Journal of Experimental Agriculture International

Volume 45, Issue 12, Page 266-272, 2023; Article no.JEAI.111684
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Effects of Bioinoculants on Total Chlorophyll Content, Yield of Soybean and Fertility Status of a Vertisols

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2023/v45i122292

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/111684

Original Research Article

Received: 25/10/2023
Accepted: 29/12/2023
Published: 30/12/2023

ABSTRACT

The field experiment was conducted at the Research Farm, Department of Soil Science and Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh (INDIA), during *kharif* season 2019-2020. The study was aimed to find out the effects of bioinoculants on total chlorophyll content, soybean yield and fertility status of a Vertisols. There were 15 treatments comprised of different beneficial microbial consortia in possible combinations applied as seed treatments with three replications in a randomized complete block design (RCBD). Amongst these, two control plots were maintained as fertilized un-inoculated control (FUI) and unfertilized un-inoculated control (UFUI). The recommended dose of fertilizers for soybean is 20 N :

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80 P_2O_5 : 20 K_2O kg ha^{-1}. The data generated on total chlorophyll contents, yield of soybean and fertility status of soilwere statistically analyzed and the results showed that the significant enhancement were noticed by the application of consortia NPK+EM+PGPR in total chlorophyll contents of soybean leaves at 25, 45 and 65 DAS over control. Similarly, significant increases in the soybean yield (seed and stover) were also found by the NPK+EM+PGPRconsortia over FUI (control). Further, results revealed that the application of consortia NPK+EM+PGPR improved the organic carbon and available NPK in a Vertisols. Therefore, it may be concluded that the NPK + EM + PGPR consortia was superior for sustainable soybean productivity and soil fertility.

Keywords: Bioinoculants, chlorophyll content, soil fertility, soybean yield, Vertisols.

1. INTRODUCTION

"Soybean (*Glycine max* L.) is an important leguminous oil seed crop, which contains 40-45% protein and 18-20% oil. The top two soybean growing states in India are Madhya Pradesh and Maharashtra with 45% and 40% shares, respectively. In Madhya Pradesh the soybean cultivation spreads over 5.2 M ha with total annual production of 6.7 M tones and productivity of 1285 kg ha^{-1}" [1,2]. "Soybean rhizosphere harbors vast proportions of soil microorganisms, whose activities largely determine the biological condition of the soil and influence the plant growth right from seed germination to maturity" [3,4]. "Different microbial consortia i.e. *Pseudomonas* as PGPR is the most efficient and effective strain with significant remarks on isolates of *P. fluorescens* and *P. putida* increasing growth and yield of different crops, especially legumes. *Rhizobium* (diazotroph) is a symbiotic N_2-fixer with roots of legume crops" [5]. "It colonizes the roots of specific legumes to form tumor like growths called root nodules, which acts as the factories of ammonia production. *Bacillus sp.* produces soluble exudates organic acids which is recognized as a major mechanism responsible for the release of phosphates from the hydroxyl apatites*.* *Frateuria aurantia* is a potassium solubilizer which increases the potassium uptake by the plant. Further, the isolates of constitutional microorganisms of EM (Effective Microbial) culture individually have already been evidenced beneficial but their consortium could be more valuable to enhance the supply of nutrients through anti-phytopathogenicity, solubilization, induced phytoresistance and phytostimulator" [6].

"The availability of nutrients in the soil for plant utilization is affected not only by the inherent soil characteristics but also by the fertilizer use and practices in a cropping system. It has been observed that a major part of the applied nutrient gets fixed and only a small part of it becomes available to the crop plants" [7]. "However, the different microbial consortia *i.e. Rhizobium, Bascillus sp.* etc. offer great promise to the crops enabling the inoculated plants for more uptake of nutrients from the soil" [8]. Therefore, the present investigation was carried to find out the effects of various bioinoculants on chlophyll content, yield of soybean and fertility status of a Vertisols.

2. MATERIALS AND METHODS

The present investigation was conducted during kharif 2019-2020 under All India Network Project on Biofertilizers, to find out the effect of bioinoculants on chlorophyll content in leaves, yield of soybean and fertility status of a Vertisol at the Research Farm, Department of Soil Science, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh (INDIA).

Table 1. Details of different treatment combinations

Treatment Combinations			
T_1	*Rhizobium*	T_9	NPK + PGPR
T_2	NPK consortium	T_{10}	PK + EM
T_3	EM culture	T_{11}	PK + PGPR
T_4	PGPR	T_{12}	NPK + EM + PGPR
T_5	PK Consortium	T_{13}	PK + EM + PGPR
T_6	*Rhizobium* + EM	T_{14}	FUI
T_7	*Rhizobium* + PGPR	T_{15}	UFUI
T_8	NPK + EM		

"The soil of the experimental site is belonging to Kheri series of fine montmorillonitic hyperthermic family of T*ypic Haplusterts* (Vertisol) with pH of 7.15, electrical conductivity 0.24 dS m^{-1} (1 : 2.5 soil : water ratio) and organic carbon 5.2 g kg^{-1}. The soil available N, P and K were 226, 15.8 and 282 kg ha^{-1}, respectively. The experiment comprised 15 treatments with three replications having 45 plots laid out under randomized complete block design (RCBD)" [6]. The different treatment combinations are presented in Table 1.

"The treatments of different liquid biofertilizers either solo and/or consortia were applied on soybean as seed treatment / basal application as per the appropriate recommendations. The biofertilizers used were diazotroph (*Rhizobium*), PSB - Phosphate Solubilizing Bacteria (*Bacillus* sp.), KSB - Potash Solubilizing Bacteria (*Fraturia aurentia*), PGPR- Plant Growth Promoting Rhizobacteria (*Pseudomonas fluorescens*) and EM - Effective microbial culture / consortium (six bacteria, two fungus and one actinomycetes) culture. Besides these, two types of control plots were maintained as fertilized uninoculated control (FUI) and unfertilized uninoculated control (UFUI) to measure the comparative effects of different microbial inoculants" [6].

The Soybean crop (variety JS 2069) was sown in the last week of June to first week of July as rainfed during *kharif* (crop duration 93-106 days, and seed rate 80 kg ha^{-1}). Insects and diseases were kept under check following suitable control measures. Soybean crop was harvested at maturity and yield data was recorded after threshing having 07-10 percent moisture content. The recommended NPK dose for soybean crop was applied as 20 N: 80 P_2O_5: 20 K_2O kg ha^{-1}. The sources of N, P and K used were urea, single super phosphate and muriate of potash.

Chlorophyll Content: Leaf chlorophyll (a, b and total) content was estimated by acetone extraction method in fresh plant leaves at 25, 45and 65 DAS by spectrophotometer at 663 nm and 645 nm wavelength for chlorophyll a and b, respectively. The content of chlorophyll a, b and total were calculated by using following formula [9]:

Chlorophyll a'(mg g^{-1} fresh leaves =

$$\frac{12.7 \times (A663) - 2.69 \times (A645) \times V}{a \times 1000 \times W}$$

Chlorophyll b'(mg g^{-1} fresh leaves) =

$$\frac{22.9 \times (A645) - 4.68 \times (A663) \times V}{a \times 1000 \times W}$$

Total Chlorophyll (mgg^{-1} fresh leaves) = Chlorophyll a + Chlorophyll b

Where is, A= Optical density; a = Length of light path in cell (1 cm); W = Weight of sample (1 gm) and V = Volume of solution

Seed and Stover Yields: The soybean crop was harvested and bundles were made plot wise, then allowed to dry in the plot for 2-3 days and weighed. After threshing, plot wise yields for straw and seeds were recorded.

Soil Fertility Status: In the present study, surface 0 -15 cm soil samples were collected from different treatment plots after harvest of soybean crop during 2019-20. Composite soil samples were air-dried at room temperature, pulverized, and sieved through a 2.0 mm sieve. The soil samples were analyzed for different basic soil properties viz. soil pH, electrical conductivity (EC), soil organic carbon and soil available nutrients (NPK) by the standard laboratory procedures.

Statistical Analysis: The data generated on total chlorophyll contents, yield of soybean and fertility status of soil were statistically analyzed to draw suitable inference as per standard method [10].

3. RESULTS AND DISCUSSION

3.1 Leaf Chlorophyll Content in Leaves of Soybean at Different Stages of Growth

The data related to total chlorophyll content at 25, 45 and 65 DAS of soybean are illustrated in Table 2. It is evident from the results that the maximum total chlorophyll content at 25 DAS was estimated with the microbial consortium of NPK+EM+PGPR for 3.53 mg g^{-1} leaf which was 24.9% increment over that of FUI (2.84 mg g^{-1} leaf), followed by PK+EM+PGPR, NPK+PGPR and Rhizo+EM for the chlorophyll content of 3.42, 3.38 and 3.31 mg g^{-1} leaf, respectively with respective response of 20.6, 19.2and 16.7over that of FUI. Similarly, NPK+ EM+PGPR treatment was attributed to maximum total chlorophyll content in soybean at 45 DAS and 65 DAS for 3.62 and 2.86 mg g^{-1} of leaf with increments of 29% and 39%, respectively over that of FUI. The rhizobial inoculation significantly increased the total leaf chlorophyll content by 70%, 62% and 90% measured at 2, 4, and 8 weeks after sowing, respectively. An increase in the leaf Fe concentration as a result of the PGPR treatments enhanced the chlorophyll concentration and prevented leaf chlorosis [11,12].

3.2 Yields of Soybean (Seed and Stover)

The data pertaining to the seed yield production of soybean are presented in Table 3and observed that the consortium of NPK+EM+PGPR achieved significantly maximum seed yield of 2295 kg ha^{-1} with relative increment of 39% over FUI (1647 kg ha^{-1}), followed by PK+ EM+PGPR, EM culture, NPK+PGPR, NPK consortium, Rhizo+EM, NPK+EM, PK+EM, PK+PGPR, Rhizo+PGPR and *Rhizobium* for grain yield of 2234, 2200, 2157, 2151, 2150, 2217, 2113, 2090, 2033 and 2029 kg ha^{-1} representing the response increment of 36, 34, 31, 31, 31, 28, 28, 27, 23 and 23%, respectively. The significant increase in grain yield was recorded due to EM application in farmyard manure as well as in soil amended with the recommended dose of NPK fertilizers in mung bean. It could be due to provides the respective nutrients to the crop along with that the EM culture organisms (acts as phytostimulators) enhanced plant growth and productivity by fixing atmospheric nitrogen. Additionally, the release of trace elements, secreted antioxidant, bioactive compounds (vitamins, hormones and enzymes), in addition to that PGPR acts as plant growth promoters [13,14,15].

The treatment NPK+EM+PGPR recorded the highest stover yield (Table 3) of soybean (4894 kg ha^{-1}) and 62% more response as compared to that of FUI (3026 kg ha^{-1}). This was followed by the effects of treatment combinations of PK+EM+PGPR, EM, NPK+PGPR, NPK consortium, PK+EM, NPK+EM, Rhizo+EM, PK+PGPR, Rhizo+PGPR and *Rhizobium* for stover yield of 4654, 4628, 4504, 4450, 4324, 4308, 4291, 4264, 4235 and 4157 kg ha^{-1}, respectively corresponding to 54, 53, 49, 47, 43, 42, 42, 41, 40 and 37% increment, respectively over that of FUI. The increase in stover yield might be attributed to the increased availability of N, P and K in soil which resulted in higher growth and development and finally yields [16,17].

3.3 Basic Soil Properties at Harvest of Soybean

The data of basic soil properties at harvest of soybean are presented in Table 4. The result revealed that the soil pH ranged between 7.09 - 7.15. There were no significant difference found amongst the different treatment. The data also indicated that the organic carbon content in soil found to increase with different microbial consortia application thereby, lower organic carbon content was found in FUI treatment UFUI treatments [18,19,20].

Table 2. Effect of bioinoculants on total chlorophyll content in leaves of soybean at different growth stages

Treatment	Total chlorophyll content (mg g^{-1} leaf)		
	25 DAS	45 DAS	65 DAS
Rhizobium	2.77	2.83	2.58
NPK consortium	3.10	3.12	2.87
EM culture	3.11	3.09	2.84
PGPR	2.80	2.79	2.54
PK consortium	2.79	2.90	2.65
Rhizobium + EM	3.31	3.26	3.01
Rhizobium + PGPR	2.97	3.16	2.91
NPK +EM	3.18	3.20	2.95
NPK+PGPR	3.38	3.40	3.15
PK+EM	3.20	3.28	3.03
PK+PGPR	3.23	3.13	2.88
NPK+EM+PGPR	3.53	3.62	3.37
PK+EM+PGPR	3.42	3.42	3.17
FUI	2.84	2.81	2.56
UFUI	2.65	2.67	2.42
SEm ±	**0.08**	**0.05**	**0.05**
CD (*p=0.05*)	**0.25**	**0.14**	**0.14**

Table 3. Effect of bioinoculants on yields (seed and stover) of soybean at harvest

Treatment	Yield (kg ha^{-1})	
	Seed	Stover
Rhizobium	2029	4157
NPK consortium	2151	4450
EM culture	2200	4628
PGPR	1905	3436
PK consortium	1872	3258
Rhizobium + EM	2150	4291
Rhizobium + PGPR	2033	4235
NPK +EM	2117	4308
NPK+PGPR	2157	4504
PK+EM	2113	4324
PK+PGPR	2090	4264
NPK+EM+PGPR	2295	4894
PK+EM+PGPR	2234	4654
FUI	1647	3026
UFUI	1127	2787
SEm ±	**119**	**361**
CD (*p=0.05*)	**346**	**1050**

Table 4. Effect of bioinoculants on soil pH, organic carbon and available nutrient (N, P and K) contents in soil (0- 15 cm depth) of soybean at harvest

Treatment	Soil pH	OC (g kg^{-1})	Available nutrients (kg ha^{-1})		
			N	P	K
Rhizobium	7.10	5.4	256	18.3	250
NPK consortium	7.12	5.7	271	17.9	295
EM culture	7.14	5.5	259	22.0	294
PGPR	7.11	5.7	227	15.8	250
PK consortium	7.15	5.5	208	22.4	301
Rhizobium + EM	7.12	5.8	260	20.5	281
Rhizobium + PGPR	7.14	5.9	250	16.1	248
NPK +EM	7.13	5.8	274	21.5	293
NPK+PGPR	7.14	6.1	266	20.7	285
PK+EM	7.14	5.9	247	21.2	290
PK+PGPR	7.10	5.8	247	21.5	265
NPK+EM+PGPR	7.09	6.2	288	23.1	313
PK+EM+PGPR	7.15	6.0	279	22.2	291
FUI	7.09	5.1	203	14.4	239
UFUI	7.10	5.2	187	13.2	231
CD (*p=0.05*)	**NS**	**0.58**	**49.1**	**4.60**	**48.7**

Further, the data on available nutrients content (NPK) of surface soil (0-15 cm depth) showed that the consortium of NPK+EM+PGPR significantly increased the soil available N content (288 kg N ha^{-1}) with response of 41.6% to that of FUI (203 kg N ha^{-1}), followed by PK+EM+PGPR, NPK+EM, NPK consortium, NPK+PGPR, Rhizo+EM, EM culture and *Rhizobium* for N content in soil of 287, 274, 271, 266, 260,259 and 256 kg N ha^{-1}, respectively and with 41.4, 35.0, 33.3, 30.7, 27.7, 27.2 and 26.1% response increment, respectively over that of FUI. It has been known to stimulate and enhance plant growth directly as they can improve and mobilize the nutrients such as nitrogen and phosphorous in available forms [21,22].

Results further revealed that the highest available P content of 22.4 kg ha^{-1} was recorded with the application of NPK+EM+PGPR along with 55.30% more response over that of FUI (14.4 kg ha^{-1}). This was followed by the effects of treatment combination, PK consortium, PK+EM+PGPR, EM culture, NPK+EM, PK+PGPR, PK+EM and NPK+PGPR for P content in soil 22.4, 22.3, 22.0, 21.5, 21.5, 21.2and 20.7 kgha^{-1}, respectively with the respective response of 55.1, 54.2, 52.7, 49.0, 48.9, 46.9and 43.4% over that of FUI. This might be attributed to many genera of bacteria such as *Bacillus*, *Pseudomonas* and several others have been reported to solubilize varying quantities of phosphorus depending on the efficiency of the strains [23,24].

It is evident from the results that the maximum available potassium content in soil of soybean at harvest was recorded with NPK+EM+PGPR of 313 kg ha^{-1} by 31% increase relative to FUI (239 kg ha^{-1}), followed by the performance of PK consortium, NPK consortium, EM culture, NPK+EM and PK+EM with values of 301, 295, 294, 293 and 290 kg K ha^{-1}, respectively and the respective response of 25.8, 23.4, 22.8, 22.5 and 21.8 % over that of FUI. The findings of Supanjani et al. [25] reported that the co-inoculation of KSB and PSB has increased P and K availability. The inoculation of *B. mucilaginosus* (KSB) increased available K over uninoculated control due to mechanism of potassium mobilization by *B. Mucilaginosus* [26,27].

4. CONCLUSION

It was concluded that the NPK+EM+PGPR treatment recorded maximum total chlorophyll content in leaves and yield of soybean followed by PK+EM+PGPR treatments at 25, 45 and 65 DAS. The consortium NPK+EM+PGPR increased the available nutrients (NPK) content 41.6% N, 55.3% P and 31.0%K, respectively overfertilized uninoculated control (FUI). Further, the microbes of EM culture can also enhanced crop productivity and sustained soil fertility.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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