RESEARCH ARTICLE Legume Research- An International Journal, Volume 47 Issue 6: 978-983 (June 2024)

Influence of Bioinoculants on Growth and Yield of Cowpea (*Vigna unguiculata* L.) under Field Condition

M.N. Prathima[1], R. Muthuraju[2], T.V. Krishna[3], B.S. Nalini[2]

10.18805/LR-4964

ABSTRACT

Background: Cowpea (*Vigna unguiculata* L.) an important and commonly grown *kharif* pulse crop in India for vegetable, grain, forage and green manuring. Considering the adverse effects of chemical fertilizers, bio-inoculants should be encouraged as natural nutrient source.

Methods: Three indigenous bio-inoculants *viz., Rhizobium sp., Bacillus megaterium* and *Glomus mosseae* were evaluated in a field experiment which was conducted during *kharif* season 2020 to study the influence of bioinoculants on growth and yield of cowpea. The experiment was laid out in randomized block design (RCBD) with ten treatments replicated thrice.

Result: The application of microbial consortia significantly influenced the number of pods bearing branches, number of nodules, fresh weight of nodules, chlorophyll content, plant nitrogen and phosphorus content and microbial activities in the cowpea rhizosphere. The present study concluded that combined application of bio-inoculants (*Rhizobium*, PSB, AM fungi) can save 25 per cent of recommended dose of fertilizer by sustaining the crop yield and improving the soil health.

Key words: *Bacillus megaterium*, *Glomus mosseae*, Microbial biomass, *Rhizobium,* Soil enzymes.

INTRODUCTION

Cowpea is an annual herbaceous legume from the genus *Vigna* and it is native to Africa (Carvalho *et al*., 2017). Cowpea grain offers key vitamins including thiamine, riboflavin, ascorbic acid, niacin and folic acid. It is low in fat and represents a fair source of fiber of about 6% (Kushwaha and Kumar, 2013). It is relatively low in sulphur containing amino acids but high in lysine and other essential amino acids, making it a good complement to the main cereal diets (Ohler and Mitchell, 1992).

Bioinoculants naturally activate microorganisms found in the soil being cheaper, effective and eco-friendly agents that gaining importance for use in crop production, restoring the soil natural fertility and protecting it against drought, soil diseases and therefore stimulate plant growth (Suhag, 2016). Bioinoculants such as *Rhizobium sp*., *Bacillus megaterium* and *Glomus mosseae* were together known to give the best results in pulses.

Rhizobium is a Gram-negative, rod-shaped bacteria generally found as root nodule-forming bacteria in legumes which fix atmospheric nitrogen with the help of nitrogenase enzyme (Somasegaran and Hoben, 2012). *Bacillus megaterium* is a potential phosphate solubilizing bacteria (PSB) found in different rhizospheric soil. *Glomus mosseae* is now known as *Funneliformis mosseae* (Schubler and Walker, 2010). It has the ability to adapt to various agricultural management.

MATERIALS AND METHODS

A field experiment of this study was conducted at Zonal Agricultural Research Station, GKVK, UAS Bangalore, during *kharif* 2020 and further microbiological and biochemical analysis were carried out in the Department of Agricultural Microbiology, UAS, GKVK, Bangalore. The experiment plot was laid out in the RCBD design with three replications. The cowpea variety selected for this study was KBC-2. The bioinoculants used for the study were *Rhizobium sp., Bacillus megaterium* and *Glomus mosseae*. Treatments are comprised of, T_1: Control, T_2: Recommended dose of fertilizer (N:P_2O_5:K_2O kg/ha: 10:20:10), T_3: 50% of N and P_2O_5+100% K_2O+*Rhizobium sp.* (*R. sp.*), T_4: 50% of N and P_2O_5+100% K_2O+*B. megaterium* (*B. m.*), T_5: 50% of N and P_2O_5+100% K_2O+*Glomus mosseae* (*G. m.*),T_6: 50% of N and P_2O_5+100% K_2O+*R.sp., B.m., G.m.*, T_7: 75% of N and P_2O_5+100% K_2O+*R. sp.*, T_8: 75% of N and P_2O_5+100%

[1]Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad-580 005, Karnataka, India.
[2]Department of Agricultural Microbiology, Gandhi Krishi Vigyana Kendra, University of Agricultural Sciences, Bangalore-560 065, Karnataka, India.
[3]Department of Genetics and Plant Breeding, All India Network Research Project on Arid Legumes, Gandhi Krishi Vigyana Kendra, University of Agricultural Sciences, Bangalore-560 065, Karnataka, India.

Corresponding Author: R. Muthuraju, Department of Agricultural Microbiology, Gandhi Krishi Vigyana Kendra, University of Agricultural Sciences, Bangalore-560 065, Karnataka, India.
Email: muthurajr@gmail.com

How to cite this article: Prathima, M.N., Muthuraju, R., Krishna, T.V. and Nalini, B.S. (2024). Influence of Bioinoculants on Growth and Yield of Cowpea (*Vigna unguiculata* L.) under Field Condition. Legume Research. 47(6): 978-983. doi: 10.18805/LR-4964.

Submitted: 10-05-2022 **Accepted:** 01-10-2022 **Online:** 17-10-2022

K_2O+*B.m.*, T_9: 75% of N and P_2O_5+100% K_2O+*G.m.*, T_{10}: 75% of N and P_2O_5+100% K_2O+*R. sp., B.m., G.m.* Observations on traits *viz.,* plant height, number of branches, number of nodules plant^{-1}, fresh weight of nodules, chlorophyll content (SPAD reading), plant N and P content, microbial biomass carbon, microbial biomass nitrogen, microbial biomass phosphorus, enumeration of N_2 fixers, phosphate solubilizers, spore load of AM fungi, enzymes such as dehydrogenase, urease, acid and alkaline phosphatase activity of soil were recorded.

Chlorophyll content (SPAD reading)

Observations were recorded from five randomly selected and tagged plants, during flowering stage of crop . The SPAD meter reading were taken from top, middle and bottom of completely opened green leaves and further average value was computed.

Nitrogen and phosphorus content in plant

Nitrogen and phosphorus content in plants were analysed at flowering and harvest stage of crop by micro kjeldhal distillation method (Piper, 1966) and vanodomolybdophosphoric yellow colour method (Piper, 1966) using spectrophotometer, respectively.

100 seed weight

One hundred seeds were counted from the produce of each treatment separately in each replication. These seeds were weighed on electronic balance mean 100 seed weight is expressed in grams.

Estimation of microbial activities in rhizospheric soil

Soil microbial biomass

Microbial biomass carbon, nitrogen and phosphorus were estimated during flowering and harvest stage of the crop. Microbial biomass carbon and nitrogen were estimated by using fumigation and extraction method proposed by Carter (1991). Microbial biomass P were estimated as per the procedure given by Brookes *et al*. (1982).

Enzyme activity

Soil enzymes like dehydrogenase, urease, acid phosphatase and alkaline phosphatase were estimated. These enzyme activities were estimated in the rhizospheric soil during flowering and harvest. Dehydrogenase activity in the samples was determined by following the procedure described by Casida *et al*. (1964). The urease activity and phosphatase activity of samples was determined by following the standard procedure of Eivazi and Tabatabai (1977).

Enumeration of functional groups of microorganisms

Enumeration of free-living nitrogen fixers and phosphate solubilizers in the rhizospheric soil was carried out using standard serial dilution and plating method. Using N free Jensen's media and Pikovskaya's medium Spore load of AM (Arbuscular Mycorrhiza) fungi was estimated using wet sieving and decanting method proposed by Gerdemann and Nicholson, (1963).

Statistical analysis

The data obtained from field experiment was statistically analysed using WASP: 2.0 (Web Agri Stat Package 2) statistical tool (www.icargoa.res.in/wasp2/index.php) and means were separated by Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Effect of bioinoculants on growth and yield attributing parameters of cowpea (*Vigna unguiculata* L.) under field condition

The growth and yield attributing parameters of cowpea during flowering and harvest were indicated in Table 1 and 2 respectively.

Growth parameters in cowpea revealed that, there is significant differences among the treatments at flowering and harvest. During flowering in treatment T_{10} (75% of N_2 and P +100% K+*R. sp., B.m., G.m.*) recorded highest plant height, highest number of branches, number of nodules, fresh weight of nodules, chlorophyll content, plant nitrogen and phosphorus content *i.e.,* 35.07, 10.44, 5.56, 1.74 g, 35.82, 2.98% and 0.31% respectively, followed by T_6: 50% of N_2 and P+100% K+*R. sp., B.m., G.m.* (33.80, 6.89, 4.44, 1.07 g, 35.48, 2.84% and 0.29% respectively). Yield attributes recorded in cowpea showed highest yield of the crop and seed index in T_{10} (9.48 q/ha and 24.89 g respectively) and followed by T_6 (9.20 q/ha and 22.91 g respectively).

Bioinoculants are known to increase the growth regulating hormones there by increase in the growth and yield attributing parameters. Similar results were obtained by Pramanik and Bera, (2012) in which they showed that the application of bioinoculants increased the yield due to production of growth regulators. Hariredy and Dawson, (2021) also mentioned that the application of inorganic fertilizer or compost along with the bioinoculants increase the growth and yield attributing parameters.

Effect of bioinoculants on functional group of microorganisms in the rhizosphere of cowpea (*Vigna unguiculata* L.) under field condition

Effect of bioinoculants on the microbial density in rhizospheric soil of different treatments during flowering and harvest in the rhizosphere of cowpea is depicted in the Table 3.

Changes in the population of the function group of microorganisms in the rhizospheric soil *viz*., nitrogen fixers, phosphate solubilizers and phosphorus mobilizers were significantly influenced during flowering and harvest by the application of bioinoculants. Highest population of N_2 fixers (5.93×10^3 and 5.82×10^3 CFU g^{-1} soil respectively), phosphate solubilizers (3.96×10^3 and 3.87×10^3 CFU g^{-1} soil respectively) and AMF spore load (169.67 and 167.53 spores/100 g of soil respectively) were noticed in the treatment T_{10} (75% of N_2 and P+100% K+*R. sp., B.m., G.m.*). The microbial load in the rhizospheric soil is gradually reduced from flowering to harvest.

Seed inoculation with bioinoculants increased the microbial population, could be due to modification of biochemical changes in soil. The microorganisms in rhizospheric soil is responsible for providing favourable physical properties, which help in the mineralization of soil nutrient leading to higher available phosphorus and potassium. The beneficial effect of microorganism on potassium availability includes minimization of the losses from leaching. Pradip, (2016) and Macik *et al*. (2020) conducted the experiment in cowpea in which application of bioinoculants improved the soil fertility and increased crop yields by nitrogen fixation, potassium and phosphorus solubilization, production of phytohormones, substances suppressing phytopathogens and guarding plants from abiotic and biotic stresses.

Effect of bioinoculants on microbial biomass carbon, nitrogen and phosphorous in the rhizosphere of cowpea (*Vigna unguiculata* L.) under field condition

Observations on the effect of bioinoculants on the soil microbial biomass carbon, nitrogen and phosphorus in the rhizosphere of cowpea during flowering and harvest are presented in Table 4.

Soil microbial biomass carbon, nitrogen and phosphorus have varied significantly among the different treatments as recorded in cowpea. Highest microbial

Table 1: Effects of bioinoculants on growth attributes of cowpea during flowering (40 DAS) under field condition.

Treatments	Plant heigh (cm)	No. of branches /plant	No. of nodules /plant	Fresh weight of nodules(g)	Chlorophyll content (SPAD reading)	Plant nutrient content	
						Nitrogen (%)	Phosphorous (%)
T_1	32.22^{de}	5.78^{f}	1.56^{f}	0.32^{e}	31.63^{j}	2.51^{f}	0.21^{h}
T_2	32.39^{d}	6.33^{de}	1.67^{f}	0.32^{e}	32.04^{i}	2.59^{ef}	0.24^{g}
T_3	31.80^{fg}	7.22^{cd}	3.56^{c}	0.45^{d}	32.27^{gh}	2.61^{e}	0.26^{d}
T_4	31.99^{ef}	6.89^{de}	1.78^{f}	0.95^{c}	33.87^{e}	2.64^{de}	0.23^{f}
T_5	33.47^{b}	7.33^{c}	2.11^{e}	0.49^{d}	33.14^{gh}	2.63^{de}	0.24^{ef}
T_6	33.80^{b}	6.89^{b}	4.44^{b}	1.07^{b}	35.48^{b}	2.84^{b}	0.29^{b}
T_7	33.66^{c}	7.00^{c}	4.38^{b}	0.95^{c}	34.38^{de}	2.71^{c}	0.28^{bc}
T_8	31.63^{g}	7.67^{d}	2.67^{d}	0.98^{c}	33.44^{ef}	2.69^{cd}	0.27^{cd}
T_9	31.07^{h}	8.11^{b}	2.40^{de}	0.59^{d}	34.36^{c}	2.68^{cd}	0.27^{cd}
T_{10}	35.07^{a}	10.44^{a}	5.56^{a}	1.74^{a}	35.82^{a}	2.98^{a}	0.31^{a}

Note: Mean values followed by the same superscript in each column do not differ significantly p≤0.05 level by DMRT.

DAS- Days after sowing. T_1: Control; T_2: Recommended dose of fertilizer; T_3: 50% of N and P_2O_5+100% K_2O +*Rhizobium sp.*(*R. sp.*); T_4: 50% of N and P_2O_5+100% K_2O+*Bacillus megaterium* (*B.m.*); T_5: 50% of N and P_2O_5+100% K_2O+*Glomus mosseae* (*G.m.*); T_6: 50% of N and P_2O_5+100% K_2O+*R. sp., B.m., G.m;* T_7: 75% of N and P_2O_5+100% K_2O+*R. sp.*; T_8: 75% of N and P_2O_5+100% K_2O+*B.m.*; T_9: 75% of N and P_2O_5+100% K_2O+*G.m.*; T_{10}: 75% of N and P_2O_5+100% K_2O+*R. sp., B.m., G.m.*

Table 2: Effects of bioinoculants on yield attributes during harvest (90 DAS) of cowpea under field condition.

Treatments	Plant height (cm)	No. of branches /plant	Yield (q/ha)	100 seed weight (g)	No. of nodules /plant	Fresh weight of nodules(g)	Plant nutrient content	
							Nitrogen (%)	Phosphorus (%)
T_1	27.71^{f}	4.33^{e}	7.61^{e}	15.87^{g}	0.98^{e}	0.23^{f}	1.09^{j}	0.10^{f}
T_2	29.67^{e}	3.67^{f}	7.67^{e}	17.17^{f}	1.08^{e}	0.33^{ef}	1.21^{h}	0.11^{ef}
T_3	30.25^{d}	5.56^{cd}	8.41^{d}	21.23^{c}	1.35^{d}	0.45^{def}	1.41^{f}	0.13^{d}
T_4	29.67^{e}	5.33^{d}	8.87^{c}	19.45^{d}	1.39^{d}	0.95^{bc}	1.60^{d}	0.12^{de}
T_5	30.13^{d}	5.75^{c}	8.94^{c}	19.89^{d}	1.83^{c}	0.49^{def}	1.48^{g}	0.11^{ef}
T_6	31.33^{b}	6.33^{b}	9.20^{b}	22.91^{b}	2.96^{b}	0.67^{cd}	2.26^{b}	0.15^{b}
T_7	30.23^{d}	5.67^{c}	8.97^{c}	21.18^{c}	1.92^{c}	9.50^{de}	1.48^{ef}	0.14^{cd}
T_8	29.37^{e}	4.35^{e}	8.98^{c}	18.67^{e}	1.94^{c}	0.98^{b}	1.55^{de}	0.13^{d}
T_9	30.83^{c}	6.49^{b}	8.99^{c}	20.97^{c}	1.44^{d}	0.59^{de}	1.87^{c}	0.12^{de}
T_{10}	33.97^{a}	8.78^{a}	9.48^{a}	24.89^{a}	3.91^{a}	1.74^{a}	2.47^{a}	0.17^{a}

Note: Mean values followed by the same superscript in each column do not differ significantly p≤0.05 level by DMRT.

DAS- Days after sowing. T_1: Control; T_2: Recommended dose of fertilizer; T_3: 50% of N and P_2O_5+100% K_2O+*Rhizobium sp.*(*R. sp.*); T_4: 50% of N and P_2O_5+100% K_2O+*Bacillus megaterium* (*B.m.*); T_5: 50% of N and P_2O_5+100% K_2O+*Glomus mosseae* (*G. m.);* T_6: 50% of N and P_2O_5+100% K_2O+*R. sp., B.m., G.m.*; T_7: 75% of N and P_2O_5+100% K_2O+*R. sp.*; T_8: 75% of N and P_2O_5+100% K_2O + *B.m.*; T_9: 75% of N and P_2O_5+100% K_2O+*G. m.*; T_{10}: 75% of N and P_2O_5 +100% K_2O+*R. sp., B.m., G.m.*

biomass carbon, nitrogen and phosphorus was recorded in the treatment T_{10}: 75% of N_2 and P+100% K+*R. sp., B.m., G. m.* (246.49, 28.76 and 53.88 mg g^{-1} soil respectively) during flowering and (238.46, 27.82 and 53.46 mg g^{-1} soil respectively) during harvest, followed by the treatment T_6: 50% of N_2 and P+100% K+*R. sp., B.m., G.m.* (236.86, 27.63 and 52.80 mg g^{-1} soil respectively) during flowering and (224.43, 26.18 and 49.72 mg g^{-1} soil respectively) during harvest.

Treatment T_{10} which received 75% RDF along with microbial consortia showed significantly higher soil microbial biomass carbon, nitrogen and phosphorous compared to the treatments that received inorganic fertilizers, single bioinoculant and control. This could be due to higher amount of organic matter inhabiting microorganisms compared to the treatment received inorganic fertilizer to supply nutrients. The results obtained were in agreement with the work carried out by Balakrishna (2001) and Latkovic *et al.* (2020).

Effect of bioinoculants on soil enzymes activityin the rhizosphere of cowpea (*Vigna unguiculata* L.) under field condition

Effect of bioinoculants on soil enzymes activity in the rhizosphere of cowpea under field condition is represented in the Table 5.

Table 3: Effect of bioinoculants on beneficial microbial population in cowpea rhizosphere under field condition at flowering (40 DAS) and harvest (90 DAS).

Treatments	N_2 fixers ($\times 10^3$ CFU g^{-1} soil)		P Solubilizers ($\times 10^3$ CFU g^{-1} soil)		Spore load (No. of AM spores/100 g soil)	
	At flowering	At harvest	At flowering	At harvest	At flowering	At harvest
T_1	3.57^{f}	3.12^{g}	2.60^{g}	2.07^{h}	131.33^{f}	130.76^{f}
T_2	3.66^{e}	3.95^{g}	2.66^{fg}	2.27^{g}	135.66^{e}	140.52^{de}
T_3	4.96^{c}	4.86^{d}	2.87^{d}	2.77^{e}	138.33^{de}	141.09^{d}
T_4	4.97^{c}	4.88^{d}	3.13^{c}	3.04^{d}	139.67^{de}	144.52^{c}
T_5	5.27^{b}	5.16^{c}	2.53^{f}	3.43^{c}	158.33^{c}	155.19^{c}
T_6	5.33^{b}	5.63^{b}	3.83^{b}	3.79^{ab}	163.33^{b}	165.19^{ab}
T_7	5.07^{c}	4.97^{d}	2.87^{d}	3.76^{ab}	144.67^{d}	139.47^{e}
T_8	4.73^{d}	4.63^{e}	3.73^{b}	3.69^{b}	145.33^{d}	143.88^{c}
T_9	4.67^{d}	4.27^{f}	2.79^{e}	2.47^{f}	162.33^{b}	165.43^{b}
T_{10}	5.93^{a}	5.82^{a}	3.96^{a}	3.87^{a}	169.67^{a}	167.53^{a}

Note: Mean values followed by the same superscript in each column do not differ significantly $p \leq 0.05$ level by DMRT.

DAS- Days after sowing. T_1: Control; T_2: Recommended dose of fertilizer; T_3: 50% of N_2 and P+100% K+*Rhizobium sp.* (*R.sp.*); T_4: 50% of N_2 and P+100% K+*B. megaterium* (*B.m.*); T_5: 50% of $N_2$and P+100% K+*Glomus mosseae* (*G.m.*); T_6: 50% of $N_2$and P+100% K+*R. sp., B.m., G.m.*; T_7: 75% of N_2 and P+100% K+*R. sp.*; T_8: 75% of N_2 and P+100% K+*B.m.*; T_9: 75% of N_2 and P+100% K+*G.m.*; T_{10}: 75% of N_2 and P+100% K+*R.sp., B.m., G.m.*

Table 4: Effect of bioinoculants on microbial biomass carbon, nitrogen and phosphorous in cowpea rhizosphere under field condition at flowering (40 DAS) and harvest (90 DAS).

Treatments	Biomass carbon (mg g^{-1} soil)		Biomass nitrogen (mg g^{-1} soil)		Biomass phosphorous (mg g^{-1} soil)	
	At flowering	At harvest	At flowering	At harvest	At flowering	At harvest
T_1	171.20^{g}	162.21^{gs}	19.97^{g}	18.92^{g}	36.64^{g}	37.93^{e}
T_2	179.26^{f}	163.29^{g}	20.91^{f}	19.05^{g}	38.86^{f}	38.96^{e}
T_3	187.23^{e}	186.27^{f}	21.84^{e}	21.73^{e}	48.91^{de}	46.04^{d}
T_4	216.16^{c}	210.45^{d}	25.22^{bc}	22.55^{d}	48.35^{e}	49.06^{c}
T_5	204.80^{d}	198.18^{e}	23.89^{d}	21.12^{f}	50.19^{bc}	51.36^{b}
T_6	236.86^{b}	224.43^{b}	27.63^{b}	26.18^{b}	52.80^{a}	49.72^{c}
T_7	214.47^{c}	199.88^{e}	25.02^{c}	23.32^{c}	49.72^{cd}	49.91^{c}
T_8	219.16^{c}	217.64^{c}	25.57^{b}	22.39^{d}	51.34^{b}	51.29^{b}
T_9	216.13^{c}	202.44^{e}	25.22^{bc}	23.62^{c}	50.40^{bc}	49.23^{c}
T_{10}	246.49^{a}	238.46^{a}	28.76^{a}	27.82^{a}	53.88^{a}	53.46^{a}

Note: Mean values followed by the same superscript in each column do not differ significantly $p \leq 0.05$ level by DMRT.

DAS- Days after sowing. T_1: Control; T_2: Recommended dose of fertilizer; T_3: 50% of N and P_2O_5+100% K_2O +*Rhizobium sp.* (*R. sp.*); T_4: 50% of N and P_2O_5+100% K_2O+*Bacillus megaterium* (*B.m.*); T_5: 50% of N and P_2O_5+100% K_2O+*Glomus mosseae* (*G. m.*); T_6: 50% of N and P_2O_5+100% K_2O+*R.sp., B.m., G.m.*; T_7: 75% of N and P_2O_5+100% K_2O+*R.sp.*; T_8: 75% of N and P_2O_5+100% K_2O+*B.m.*; T_9: 75% of N and P_2O_5+100% K_2O+*G.m.*; T_{10}: 75% of N and P_2O_5+100% K_2O+*R.sp., B.m., G.m.*

Table 5: Effect of bioinoculants on soil enzyme activity in cowpea rhizosphere under field condition at flowering (40DAS) and harvest (90 DAS).

Sl. no.	Dehydrogenase (mg TPF g^{-1} of soil h^{-1})		Urease (mg NH_4^+-N g^{-1} of soil h^{-1})		Acid phosphatase (mg PNP g^{-1} of soil h^{-1})		Alkaline phosphatase (mg PNP g^{-1} of soil h^{-1})	
	At flowering	At harvest	At flowering	At harvest	At flowering	At harvest	At flowering	At harvest
T_1	1.40^{f}	1.09^{d}	10.84^{h}	9.44^{g}	12.52^{f}	11.75^{g}	8.68^{f}	6.22^{g}
T_2	2.08^{de}	1.13^{d}	11.28^{h}	11.23^{f}	14.44^{ef}	13.87^{f}	8.53^{f}	9.01^{f}
T_3	2.15^{cde}	1.95^{bc}	15.69^{ef}	14.56^{c}	23.82^{c}	20.88^{c}	11.09^{e}	9.31^{f}
T_4	2.02^{de}	1.80^{c}	14.35^{g}	11.97^{e}	18.81^{de}	16.74^{e}	9.00^{f}	10.21^{e}
T_5	1.87^{e}	1.84^{c}	16.37^{de}	13.77^{d}	17.11^{cd}	16.68^{e}	14.50^{c}	8.88^{f}
T_6	5.19^{b}	4.90^{a}	19.97^{b}	16.13^{b}	25.20^{b}	23.91^{b}	18.04^{b}	17.69^{b}
T_7	2.34^{cd}	2.02^{bc}	17.53^{c}	14.64^{c}	20.27^{b}	19.73^{d}	14.56^{c}	12.90^{cd}
T_8	2.45^{c}	2.03^{bc}	14.99^{fg}	12.08^{e}	21.64^{b}	20.62^{c}	15.66^{c}	12.94^{c}
T_9	2.49^{c}	2.18^{b}	16.93^{cd}	11.75^{ef}	22.89^{c}	21.95^{c}	13.16^{d}	12.08^{d}
T_{10}	6.39^{a}	5.17^{a}	21.56^{a}	19.36^{a}	27.60^{a}	25.51^{a}	21.97^{a}	18.75^{a}

Note: Mean values followed by the same superscript in each column do not differ significantly p≤0.05 level by DMRT.

DAS- Days after sowing. T_1: Control; T_2: Recommended dose of fertilizer; T_3: 50% of N and P_2O_5+100% K_2O+*Rhizobium sp.*(*R. sp.*); T_4: 50% of N and P_2O_5+100% K_2O+*Bacillus megaterium* (*B.m.*); T_5: 50% of N and P_2O_5+100% K_2O+*Glomus mosseae* (*G.m.*); T_6: 50% of N and P_2O_5+100% K_2O+*R. sp., B.m., G.m.*; T_7: 75% of N and P_2O_5+100% K_2O+*R. sp.*; T_8: 75% of N and P_2O_5+100% K_2O+*B.m.*; T_9: 75% of N and P_2O_5+100% K_2O+*G.m.*; T_{10}: 75% of N and P_2O_5+100% K_2O+*R. sp., B.m., G.m.*

Significant difference was recorded with respect to the soil enzymes in the rhizosphere during flowering in cowpea. Highest dehydrogenase, urease, acid and alkaline phosphatase were recorded during flowering in the treatment T_{10}: 75% of $N_2$and P+100% K+*R. sp., B.m., G.m.* (6.39 mg TPF g^{-1} of soil h^{-1}, 21.56 mg NH_4^+-N g^{-1} of soil h^{-1}, 27.60 mg PNP g^{-1} of soil h^{-1} and 21.97 mg PNP g^{-1} of soil h^{-1} respectively) followed by the treatment T_6: 50% of $N_2$and P +100% K+*R. sp., B.m., G.m.* (5.19 mg TPF g^{-1} of soil h^{-1}, 19.97 mg NH_4^+-N g^{-1} of soil h^{-1}, 25.20 mg PNP g^{-1} of soil h^{-1} and 18.04 mg PNP g^{-1} of soil h^{-1} respectively).

Higher enzyme activity in soil in above mentioned treatment might be due to the application of bioinoculants that resulted in higher biomass production and extensive root exudates in the rhizosphere and this might have promoted the build-up of microbial population in soil. Application of bioinoculants induces better proliferation of roots and is responsible for the higher soil enzymes activity. Similar results were reported by Abd-Elgwad, (2019) in cowpea with higher dehydrogenase due to microbial inoculation and Kaur *et al.* (2017) in greenpea there was higher dehydrogenase, urease and alkaline phosphatase in soil due to addition of co-inoculation of bioinoculants. The lower enzymes activity in soil was observed in control with no bioinoculants seed inoculation might be due to restricted root growth and lesser exudates from roots which might have caused lower enzymes activity in soil.

CONCLUSION

Modern agriculture involves the use of hazardous chemical fertilizers, pesticides and high yielding varieties. These bioinoculants will help to overcome the usage of these chemicals by providing essential nutrients to plants and improve plant resistance against diseases. Hence from the present study conclude that the use of bioinoculants will partly replace the inorganic fertilizers.

Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Abd-Elgwad, S.A.A. (2019). Effect of microbial inoculation and bentonite amendments on growth, enzyme activity and yield of cowpea cultivated in sandy soil. Environ. Biodivers. Soil Sec. 3: 63-72.

Balakrishna, A.N. (2001). Effect of Agricultural Practices on Native VA Mycorrhizal Fungi and Microbial Biomass in Soil. Ph. D. Thesis, GKVK, UAS, Bangalore.

Brookes, P.C., Powlson, D.S. and Jenkinson, D.S. (1982). Measurement of microbial biomass phosphorous in soil. Soil Biol. Biochem. 14: 319-329.

Carter, M.R. (1991). Ninhydrin reactive N released by the fumigation extraction method measure of microbial biomass under field conditions. Soil Biol. Biochem. 23: 139-143.

Carvalho, M., Lino Neto, T., Rosa, E. and Carnide, V. (2017). Cowpea: A legume crop for a challenging environment. J. Sci. Food Agric. 97(13): 4273-4284.

Casida, L.E., Klein, D.A. and Snatoro, T. (1964). Soil dehydrogenase activity. Soil Sci. 98: 371-376.

Eivazi, F. and Tabatabai, M.A. (1977). Phosphatases in soils. Soil Biol. Biochem. 9: 167-172.

Gerdemann, J.W. and Nicolson, T.H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. soc. 46(2): 235-244.

Harireddy, Y.V. and Dawson, J. (2021). Effect of biofertilizers and levels of vermicompost on growth and yield of cowpea (*Vigna unguiculata* L.). Pharm. Innov. J. 10(6): 985-988.

Kaur, H., Gosal, S.K. and Walia, S.S. (2017). Synergistic effect of organic, inorganic and biofertilizers on soil microbial activities in rhizospheric soil of green pea. Annu. Res. Rev. Biol. 12(4): 1-11.

Kushwaha, A. and Kumar, A. (2013). A Comprehensive review on physical properties, nutritional composition, culinary uses and processing of cowpea (*Vigna unguiculata* L.). Int. J. Pure App. Biosci. 1(5): 51-69.

Latkovic, D., Maksimovic, J., Dinic, Z., Pivic, R., Stanojkovic, A. and Stanojkovic-Sebic, A. (2020). Case study upon foliar application of biofertilizers affecting microbial biomass and enzyme activity in soil and yield related properties of maize and mheat grains. Biol. 9(12): 452.

Macik, M., Gryta, A. and Frąc, M. (2020). Biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms. Adv. Agron. 162: 31-87.

Ohler, T.A. and Mitchell, C.A. (1992). Evaluation of cowpea [*Vigna unguiculata* (L.) Walp] as a candidate species for inclusion in bioregenerative life-support systems. American Society for Space and Gravitational Biology Bulletin. 6: 37.

Piper, C.S. (1966). Soil and Plant Analysis. Hans Pub: Bombay. 7: 368-369.

Pradip, T. (2016). Effect of potassium humate and bio-inoculants on cowpea [*Vigna unguiculata* (L.) Walp] influence of soil fertility, enzymatic activity and microbial population in soil. Int. J. Agric. Sci. 8(53): 0975-3710.

Pramanik, K. and Bera, A.K. (2012). Response of biofertilizers and phytohormone on growth and yield of chickpea (*Cicer arietinium* L.). J. Crop Weed. 8(2): 45-49.

Schubler, A. and Walker, C. (2010). The *Glomeromycota.* A Species List with New Families and New Genera. Libraries at The Royal Botanic Garden Edinburgh, Edinburgh, UK; The Royal Botanic Garden Kew, Kew, UK; Botanische Staatssammlung Munich, Munich, Germany; and Oregon State University, Corvallis. pp: 1-56.

Somasegaran, P. and Hoben, H.J. (2012). Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. Springer Science and Business Media. pp. 1-6.

Suhag, M. (2016). Potential of biofertilizers to replace chemical fertilizers. Int. advanced Res. J. Sci. Eng. Technol. 3(5): 163-167.