Original Research Article

Effect of Seed priming, Beejamrit, Jeevamrit and Micronutrients on Symbiotic traits,Growthattributes,Dehydrogenaseactivity(DHA), Leghaemoglobincontent, Protein content and Grain yield of chickpea

ABSTRACT

A field experiment was conducted during *rabi* season 2023-24 to study the effect of Seedpriming,Beejamrit, Jeevamrit and Micronutrients (Zn and Mo) in combination with *Rhizobium* and PSB inoculation on symbiotic traits, growth attributes, dehydrogenase activity (DHA) of rhizospheric soil and leghaemoglobin content in root nodules, protein content in seed and grain yield of chickpea crop grown with one irrigation in Vertisol. The experiment was laid out in randomized block design with 10 treatments and replicated thrice. The symbiotic traits, growth attributes at 50 DAS and grain yield was significantly increased by the application of RDF + *Rhizobium* + PSB + Ammonium Molybdate @ 1 gm kg^{-1} seed with inoculation (T_5), followed by treatment T_{10}(50% RDF + *Rhizobium* + PSB + Jeevamrit spray + 0.05% AM + 0.25% $ZnSO_4$+ 0.25% Lime sprayat 35 and 55 DAS). The T_5treatmentproduces about 26% higher grain yield over theRDF + *Rhizobium* + PSB (T_1).The treatment T_5 recorded highest protein content in seed i.e. 19.50%, followed by the treatment T_{10}(19.31%). DHA in rhizosphere soil and leghaemoglobin content in root nodules at 50 DAS was found maximum in RDF + *Rhizobium* + PSB + AM 1 gm kg^{-1}seed with inoculation withthevalues31.20µgTPFg^{-1} soilhr^{-1}and3.10mg g^{-1}respectively,followedby50%RDF + *Rhizobium* + PSB + Jeevamrit spray + 0.05% AM + 0.25% $ZnSO_4$+ 0.25% Lime spray at 35 and 55 DAS (T_{10}) with values 30.60 µg TPF g^{-1} soil $hr^{-1}$and 3.08 mg g^{-1}respectively. The study indicated necessity of application of molybdenum wherever deficient in Vertisol under intensive (Soybean-Chickpea)cultivation oflegumes,asitisdirectlyinvolvedinBNFthroughnitrogenase enzyme activity.

Key words: Seed priming, Beejamrit, Jeevamrit, Micronutrients, Dehydrogenase activity, Leghaemoglobin, Protein content,Chickpea.

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a cool- season long-day legume crop and has a diverse use with specific consumer preferences in the global market. It is also known as Gram or Bengal gram. During 2021-2022, India produced13.75milliontonnesofchickpeafrom an area of 10.91 million ha and productivity of 1260kgha^{-1}.Witha2.03millionhaareaand3.03 million tonnes of production, Madhya Pradesh is the major chickpea growing state in India.(Source:DA&FW,2021-2022; Directorate of Economics and Statistics).

The yield levels of chickpea have been generally low which might be attributed to its major cultivation under rainfed conditions with less/imbalance use of fertilizers, limited seed inoculation (10% approximately) with *Rhizobium* and phosphorus solubilizingbacterial cultures (Sharma and Gupta, 2005)and also due to its susceptibility to wilt, insect, pest and diseases. Further, due to intensive cultivation of soybean-chickpea in Vertisol of M.P., some of the micronutrients deficiencies are also observed which might be affecting the productivity.

BeejamritreferstoBeej,whichmeansseed, is dipped into Amrit which means magical liquid. It is a homemade organic inputoriginally made up of cow dung and cow urine.AccordingtoChadha*etal.*(2012),itisbelieved to protect seeds and plants from pest infestation and illnesses, especially those that are transmitted by seeds. Jeevamrit is traditional fermented liquid organic manure that is frequently employed as a soil microbial enhancer in natural farming. It is abundant in beneficial microflora, macronutrients, essential micronutrients, growth-promoting factors like IAA and GA, numerous vitamins, and essential amino acids (Nitin and Purohit, 2021). Seed priming is a pre-sowing approach thatinfluences seedling development by modifying pre-germination metabolic activity prior to radicle emergence, resulting in rapid, uniform emergence and improved plant performance to attain high vigor and yield. Micronutrients are needful elements for normal growth of plants that are needed at little amount. Zinc is an important micronutrient involved in distinct biochemical processes in plants, such as respiration, photosynthesis, chlorophyll biosynthesis, and the synthesis and degradation of proteins, lipids, carbohydrates, and nucleic acids (Nishizawa, 2005). Mo is involved in important functions like nitrogen metabolism, nitrogen-fixation, and transportation of sulphur-containing amino acids in legumes (Togay *etal.,* 2008). Micronutrient deficiency causes significant yield loss in chickpea, and the application of deficient micronutrients is often recommended to maintain the desired yieldlevel (Montenegro *et al.,* 2010).

Thus, keeping this in view, the experiment was conducted to assess the effect of Seed priming, Beejamrit, Jeevamrit and Micronutrients (Zn and Mo) in combination with*Rhizobium*andPSBinoculationongrowth, yield, protein content in seed, DHA of rhizosphere soil and leghaemoglobin content in root nodules of chickpea.

2. MATERIALSANDMETHODS

Afieldexperimentwasconductedat Research Farm area at Department of Soil Science, R.A.K. College of Agriculture, Sehore (M.P.) during *rabi* season 2023-24 to study the effect of Seed priming, Beejamrit, JeevamritandMicronutrients(ZnandMo)incombination with *Rhizobium* and PSB inoculation on symbiotic traits, growth attributes, grain yield, protein content in seed, dehydrogenase activity (DHA) of rhizosphere soil andleghaemoglobincontentinrootnodules of chickpea crop grown with one irrigation in Vertisol having soil pH 7.4, EC 0.36 dSm^{-1}, available N: 204.50 kg ha^{-1}, available P: 17.32 $kgha^{-1}$,availableK:515.20$kgha^{-1}$,available S: 10.72 mg kg^{-1} and available Zn: 0.48 mg kg^{-1} and 0.02 mg kg^{-1} Mo. The experiment was laid out in randomized block design with 10 treatments replicated thrice. The treatments comprised of T_1: RDF + *Rhizobium* (Rh) + Phosphate Solubilizing Bacteria (PSB) [Recommended doses of fertilizer (RDF):20:60:20:20N,P_2O_5,K_2OandSinkgha^{-1}respectively], T_2: RDF + Rh + PSB + Seed primingwithwater,T_3:RDF+Rh+PSB+Beejamrit treatment (seed primed), T_4: RDF + Rh+PSB+Seedprimingwith0.05%MoasAmmonium Molybdate (AM), T_5: RDF + Rh + PSB + AM 1 gm kg^{-1} seed with inoculation, T_6: RDF + Rh + PSB + Jeevamrit spray at 35 and55 days after sowing (DAS), T_7: T_1 + 0.05% AM spray at 35 and 55 DAS, T_8: T_1+ 0.5% $ZnSO_4$+ 0.25% Lime spray at 35 and 55 DAS, T_9:T_1 +0.05%AM+0.25%$ZnSO_4$+0.25%Lime spray at 35 and 55 DAS and T_{10}: 50% RDF + Rh + PSB + Jeevamrit spray + 0.05% AM + 0.25% $ZnSO_4$+ 0.25% Lime spray at 35 and 55 DAS. Before sowing, the seed were treated with *Rhizobium* and PSB cultures @ 5g eachkg^{-1}seed.Beejamrit is prepared by taking 20 litre of water + 5 kg of cow dung + 5 litre of cow urine + 50 gm lime and 1 kg soil from the bund or from shady area. Seeds were treated with Beejamrit by dipping seed for 4 hour prior to sowing. Forseedpriming,theseedswere soaked for 4 hours before sowing.Jeevamrit is prepared by taking 10 kg cow dung + 10 litre cow urine + 2 kg gram flour + 2 kg jaggery + handful of soil + 200 litre water. Jeevamrit (200 l ha^{-1} at 35 DAS and 400 l ha^{-1} at 55 DAS) application in treatment plots, as spray was done in standing crop.The observation on symbiotic traits at 50 DAS (number of root nodules $plant^{-1}$ and dry weight of root nodules $plant^{-1}$),growth attributes i.e.,dryweight $plant^{-1}$ at 50 DAS and plant height and number of branches $plant^{-1}$ at 50 DAS and maturity, protein content in seedand grain yield at harvest. Dehydrogenase activity (DHA) in rhizosphericsoil andleghaemoglobin content inrootnodulesat50DASwererecordedusing standard procedure described by Klein *et al.* (1971) and Wilson and Reisenauer (1963) respectively.

3. RESULTSANDDISCUSSION

3.1 EffectonSymbioticTraits:

The data of symbiotic traits i.e., number of root nodules $plant^{-1}$ and dry weight of root nodules $plant^{-1}$ at 50 DAS (Table 1) indicates significant effect by different treatments over control. The treatment T_5(RDF + Rh + PSB + AM 1 gm kg^{-1} seed with inoculation) recorded maximum number of root nodules $plant^{-1}$(25.81), which was statistically at par with $T_9$and T_{10}treatments. Similarly, the dry weight of root nodules $plant^{-1}$ was also highest in RDF + Rh + PSB + AM 1 gm kg^{-1} seed with inoculation (T_5) with a value of 40.00 mg, followedbythetreatmentT_{10}(50%RDF+Rh+ PSB + Jeevamrit spray + 0.05% AM + 0.25% $ZnSO_4$+ 0.25% Lime spray at 35 and 55 DAS) i.e., 38.64 mg at 50 DAS. The minimumnumber of root nodules $plant^{-1}$ and dry weightof root nodules $plant^{-1}$ was recorded in RDF + Rh+PSB(T_1)i.e.,15.41and28.01mg respectively. The increased microbial activity through effective inoculated rhizobia and P solubilizing bacteria along with micronutrients molybdenum and Zn application may be the cause of rise in number of root nodules and dry weight of nodules. Molybdenum plays an important role in nitrogen metabolism as it is involved in process of nitrogen fixation, nitrate reduction and in the transport of nitrogen in plants. Zinc is required for synthesis of tryptophan, which is essential for the formation of nodules and for promoting plant cell differentiation. Jeevamrit application might favor microbial activities in the rhizosphere, which resulted in increased nodulation. These findings are in accordance with the results of Gupta and Sahu (2012), Gangwar and Dubey (2012) and Khandkar *et al.* (2019).

3.2 EffectonGrowthAttributes:

The data of growth attributes i.e., plant height, number of branches plant^{-1} and total dry weightplant^{-1}(Table1)indicatessignificant effect by different treatments over control. The highest plant height and number of branches plant^{-1} at 50 DAS and maturity was recorded under RDF + Rh + PSB + AM 1 gm kg^{-1} seed with inoculation (T_5) with a value of 29.42 cm (At 50 DAS), 35.04 cm (At Maturity) and 3.31 (At 50 DAS) and 4.30 (At Maturity) respectively, followed by 50% RDF + Rh +PSB + Jeevamrit spray + 0.05% AM + 0.25% $ZnSO_4$+ 0.25% Lime spray at 35 and 55 DAS (T_{10}).TheRDF+Rh+PSB+AM1gmkg^{-1}seedwithinoculation(T_5)recordedhighesttotal dryweightplant^{-1}at50DAS(2.28g),whichwas statistically at par with $T_9$and T_{10}treatments. This could be explained byincreased nitrogenase and nitrate reductase activitiesasaresultofusingmolybdenumalong with *Rhizobium* + PSB, which could boost nitrogen fixation. Increased phosphorus solubilization, improved nitrogen fixation and increased Mo nutrition, all these contributed to legume crop's quicker development, which raised the plant height and number of branches. Zinc plays an important role to synthesize the plant growth regulator such as auxin, which takes active role in enlargement and elongation of plant height and also helps in increasing the number of branches of crop. Jeevamrit contains beneficial microorganisms and plant growth promoting substances like IAA and GA, which promotes growth such as plant height and number of branches of crop. These findings are in accordance with the results of Nagaraju and Mohankumar (2010), Sutar *et al.* (2019) and Bharadwaj *et al.* (2021).

3.3 EffectonGrain Yield(kg ha^{-1}):

The grain yield is the most important character of any treatment which represents the superiorityofanytreatment.Highestgrainyield was observed under RDF + Rh + PSB + AM 1 gm kg^{-1} seed with inoculation (T_5) with a value of 1333.3 kg ha^{-1}, followed by the treatment T_{10}(50% RDF + Rh + PSB + Jeevamrit spray + 0.05%AM+0.25%$ZnSO_4$+0.25%Limespray at 35 and 55 DAS) with value of 1250.0 kgha^{-1}andT_9(T_1+0.05% AM+ 0.25%$ZnSO_4$+0.25%Limesprayat35and55DAS)with grain yield 1229.3 kg ha^{-1} (Table 1). Lowest grainyieldwasfoundinRDF+Rh+PSB(T_1) i.e. 1058.3 kg ha^{-1}. The grain yield of treatment $T_5$was about 26 % more than the treatment T_1. These responses may be attributed to use ofMo-treated seed which enhances nitrogen fixation with increased nitrogenase activity and also enhances N use efficiency through nitrate reductase because Mo is a key component of both of these enzymes. Enhanced N contentalso improves chlorophyll production, which boosts photosynthesis and increases plant attributes, which in turn raised seed yields.Zn influences the synthesis of IAA in plants which indirectly improves the growth, development and symbiotic parameters and ultimately the seed yield of the crop. Jeevamrit contains beneficial microbes viz. bacteria, fungi, yeast, actinomycetesandsomephotosyntheticbacteria which are beneficial for plant nutrient availability which increases seed yield. Together, all these factors contribute in better crop growth and yield attributes, which leads to higher seed yield under these treatments. These findings are supported by Poonia and Pithia (2014), Poojar *et al.* (2022), and Singh *et al.* (2023).

3.4 Effecton Protein content in seed (%):

Protein content in chickpea seed is directly related to nitrogen content in seed. The data of protein content in chickpea seed (Table 1) indicates significant effect of different treatments over control. The treatment T_5 (RDF + *Rhizobium* + PSB + Ammonium molybdate 1 gm kg^{-1} seed with inoculation) recorded highest protein content in seed (19.50%), followed by the treatment T_{10} (50% RDF + *Rhizobium* + PSB + Jeevamrit spray + 0.05% AM + 0.25% $ZnSO_4$ + 0.25% Lime spray at 35 and 55 DAS) with 19.31%protein content in seed and T_9 (T_1 + 0.05% AM + 0.25% $ZnSO_4$ + 0.25% Lime spray at 35 and 55 DAS) with 19.18%protein content in seed. This may be the result of application of molybdenum along with the efficient use of *Rhizobium* + PSB, which boosted BNF. Mo is necessary for the nitrogenase and nitrate reductase enzymes, as well as for the quicker nitrate transformation that occurs inside the plant systems. Since nitrogen is a component of amino acids, which are known to be the building blocks of protein, the notable increase in protein content under these treatments may be the result of a continuous supply of nitrogen from various organic and inorganic sources. The results are in agreement with the findings of Gupta and Sahu (2012) and Gangwar and Dubey (2012).

3.5 EffectonDehydrogenaseActivity(DHA):

Dehydrogenase activity (DHA) reflects the total range of oxidative activity of soil microflora.ThetreatmentT$_5$(RDF+Rh+PSB+ Ammonium Molybdate @ 1 gm kg^{-1} seedwith inoculation) recorded highest dehydrogenaseactivityinrhizospheresoili.e.,31.20 µg TPF g^{-1}soil hr^{-1}, followed by the treatment T$_{10}$(50% RDF + Rh + PSB + Jeevamrit spray+0.05% AM+0.25%ZnSO$_4$+ 0.25% Lime spray at 35 and 55 DAS) with the value of 30.60 µg TPF g^{-1} soil hr^{-1} as shown in Figure1.ThisaccelerationinDHAcouldbe attributed due to increased microbial activity in the rhizosphere might be due to application of effective *Rhizobium*, PSB,along with ammonium molybdate and jeevamrit which increases the microbial activity in the rhizosphere soil. These findings are in accordance with the results of Bidyarani *et al.* (2016), Gupta *et al.* (2020) and Swami *et al.* (2021).

3.6 Effect on Leghaemoglobin content in root nodules:

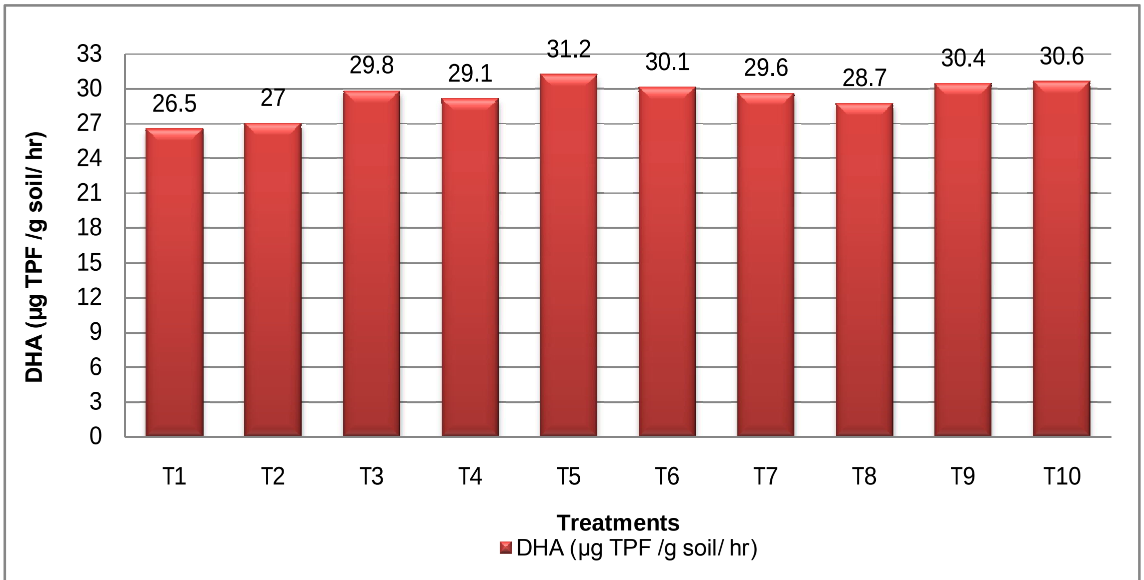
Highest leghaemoglobin content in root noduleswasobservedunderRDF+Rh+PSB+ Ammonium Molybdate @ 1 gm kg^{-1} seedwith inoculation (T$_5$) i.e., 3.10 mg g^{-1}, followed by the treatment T$_{10}$(50% RDF + Rh + PSB + Jeevamrit spray+0.05% AM+0.25%ZnSO$_4$+ 0.25% Lime spray at 35 and 55 DAS) with the value of 3.08 mg g^{-1} as shown in Table 1. This might be ascribed to the use of efficient strainof *Rhizobium,* which produces more nodulation and aids in biological nitrogen fixation. Further the use of Mo, which is an essential component of nitrogenase enzyme, led to increase leghaemoglobin content in root nodules. Mo is an important component of nitrate reductaseand nitrogenase, which stimulate BNF and nodule formation. Zinc is an important micronutrient for nodulation and nitrogen fixation and also involved in leghaemoglobin synthesis. Jeevamrit application might favor microbial activities in the rhizosphere which resulted in increased nodulation and ultimately enhance leghaemoglobin content in nodules. The findings are in accordance with the results of Tagore *et al.* (2013) and Edulamudi *et al.* (2017).

Table 1:EffectofSeedpriming,Beejamrit,JeevamritandMicronutrientsonsymbiotictraits, growth attributes, grain yield, protein content in seed and leghaemoglobin content in root nodules of chickpea crop.

Treatments	**Number of root nodules plant^{-1}at 50 DAS**	**Dry weightof root nodules plant^{-1}(mg) at 50 DAS**	**Plantheight (cm)**		**Number of branchesplant^{-1}**		**Total dry weight plant^{-1}(g) at 50DAS**	**Grain yield(kgha^{-1})**	**Protein content in seed (%)**	**Leghaemoglobin content in root nodules (mg g^{-1}) at 50 DAS**
			50 DAS	**Maturity**	**50 DAS**	**Maturity**				
T_1:RDF+ Rh + PSB	15.41	28.01	23.15	28.86	2.27	2.68	1.43	1058.3	18.56	2.94
T_2: RDF + Rh +PSB +Seedpriming with water	17.60	32.11	23.22	30.02	2.31	3.15	1.55	1138.0	18.68	2.99
T_3: RDF + Rh +PSB +Beejamrit treatment (seedprimed)	21.03	36.15	27.28	31.71	2.48	3.64	1.81	1225.0	18.75	3.06
T_4: RDF + Rh +PSB +Seedpriming with 0.05% Mo as AM	19.90	36.00	26.01	33.07	2.43	3.52	1.60	1212.7	19.12	3.04
T_5: RDF + Rh +PSB +AM1gmkg^{-1} seed with inoculation	25.81	40.00	29.42	35.04	3.31	4.30	2.28	1333.3	19.50	3.10
T_6: RDF + Rh +PSB +Jeevamrit sprayat35and55 DAS	18.01	33.04	24.81	33.29	2.40	3.22	1.68	1172.7	18.75	3.04
T_7: T_1+ 0.05%AMsprayat35and55 DAS	21.31	35.51	26.35	33.68	2.51	3.46	1.81	1228.3	19.0	3.06
T_8: T_1+ 0.5%$ZnSO_4$+0.25%Limesprayat 35 and 55 DAS	19.40	33.28	25.00	31.42	2.35	3.41	1.74	1208.0	18.87	3.02
T_9:T_1+ 0.05%AM +0.25%$ZnSO_4$+0.25%Limesprayat 35 and 55 DAS	24.00	37.51	27.75	34.00	2.78	3.78	2.01	1229.3	19.18	3.07
T_{10}: 50% RDF+Rh+ PSB +Jeevamrit spray + 0.05%AM +0.25%$ZnSO_4$+0.25%Limesprayat 35 and 55 DAS	24.51	38.64	28.0	34.78	3.00	4.02	2.16	1250.0	19.31	3.08
CD @5%	2.69	4.17	2.96	3.47	0.42	0.67	0.29	139.73	0.55	0.06

RDF:Recommendeddoseoffertilizer,Rh:*Rhizobium*,PSB:Phosphatesolubilizingbacteria,AM: Ammonium Molybdate, DAS: Days after sowing

Figure 1:- Effect of different treatments on Dehydrogenase activity (DHA) of rhizospheric soil(µg TPF g^{-1} soil hr^{-1}) at 50 DAS in chickpea.



4. CONCLUSION

The present experiment investigation revealed thatthetreatmentT_5:RDF+*Rhizobium*+PSB+ Ammonium molybdate 1 gm kg^{-1} seed with inoculation found significantly beneficial for all the observations, followed by the treatment T_{10}: 50% RDF + *Rhizobium* + PSB + Jeevamrit spray+0.05%AM+0.25%$ZnSO_4$+0.25%Lime spray at 35 and 55 DAS. The use of treatment T_{10}can save 50% RDF without significant yield difference.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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