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RESPONSE OF BIOFERTILIZERS ON THE GROWTH AND YIELD OF BLACKGRAM (*VIGNA MUNGO* L.)

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Abstract
An experiment was conducted to determine the effect of biofertilizers on growth and yield of blackgram in field condition. The experiment was a randomized complete block design with five replication. The different inoculation (single and dual) of biofertilizers *Azotobacter, Azospirillum, Rhizobium,* phosphobacteria were incorporated into the top 15 cm of the soil. During the experiment period the plant samples were analysed, such as root length, shoot length, fresh and dry weight, leaf number, leaf area, root nodules and the biochemical content such as chlorophyll 'a', 'b', total chlorophyll, carotenoid, protein content, nodules and yield were analysed. The results revealed that addition the combination inoculation of *Rhizobium* + phosphobacteria significantly increased growth and yield of blackgram compared with control (without biofertilizers).

Key Words: Azotobacter; *Azospirillum*; *Rhizobium*; phosphobacteria; Growth parameter; Biochemical content.

Introduction

Today, global agriculture is at crossroads and this is the consequence of climatic change, increased population pressure and detrimental environmental impacts and new mechanism must be found to ensure food security through sustainable crop production system that will supply adequate nutrition without harming the agroecosystem.

Biofertilizers are commonly called microbial inoculants which are capable of mobilizing important nutritional elements in the soil from non-usable to usable form by the crop plants through their biological processes. For the last one-decade, biofertilizers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility status and for enhancement of crop production by their biological activity in the rhizosphere. Extensive researches were carried out on the use of bacteria (*Azotobacter, Azospirillum*, *Rhizobium,* phosphobacteria) and VAM fungi as biofertilizers to supplement nitrogen and phosphorus fertilizers and observed considerable improvement in the growth of several crop plants [1-5]. Dual inoculation of VAM and bacteria biofertilizers proved more effective in increasing the growth of different crop plants [6-10].

In recent years, biofertilizers have emerged as a promising component of integrating nutrient supply system in agriculture. Our whole system of agriculture depends in many important ways, on microbial activities and there appears to be a tremendous potential for making use of microorganisms in increasing crop production. Microbiological fertilizers are an important part of environment friendly sustainable agricultures practices [11]. Biofertilizers include mainly the nitrogen fixing, phosphate solubilizing and plant growth-promoting microorganisms [12]. Among, biofertilizers benefiting the crop production are *Azotobacter*, *Azospirillum*, blue green algae, *Azolla,*P-solubilizing microorganisms, mycorrhizae and sinorhizobium [13]. In this field, many experiments were conducted to study the effect of biofertilizers alone or in combination with other chemical fertilizers [14-19]. Pulses play a vital role in Indian agriculture. Pulses are important sources of food. They are very rich in protein, particularly to the vegetarian who constitute the bulk of population in India. Blackgram is an annual food legume. It is very nutritious and is recommended for diabetics.

Materials and Methods

Seeds of blackgram (*Vigna mungo* L.) obtained from Rice Research Institute, Aduthurai, Thanjavur District,

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Tamil Nadu. The different types of biofertilizers (*Rhizobium*, *Azotobacter, Azospirillum* and phosphobacteria, were obtained from Ramvijay biofertilizer, Puducherry. In this experiments blackgram seeds were sowed in the plotted field biofertilizers were mixed with sand (10 kg/acre) and applied to different combination of the field (T_0 - Control (Untreated); T_1 - *Azotobacter;* T_2 – *Azospirillum;* T_3 – Phosphobacteria; T_4 –*Rhizobium;* T_5 – *Rhizobium* + *Azotobacter;* T_6 – *Rhizobium* + *Azospirillum* and T_7 – *Rhizobium* + phosphobacteria). The experiment was carried out based on randomized complete block design with four replications.

The plants were sampled at 15, 30 and 45 DAS. At each plot, five plants were took and shoot length (cm), root length (cm), number of leaves, fresh weight, dry weight and root nodules was analysed. The pigment and biochemical content chlorophyll 'a', 'b', total chlorophyll [20], carotenoid [21] and protein [22] were estimated.

The effects of different types of biofertilizers *viz., Azotobacter*, *Azospirillum, Rhizobium* and phosphor bacteria combined inoculation of *Rhizobium* with *Azotobacter, Rhizobium* with *Azospirillum* and *Rhizobium* with phosphobacteria on the seed germination percentage, root length, shoot length, fresh weight and dry weight, root nodule pigment content, protein content and yield of blackgram.

The results showed that biofertilizer, had significantly effects on germination percentage shoot, root length, fresh, dry weight of plants at the inoculation of *Rhizobium* + phosphobacteria treatment 30% greater than those obtained in control (Figs. 1-5). In addition biofertilizer increased in pigment content, such as chlorophyll 'a', 'b', total chlorophyll, carotenoid and protein content significantly increased in *Rhizobium* + phosphobacteria treatment when compared to control (Figs. 6-10). The *Rhizobium* + phosphobacteria inoculation significantly increased in nodules, yield of blackgram when compared to control (Fig. 11).

Results

Fig. 1. Effect of biofertilizers on seed germination percentage of *Vigna mungo* (L.) Hepper, Fig. 2. Effect of biofertilizers on shoot length of *Vigna mungo* (L.) Hepper, Fig. 3. Effect of biofertilizers on root length of *Vigna mungo* (L.) Hepper, Fig. 4. Effect of biofertilizers on fresh weight (mg/g fr. wt.) of *Vigna mungo* (L.) Hepper

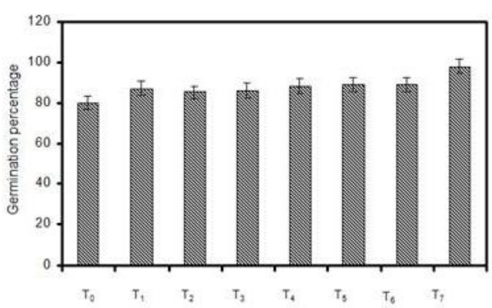


Fig. 1

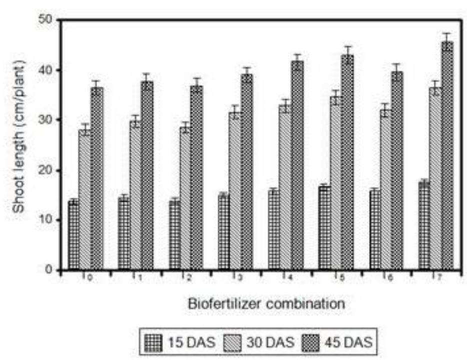
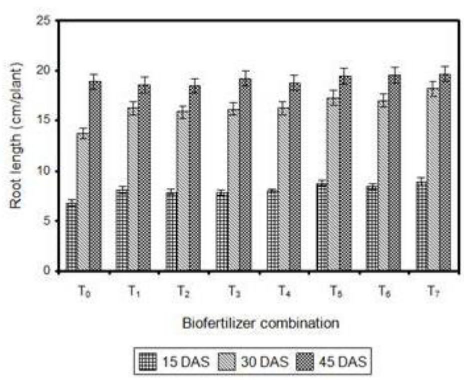


Fig. 2



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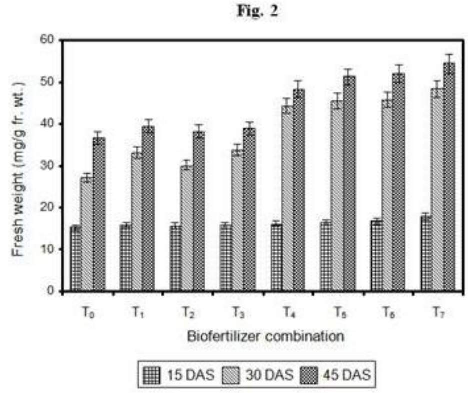
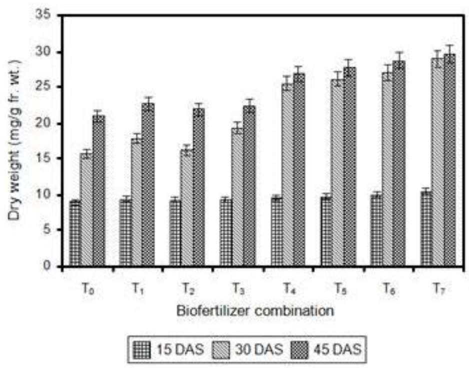


Fig. 4

Fig. 5. Effect of biofertilizers on dry weight (mg/g fr. wt.), Fig. 6. Effect of biofertilizers on chlorophyll ‘a’ (mg/g fr. wt.) content, Fig. 7. Effect of biofertilizers on chlorophyll ‘b’ (mg/g fr. wt.) content, Fig. 8. Effect of biofertilizers on total chlorophyll (mg/g fr. wt.) content of *Vigna mungo* (L.) Hepper



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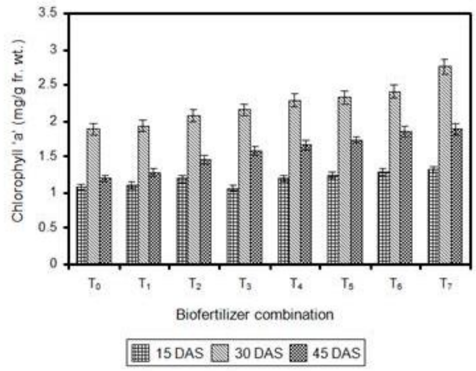
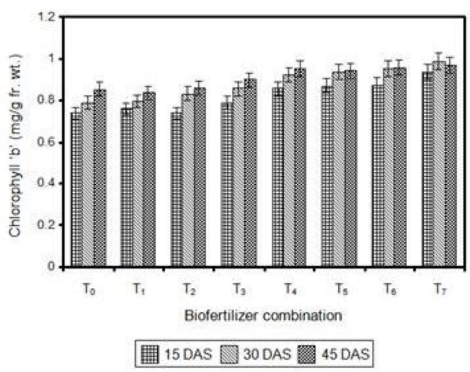


Fig. 6



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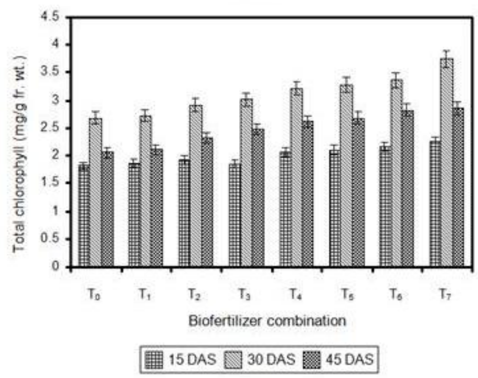

Fig. 8

Fig. 9. Effect of biofertilizers on carotenoid (mg/g fr. wt.) content, Fig. 10. Effect of biofertilizers on protein (mg/g fr. wt.) content, Fig. 11. Effect of biofertilizers on number of nodules, and yield parameters (Number of pods/ plants) of *Vigna mungo* (L.) Hepper

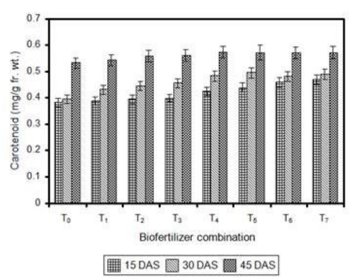

Fig. 9

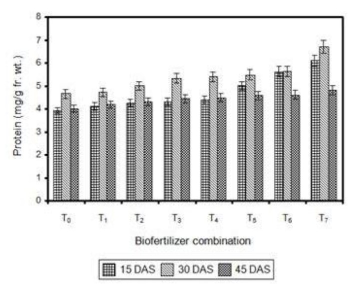

Fig. 10

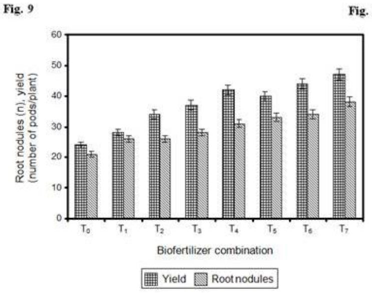

Fig. 11

Discussion

Application of biofertilizers is an acceptable approach for higher yield with good quality and safe for human consumption. Our results show that either single or mixed inocula gave positive response to the studied parameters. This response was accompanied by significant increase in fresh and dry weight and the other parameters. Growth parameters increased due to the mixed biofertilizers treatments. This primitive effect of biofertilizers treatments is the same line with those obtained by [23] who stated that vegetative growth parameters increased in the biofertilizers treatments compared to control. In the present investigation the single strain inoculant was not always as good as the mixed inoculant strains in terms of biomass accumulation and N_2 fixation as confirmed by [24] who found that single inoculation of rhizobia performed lower in terms of N_2 fixation and N accumulation.

Treatment with plant growth promoting rhizobacteria increase germination percentage, seedling vigor, emergence, root and shoot growth, total biomass of plants, seed weight, early flowering, grains fodder and fruit yields etc [25, 26]. Various mechanisms have been suggested to explain the phenomenon of plant growth promotion. These include increase in the nitrogen fixation, production of auxins, gibberellins, cytokinins, ethylene solubilization of phosphorus, oxidation of sulfur, increase in availability of nitrate, extra cellular production of antibiotics, lytic enzyme, hydrocyanic acid, increase in root permeability, strict competition for the available and root sites, suppression of deleterious rhizobacteria and enhancement in the uptake of essential plant nutrients etc., [27-29]. Plant growth promoting rhizobacteria (PGPR) may be important for plants growth stimulation when other potentially deleterious rhizosphere microorganisms are present in the rhizosphere [30, 31].

In the present study, single and combined inoculation promoted early days dry weight and shoot length in blackgram when compared to control. The inoculated plants, both root and shoot length increased significantly than control. This complies with the finding of [32] who reported that root elongation is associated with the production of IAA in early stages. The IAA content was increased in inoculated plants as compared to control. [33] also reported that increased root length, shoot length after inoculation was due to bacterial phytohormones. Co-inoculation resulted in more root and shoot length than single strain. This may be attributed to synergistic effects [34].

The values of plant dry weight and N were significantly higher in mixed strain inoculation that single strain at stated by [35] who mentioned that rhizobia inoculation showed as positive response to inoculation in terms of nodules number and dry weight and also enhanced plant growth and N values when compared to control.

Treatment with biofertilizer enhances the chlorophyll content of *Vigna mungo.* In present study, the chlorophyll content is maximum in mixed inoculation of *Rhizobium* with phosphobacteria and minimum in control. The beneficial effect of bacterial inoculation as an increased chlorophyll content might have been due to the supply of high amount of nitrogen to the growing tissues and organs supplied by N_2 fixing *Azospirillum* and *Azotobacter* [36]. The effect of *Azospirillum* on various growth characters in okra where the treatment with *Azospirillum* resulted in significant increase in total chlorophyll content.

The increased amount of chlorophyll content in leaves indicates the photosynthetic efficiency, thus it can be used as one of the criteria for quantifying photosynthetic rate [37]. The chlorophyll content might be due to synergistic interaction of biofertilizers. The stimulative effect of these filtrates could be attributed to elevated level of GA of the filtrates which is known to inhibit chlorophyllase activity [38]. Moreover, [39] stated that cyanobacteria and microalgae active compounds including plant growth regulators which can be used for treatments to decrease senescence, transpiration as well as to increase leaf chlorophyll, protein content and root/shoot development.

Protein content was increased at all the biofertilizers inoculation. The highest protein content was showed in T_7, with combined effect of *Rhizobium* and phosphobacteria. It was found that uptake of nitrogen and phosphorous is increased with application of phosphate solubilizing bacteria [40]. The trend of variation in protein content due to absorption of nitrogen and phosphorus content by plants. These results are in accordance with those obtained by [41, 15, 42-45].

Protein content in seed was progressively increased with increasing level of nitrogen. The same results have reported by many workers [44-46]. The trend of variation in protein content was similar to that of nitrogen content, because protein content was computed by multiplying the nitrogen content in seeds with 6.25 [47].

The number of nodules increased significantly in treatments with combined inoculation. Number of nodules reported to be the maximum in combined effect of *Rhizobium* with phosphobacteria than that of single inoculation. Higher concentration of IAA and GA produced by microbes may be other cause for more nodulation [48]. Nodulation pattern was comparable in all treatments. This could be due to the sufficient native and inoculated *Rhizobium* population of the associated species and their efficiency. The trend of higher nodulation pattern with the uses of organic manure, former proves their synergistic effect in soil due to the interaction between fertilizer levels and manorial forms. The findings are in line with those of [49, 50].

The yield of blackgram shown minimum in control and maximum in biofertilizers inoculation of *Rhizobium* with phosphobacteria. The single inoculation biofertilizers could not increase the yield as compared to dual inoculation. The dual inoculation of phosphobacteria and *Rhizobium* gave highest yield. It is evident that the increases in plant height, leaf number and leaf area have contributed to increased yield. Similar results were reported earlier by [51-54] who stated that combined inoculation overcome the single inoculation with *Rhizobium*.

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