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Effect of *Rhizobium* on the crop productivity of Chickpea (*Cicer arietinum* L.) and soil fertility

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Abstract

The development, improvement, and yield of chickpea (*Cicer arietinum* L.) are firmly affected by abiotic factors, for example, salinity and dry season in the arid conditions. The utilization of effective PGPR in the production of chickpea is the best answer to defeat those stresses. In the present study, 16 rhizobial strains were isolated and purified from the root nodules of chickpea genotype developed on middle salinated soils with various chickpea cultivation antecedents, three of them were increasingly productive in salt resilience and demonstrated higher nodulation capacities. Neighborhood chickpea genotype Phule-G-12313 was inoculated with specific *Rhizobium* bacterial strains before planting them to the field condition. Inoculation of plants with the strains of *Rhizobium* species RB1, RB6 and RB9 increase significantly shoot, root dry matter and also the nodule number by 19, 14, and 25% over the un-inoculated plants. The shoot length expanded by 50%, root length by 40%, shoot dry weight by 35%, and root dry weight by 60%. The pod number was increased significantly by 30% due to inoculation and yield up to 50%, as compared to the control plant which was not inoculated with any strain. The powerful indigenous rhizobial strains isolated in this examination from chickpeas on middle salinated soils of Bhopal have the characters of expansive host range, the efficiency of high nodulation, the efficiency of nitrogen fixation, incredible salt tolerance. The amount of soil nitrogen, phosphorus, and carbon at the end of the examination were positive in every treatment as compared to the control. In this investigation, we are concentrating on the symbiotic relationship between chickpea and its advantageous nitrogen-fixing bacterial strains and how it will impact the plant productivity and fertility of the soil.

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Chickpea (*Cicer arietinum* L.) is a significant vegetable nourishment crop in arid areas considering as a fundamental source of protein, lipid, starch, and nutrients for human beings. Though, the production of chickpea is severely limited in arid regions because of the salinity of soil and dry season condition. Additionally, low soil fertility and severe atmosphere are the significant components limiting the production of leguminous crops.

In India, Chickpea is developed in rainy areas as they are best suited for its production. Chickpea producing states in India are Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra, and Andhra Pradesh. Madhya Pradesh produces a significant offer of 42% in the Indian generation of around 6 million tons. Andhra Pradesh, Uttar Pradesh, Maharashtra, and Rajasthan pursue Madhya Pradesh with contributing around 11%, 12%, 13% and 9% of generation individually.

The best test for our arable agriculture in the long haul is the upkeep, and ideally the improvement of soil quality. Leguminous cropping systems that expansion soil richness and simultaneously improve plant efficiency and counteract disintegration and desertification are of significant enthusiasm for some countries in the world[6]. The integration of leguminous crops in the crop rotation system boosts the yield of wheat which is the primary crop, yet crop yield has been diminishing because of compounding soil conditions[12].

Chickpea (*Cicer arietinum* L.) is a high proteinaceous leguminous plant grown in India. It is grown an about 9.21 million ha with a production of 8.88 million tons with a productivity of 995 kg/ha. The majority of chickpea plants were affected by diseases that may be seed or soil-borne caused by *Fusarium oxysporum*, *Rhizoctonia solani*, and *Botrytis cinerea*. Annual chickpea yield losses implicating Fusarium-wilt vary from 10-15% [3]. *Rhizobium* also effectively controls various soil-borne plant pathogenic fungi such as *Fusarium oxysporum*. Amongst the soil bacteria, *Rhizobium* which belongs to the Rhizobiaceae family[22] has a beneficial effect on the growth of plants.

Rhizobia infect the roots of chickpea and induce the formation of nodules in which the rhizobia fix nitrogen. Viable bacteria are often isolated from the nodules to identify the infecting strains. Identification of the infecting strain is essential for studies of symbiotic properties such as host range specificity and nodulation competitiveness. The rhizosphere or zone of influence around plant roots possesses various microorganisms such as bacteria, actinomycetes, fungi, and algae which affect the physical, chemical and biological properties of soil.

With the capacity of organic nitrogen fixation (N) in relationship with rhizobial strains, chickpea could be considered as an excellent rotation and intercropping crop by improving the fertility of soil and structure, and diminishing soil disintegration in agricultural production framework[19]. Even though legume *Rhizobium* symbiotic nitrogen (N) fixation is a significant natural character and the base of improving soil fertility, a valid example is an absence of powerful rhizobial inoculants adjusting to salinated soils. Past examinations have demonstrated that salinity and drought pressure

prompted a huge decrease in plant biomass collection (root and shoot), nodule improvement and nitrogenase action just as firmly declined the yield in chickpea[6,8]. Chickpea can reestablish the fertility of soil because of the profound penetrating root system which enables them to use the constrained accessible moisture[21].

There are confirmations that specific strains of microscopic organisms in the root nodules of leguminous plants can help endure toxic salinity levels. The salt resistance capacities of rhizobia may importantly affect the fruitful association of *Rhizobium* leguminous plants under salinity conditions. It targets acquiring naturally safe nourishment and better return without disturbance of the environment and at the same time, improves the quality of the soil. Due to the lack of chickpea cultivation, indigenous *Rhizobium* strains in most soils is low and the rhizobial inoculants from chickpea developments of various areas couldn't adjust to salinated soils. In this manner, it is extremely urgent to choose efficient rhizobial strains, which are very much adjusted to salinated soils for creating chickpea generation and improving chickpea yield and soil richness.

The morphology and physiology of *Rhizobium* will vary from free-living conditions to the asteroid of nodules. In addition to fixing the atmospheric nitrogen through nodulation, it shares many characteristics with other PGPRs including hormone production and solubilization of organic and inorganic phosphate. Through plant-growth-promoting substances, it helps in root expansion, improves uptake of plant nutrients, protects plants from root diseases and most importantly improves biomass production of fast-growing at wasteland[9].

In this examination series of rhizobial strains were isolated and purified with serial dilution, from salinated soils and nearby chickpea variety, and their nodulation effectiveness was tried both in soil pot and field condition. Besides, the inoculation bacterial strains were able to persist in the rhizosphere indicating colonization on root and inside nodules. The present examination shows that plant-growth-promoting rhizobacteria (PGPR) inoculation ought to be incorporated with the chickpea production programme.

Study site and soil examining :

Soil for pot test was examined from an irrigated agriculture site situated in Morena district. The study area was situated in the northern part of Madhya Pradesh (23° 152 to 26° 452 N, 70° 352 E) with an altitude ranging from 160-250 m. In these soils, cotton has been cultivated for a long time under a consistent monoculture production framework and under flood water system without appropriate drainage system however utilizing a characteristic stream system. As per the WRB-FAO [23] grouping, the soils of marked fields were recognized as Calcisol (sediment soil serozem) including a horizon of calcic inside 50 cm of the surface. The physical and chemical properties of soil of the recognized site were abridged in Tables 3 and 4. The surface soil horizon was calcareous saline while the more profound soil horizon was just somewhat alkaline. The organic matter of the orchic

horizon is very low. The atmosphere is semiarid and extremely hot with mean yearly air temperatures of 48° C and 42° C and means yearly rainfalls of 500-600 mm. The traditional culturing comprised of moldboard plowing to 30 cm depth after harvest and offset disking, to a depth of 20 cm, preceding planting in the spring. Soil tests of 0-30 cm depth were taken with a soil auger (3.5 cm width). Soil samples were gathered toward the starting March (spring), and end of the trials July (summer). The centers were pooled; field-moist (clammy) soils were sieved (<2mm) after collection. The samples of soil were kept in dark polyethylene packs and put away at 4°C. These fresh field-moist samples, which were sieved utilized for the study after incubation. The rate of conventional fertilizers, N, P, K input rates range from 200, 140 and 70 kg ha^{-1} yr^{-1} individually in all plots of the test.

Plant and microorganisms :

Seeds of the chickpea "Phule-G-12313" were obtained and treated with sixteen treatments of rhizobial isolates. Every single bacterial strain was recently isolated from the rhizosphere of chickpea developed in salinated soil of Morena.

Isolation, characterization, purification, sequencing, and inoculation of rhizobial strains :

Root nodules from neighborhood chickpea genotype Phule-G-12313 grown in pots loaded up with salinated soil brought from field locales in Morena district in Madhya Pradesh. They were utilized to isolate and purify rhizobial strains. There were a couple of long stretches of chickpea development history in Morena locale's test field, where the soil had a higher profuseness of indigenous *Rhizobium* and marginally more salinity. The techniques of *Rhizobium* isolation, characterization and purification followed as detailed in the following methodology. Initially, the root nodules were washed in 95% ethanol for 3 minutes, at that point sanitized in 0.2% $HgCl_2$ for 5 minutes and flooded with sterile water for 5-6 times. From that point forward, we crush the nodules with the help of a sterilized glass rod to obtain and milky solution. And after completion of this process, we follow the dilution method and spread 200 µL of the bacterial solution on the YEMA medium. At that point the YEMA medium was incubated for 5 to 6 days under 28° C. The bacterium looked like *Rhizobium* was picked to purify and pure-culture of *Rhizobium* was obtained. After the gram staining technique and biochemical characterization, the isolated rhizobial strains were characterized dependent on the shape of the bacterium under the magnifying instrument.

The process of inoculation was carried out under laminar airflow utilizing the specially designed big tubes cylinder paper culture framework. In the first place, the filter paper with a length around 2/3 of the cylinder was put into the cylinder. Each cylinder was included a 90 mL low N supplement arrangement. The fixed cylinders were sterilized for 30 minutes under 121° C.

Salt tolerance of rhizobial isolates :

To decide the ideal salt focus for development, bacterial strains were cultured

in the YEMA medium enhanced with various amounts of NaCl: 2%, 3%, 4%, and 5% NaCl (w/v). The rate of growth and development of these isolates was determined with a spectrophotometer following 24, 48, 72 hours of the incubation period.

Germination of seeds :

The seeds of chickpea were first arranged to wipe out broken, little seeds and afterwards they were surface sterilized with a solution of 75 mL chloride + 25 mL water for 2–3 min, washed thoroughly with distilled water. Surface sterilized seeds were transferred on a piece of paper and soaked in 0.5 mM $CaSO_4$ and germinated in darkness for seven days in a dark room at 25^o C.

Growth of plants in pots :

For the inoculation of seeds, *Rhizobium* strains were grown in YEMA broth. One ml of bacterial culture was pelleted by centrifugation and cell pellets were washed with 1 ml phosphate buffer saline (PBS; 20 mM sodium phosphate, 150 mM NaCl, pH 7.4) and again suspended into PBS. The suspension utilized for the inoculation was changed to the final concentration of approximately 10^{-7} CFU mL^{-1}. Uniform seedlings were first put with sterile forceps into bacterial suspension for 15 minutes and were then transplanted into pots loaded up with salinated soil (500 g each pot). Two chickpea seedlings were transplanted into each pot; however, later one seedling was evacuated. Two treatments will be followed for the pot experiment: seeds without bacterial inoculation and seeds with bacterial inoculation. Plants were grown at 25-30° C during the day and 18-20° C at night and following a month and a half the shoot and root length and dry matter of chickpea were estimated.

Field analysis :

The examination was completed on a regular salinated soil, without chickpea developments in its history, at the field site of experimental station Morena, district of Madhya Pradesh. The examination was in a randomized total square structure. There were four treatments including three treatments with *Rhizobium* (inoculation with RB1, RB6 and RB9 and control without inoculation) and local chickpea genotype Phule-G-12313, three replications for each and with a total number of twelve plots. Each plot was 10 m^2. This analysis utilized the inoculants separated from the past tube study. These three rhizobial strains had the highest activity of nitrogenase and utilized peat and plant debris blend (1:1; w/w).

Note down that the chickpea genotype Phule-G-12313 was utilized in this analysis. The density of plantation was 60cm x 15cm. The control of pest, weeds, and irrigation were carried out on the conventional direction for chickpea. There were two harvests. The flowering stage was identified as the first stage of harvest. One plant for each plot was collected. The parameters analyzed were shoot biomass, N_2 content, the height of the plant, the pod number, the nodule number, and nodule dry weight. The subsequent harvest was at the development stage. The plants were harvested by their development time and were utilized to evaluate the yield based on the plot yield.

Soil compound and physical investigation:

The samples were air-dried and analyzed for the total amount of C, N, P, K, and soil humus. The distribution of soil particles was resolved to utilize natrium phosphate. The complete carbon content (C_{total}) was distinguished by basic examination while total nitrogen (N_{total}) content was estimated by the Kjeldahl technique.[15] The total phosphorus content (P_{total}) in the soil was observed by molybdenum blue strategy and potassium (K) was estimated by utilizing the Flame Photometric Method. The Atomic Absorption Spectrophotometer (AAS) was utilized to quantify calcium chloride ($CaCl_2$). The pH of soil was estimated by the soil testing kit.

Statistical analysis :

The outcomes are mean±SD from three investigations performed in triplicate. Correlation of qualities between various bacterial strains in similar conditions was performed utilizing a single direction ANOVA to discover factually huge contrasts. In situations where the null hypothesis (all populations implies are equivalent) was rejected at the alpha=0.05 level. Significant differences between the tested strains were considered at the degree of p values <0.05.

The production of chickpea in Morena is good on the grounds but there are such huge numbers of elements in soil restricting chickpea development, for example, drought, salinity and suitable rhizobial strains. Leguminous plants could fix N_2 organically, yet simply after they form root nodules with *Rhizobium*. The symbiotic interaction of *Rhizobium*-legume has received a great attention as it is broadly sent in agricultural practices for the sustainability of crop yield and regeneration of fertility of the soil.

The utilization of specific rhizobial strains that stimulate the growth of plants permits a significant decrease in the utilization of agrochemicals which are currently being utilized for stimulation of growth of plants. This will decidedly influence the percent emergence of seedlings and further development in soils with a poor structure. Microorganisms in the rhizosphere respond to the numerous root exudates discharged by plant roots. Their positive interaction helps them in the uptake of nutrients in plants, additionally the adjustment of plants to unfavorable soil synthetic conditions and sensitivity to diseases. Soil valuable microorganisms have been examined intensively on account of their higher impact on farming productivity.

The present investigation report clearly shows that the length of root and shoot of the control and all other plants of chickpea treated with rhizobial isolates increased progressively with the time. The majority of chickpea plants were affected by diseases that may be seed or soil-borne caused by *Fusarium oxysporum, Rhizoctonia solani,* and *Botrytis cinerea*. Annual chickpea yield losses implicating Fusarium-wilt vary from 10-15%. *Rhizobium* also effectively controls various soil-borne plant pathogenic fungi such as *Fusarium oxysporum.*

Sixteen bacterial strains of *Rhizobium* species recently separated from root nodules of chickpea were screened for their salt resistance capacities. Many rhizobial strains

Table-1. The effect of Rhizobial strains on the root-shoot ratio and nodule number of chickpea grown under saline soil

Rhizobial Strains	Nodule Number	Shoot length (cm)	% increase	Root length (cm)	% increase	Total length (cm)	% increase	Shoot dry mass(g)	Root dry mass(g)
Control	4	17.5±.14		10.6±14		28.1		0.186	0.080
RB1	12	22.5±.85	25.57	12.0±.08	13.2	34.5	18.5	0.246	0.113
RB2	12	26.5±.87	51.42	14.8±.78	39.6	41.3	46.97	0.297	0.125
RB3	13	22.5±1.1	25.57	15.2±.02	43.39	37.7	34.16	0.274	0.120
RB4	15	26.6±.91	52.0	11.5±.83	8.49	38.1	35.58	0.286	0.122
RB5	14	21.2±1.2	21.14	11.6±.23	9.43	32.8	16.72	0.245	0.112
RB6	11	25.5±.44	45.7	15.5±.74	46.2	41.0	45.9	0.290	0.126
RB7	10	19.6±.17	12.0	14.4±.23	35.8	34.0	20.99	0.256	0.118
RB8	13	21.7±.85	24.0	10.9±.59	2.83	32.6	16.01	0.242	0.110
RB9	16	18.8±.36	7.43	13.2±.29	24.5	32.0	13.87	0.240	0.108
RB10	15	19.6±.23	12.0	14.8±.89	40.9	34.4	22.42	0.254	0.115
RB11	12	21.3±.57	21.7	16.2±.12	52.8	37.5	33.45	0.277	0.119
RB12	10	22.4±1.2	28.0	12.0±.89	14.28	34.4	22.42	0.255	0.117
RB13	14	19.7±.19	12.57	11.4±.76	7.54	31.1	10.67	0.235	0.98
RB14	10	25.3±.66	44.57	15.6±.12	47.16	40.9	45.55	0.289	0.127
RB15	11	26.2±.91	49.7	12.0±.12	13.2	38.2	35.94	0.266	0.118
RB16	13	23.1±.17	32.0	12.6±.34	18.86	35.7	27.04	0.252	0.121

Significantly different from the un-inoculated control at $p<0.05$

were ready to grow under 3% NaCl, and just 10 strains had the option to endure up to 5% NaCl. The outcomes indicated that the seed dormancy enforced by the salinity of soil (5% NaCl) was significantly reduced and the germination was advanced by selected rhizobial strains from 54 up to 90%. Those 16 strains were taken for additional examinations on their impact on the development and advantageous execution of chickpea under salt stress conditions. The consequences of the study demonstrated that salt-tolerant rhizobial strains stimulated root, shoot development and nodulation of chickpea influenced by salt stress are shown in Table-1.

The salt tolerant rhizobia had great symbiotic relationship with the variety of chickpea Phule-G-12313, which has been selected as salt tolerant variety.

Without inoculation, no root nodules were shaped in the tested chickpea genotype Phule-G-12313; with inoculation, the nodulation rates in all were 100%. The rhizobial strains RB3, RB9, and RB10 eased effectively

the reductive impact of salt stress on the level of germination (up to 70%) and growth of the seedling. Inoculation with rhizobial inoculants made numerous nodules framed, yet besides expanded chickpea shoot and root biomass. The shoot length expanded by 52%, root length by 9.3%, shoot dry mass by 34%, and root dry mass by 42% after inoculation with bacterial strain RB4. After a comparative analysis, all the bacterial strains show similar results. The progressively impressive outcome was discovered when inoculation of chickpea with *Rhizobium* strains RB9 and RB10, which essentially expanded shoot and root dry mass by 30-35% and 35-38% over the un-inoculated plants, separately. Inoculation altogether increased the pod number and pod weight as compared to the control plant (Table-2). The growth and development of chickpea rely upon the rhizobia affiliation and plant genotype which together impacting the harmonious performance.

The bacterial strains adapted to drought stress because of the symbiotic association in the root-nodules of chickpea plant and also relieve the decreased development and yield of chickpea forced by drought stress. The three best strains RB4, RB9, and RB10 demonstrated a high stimulatory impact on the root and shoot development of chickpea seedlings which further were utilized for field tests.

The colonization of root related valuable microorganisms in the rhizosphere is significant for their advantageous impact on plant development, particularly under pressure soil conditions[25]. It has been likewise seen that the survival of rhizobia in the plant root and soil is influenced by supplement insufficiency, salinity, dry season and acidity[20]. In a prior report, Lowendorf[16] announced that salinity repressed survival and expansion of Rhizobium species in the soil and rhizosphere and also to the contamination process. This examination on the survival of salt-tolerant RB4, RB9 and RB10 strains in the rhizosphere of chickpea developed under saline soil condition shows that screening for salt-tolerant rhizobial strains is fundamental to improve cooperative execution of chickpea under salt stress condition. They can enhance plant development, lighten salt stress and able to survive in the rhizosphere of the plant under extreme soil conditions.

Table-2. The salt-tolerant *Rhizobium* species and its impact on the chickpea under field conditions

Rhizobial Strains	Nodule Number	Nodule weight g/plant	Root dry weight g/plant	Shoot dry weight g/plant	Yield of chickpea dt/ha
Phule-G-12313 Control	12	16±.004	1.65±1.4	17.50±1.5	12.5±1.2
Phule-G-12313 RB4	28	56±.008	2.86±0.6	21.43±2.4	19.6±1.5
Phule-G-12313 RB9	26	42±.015	2.54±0.7	25.56±2.8	18.2±1.7
Phule-G-12313 RB10	38	49±.012	2.60±0.5	22.25±3.2	22.4±1.4

Significantly different from the un-inoculated control at $p<0.05$

A field test was completed by applying rhizobial inoculants RB4, RB9 and RB10, which indicated the most elevated nitrogenase activity on the salinated soil of district Morena. The results showed that the legumes inoculated with rhizobial culture form root nodules and improve the soil fertility which ultimately increases the number of microbes in the soil. Due to this process, the action of *Rhizobium* also improves which upgrade N_2 fixation and stimulate root growth, to increase the yield of leguminous plants. The inoculation impact on yield increment mostly relies upon the challenge for nodulation of *Rhizobium* and the efficiency of nitrogen fixation. To improve the effects of *Rhizobium* application, the key measure is to choose rhizobial strains with good adaptation to local soils, a challenge for efficient nitrogen fixation and nodulation.

It was also observed that un-inoculated seeds after germination gave lesser biological yield as compared to inoculated seeds. After this study, we can easily say that the inoculation of *Cicer arietinum* seeds with rhizobial strains was very significant in case of the production of nodules, length of root shoot ratio and germination of seeds. The inoculated seeds after germination give significantly higher root shoot length at a percentage of about 50% as compared to the un-inoculated control. Even though inoculation with *Rhizobium* could essentially advance chickpea development and increases pod number. In the meantime, inoculation with *Rhizobium* could fundamentally influence the reproductive development of chickpeas.

This may be contemplated that inoculation with *Rhizobium* could improve N_2 nourishment, advance vegetative growth and especially root development, also benefit root uptake from the soil in chickpea. The above results are closely related with the findings of Khalequzzaman and Hossain[14], Ali *et al.*,[1], who also reported that length of root shoot ratio increased in plants which are already inoculated with rhizobial culture as compared to the un-inoculated plants.

Deep penetrating and well-organized root system of chickpea and interaction of Rhizobium-legume cause to improve the physical and chemical properties of soil (Table 3 and 4). The soil bulk density diminished in the 0-30 cm layer from 0.02-0.05 g cm^{-3}, the quantity of water-stable totals increased from 2.5 to 3.6%, the permeability of soil increases from 2.2 to 14.5% and organic matter of soil (humus) also increased from 0.02-0.04%. Absolute nitrogen, carbon and phosphorous substance in the soil expanded for 0.023-0.035, 0.055-0.086 and 0.007-0.016 $g.kg^{-1}$, separately. The results show that inoculation of chickpea seeds with *Rhizobium* strains can impressively improve the physical and chemical properties of soil than that of control. These outcomes recommend inoculation of chickpea seed with *Rhizobium* microbes has the gainful impacts on both harvest creation and soil fruitfulness.

The all-out C, N and P fixations in soil under conventional culturing framework relied upon chickpea relationship with rhizobial strains (Table-3). The developing of chickpea genotypes without inoculation with *Rhizobium* had a huge impact on the fertility of soil as compared with *Rhizobium* inoculated ones. Inoculations of chickpea genotypes with

Rhizobium strains have prompted increment soil carbon, nitrogen, and phosphorous substance. Lower concentrations of N and P content under chickpea is grown without *Rhizobium* can rely upon the diminished microbial movement thus the decreased C contribution to the soil. Schimmel and Bennett[18] find N mineralization by soil microorganisms is the key occasion in the N cycle making mineral N bio-accessible, while plants just take-up mineral N. Changes in N elements in soils are firmly associated with modifying in microbial activities engaged with N cycle by biotic and abiotic factors[2]. It has been proved that accumulation of N by chickpea and with the symbiotic association of *Rhizobium* strains substantially affect the organic matter of soil yet besides on microbial exercises under stressed condition[10]. We have seen that soil supplements were expanded under chickpea developed in relationship with *Rhizobium* strains RB4, RB9, and RB10. It is most presumably identified with more prominent release of root exudates and accessibility of N and C substrates, because of legumes broad establishing root system[5], and the accessibility of mineral supplements in soil which are of impressive significance to increasing soil microbial populations[11]. Chickpea had a flexible ability to deliver more prominent root exudates and enhance the soil with nitrogen through the process of nitrogen-fixing[21].

Results obtained from the field analysis showed that isolated rhizobial strains could infect more and more chickpea genotypes with successful fixation of N and flexibility against salinity and drought conditions. The morphology and physiology of *Rhizobium* will vary from free-living conditions to the asteroid of nodules. In addition to fixing the atmospheric nitrogen through nodulation, it shares many characteristics with other PGPRs including hormone production and solubilization of organic and inorganic phosphate. Through plant-growth-promoting substances, it helps in root expansion, improves uptake of plant nutrients, protects plants from root diseases and most importantly improves biomass production of fast-growing at wasteland[9]. It is significant to generally apply the successful rhizobial inoculants in district Morena, altogether not exclusively to increase N nourishment, improve crop generation and boost soil N substance and fertility of the soil. But we have to decrease the number of chemical fertilizers, especially N fertilizers, at long last aim to improve the nature of environmental conditions.

Table-3. Chemical analysis of soil

Treatments with Rhizobial strains	C g kg^{-1}	N g. kg^{-1}	P g kg^{-1}
Before experiment	0.805±.0.01	0.055±0.011	0.158±0.020
Phule-G-12313 Control	0 .816±0.03	0.077±0.007	0.165±0.030
Phule-G-12313 RB4	0.856±0.02	0.088±0.012	0.174±0.020
Phule-G-12313 RB9	0.942±0.01	0.096±0.009	0.178±0.011
Phule-G-12313 RB10	0.940±0.03	0.089±0.006	0.176±0.010

Significantly different from the un-inoculated control at $p<0.05$

Table-4. Physical analysis of soil

Treatments with Rhizobial strains	Bulk density of soil g. cm^{-3}		Content of humus, %	
	0-30cm	30-50 cm	0-30 cm	30-50 cm
Before experiment	1.520±0.04	1.620±0.04	0.943±0.03	0.840±0.04
Phule-G-12313 Control	1.448±0.02	1.548±0.06	0.973±0.06	0.873±0.05
Phule-G-12313 RB4	1.442±0.05	1.542±0.04	0.978±0.07	0.872±0.07
Phule-G-12313 RB9	1.438±0.03	1.538±0.03	0.992±0.11	0.869±0.06
Phule-G-12313 RB10	1.443±0.06	1.543±0.02	1.003±0.07	0.874±0.08

Most fields of Morena have salinated soils (9999 ha). The increasing salinity of irrigated land is perceived as the major problem of agriculture in the particular district and the essential regions under harvest generation in the nation are influenced by various degrees of soil salinity.

The profusion of native *Rhizobium* in these regions is low because of the absence of cultivation of chickpea, the high temperature in summer and chilly climate in winter seasons. Inoculation with Rhizobium is a way to deal with nitrogen fixation and also increase nitrogen nutrition and advance yield in chickpea. In this manner, inoculation with effective rhizobial inoculants may be significant ways to deal with improve chickpea generation on salinated soils in Morena. This investigation uncovered that screening for salt-tolerant rhizobial strains is fundamental to improve advantageous symbiosis of chickpea under salt pressure conditions just as improve soil richness. The use of inoculation procedures with rhizobial inoculants in leguminous plant generation has extraordinaryly affordable, natural and environmental advantages. Moreover, the examination shows the capability of phytohormone delivering strains RB4, RB9 and RB10 as a promising possibility for the advancement of bio-fertilizer alongside nodulating strains to get a supportable yield of chickpea with least contributions at negligible land.

The above results are closely related to the findings of Ali *et al.*,[1], who also reported that length of root shoot ratio increased in plants which are already inoculated with rhizobium culture as compared to the un-inoculated plants. Yadegari *et al.*,[24] reported an increase in the number of root nodules and also plant yield after inoculation. Rashid *et al.*,[17] reported the increase in several root nodules by the application of bio-fertilizer in combination with nitrogen fertilizer. Khanam *et al.*, and Bhuiyan *et al.*,[4] reported an increase in the number of nodules and seed yield of the rhizobial inoculation. Fatima *et al.*,[7] stated that the effect of plant growth was highly significant with an increase in the length of both root shoots.

Our present study was concluded that the chickpea seeds were well grown in Rhizobium based bio-fertilizer as compared to control. The essential nutrients present in the

bio-fertilizer are available in sufficient amounts that are easily available to plants and eco-friendly. This bio-fertilizer can be recommended as an effective fertilizer which in turn boosts the productivity of chickpea. The *Rhizo-bium* based bio-fertilizer will increase the efficiency of root and shoot systems by providing the essential nutrients for growth and development. This process could be adopted in chickpea growing areas to enhance the rate of growth and productivity in the said fields and also help in reducing fertilizers which are harmful to us and also to our environment.

Additional information:

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