers are cost effective and renewable source of plant nutrients to supplement the part of chemical fertilizers. Biofertilizers are beneficial microorganisms which are one of the low cost inputs and have the ability to mobilize the nutrients from non-available to available forms besides producing growth promoting and antifungal substances [8]. Thus, they ensures saving a substantial amount of chemical fertilizers which eventually reduce the cost of production.

II. MATERIAL AND METHODS

The seeds of green gram *Vigna radiata* were procured from Tamil Nadu Agriculture Research Center, Kovilpatti, Tamil Nadu. The green gram seeds with uniform size, colour and weight were chosen for the experimental purpose and surface sterilized with mercuric chloride. Seeds were pre-soaked for 12 hours in distilled water and were sown in sterilized soil mixture. The soil mixture was prepared by mixing black soil, red soil and sand in the ratio of 1:1:1. Biofertilizers such *Azospirillum*, PSB alone, *Azospirillum* combined with PSB and chemical fertilizer, Urea were applied 10g each at the top soil of the pots.

The growth parameters such as seed germination [9], germination index [10], seedling vigour index I (SVI I) and II (SVI II) [11], shoot length, root length, fresh weight and dry weight were studied in the treated and untreated control plants. The number of seeds germinated in each treatment was counted on 7 day after sowing. The final count of germination was recorded on the 7th day and number of normal seedlings was expressed as per cent germination. Germination Index was calculated with the number of germinated seeds and day first count to the days of last count. Seedling Vigour Index was calculated with the help of data recorded on germination percentage and seedling growth. Seedling Vigour Index II was calculated with the help of data recorded on germination percentage and seedling dry weight.

Plants were uprooted without causing any damage to the seedlings and it was thoroughly washed with tap water in order to remove soil and debris particle. Then the root length and shoot length was measured with the help of meter scale and expressed in centimeter. The fresh weight of whole plant parts (shoot, leaves and root) was weighed using electronic balance. The fresh undamaged whole plant system of seedlings were kept in the oven at 80°C 4-6 hours and the dried seedlings were weighed using electronic balance. The biochemical characters such as chlorophyll and carotenoid [12], soluble sugar [13], soluble protein [14], free amino acids [13] and nitrate reductase activity [15] were analyzed. The data were reported as mean ± SE and in the figure parentheses represent the percent activity. Values are expressed as means ± standard deviation of three independent data.

III. RESULTS AND DISCUSSION

A. Growth Characters

The nursery experiment revealed that there was a considerable improvement in growth of *Vigna radiata* with biofertilizer and chemical fertilizer with reference to seed germination, seedling vigour index, shoots length, root length, plant fresh weight and plant dry weight. The growth characters were higher in the plants treatment with biofertilizers and urea over the control plants. The results proved that combined inoculation of *Azospirillum* beneficial microorganism increased the plant growth response in green gram. Little difference was shown in seed germination and germination index among different fertilizers. But the effect was comparatively higher than control. The values of the vigour index of the green gram also differed according to the nature of microbes. The low amount of vigour index was shown by the control plant and the high amount of vigour index was occur in the application of soil to *Azospirillum* + PSB followed by Urea. The drift was similar in both in SVI- I (based on seedling length) and SVI- II (based on seedling dry weight) (Table I).

The effect of inoculation of *Azospirillum*, PSB and urea on the root and shoot length in nursery plants was tested. Results indicated that the root length was higher in all treated plants over the control. It was observed that the plant grown with *Azospirillum* + PSB produced taller root (12.5cm) followed by urea. The experiment revealed that the dual inoculation of *Azospirillum* + PSB improved the shoot length compared to control and biofertilizer alone. The fresh weight was comparatively maximum 2.79g in plants treated with *Azospirillum* + PSB followed by urea. There was not much variation in the fresh weight of plants treated with *Azospirillum* + PSB and alone. The analysis of the dry weight of plant was ranging from 0.29- 0.59g. The maximum dry weight was found in plant treated with *Azospirillum* + PSB followed by urea (Table II). In general, the dual inoculation of *Azospirillum* + PSB gave a better response in plant growth and development of green gram. It is also comparable to the chemical fertilizers in all growth characters.

The effect of biofertilizers (*Azospirillum*, PSB combined and alone) and chemical fertilizer (Urea) on morphological and biochemical parameters in green gram is discussed. The synergistic effect of biofertilizers and chemical fertilizers on green gram was tested under nursery condition. The experiment revealed that the inoculation of *Azospirillum* and PSB improved the morphological as well as biochemical characters in green gram. The result clearly indicated that the combined inoculation of biofertilizers and chemical fertilizer significantly improved the both morphological and biochemical parameters. These improvements are due to various dynamic properties of biofertilizers and chemical fertilizer. The seedling vigour index and dry weight of seedlings which really indicate the overall seed quality has varied significantly with the treatments and these values were significantly higher with application of organic manure [16]. The increase in plant weight with application of organic manures may be due to better nutrient availability and its uptake by mother plant. This might have lead to accumulation of higher quantities of seed components like calcium carbonate and increased the lipid metabolisms which help in increasing the protein content in seed. These results are in akin with findings in barley [17].

Biofertilizer as a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers have a natural mechanism to supply nutrients to plants by solubilizing phosphorus, nitrogen fixation and by synthesis of plant growth promoting substances. There are microbes present in biofertilizers that increase the soil natural nutrient cycle and help in building soil organic matter and maintain the soil fertility [18]. Phosphate solubilizing bacteria of *Pseudomonas* and *Bacillus* species are producing phytohormones *i.e.* auxins, inhibition of deleterious pathogens or nutrient mobilization and ammonification. The plant growth benefits due to the addition of PSB include increases in germination rate, root growth, yield, leaf area, chlorophyll content, tolerance to drought, shoot and root weight. Further, the inoculation of PSB as biofertilizers enhanced the P accumulation and biomass production of plants [7]. The action of PSB was not only due to the release of plant available phosphorus but also the production of biologically active substances like Indole acetic acid, gibberellins; cytokinins production was also correlated with P solubilization. The favourable effect of the inoculants on plant growth and nutrient uptake was due to the production of growth promoting substances by PSB [19]. The available phosphorus content of the soil, nodulation, root and shoot biomass, straw and grain yield were increased due to PSB inoculation as compared to the inoculated controls.

B. Biochemical Characters

It was observed that the total chlorophyll content of green gram showed much significant in urea and biofertilizers treatment. Total chlorophyll content was more in plant treated with urea followed by *Azospirillum* + PSB. The carotenoid content was more in plants treated with *Azospirillum* + PSB and urea and least with control in plants. The control plant showed the carotenoid content only 0.79 mg/g LFW. The protein content was comparatively more in plants treated with dual inoculation of biofertilizers followed by urea treatment. The result revealed that there was marked difference in the glucose content among treatments. Among them, glucose content was higher in plants treated with *Azospirillum* and PSB and urea and least in control. Application of biofertilizers and urea increased the free amino acid in leaves of green gram. NR activity was estimated in leaves of treated and control plants. The results indicated that there was not much variation in NRA among treatments in green gram. But effect was far better than control plants (Table III).

The significant difference was observed in growth and biochemical parameters in *Abelmoschus esculentus* with reference to treatment of *Azospirillum* with different carrier materials. The response was varied with respect to carrier materials. The coir pith formulation had superior effect in all the growth response. The effect was mainly due the incorporation of *Azospirillum* strain and addition to this the nature of carrier material also have the beneficiary effect in the response. So, the nature of formulation by means of nature of carrier also determines the positive efficacy of biofertilizers. The success of application of biofertilizers is mainly based on the delivery system or nature of carrier material. The carrier material determines the shelf life and survival of bacterial strain in the soil. The survival is very important in the biofertilizer application because the applied strain should have the optimum population the rhizosphere region of applied crop plants [20].

The application of organic fertilizers can not only enhances the synthesis and amount of chlorophylls but also increases the rate of photosynthesis [21]. Application of organic manures not only influenced the growth and yield of wheat, but it also helped in enhancing the seed quality parameters [22]. The highest protein content in okra fruit was recorded with application of N through FYM, vermicompost, poultry manure and urea over control [23]. The use of biofertilizers in combination with chemical fertilizers and organic manures offers a great opportunity to increase the production as well as quality of cauliflower. Among the nitrogen fixing bacteria, *Azotobacter*, not only provides nitrogen, but also synthesizes growth promoting hormones such as IAA and GA. *Azospirillum* also helps in plant growth and increases the yield of crops by improving root development, mineral uptake etc. The positive role of these biofertilizers has been recorded in many vegetables and spice crops by different scientists. To maintain long term soil health and productivity there is a need for integrated nutrient management through manures and biofertilizers apart from costly chemical fertilizers for better yield of the crop [24]. Biochemical parameters of chlorophyll, total carbohydrate, total protein and total fat contents found higher in biofertilizer enriched vermicompost treatments. Increased amount of chlorophyll contents seems to correlate the increased photosynthetic properties [25]. Application of PSB also increased the physiological parameters like total chlorophyll, protein, amino acids, glucose and NR activity. The response was varied based on the nature of carrier material used for preparation of bioinoculants [26]. PSB application significantly increased the biochemical parameters like the Chlorophyll a, and b, carotenoid, protein and abscorbic acid [27]. The wheat plants inoculated with PSB showed greater activity of the nitrate reductase enzyme [28].

IV. CONCLUSION

The application of biofertilizers has major beneficial effect on the growth of green gram. The growth characters such as seed germination, germination Index, Seedling Vigour Index I & II, root length, shoot length, plant fresh weight and plant dry weight and biochemical characters such as total chlorophyll, glucose, protein, amino acids and NRA activity were higher in plants treated with biofertilizers and chemical fertilizer. The effect was varied with nature of fertilizers used in the nursery. The response was superior in the *Azospirillum* + PSB followed by Urea and biofertilizers alone over the control plants. The beneficial effect biofertilizers were comparable to inorganic fertilizer. By the application of biofertilizers, can able to reduce the application of chemical fertilizers for sustainable growth and development of green gram.

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Table I: Effect of biofertilizers and chemical fertilizer on the germination growth and Vigour Index of green gram

S. No.	Treatment	Seed Germination (%)	Germination Index	Seedling Vigour Index I	Seedling Vigour Index II
1	Control	74	0.55	11.10 ±0.17 (100)	0.21 ±0.05 (100)
2	*Azospirillum*	76	0.73	15.96 ±0.22 (143)	0.31 ±0.03 (147)
3	PSB	81	0.95	17.82 ±0.05 (160)	0.34 ±0.04 (162)
4	*Azospirillum* +PSB	92	1.27	22.63 ±0.04 (2103)	0.54 ±0.05 (257)
5	Urea	87	1.03	20.88 ±0.16 (188)	0.44 ±0.11 (210)

Table II: Effect of biofertilizers and chemical fertilizer on growth characters of green gram

S. No.	Treatment	Root Length (cm)	Shoot Length (cm)	Fresh weight (g)	Dry weight (g)
1.	Control	6.8 ± 0.216 (100)	15.0 ± 0.471 (100)	1.20 ± 0.317 (100)	0.29 ± 0.253 (100)
2.	*Azospirillum*	7.5 ± 0.408 (110)	21 ± 0.816 (140)	1.69 ± 0.145 (141)	0.41 ± 0.028 (195)
3.	PSB	8.5 ± 0.408 (125)	22 ± 0.816 (146)	1.69 ± 0.417 (142)	0.42 ± 0.169 (145)
4.	*Azospirillum* + PSB	12.5 ± 0.408 (183)	24.6 ± 0.471 (164)	2.79 ± 0.118 (232)	0.59 ± 0.264 (203)
5.	Urea	11.5 ± 0.573 (169)	24.0 ± 0.632 (160)	2.17 ± 0.102 (181)	0.51 ± 0.266 (176)

Table III: Effect of biofertilizers and chemical fertilizer on biochemical characters of green gram

S. No.	Treatment	Total Chlorophyll (mg/g LFW)	Carotenoid (mg/g LFW)	Glucose (mg/g LFW)	Protein (mg/g LFW)	Amino acids (mg/g LFW)	NRA (µ moles g/ LFW)
1	Control	1.55 ± 0.344 -100	0.79 ± 0.143 -100	1.13 ± 0.4 -100	2.13 ± 0.01 -100	2.26 ± 0.200 -100	0.39 ± 0.014 -100
2	*Azospirillum*	2.92 ± 1.843 -188	0.99 ± 0.053 -125	1.75 ± 0.05 -154	2.95 ± 0.22 -124	2.36 ± 0.293 -104	0.4 ± 0.0345 -102
3	PSB	3.92 ± 0.813 -253	0.99 ± 0.022 -125	2.98 ± 0.04 -262	3.73 ± 0.01 -175	2.49 ± 0.308 -110	0.44 ± 0.014 -112
4	*Azospirillum* + PSB	4.58 ± 0.617 -295	1.12 ± 0.015 -141	3.37 ± 0.05 -297	4.95 ± 0.01 -232	5.41 ± 0.051 -239	0.5 ± 0.0105 -128
5	Urea	5.62 ± 0.613 -362	1.12 ± 0.054 -140	3.09 ± 0.6 -272	3.94 ± 0.025 -184	5.15 ± 0.416 -227	0.48 ± 0.069 -120