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Vijaykumar Gangaraddi
Department of Agricultural Microbiology, UAS, GKVK, Bengaluru, Karnataka, India

GP Brahmaprakash
Department of Agricultural Microbiology, UAS, GKVK, Bengaluru, Karnataka, India

Correspondence
Vijaykumar Gangaraddi
Department of Agricultural Microbiology, UAS, GKVK, Bengaluru, Karnataka, India

Evaluation of selected microbial consortium formulations on growth of green gram (*Vigna radiata* L.)

Vijaykumar Gangaraddi and GP Brahmaprakash

Abstract
A Greenhouse investigation was carried out to evaluate the selected formulations (alginate based, fluid bed dryer based, lignite and liquid formulations) of Agriculturally Important Microorganisms (AIMs) *viz*., *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* on growth and nutrient uptake of green gram (*Vigna radiata* L.). The plant growth parameters such as higher plant height (21.63 cm), maximum number of leaves (11.33), total chlorophyll content (3.08 mg/gm of leaf), total nitrogen uptake (81.36 mg/plant), total phosphorus uptake (12.42 mg/plant) and total biomass content (5.20 g/plant) were recorded in plants receiving triple inoculants in liquid formulation. The present study revealed that the microbial inoculants in liquid formulation influenced more growth and nutrient uptake when compared to other test formulations used in the present study.

Keywords: Rhizobium sp., *Bacillus megaterium*, *Pseudomonas fluorescens*, consortium, formulations, green gram

Introduction

Group of microorganisms that benefit plants are collectively called as Agriculturally Important Microorganisms (AIMs). These AIMs benefit crop plants more when they are applied as consortia than applying as single inoculants (Antoun and Prevost, 2005; Kennedy, 2005; Sahara and Nehra, 2011) [3, 10, 23]. In order to achieve an increased impact of these AIMs towards enhancement of plant growth parameters, the use of mixed inoculants over single inoculants that interact synergistically is recommended.

Biofertilizers containing live microorganisms and a formulation of some carrier material enables easy handling and long term storage and effectiveness of biofertilizer. These are most commonly applied for the fixation of atmospheric di-nitrogen and to enhance the availability and uptake of mineral nutrients for growth and development of plants. A good formulation increases the survival of inoculants over time of storage.

There is a necessity to use microbial inoculants formulations as an integral part of sustainable agricultural practices. This can be achieved by increasing and extending the role of microbial inoculation. Later, which helps in minimizing the adverse environmental effects.

Inoculation with different microbial consortium formulations to Leguminous crops such as green gram (*Vigna radiata* L.) helps in better growth and development. Green gram is a short duration crop grown in almost all parts of India (Peter and Bhalerao, 2015) [18]. These are not expensive, rich source of proteins and these respond very well to microbial inoculants. Hence, the aim of the investigation was to enhance the plant growth parameters of green gram with the help of Agriculturally Important Microorganisms (AIMs) in different formulations.

Material and Methods

Preparation of consortium formulations

Different inoculants (*Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens*) consortium formulations (alginate based, fluid bed dryer based, lignite and liquid formulations) were prepared in 8 combinations- T_1 (un-inoculated control), T_2 (*Rhizobium* sp.) T_3 (*Bacillus megaterium*), T_4 (*Pseudomonas fluorescens*), T_5 (*Rhizobium* sp + *Bacillus megaterium*), T_6 (*Rhizobium* sp + *Pseudomonas fluorescens*) T_7 (*Bacillus megaterium*+ *Pseudomonas fluorescens*) and T_8 (*Rhizobium* sp + *Bacillus megaterium* + *Pseudomonas fluorescens*) in the laboratory based on their compatibility between each other (Vijaykumar and Brahmaprakash, 2018; Sneha and Brahmaprakash, 2017) [28, 25].

A pot experiment in a greenhouse was carried out to evaluate the effectiveness of microbial inoculants in different formulations on growth of green gram (cv KKM-3) at the Department of Agricultural Microbiology, University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra Campus, Bengaluru-560 065 (Latitude of 12º 58' N and longitude of 77º 38' E).

Seed treatment

Seeds of green gram (*Vigna radiata* L.) were treated with four different formulations of single, dual and triple inoculants comprising *Rhizobium* Sp., *Bacillus megaterium* and *Pseudomonas fluorescens*.

Green gram (*Vigna radiata* L.) seeds were treated with the inoculant formulations were sown in experimental pots at the rate of ten seeds per pot. After a week of germination, seedlings were thinned to retain two seedlings per pot. All the pots were maintained at field capacity. Plants were harvested at 50 per cent flowering.

Nutrients

The recommended dose of fertilizer for green gram (*Vigna radiata* L.) is 20: 40: 00 kg of NPK per acre. Appropriate dose of nitrogen was supplied through urea; phosphorus was supplied through single super phosphate.

Observation

After establishment of green gram (*Vigna radiata* L.) in the pot under greenhouse conditions, the growth parameters of crop plants were taken at selected intervals.

Pre-harvest observations include, plant height, number of leaves were recorded in the green gram (*Vigna radiata* L.) at the interval of 15 days interval up to 50 per cent of flowering stage.

Total Chlorophyll content

Thirty Days after Sowing (DAS), total chlorophyll content was estimated as suggested by Shoef and Lium (1976) [24].

Post-harvest observations include, total nitrogen uptake, total phosphorus uptake and total biomass content were recorded.

Total biomass content

Total biomass was recorded after harvest and till attaining a constant weight in oven at 60 ºC.

Estimation of nitrogen concentration

Nitrogen concentration in the root and shoot of green gram plants were estimated by Micro Kjeldhal method as given by Subbiah and Asija, 1956 [27].

Estimation of phosphorus concentration

The procedure used for estimation of phosphorus concentration in plant sample as given by Black (1965) [6].

Statistical analysis

Statistical data analysis was done by Complete Randomized Design (CRD) and means were compared by the Duncan's Multiple Range Test (Little and Hills, 1978) [14].

Results and Discussion

Plant height

After fifteen and 30 days of sowing, higher plant height was recorded in treatment T_8 receiving triple inoculants in liquid formulation followed by lignite formulation, alginate based formulation and fluid bed dryer based formulation (Table 1).

Inoculation with PGPRs in combinations as consortia enhances the plant growth (Plate. 1) these findings are similar with earlier reports made by Ravikumar (2012) [21], Ray and Valsalakumar, 2009 [22]. In chickpea (Almas *et al*., 2006) [1], black gram (Rathi *et al*., 2009) [20] and cowpea (Lakshmi, 2013 and Lavanya, 2014) [12, 13].

Table 1: Effect of different inoculant formulations on plant height of green gram

Treatments	Plant height (cm)									
	15 DAS					30 DAS				
	ABF	FBD BF	LGF	LQF	Main effect of treatments (T)	ABF	FBD BF	LGF	LQF	Main effect of treatments (T)
T_1	8.58^{o}	8.57^{o}	8.55^{o}	8.55^{o}	8.56^{h}	13.73^{q}	13.78^{pq}	13.72^{q}	13.75^{q}	13.75^{h}
T_2	9.63^{j}	8.89^{n}	10.03^{igh}	10.35^{f}	9.72^{e}	15.03^{n}	14.06^{p}	15.84^{l}	16.07^{l}	15.25^{e}
T_3	9.31^{k}	9.12^{ml}	9.67^{j}	9.24^{kl}	9.34^{g}	14.69^{o}	13.55^{rq}	14.71^{o}	15.52^{m}	14.62^{g}
T_4	9.51^{j}	8.86^{n}	9.88^{i}	9.95^{ih}	9.55^{f}	14.71^{o}	13.26^{r}	16.00^{l}	16.48^{k}	15.11^{f}
T_5	10.44^{f}	9.22^{kl}	10.44^{f}	10.67^{de}	10.19^{d}	17.77^{ih}	17.22^{j}	17.97^{gh}	18.66^{e}	17.91^{d}
T_6	10.82^{d}	8.97^{nm}	10.79^{d}	11.25^{c}	10.46^{b}	17.62^{i}	15.99^{l}	19.54^{c}	19.82^{c}	18.24^{b}
T_7	10.04^{gh}	10.13^{g}	10.33^{f}	10.61^{e}	10.28^{c}	18.20^{gh}	17.80^{ih}	18.38^{ef}	18.50^{e}	18.22^{bc}
T_8	11.68^{b}	10.32^{f}	11.77^{b}	12.13^{a}	11.48^{a}	20.94^{b}	19.08^{d}	21.08^{b}	21.63^{a}	20.68^{a}
Main effect of formulations (F)	10.00^{c}	9.26^{d}	10.18^{b}	10.34^{a}		16.59^{c}	15.59^{d}	17.16^{b}	17.55^{a}	
	LSD at 1%					LSD at 1%				
T	0.08					0.14				
F	0.06					0.10				
T x F	0.16					0.29				

Note: ABF; Alginate Based Formulation, FBDBF; Fluid Bed Dryer Based Formulation, LGF; Lignite Formulation, LQF; Liquid Formulation

T1	Control	T5	*Rhizobium* sp. + *Bacillus megaterium*
T2	*Rhizobium* sp.	T6	*Rhizobium* sp. + *Pseudomonas fluorescens*
T3	*Bacillus megaterium*	T7	*Bacillus megaterium* + *Pseudomonas fluorescens*
T4	*Pseudomonas fluorescens*	T8	*Rhizobium* sp + *Bacillus megaterium* + *Pseudomonas fluorescens*

Plate 1: Green gram plant height as influenced by triple inoculants in different formulations in comparison with control

Number of leaves

Higher number of leaves recorded in treatment T_8 receiving triple inoculants in liquid formulation followed by lignite, alginate based and fluid bed dryer based formulations (Table 2). Number of leaves indicates the amount photosynthetic activity and subsequent biomass accumulation, more the number of leaves more the photosynthesis and more the growth. Similar findings were reported by many workers Mishra *et al*., 2009 [16] and Sneha and Brahmaprakash, 2017 [25].

Table 2: Effect of different inoculant formulations on number of leaves in green gram

Treatments	15 DAS					30 DAS				
	ABF	**FBD BF**	**LGF**	**LQF**	**Main effect of treatments (T)**	**ABF**	**FBD BF**	**LGF**	**LQF**	**Main effect of treatments (T)**
T_1	3.33^{i}	3.33^{i}	3.33^{i}	3.33^{i}	3.33^{h}	6.67^{n}	6.67^{n}	6.67^{n}	6.67^{n}	6.67^{g}
T_2	4.67^{ef}	4.33^{fg}	5.00^{dc}	5.00^{de}	4.75^{de}	8.33^{hi}	8.33^{hi}	9.00^{g}	9.00^{g}	8.66^{d}
T_3	4.33^{fg}	3.67^{ij}	4.33^{fg}	4.33^{fg}	4.16^{fg}	7.67^{lm}	7.00^{mn}	7.67^{lm}	7.67^{lm}	7.50^{f}
T_4	4.67^{ef}	3.67^{ij}	4.00^{gh}	4.33^{fg}	4.17^{f}	8.67^{gh}	8.00^{jk}	8.67^{gh}	9.00^{g}	8.58^{de}
T_5	5.33^{cd}	5.00^{de}	5.33^{cd}	5.00^{de}	5.16^{cd}	10.33^{cd}	10.00^{ef}	10.33^{cd}	10.67^{bc}	10.33^{bc}
T_6	5.33^{cd}	5.00^{de}	5.33^{cd}	5.67^{bc}	5.33^{bc}	10.33^{cd}	10.00^{ef}	10.33^{cd}	10.67^{bc}	10.33^{bc}
T_7	5.33^{cd}	5.33^{cd}	5.33^{cd}	5.67^{bc}	5.41^{ab}	10.33^{cd}	10.33^{cd}	10.33^{cd}	10.67^{bc}	10.41^{ab}
T_8	5.67^{bc}	5.67^{bc}	6.00^{ab}	6.33^{a}	5.91^{a}	10.67^{bc}	10.67^{bc}	11.00^{ab}	11.33^{a}	10.92^{a}
Main effect of formulations (F)	4.83^{ab}	4.50^{bc}	4.83^{ab}	4.96^{a}		9.13^{c}	8.88^{d}	9.25^{ab}	9.46^{a}	
	LSD at 1%					LSD at 1%				
T	0.57					0.63				
F	0.41					0.44				
T x F	1.15					1.25				

Note: ABF; Alginate Based Formulation, FBDBF; Fluid Bed Dryer Based Formulation, LGF; Lignite Formulation, LQF; Liquid Formulation

T1	Control	T5	*Rhizobium* sp. + *Bacillus megaterium*
T2	*Rhizobium* sp.	T6	*Rhizobium* sp. + *Pseudomonas fluorescens*
T3	*Bacillus megaterium*	T7	*Bacillus megaterium* + *Pseudomonas fluorescens*
T4	*Pseudomonas fluorescens*	T8	*Rhizobium* sp + *Bacillus megaterium* + *Pseudomonas fluorescens*

Total chlorophyll content

Green gram plants receiving triple inoculants recorded highest total chlorophyll content (Fig. 1) in liquid formulation (3.08 mg/g of leaf) followed by alginate based formulation (3.00 mg/g of leaf), lignite based formulation (2.84 mg/g of leaf) and fluid bed dryer based formulation (2.83 mg/g of leaf). Higher chlorophyll content indicates the higher photosynthetic activity and subsequently it represents the amount of fixed carbohydrates and it is directly proportional to the biomass accumulation. Maximum total chlorophyll content was recorded in triple inoculants where all the inoculants could perform synergistic activity and make necessary micronutrient for chlorophyll productions such as, iron (Fe), manganese (Mn), zinc (Zn) made available to the plants by these inoculants (Arumugam *et al*., 2010; Shoef and Lium, 1976 and Stefan *et al*., 2013) [4, 24, 26].

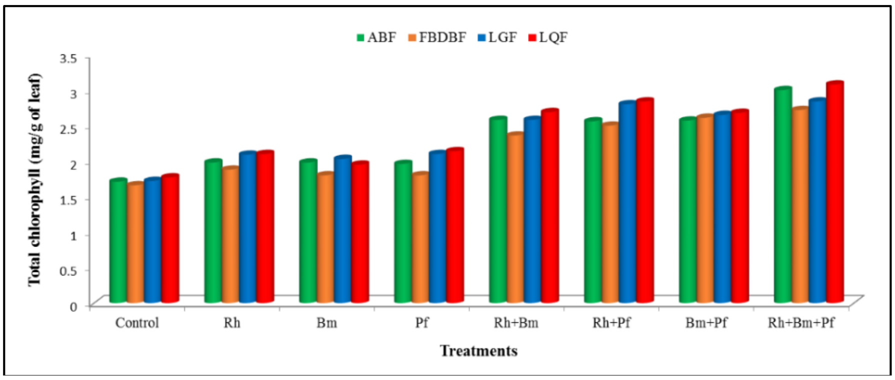

Fig 1: Total chlorophyll content of green gram as influenced by different inoculant formulations Rh; *Rhizobium* sp., B; *Bacillus megaterium* P; *Pseudomonas fluorescens*

Total nitrogen uptake

Maximum total nitrogen uptake was recorded in triple inoculants followed by dual inoculants and single inoculants (Table 3). The increased nitrogen uptake might be due to the increased availability of fixed atmospheric di-nitrogen by potential symbiotic N-fixing *Rhizobium* sp (Jain *et al*., 2007; Mehboob *et al*., 2013; Rajwar *et al*., 2013) [9, 15, 19].

Total phosphorus uptake

Total phosphorus uptake in green gram was recorded significantly higher in treatment received triple inoculants followed by dual inoculants and single inoculants (Table 3).

Table 3: Effect of different inoculant formulations on total nitrogen and phosphorus uptake by green gram

Treatments	Total Nitrogen Uptake (mg/plant)					Total Phosphorus Uptake (mg/plant)				
	ABF	**FBD BF**	**LGF**	**LQF**	**Main effect of treatments (T)**	**ABF**	**FBD BF**	**LGF**	**LQF**	**Main effect of treatments (T)**
T_1	36.87^{p}	36.83^{p}	37.12^{p}	39.95^{p}	37.69^{h}	5.34^{pq}	5.21^{q}	5.35^{pq}	5.43^{pq}	5.33^{h}
T_2	64.24^{lm}	59.73^{o}	66.39^{jlm}	71.20^{fgh}	65.39^{e}	7.04^{on}	6.72^{op}	7.40^{omn}	7.87^{olm}	7.26^{g}
T_3	63.09^{no}	58.70^{o}	64.72^{lmk}	69.01^{ghi}	63.88^{f}	7.97^{olm}	7.35^{omn}	8.35^{jlm}	8.87^{jhl}	8.13^{ef}
T_4	61.29^{on}	57.09^{o}	64.65^{lmk}	68.43^{igh}	62.87^{g}	8.66^{jlm}	8.13^{olm}	9.19^{jhl}	9.74^{jhf}	8.93^{de}
T_5	71.70^{fge}	68.08^{jgh}	75.5^{1cd}	79.50^{ab}	73.70^{b}	9.14^{jhl}	8.83^{jhl}	9.79^{hfi}	10.12^{hfd}	9.47^{d}
T_6	66.96^{jlh}	63.61^{jlm}	70.46^{fge}	74.54^{cdb}	68.89^{d}	9.81^{hfi}	9.44^{jhf}	10.38^{cfd}	10.87^{cdb}	10.12^{c}
T_7	67.99^{jlm}	65.03^{nm}	71.99^{fgh}	76.02^{cde}	70.26^{c}	10.56^{cfd}	10.08^{hfd}	11.03^{cab}	11.72^{cab}	10.85^{b}
T_8	73.68^{fde}	70.29^{fgh}	77.71^{cab}	81.36^{a}	75.76^{a}	11.30^{cad}	10.92^{cdb}	12.04^{ab}	12.42^{a}	11.67^{a}
Main effect of formulations (F)	63.23^{c}	59.92^{d}	66.07^{b}	70.00^{a}		8.73^{bc}	8.33^{cd}	9.19^{ab}	9.63^{a}	
	LSD at 1%					LSD at 1%				
T	1.55					0.70				
F	1.10					0.50				
T x F	3.11					1.41				

Note: ABF; Alginate Based Formulation, FBDBF; Fluid Bed Dryer Based Formulation, LGF; Lignite Formulation, LQF; Liquid Formulation

T1	Control	T5	*Rhizobium* sp. + *Bacillus megaterium*
T2	*Rhizobium* sp.	T6	*Rhizobium* sp. + *Pseudomonas fluorescens*
T3	*Bacillus megaterium*	T7	*Bacillus megaterium* + *Pseudomonas fluorescens*
T4	*Pseudomonas fluorescens*	T8	*Rhizobium* sp + *Bacillus megaterium* + *Pseudomonas fluorescens*

The increased phosphorus uptake might be due to the solubilization of unavailable form of phosphorus around the rhizosphere by phosphorus solubilizer bacterium *Bacillus megaterium* with the co-inoculation of *Rhizobium* sp and *Pseudomonas fluorescens*. Results are in agreement with reporters, Charana and Yoon 2013 [7]; Hussain and Noorka (2012) [8]; Lavanya, 2014 [13].

Total biomass content

Higher total biomass was observed (Fig. 2) in treatment T_8 received triple inoculants in liquid formulation followed by lignite, alginate based and FBD based formulations. Higher the accumulation of biomass indicates higher the growth. The current results are in agreement with Amit *et al*., 2010 [2]; Bansal, 2009 [5]; Kumar *et al*., 2015 [11]; Peter and Satish (2015) [18].

From the investigation it was concluded that treatments received triple inoculants in liquid formulation showed higher plant growth compared to other test formulations and un-inoculated control. The microbial load in the liquid formulation might have positively influenced on quick and effective colonization into the rhizosphere.

Comparatively increased nutrient uptake and total biomass content was observed in plants treated with triple inoculants in liquid formulations followed by lignite based, alginate based and fluid bed dryer based formulations which signifies the effective release of microbial inoculants and their subsequent colonization in liquid formulation compared to other test formulations and un-inoculated control.

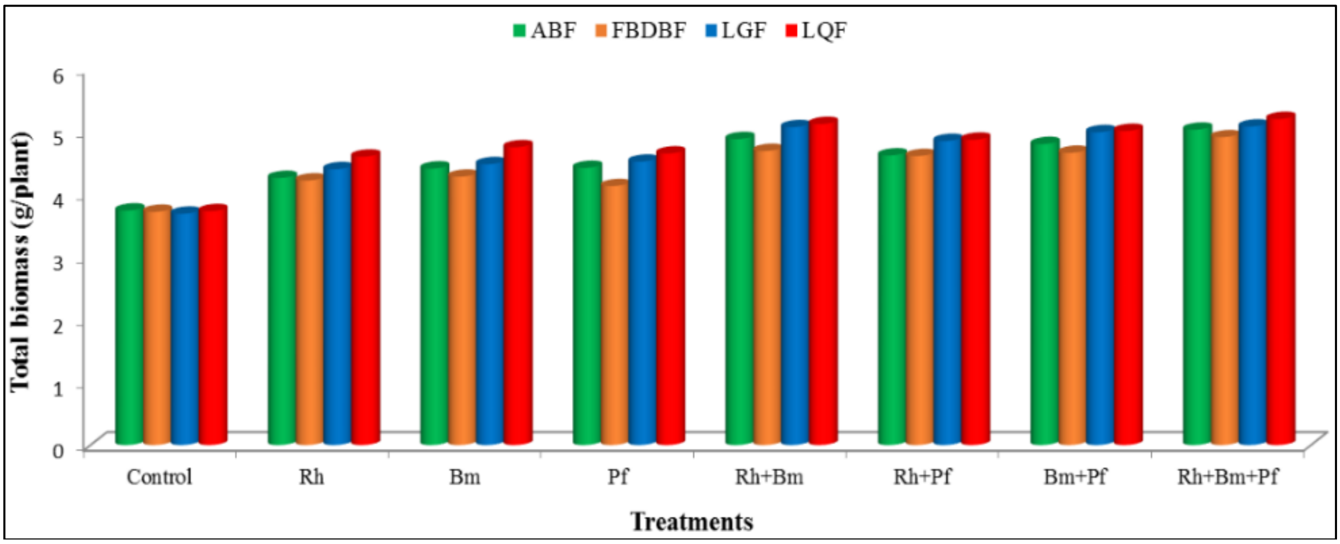

Fig 2: Total biomass of green gram as influenced by different inoculant formulations Rh; *Rhizobium* sp., B; *Bacillus megaterium* P; *Pseudomonas fluorescens*

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