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Co-inoculation with *Mesorhizobium ciceri* and PGPR can improve chickpea (*Cicer arietinum* L.) growth in rain-fed and irrigated conditions

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ABSTRACT
An experiment was conducted to evaluate the effect of co-inoculation with *Rhizobium* and plant growth promoting rhizobacteria (PGPR) on chickpea growth under rain-fed and irrigated conditions. Treatments included inoculation with PGPR, co-inoculation with two strain of *Mesorhizobium ciceri* (SWRI3 and SWRI17), co-inoculation with SWRI3+SWRI17, co-inoculation with PGPR+SWRI3+SWRI17 and application of 25 kg nitrogen ha^{-1} and no fertilizing as a control. Growth components analysis was estimated from growth curves fitted to the relationships between the measured variables and the temperature index measured based on growing degree days. Drought stress due to rainfed condition, caused significant reduction in crop growth parameters; however, bio-fertilizer improved growth significantly. The maximum LAI in the rainfed condition was 0.29 while co-inoculation with PGPR+SWRI3+SWRI17 increased it to 0.54. Although, bio-fertilizers increased the time chickpea plants needed to reach their maximum LAI. In the irrigated condition, the highest CGR, TDM and seed yieldirrigated conditionwere 0.42 g m^{-2} d^{-1}, 32.34g and 2335 kg ha^{-1} respectively and decreased to 0.23 g m^{-2} d^{-1}, 18.34g and 1438 kg ha^{-1} respectively in rain-fed condition that were also obtained from co-inoculation with all bio-fertilizers (PGPR+SWRI3+SWRI17) in both conditions. Overall, results suggest that using bio-fertilizer helps to alleviat drought stress in rainfed condition and could be recommended in semi-arid environment to maximize chickpea yield.
Keywords: Bio-fertilizer, Drought, Rhizobium, Seed yield and Total dry weight.

اثر تلقیح باکتری *Mesorhizobium ciceri* و PGPR بر بهبود رشد نخود (*Cicer arietinum* L.) در شرایط آبیاری و دیم

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چکیده
به‌منظور بررسی اثر تلقیح نخود با باکتری ریزوبیوم و ریزوباکتری‌های محرک رشد گیاه (PGPR) بر رشد در شرایط آبیاری و دیم آزمایشی به مرحله اجرا درآمد. تیمارهای آزمایش شامل شاهد (عدم کاربرد کود زیستی و شیمیایی)، تلقیح بذر با PGPR، تلقیح بذر با دو نژاد باکتری *Mesorhizobium ciceri* (SWRI13 و SWRI17)، تلقیح همزمان بذر با کودهای SWRI13+SWRI17، تلقیح همزمان بذر با کودهای PGPR+SWRI3+SWRI17 و مصرف ۲۵ کیلوگرم بر هکتار نیتروژن بود. تجزیه‌وتحلیل مؤلفه‌های رشد بر اساس منحنی رشد برازش داده‌شده با روابط بین پارامترهای اندازه‌گیری شده تخمین زده شد و شاخص دما بر پایه درجه روز رشد (GDD) اندازه‌گیری گردید. تنش خشکی ناشی از شرایط دیم، باعث کاهش معنی‌دار پارامترهای مرتبط با رشد شد؛ درحالی‌که کودهای زیستی به‌صورت معنی‌داری رشد را بهبود بخشیدند. در شرایط دیم حداکثر میزان LAI، ۰/۲۹ بود درحالی‌که تلقیح همزمان PGPR+SWRI3+SWRI17، شاخص سطح برگ را به ۰/۵۴ افزایش داد. هرچند کودهای زیستی زمان رسیدن گیاه نخود به حداکثر میزان LAI را افزایش دادند. در شرایط آبیاری، بالاترین میزان CGR، TDM و عملکرد دانه به ترتیب ۰/۴۲گرم بر مترمربع در روز، ۳۲/۳۴ گرم و ۲۳۳۵ کیلوگرم بر هکتار بود که این پارامترها در شرایط دیم به ترتیب به ۰/۲۳ گرم بر مترمربع در روز، ۱۸/۳۴ گرم و ۱۴۳۸ کیلوگرم بر هکتار کاهش یافت که در هر دو شرایط از تلقیح همزمان با کودهای زیستی (PGPR+SWRI3+SWRI17) به‌دست‌آمده بودند. به‌طورکلی، نتایج نشان داد که کاربرد کودهای زیستی به کاهش تنش خشکی در شرایط دیم کمک می‌کند و می‌تواند در مناطق نیمه‌خشک برای به دست آوردن بالاترین میزان عملکرد نخود توصیه شود.
واژه‌های کلیدی: خشکی، ریزوبیوم، عملکرد دانه، کود زیستی، وزن خشک کل

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Introduction

Pulse crops are the main sources of protein in several arid and semi-arid regions around the world and have a major role in the agricultural economy of these regions (Tuba-Bicer *et al*., 2004). Chickpea (*Cicer arietinum* L.) is one of the most important legume crops in both rainfed and irrigated cropping systems which are widely cultivated in arid and semi-arid areas (Singh, 1997). In 2013, the world's total harvested area of chickpea was 13.54 million ha, with a total production of 13.1 million tons. The average chickpea yield in Iran is 496 kg ha^{-1} and it is significantly low compared to the global average of 967 kg ha^{-1} (FAOSTAT, 2014). Many factors, such as lack of certified seed and poor crop management contribute to the low yield (Sabaghpour *et al*., 2006), but drought stress is also a major constraint to achieve higher yield in chickpea especially in rainfed fields (Leport *et al*., 1999). Drought stress can occur in both plant vegetative and reproductive growth stage. Due to its dependence on uncertain precipitation, rainfed cultivation is regarded as the most vulnerable agricultural endeavor. Since about 95 % of chickpea cultivated area in Iran is rainfed, the lack of rainfall during flowering, poding and seed filling is the main factor result in low productivity of chickpeas in Iran (Sabaghpour *et al*., 2006). Therefore, appropriate field management of rainfed areas such as fertilizing, could have great role in sustainable cropping and avoide yield loss. In a research conducted in a semi-arid region, PGPR positively affected growth and nutrient uptake of cotton and pea (Egamberdiyeva & Höflich, 2004).

Legumes can provide the required nitrogen through a symbiotic relationship with rhizobium bacteria.These bacteria are in the root nodules of legume plants that can convert atmospheric nitrogen into the usable form for plants (Sivaramaiah *et al*., 2007). In addition to rhizobium, there are also groups of soil free living bacteria that have beneficial effects on plant growth. These bacteria are called plant growth promoting rhizobacteria (PGPR). PGPR is capable to increase the growth and yield of crops through direct and indirect mechanisms. Direct mechanisms by PGPR increase plant growth through synthesis of growth stimulants by bacteria or enhance nutrient absorption. Indirect ways to increase crop yield by PGPR includes reduce or eliminate the harmful effects of pathogens, destruction and increase the liquidity of the nutrient and the production of plant hormones (Qureshi *et al*., 2009; Verma *et al*., 2010). Effects of these bacteria on growth and yield of various crops are examined. In a study on the effect of rhizobium inoculation on yield and protein content of 6 chickpea cultivars, the total number of rhizobium nodules per plant, seed weight, yield and seed protein content was increased significantly compared to control (Elsheikh & Hadi, 1999). Besides PGPR inherent capabilities, promoting plant growth and development could be also affected by the interaction with host plant and soil environment. Therefore, the efficacy of PGPR in rainfed is likely different in irrigated condition.

Quantitative analysis of plant growth is a method for explaining and interpreting the responses of plants to environmental conditions during its life. By these methods, a better understanding of how photosynthetic material transfer to different organs and its accumulation during the growing season by dry matter measuring can be obtained. *Classical* (or intervals) and *functional* approaches are two distinct methods which have been suggested for plant growth analysis. The problems with the interval method are computing growth analysis indices using two sampling dates and therefore assume linear growth between the two sampling dates and that sampling errors can have a relevant effect on the value of indices itself, so high number of replications are required. However, functional approach typically is more robust functional approach, even if some minor scale effect can be hidden because original values of crop data are substituted by their interpolated values. Through this approach, a function must be fit for root, shoot and leaf biomass and area over the time. Once a mathematical dynamic expression of an appropriate growth model is obtained, it is possible to calculate the instantaneous value of growth rate at any time (Anonymous, 2011) and derive different parameters and, most of the model parameters have a biological meaning. The purpose of this study was to assess the effect of seed inoculation with *rhizobium* bacteria and plant growth promoting rhizobacteria (PGPR) on chickpea yield and some growth indices under rainfed and irrigated conditions using functional growth analysis approach.

Materials and Methods

Field experiments

Field experiment was conducted at the research farm of the University of Zanjan, Zanjan, Iran (36° 41' N and 48° 29' E and altitude 1663 m) during the growing season of 2012. This region is characterized by a semi-arid cool climate, with 11°C annual mean temperature of and 293 mm mean precipitation for the past 30 years. Mean daily temperature and precipitation data recorded near the experimental area during the growing season are given in Table 1. Soil properties of the experimental site including pH, electrical conductivity (EC), organic matter content, total nitrogen (N), phosphorus (P) and potassium (K) were 7.6, 1.2 (dS/m), 1.75%, 0.2%, 8 mg/kg and 156 mg/kg, respectively. Chickpea (cv. Arman) was sown at 20 seeds m^{-2} density, at 0.50 m row spacing, on April 8, 2012. Each plot had five rows of 5 m with 2.5 m row spacing.

Table 1. Air temperature and precipitation at the experimental site during the chickpea growth in 2012.

Month	Air temperature(^{0}C)			Total precipitation(mm)
	Maximum	Minimum	Mean	
April	15.4	2.6	9.0	94.3
May	22.3	7.2	14.7	55
June	26.7	11.1	18.9	17.7
July	30.2	13.7	21.9	1.1
August	32.8	15.8	24.3	0.2

Experimental treatments

The experiment was arranged in a split plot based on randomized complete block design with four replications. Irrigated condition (rainfed and irrigated) was the main plots and different fertilizers were the subplots.

Fertilizer treatments included seed inoculation with *Mesorhizobium ciceri* strain SWRI3 and SWRI17, seed inoculation with PGPR, Co-inoculation with rhizobium strains SWRI3+SWRI17 and co-inoculation with all bio-fertilizers (PGPR+SWRI3+SWRI17). Nitrogen application at the rate of 25 kg ha^{-1} and no fertilizing were considered as control treatments.

Seed inoculation

The SWRI3 and SWRI17 bacteria were two strains of *Mesorhizobium ciceri*. Plant growth promoting rhizobacteria (PGPR) was a combination of *Azotobacter chroococcum* strain 12, *Azospirillum lipoferum* strain OF and *Pseudomonas flourescens* strain 169. The inoculant was content of 10^8 alive and active bacteria per ML of liquid. All bacteria used in this study were natural and native to the soils of Iran and isolated in the Soil and Water Research Institute (SWRI). To inoculation with the bio-fertilizers, seeds were soaked in inoculum formulated solution 12 hours before sowing. Before planting, seeds were placed in a clean cloth to dry and immediately were planted.

Sampling and data collection

(i) Growth indices

Destructive samplings for growth indices were carried out 14 days after emergence (DAE) and repeated weekly during the growing season. The growing degree days (GDD) accumulated after planting were calculated by the equation 1(McMaster & Wilhelm, 1997).

$$GDD = \sum [\{(T_{max} + T_{min})/2\} - T_{base}] \qquad \text{Eqn 1}$$

Where T_{max} and T_{min} are daily maximum and minimum air temperature, respectively, and T_{base} is the base temperature. A base temperature of 4.5◦C was considered for chickpea (Soltani *et al*., 2006).

At each sampling date, all chickpea plants from a 50-cm length of the three middle rows of each plot were harvested by cutting at the soil surface. Plants were then divided into leaf, stem and pod. The areas of green leaves were measured using leaf area meter (model: VM-900 E/K). All plant parts were oven-dried at 70 ºC for 48h until a constant weight was reached. These data was used to calculate crop growth rate (CGR), leaf area index (LAI), and total dry matter (TDM) accumulation. LAI and CGR were calculated by the following equations (Hunt, 1990):

$$LAI = \frac{LA}{GA} \qquad \text{Eqn 2}$$

$$CGR = \frac{W_2 - W_1}{GA(GDD_2 - GDD_1)} \qquad \text{Eqn 3}$$

Where LA is leaf area (cm^2) of sampled area, GA is sampled area (cm^2), W_1 is dry weight (g) per sampled area at a given sampling date, W_2 is dry weight (g) at the next consecutive sampling date, and GDD_1 and GDD_2 are growing degree days at a given and the next sampling date.

(ii) Chickpea yield

Chickpea plants were harvested manually at physiological maturity. The crop was hand clipped from a 3-m section of the centre of three rows in each plot and dried at 70 ºC to a constant weight.

Data analysis

To display the leaf growth (in term of leaf area index, LAI) through the growing season, Gaussian model (equation 4) was fitted to leaf area data:

$$LAI = LAI_{max} \exp\left(-0.5\left(\frac{T - T_{max}}{T_L}\right)^2 \right) \quad \text{Eqn 4}$$

Where LAI is the chickpea leaf area index, LAI_{max} is maximum leaf area index over the growing season, T is the GDD (independent variable), T_{max} represents the time to reach maximum LAI, and T_L is the transition time to linear LAI. To display the CGR through the growing season, 3 parameter version of the Gompertz growth equation (equation 5) (Gompertz, 1832) was fitted to the CGR data calculated for each treatment.

$$CGR = CGR_{max} \exp\left(-0.5\left(\frac{T - T_{max}}{T_L}\right)^2 \right) \quad \text{Eqn 5}$$

A logistic equation (equation 6) (Richards, 1959) was fitted to the TDM data measured in each treatment separately.

$$DM = \frac{DM_{max}}{1 + \exp(-b(t - t_m))} \quad \text{Eqn 6}$$

Where DM is the chickpea dry matter, DM_{max} is maximum dry matter over the growing season, b is a constant that determines the curvature of the growth pattern, t is the GDD (independent variable) and t_m is the inflection point at which the growth rate reaches its maximum value. At the GDD t_m, RGR is b/2. The dry matter at t_m is half of its maximum value, DM_{max}.

A non-linear regression fitting routine was applied for parameters estimation using the Levenverg-Marquardt algorithm by SigmaPlot 11.0 (SigmaPlot, 2008). Multiple initial values were used to ensure that the solution was global rather than local. Goodness of fit was based on root mean square error (RMSE):

$$\mathrm{RMSE} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)^2} \quad \text{Eqn 7}$$

The adjusted coefficient of determination (R^2_{adj}) as a measure of the observed variability explained by the model, where a larger value indicates a better fit:

$$R^2_{adj} = 1 - \frac{RMSE}{TotalMS} \quad \text{Eqn 8}$$

The difference between curves (e.g. LAI in rain-fed vs. irrigated condition) was tested statistically using F-test. Yield data were subjected to an analysis of variance using PROC GLM in SAS Software (Version 9.1, SAS Institute Inc., and Cary, NC). Before analyzing the data, the assumption of variance homogeneity was tested using residual plots and the Kolmogorov-Smirnov normality test. If the analysis of variance indicated significant differences, means were compared using a Fisher's Protected LSD (P≤0.05).

Results

Chickpea Leaf Area Index (LAI)

Parameter values of the Gaussian growth function fitted to LAI data of chickpea are given in Table 2. The equivalent visual illustration of the fitting is shown in Figure 1. The function described the dynamics of change in LAI of chickpea in all treatments with reasonable accuracy (Figure 1). The R^2 value range of these models was 0.70- 0.97. Comparison of the curve in control treatment (without fertilizer application) with curves in different fertilizer treatments by F-test showed that LAI significantly affected by fertilizer treatments in irrigated conditions (Table 3). Without fertilizer application, the maximum LAI was 0.36 and 0.29 in the irrigated and rainfed conditions, respectively, however, application of of 20 kg N ha^{-1}, seed inoculation with *M. ciceri* strain of SWRI3, *M. ciceri* strain SWRI17, PGPR, co-inoculation with rhizobium strains SWRI3+SWRI17 and PGPR+SWRI-3+SWRI-17increased LAI to 0.78, 0.92, 0.97, 0.99, 1.03 and 1.30 in the irrigated chickpea, respectively. In the rainfed condition, all treatments had LAI higher than control, except for N treatment. Inoculating with *M. ciceri* strain of SWRI3, *M. ciceri* strain SWRI17, PGPR, co-inoculation with rhizobium strains SWRI3+SWRI17 and PGPR+SWRI3+SWRI17, the maximum LAI reached to 0.32, 0.34, 0.39, 0.42 and 0.54, respectively (Figure 1 and Table 2).

Statistical comparison of LAI in the two different moisture conditions (rainfed vs. irrigated) is shown in Table 4. In general, the value of LAI at the given time of the season in the rainfed condition was significantly lower than irrigated condition (Figure 1). The highest LAI in the irrigated condition was 1.30 while under rainfed condition, it decreased to 0.54 (Figure 1).

Time needed to reach the maximum LAI (LAI_{max}, equation 4) was also different between treatments. Without fertilizer application, chickpea plants required 405 and 310 GDD to make their maximum LAI in irrigatied and rainfed condition, respectively. In general, time to maximum LAI increased with fertilizing (Table 3). For example, using PGPR+SWRI3+SWRI17 increased this time to 450 and 352 GDD in irrigation and rainfed condition, respectively.

Table 2. Chickpea LAI parameters affected by different fertilizers in irrigation and rainfed conditions (see Eqn 3). Values in the parentheses are standard errors.

Fertilizer levels	Condition	LAI_{max}	T_L	T_{max}	RMSE	R^2
Control	Irrigated	0.36(0.09)	176.02(55.04)	405.19(17.15)	0.010	0.94
	Rain-fed	0.29(0.09)	257.84(79.79)	310.73(18.45)	0.006	0.96
Urea (46%N)	Irrigated	0.78(0.13)	198.28(42.53)	454.71(40.00)	0.054	0.70
	Rain-fed	0.28(0.01)	236.26(19.63)	303.81(15.88)	0.006	0.96
SWRI3	Irrigated	0.92(0.08)	189.98(21.00)	447.78(20.13)	0.033	0.90
	Rain-fed	0.32(0.02)	222.62(20.03)	319.04(16.40)	0.008	0.95
SWRI17	Irrigated	0.97(0.08)	185.42(18.96)	445.82(18.34)	0.032	0.92
	Rain-fed	0.34(0.03)	217.85(24.49)	315.42(20.19)	0.011	0.92
PGPR	Irrigated	0.99(0.08)	186.83(19.56)	448.77(18.88)	0.034	0.91
	Rain-fed	0.39(0.03)	205.84(20.57)	340.65(17.53)	0.012	0.93
SWRI3+SWRI17	Irrigated	1.03(0.11)	191.65(24.89)	450.77(23.77)	0.043	0.87
	Rain-fed	0.42(0.02)	212.67(13.16)	345.11(11.10)	0.008	0.97
PGPR+SWRI3+SWRI17	Irrigated	1.30(0.13)	164.74(19.95)	450.08(19.99)	0.050	0.90
	Rain-fed	0.54(0.04)	198.58(16.91)	351.83(14.78)	0.014	0.95

Control: no chemical and biological fertilizer application, SWRI3 and SWRI17: seed inoculation with *Mesorhizobium ciceri* strain of SWRI3 and SWRI17 respectively and PGPR: combination of *Azotobacter chroococcum* strain 12, *Azospirillum lipoferum* strain OF and *Pseudomonas flourescens* strain 169.

Table 3. F-test (P-value) of the comparison of different growth curves (leaf area index, crop growth rate and total dry matter) of chickpea in irrigation vs. rainfed conditions.

Fertilizer levels	LAI	CGR	TDM
		P-value	
Control	0.0089	0.0099	0.0477
Urea (46%N)	0.0084	0.0001	0.0061
SWRI3	0.0001	0.0001	0.0028
SWRI17	0.0001	0.0009	0.0140
PGPR	0.0002	0.0003	0.0213
SWRI3+SWRI17	0.0007	0.0001	0.0058
PGPR+SWRI3+SWRI17	0.009	0.0003	0.0490

Control: no chemical and biological fertilizer application, SWRI3 and SWRI17: seed inoculation with *Mesorhizobium ciceri* strain of SWRI3 and SWRI17 respectively and PGPR: combination of *Azotobacter chroococcum* strain 12, *Azospirillum lipoferum* strain OF and *Pseudomonas flourescens* strain 169.

Table 4. F-test (P-value) of the comparison control with other fertilizers in irrigation vs. rainfed conditions.

Fertilizer levels	P-value					
	LAI		CGR		TDM	
	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed
Urea (46%N)	0.0481^{*}	0.6046^{ns}	0.0057^{**}	0.0499^{*}	0.0115^{*}	0.0002^{**}
SWRI3	0.0008**	0.10086^{ns}	0.0060^{**}	0.0484^{*}	0.0261^{*}	0.5894^{ns}
SWRI17	0.0005^{**}	0.07896^{ns}	0.0001^{**}	0.0010^{**}	0.0215^{*}	0.0023^{**}
PGPR	0.0004^{**}	0.01098^{*}	0.0016^{**}	0.0002^{**}	0.2600^{ns}	0.0121^{*}
SWRI3+SWRI17	0.0011^{**}	0.00029^{**}	0.0003^{**}	0.0001^{**}	0.0036^{**}	0.2171^{ns}
PGPR+SWRI3+SWRI17	0.0005^{**}	0.00018^{**}	0.0252^{*}	0.0001^{**}	0.0399*	0.0078^{**}

Control: no chemical and biological fertilizer application, SWRI3 and SWRI17: seed inoculation with *Mesorhizobium ciceri* strain of SWRI3 and SWRI17 respectively and PGPR: combination of *Azotobacter chroococcum* strain 12, *Azospirillum lipoferum* strain OF and *Pseudomonas flourescens* strain 169.

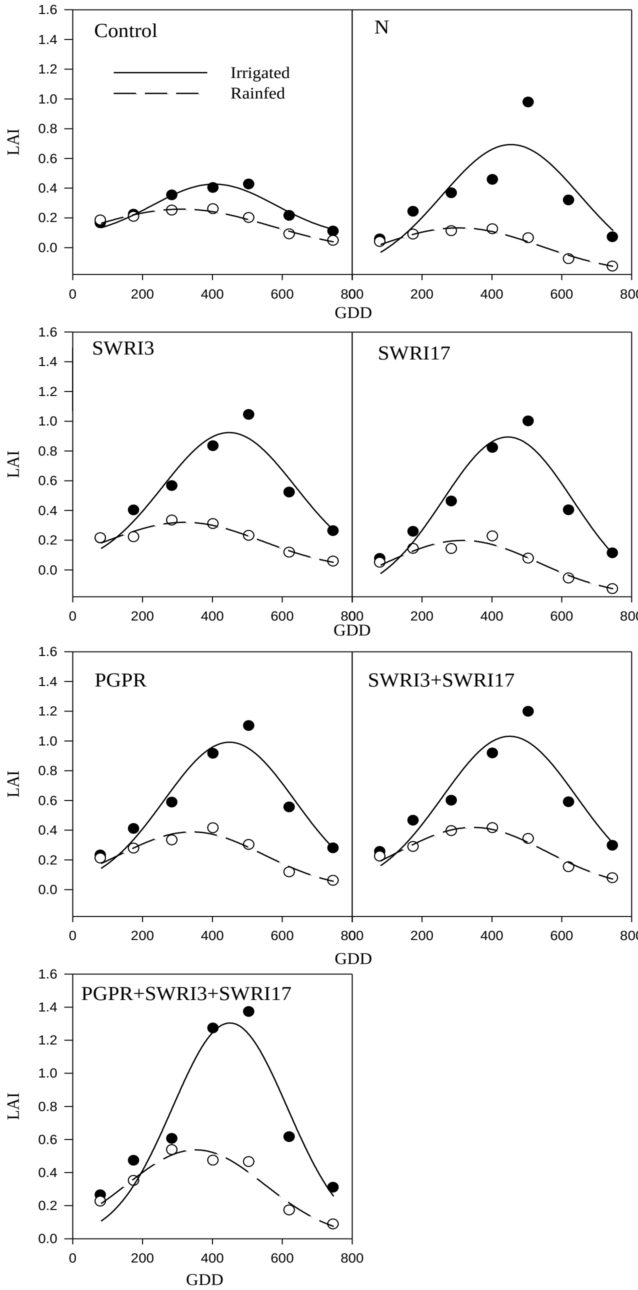

Figure 1. Observed (points) and predicted (lines) LAI_of chickpea affected by different fertilizers in irrigation and rainfed conditions. Parameter estimates are given in Table 2. Control: no chemical and biological fertilizer application, N: 46% N urea fertilizer (25 kg ha^{-1}), SWRI3 and SWRI17: seed inoculation with *Mesorhizobium ciceri* strain of SWRI3 and SWRI17 respectively and PGPR: combination of *Azotobacter chroococcum* strain 12, *Azospirillum lipoferum* strain OF and *Pseudomonas flourescens* strain 169.

Crop Growth Rate (CGR)

The dynamics of CGR during the season have been shown in the Figure 2 and stimated parameters were presented in Table 3. All growth functions properly described the dynamics of crop growth rate during the season (R^2 > 0.79), except for control treatment which has slightly low R^2 in irrigated condition (R^2=0.54). The drought related to rainfed condition, caused a significant reduction in crop growth rate in all treatments (Table 5). The maximum CGR in irrigated condition in control urea, SWRI3, SWRI17, PGPR, SWRI3+SWRI17 and PGPR+SWRI3+SWRI17 treatments were 0.13, 0.34, 0.39, 0.36, 0.39, 0.41 and 0.42 g m^{-2} d^{-1}, respectively. In rainfed condition, CGR in these treatments were 0.08, 0.19, 0.09, 0.14, 0.22, 0.19 and 0.23 g m^{-2} d^{-1}, respectively (Table 5). According to Table 4, CGR in all treatments were significantly different in both irrigated and rainfed conditions. The highest CGR (0.42 $gm^{-2}d^{-1}$) was observed in co-inoculation with all bio-fertilizers (PGP +SWRI SWRI17) treatment in irrigated condition which about 69% compared to the control (Table 5). In rainfed condition, the maximum CGR (0.23 $g\ m^{-2}\ d^{-1}$) was observed in the same treatment which was about 65 % higher than control (Table 5).

Total Dry Matter (TDM)

Parameter values of the logistic growth function fitted to the dry weight data of chickpea are given in Table 6. The equivalent visual illustration of the fitting is also shown in Figure 3. The function accurately described the dynamics of changes in dry weight of chickpea (R^2 > 0.81) with the exception for_PGPR treatment in irrigated condition.

In this study, there was a significant difference between total dry matter in irrigated and rainfed conditions (Table 6). The maximum TDM in control, urea, SWRI3, SWRI17, PGPR, SWRI3+SWRI17 and PGPR+SWRI3+SWRI17 treatments were 4.09, 13.80, 13.10, 15.23, 29.82, 22.29 and 32.34 $g\ m^{-2}$, respectively in irrigated condition, and 2.16, 3.09, 4.36, 4.56, 15.15, 11.75 and 18.34 $g\ m^{-2}$, respectively in rainfed condition (Table 6). There was also significant difference between control and other treatments, in irrigated and rainfed conditions (Table 4). Maximum TDM (32.34 $g\ m^{-2}$) was observed when irrigated chickpea plant treated with PGPR+SWRI3+SWRI17 (Table 6). Under rainfed condition, the highest total dry matter (18.4 $g\ m^{-2}$) was also found in PGPR+SWRI-3+SWRI-17 treatment.

Table 5. Parameters of chickpea CGR affected by different fertilizers in irrigatied and rainfed conditions (see eqution 5). Values in the parentheses are standard errors.

Fertilizer levels	Condition	CGR_{max}	T_L	T_{max}	RMSE	R^2
Control	Irrigated	0.13(0.03)	201.41(63.43)	470.33(59.21)	0.014	0.54
	Rain-fed	0.08(0.01)	158.36(21.88)	443.95(22.09)	0.004	0.88
Urea (46%N)	Irrigated	0.34(0.03)	107.65(11.29)	383.29(11.08)	0.010	0.95
	Rain-fed	0.19(0.01)	122.99(7.75)	375.84(7.59)	0.003	0.98
SWRI3	Irrigated	0.39(0.06)	92.76(15.26)	391.04(15.97)	0.043	0.87
	Rain-fed	0.09(0.01)	142.32(19.02)	401.91(19.06)	0.009	0.89
SWRI17	Irrigated	0.36(0.05)	124.51(19.68)	383.96(19.42)	0.042	0.86
	Rain-fed	0.14(0.01)	245.69(36.03)	423.08(29.00)	0.016	0.79
PGPR	Irrigated	0.39(0.05)	164.15(24.87)	413.36(24.66)	0.053	0.83
	Rain-fed	0.22(0.03)	141.05(25.60)	398.69(25.59)	0.032	0.80
SWRI3+SWRI17	Irrigated	0.41(0.04)	100.69(11.86)	363.59(11.16)	0.032	0.94
	Rain-fed	0.19(0.03)	110.61(18.37)	387.93(18.15)	0.022	0.87
PGPR+SWRI3+SWRI17	Irrigated	0.42(0.02)	140.88(14.12)	389.12(14.01)	0.021	0.94
	Rain-fed	0.23(0.02)	175.99(29.56)	388.97(28.21)	0.23	0.79

Control: no chemical and biological fertilizer application, N: 46% N urea fertilizer , SWRI3 and SWRI17: seed inoculation with *Mesorhizobium ciceri* strain of SWRI3 and SWRI17 respectively and PGPR: combination of *Azotobacter chroococcum* strain 12, *Azospirillum lipoferum* strain OF and *Pseudomonas flourescens* strain 169.

Table 6. Parameters of chickpea TDM affected by different fertilizers in irrigation and rainfed conditions (see euation 6). Values in the parentheses are standard errors.

Fertilizer levels	Condition	TDM_{max}	T_L	T_{max}	RMSE	R^2
Control	Irrigated	4.09(5.26)	306.03(291.43)	432.33(828.63)	0.315	0.81
	Rain-fed	2.16(0.38)	275.67(100.53)	444.09(1341.41)	0.241	0.98
Urea (46%N)	Irrigated	13.80(6.42)	56.24(12.51)	321.70(50.24)	0.236	0.99
	Rain-fed	3.09(0.89)	74.95(48.19)	412.06(70.75)	0.128	0.87
SWRI3++	Irrigated	13.10(10.51)	275.11(96.87)	376.45(414.17)	0.317	0.97
	Rain-fed	4.36(0.50)	231.28(84.14)	336.10(120.52)	0.117	0.95
SWRI17	Irrigated	15.23(0.40)	137.46(20.25)	362.64(27.82)	0.204	0.99
	Rain-fed	4.56(0.28)	150.55(50.09)	248.67(51.75)	0.172	0.94
PGPR	Irrigated	29.82(119.09)	86.83(84.36)	299.54(79.34)	0.460	0.61
	Rain-fed	15.15(62.26)	334.66(129.58)	340.21(1108.62)	0.313	0.97
SWRI3+SWRI17	Irrigated	22.29(2.04)	213.87(43.49)	354.81(107.52)	0.242	0.99
	Rain-fed	11.75(0.21)	121.43(39.16)	193.22(35.46)	0.186	0.94
PGPR+SWRI3+SWRI17	Irrigated	32.34(167.50)	377.17(262.38)	310.59(2763.01)	0.457	0.93
	Rain-fed	18.34(0.00)	317.17(0.00)	358.59(0.00)	0.457	1.00

Control: no chemical and biological fertilizer application, SWRI3 and SWRI17: seed inoculation with *Mesorhizobium ciceri* strain of SWRI3 and SWRI17 respectively and PGPR: combination of *Azotobacter chroococcum* strain 12, *Azospirillum lipoferum* strain OF and *Pseudomonas flourescens* strain 169.

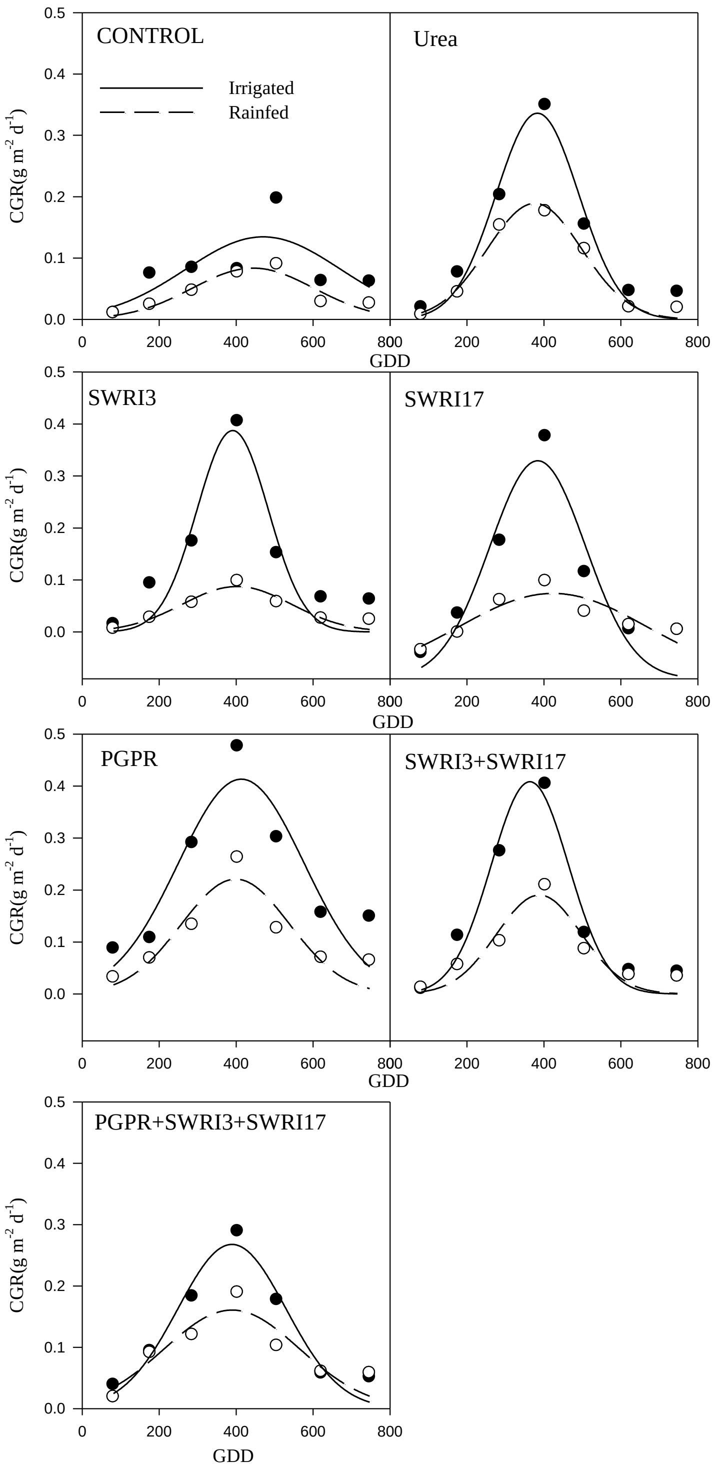

Figure 2. Observed (points) and predicted (lines) growth rate of chickpea affected by different fertilizers in irrigation and rainfed conditions. Parameter estimates are given in Table 4. Control: no chemical and biological fertilizer application, N: 46% N urea fertilizer (25 kg ha^{-1}), SWRI3 and SWRI17: seed inoculation with *Mesorhizobium ciceri* strain of SWRI3 and SWRI17 respectively and PGPR: combination of *Azotobacter chroococcum* strain 12, *Azospirillum lipoferum* strain OF and *Pseudomonas flourescens* strain 169.

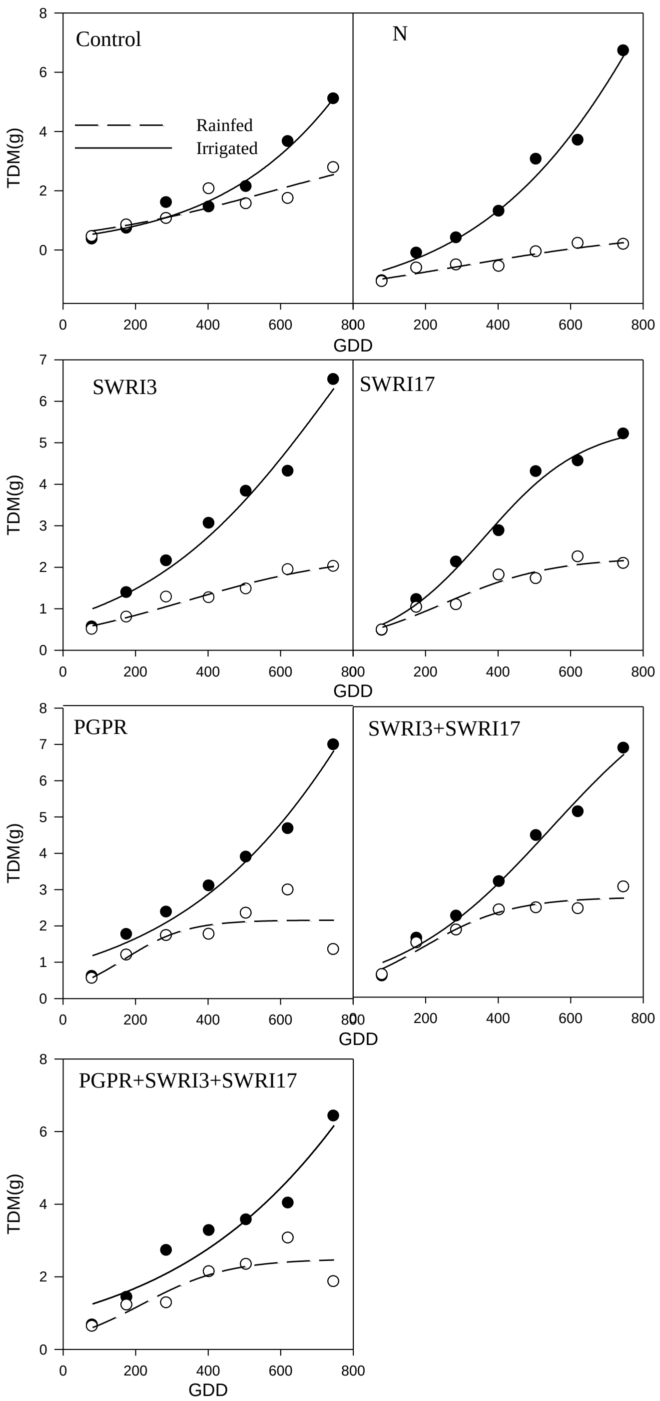

Figure 3. Observed (points) and predicted (lines) total dry weight of chickpea affected by different fertilizers in irrigation and rainfed conditions. Parameter estimates are given in Table 6. Control: no chemical and biological fertilizer application, N: 46% N urea fertilizer (25 kg ha^{-1}), SWRI3 and SWRI17: seed inoculation with *Mesorhizobium ciceri* strain of SWRI3 and SWRI17 respectively and PGPR: combination of *Azotobacter chroococcum* strain 12, *Azospirillum lipoferum* strain OF and *Pseudomonas flourescens* strain 169.

Seed yield: The effects of irrigation, fertilizers and interaction effects were significant on chickpea seed yield (P<0.05). Average seed yield in irrigation and rainfed conditions were 1723 and 857 kg ha^{-1}, respectively. The highest and lowest seed yield in irrigated condition was observed in PGPR+SWRI3+SWRI17 and control treatments, respectively (Figure 4). In rainfed condition, the highest and lowest seed yield was observed in PGPR+SWRI3+SWRI17 and control treatments, respectively (Figure 4).

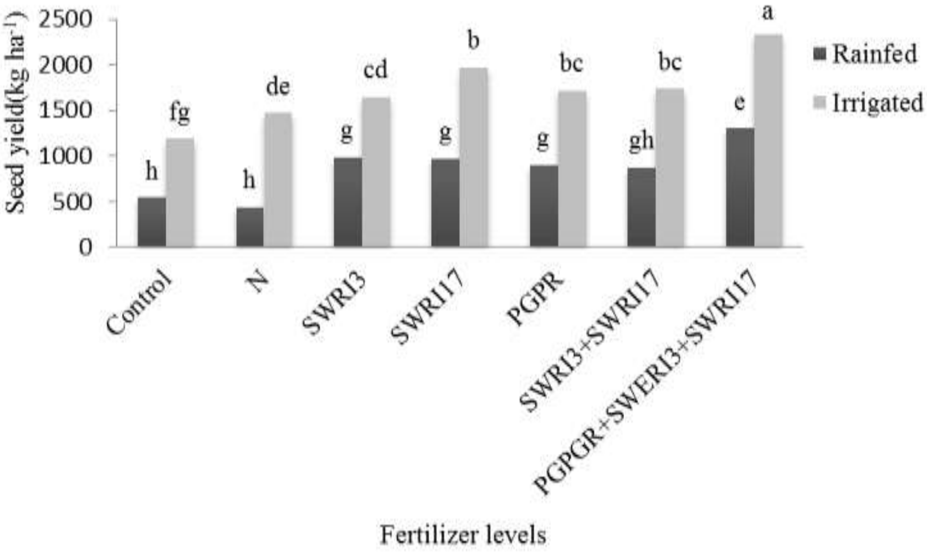

Figure 4. Chickpea seed yield affected by different fertilizers in irrigated and rainfed conditions. Means with the same letters are not significantly different at 5% of probability level.Control: no chemical and biological fertilizer application, N: 46% N urea fertilizer (25 kg ha^{-1}), SWRI3 and SWRI17: seed inoculation with *Mesorhizobium ciceri* strain of SWRI3 and SWRI17 respectively and PGPR: combination of *Azotobacter chroococcum* strain 12, *Azospirillum lipoferum* strain OF and *Pseudomonas flourescens* strain 169.

Discussion

Chickpea Leaf Area Index (LAI)

In this study, leaf area index reduced in all fertilizer treatments due to drought caused by rain-ed condition (Figure 1). Since photosynthetic material produced mainly by green leaves, every changes in plant leaf area had great influences on grain yield. High LAI would ensure faster crop growth rate and biomass production (Karimi and Siddique, 1991), therefore, the ability of crop plants to produce enough LAI is crucial for efficient PAR interception. Chickpea characterized as a low LAI crop plant and water deficit in rainfed condition could have additional negative effect on plant LAI; however, our results showed that bio-fertilizers could increase chickpea plants LAI significantly (Table 4). Among the treatments, co-inoculation with PGPR+SWRI3+SWRI17 showed the highest LAI (Table 2).

In chickpea as well as most legumes, leaf area expansion is slow especially in early growth stage; therefore, large amount of solar radiation wasted due to low leaf area. With bio-fertilizers application, the rate of leaf area expansion increased significantly and as a result, chickpea plant had more LA than control at the given time of the season in these plots. Additionally, expansion of plant growth period with increasing total PAR converted to carbohydrates can increase crop yield. In this study, bio-fertilizer applications increased time required to plant reach their maximum leaf area, compared to the control treatment. So, with bio-fertilizers application chickpea plants can form their canopy in shorter time and use the radiation more efficiently for longer time during growth season.

Chickpea Crop Growth Rate (CGR)

In this study, crop growth rate was reduced in all fertilizer treatments due to drought caused by rainfed condition (Figure 2). Reduction in crop growth rate began early in stressful conditions. Low turger pressure, stomatal closure, leaf area reduction and ultimately the loss of nutritious uptake in stress condition can decrease the CGR (Neumann, 1995; Pardo *et al*., 2000). In this study, in both rainfed and irrigated conditions, co-inoculation with all bio-fertilizers (PGPR+SWRI3+SWRI17) showed maximum crop growth rate (Table 5). Nanda *et al*. (1995) indicated that maize inoculation with bio-fertilizers (*Azotobacter* and *Azospirillum)* induced a significant increase in plant growth rate. They concluded that improvement in nutritious materials assimilation rate is the main reason for increasing the plant growth rate. Improvement of physical structure in soil and increases mineral materials and nitrogen for symbiotic plants with bio-fertilizers has been reported previously (Wu *et al*., 2005).

Chickpea Total Dry Matter (TDM)

Total dry matter (TDM) in plants decrease under drought stress (Acosta Gallegos & Shibata, 1989). In our study, a great decrease in dry matter accumulation was observed in rainfed condition (Figure 3). Since a large amount of solar radiation is not absorbed due to the low LAI, decrease in LAI could be main reason of low TDM in rainfed condition. Our result showed that bio-fertilizers application can alleviate rainfed condition. The highest TDM was observed from co-inoculation with all bio-fertilizers (PGPR+SWRI3+SWRI17) treatment in both irrigated and rainfed conditions (Table 6). Bashan *et al*. (2004) reported that inoculation of seeds with *Azospirillum* could result in significant changes in various growth parameters, such as increase in total plant biomass. The increase in dry matter accumulation with seed priming with PGPR and *Rhizobium leguminozarum* indicates the favorable response of chickpea to seed priming. Similar observations were also made by Lucas *et al*. (2004) in soybean, and Peix *et al*. (2001) in chickpea.

Chickpea Seed Yield

In rainfed condition, especially in arid and semi arid area, crop could not reach to their potential yield due to water deficit. In an experiment, results showed that the seed yield reduced from 2766 to 909 kg ha^{-1} because of rainfed condition. That was equal to 67%reduction (Onyari *et al*., 2003). Simillar result was seen for chickpea in this study; however, negative effect of water deficit was alleveated by bio-fertilizer applications. The use of organic fertilizers (either single or combination) produced more seed yield in both irrigatied and rainfed conditions compared to the control and N application. For example, co-inoculation with PGPR+SWRI3+SWRI17 increased seed yield 49 and 37% compared to control and N application respectively in irrigated condition. The respected values were 62 and 69% respectively in rainfed condition. It seems that increasing the amount of biological nitrogen fixation and nutrient uptake efficiency by rhizobium bio-fertilizers and increasing the availability of plant nutrients like nitrogen and phosphorus and enhancement of root growth by plant growth promoting rhizobacteria, increased seed yield in irrigated and rainfed conditions (Dashti *et al*., 1998; Sabaghpour *et al*., 2006).

Conclusion

In this experiment, the effect of seed inoculation with rhizobium and plant growth promotion rhizobacteria on chickpea growth parameters were studied at irrigation and rainfed conditions. Drought caused by rainfed condition caused a significant reduction in the studied parameters; however, negative effect of water deficit was alleviated with bio-fertilizers application. Among the studied treatments, seed co-inoculation with PGPR + SWRI3 + SWRI17 showed highest LAI, CGR, TDW and seed yield in both rainfed and irrigated conditions. Therefore, we can conclude that application of these bio-fertilizers can be recommended as cultural methods to improve chickpea yield in both rainfed and irrigated area.

REFERENCES

1. Acosta Gallegos, J. A & Shibata, J.K. (1989) .Effect of water stress on growth and yield of indeterminate dry-bean (*Phaseolus vulgaris* L.) cultivars. *Field Crop Research*, 20, 81-93.
2. Anonymous. (2011). http://www.diprove.unimi.it/groups/agro_rg3.htm. Accessed: October 10, 2015.
3. Bashan, Y. & Holguinde Bashan, L. E. (2004). Azospirillum- plant relationships: physiological, molecular, agricultural and environmental advances. *Canadian Journal of Microbiology*, 50, 521–577.
4. Dashti1, N., Zhang, F., Hynes, R. & Smith, D. L. (1998). Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean [*Glycine max* (L.) Merr.] under short season conditions. *Plant and Soil*, 200, 205–213.
5. Egamberdiyeva, D. & Höflich, G. (2004). Effect of plant growth-promoting bacteria on growth and nutrient uptake of cotton and pea in a semi-arid region of Uzbekistan. *Journal of Arid Environments,* 56, 293-301.
6. Elsheikh, E. A. E. & Hadi, E. A. E. l. (1999). Effect of Rhizobium inoculation and nitrogen fertilization on yield and protein content of six chickpea (*Cicer arietinum* L.) cultivars in marginal soils under irrigation. *Nutrient Cycling in Agroecosystems*, 54, 57–63.
7. FAOSTAT. (2014). Agriculture Data. http://faostat.fao.org/site/567/default.aspx#ancor. Accessed: May 12, 2015.

8. Gompertz, B. (1832). On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. *Philosophical* Transactions *of the* Royal Society *of London*, 123, 513-585.
9. Hunt, R. (1990). *Basic Growth Analysis*. Unwin-Hyman, London.
10. Leport, L., Turner, N. C., French, R. J., Barr, M. D., Dua, R., Davies, S. L., Tennant, D.& Siddique, K. H. M. (1999). Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. *European Journal of Agronomy*, 11, 279-291.
11. Lucas, J. A., Probanza1, A., Ramos, B., Barriuso, J. & Gutierrez Mañero, F. J. (2004). Effects of inoculation with plant growth promoting rhizobacteria (PGPRs) and Sinorhizobium fredii on biological nitrogen fixation, nodulation and growth of *Glycine max* cv. Osumi. *Plant and Soil*, 267,143–153.
12. McMaster, G. S. & Wilhelm, W. W. (1997). Growing degree-days: one equation, two interpretations. *Agricultural and forest meteorology*, 87, 291–300.
13. Nanda, S. S., Swain, K. C., Panda, S. C., Mohanty, A. K. & Alim, M. A. (1995). Effect of nitrogen and bio-fertilizers in fodder maize under rain-fed upland conditions of Orissa. *Current Agriculture Research*, 8, 45-47.
14. Neumann, P. M. (1995). The role of cell wall adjustment in plant resistance to water deficits. *Crop Science*, 35, 1258-1266.
15. Onyari, C. A. N., McKenzie, B. A. & Hill, G. H. (2003). The effect of irrigation and sowing date on crop yield and yield components of chickpea(*Cicer arietinum* L.) under semi-arid conditions in keniya. *Journal of applied bioscience,* 34, 2156-2165.
16. Pardo, A., Amato, M. & Chiaranda, F. Q. (2000). Relationships between soil structure, root distribution and water uptake of chickpea (*Cicer arietinum* L.) on plant growth and water distribution. *European Journal of Agronomy*, 13, 39-45.
17. Peix, A., Rivas-Boyero, A., Mateos, P. F., Rodriguez-Barrueco, C., MartõÂnez-Molina, E. & Velazquez, E. (2001). Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediterraneum* under growth chamber conditions. *Soil Biology & Biochemistry*, 33, 103-110.
18. Qureshi, M. A., Shakir, M. A., Naveed, M. & Ahmad, M. J. (2009). Growth and yield response of chickpea to co-inoculation with *Mesorhizobium ciceri* and *Bacillus megaterium*. *Journal of Animal & Plant Sciences*, 19, 205-211.
19. Richards, F. J. (1959). A flexible growth function for empirical use. *Journal of Experimental Botany*, 10 , 290-300.
20. Sabaghpour, H., Mahmoudi, A. A., Saeed, A., Kamel, M. & Malhotra, R. S. (2006). Study on chickpea drought tolerance lines under dryland condition of Iran. . *Indian Journal of Crop Science*, 1, 70-73.
21. SIGMAPLOT. [computer software]Sigmaplot for Windows, Release 11.0. (2008). Systat Software Inc.
22. Singh, K. B. (1997). Chickpea (*Cicer arietinum* L.). *Field Crop Research*, 53,161–170.
23. Sivaramaiah, N., Malik, D. K. & Sindhu, S. S. (2007). Improvement in symbiotic efficiency of chickpea (*Cicer arietinum*) by coinoculation of Bacillus strains with *Mesorhizobium* sp. *Cicer. Indian Journal of Microbiology*, 47, 51–56.
24. Soltani, A., Robertson, M. J., Mohammad-Nejad, Y. & Rahemi-Karizaki, A. (2006). Modeling chickpea growth and development: leaf production and senescence. *Field Crops Research*, 99, 14-23.
25. Tuba-Bicer, B., Narin-Kalender, A.& Sakar, D. (2004). The effect of irrigation on spring-sown Chickpea. *International journal of agricultural reaserch*, 3, 154-158.
26. Verma, J. P., Yadav, J., Tiwari, K., Lavakush, N. & Singh, V. (2010). Impact of plant growth rhizobacteria on crop production. *International journal of agricultural research*, 5, 954-983.
27. Wu, S. C., Caob, Z. H., Lib, Z. G., Cheunga, K. C. & Wong, M. H. (2005). Effects of bio-fertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma*, 125, 155–166.