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Impact of different levels of chemical and biofertilizer application on biochemical components of *Vigna mungo*

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Abstract
Agricultural practices are generally soil dependent and also one of the most significant driving factors to support the livelihood as well as economic development of the country. In present time agricultural practices, farmers frequently add fertilizers above the required rate to ensure high crop yield. By supplying plants with nutrients, they serve as catalysts for their optimal growth and production but gradually, chemical fertilizers begin to show their ill impacts on the environment. With raising perception of the ill impacts of indiscriminate use of chemical fertilizers, the tendency of farmers toward environmentally sustainable technology is rising. Today, biofertilizers are important and frequently in use because of their role in maintenance of soil health, environmental pollution reduction and also reduce the use of chemicals in agricultural practices. A poly pot experiment was carried out to study the impact of different levels of chemical and biofertilizer application on biochemical components of *Vigna mungo* and found that the combined application of both fertilizers significantly enhanced the value of experimental parameters as compared to the control as well as their single treatments. Therefore, it concluded from the study that it could be reduce the dependency on chemical fertilizers alone to improve the crop growth and yield for sustainable agricultural practices.

Keywords: agricultural practices, biofertilizer, chemical fertilizer, *Vigna mungo*

Introduction

Plants, whether cultivated on the field or in a jar, utilize minerals nutrients for their nourishment. Nutrients must be accessible in adequate and steady amounts for optimum growth of plants (Chen, 2006) [7]. In mineral nutrients, both nitrogen and phosphorus are essential to nearly all the biochemical substances that make existence possible for plants. Adequate nitrogen availability is compulsory for proper growth and yield of plant (Mozumder *et al*., 2003) [17]. Less nitrogen amount inevitably contribute to less biochemical machinery to catalyze plant metabolism and reduced the crop leaf area, seed development and photosynthetic assimilation (Sinclair and Vadez, 2002) [22]. After the nitrogen, phosphorus is the second most important nutrient and it is a major component of compounds with production, development of roots, flowering and ripening processes (Sompong *et al*., 2010) [23]. It is completely clear that phosphorus and nitrogen are essential elements in their systemic, physiological and biochemical roles which contribute to the growth of crops (Sinclair and Vadez, 2002) [22]. Farmers inoculate the agricultural fields with different kinds of fertilizers to secure a preferable yield. India is one of the most important fertilizer generating and consumption nations. Many experiments have shown that the usage of phosphorus fertilizers typically has a significant effect on crop yield as its reduction restricts plant reaction to certain nutrients (Akinrinde and Adigun, 2005) [1]. Increasing high inputs of chemical fertilizers have not only left soil degraded, contaminated and less competitive over the past 150 years but have also posed intensive health and environmental hazards. Hence reliance on chemical fertilizers for potential agricultural development will lead in more degradation of soil fertility and water pollution possibilities. Biofertilizer, on the other hand, is the term to denote all the nutrients input of biological origin that serve as manure for crop plants. These are preparations containing latent or live cells of effective nitrogen fixing strains and/or solubilizing phosphates. Application of the beneficial microorganisms in agricultural practices started around 60 years ago and it is now revealed that such beneficial microbes may even improve plant resistance to adverse environmental pressures such as water and nutrient shortage and heavy metal pollution (Itelima, 2018) [12]. In India, DBT and ICAR, New Delhi is making continued efforts to popularize biofertilizers. Government extension programs have been active in technology transition including 'Lab to Land system'. Therefore, biofertilizers are gaining quickness due to the increasing insistence on maintenance of soil health, reducing the environmental pollution and cut down on the use of chemicals in agriculture that will lead to sustainable agricultural production (Rashid *et al*., 2016) [20]. They have positive implications for plant development (Vessey, 2003) [24]. In the present study, experimental crop was *Vigna mungo* L. Hepper (var. Azad-3) also locally known as urd bean and black gram which is an annual herbaceous self-fertilized crop. Plants are rising to a height of 30 to 100 cm containing trifoliate leaves along with yellow colored flowers formed in axillary racemes. It is a good source of quickly digestible protein to the vegetarian population of the country. Experimental chemical fertilizer was DAP (Diammonium Phosphate) which is an excellent source of phosphorus as well as nitrogen for plant nutrition. It is the world's most widely used phosphorus fertilizer which contains approximately 18% N and 46% P_2O_5 whereas, biofertilizer was PSB (Phosphate Solubilizing Bacteria) which have the capacity to transform inorganic inaccessible phosphorus source to soluble forms HPO_4^{--} and $H_2PO_4^-$ via the process of organic acid processing, chelation and ion exchange reactions can solve the fixed phosphorus in the soil and make it accessible to plants. Consequently, the usage of PSB in agricultural use will not only mitigate the high costs of producing phosphate fertilizers but would also generate insoluble in the fertilizers and soils on which they are added (Chang and Yang, 2009 [5]; Banerjee *et al*., 2010 [3]).

The main purpose of the present experimental study was to reduce the dependency on chemical fertilizers to improve crop growth and yield along with its replacement with biofertilizers as they are ecofriendly and comparatively less cost inputs in sustainable agricultural system. There are many studies that showed the combined application of chemical fertilizers and biofertilizers significantly influenced the growth and development of many agricultural crops and improve their yield thus, reduced the dependency on chemical fertilizers. Reduction in the use of chemical fertilizers up to 50 percent noticed through the biofertilizer application in of fennel plants without any reduction in growth of plants and yield (El-kkoly *et al*., 2000 [9]; Mahfouz *et al*., 2007 [16]; Dadkhah, 2012 [8]). Application of PSB with phosphoric fertilizer had a significant effect on growth and yield of *Zea mays* (Rathor *et al*., 2018 [21]). Thus present study was undertaken to identify specific level of fertilizers to obtain maximum value of biochemical components of *Vigna mungo* plants.

Materials & Methods

To study the impact of different levels of chemical and biofertilizer application on biochemical components of *Vigna mungo*, a poly pot experiment, based on randomized complete block design (RCBD) with three replicates of 10 plants per treatment was carried out during kharif season (2017) at department of Botany, D. V. College, Orai (Jalaun) in Bundelkhand region of Uttar Pradesh, India which is predominantly drought prone area, lies in south-west corner of Uttar Pradesh and extended 24º11′ and 26º27′ N latitude and 78º17′ and 81º34′ E longitudes with an average altitude ranging 250 to 300 m above MSL (mean sea level) (Kiran *et al*., 2009 [14]).

Collection of Materials

For the present study purpose, black soil, locally called as ‘kabar’ was collected from neighboring localities of Orai. Experimental crop seed (certified) and PSB culture were obtained from kendriya krashi beej bhandar, Orai whereas, DAP obtained from IFFCO, Orai.

Soil Treatments

There were total sixteen soil treatments (T_1 to T_{15}) prepared containing different doses of DAP (as 0.25 g, 0.50 g and 1.00 g of DAP per kg of soil) and PSB (as 2.50 g, 5.00 g and 10.00 g of PSB per kg of soil) separately and in combination of each other with a control treatment (T_0) i. e. without fertilizer application (Table-1). Fixed doses of experimental fertilizers were mixed well with calculated amount of soil to prepare different fertilizer levels (Table-1).

Sowing of Seeds

Plants were raised in black coloured 12 inches poly flower pots with a bottom and few on the upper edge drainage holes. Before sowing, *Vigna* seeds were surface sterilized with 0.1% $HgCl_2$ for two minutes and thoroughly washed with distilled water. Then, the seeds were sown in poly pots with treated soil according to the Table-1. As many as 10 seeds were sown in each pot. Thinning of plants was done when required.

Table 1: Different levels of chemical fertilizer (DAP) and biofertilizer (PSB)

S. No.	Treatments	Doses (in per kg of soil)
1	T_0	Control (Without fertilizer)
2	T_1	0.25 g of DAP
3	T_2	0.50 g of DAP
4	T_3	1.00 g of DAP
5	T_4	2.50 g of PSB
6	T_5	5.00 g of PSB
7	T_6	10.00 g of PSB
8	T_7	0.25 g DAP×2.50 g PSB
9	T_8	0.25 g DAP×5.00 g PSB
10	T_9	0.25 g DAP×10.00 g PSB
11	T_{10}	0.50 g DAP×2.50 g PSB
12	T_{11}	0.50 g DAP×5.00 g PSB
13	T_{12}	0.50 g DAP×10.00 g PSB
14	T_{13}	1.00 g DAP×2.50 g PSB
15	T_{14}	1.00 g DAP×5.00 g PSB
16	T_{15}	1.00 g DAP×10.00 g PSB

Observations and Data analysis

Experimental biochemical components of *Vigna* crop were observed, analyzed and recorded at requisite time intervals from germination to harvesting. Chlorophyll content of fresh leaves was determined by Petering method (1940) [19]. Phosphorus content of plant samples was determined by the molybdenum reduced phosphomolybdic acid blue colour method in H_2SO_4 system. The blue colour was determined in Elico-CL-20A photo-electric colorimeter using red filter. Potassium content of the samples was measured turbidimetrically. The turbidity produced at 20ºC under standardized conditions was estimated in Elico-CL-20A photo-electric colorimeter using red filter. The nitrogen content of the samples was determined by semi-micro-kjeldahl method and the crude protein content of samples was calculated by the correction factor (N × 6.25) (Chang *et al*., 1989 [6]). Catalase was assayed by the permanganate titration method of the Euler and Josephson (1927) [10].

All these parameters were recorded at requisite time intervals (30 and 60 DAS) of crop growing period. Average values of the experimental parameters were shown in Table-2A and 2B. The data for the various experimental parameters in the present study were ANOVA tested and the significance of differences between control and each treatment was determined by using the value of least significant difference (L.S.D.) at P=0.05 and P=0.01.

Results

Overall maximum value of the experimental parameters recorded with combined application of both DAP and PSB fertilizers. The interaction levels of DAP and PSB significantly influenced the biochemical parameters tested in *Vigna mungo* plants. The application of interaction 0.50 g DAP×10.00 g PSB per kg soil (T_{12}) level showed overall maximum chlorophyll content (1.82 mg and 3.66 mg at 30 and 60 DAS respectively), phosphorus content of leaves (0.39 and 0.54 percent at 30 and 60 DAS respectively) and stem (0.34 and 0.47 percent at 30 and 60 DAS respectively), potassium content of leaves (0.67 and 1.07 percent at 30 and

60 DAS respectively) and stem (0.61 and 0.85 percent at 30 and 60 DAS respectively), nitrogen content of leaves (1.97 and 2.77 percent at 30 and 60 DAS respectively) and stem (1.97 and 2.78 percent at 30 and 60 DAS respectively), crude protein content of leaves (12.33 and 17.33 percent at 30 and 60 DAS respectively) and stem (12.41 and 17.42 percent at 30 and 60 DAS respectively) as well as catalase activity in leaves (3.12 and 3.49 unit/g at 30 and 60 DAS respectively) and stem (3.25 and 3.54 unit/g at 30 and 60 DAS respectively) of *V. mungo* plants as compared to the control (Table 2A and 2B).

Table 2A: Impact of different levels of DAP and PSB application on biochemical components of *Vigna mungo* leaves

Fertilizer	Treatment Level	Chlorophyll (mg/100g Fresh weight of leaf)		Phosphorus content (%)		Potassium content (%)		Nitrogen content (%)		Crude protein content (%)		Catalase activity (unit/g fresh weight)	
		30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Control	T_0	1.25	2.63	0.19	0.33	0.41	0.75	1.78	2.27	11.07	14.21	1.91	2.97
DAP	T_1	1.37	3.08	0.27	0.39	0.49	0.83	1.84	2.51	11.49	16.01	2.27	3.23
	T_2	1.31	2.81	0.23	0.35	0.42	0.78	1.81	2.45	11.34	15.33	2.21	3.19
	T_3	1.35	2.92	0.24	0.38	0.44	0.81	1.82	2.48	11.41	15.43	2.21	3.21
PSB	T_4	1.28	2.71	0.22	0.35	0.44	0.81	1.80	2.55	11.30	15.93	2.11	3.01
	T_5	1.35	2.80	0.23	0.36	0.44	0.82	1.84	2.56	11.49	15.96	2.23	3.12
	T_6	1.43	3.07	0.24	0.38	0.49	0.84	1.86	2.59	11.63	16.18	2.39	3.22
DAP×PSB	T_7	1.41	3.13	0.28	0.41	0.51	0.86	1.86	2.61	11.61	16.25	2.35	3.27
	T_8	1.49	3.21	0.32	0.44	0.53	0.89	1.87	2.63	11.72	16.43	2.41	3.36
	T_9	1.53	3.35	0.32	0.45	0.54	0.91	1.89	2.64	11.81	16.53	2.63	3.42
	T_{10}	1.65	3.45	0.31	0.48	0.58	0.94	1.91	2.69	11.91	16.49	2.69	3.41
	T_{11}	1.73	3.52	0.37	0.51	0.61	0.96	1.96	2.75	12.24	17.18	2.86	3.46
	T_{12}	1.82	3.66	0.39	0.54	0.67	1.07	1.97	2.77	12.33	17.33	3.12	3.49
	T_{13}	1.52	3.32	0.29	0.45	0.54	0.91	1.87	2.67	11.74	16.69	2.50	3.36
	T_{14}	1.61	3.44	0.34	0.48	0.58	0.93	1.91	2.68	11.92	16.74	2.71	3.41
	T_{15}	1.68	3.55	0.33	0.50	0.61	0.94	1.95	2.71	12.19	16.97	2.88	3.52
L.S.D.	P=0.05 DAP	0.05	0.11	0.03	0.02	0.03	0.05	0.03	0.03	0.13	0.43	0.05	0.03
	P=0.01 DAP	0.06	0.13	0.03	0.02	0.03	0.06	0.03	0.03	0.16	0.54	0.06	0.04
	P=0.05 PSB	0.05	0.11	0.03	0.02	0.03	0.05	0.03	0.03	0.13	0.43	0.05	0.03
	P=0.01 PSB	0.06	0.13	0.03	0.02	0.03	0.06	0.03	0.03	0.16	0.54	0.06	0.04
	P=0.05 DAP×PSB	0.14	0.29	0.08	0.05	0.08	0.14	0.08	0.08	0.36	1.18	0.13	0.09
	P=0.01 DAP×PSB	0.17	0.34	0.09	0.06	0.09	0.17	0.09	0.09	0.42	1.39	0.15	0.11

Table 2B: Impact of different levels of DAP and PSB application on biochemical components of *Vigna mungo* stem

Fertilizer	Treatment Level	Phosphorus content (%)		Potassium content (%)		Nitrogen content (%)		Crude protein content (%)		Catalase activity (unit/g fresh weight)	
		30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Control	T_0	0.17	0.31	0.37	0.61	1.61	2.26	10.02	14.06	2.03	3.14
DAP	T_1	0.24	0.36	0.43	0.66	1.79	2.57	11.21	16.13	2.46	3.31
	T_2	0.19	0.32	0.39	0.53	1.78	2.46	11.12	15.26	2.76	3.19
	T_3	0.21	0.34	0.42	0.64	1.79	2.54	11.19	15.89	2.23	3.27
PSB	T_4	0.19	0.32	0.39	0.64	1.62	2.39	10.17	14.92	2.13	3.19
	T_5	0.19	0.32	0.41	0.65	1.65	2.44	10.31	15.52	2.43	3.22
	T_6	0.22	0.34	0.43	0.69	1.66	2.53	10.42	15.87	2.46	3.28
DAP×PSB	T_7	0.25	0.38	0.45	0.68	1.81	2.61	11.24	16.28	2.46	3.34
	T_8	0.28	0.41	0.46	0.71	1.83	2.66	11.44	16.63	2.66	3.41
	T_9	0.31	0.41	0.49	0.75	1.86	2.68	11.61	16.78	2.80	3.46
	T_{10}	0.28	0.43	0.53	0.73	1.88	2.64	11.78	16.52	3.01	3.42
	T_{11}	0.33	0.46	0.55	0.79	1.95	2.74	12.20	17.11	3.08	3.47
	T_{12}	0.34	0.47	0.61	0.85	1.97	2.78	12.41	17.42	3.25	3.54
	T_{13}	0.25	0.41	0.49	0.71	1.83	2.62	11.43	16.39	2.76	3.36
	T_{14}	0.31	0.44	0.52	0.76	1.87	2.69	11.71	16.84	2.94	3.46
	T_{15}	0.32	0.46	0.56	0.81	1.89	2.75	11.82	17.23	3.15	3.51
L.S.D.	P=0.05 DAP	0.03	0.02	0.02	0.05	0.02	0.03	0.15	0.38	0.23	0.04
	P=0.01 DAP	0.03	0.02	0.02	0.06	0.02	0.03	0.19	0.47	0.29	0.05
	P=0.05 PSB	0.03	0.02	0.02	0.05	0.02	0.03	0.15	0.38	0.23	0.04
	P=0.01 PSB	0.03	0.02	0.02	0.06	0.02	0.03	0.19	0.47	0.29	0.05
	P=0.05 DAP×PSB	0.08	0.05	0.05	0.14	0.05	0.08	0.42	1.04	0.63	0.12
	P=0.01 DAP×PSB	0.09	0.06	0.06	0.17	0.06	0.09	0.50	1.22	0.74	0.14

(DAS= Days after sowing; L.S.D = Least significant difference)

Discussion and conclusion

Phosphorus solubilizing bacteria supported the phosphorus solubilization process in the soil to increase the phosphorus availability to plants (Khan *et al*., 2007) [13]. Maximum value for chlorophyll, carbohydrate and protein content in *stevia rebaudiana* observed with the combination of biofertilizer and chemical fertilizer as compared to control (Patil, 2010) [18]. *Echinockloa frumentacea* found improved values of morphological and yield parameters in combined application of biofertilizer and synthetic fertilizer than using either method alone (Chandrasekar *et al*., 2005) [4].

In the present study, PSB and DAP both positively influenced the growth of *V. mungo* plants over the control. PSB in addition to phosphate solubilization, are also capable of producing growth hormones such as auxins, cytokinins and gibberellins, vitamins, antibiotics, siderophores etc. and due to this property, they enhance plant growth and suppress the soil born pathogens and also improve physico-chemical properties of the soil and sustain soil fertility (Baliah, 2018) [2]. Biological nitrogen fixation (BNF), a major source of nitrogen for farmers who use limited fertilizers, is one of the probable solutions and plays a key role in sustainable legumes and also non-leguminous development (Itelima *et al*., 2018) [12]. In legumes, BNF is the most sustainable and lowest cost source of nitrogen (Hellal *et al*., 2013) [11]. Therefore, biofertilizers provide ecofriendly organic agro-input and are more cost-effective than chemical fertilizers. Biofertilizers with reduced amount of chemical fertilizers recommended for better yield of chickpea (Kumar *et al*., 2015) [15].

Thus, it concluded from the present study that the interaction 0.50 g DAP×10.00 g PSB per kg soil level (T_{12}) was significantly influenced the chlorophyll, phosphorus, potassium, nitrogen, crude protein content as well as catalase activity in samples of *V. mungo* plants. The soil treatment with combined application of chemical fertilizer (DAP) and biofertilizer (PSB) was found significant on biochemical components of *V. mungo* plants as compared to the control. Therefore, the combination of chemical fertilizer and biofertilizer can reduce the dependency on chemical fertilizers alone to improved crop growth and yield for sustainable agricultural system. It is suggested from the study that biofertilizer application could be applied as enhancer for agricultural crops to replace or minimize the quantity of chemical fertilizers in agricultural system to save and protect the environment.

The present investigation is the primary stage of study to analyze the response of different levels of chemical and biofertilizer on biochemical components of *V. mungo* crop. In Future, studies may be conducted to examine the same on different crop growth and yield with the application of different chemical fertilizer and biofertilizer at different levels to enhance the crop production.

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