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Biofertilizers formulations and their effect on morphological characters in greengram (*Vigna radiata* L.)

K Shravani, S Triveni, PC Latha and V Ramulu

Abstract
The investigations to evaluate the effect of different formulations of biofertilizers on plant growth parameters of Greengram, variety (MGG –295) and the field experiment was conducted at water technology centre fields, college farm, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana during *kharif* 2016 – 2017. From the results, the plant height and Dry matter production at 30, 45, 60 DAS and at harvest stage respectively (27.40, 39.00, 46.33, 47.07 cm) and (8.60, 15.80, 22.30 and 18.30 g plant^{-1}) significantly increased in seed treatment with liquid based bio fertilizers with combination of 100 % RDF and LBBF Seed treatment when compared to all other treatments. The significantly highest root length of greengram was noticed due to combined seed treatment with carrier based *Rhizobium* and PSB bio-inoculants at 30, 45, 60 DAS and at harvest stage was recorded 12.63, 17.60, 22.83 and 23.50 cm respectively. The soil treatment with LBBF along with 100 % RDF application recorded significantly highest number of root nodules and nodule dry weight i.e. (18) and (30) nodules plant^{-1} and 0.18 and 0.30 nodule dry weight g plant^{-1} at 30 and 45 DAS respectively when compared to all other treatments. These results provide baseline information to promote an eco-friendly and sustainable agriculture.

Keywords: liquid biofertilizers, biofertigation, microbial inoculants (*Rhizobium* and PSB)

Introduction
India is an agricultural based country. In order to feed the ever growing populations, India has to increase the per unit area productivity. According to the United Nations Food and Agriculture Organization (FAO) estimations, the average demand for the agricultural commodities will be 60% higher in 2030 than present time and more than 85% of this additional demand will be from developing countries, for over half a century, the world has relied on the concept of increasing crop yields to supply an ever increasing demand of food. Therefore, vertical expansion of food production is necessary (Mia *et al.*, 2010) [4]. In order to increase the unit area productivity of agricultural land, the role of different crop nutrients in contributing increased crop yields is vital. Among the crop nutrients, nitrogen as well as phosphorus play an important role in increasing the productivity.

For sustainable agriculture emphasis should be given for using more organic manure and curtail use of chemical fertilizers. Under dwindling supply of traditional organic manures like FYM, composts and biofertilizers can have appreciable contribution to substitute FYM and can be an excellent option for chemical fertilizers too, particularly in the crops like pulses whose nutrient requirement is low. Pulses are the most important crops in our country and the main source of vegetable. Among the pulses green gram (*Vigna radiata* L.) is one of the most important and extensively cultivated pulse crops in India for grain, forage and green manure purpose.

Biofertilizers contains microorganisms which promote the adequate supply of nutrients to the host plants to ensure their proper development of growth and regulation in their physiology. Living microorganisms are used in the preparation of biofertilizers (Mishra *et al.*, 2013) [5]. Shelf life is the first and foremost problem of the carrier based biofertilizer which is up to 3 months and it does not retain throughout the crop cycle. LBF on the other hand facilities the long survival of the organisms by providing the suitable medium which is sufficient for the entire crop cycle (Mahdi *et al.*, 2010) [3].

Liquid biofertilizer is increasingly available in the market as one of the alternatives to chemical fertilizer and pesticide. One of the benefits from biofetilizer is a contribution from population of microorganisms available. Traditionally liquid biofertilizer produced from fermentation of effective microorganisms (EM) was recommended to be used within three months. Nowadays, the production of ready to use liquid biofertilizer from EM is becoming

available in market. A preparation comprising requirements to preserve organisms and deliver them to the target regions to improve their biological activity. Sahai *et al*. (2011) [7] and Patel (2012) [6] reported that dual seed inoculation with *Rhizobium* and PSB benefitted the plants by providing atmospheric N and rendering the insoluble phosphorus into available form. The enhanced availability of P favoured N fixation and rate of photosynthesis and consequently led to increased plant growth parameters in Greeengram.

Material and Methods

The field experiment was conducted at water technology centre fields, college farm, College of Agriculture, PJTSAU, Rajendranagar, during *kharif* 2016 – 2017. The location is geographically situated at 17° N Latitude and 78° E longitude at an altitude of 542.3 m above MSL. The soil of an experimental site was sandy loam in texture, moderate in organic carbon, low in nitrogen and medium in available phosphorus and high in potash and slightly alkaline (pH 7.6) in chemical reaction.

Seed source: The seed of greengram (Cv. MGG 295) was obtained from Agriculture Research Station, Madira, Khammam.

Seed treatment with microbial inoculants (Carrier): After quality testing of the bioformulation, the good quality bioinoculants were used for the field experimentation to study the performance on yield of greengram crop. Twenty grams of jaggery was dissolved in 200 ml of water. Jaggery solution was prepared as per the volume of seed. The *Rhizobium &* PSB cultures were thoroughly mixed for slurry preparation. Seeds were treated with this mixture carefully, so that seed coat was not injured and a uniform coating was made. Treated seeds were dried under shade on gunny bags and then used for sowing.

Seed treatment with microbial inoculants (Liquid): Inoculums of PSM & *Rhizobium* were prepared by dissolving 10 g of jaggery in one litre of boiled water and subsequently cooled and then added to the broth culture in required quantity, so as to obtain at least 1.0 - 1.5 x 10^8 cells per ml.

Soil application of liquid culure based bioferilizers: PSB and *Rhizobium* culture was applied in the soil @ 1 l ha^{-1} in 10 days interval for the treatment T_8.

The experiment was laid out in a Randomized Block Design with 10 treatment combinations. The treatments are T_1: 100 % RDF, T_2 : 100 % RDF + CBBF Seed treatment, T_3: 100 % RDF + CBBF Soil treatment, T_4: 100 % RDF + LBBF Seed treatment, T_5: 100 % RDF + LBBF Soil treatment, T6: 100 % RDF + LBBF Drip fertigation, T_7: 100 % RDF + LCBF Seed treatment, T_8: 100 % RDF + LCBF Soil treatment, T_9: 100 % RDF + LCBF Drip fertigation, T_{10} Control: Only biofertilizers. Sowing was done on 15th July, 2016 by hand dibbling two to three seeds at each hill at a recommended spacing of 30 cm × 10 cm. Recommended dose of fertilizer for greengram is 20: 50: 00 N P K kg ha^{-1}. Fertilizer *viz.*, nitrogen, phosphorus were applied in respective plots as per the recommendation by using the urea and SSP.

The fertilizer solution was prepared by dissolving the required quantity of fertilizer with water in 1:5 ratio and liquid biofertilizers *Rhizobium* (10 ml) + PSB (10ml) injected into the irrigation system through venturi assembly. Fertigation interval was scheduled once in 7 days interval. The recommended doses (20: 50: 00 NPK kg ha^{-1}) of inorganic fertilizers *i.e.,* urea (46 % N) and single super phosphate (16 % P_2O_5) were applied as basal to the surface irrigated treatments except fertigation treatment (T_6 & T_9) combinations. Data was collected for greengram nutrient uptake and seed yield and haulm yield at harvest stage. The nutrient content uptake values obtained as percentage in the analysis was multiplied by the respective dry matter content for computing N, P and K uptake expressed in kg ha^{-1}.

$$\text{Nutrient uptake (kg ha}^{-1}\text{)} = \frac{\text{Percentage of nutrient content} \times \text{Total dry matter production (kg ha}^{-1}\text{)}}{100}$$

Results and Discussion

Impact of biofertlizers on morphological parameters of greengram

Plant height (cm)

Plant height is one of the most important growth parameters of any crop. It is an index of growth and development, representing the dry matter build up by the plant over a period of time. The data regarding plant height of greengram was influenced by the application of different formulations of biofertilizers (Table 1 and illustrated in fig: 1).

Significant differences in plant height of greengram was observed due to the effect of different formulations of biofertilizers application. The plant height was significantly increased in seed treatment with liquid based biofertilizers with combination of 100 % RDF and LBBF Seed treatment i.e the plant height at 30, 45, 60 DAS and at harvest was varied from 12.83 to 27.40 cm, 20.00 to 39.00 cm, 23.43 to 46.33 cm and 24.07 to 47.07 cm, respectively. when compared to all other treatments at all crop growth stages of greengram, followed by treatment T_6 (100 % RDF and LBBF Drip fertigation) and T_2 (100 % RDF and CBBF seed treatment) both were on par with each other at all crop growth stages of greengram.

It's may be due to the dual seed inoculation with *Rhizobium* and phosphobacteria of liquid based biofertlizers which is coated uniformly on greengram seed coat, leads to better uptake and translocation of plant nutrients to growing plants. These findings lend support to the report of Sahai *et al*. (2011) [7] and Patel (2012) [6] who reported that dual inoculation benefitted the plants by providing atmospheric N and rendering the insoluble phosphorus into available form. The enhanced availability of P favoured N fixation and rate of photosynthesis and consequently led to better plant height and branches per plant.

Table 1: Influence of different formulations of biofertilizers application on plant height at 30, 45, 60 DAS and at harvest stage of greengram.

Treatments	Plant height (cm)			
	30 DAS	45 DAS	60 DAS	At harvest
T_1 : 100 % RDF	15.30	23.00	32.67	32.90
T_2 : 100 % RDF + CBBF Seed treatment	21.40	33.00	39.17	40.00
T_3 : 100 % RDF + CBBF Soil treatment	16.80	26.00	36.13	36.70
T_4 : 100 % RDF + LBBF Seed treatment	27.40	39.00	46.33	47.07

T_5 : 100 % RDF + LBBF Soil treatment	18.33	28.00	36.50	36.83
T_6 : 100 % RDF + LBBF Drip fertigation	23.17	35.00	41.33	41.93
T_7 : 100 % RDF + LCBF Seed treatment	18.50	29.00	37.17	38.00
T_8 : 100 % RDF + LCBF Soil treatment	15.67	25.00	34.33	34.83
T_9 : 100 % RDF + LCBF Drip fertigation	20.50	32.00	38.50	39.50
T_{10} Control : Only biofertilizers	12.83	20.00	23.43	24.07
SE(m)	1.00	0.95	1.17	5.56
CD (P=0.05)	3.00	2.85	3.51	3.57
CV	9.15	5.68	5.55	5.56

Legend: RDF: Recommended dose of fertilizers (20 - 50 - 0) N - P - K kg ha^{-1},
CBBF: based biofertilizers, LBBF: Liquid based biofertilizers,
LCBF: Liquid culture based biofertilize

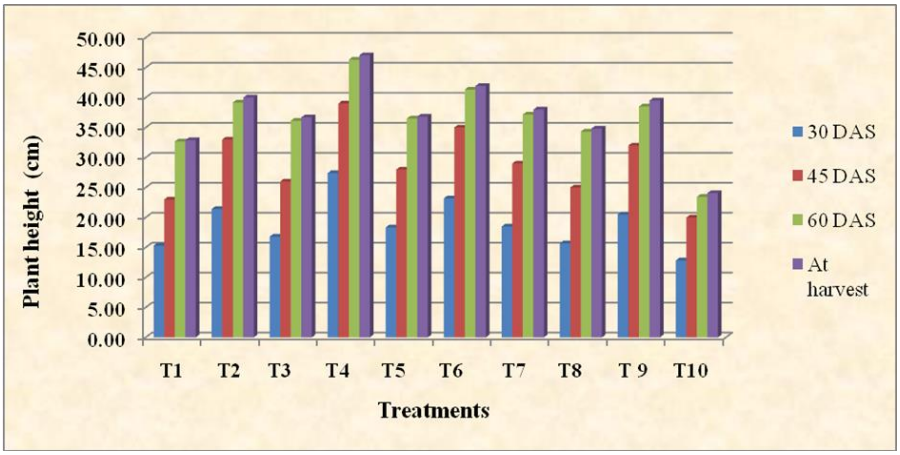

Fig 1: Influence of different formulations of biofertilizers application on plant height at 30, 45, 60 DAS and at harvest stage of greengram.

Root length (cm)

It was evident from the results that the significantly highest root length of greengram was noticed due to combined seed treatment with carrier based *Rhizobium* and PSB bioinoculants at 30, 45, 60 DAS and at harvest stage was recorded 12.63, 17.60, 22.83 and 23.50 cm respectively when compared to the other treatments (Table 2 and illustrated in fig: 2). The treatments T_6 (100 % RDF and LBBF drip fertigation), T_4 (100 % RDF and LBBF seed treatment) and T_3 (100 % RDF and CBBF soil treatment) were found to be statistically at par with each other at all stages of greengram crop. The minimum root length was recorded with treatment T_{10} (Control).

The significantly increased root length was noticed with T_2 treatment might be due to combined seed treatment with carrier based *Rhizobium* and PSB bioinoculants. Similar results found by (Ahmed *et al*., 2006) [1] seed inoculation with (*Rhizobium* and PSB) 15 kg N ha^{-1} was significantly increase the root length of greengram.

Table 2: Influence of different formulations of biofertilizers application on root lengthat 30, 45, 60 DAS and at harvest stage of greengram.

Treatments	Root length (cm)			
	30 DAS	45 DAS	60 DAS	At harvest
T_1 : 100 % RDF	06.27	09.87	15.63	16.37
T_2 : 100 % RDF + CBBF Seed treatment	12.63	17.60	22.83	23.50
T_3 : 100 % RDF + CBBF Soil treatment	08.08	12.00	16.20	17.00
T_4 : 100 % RDF + LBBF Seed treatment	09.20	13.30	18.40	19.00
T_5 : 100 % RDF + LBBF Soil treatment	08.03	12.23	16.70	17.50
T_6 : 100 % RDF + LBBF Drip fertigation	10.40	15.13	20.03	20.80
T_7 : 100 % RDF + LCBF Seed treatment	08.32	13.00	17.20	18.00
T_8 : 100 % RDF + LCBF Soil treatment	07.10	11.47	16.00	16.80
T_9 : 100 % RDF + LCBF Drip fertigation	09.07	13.03	17.93	18.57
T_{10} Control : Only biofertilizers	05.37	08.43	12.17	13.00
SE(m)	0.53	0.93	0.92	0.86
CD (P=0.05)	1.59	2.79	2.75	2.58
CV	10.87	12.78	9.20	8.29

Legend: RDF: Recommended dose of fertilizers (20 - 50 - 0) N - P - K kg ha^{-1}, CBBF: Carrier based biofertilizers,: Liquid based biofertilizers, LCBF: Liquid culture based biofertilizers.

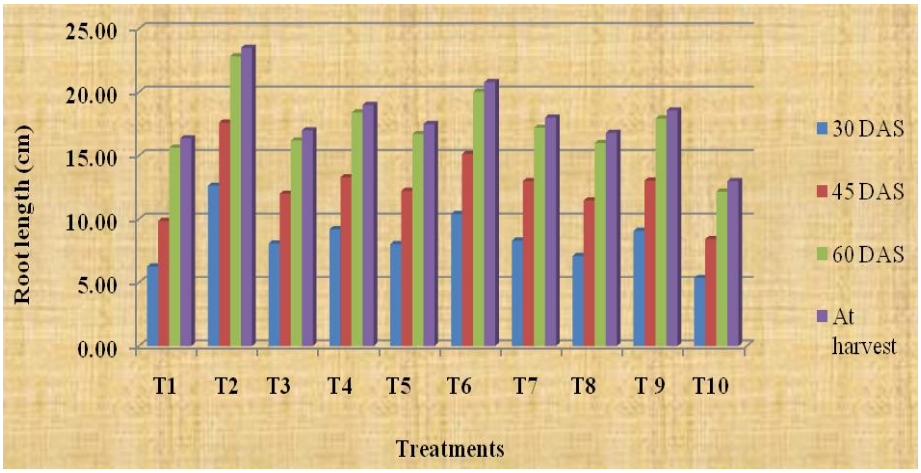

Fig 2: Influence of different formulations of biofertilizers application on root length at 30, 45, 60 DAS and at harvest stage of greengram.

Nodulation

It perusal of the data indicates that the nodulation in greengram was increased with advancement in age of the crop up to 45 DAS. Number of nodules and dry weight of nodules of greengram at 30 and 45 DAS differed significantly influenced by the application of different formulations of biofertilizers.

Number of nodules plant^{-1}

Nodulation in greengram was significantly influenced due to soil application with liquid based biofertilizers of *Rhizobium* and PSB obtained data presented in (Table 3 and illustrated in Fig: 3). It revealed that the soil treatment with LBBF along with 100 % RDF application recorded significantly highest number of root nodules i.e. (18) and (30) nodules plant^{-1} at 30 and 45 DAS respectively when compared to all other treatments. But it was on par with treatment T_6 (100 % RDF and LBBF drip fertigation), T_2 (100 % RDF and CBBF seed treatment) and T_3 (100 % RDF and CBBF soil treatment) at 30 and 45 DAS of crop. Nodule number found significantly higher in all treatment combinations when compared to control and 100 % RDF was registered the lowest number of nodules plant^{-1}.

Table 3: Influence of different formulations of biofertilizers application on nodulation at 30 and 45 DAS of greengram crop.

Treatments	Nodule number per plant^{-1}	
	30 DAS	45 DAS
T_1 : 100 % RDF	12.60	24.60
T_2 : 100 % RDF + CBBF Seed treatment	16.80	28.50
T_3 : 100 % RDF + CBBF Soil treatment	15.50	27.40
T_4 : 100 % RDF + LBBF Seed treatment	15.80	27.60
T_5 : 100 % RDF + LBBF Soil treatment	17.50	29.60
T_6 : 100 % RDF + LBBF Drip fertigation	17.00	29.00
T_7 : 100 % RDF + LCBF Seed treatment	16.00	27.80
T_8 : 100 % RDF + LCBF Soil treatment	14.80	27.00
T_9 : 100 % RDF + LCBF Drip fertigation	16.50	28.30
T_{10} Control : Only biofertilizers	11.00	22.50
SE(m)	0.79	0.99
CD(P=0.05)	2.37	2.98
CV	8.98	6.33

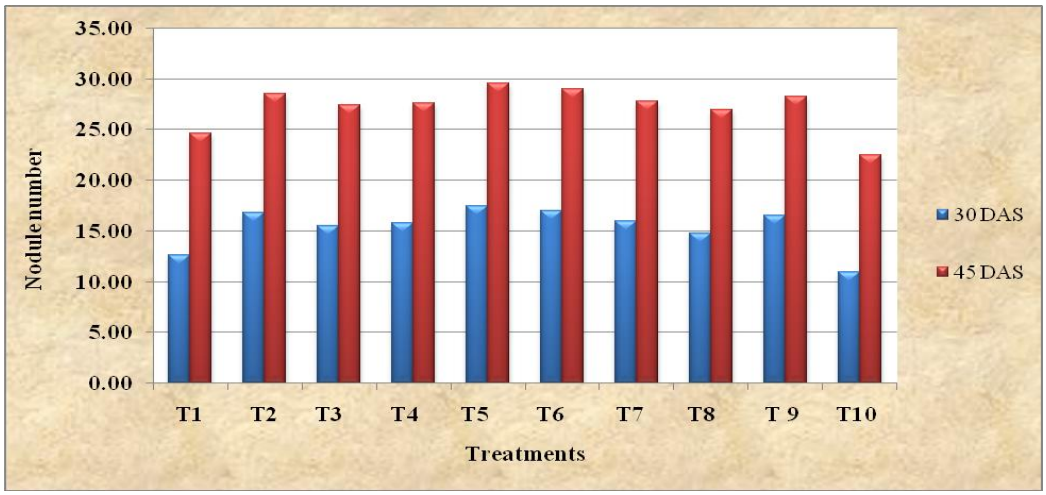

Fig 3: Influence of different formulations of biofertilizers application on nodulation at 30 and 45 DAS of greengram crop.

Nodule dry weight g plant^{-1}

Soil application with liquid based biofertilizers co-inoculation of *Rhizobium* and PSB was significantly influenced the nodule dry weight in greengram (Table 4 and illustrated in Fig: 4). It indicates that maximum nodule dry weight was registered in response to treatment 100 % RDF along with soil application of LBBF at 30 and 45 DAS were 0.18 and 0.30 g plant^{-1} respectively when compared to remaining treatments. On the other hand, the significantly lowest dry weight of root nodules was observed with treatment T_{10} (Control) at all crop growth stages *viz*., 30 and 45 DAS was 0.09 and 0.21 g plant^{-1} respectively.

T_5 recorded significantly higher number of root nodules and nodule dry weight in greengram, due to combined inoculation of PSB in combination with *Rhizobium* formed more proportion of effective nodules and resulting in better nitrogen fixation by facilitating *Rhizobium*. It leads to more nitrate reductase and nitrogenase activity in root nodules which enhanced the availability of N and P to the plant promoting better growth and development leading to more number of pod, pod length and more number of seeds per pod. These results are in conformity with the findings of Sahai and Chandra (2011) [7] who reported that the liquid inoculants of *Mesorhizobium* spp. and PGPR gave better nodulation in chick pea than carrier based inoculants registered increased in nodule number by 35 and 31 % and nodule dry weight by 2.9 and 2.7 %, over carrier respectively.

Table 4: Influence of different formulations of biofertilizers application on nodul dry weight at 30, 45 DAS of greengram crop.

Treatments	Nodule dry weight (g)	
	30 DAS	45 DAS
T_1 : 100 % RDF	0.12	0.23
T_2 : 100 % RDF + CBBF Seed treatment	0.14	0.29
T_3 : 100 % RDF + CBBF Soil treatment	0.15	0.27
T_4 : 100 % RDF + LBBF Seed treatment	0.15	0.28
T_5 : 100 % RDF + LBBF Soil treatment	0.18	0.30
T_6 : 100 % RDF + LBBF Drip fertigation	0.16	0.29
T_7 : 100 % RDF + LCBF Seed treatment	0.13	0.28
T_8 : 100 % RDF + LCBF Soil treatment	0.11	0.26
T_9 : 100 % RDF + LCBF Drip fertigation	0.12	0.29
T_{10} Control : Only biofertilizers	0.09	0.21
SE(m)	0.01	0.01
CD(P=0.05)	0.03	0.03
CV	13.84	6.56

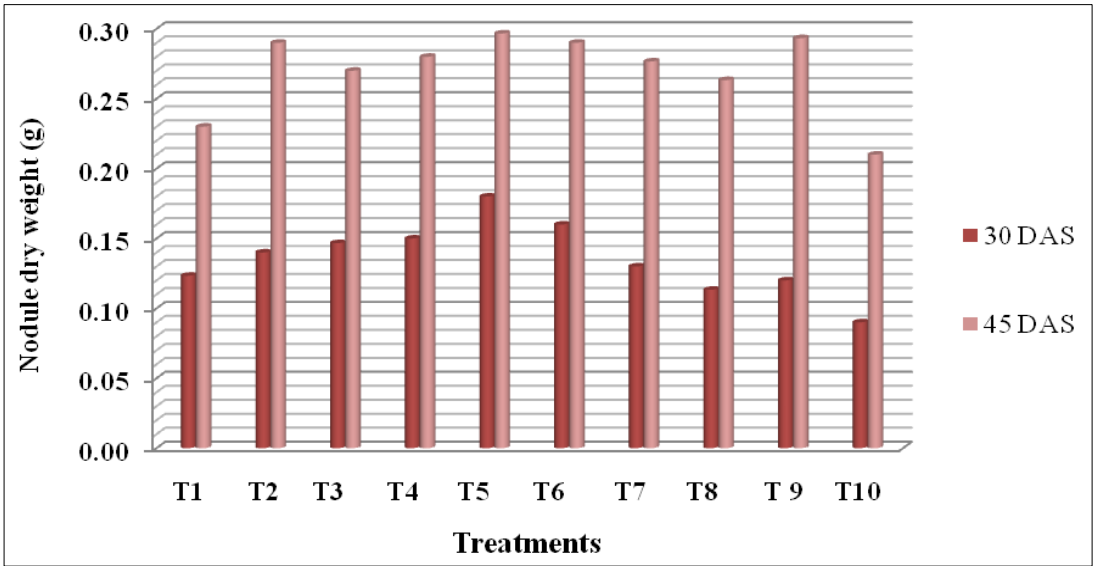

Fig 4: Influence of different formulations of biofertilizers application on nodule dry weight at 30, 45 DAS of greengram crop

Dry matter production (Plant^{-1})

The dry matter production of greengram was increased in advancement with the age of crop up to 60 DAS and thereafter, it was noticed in decreased at harvest due to leaf shedding. The data further revealed that the dry matter accumulation was affected significantly due to seed treatment with liquid biofertilizers of *Rhizobium* and PSB at all the growth stages of greengram was 8.60, 15.80, 22.30 and 18.30 g plant^{-1} at 30, 45, 60 DAS and at harvest respectively when compared to all other treatments, but it was on par with the treatment T_6 i.e. 100 % RDF along with LBBF drip fertigation (Table 5 and illustrated in Fig: 5).

The lowest dry matter production plant^{-1} was recorded with treatment T10 (Control) at all the growth stages *viz*. 30, 45, 60 DAS and harvest were 4.60, 8.80, 14.80 and 11.20 g plant^{-1} respectively.

Maximum total dry matter accumulation (g) plant^{-1} recorded in seed treatment with liquid based biofertilizers of *Rhizobium* and PSB than all other treatments, might be reason for more availability of nutrients resulting in increased growth parameters i.e. plant height, branches, leaf area, number and dry weight of root nodules, which ultimately turn into increased dry matter accumulation plant^{-1}. This results were correlated with the findings of (Dorle *et al*., 2015) [2].

Table 5: Influence of different formulations of biofertilizers application on plant dry matter at 30, 45, 60 DAS and at harvest stage of greengram.

Treatments	Plant dry weight (g)			
	30 DAS	45 DAS	60 DAS	At harvest
T_1 : 100 % RDF	05.20	10.50	17.60	12.90
T_2 : 100 % RDF + CBBF Seed treatment	07.40	12.50	21.00	15.80
T_3 : 100 % RDF + CBBF Soil treatment	06.00	11.20	19.00	14.00
T_4 : 100 % RDF + LBBF Seed treatment	08.60	15.80	22.30	18.20
T_5 : 100 % RDF + LBBF Soil treatment	06.20	11.30	19.50	14.40
T_6 : 100 % RDF + LBBF Drip fertigation	08.00	13.60	21.60	16.30
T_7 : 100 % RDF + LCBF Seed treatment	06.50	11.50	19.80	14.60
T_8 : 100 % RDF + LCBF Soil treatment	05.80	10.83	18.50	13.50
T_9 : 100 % RDF + LCBF Drip fertigation	07.00	12.00	20.60	15.20
T_{10} Control : Only biofertilizers	04.60	8.80	14.80	11.20
SE(m)	0.46	0.66	1.10	0.77
CD(P=0.05)	1.37	1.98	3.30	2.32
CV	12.18	9.72	9.80	9.21

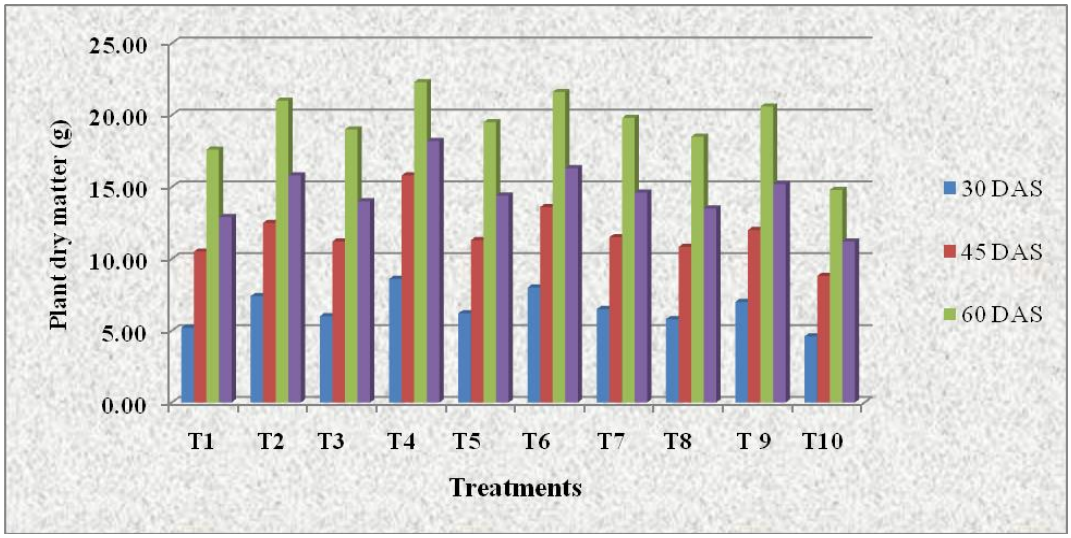

Fig 5: Influence of different formulations of biofertilizers application on plant dry matter at 30, 45, 60 DAS and at harvest stage of greengram

Conclusion

Hence our study was clearly highlighted that combined inoculation of liquid biofertilizers such as *Rhizobium* & PSB could enhance the morphological parameters such as height of the plant, Root length, number of nodules plant^{-1}, nodule dry weight g plant^{-1}, and Dry matter production plant^{-1}. Compared to control, could be the collective effect of liquid biofertilizer. To combat the threat of global food crises the alternative technologies in the agriculture like liquid biofertilizers are obligatory. Liquid biofertilizers of course have the capacity to replace the traditional chemical fertilizers and carrier based biofertilizers and play a major role in restoring the soil health, but a lot of measures in terms of technology, government support, subsides, and constructive awareness by well trained technicians among the agrarians are emphasized.

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