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Effect of *Liquid Rhizobium* with Organic Bio-stimulants on Growth, Yield Attributes and Yield of Leguminous Blackgram [*Vigna mungo* (L.) Hepper]

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ABSTRACT

Background: Blackgram [*Vigna mungo* (L.) Hepper] is one of the most important cultivated legume crops with high nutritive value and agricultural importance. The productivity of blackgram is not adequate to meet the domestic demand of the growing Indian population. Consequently, there is an urgent need for enhancement of productivity through proper agronomic practices. With this background, a research was conducted to investigate the effect of liquid rhizobium with organic bio-stimulants on growth, yield attributes, yield and economics of irrigated blackgram.

Methods: A field experiment was laid out in randomized block design with three replications during 2021 (*Kharif* and *Rabi*) season. The treatments include 100% RDF foliar application of Dasagavya, liquid rhizobium, fish amino acid, panchagavya, pink pigmented facultative methylotrophs (PPFM) and sea weed extract with different concentration (1% and 3%, respectively) in addition to control.

Result: The experiment results revealed that the application of 100% RDF + liquid rhizobium @ 1% recorded higher growth characters *viz.,* plant height (cm), number of branches plant^{-1}, dry matter production (kg ha^{-1}), leaf area index, yield attributes, grain and haulm yield (kg ha^{-1}). Correlation and regression analysis also indicated that the yield attributes had a positive impact on the grain yield.

Key words: Blackgram, Growth, Liquid rhizobium, Organic bio stimulants, Yield, Yield attributes.

INTRODUCTION

Pulses are important crops in India because of its low cost and high quality protein. They play a major role in providing a balanced protein component in the diet of the people. Pulses contain higher level of quality protein, nearly three times as much as cereals; therefore, they are the cheapest and richest source of protein and essential amino acids and thus are a major protein source of the vegetarian diet. Besides, the crops enrich soil fertility and health in terms of the addition of nitrogen and organic matter. Among pulses, blackgram [*Vigna mungo* (L.) Hepper], occupies a unique place for its use as vegetable and it is grown both as a pure and mixed crop along with maize, cotton, sorghum and other millets (Ajaykumar *et al*., 2022).

In India, it occupies an area of 3.06 million hectares with a production of 1.70 million tones and an average productivity of 555 kg ha^{-1} (India Stat, 2021). One of the important reasons for low productivity is the poor fertility of soil. The problem is compounded by the fact that the majority of the farmers in irrigated areas are resource poor with low risk bearing capacity and they generally do not apply recommended dose of fertilizers, either through organic or inorganic sources. This imbalanced nutrient supply adversely affects the seed yield of blackgram soil health and even the profit to the farmers (Subramani and Solaimalai, 2000).

Organic substances are known to influence a wide array of physiological parameters like alteration of plant architecture, assimilate partitioning, promotion of photosynthesis, uptake of nutrients (mineral ions), enhancing nitrogen metabolism, promotion of flowering, uniform pod formation, increased mobilization of assimilates to defined sinks, improved seed quality, induction of synchrony in flowering and delayed senescence of leaves (Pradeep and Elamathy, 2007). These organic bio stimulants, when applied as foliar spray at proper crop growth stage in optimum concentration could play a significant role in increasing crop yield and quality of produce in different field crops (Jha *et al.,* 2009). Bio fertilizers

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comprised mostly the nitrogen fixing, phosphate solubilizing and plant growth-promoting microorganisms. Role of foliar applied Panchagavya and dhasagavya in production of many plantation crops had been well documented in India. The use of fermented, liquid organic fertilizers, effective microorganisms (EM) as foliar fertilizers have been introduced to modern agriculture in recent years to produce food with good quality and safety (Galindo *et al*., 2007).

Fish amino acid is a liquid and great value to both plants and microorganisms in their growth, because it contains and abundant amount of nutrients and various types of amino acids (will constitute a source of nitrogen (N) for plants). Seaweed concentrates are beneficial effects on plants as they contain growth promoting hormones (IAA, IBA and Cytokinins) and different trace elements, vitamins and amino acids (Khan *et al.* 2009). Integrated use of sea weed liquid fertilizer in combination with the chemical fertilizer and their proper management for better growth and yield is very essential.

In green gram, foliar application of liquid bio fertilizers during vegetative and flower bud initiation stages increased the number of flowers, pods and seeds per plant and seed yield. Foliar application of organic substance increased the chlorophyll content and promoted epicotyls elongation of soybean, mungbean and pea (Senthil *et al*., 2003). Exogenous application of PPFM (*Methylobacterium* species are a group of bacteria known as *pink-pigmented facultative methylotrophs*) produces some benefit in alleviating the adverse effects of drought stress and also improves germination, growth, development, quality and yield of crop plants (Hayat *et al*., 2010).

Based on the available background knowledge, the present investigation was carried out to develop suitable nutrient management technology involving liquid rhizobium and organic bio stimulants to enhance the productivity of irrigated blackgram.

MATERIALS AND METHODS

A field experiment was conducted during the year of 2021 (*Kharif* and *Rabi* season) in a Vanavarayar Institute of Agriculture, Pollachi (10°N and 77°E with an altitude of 210.7 m above MSL). The experiment was laid out in randomized block design with seven treatments and three replications. The treatments were T_1 - 100% RDF (Recommended dose of fertilizer) along with foliar application of Dhasagavya @ 3%, T_2 - 100% RDF + liquid rhizobium @1%. T_3- 100% RDF + Fish amino acid @1 %, T_4 - 100% RDF + Panchagavya @ 3%, T_5 - 100% RDF + PPFM @ 1%, T_6- 100% RDF + Sea weed extract @ 3 %, T_7- control. Organic bio fertilizers were purchased from Tamil Nadu Agricultural University, Coimbatore. Blackgram variety VBN 8 was used for the study.

The recommended doses of N, P_2O_5, K_2O were 25, 50, 25 kg ha^{-1}, respectively. Full dose of nitrogen, phosphorus, potassium in the form of urea, DAP, MOP were applied basal as per treatments. In addition to this, gypsum 20 kg ha^{-1} and soil application of 25 kg $ZnSO_4$ ha^{-1} were applied. Liquid bio fertilizers and organic bio stimulants were given as foliar spray at 30 and 45 days after sowing of blackgram. All other agronomic practices were adopted as per the need of the crop.

The growth characters *viz*., plant height, number of branches/plant, leaf area index (LAI) and dry matter production (DMP) were recorded. The maximum plant height was measured from the base of the stem to the tip of the longest trifoliate leaf. Numbers of branches were counted by manual and LAI was measured by using leaf area meter (LICOR 3000). DMP of various plant parts was arrived at by taking the sum of all the plant parts after keeping the sample in oven at 80°C for 48 hours.

Yield attributes *viz*., number of pods $plant^{-1}$, number of seeds pod^{-1}, pod weight, pod length, 100 seed weight, grain and haulm yield were recorded during harvest stage. Harvest index (HI) was also arrived by the ratio of economic yield to the biological yield. The data on the different parameters was analyzed statistically by adopting Fisher's method of ANOVA suggested by Gomez and Gomez (1984).

Quantitative variables analysis

Benefit-cost ratio (BCR), correlation and multiple linear regressions were employed to study the value of money or profitability, relationship between the various parameters (variables) and grain yield. Benefit-cost ratio was computed by dividing the present value of the net returns (Rs/ha) by present value of total cost (Rs/ha) (Shively, 2012). Correlation is a method for determining the relationship between two variables. The correlation coefficient formula enables the calculation of the connection between two variables and the resulting number describes the accuracy of the expected and actual values. In this article, correlation was employed to identify the relations among grain yield, plant height, number of branches $plant^{-1}$, number of pods $plant^{-1}$, DMP, leaf area index, number of seeds pod^{-1}, pod weight and its length (Ajaykumar *et al*., 2022). It was computed using the equation:

$$r_{xy} = \frac{S_{xy}}{S_x S_y} = \frac{\Sigma (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{(\Sigma (x_i - \bar{x})^2 (\Sigma (y_i - \bar{y})^2}}$$

Where,

r_{xy} = Coefficient of the linear relationship between the variables x and y.

S_x and S_y = Sample standard deviation.

S_{xy} = Sample covariance.

x_i and y_i = Values of x and y variables in the sample of the population.

$\bar{x}$ and $\bar{y}$ are the sample mean.

Another econometric tool used in this study was regression which examines the relationship between a dependent variable and a collection of independent variables (Pillai *et al.*, 2010). Regression was estimated by

$$Y_i = \alpha + \beta X_i + u_i$$

Where,

y_i = Dependent variable (grain yield).

x_i = Independent variables (plant height, number of branches $plant^{-1}$, number of pods $plant^{-1}$, leaf area index, number of seeds pod^{-1}, pod weight and its length).

β= Slope coefficient of the respective independent variable.
α = Constant or intercept term and is the error term.
This formal mode of regression equation could be rewritten as,
Grain yield = α+ β_1 plant height (cm) + β_2 no. of branches plant^{-1} + β_3 DMP (kg\ha) + β_4 LAI + β_5 number of pods plant^{-1} + β_6 number of seeds pod^{-1} + β_7 pod weight plant^{-1} + β_8 pod lenghth (cm)

RESULTS AND DISCUSSION

Effect on growth characters

The data on growth characters of blackgram are presented in Table 1. Various plant growth characters of blackgram crop were significantly influenced by liquid rhizobium with organic bio stimulants.

100% RDF along with foliar application of 1% liquid rhizobium registered increased plant height of 40.9 cm, 42.0 cm at harvest stage during *kharif* and *rabi* and it was followed by foliar spraying of panchagavya @ 3 % and sea weed extract @ 3 %. According to Gardner *et al*. (1991), nitrogen nutrients are needed by plants for the synthesis of amino acids and proteins, especially at plant growth points to accelerate the process of plant growth such as cell division and cell elongation to increase plant height. Spraying of panchagavya and sea weed extract stimulated the growth and plant height by increasing the auxin and cytokinin content in blackgram (Kumar *et al*., 2011).

Significantly higher number of branches plant^{-1} was found with the application of 100% RDF + 1% liquid rhizobium followed by 100 % RDF + panchagavya @ 3%. This helped to increase the cell division activity, cell expansion and elongation, ultimately leading to more number of branches plant^{-1}. Nitrogen can stimulate vegetative growth of plants as well as the number of branches. The influence of seaweed extract, dhasagavya and fish amino acid on the endogenous levels of hormones enhanced the photosynthesis and nitrate assimilation (Senthil *et al*., 2003).

The increase in DMP was significantly more with 100% RDF + foliar application of 1% liquid rhizobium (2995 and 3087 kg/ha) and 3% panchagavya (2713 and 2801 kg/ha) during *kharif* and *rabi*. It might have contained microbial metabolites in appreciable amount that help in maintaining the opening of stomata for longer period both in optimum and adverse conditions during the crop growth which led to increased DMP. The increased availability of nitrogen due to the presence of nitrogen fixing bacteria (Jeyakumar *et al*., 2008). Also, the biosynthesis of plant growth promoting substances like cytokinins, IAA, GA, vitamins and antibiotic substances by the microbial inoculants acted in synergy to it (Zhang *et al*., 2008).

LAI is an important indicator of total photosynthetic surface area available to the plant for the production of photosynthates, which accumulate in the developing sink. Higher LAI was recorded under 100% RDF + 1% liquid rhizobium (4.92, 5.87 during *kharif* and *rabi*), which was followed by 100% RDF + 3% panchagavya (Table 1). Liquid bio fertilizers played an important role in increasing the number of leaves, leaf elongation, chlorophyll content and thus led to increase in leaf area index. The overall improvement in the growth of blackgram with the addition of seaweed extract, dhasagavya and fish amino acid could be ascribed to their pivotal role in several physiological and biochemical processes, *viz*., root development, photosynthesis, energy transfer reaction and symbiotic biological N fixation process. The similar results were also reported by Subramani and Solaimalai (2000).

Yield attributes and yield

A perusal of data revealed that yield attributes and the yield increased significantly with the foliar application of Liquid rhizobium and organic bio stimulants in blackgram over control.

100% RDF along with foliar application of 1% liquid noticed significantly increased number of pods plant^{-1} (37.5 and 37.7), number of seeds pod^{-1} (9.0 and 9.2), pod weight (15.62 and 16.03 g) and pod length (5.84 and 5.91 cm) during *kharif* and *rabi*. This was followed by 100 % RDF + 3 % panchagavya (Table 2). This was due to the enhanced root and shoot development, solar radiation interception and nutrients uptake. Further, the translocation and accumulation of photosynthates in the economic sinks resulted in increased yield attributes and biological yield of blackgram.

Grain yield (1004 and 1063 kg ha^{-1} during *kharif* and *rabi*, respectively) and haulm yield (1936 and 2108 kg ha^{-1})

Table 1: Effect of liquid rhizobium and organic bio stimulants on growth characters of irrigated blackgram.

T. no	*Kharif*				*Rabi*			
	Plant height (cm)	No. of branches plant^{-1}	DMP (kg ha^{-1})	LAI	Plant height (cm)	No. of branches plant^{-1}	DMP (kg ha^{-1})	LAI
T_1	36.1	5.23	2419	4.25	37.2	6.13	2509	5.15
T_2	40.9	6.64	2995	4.92	42.0	7.56	3087	5.87
T_3	34.8	4.99	2214	4.08	36.0	5.93	2308	5.02
T_4	38.6	6.03	2713	4.69	39.7	6.95	2801	5.59
T_5	30.3	4.42	1873	3.51	31.5	5.37	1968	4.46
T_6	36.7	5.34	2500	4.32	37.9	6.22	2588	5.20
T_7	28.4	4.13	1554	3.24	29.6	5.08	1649	4.19
SEd	0.51	0.11	59.1	0.10	0.60	0.15	61.2	0.11
CD (P = 0.05)	1.10	0.22	121.2	0.22	1.21	0.32	124.6	0.23

were significantly higher with application of 100% RDF + 1% liquid rhizobium and 100 % RDF + 3% panchagavya. In addition to that, the effect of 3 % Seaweed extract was on par with 3% dhasagavya (Table 3). The least grain and straw yield was observed in control plot. The overall effect of rhizobium in increasing the grain yield of this crop was primarily due to the enhanced availability of nutrients, through nitrogen fixation by bacteria production of plant growth promoting (PGP) substances and vitamins, especially B_{12} produced in the rhizosphere soils. As observed in the present investigation, liquid bio fertilizers and organic bio stimulants play a favorable role in improving various metabolic activities through enhanced nucleic acid metabolism and protein synthesis (Vardhini and Rao, 1998).

Correlation and regression analysis

The correlation results were represented in Table 4. Its results revealed that all the variables included in the model were positively significant at one percent level of significance and these signs emphasize all the variables would attribute to the grain yield of the blackgram. The correlation coefficients of the grain yield with plant height (0.83), number of branches plant^{-1} (0.55), DMP (0.92), leaf area index (0.66), number of pods plant^{-1} (0.89), number of seeds pod^{-1} (0.89), pod weight (0.98) and pod length (0.81) show that all the attributes were positively related and that strongly proves when there is an increment in these variables, there would be an increase in the yield of the blackgram. So, all these variables are included as the independent variables in the multiple linear regression model (Xiaolu *et al*., 2019). The multiple linear regressions were estimated to measure the relationship and the change in magnitude of the grain yield due to the other prescribed parameters (Table 5). The multiple linear regression equation could be written as,

Grain yield = -101.07 + 1.11 plant height + 0.83 no. of branches plant^{-1} + 1.28 DMP (kg/ha) + 0.19 LAI + 2.08 number of pods plant^{-1} + 2.54 number of seeds pod^{-1} + 0.18 pod weight plant^{-1} + 0.07 pod lenght plant^{-1}

The R^2 (0.81) depicts a good sign of model fit which implies that 81 percent of the grain yield was caused by the independent variables. All the variables except plant height except plant height and DMP were found statistically significant (Table 5). The slope coefficient of the number of branches has shown that when there is one percent increase number of branches, there would be a significant increase in the grain yield by 0.83 percent, other variables being held constant. Likewise, when there is a one percent increase in the variables viz., leaf area index, number of pods plant^{-1}, number of seeds pod $^{-1}$, pod weight and pod length, there would an increase in the yield by 0.19, 2.08, 2.54, 0.18 and

Table 2: Effect of liquid rhizobium and organic bio stimulants on yield attributes of irrigated blackgram.

T. no	*Kharif*					*Rabi*				
	No. of pods plant^{-1}	No. of seeds pod^{-1}	Pod weight (g) plant^{-1}	Pod length (cm)	TW (g)	No. of pods plant^{-1}	No. of seeds pod^{-1}	Pod weight (g) plant^{-1}	Pod length (cm)	TW (g)
T_1	31.8	8.1	14.32	5.72	4.3	32.0	8.1	14.66	5.76	4.3
T_2	37.5	9.0	15.62	5.84	4.4	37.7	9.2	16.03	5.91	4.4
T_3	29.2	7.6	13.98	5.52	4.2	29.2	7.6	14.18	5.57	4.2
T_4	35.1	8.6	15.21	5.80	4.4	35.3	8.7	15.71	5.84	4.4
T_5	27.5	7.3	13.52	5.37	4.1	27.3	7.3	13.74	5.41	4.1
T_6	32.9	8.3	14.95	5.75	4.3	32.9	8.4	15.35	5.81	4.3
T_7	22.3	6.4	11.94	5.00	4.0	22.9	6.7	12.34	5.17	4.0
SEd	1.02	0.14	0.30	0.10	0.1	1.06	0.15	0.30	0.11	0.1
CD (P = 0.05)	2.10	0.30	0.61	0.21	NS	2.20	0.32	0.63	0.23	NS

Table 3: Effect of liquid rhizobium and organic bio stimulants on grain yield (kg ha^{-1}), haulm yield (kg ha^{-1}) and harvest Index (%) of irrigated blackgram.

T. no	*Kharif*			*Rabi*		
	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest index (%)	Grain yield (kg ha^{-1})	Haulm yield (kg ha^{-1})	Harvest index (%)
T_1	864	1514	2.74	936	1666	2.79
T_2	1004	1936	2.94	1063	2108	2.99
T_3	778	1324	2.71	852	1478	2.75
T_4	951	1714	2.81	1007	1888	2.88
T_5	669	1266	2.73	812	1402	2.77
T_6	886	1533	2.76	946	1703	2.81
T_7	495	824	2.65	746	1313	2.70
SEd	23.2	74.8	0.05	24.8	75.8	0.05
CD (P =0.05)	48.3	151.4	0.11	50.2	156.2	0.12

Table 4: Correlation between yield attributes and yield (Pooled data).

Variables	Grain yield (kg/ha)	Plant height (cm)	No. of branches /plant	DMP (kg/ha)	LAI	No. of pods plant^{-1}	No. of seeds pod^{-1}	Pod weight plant^{-1}	Pod length (cm)
Grain yield (kg/ha)	1								
Plant height (cm)	0.83	1							
No. of branches plant^{-1}	0.55	0.44	1						
DMP (kg/ha)	0.92	0.79	0.50	1					
LAI	0.66	0.55	0.20	0.57	1				
No. of pods plant^{-1}	0.89	0.79	0.49	0.89	0.57	1			
No. of seeds pod^{-1}	0.89	0.81	0.47	0.86	0.59	0.85	1		
Pod weight plant^{-1}	0.98	0.86	0.55	0.93	0.63	0.90	0.92	1	
Pod length (cm)	0.81	0.68	0.57	0.82	0.50	0.81	0.80	0.84	1

Table 5: Multiple linear regression estimates the blackgram yield.

Variables	Coefficients	Std. error	t-stat	P-value	Significance
Intercept	-101.07	8.16	-12.39	0.00	**
Plant height (cm)	1.11	0.69	1.61	0.15	NS
No. of branches plant^{-1}	0.83	0.12	6.92	0.00	**
DMP (kg/ha)	1.28	0.78	1.64	0.14	NS
LAI	0.19	0.08	2.38	0.02	*
No. of pods plant^{-1}	2.08	0.92	2.26	0.03	*
No. of seeds pod^{-1}	2.54	1.12	2.27	0.03	*
Pod weight plant^{-1}	0.18	0.07	2.57	0.00	**
Pod length (cm)	0.07	0.03	2.33	0.03	*

Note: *Significant at 5% level of significance; **Significant at 1% level of significance.
NS - Non significant.

0.07 per cent respectively (Tittonell *et al*., 2007). There is strong econometric evidence that the number of branches and pod weight have a significant impact on the grain production of black gram.

CONCLUSION

Both the seasons of experiments concluded that the application of 100% RDF along with foliar spraying of 1% liquid rhizobium significantly increased growth characters, yield attributes and yield compared to all other treatments. Subsequently, it was followed by the application of 100% RDF along with 3% panchagavya. The correlation and regression results showed that all the parameters had a positive relation on the grain yield and thus variables should be focused to enhance the productivity of the blackgram.

Conflict of interest: None.

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