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Effect of *pink-pigmented facultative methylotrophs*, PGRs and Nutrients on Growth, Yield and Economics of Irrigated 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 experiment was conducted in a farmer's field at Pudhupalayam, Coimbatore to investigate the effect of foliar application of PPFM, plant growth regulating compounds and nutrients 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 2019 (*kharif* and *rabi*) season. The treatments include 100% recommended dose of NPK along with foliar application of diammonium phosphate (DAP), brassinolide (Br), salicylic acid (SA) and pink pigmented facultative methylotrophs (PPFM) with different concentration (1%, 2%, 1 ppm, 2 ppm, 50 ppm, 100 ppm, respectively) in addition to control. PPFM and PGRs were sprayed at 30 and 45 days after the sowing of blackgram.

Result: The experiment results of the two seasons study revealed that the application of 100% RDF + PPFM @ 2% recorded higher growth characters *viz.,* plant height (cm), number of branches plant^{-1}, leaf area index and yield attributes *viz*., number of pods plant^{-1}, number of seeds pod^{-1}, pod weight (g), pod height (cm), 100 seed weight (g) and yield *viz*., grain yield (kg/ha), straw yield (kg/ha) and harvest index (%). As well as the same treatment recorded higher net return and B:C ratio. Correlation and regression analysis also indicated that the yield attributes had a positive impact on the grain yield with a magnitude of 1.91 and 1.67, respectively. Therefore, application of 100% RDF+2% PPFM spray can be recommended as the best technology to improve the yield and economics of blackgram.

Key words: Blackgram, DAP, Economics, Growth, Growth regulator, PPFM, 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. 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.

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, 2019).

Pulses are also known to increase nitrogen percentage in the soil by fixing atmospheric nitrogen. Therefore, it plays an important role in maintaining soil fertility. But, the average productivity of the crop is far below. Major hurdles in increasing the productivity are the poor fertility of the soil in which the crop is grown and improper fertilization. Mineral nutrition plays a key role in exploiting the genetic potential of pulse crops. Phosphorous (P) is an important mineral element for grain legumes as it helps in root development, participates in synthesis of phosphate and phosphoproteins, takes part in energy fixing and releasing process in plants. Most of the applied P gets fixed, only 10-18% is utilized by the current crop. Significant response of legumes to phosphate nutrition has been reported by several workers (Singh and Yadav, 2008).

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Growth regulating substances/growth regulators 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).

In green gram, foliar application of salicylic acid (SA) during vegetative and flower bud initiation stages increased the number of flowers, pods and seeds per plant and seed yield. Foliar application of 0.5 ppm brassinolide increased the chlorophyll content and promoted epicotyls elongation of soybean, mungbean and pea (Senthil *et al*., 2003).

Exogenous application of pink-pigmented facultative methylotrophs (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 PPFM, plant growth regulators and nutrients to enhance the productivity of irrigated black gram.

MATERIALS AND METHODS

Details of the experimental site

A field experiment was conducted during the year of 2019 (*kharif* and *rabi* season) in a farmer's field at Pudhupalayam, Coimbatore (11°N and 77°E with an altitude of 426.7 m above MSL). The initial analysis of the soil of the experimental site revealed that the soil was slightly alkaline (pH= 7.81) with low in soluble salts (EC= 0.41 dSm^{-1}), medium in organic carbon content (0.54%), low in available N (218 kg ha^{-1}), medium in P_2O_5 (16.4 kg ha^{-1}) and high in K_2O (428 kg ha^{-1}). The irrigation water was found to be neutral in reaction (pH= 7.7) with medium level of the soluble salts (EC= 1.17 dSm^{-1}).

Experimental details

The experiment was laid out in randomized block design with nine treatments and three replications. The treatments were T_1 - 100% RDF (Recommended dose of fertilizer) along with foliar application of diammonium phosphate (DAP) at 1%, T_2- 100% RDF along with foliar application of DAP at 2%, T_3- 100% RDF along with foliar application of Brassinolide (BRs) at 1 ppm, T_4 - 100% RDF along with foliar application of BRs at 2 ppm, T_5 - 100% RDF along with foliar application of SA at 50 ppm, T_6- 100% RDF along with foliar application of SA at 100 ppm, T_7- 100% RDF along with foliar application of PPFM at 1%, T_8- 100% RDF along with foliar application of PPFM at 2%, T_9- control. Brassinolide was purchased from Star Bio Science with the commercial name of Brass-16 (0.01% SC). Salicylic acid, DAP and PPFM was purchased from Tamil Nadu Agricultural University, Coimbatore. Blackgram variety VBN 7 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. PPFM and plant growth regulators (PGRs) treatments 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.

Data collection

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 height, 100 seed weight, grain and straw yield were recorded during the harvest stage. Harvest index (HI) was also arrived by the ratio of economic yield to the biological yield.

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 the statistical tool which measures the linear relationship between two or more variables in the system. In this article, correlation was performed to identify the relations among grain yield, 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. It was computed using the equation,

$$r_{xy} = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}}$$

Where,

r_{xy} is the coefficient of the linear relationship between the variables x and y; x_i is the values of x variable in the sample of the population; x_i is the mean of x; y_i is the values of y variable; y_i is the mean of y. Another economic tool used in this study is regression which examines the relationship between a dependent variable and a collection of independent variable (Leona *et al*., 2013). Regression was estimated by

$$y_i = \alpha + \beta x_i + e_i$$

Where,

y_i is the dependent variable (grain yield); x_i is the 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 is the slope coefficient of the respective independent variable; α is the constant or intercept term and e is the error term. This formal mode of regression equation could be rewritten as,

Grain yield = $\alpha + \beta_1$ plant height + β_2 no. of branches plant^{-1} + β_3 LAI + β_4 no. of pods plant^{-1} + β_5 no. of seeds pod^{-1}+ β_6 pod weight + β_7 pod length

Statistical analysis

The data on the different parameters was analyzed statistically by adopting Fisher's method of ANOVA suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Effect on growth characters

Growth characters of blackgram were significantly influenced by *Methylobacterium,* PGRs and nutrients (Table 1). RDF of 100% along with foliar application of 2% PPFM increased the plant height of 40.8 and 41.9 cm at harvest stage during *kharif* and *rabi*, respectively and it was followed by foliar spraying of SA @ 100 ppm. Spraying of *methylobacterium* stimulated the growth and plant height by increasing the auxin and cytokinin content in blackgram.

Significantly higher number of branches plant^{-1} was found with the application of 100% RDF + 2% PPFM followed by 100% RDF + 100 ppm SA. This helped to increase the cell division activity, cell expansion and elongation, ultimately leading to more number of branches plant^{-1}. The influence of SA and BRs 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 2% PPFM and SA (100 ppm) during *kharif* and *rabi*. This might be possible due to the increased availability of nitrogen due to the presence of nitrogen fixing facultative methylotrophs (Jeyakumar *et al*., 2008). Also, the biosynthesis of the plant growth promoting substances like cytokinins, indole acetic acid (IAA), gibberellic acid (GA), vitamins and antibiotic substances by the PPFM microbial inoculants acted in synergy to it. Foliar application of salicylic acid and brassinolides could partially alleviate the detrimental effect of moisture stress on the growth of soybean by improving the antioxidant system and promoting dry matter accumulation (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 + 2% PPFM (4.81, 5.98 during *kharif* and *rabi*), which was followed by 100 % RDF + 100 ppm SA (Table 1). Growth regulators played an important role in increasing the number of leaves, leaf elongation and chlorophyll content and thus led to increase in LAI. The overall improvement in the growth of blackgram with the addition of PPFM and PGRs 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 (Subramanian and Solaimalai, 2000).

Yield attributes and yield

A perusal of data (Table 2) revealed that yield attributes and the yield increased significantly with the foliar application of PPFM, PGRs and nutrients in blackgram over control.

Foliar application of 2% PPFM along with 100% RDF significantly increased the number of pods plant^{-1} (36.5 and 37.7), number of seeds pod^{-1} (34.1 and 35.2), pod weight (15.82 and 16.14 g) and pod height (5.95 and 6.02 cm) during *kharif* and *rabi* season, respectively. This was followed by 100% RDF + 100 ppm SA (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.

Table 1: Effect of PPFM, PGRs and nutrients on growth characters of irrigated blackgram.

Treatments	*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	30.2	4.31	1862	3.40	31.4	5.48	1979	4.57
T_2	34.7	4.88	2203	3.97	35.9	6.04	2319	5.13
T_3	30.7	4.39	1976	3.66	31.8	5.52	2089	4.79
T_4	36.0	5.12	2408	4.14	37.1	6.24	2520	5.26
T_5	32.2	4.67	2092	3.85	33.3	5.81	2206	4.99
T_6	38.5	5.92	2702	4.58	39.6	7.06	2812	5.70
T_7	36.6	5.23	2489	4.21	37.8	6.33	2599	5.31
T_8	40.8	6.53	2984	4.81	41.9	7.67	3098	5.98
T_9	28.3	4.02	1543	3.13	29.5	5.19	1660	4.30
SEd	0.53	0.13	59.2	0.11	0.61	0.17	61.4	0.13
CD (P=0.05)	1.12	0.27	123.8	0.24	1.23	0.35	125.4	0.26

Grain yield (992 and 1051 kg ha^{-1}) and straw yield (1924 and 2096 kg ha^{-1}) during *kharif* and *rabi* season, respectively were significantly higher with application of 100% RDF + 2% PPFM and 100% RDF + 100 ppm SA. In addition to that, the effect of 1% PPFM was on par with 2 ppm BRs (Table 3). The least grain and straw yield was observed in control plot. This might be due to increased yield attributes *viz*., number of pods plant^{-1}, number of seeds pod^{-1}, pod weight (g) and pod height (cm). As observed in the present investigation, salicylic acid and brassinolide play a favourable role in improving various metabolic activities through enhanced nucleic acid metabolism and protein synthesis (Vardhini and Rao, 1998).

PPFM and PGRs enhanced the early root growth and cell multiplication, leading to more absorption of other nutrients from deeper layers of soil. This ultimately resulted in increased plant growth attributes and finally increased the crop yield. The increased yield attributes and yield might be due to the increased supply of the major nutrients by translocation of photosynthates, accumulated under the influence of organic and inorganic sources of nutrients. Increased yield attributes and yield by various workers have been reported at different places (Balachandar *et al*., 2003; Rathore *et al*., 2010).

Economics

Higher crop productivity with lesser cost of cultivation could result in better economic parameters like gross return, net returns and B: C ratio (Table 4). Significantly higher gross returns (Rs. 51074 ha^{-1} and Rs. 52191 ha^{-1}) and net return (Rs. 35016 ha^{-1} and Rs. 36133 ha^{-1}) were recorded with 100% RDF along with foliar application of 2% PPFM during *kharif* and *rabi* season, respectively. However, this was followed by 100% RDF along with foliar application of SA @ 100 ppm. Higher B:C ratio (2.98 and 3.04) was observed with the application of 100% RDF + 2% PPFM and 100% RDF + 100 ppm SA, noticing 2.92 and 2.95 of B:C ratio during *kharif* and *rabi*, respectively. The increased net return could be explained on the basis of increased yield under the influence of PPFM and PGRs in the present investigation. The significant increase in net return and benefit cost ratio was due to the foliar application of *Methylobacterium* with PGRs (Kumawat *et al.,* 2013).

Table 2: Effect of PPFM, PGRs and nutrients on yield attributes of irrigated blackgram.

Treatments	*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	26.0	6.9	13.31	5.41	4.1	26.7	7.2	13.52	5.47	4.1
T_2	28.7	7.6	14.36	5.72	4.2	29.3	8.0	14.58	5.75	4.2
T_3	26.5	7.1	13.72	5.48	4.1	27.2	7.4	13.85	5.52	4.1
T_4	30.8	7.9	14.52	5.83	4.3	31.9	8.2	14.77	5.87	4.3
T_5	28.2	7.4	14.18	5.63	4.2	29.1	7.7	14.29	5.68	4.2
T_6	34.1	8.4	15.41	5.91	4.4	35.2	8.8	15.82	5.95	4.4
T_7	31.9	8.1	15.15	5.86	4.3	32.8	8.5	15.46	5.92	4.3
T_8	36.5	8.8	15.82	5.95	4.4	37.6	9.3	16.14	6.02	4.4
T_9	21.3	6.2	12.14	5.11	4.0	22.8	6.8	12.45	5.38	4.0
SEd	1.05	0.15	0.30	0.11	0.1	1.08	0.17	0.31	0.12	0.1
CD (P=0.05)	2.12	0.31	0.62	0.23	NS	2.23	0.36	0.64	0.24	NS

Table 3: Effect of PPFM, PGRs and nutrients on grain yield (kg ha^{-1}), straw yield (kg ha^{-1}) and harvest index (%) of irrigated blackgram.

Treatments	*Kharif*			*Rabi*		
	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest index (%)	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest index (%)
T_1	657	1254	2.73	734	1301	2.77
T_2	813	1343	2.68	889	1579	2.78
T_3	680	1285	2.75	753	1299	2.73
T_4	852	1502	2.74	924	1654	2.79
T_5	766	1312	2.71	840	1466	2.75
T_6	939	1702	2.81	995	1876	2.88
T_7	874	1521	2.76	934	1691	2.81
T_8	992	1924	2.94	1051	2096	2.99
T_9	483	812	2.65	734	1301	2.70
SEd	24.3	75.2	0.05	25.1	76.8	0.05
CD (P =0.05)	49.2	152.6	0.12	50.3	158.4	0.13

Table 4: Effect of PPFM, PGRs and nutrients on economics of irrigated blackgram.

Treatments	*Kharif*			*Rabi*		
	Gross return (Rs.ha^{-1})	Net return (Rs.ha^{-1})	B:C Ratio	Gross return (Rs.ha^{-1})	Net return (Rs.ha^{-1})	B:C ratio
T_1	40066	22013	2.21	41183	23130	2.27
T_2	44501	26448	2.48	45618	27565	2.58
T_3	41282	23229	2.26	42399	24346	2.30
T_4	44762	27832	2.64	45879	28949	2.70
T_5	36185	20816	2.33	37302	21933	2.36
T_6	47299	31491	2.92	48416	32608	2.95
T_7	50543	32490	2.87	51660	33607	2.91
T_8	51074	35016	2.98	52191	36133	3.04
T_9	25865	11812	1.82	26982	12929	1.87

Data was not statistically analyzed.

Table 5: Correlation between yield attributes and yield (two seasons of mean data).

Variables	Grain yield (kg ha^{-1})	Plant height (cm)	No. of branches plant^{-1}	LAI	No. of pods plant^{-1}	No. of seeds pod^{-1}	Pod weight (g) plant^{-1}	Pod length (cm)
Grain yield (kg ha^{-1})	1	0.83	0.78	0.68	0.86	0.87	0.78	0.76
Plant height (cm)		1	0.69	0.51	0.93	0.90	0.84	0.75
No. of branches plant^{-1}			1	0.89	0.65	0.75	0.57	0.52
LAI				1	0.48	0.63	0.44	0.41
No. of pods plant^{-1}					1	0.89	0.86	0.74
No. of seeds pod^{-1}						1	0.83	0.72
Pod weight (g) plant^{-1}							1	0.70
Pod length (cm)								1

Table 6: Multiple linear regression estimates the blackgram yield.

Source	Coefficients	t-stat	P-value	Standard error	Significance
Intercept	-822.72	-6.43	0.00	0.217	**
Plant height (cm)	1.34	0.97	0.34	3.01	NS
No. of branches plant $^{-1}$	1.67	2.99	0.01	0.69	**
LAI	0.24	2.75	0.01	0.89	**
No. of pods plant $^{-1}$	1.24	3.05	0.00	1.35	**
No. of seeds pod^{-1}	1.91	2.45	0.02	0.03	*
Pod weight (g) plant^{-1}	0.12	0.20	0.04	1.86	*
Pod length (cm)	0.06	2.87	0.01	0.73	**

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

Correlation and regression analysis

The correlation results (Table 5) showed that all the variables included in the model were positively significant at 1% 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 the plant height (0.83), number of branches plant^{-1}(0.78), number of pods plant^{-1}(0.86), leaf area index (0.68), number of seeds pod^{-1} (0.87), pod weight (0.78) and pod length (0.76) show that all the attributes were positively related and that ironicallyproves when there is an increment in these variables, there would be a hike in the yield of the blackgram. All these variables are included as the independent variables in the multiple linear regression model (Xiaolu *et al*., 2019). The multiple linear regression was estimated to measure the relationship and the change in magnitude of the grain yield due to the other prescribed parameters (Table 6). The multiple linear regression equation could be written as,

Grain yield = -822. + 1.34* Plant height + 5.97* no. of branches plant^{-1} + 1.24*LAI + 2.24*no. of pods plant^{-1} + 1.91* no. of seeds pod^{-1} + 0.42* pod weight + 0.16* pod length

The R^2 (0.97) depicts a good sign of model fit which implies that 97% of the grain yield was caused by the independent variables. All the variables except plant height

were found statistically significant (Table 6). The slope coefficient of the number of branches has shown that when there is one percent increase in branches, there would be a significant increase in the grain yield by 1.67%, other variables being held constant. Likewise, when there is a 1% 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 0.24, 1.24, 1.91, 0.12 and 0.06%, respectively.

CONCLUSION

Both the seasons of experiments concluded that the application of 100% RDF along with foliar spraying of 2% PPFM significantly increased the growth characters, yield attributes and yield compared to all other treatments. Subsequently, it was followed by the application of 100% RDF along with 100 ppm SA. 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. Hence it can be recommended that 100% recommended dose of fertilizer along with foliar application of 2% PPFM (*pink-pigmented facultative methylotrophs*) was found to be optimum for obtaining maximum yield and economic returns of irrigated blackgram in the Western zone of Tamil Nadu.

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