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Effects of Inoculation of *Sinorhizobium ciceri* and Phosphate Solubilizing Bacteria on Nodulation, Yield and Nitrogen and Phosphorus Uptake of Chickpea (*Cicer arietinum* L.) in Shoa Robit Area

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Abstract

A field experiment was conducted during the 2006/07 growing season to assess the effects of inoculation of *Sinorhizobium ciceri* and phosphate solubilizing bacteria on the performance of chickpea variety" DZ-10-11" in Shoa Robit area, Ethiopia. Three levels of NP fertilizer and four levels of inoculants were used for the experiment. Treatments were laid down in a Randomized Complete Block Design (RCBD) in a factorial combination with three replications. The result of this study revealed that inoculation of *Sinorhizobium ciceri* alone increased dry matter yield by 156.58% and nodule number by 117.96% over the control whilst the addition of 18/20 kg NP ha^{-1} as urea and DCB resulted in 149.6% increase of dry matter yield and 143.6% increase in nodule number per plant over the uninoculated control. There was also a marked increase in nodule dry weight (200%), as a result of *Sinorhizobium ciceri*+ 18/20 kg NP ha-1 as urea and DCB, indicating the importance of phosphorus for nodule tissue development. Similarly inoculation of *Pseudomonas sp.*+ 18/20 kg NP ha^{-1} as urea and DCB also increased nodule dry weight, nodule number, nodule volume and seed yield by 240%, 188.52%, 151.81% 142.95% respectively over the control, indicating the efficiency of the bacteria in solubilizing phosphate in DCB. On the other hand inoculation of *Sinorhizobium ciceri*+ *Pseudomonas sp*. with 18/20 kg NP ha^{-1} as urea and DCB increased nodules number per plant by 208.8% and nodule dry weight by 220% and nodule volume by 221.24%, dry matter by 172.09% over uninoculated control at mid flowering stage of chickpea. Similarly inoculation of *Sinorhizobium ciceri*+*Pseudomonas sp*. With 18/20 kg NP ha-1 as urea and DAP increased nodule number, nodule dry weight, nodule volume and dry matter by 271.59%, 220%, 241.97%, 181.40% respectively over uninoculated control at mid flowering stage.

Introduction

Chickpea (*Cicer arietinum* L) belongs to the family *Fabaceae* (earlier Leguminoseae) and sub family papilonaceae [1]. It is most probably originated in an area of present day south-eastern Turkey and adjoining Syria where three mild annual species of *Cicer* viz *C. bijigum, C. aerhinosperum, and C. reticulatum* are found [2].

Chickpea is one of the most important cool season food legumes in Ethiopia and grown on heavy black soils (Vertisols). It is mainly cultivated between 1400-2300 m.a.s.l where annual rainfall ranges from 700-1200 mm. Chickpea, being a legume, can be used to restore fertility in crop rotation [3]. Hence, the farmers in Ethiopia commonly rotate chickpea with cereals such as wheat, barley and teff. Despite the above fact, chickpea yield in the country is extremely low. The national average yield is 0.8-0.9 t ha^{-1} [4], whereas at farmer's field the average yield is 0.6 t ha^{-1}.

Being a legume crop, chickpea can obtain a significant portion of its nitrogen requirement through symbiotic N_2 fixation when grown in association with effective and compatible *Rhizobium* strains [5]. Most Ethiopian soils, similar to the agricultural soils of other countries in the tropics, are generally low in nitrogen (N) and phosphorus (P). These two nutrients are often limiting the crop production in Ethiopia [6]. For pulse production, P is the major limiting nutrient followed by N. This is because P not only affects legume growth, but also nodule formation and development [7-9]. The phosphate solubilizing microorganisms have the capacity to dissolve the insoluble phosphatic compounds present in the soil and also solubilize rock phosphate, bone meal and basic slag [10,11]. The field experiments done on inoculation of P-solubilizing bacteria in various crops have shown 10-15% increases in crop yields in 10 out of 37 experiments [12].

Various authors reported increased yield responses of pulses to seed inoculation of *Rhizobium* [13-15] and phosphate-solubilizing bacteria (PSB) [16]. When inoculated, these organisms colonize the rhizosphere and enhance plant growth by providing it with N and P [17].

In Ethiopia, there is very little information on combined or dual inoculation of *Rhizobium* and PSB on crop productivity [18]. Hence, it is of great practical importance to study the combined effect of these organisms on nodulation, plant growth and nutrition and legume crop yields. Adoption of such technologies by farmers will help in minimizing production costs and at the same time, avoid the environmental hazards [19]. Therefore, in view of this, a field study on chickpea was carried out at farmers' field in Shoa Robit with the specific objective to study the effects of inoculation of *Sinorhizobium ciceri* and phosphate solubilizing bacteria and their interaction on nodulation, growth, yield, and nitrogen and phosphorus uptake of chickpea.

Material and Methods

Description of the study area: The study was conducted on a

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farmer's field under Merye Peasant Association around Shoa Robit, the capital of Kewet woreda located 11°55'N and 37°20'E at 1300 m.a.s.l, in North Shoa of the Amhara National Regional Sate. It is located at a distance of about 225 km to the North east of Addis Ababa on the way to Dessie. The Kewet woreda is classified under hot to warm moist agro-ecological zone. The annual rainfall, from meteorological records of last 14 years, is 1023.8 mm and the temperature ranges from 8°C to 37°C with a mean daily minimum and maximum temperatures of 16.6°C and 31°C [20]. The soil of the area is typically dark grey when dry and very dark grey-brown when moist with clay texture. The clay is montmorillonite, so the soils have high shrinkage capacity when dry and high swellings when wet for long time. The farmer's field, where the experiment was conducted, is located around 7 km away from Shoa Robit town.

There are two distinct growing seasons in the area viz., 'Belg' (March-July) and 'Meher' (August-December). The study was conducted during the Meher 2006/07 cropping season under rainfed condition with supplemental irrigation when required.

Experimental procedures

Soil sampling, preparation and analysis: Pre-sowing surface soil samples were collected at 0-30 cm diagonally from five spots in the experimental field, then were composited and processed for soil analysis before sowing. Composite soil samples were analysed for organic matter, total N by Kjeldahl digestion and distillation method; available soil P was extracted and analysed; soil texture, cation exchange capacity and soil pH were measured using standard laboratory procedures. At harvest, soil samples were collected at 0-30 cm from each plot and composited treatment-wise for determination of available P.

Treatments: Urea as a source of nitrogen and two sources of P namely, dried and crushed bone (DCB) and diammonium phosphate (DAP) were used for the field experiment. They were used in combination with inoculants as per the treatment. Lignite -based inoculants of *Sinorhizobium ciceri* (strain EAL 001) and local isolate of phosphate-solubilizing bacteria, *Pseudomonas sp*. singly or in combination and with or without N and P sources (urea, DCB and DAP) were used to get the treatments.

Experimental design: The experiment consisted of 12 treatments with a factorial combination of 3 levels of NP fertilizer (0/0, 18/20 as urea and DAP and 18/20 as urea and DCB kg ha^{-1}), and with four levels of inoculants (Uninoculated, *Sinorhizobium ciceri* (EAL 001), *Pseudomonas sp*. and *Sinorhizobium ciceri* (EAL 001)+*Pseudomonas sp*.). Treatments were laid down in a Randomized Complete Block Design (RCBD) with three replications making a total number of 36 plots. The full dose of N and P fertilizers were applied using row methods of application at planting time.

As per design of the experiment, field layout was prepared and each treatment was assigned randomly to experimental units within a block. The size of each plot was 2.8×4 m (11.2 m^2). The spacing between blocks and plots was 1.5 m and 0.5 m, respectively. The chickpea variety 'DZ-10-11' was used for planting. This variety was chosen on the basis of its resistance for ascochyta blight disease and better performance for many years in the mid altitudes areas of Ethiopia.

Seed inoculation: Seeds were inoculated with lignite-based inoculants of *Sinorhizobium ciceri* (EAL 001, Mojo isolate) and/or phosphate solubilizing bacteria, *Pseudomonas sp*. (Jimma isolate) at the rate of 7 g/kg of seeds as per the treatment. Carrier based cultures were mixed with small amount of 10% solution of sucrose in cool and clean water to form a thick slurry. The slurry was poured over the dry seeds so as to uniformly coat the seeds with the inoculant. For combined inoculation, inoculants of *Sinorhizobium ciceri* and phosphate solubilizers were mixed in equal proportions (7 g/kg seed) and applied to the seeds in the similar manner. All inoculations were done just before planting under shade to maintain the viability of microbial cells.

Sowing: The experiment was planted on September 28, 2006. Chickpea seeds of variety DZ-10-11 were sown in seven rows per plot at 40 cm row to row and 10 cm plant to plant distance.

Agronomic practices: The experimental field was weeded two times during the growing season. The first weeding was done 15-days after planting of chickpea to avoid competition during early stage of crop growth. The second weeding was undertaken one month later. Weeds, in general were not a serious constraints to chickpea. At about the poding stage of the crop, the insecticide Selectron was sprayed at the rate of 1.04 liter ha^{-1} to control ball worms.

Data collected

Nodulation: The data on nodulation parameters were taken at mid flowering stage of chickpea. Five competitive plants were randomly taken from second border rows from each side of the plot for nodulation parameters (number of nodules, nodule volume and nodule dry weight per plant) and dry weight of plants at mid flowering. In each plot, whole root system of a plant was completely exposed and carefully uprooted for nodulation parameters. The roots were gently washed under running tap water over a sieve to avoid loss of detached roots. The nodules from all the plants were removed and separately spread on the sieve for some minutes until the water drained off. The total number of nodules was counted and the mean value of five plants was recorded as the average number of nodules per plant. The color on inside of nodules was observed by cutting with the help of a sharp blade.

The collected nodules were immersed in a previously measured volume of water in a measuring cylinder. The volume of water displaced by nodules from 5 plants was considered as nodule volume and converted to average nodule volume per plant. After determination of nodule volume, the nodules were dried in an oven at 70°C to constant weight to determine nodule dry weight per plant. The average of five plants was taken as nodule dry weight per plant.

Dry matter: Dry matter of plants was determined at mid flowering stage of the crop from plants sampled for nodulation. The sampled plants were placed in labeled perforated paper bags and oven-dried at 70°C to a constant weight. The average dry weight of five plants was measured to determine dry weight per plant.

Yield and yield components: At physiological maturity, five competitive plants from net area were sampled for the determination of number of pods per plant. Number of seeds per pod was determined for 20 randomly sampled pods from the same five plants.

One hundred seed weight was also determined by counting 100 seeds and weighing on a sensitive electronic balance. Harvest index was determined as the ratio of grain yield with above ground dry biomass per plot. Yield per plot was determined by harvesting the chickpea from the central three rows of a net size of 1.2×3 m (3.6 m^2) leaving the boarder rows and 0.5 m row length on every end of each row. Chickpea plants were harvested from each plot at physiological maturity, the harvested plants was sun-dried in the open air for 3-4 weeks, weighed to determine above ground biomass yield and then threshed and weighed to determine the grain yield of each plot. Finally, yield per plot

was converted to per ha basis. Straw yield was calculated by subtracting grain yield from the corresponding above ground biomass yield.

Plant tissue sampling and analysis for N and P: At physiological maturity, five non-border plants were harvested and partitioned into grain and straw. The grain and straw sample materials was separately air-dried, oven dried at 70°C to a constant weight, ground to pass a 1 mm sieve and saved for laboratory analysis of grain and straw N and P concentration.

Phosphorus in grain and straw sub-samples was determined using metavanadate method. Samples were calcinated in the furnace overnight at 450°C and the ash was dissolved in 20% nitric acid (HNO_3) to liberate organic P. The phosphorus in the solution was determined colorimetrically using molybdate and metavanadate for color development. The reading of phosphorus was made at 460 nm in spectrophotometer. Total N in the grain and straw sub-samples were quantitatively determined by a Kjeldahl procedure that included a salicylic acid predigest ion step to convert nitrate to ammonium.

Phosphorus uptake by grain and straw were determined from the phosphorus content of the respective parts after multiplying with the grain yield and straw yield, respectively. Total phosphorus uptake was then calculated as the summation of grain and straw uptake. Similarly, N uptake in the grain was determined after multiplying nitrogen content of the grain by grain yield, and straw nitrogen uptake was determined by multiplying nitrogen content in the straw by straw yield. Total nitrogen was recorded as the sum of grain N uptake and straw N uptake.

Statistical analysis

All data collected were subjected to the analysis of variance (ANOVA) appropriate to factorial experiments in Randomized complete Block Design using SAS software (SAS Inistitute, 1989 [21]).

Results

Some selected physical and chemical properties of the soil of experimental site before planting

Results of the laboratory analysis (Table 1) for the soil sample taken before planting indicated that the textural class of the experimental soil was loam. It had high N, 1.26% while the available P content was 7.64 ppm, the P level of the soil can be rated as low.

The percent organic carbon content of the soil sample is low (1.74%). The organic matter content of the soil sample is computed by multiplying the organic carbon content with a conversion factor 1.724 and the result showed that medium organic matter content of the soil (2.99%). The pH value of soil is 8.3 (in 1:2.5 soil: water suspension) which according to Landon (1984) was rated as high and shows the cation exchange capacity of the soil was medium (29.57 cmol (+) kg^{-1}.

Particle size distribution (%)			Textural class	pH (1:2.5 H_2O)	OC (%)	TN (%)	AP (ppm)	CEC (Cmol (+)/kg)	PBS
Sand	Silt	Clay							
26	42	32	Loam	8.3	1.74	1.26	7.64	29.57	89

OC: Organic carbon; TN: Total nitrogen; AP: Available phosphorous; CEC: Cation exchange capacity; PBS: Percent base saturation

Table 1: Selected physical and chemical properties of the soil of experimental site before planting.

Number of nodules per plant

As presented in table 2, the analysis of variance test showed a significant statistical difference among treatments in relation to number of nodules per plant. The highest number of nodules (140.60 nodules $plant^{-1}$) were recorded with inoculation of *Sinorhizobium ciceri*+ *Pseudomonas sp*. along with 18/20 kg NP ha^{-1} as urea and DAP, followed by *Pseudomonas* sp.+18/20 kg NP ha^{-1} as urea and DAP (108.10 nodules $plant^{-1}$), *Sinorhizobium ciceri*+*Pseudomonas sp*.+18/20 kg NP ha^{-1} as urea and DCB (108.07 nodules $plant^{-1}$) and *Pseudomonas sp*. alone (104.93 nodules $plant^{-1}$) which were significantly higher than uninoculated control. Compared to the control all the treatments recorded higher nodule number per plant, however, inoculation of *Sinorhizobium* without urea+DCB/DAP and uninoculated treatment with urea and DCB/DAP gave nodules at par with the control.

Nodule dry weight

F-test indicated significant difference ($P \leq 0.01$) among treatments with respect to nodule dry weight per plant (Table 2). Maximum nodule dry weight (1.20g $plant^{-1}$) was recorded with inoculation of *Pseudomonas sp*. along with 18/20 kg NP ha^{-1} applied as urea and DCB and *Sinohizobium ciceri*+18/20 kg NP ha^{-1} as urea and DAP (1.20 g $plant^{-1}$) followed by *Pseudomonas sp*.+18/20 kg NP ha^{-1} as urea and DAP (1.1 g $plant^{-1}$) and (*Sinorhizobium ciceri* + *Pseudomonas sp*.)+18/20 kg NP ha^{-1} as urea and DAP/DCB.

Although inoculation of *Sinohizobium ciceri* alone and combined inoculation of *Sinorhizobium ciceri* and *Pseudomonas sp*. in the absence of P source resulted only in marginal increase in nodule dry weight over the untreated control, but inoculation of *Pseudomonas sp*. alone in the presence or absence of P source showed a considerable increase in nodule dry weight over uninoculated control. In general, the treatments having P solubilizers alone or with *Sinorhizobium ciceri* with phosphorous source recorded higher nodule dry weight as compared to treatments without P solubilizers (Table 2). Therefore, the results have indicated that due to increased availability of phosphorus from soil or DAP/DCB which resulted due to solubilization of P by inoculated phosphate solubilizers, the infection of roots by inoculated rhizobia increased and resulted in higher number and mass of nodules. Application of 18/20 kg NP ha^{-1} as urea and DAP/DCB, inoculation of *Sinorhizobium* cicer without N and P source and *Sinorhizobium ciceri* and phosphorous solublizing bacteria without N and P source were at par with the uninoculated control.

Nodule volume

Nodule volume was significantly ($P < 0.01$) affected by application of treatments (Table 2). The highest nodule volume (4.67 ml) was recorded with inoculation of (*Sinorhizobium ciceri*+*Pseudomonas sp*.)+18/20 kg N P ha^{-1} as urea and DAP followed by (*Sinorhizobium ciceri* + Pseudomonas)+18/20 kg N P ha^{-1} as urea and DCB (4.27ml) and *Pseudomonas sp*.+18/20 kg N P ha^{-1} as urea and DCB (2.93ml). However, all other treatments gave nodule volume at par with the control.

Effect on dry matter yield per plant at mid flowering

The dry matter production at mid flowering stage of chickpea was significantly ($P \leq 0.05$) influenced by different treatments (Table 2). Among the treatments, inoculation of (*Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp*.)+18/20 kg N P ha^{-1} as urea and DAP (7.8 g $plant^{-1}$) and (*Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp*.)+18/20 kg N P ha^{-1} as urea and DCB (7.4 g $plant^{-1}$) were significantly ($P \leq 0.05$)

Treatments	Number of nodule plant^{-1}	Nodule dry wt. plant^{-1}(g)	Nodulevol.plant^{-1} (ml)	Dry matter plant-1(g)
Uninoculated	51.77f	0.5d	1.93bc	4.30d
18/20 kg NP ha^{-1} as urea and DAP	64.67def	0.7dc	2.067bc	4.27d
18/20 kg NP ha^{-1} as urea and DCB	57.23f	0.7dc	2.43bc	5.93abcd
Sinorhizobium	61.07ef	0.8bcd	1.97bc	6.73abc
Sinohizobium+18/20 kg N P ha^{-1} as urea and DAP	90.13bcd	1.2a	2.60bc	5.33bcd
Sinorhizobium +18/20 kg N P ha^{-1} as urea and DCB	74.33cdef	1.0abc	1.53c	6.43abcd
Pseudomonas sp.	104.93b	1.0abc	2.47bc	5.07cd
Pseudomonas sp.+18/20 kg N P ha^{-1} as urea+DAP	108.10b	1.1ab	2.7bc	5.17bcd
Pseudomonas sp.+18/20kg NP ha^{-1} as urea and DCB	97.60bc	1.2a	2.93b	5.57abcd
Sinorhizobium +Pseudomonas sp.	84.33bcde	0.8bcd	2.53bc	4.20d
Sinorhizobium +Pseudomonas sp.+18/20 kg NP ha^{-1}as urea and DAP	140.60a	1.1ab	467a	7.80a
Sinorhizobium +Pseudomonas sp.+18/20 kg NP ha^{-1}as urea and DCB	108.07b	1.1ab	4.27a	7.40ab
LSD (5%)	27.02	0.3387	1.25	2.31
CV (%)	18.36	21.43	27.53	23.99

CV: Coefficient of variance, DCB: Dried and crushed bone, DAP: Diammonium phosphate, N: Nitrogen and P: Phosphorous. Means within a column followed by the same letter(s) are not significantly different.

Table 2: Nodulation of chickpea and dry matter yield plant^{-1} at mid flowering stage as affected by inoculation of *Sinorhizobium ciceri+Pseudomonas sp*.

Treatments	SY (kg/ha)	HSW (g/p)	Harvest index	Percent yield
Uninoculated	790.83f	12.67bc	0.4c	100
18/20 kg NP ha^{-1} as urea and DAP	1176.47bcd	12.83abc	0.5b	148.76
18/20 kgNP ha^{-1} as urea and DCB	1081.00d	13.40abc	0.5b	136.69
Sinorhizobium ciceri, EAL 001	879.63ef	14.73a	0.47b	111.22
Sinohizobium ciceri, EAL 001 +18/20 kg NP ha-1 as urea and DAP	790.73f	14.37abc	0.47b	99.98
Sinorhizobium ciceri, EAL 001 +18/20kg NP ha^{-1} as urea and DCB	941.10e	13.47abc	0.47b	119.00
Pseudomonas sp.	1113.00cd	14.60ab	0.5b	140.73
Pseudomonas sp.+18/20 kg NP ha^{-1} as urea+DAP	1209.43abc	12.90abc	0.5b	152.93
Pseudomonas sp.+18/20 kg NP ha^{-1} as urea and DCB	1130..47bcd	12.90abc	0.47b	142.94
Sinorhizobium ciceri, EAL 001+*Pseudomonas sp.*	856.63ef	12.40c	0.5b	108.32
Sinorhizobium ciceri, EAL 001+*Pseudomonas sp.*+18/20kg NP ha^{-1}as urea and DAP	1318.53a	13.03abc	0.57a	166.72
Sinorhizobium ciceri, EAL 001+*Pseudomonas sp.*+18/20 kg NP ha^{-1} as urea and DCB	1241.73ab	13.47abc	0.6a	157.02
LSD (5%)	118.81	1.98	0.07	-
CV (%)	6.72	8.75	7.80	-

Means within a column followed by the same letter(s) are not significantly different. NPP: Number of pods per plant; NSP: Number of seeds per pod; SY: Seed yield; HSW: Hundred seed weight; Nr: Number; AGW: Above ground weight; DCB: Dried and crushed bone; DAP: Diammonium phosphate; N: Nitrogen and P: Phosphorous

Table 3: Effects of inoculation of *Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp.* on number of pods per plant, number of seeds per pod, seed yield, percent yield increase and hundred-seed weight of chickpea.

0.05) increased dry matter yield per plant over the uninoculated control. The increase in dry matter yield due to combined inoculation of *Sinorhizobium* and phosphate solubilizing bacteria might be due to synergetic effect, which enhanced nitrogen and phosphorus availability to the plant. In agreement with the present finding, Application of 18/20 kg NP ha^{-1} as a source urea and DAP gave dry matter yield in par with the control.

Inoculation of *Sinorhizobium ciceri*, EAL 001 alone (6.73 g plant^{-1}) increased dry matter yield significantly over the control (4.3 g plant^{-1}). The increased dry matter yield due to inoculation of *Sinorhizobium ciceri*, EAL 001 alone could be the result of increased nitrogen fixation and its supply to chickpea, which enhanced crop growth.

Effect on number of pods per plant and seed per pod

The number of pods per plant was significantly different ($P \leq 0.01$) among the treatments (Table 3). All the treatments gave higher number of pods per plant over the control. The highest number of pods per plant (122.63) was recorded with inoculation of (*Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp.*)+18/20 kg NP ha^{-1} as urea and DAP followed by (*Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp.*)+18/20 kg NP ha^{-1} as urea and DCB (119.87) and *Pseudomonas sp.*+18/20 kg NP ha^{-1} as urea and DAP (106.80) as compared to the control.

Uninoculated treatment with 18/20 kg N P ha^{-1} as urea and DAP or DCB, *Sinorhizobium ciceri*, EAL 001+18/20 kg NP ha^{-1} as urea and DCB and *Pseudomonas sp* alone also gave a marginal increase in number of pods per plant over the control. However, other treatments were at par with the control.

Seeds per pod were not significantly ($P \geq 0.05$) affected by inoculation of *Sinorhizobium ciceri*, EAL 001 and *Pseudomonas sp*. with or without the P source.

Effect on seed yield and hundred-seed weight

Results (Table 3) showed that seed yield was significantly affected by application of different treatments. Inoculation of *Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp*. in the presence of 18/20 kg NP ha^{-1} as urea and DAP (1318.53 kg ha^{-1}) was superior to all other treatments followed by inoculation of *Pseudomonas sp*. and *Sinorhizobium ciceri*, EAL 001 in the presence of 18/20 kg NP ha^{-1} as urea and DCB (1241.73 kg ha^{-1}) and *Pseudomonas sp*. with 18/20 kg NP ha^{-1} as urea and DAP (1209.43 kg ha^{-1}). These three treatments showed significant ($P < 0.01$) increase in seed yield over the uninoculated control. Inoculation of phosphate solubilizing bacterial isolates alone with or without DCB/DAP as P source and uninoculated+18/20 kg NP ha^{-1} as urea and DAP

Treatments	Total N uptake (kg ha^{-1})	Total P uptake (kg P ha^{-1})	Olsen P (ppm)
Uninoculated	5.7	4.1	8.6
18/20 kg NP ha^{-1} as urea and DAP	7.2	4.8	9.5
18/20 kg NP ha^{-1} as urea and DCB	7.9	5.8	10.4
Sinorhizobium ciceri, EAL 001	6.5	4.2	10
Sinorhizobium ciceri, EAL 001+18/20 kg NP ha^{-1} as urea and DAP	9.1	6.3	9.2
Sinorhizobium ciceri, EAL 001+18/20 kg NP ha^{-1} as urea and DCB	6.0	6.3	10.4
Pseudomonas sp.	9.2	6.2	8.4
Pseudomonas sp.+18/20 kg NP ha^{-1} as urea andDAP	10.1	6.8	9.9
Pseudomonas sp.+18/20 kg NP ha^{-1} as urea and DCB	7.7	5.2	8.5
Sinorhizobium ciceri, EAL 001+*Pseudomonas sp.*	6.7	4.8	9.0
Sinorhizobium ciceri, EAL 001+*Pseudomonas sp.*+18/20 kg NP ha^{-1} as urea and DAP	7.9	5.4	11.5
Sinorhizobium ciceri, EAL 001+*Pseudomonas sp.*+18/20 kg NP ha^{-1} as urea and DCB	8.8	5.7	9.0

Table 4: Uptake of nitrogen, phosphorus and available phosphorus content of the soil after harvest as influenced by inoculation of Sino*Rhizobium* ciceri, EAL 001 and phosphate solubilizing bacteria.

or DCB also gave a marginal increase over the control, however, the remaining treatments are statistically at par each other and with the control.

As presented in table 3, inoculation of (*Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp*).+18/20 kg NP ha^{-1} as urea and with the cheap source of phosphorous as dried and crushed bone resulted 57.02% yield increase over the control. In general, the response to inoculation of phosphate solubilizing bacteria singly or in combination with *Sinorhizobium ciceri*, EAL 001 was found to be greater in the presence or absence of DCB and DAP as phosphorus source compared to inoculation of *Sinorhizobium ciceri*, EAL 001 with or without phosphorous source . Hundred-seed weight on the other hand was not significantly ($P \geq 0.05$) affected by any of the treatments (Table 3).

Harvest index

Harvest index was observed to be significantly ($P \leq 0.01$) affected by the treatments (Table 3). The highest value of harvest index (0.60) was recorded with inoculation of *Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp*. in the presence of 18/20 kg NP ha^{-1} as urea and DCB followed by *Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp*. in the presence of 18/20 kg NP ha^{-1} as urea and DAP (0.57). The other treatments were at par with each other, however, significantly different from the control.

Total nitrogen uptake

As presented in table 4, inoculation of *Pseudomonas sp*.+18/20 kg NP ha^{-1} as urea and DAP (10.1 kg N ha^{-1}) resulted the highest total nitrogen uptake of chickpea compared to the control and followed by inoculation of *Pseudomonas sp*. alone (9.2 kg N ha^{-1}) and inoculation of. *Sinorhizobium ciceri*, EAL 001 *Pseudomonas sp*.+18/20 kg NP ha^{-1} as urea and DCB (8.8 kg N ha^{-1}), and *Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp*.+18/20 kg NP ha^{-1} as urea and DAP (7.9 kg N ha^{-1}). Application of 18/20 kg NP ha^{-1} as urea and DCB also resulted in the total nitrogen uptake of Chickpea in similar manner with *Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp*.+18/20 kg NP ha^{-1} as urea and DAP. However, inoculation of *Sinorhizobium ciceri*, EAL 001 alone with 18/20 kg NP ha^{-1} as urea and DCB resulted in the total nitrogen uptake of chickpea at par with the control.

Total phosphorus uptake

Total phosphorus uptake was markedly affected due to various treatments (Table 4). The maximum total P uptake was observed due to inoculation of *Pseudomonas sp*.+18/20 kg N P ha^{-1} as urea and DAP (6.8 kg P ha^{-1})followed by *Sinorhizobium ciceri*, EAL 001+18/20 kg NP ha^{-1} as urea and DAP (6.3 kg P ha^{-1}) and *Pseudomonas sp*. alone (6.2 kg P ha^{-1}). Inoculation of *Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp*+18/20 kg NP ha^{-1} as urea and DAP or urea and DCB also resulted in a higher total phosphorus uptake as compared to the control. The increased total P uptake as a result of inoculation of *Sinorhizobium ciceri*, EAL 001 and *Pseudomonas sp*. could be due to increased availability of N and P which enhances crop growth.

Effect on available phosphorus at harvest

As presented in table 4, available phosphorus immediately after harvesting chickpea was found to be highest with inoculation of *Sinorhizobium ciceri*, EAL 001+*Pseudomonas sp*.+18/20 kg NP ha^{-1} as urea and DAP (11.5 ppm P) followed by *Sinorhizobium ciceri*, EAL 001+18/20 kg NP ha^{-1} as urea and DCB and application of 18/20 kg NP ha^{-1} as urea and DCB. (10.4 ppm P each).

Application of 18/20 kg N P ha^{-1}as urea and DAP (9.5 ppm P) resulted higher available phosphorous immediately after harvest as compared to control.

In all treatments the available phosphorus at harvest was higher than the soil P status (7.64 ppm) before planting. Inoculation of *Pseudomonas sp*. either singly or in combination with *Sinorhizobium ciceri*, EAL 001 in the presence or absence phosphorus source increased the available phosphorus immediately after crop harvest.

Discussion

Microbial processes such as biological nitrogen fixation, phosphate solubilization and cellulose degradation etc., could supplement the nutrient requirements of crops. The contributions of these microbial processes are enhanced by introducing efficient microbes in the rhizosphere. For instance, symbiotic nitrogen fixation rates could be markedly increased by introducing highly efficient, competitive and persistent strains of Rhizobia [22]. Similarly, besides solubilizing the native phosphorus sources in the soil, phosphate solubilizing microorganisms could increase the effectiveness of mineral P fertilization [23]. Inoculation of seeds or soil with efficient nitrogen fixing and phosphate solubilizing microorganisms change the rhizosphere population, consequently affecting plant growth.

The results of field study on the effects of inoculation of *Sinorhizobium ciceri*, EAL 001 and *Pseudomonas sp*. on chickpea in presence or absence of N P sources showed positive response for most of the parameters. The nodulation parameters such as nodule number per plant, nodule volume per plant and nodule dry weight were significantly affected by single or combined inoculation of *Sinorhizobium ciceri*, EAL 001 and *Pseudomonas sp*. on chickpea. It also increased seed yield (166.7%) compared to the control. This implies that interaction between the two

organisms, PSB and *Sinorhizobium ciceri*, EAL 001, benefited the crop in terms of growth and yield.

The response to inoculation was also more when DAP used in combination with *Pseudomonas sp*. and *Sinorhizobium ciceri*, EAL 001. The results indicated that the integrated use of chemical fertilizers and inoculants increase the growth, yield and yield parameters due to solubilization of fixed P in the soil. Therefore, the results of the study indicated that single or combined inoculation of *Sinorhizobium ciceri*, EAL 001 and *Pseudomonas sp*. on chickpea was beneficial for initiating formation of effective nodules under Shoa Robit soil conditions. The dry matter production at mid flowering, yield and yield components were significantly affected by single or combined inoculation of *Sinorhizobium ciceri*, EAL 001 and *Pseudomonas sp*. in the presence of N P sources on chickpea but, hundred seed weight, and seed per pod, were not affected by the treatments. Therefore, it can be concluded that use of effective inoculants is promising under Shoa Robit conditions in relation to growth and yield of chickpea. Total nitrogen uptake, total phosphorous uptake and available phosphorous at harvest increased due to the treatments.

In all treatments the available phosphorus at harvest was higher than the soil P status before planting (7.64 ppm). The increased in available phosphorus because of inoculation with phosphate solubilizing bacteria could be explained by solubilization of native phosphate by these organisms. Therefore the uses of inoculants made from effective strains will not only increase crop yield where fertilizer uses is negligible but it will also help in maintaining and enhancing soil fertility. However, the response to microbial inoculants is dependent on several soil factors including organic matter, temperature, moisture, aeration and nutrient status of the soils. Therefore the results of present study need to be evaluated and re-confirmed by conducting extensive field trials under varying soil fertility conditions with different sources and rates of N P fertilizers in combination with *Sinorhizobium ciceri*, EAL 001 and *Pseudomonas sp*.

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References

1. Kupich FK (1977) The Delimitation of the Tribe *Viceae* (Leguminosae) and the Relationship of *Cicer arietinum* L. Botanical Journal of the Linnaean Society 74: 131-162.
2. Saxena MC, Singh KB (1987) The Chickpea CAB International, the International Center for Agricultural Research in the Dry Areas. Aleppo Syria.
3. Baldev B (1988) Cropping patterns in pulse crops (Eds) Oxford and IBH Publishing Co., Pvt. Ltd., New Delhi 513-517.
4. CSA (Centeral Statisical Authority) (2000) Time Series Data on Production and Yield of Major Crops. Statistical Bulletin, Addis Ababa 56: 11.
5. Kyei-Boahen ES, Slinkard AE, Walley FL (2002) Evaluation of Rhizobial Inoculation Methods for Chickpea. Agronomy Journal 94 : 851-859
6. Asgelil Dibabe (2000) Effect of fertilizer on the yield and nodulation pattern of Faba bean on a nitosol of Adet Northwestern Ethiopia. Ethiopian Journal of Natural Resources 2: 237-244.
7. Andrew CS, Jones RK (1978) The Phosphorus Nutrition of Tropical Forage Legumes. E.J. (Eds.). Mineral nutrition of legumes in tropical and subtropical soils, CSIRO, Brisbane 295-311.
8. Haque I, Nnadi LA, Saleem MMA (1986) Phosphorus Management with Special Reference to Forage Legumes in Sub-Saharan Africa (Eds.) Potentials of Forage Legumes in Farming Systems of Sub-Saharan Africa. ILCA, Addis Ababa, Ethiopia 100-119.
9. Uloro Y, Richter C (1999) Phosphorus Efficiency of Different Varieties of *Phaseolus vulgaris* L. and *Sorghum bicolor* (L.) moench on an Alfisol in the Eastern Ethiopian Highlands. Ethiopian Journal of Natural Resources 1: 187-200.
10. Kucey RMN, Tanzen HH, ME Legett (1989) Microbiologically Mediated Increases in Plant Available Phosphorus. Advances in Agronomy 42: 199-221.
11. Asfaw Hailemariam (1997) The Effect of Phosphorus Solubilizing Fungus on the Growth and Yield of Teff. Ethiopian Science and Technology Research Report. Addis Ababa, Ethiopia 16-21.
12. Tandon HL (1987) Phosphorous Research and Production in India. Fertilizer Development and Consultation Organization, New Delhi, 160.
13. Dorosinksy LM, Kadyrov AA (1975) Effect of inoculation on nitrogen fixation by chickpea, its crop and content of protein. Mikrobiologiya 44: 1103-1106.
14. Moawad H, Badr El-Din SMS, Khalfallah MA (1988) Field Performance of Rhizobial Inoculants for Some Important Legumes in Egypt. In: Beck, D.P. and L.A. Materon (Eds. 89). Proceedings of a Workshop on Biological Nitrogen Fixation on Mediterranean-Type Agriculture, April 14-17, 1986, ICARDA, Syria.
15. Beyene D (1988) Biological Nitrogen Fixation Research on Grain Legumes in Ethiopia: An Overview. Pp 73-78. In: Beck, D.P. and Materon, L.A. (Eds.). Proceedings of a Workshop on Biological Nitrogen Fixation on Mediterranean-Type Agriculture, April 14-17, 1986, ICARDA, Syria.
16. Gaur AC (1985) Phosphate-Solubilizing Microorganisms and Their Role in Plant Growth and Crop Yields. Proceedings of Soil Biology Symposium, Hissa-R, India 125-135.
17. Kundu BS, Gaur AC (1980) Establishment of Nitrogen Fixing and Phosphate Solubilizing Bacteria in the Rhizosphere and Their Effect on Yield and Nutrient Uptake of Wheat Crop. Plant and Soil 57: 223-230.
18. Haile W (1999) Isolation and Characterization of Phosphate solublizing bacteria from Some Ethiopian Soils and their Effect on the Growth of Faba Bean. M.Sc. Thesis, Addis Ababa University, Addis Ababa 65.
19. Galal YGM, El-Ghandour IA, El-Akel EA (2002) Stimulation of Wheat Growth and N Fixation through *Azospirillum* and *Rhizobium* Inoculation. A Field Trial with ^{15}N Techniques. Plant Nutrition 92: 666-667.
20. Kewet Wereda Bureau of Agriculture (1995) Annual Report on Agriculture and Rural Development 15.
21. SAS Institute (1989) SAS/STAT User's Guide. SAS Publ., Cary, NC.
22. Materon LA, Keatinge JDH, Beck DP, Yurtsever N, Altuntas S (1995) The Role of Rhizobial Biodiversity in Legume Crop Productivity in the West Asian Highlands. II. *Rhizobium leguminosarum*. Journal of Experimental Agriculture 31: 485-491
23. Mikanova O, Novakova J (2002) Evaluation of the P-Solubilizing Activity of Soil Microorganisms and its Sensitivity to Soluble Phosphate. Rostlinna Vyroba 48: 397-400.