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Research Article

Effect of *Rhizobium* and Phosphate Solubilizing Bacterial Inoculants on Symbiotic Traits, Nodule Leghemoglobin, and Yield of Chickpea Genotypes

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A field experiment was carried out during the *rabi* season of 2004-05 to find out the effect of *Rhizobium* and phosphate solubilizing bacterial (PSB) inoculants on symbiotic traits, nodule leghemoglobin, and yield of five elite genotypes of chickpea. Among the chickpea genotypes, IG-593 performed better in respect of symbiotic parameters including nodule number, nodule fresh weight, nodule dry weight, shoot dry weight, yield attributes and yield. Leghemoglobin content (2.55 mg g^{-1} of fresh nodule) was also higher under IG-593. Among microbial inoculants, the *Rhizobium* + PSB was found most effective in terms of nodule number (27.66 nodules $plant^{-1}$), nodule fresh weight (144.90 mg $plant^{-1}$), nodule dry weight (74.30 mg $plant^{-1}$), shoot dry weight (11.76 g $plant^{-1}$), and leghemoglobin content (2.29 mg g^{-1} of fresh nodule) and also showed its positive effect in enhancing all the yield attributing parameters, grain and straw yields.

1. Introduction

Pulses are the second most important group of crops after cereals. Developing countries contribute about 74% to the global pulses production and the remaining comes from developed countries. India, China, Brazil, Canada, Myanmar, and Australia are the major pulse producing countries with relative share of 25%, 10%, 5%, 5%, and 4%, respectively. In 2009, the global pulses production was 61.5 million tonnes from an area of 70.6 million hectares with an average yield of 871 kg/ha. Dry beans contributed about 32% to global pulses production followed by dry peas (17%), chickpea (15.9%), broad bean (7.5%), lentil (5.7%), cowpea (6%), and pigeonpea (4.0%). India is the largest producer and consumer of pulses in the world contributing around 25–28% of the total global production. About 75% of the global chickpea (*Cicer arietinum* L.) area falls in India [1]. Chickpea is one of the major post rainy seasonpulse crops in Madhya Pradesh, which occupies 3.09 m ha with production of 3.30 mt and productivity of 1071 kg ha^{-1} [2]. The poor productivity of chickpea in this region is mainly due to imbalance application of nutrients and use of traditional varieties. Under such situations, use of *Rhizobium* and phosphate solubilizing bacteria (PSB) had shown advantage in enhancing chickpea productivity [3, 4]. Microbial inoculants are cost effective, ecofriendly, and renewable sources of plant nutrients [5]. *Rhizobium* and PSB assume a great importance on account of their vital role in N_2-fixation and P-solubilisation. The introduction of efficient strains of P-solubilizing species of *Bacillus megaterium* biovar *phosphaticum*, *Bacillus polymyxa*, *Pseudomonas striata*, *Aspergillus awamori*, and *Penicillium digitatum* in the rhizosphere of crops and soils has been reported to help in increasing phosphorus availability in the soil [6]. Since the information on response of elite genotypes of chickpea to inoculation with *Rhizobium* and phosphate solubilizing bacterial inoculants is meager under such situation, therefore, an experiment was designed to assess the productivity of chickpea genotypes in combinations with microbial inoculants in *Malwa* Region of Madhya Pradesh.

2. Material and Methods

2.1. Experimental Site. The field experiment was conducted at the Experimental Farm of College of Agriculture, Indore, Madhya Pradesh (22°43′ N, 75°56′ E and 555.7 m above mean sea level). The soil of the experimental site belongs to sarol series, which is a member of a fine montmorillonitic family of Vertic Ustochrept and Vertic Chromusters. The soil characteristics of the experimental site before start of the study were analysed and presented in Table 1.

2.2. Treatments Details and Crop Culture. The experiment was conducted during the winter (*rabi*) season of 2004-05. The experiment was laid out in split-plot design with three replications. The experiment was conducted with twenty treatment combinations comprising five genotypes, namely, IG-226, IG-370, IG-379, JG-412, and IG-593, in main plots and four microbial inoculants (no inoculum, *Rhizobium*, PSB, and *Rhizobium* + PSB) in sub-plots. The chickpea genotypes were collected from college of Agriculture, Indore and microbial inoculants from JNKVV, Jabalpur. The gross plot size was 5 m × 2.40 m^2. Chickpea crop was sown on a fine seed bed prepared after presown irrigation with a seed rate of 100 kg ha^{-1}. *Rhizobium* inoculant at 5 g kg^{-1} seed was applied as seed treatment, whereas, phosphate solubilizing bacterial inoculants were applied in soil at 3 kg ha^{-1} prior to sowing. The chickpea crop was given irrigation at 40 days after sowing.

2.3. Soil Analysis. Soil samples from surface soil (0–15 cm) were taken for chemical analysis after harvesting of rice crop. Random cores were taken from each plot with a 5 cm diameter tube auger and bulked. The moist soil samples were sieved (2 mm) after removing plant material and roots. Similarly, initial soil samples were collected from 10 random places of experimental site before start of study. All chemical results are means of triplicate analyses and are expressed on oven-dry basis. Soil was analyzed for pH in 1 : 2.5 soil : water suspension [7], SOC by the method of Walkley and Black [8], Kjeldahl N by FOSS Tecator (Model 2200), available P following the method of Bray and Kurtz [9], available K by 1 N NH_4OAc using a flame photometer [7], and available S by using 0.15% $CaCl_2$ [10]. *Rhizobium* population in the soil samples was enumerated by plant infection technique of Toomsan et al. [11] and phosphate solubilizing bacteria (PSB) by dilution plate count method as described by Sundara Rao and Sinha [12].

2.4. Nodulation, Growth, and Yield of Crops. Five plants were randomly selected and removed from each plot and recorded the nodule fresh weight at 35, 55, and 75 days after sowing (DAS). After removal of nodules, the plants were first sun dried for 3 days and then oven dried at 65°C for 48 hours to obtain dry weight. Leghemoglobin content in nodular tissues collected at 35, 55, and 75 DAS was determined by the procedure outlined by Beau [13]. The nodules were dried in oven at 65°C for 78 hours for dry weight of nodules. Plants were harvested at physiological maturity and plant height, and number of branches per plant, number of pods per plant, and number of seeds per pod and test weight (1000 seed weight) were recorded from 10 randomly selected plants at the time of harvest. Total dry matter and grain yield were also recorded for each plot.

Table 1: Soil properties before the start of the study.

Soil parameter	Value
pH	7.8
Electrical conductivity, dS m^{-1}	0.23
Soil organic carbon, %	0.45
Available nitrogen, kg ha^{-1}	204
Available phosphorous (Olsen' P), kg ha^{-1}	9.58
Available potassium, kg ha^{-1}	576
Available sulphur, kg ha^{-1}	12.88

2.5. Statistical Analysis. Effect of treatments were evaluated by split-plot analysis of variance (ANOVA) with chickpea genotypes as main and microbial inoculants as subfactors. Analysis of variance was performed using the program SPSS 11.0 for windows. The significance of the treatment effect was determined using F-test. When ANOVA indicated that there was a significant value, multiple comparisons of mean value were performed using the least significant difference method (LSD).

3. Results and Discussion

3.1. Symbiotic Traits. The data on mean nodule number, nodule fresh weight, nodule dry weight, and shoot dry weight at 35, 55 and 75 days after sowing (DAS) are presented in Figures 1(a), 1(b), 1(c), 1(d) and Table 2. The analysis of data revealed that among the genotypes, IG-593 exhibited the highest nodule number, namely, 19.91, 27.04, and 25.29 $plant^{-1}$, nodule fresh weight, namely, 86.79, 127.48, and 100.16 mg $plant^{-1}$ and dry weight of nodules, namely, 46.16, 67.29, and 65.68 mg $plant^{-1}$ at 35, 55, and 75 DAS, respectively.

Genotype IG-593 recorded maximum shoot dry weight, namely, 2.56, 12.55, and 25.53 g $plant^{-1}$ and the minimum in IG-370, namely, 1.67, 8.03, and 14.98 g $plant^{-1}$ at 35, 55, and 75 DAS, respectively. The data on symbiotic traits of chickpea genotypes indicated that shoot dry weight increased progressively and nodule number, nodule fresh weight, nodule dry weight also followed the similar trend at 35 and 55 DAS, but the decline was noted in nodule number, fresh weight, and dry weight of nodules at 75 DAS. This was mainly due to decay of nodular tissues at pod formation, which start from 60 to 65 DAS.

Coinoculation of *Rhizobium* and PSB recorded significantly higher nodule number and its fresh as well as dry

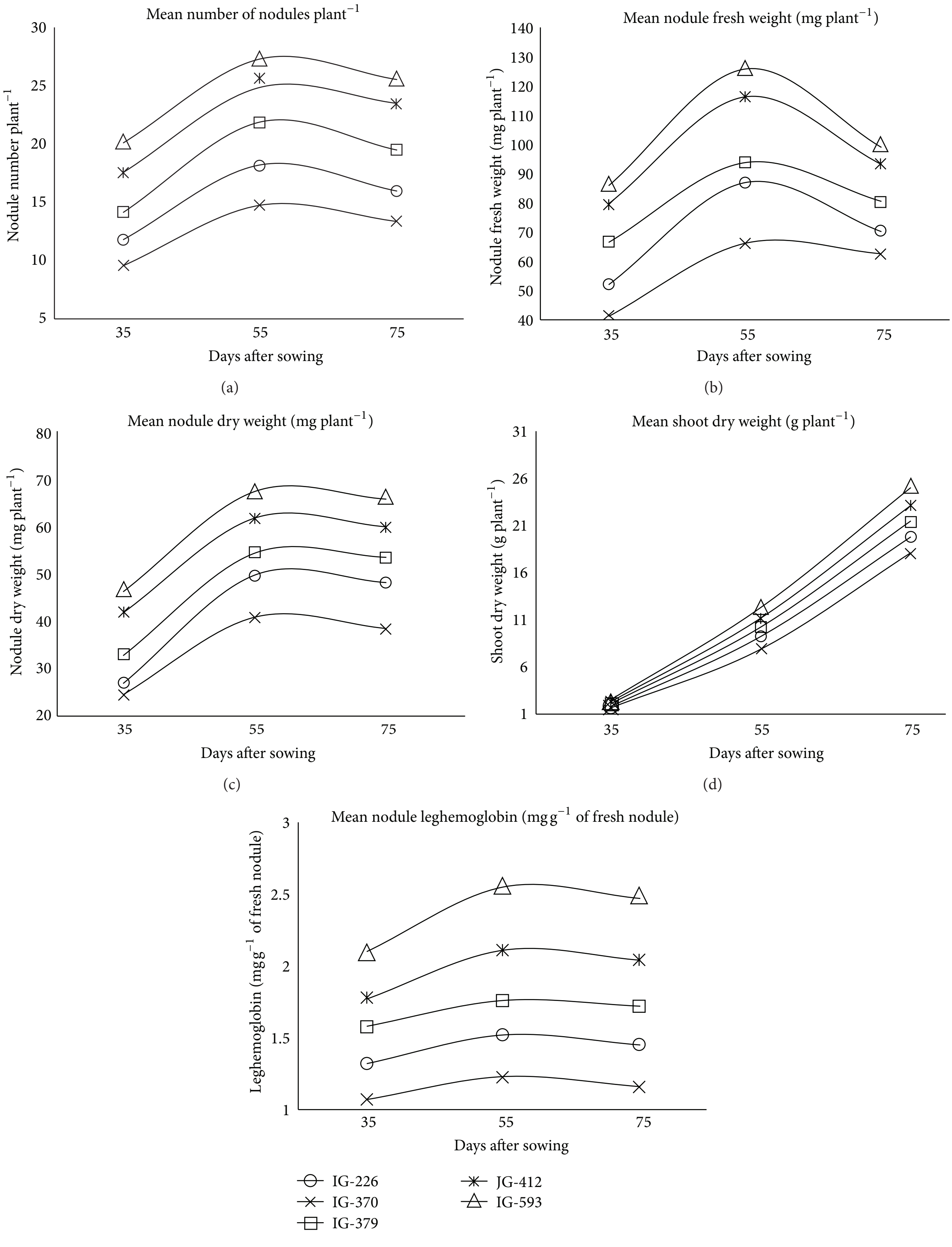

Figure 1: ((a)–(d)) Symbiotic parameters and (e) leghemoglobin content in nodular tissues of chickpea genotypes at different intervals.

Table 2: Symbiotic parameters and leghemoglobin content in nodular tissues at different intervals.

Chickpea genotypes	Nodule number plant^{-1}			Nodule fresh weight (mg plant^{-1})			Nodule dry weight (mg plant^{-1})			Shoot dry weight (g plant^{-1})			Nodule leghemoglobin (mg g^{-1} of fresh nodule)		
	Days after sowing			Days after sowing			Days after sowing			Days after sowing			Days after sowing		
	35	55	75	35	55	75	35	55	75	35	55	75	35	55	75
Chickpea genotype															
IG-226	11.7	18.1	15.8	52.3	87.9	70.8	26.8	49.7	48.1	1.8	9.4	20.2	1.3	1.5	1.5
IG-370	9.5	14.6	13.3	41.2	66.7	63.0	24.5	40.8	38.4	1.7	8.0	18.5	1.1	1.2	1.2
IG-379	14.0	21.7	19.3	67.0	94.8	81.3	32.7	54.3	53.4	2.1	10.4	21.9	1.6	1.8	1.7
JG-412	17.4	24.6	23.3	80.5	117.8	94.2	41.7	61.7	59.9	2.3	11.4	23.6	1.8	2.1	2.0
IG-593	19.9	27.0	25.3	86.8	127.5	100.2	46.2	67.3	65.7	2.6	12.6	25.5	2.1	2.6	2.5
SEm±	1.2	1.2	0.9	2.0	3.1	2.2	0.6	1.3	0.6	0.04	0.3	0.5	0.1	0.7	0.1
LSD (P = 0.05)	3.9	3.6	2.8	6.6	10.1	7.3	1.9	4.1	2.0	0.1	1.0	1.7	0.2	0.2	0.3
Microbial inoculants															
Control	9.4	15.6	14.8	46.2	56.9	54.1	24.3	39.0	38.0	1.9	9.2	16.6	1.2	1.5	1.5
Rhizobium	18.9	25.4	24.7	67.1	120.9	102.1	40.3	64.6	63.1	2.2	10.8	24.6	1.7	2.0	1.9
PSB	10.0	16.8	15.7	54.8	64.6	61.6	26.3	42.1	40.9	2.0	9.6	20.7	1.3	1.5	1.5
Rhizobium + PSB	20.7	27.7	26.7	95.1	144.9	120.4	46.6	74.3	70.5	2.3	11.8	26.5	2.0	2.3	2.3
SEm±	0.6	0.8	0.7	3.1	3.9	2.8	0.8	1.3	1.3	0.05	0.2	0.3	0.04	0.05	0.1
LSD (P = 0.05)	1.7	2.2	1.9	9.1	11.2	8.0	2.3	3.7	3.8	0.1	0.4	0.8	0.1	0.1	0.2

PSB: phosphate solubilising bacteria.

weight than *Rhizobium* and PSB alone. The increase in nodulation might be due to synergistic effect of the two types of microorganisms for biological nitrogen fixation as against their individual application. Results of the similar kind have also been reported by Rudresh et al. [4]. It is also due to the fact that phosphate solubilizing bacteria by virtue of their property of producing organic acids solubilize insoluble or fixed form of phosphorus in the rhizosphere and make it available to the growing plants, which promotes root development in plants [14]. In the present study, a significant response of dual inoculation with *Rhizobium* and PSB was observed with respect to shoot dry weight per plant. Observations of the similar kind have been recorded by Gupta and Namdeo [15] and Barea et al. [16].

3.2. Leghemoglobin Content in Root Nodules. The results given in Figure 1(e) and Table 2 showed that the leghemoglobin content in chickpea root nodules increased with the advancement of crop age and was maximum at 55 DAS and thereafter decline at 75 DAS. Among the genotypes, IG-593 possessed the highest nodule leghemoglobin of 2.10, 2.55, and 2.47 mg g^{-1} of fresh nodule and lowest 1.07, 1.23, and 1.45 mg g^{-1} of fresh nodule in IG-370 at 35, 55 and 75 DAS, respectively. In the present study, coinoculation of *Rhizobium* and PSB performed better than *Rhizobium* and PSB alone with respect to leghemoglobin content in the nodular tissues of chickpea crop. In case of microbial inoculants, higher leghemoglobin content in nodular tissues was observed in *Rhizobium* + PSB in comparison to their individual inoculation. The better nodulation under chickpea genotype IG −593 might be resulted in higher content of leghemoglobin in nodular tissues. Similarly, higher leghemoglobin content in *Rhizobium* + PSB was mainly due to better root and nodules development [17].

3.3. Yield Attributes. The significant differences were found in yield attributing parameters due to genotypes and microbial inoculants, while their interaction effect was nonsignificant (Table 3). Among the genotypes, IG-593 exhibited the highest mean number of branches and pods per plant and seeds per pod, that is, 15.95, 63.96 $plant^{-1}$ and 1.59 pod^{-1}, respectively, and the lowest values were recorded in IG-370, that is, 10.23, 34.71 $plant^{-1}$ and 1.13 pod^{-1}, respectively. It was further noted that among the genotypes, IG-593 produced the tallest plant (42.07 cm), while the genotype IG-370 had shortest plant (30.63 cm). Variation in the above parameters is bound to occur due to difference in genetic makeup and inherited characters in different genotypes. The results corroborate with the findings of Singh et al. [18] and Tiwari et al. [19].

The data further indicated that IG-593 had highest test weight (333.80 g) followed by JG-412 (249.13 g), IG-379 (191.48 g), IG-370 (158.68 g), and IG-226 (152.34 g). The variation in test weight among the genotypes is likely to occur due to difference in seed size of the individual genotype. A high value of test weight indicated the boldness of seeds, while the lower values indicated small seeds. Large/small seed size of chickpea is basically a genotypic character [19]. In the present study, seed inoculation with *Rhizobium* + PSB significantly increased the plant height, number of branches, number of pods per plant, number of seeds per pod, and 1000 seed weight (test weight) over no inoculation. The higher growth and yield attributes under *Rhizobium* + PSB inoculation were mainly due to more availability of N, P, K, and S in the soil for chickpea plants [20–22]. Moreover, growth promoting substances (phytohormones) are produced by these organisms which further promote plant growth [23–25]. Further, inoculation of *Rhizobium* and PSB alone produced significantly higher number of pods per plant and test weight. These results are in close agreement with Takankhar et al. [26] and Khoja et al. [27].

3.4. Grain and Straw Yield. Critical examination of the data in Table 3 revealed that genotype IG-593 produced the highest grain and straw yields (2 286 and 2 728 Kg ha^{-1}) followed by JG-412 (1 995 and 2 291 Kg ha^{-1}) and lowest in IG-370 (1 475 and 1 613 Kg ha^{-1}). The observed variation in seed and straw yields in the present investigation seems to be due to genetic difference in yield potential of different genotypes and also due to variable response of different genotypes to microbial inoculants [19, 28].

Significant differences in grain and straw yields were also recorded due to microbial inoculation. The grain and straw yields increased due to microbial inoculation, and the highest seed and straw yields were obtained in inoculation of *Rhizobium* + PSB, that is, 2 150 and 2 461 Kg ha^{-1}, and the lowest in the case of control, that is, 1 587 and 1 901 Kg ha^{-1}, respectively. The increase in grain and straw yield might be attributed to the increased availability of N and P in soil which resulted in higher growth and development and finally yields [20, 23, 29–31].

3.5. Soil Characteristics. Soil pH and EC remain unaffected under different chickpea genotypes and microbial inoculation. However, highest value of soil organic carbon (SOC) was observed under IG-593 (0.53%) and the lowest under IG-370 (0.47%). The significant variations in available N, P, K, and S were also recorded due to chickpea genotypes and microbial inoculation. The highest value of available N (219.25 kg ha^{-1}), available P (12.18 kg ha^{-1}), available K (568.6 kg ha^{-1}), and available S (13.6 kg ha^{-1}) was recorded after harvest of chickpea genotype 1G-593 and the lowest values of available N, P, K and S were recorded in IG-370. In case of microbial inoculation, the highest values of available N (220.3 kg ha^{-1}), available P (14.1 kg ha^{-1}), available K (556.9 kg ha^{-1}), and available S (13.41 kg ha^{-1}) were recorded under inoculation of both *Rhizobium* and PSB; however, the lowest values of available N, P, K, and S were under no-inoculation (control). The variations in available nutrients under different genotype might be due to variations in compatibility between soil microflora and chickpea genotypes. However, *Rhizobium* and PSB inoculation had resulted in better plant growth, nodulation, and rhizospheric environment which finally resulted in more availability of plant nutrients (NPKS) in the soil [28, 32, 33].

Table 3: Yield attributes, grain and straw yields of chickpea genotypes.

Chickpea genotypes	Plant height (cm)	Number of branches plant^{-1}	Number of pods plant^{-1}	Number of seeds pod^{-1}	Test weight (g)	Mean yield (Kg ha^{-1}) Grain	Straw
			Chickpea genotype				
IG-226	33.4	12.4	38.6	1.25	152.3	1622	1894
IG-370	30.6	10.2	34.7	1.13	158.7	1475	1613
IG-379	34.5	13.3	42.8	1.30	191.5	1922	2199
JG-412	39.0	14.4	48.4	1.33	249.1	1995	2291
IG-593	42.1	16.0	64.0	1.38	333.8	2286	2728
SEm±	1.4	0.3	0.9	0.058	2.0	88	91
LSD (P = 0.05)	4.5	0.9	3.0	0.191	6.5	288	299
			Microbial inoculants				
Control	34.1	11.4	37.9	1.1	213.1	1587	1901
Rhizobium	36.7	13.6	48.9	1.3	220.0	1967	2303
PSB	34.2	12.1	43.5	1.3	216.0	1764	2089
Rhizobium + PSB	37.1	16.0	55.7	1.4	220.7	2150	2461
SEm±	0.8	0.3	0.8	0.6	1.4	59	52
LSD (P = 0.05)	2.4	0.8	2.2	0.2	4.0	170	152

PSB: phosphate solubilising bacteria.

Table 4: Soil fertility as influenced by chickpea genotypes and microbial inoculants.

Chickpea genotypes	pH	EC dS m^{-1}	SOC (%)	Available nutrients in soil (kg ha^{-1}) N	P	K	S	*Rhizobium* population ($\times 10^4$ g^{-1} of soil)	PSB population ($\times 10^5$ g^{-1} of soil)
				Chickpea genotype					
IG-226	7.75	0.36	0.49	198.5	10.6	545.6	12.5	9.68	4.23
IG-370	7.68	0.35	0.47	192.8	10.4	544.8	12.4	9.29	4.03
IG-379	7.76	0.36	0.49	194.5	11.4	546.4	13.0	9.99	4.42
JG-412	7.79	0.37	0.53	210.3	11.6	547.1	13.4	10.60	4.53
IG-593	7.79	0.38	0.53	219.3	12.2	568.6	13.6	10.99	5.09
SEm±	—	—	0.01	1.5	0.4	1.6	0.4	0.24	0.13
LSD (P = 0.05)	NS	NS	0.02	4.8	1.4	5.1	1.2	0.78	0.41
				Microbial inoculants					
Control	7.71	0.36	0.48	193.3	9.1	545.7	12.5	9.37	4.12
Rhizobium	7.76	0.37	0.51	206.8	9.2	552.7	13.0	10.79	4.14
PSB	7.73	0.36	0.48	191.9	12.5	546.7	13.0	9.40	4.77
Rhizobium + PSB	7.81	0.37	0.54	220.3	14.1	556.9	13.4	10.86	4.80
SEm±	—	—	0.01	2.6	0.2	1.9	0.3	0.08	0.03
LSD (P = 0.05)	NS	NS	0.02	7.5	0.6	5.5	0.7	0.24	0.10

SOC: soil organic carbon, EC: electrical conductivity.
Initial rhizobial count: 9.56×10^3 g^{-1} of soil and phosphate solubilizing bacterial (PSB) count: 4.36×10^4 g^{-1} of soil.

3.6. Microbial Population. The data on *Rhizobium* and phosphate solubilising bacterial counts are given in Table 4. Significant variations in *Rhizobium* and phosphate solubilising bacteria (PSB) was observed due to both chickpea genotypes and microbial inoculation; however, their interactions were nonsignificant. Among chickpea genotypes, significantly highest *Rhizobium* population was recorded in IG-593 (10.99×10^4 g^{-1} of soil) followed by JG-412 (10.60×10^4 g^{-1} of soil) and least in IG-370 (9.29×10^4 g^{-1} of soil). Similarly, PSB population was significantly higher in 1G-593 (5.09×10^5 g^{-1} of soil) over rest of genotypes. In case of microbial inoculation, the highest values of *Rhizobinum* (10.86×10^4 g^{-1} of soil) and PSB (4.80×10^5 g^{-1} of soil) were recorded in 1G-593 and lowest under control (no-inculcation). The highest values of microbial counts in 1G-593 might be due to greater compatibility of this genotype with inoculated microbial strains. However, inoculation of both *Rhizobinum* and PSB might have given added advantage over native microbial population [34].

4. Conclusion

Based on above results, it can be concluded that the chickpea genotype IG-593 is superior over the remaining genotypes with respect to nodulation, yield attributing parameters, nodule leghemoglobin content, and yield under limited irrigation in vertisols of Malwa Region. Use of *Rhizobium* and PSB inoculation had also shown advantage over no-inoculation. Thus, chickpea genotype IG-593 and inoculation of *Rhizobium* and PSB may be recommended to realize higher yield of chickpea in this region.

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