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Physiological and Biochemical Characters of Blackgram as Influenced by Liquid Rhizobium with Organic Biostimulants

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ABSTRACT

Background: 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 management practices. With this background, a research experiment was conducted to investigate the effect of liquid rhizobium with organic bio-stimulants on physiological and biochemical characters, antioxidant enzymes and yield of 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% recommended dose of NPK along with 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. Organic bio stimulants were sprayed at 30 and 45 days after the sowing of blackgram.

Result: The experiment results revealed that the application of 100% RDF+Liquid Rhizobium @ 1% registered maximum physiological and biochemical characters *viz.,* CGR, total chlorophyll content, soluble protein content, nitrate reductase activity, catalase activity, peroxidase activity and number of root nodules plant^{-1}, yield attributes *viz.*, number of pods plant^{-1}, number of seeds pod^{-1}, grain yield (kg ha^{-1}), 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: Biochemical, Blackgram, Liquid rhizobium, Organic bio-stimulants, Physiological, Yield.

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. 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).

The yield of blackgram is low due to various reasons includes poor management practices, various physiological, biochemical as well as inherent factors associated with the crop. 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).

Role of foliar applied panchagavya and dhasagavya in production of many plantation crops had been well documented in India (Selvaraj, 2003). 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 (FAA) 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

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and amino acids (Khan *et al.* 2009). 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 black gram.

MATERIALS AND METHODS

A field experiment was conducted during the year of 2021 (*kharif* and *rabi* season) in a Vanavarayar Institute of Agriculture, Pollachi. The experiment was laid out in randomized block design with seven treatments and three replications. The treatments were T_1 - 100% RDF along with foliar application of dhasagavya at 3 %, T_2 - 100% RDF along with foliar application of liquid rhizobium at 1%. T_3- 100% RDF along with foliar application of Fish amino acid at 1%, T_4 - 100% RDF along with foliar application of Panchagavya at 3%, T_5 - 100% RDF along with foliar application of PPFM at 1%, T_6- 100% RDF along with foliar application of Sea weed extract at 3%, T_7 - control. Liquid bio fertilizers and organic bio stimulants 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, SSP, MOP were applied basal as per treatments. 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 physiological biochemical characters *viz*., crop growth rate, chlorophyll content and soluble protein were estimated. The CGR was computed using the formula suggested by Watson (1958). Chlorophyll content of leaves was recorded as described by Yoshida *et al.*, (1976). Soluble protein content of the leaf was estimated at by using folinciocalteau reagent by adopting the procedure described by Lowry *et al.,* (1950). Nitrate reductase activity (NRase activity) (Nicholas *et al.*, 1976), Catalase activity (Gopalachari, 1963), Peroxidase activity (Gurumurthy *et al.* 2019) were also estimated. Yield attributes *viz*., number of pods $plant^{-1}$, number of seeds pod^{-1}, grain and haulm yield were recorded during harvest stage. 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

Correlation and multiple linear regressions were employed to study the value of money or profitability, the relationship between the various parameters (variables) and grain yield. The Pearson Correlation Coefficient (PCC) is the most prevalent sort of correlation coefficient and it creates a relationship between expected and observed values after a statistical investigation. In this article, the correlation was employed to identify the relations among grain yield (kg ha^{-1}), Crop growth rate (g. m^{-2} day^{-1}), Total chlorophyll content (mg g^{-1}), Soluble protein content (mg g^{-1}), Nitrate Reductase activity (µg NO_2 g^{-1} h^{-1}), number of pods $plant^{-1}$ and number of seeds pod^{-1} (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 $\bar{y}$ = 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 grain yield (kg ha^{-1}), crop growth rate (g. m^{-2} day^{-1}), total chlorophyll content (mg g^{-1}), soluble protein content (mg g^{-1}), nitrate reductase activity (µg NO_2 g^{-1} h^{-1}), number of pods $plant^{-1}$ and number of seeds pod^{-1}).

β = Slope coefficient of the respective independent variable.

α = Constant or intercept term.

u_i = The error term.

This formal model of regression equation could be rewritten as,

Grain yield (kg ha^{-1}) = $\alpha + \beta_1$ crop growth rate (g. m^{-2} day^{-1}) $+\beta_2$ total chlorophyll content (mg g^{-1}) + β_3 soluble protein content (mg g^{-1}) + β_4 nitrate reductase activity (µg$NO_2$$g^{-1}$ h^{-1}) $+\beta_5$ number of pods $plant^{-1}$ + β_6 number of seeds pod^{-1}+ u_i.

RESULTS AND DISCUSSION

Physiological and biochemical characters

Liquid bio fertilizer and organic bio stimulants influenced the crop growth rate, total chlorophyll content and soluble protein content during vegetative and flowering stages. Maximum crop growth rate was registered at 1% liquid rhizobium (4.75 and 6.33 g.m^{-2} day^{-1}) at 60 DAS. Which was followed by 3% panchagavya and 3% seaweed extract. Liquid bio fertilizer are excellent source of macro and micro nutrients, trace elements, amino acid, Plant growth promoting hormones, vitamins, antibiotics, carbohydrates, proteins and other organic matters exhibits plant growth stimulating property under diluted condition (Moshe *et al.*, 2015) which increased the crop growth. Total chlorophyll content was significantly improved with 1% liquid rhizobium application with value of 3.02 mg g^{-1} (vegetative stage) and 3.60 mg g^{-1} (flowering stage). Furthermore, it was followed by foliar application of 3% panchagavya (Fig 1). The increased chlorophyll content in PGPR sprayed plants may be due to the fact that nitrogen is a constituent of chlorophyll molecule (Teotia *et al.*, 2000).

Soluble protein content of the leaf, being a measure of RuBP carboxylase activity, was considered as an index for photosynthetic efficiency. Rubisco enzyme forms nearly 80 per cent of the soluble protein in leaves of many plants (Joseph *et al.,* 1981). In the present study, higher soluble protein content (12.51 mg g^{-1} at vegetative stage and 12.74 mg g^{-1} at flowering stage) was observed under application 100% RDF + 1% liquid rhizobium followed by foliar spray of 3% panchagavya. 100% RDF + sea weed extract 3% noticed higher soluble protein content with value 12.34 mg g^{-1} and 12.52 mg g^{-1} (vegetative stage and flowering stage) over than rest of the treatments (Fig 1). The positive effect of liquid bio fertilizers on soluble protein content might be due to its involvement in the expression and activation of genes responsible for synthesis of rubisco and rubiscoactivate (Xia *et al.,* 2009), thereby increasing the capacity of CO_2 assimilation in the Calvin cycle. The rubisco content per unit leaf area was positively correlated with that of soluble protein content of the leaf (Balachandar *et al.*, 2003).

Antioxidant enzymes

Antioxidant enzymes activities are significantly influenced by application of liquid rhizobium and organic bio stimulants. The data on antioxidant enzymes are presented Table 1. Nitrate reductase (NR) is an important enzyme for nitrogen assimilation and is a key point of metabolic regulation in crop plants. Thus, NRase is intimately associated with the plant growth and development (Sinha and Nicholas, 1981). Peroxidases, one of the key antioxidant enzymes, are widely distributed in nature and catalyze oxidation of various electron donor substrates concomitant with the decomposition of H_2O_2. Catalase was a main enzyme to eliminate H_2O_2 in the mitochondrion and microbody (Shigeoka *et al.*, 2002). The reduction trend of H_2O_2 content revealed that, gradual increase of catalase enzyme activity during vegetative and flowering stage of the investigation. In the present study, high NRase activity, POD and catalase enzymes activity were observed with application of 1% liquid

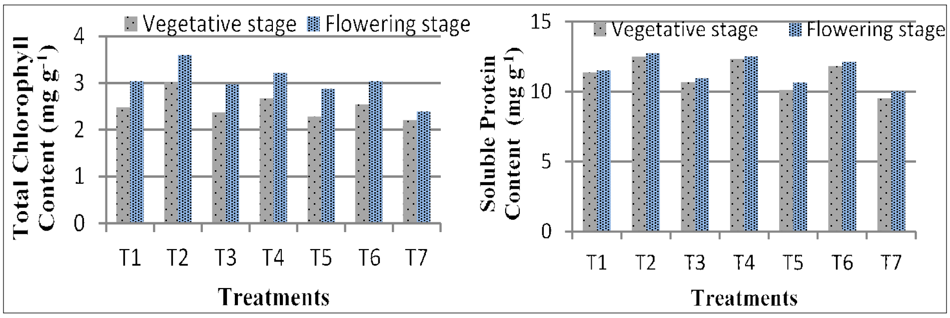

Fig 1: Effect of liquid rhizobium and organic bio stimulants on total chlorophyll content and soluble protein content (mg g^{-1}) in irrigated blackgram (pooled data).

Table 1: Effect of liquid rhizobium and organic bio stimulants on antioxidant enzymes of irrigated blackgram (Pooled data).

T. no	Vegetative stage			Flowering stage		
	Nitrate reductase activity (µg NO_2 g^{-1} h^{-1})	Catalase activity (µg of H_2O_2 g^{-1} min^{-1})	Peroxidase activity (µ mole min^{-1} protein^{-1})	Nitrate reductase activity (µg NO_2 g^{-1} h^{-1})	Catalase activity (µg of H_2O_2 g^{-1} min^{-1})	Peroxidase activity (µ mole min^{-1} protein^{-1})
T_1	45.01	12.71	10.01	77.04	14.92	10.89
T_2	51.94	8.14	11.70	98.56	8.64	8.63
T_3	42.31	13.89	9.85	73.41	13.72	10.12
T_4	47.85	10.97	10.91	91.09	11.03	9.57
T_5	38.63	15.07	9.69	69.57	16.12	11.96
T_6	46.52	11.48	10.32	85.68	12.54	11.35
T_7	27.69	16.87	8.34	58.03	17.91	12.74
SEd	1.56	0.81	0.76	1.65	0.85	0.78
CD (P = 0.05)	3.84	1.92	1.57	3.98	1.89	1.60

rhizobium and it was followed by 3% panchagavya. The absolute control recorded the minimum catalase activity and control recorded the maximum value (16.87 and 17.91 µg of H_2O_2 g^{-1} min^{-1}). Catalases also played an important role in the fine regulation of reactive oxygen speciesin the cell through activation and deactivation of several apoplastic enzymes might also generate reactive oxygen species under normal conditions (Sairam *et al.,* 1996).

Effect on nodulation

Number of completely developed nodules in all the treatments was calculated manually. 100% RDF along with foliar application of 1% liquid rhizobium noticed significantly increased number of nodules plant^{-1} (22.67 and 23.05) during *kharif* and *rabi*. This was followed by 100% RDF + 3% panchagavya (Fig 2). Being, a leguminous crop, blackgram fulfills major part of nitrogen requirement by symbiotic nitrogen fixation with the help of bacterium called *Rhizobia. Rhizobium* involve in symbiotic biological nitrogen fixation; survival in soil, *Rhizosphere* colonization, infection and nodule development and energy transformation during Nitrogen fixation in root nodules (O:Hara *et al.,* 1988). Symbiotic nitrogen fixation is well known process exclusively driven by bacterial nitrogenase enzyme which specifically reduces atmospheric nitrogen to ammonia in the symbiotic root nodules (Leigh, 2002).

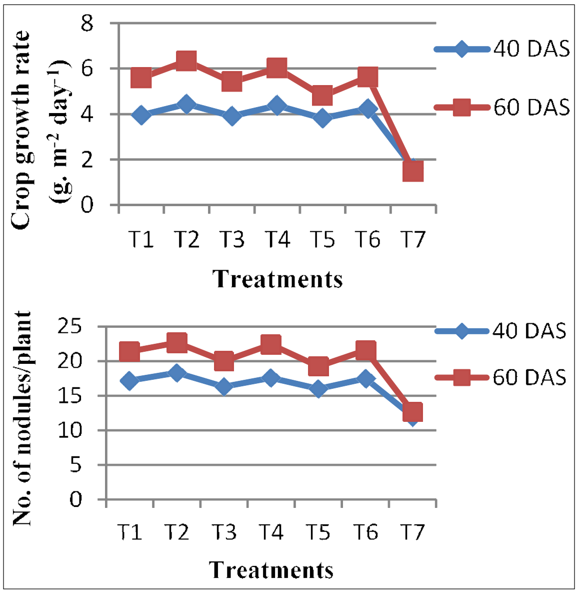

Fig 2: Effect of liquid rhizobium and organic bio stimulants on crop growth rate (g m^{-2} day^{-1}) and no. of nodules/plant in irrigated blackgram (pooled data).

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 (Table 2). 100% RDF along with foliar application of 1% liquid rhizobium noticed significantly increased number of pods plant^{-1} (37.5 and 37.7), number of seeds pod^{-1} (9.0 and 9.2), grain yield (1004 and 1063 kg ha^{-1} during *kharif* and *rabi*, respectively) and haulm yield (1936 and 2108 kg ha^{-1}) and which was followed by 100% RDF + 3% panchagavya. 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.

In addition to that, the effect of 3% Seaweed extract was on par with 3% dhasagavya. The least grain and haulm 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 (Vardhini and Rao, 1998).

Correlation and regression analysis

The correlation results revealed that all the variables included in the model were positively significant at a one percent level of significance (Table 3). The correlation coefficients of the grain yield with crop growth rate (0.93), total chlorophyll content (0.94), soluble protein content (0.79), nitrate reductase activity (0.52), number of pods

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

T. no	*Kharif*				*Rabi*			
	No. of pods plant^{-1}	No. of seeds pod^{-1}	Grain yield (kg ha^{-1})	Haulm yield (kg ha^{-1})	No. of pods plant^{-1}	No. of seeds pod^{-1}	Grain yield (kg ha^{-1})	Haulm yield (kg ha^{-1})
T_1	31.8	8.1	864	1514	32.0	8.1	936	1666
T_2	37.5	9.0	1004	1936	37.7	9.2	1063	2108
T_3	29.2	7.6	778	1324	29.2	7.6	852	1478
T_4	35.1	8.6	951	1714	35.3	8.7	1007	1888
T_5	27.5	7.3	669	1266	27.3	7.3	812	1402
T_6	32.9	8.3	886	1533	32.9	8.4	946	1703
T_7	22.3	6.4	495	824	22.9	6.7	746	1313
SEd	1.02	0.14	23.2	74.8	1.06	0.15	24.8	75.8
CD (P=0.05)	2.10	0.30	48.3	151.4	2.20	0.32	50.2	156.2

Table 3: Correlation between physio-biochemical parameters and yield attributes (Pooled data).

	Grain yield (kg ha^{-1})	Crop growth rate (g. m^{-2} day^{-1})	Total chlorophyll content (mg g^{-1})	Soluble protein content (mg g^{-1})	Nitrate reductase activity (µg NO_2 g^{-1} h^{-1})	Number of pods $plant^{-1}$	Number of seeds pod^{-1}
Grain yield (kg ha^{-1})	1						
Crop growth rate (g. m^{-2} day^{-1})	0.93	1					
Total chlorophyll content (mg g^{-1})	0.94	0.49	1				
Soluble protein content (mg g^{-1})	0.79	0.38	0.29	1			
Nitrate reductase activity (µg NO_2 g-1 h^{-1})	0.52	0.48	0.44	0.63	1		
Number of pods $plant^{-1}$	0.89	0.57	0.49	0.38	-0.52	1	
Number of seeds pod^{-1}	0.29	0.55	0.51	0.79	0.19	0.65	1

Table 4: Multiple linear regression estimates the blackgram yield.

Variables	Coefficients	Std. Error	t-stat	P-value	Significance
Intercept	-174.97	57.77	-3.03	0.00	**
Crop growth rate (g. m^{-2} day^{-1})	7.46	2.31	3.23	0.00	**
Total chlorophyll content (mg g^{-1})	1.91	0.47	4.06	0.00	**
Soluble protein content (mg g^{-1})	0.63	0.29	2.18	0.03	*
Nitrate reductase activity (µg NO_2 g^{-1} h^{-1})	3.11	1.90	1.64	0.23	NS
Number of pods $plant^{-1}$	1.51	0.31	4.81	0.00	**
Number of seeds pod^{-1}	1.02	0.79	1.29	0.29	NS
R^2			0.77		
Observations			56		

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

$plant^{-1}$ (0.89) and number of seeds pod^{-1} (0.29) showed 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 black gram. 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 4). The multiple linear regression equation could be written as,

Grain yield = -174.97 + 7.46 crop growth rate (gm^{-2} day^{-1}) + 1.91 otal chlorophyll content (mg g^{-1}) + 0.63 soluble protein content (mg g^{-1}) + 3.11 nitrate reductase activity (ug NO_2 g^{-1} h^{-1}) + 1.51 number of pods $plant^{-1}$ + 1.02 number of seeds pod^{-1}.

The R^2 (0.77) depicts a good model fit, implying that the independent variables caused 77 per cent of the grain yield. All the variables except nitrate reductase activity and number of seeds pod^{-1}were found statistically significant (Table 4). The slope coefficient of the crop growth rate has shown that when there is one per cent increase incrop growth rate, there would be a significant increase in the grain yield by 7.46 per cent, other variables being held constant. Likewise, when there is a one per cent increase in the variables *viz.*, total chlorophyll index, soluble protein content and number of pods $plant^{-1}$, there would an increase in the yield by 1.91, 0.63 and 1.51 per cent, respectively. There is strong econometric evidence that the crop growth rate, total chlorophyl content and number of pods $plant^{-1}$ significantly impact the grain yield of blackgram.

CONCLUSION

Both the seasons of experiments concluded that the application of 100% RDF along with foliar spraying of 1% liquid rhizobium significantly increased physiological and biochemical characters 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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