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ORIGINAL ARTICLE

The Effect of deficit irrigation and *Azotobacter Chroococcum* and *Azospirillum brasilense* on grain yield, yield components of maize (S.C. 704) as a second cropping in western Iran

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ABSTRACT
In order to investigate the effect of deficit irrigation and bio-fertilizer on yield and its components of maize, an experiment was conducted as split plot in randomized complete bock design with three replications in Agricultural Research Center, Ilam, Iran in 2010-2011 growing season. Three levels of deficit irrigation (80, 100 and 120 evaporation (mm) from pan (Class A)) were assigned as main plots and bio-fertilizer (non-inoculation, inoculation with Azotobacter Chroococcum, Azospirillum brasilense and double-inoculation of Azotobacter and Azospirillum) were randomized in subplots. The effect of levels of deficit irrigation on plant height, number of rows per ear, number of grain per row, 1000-grain weight, grain yield, biological yield and protein content was significant. 80 mm had the highest plant height, number of grains per row, grain yield and biological yield compared with other treatments. The effect of bio-fertilizer on plant height, number of grain per row, 1000-grain weight, grain yield, biological yield and protein content was significant was significant. Dual inoculation with Azotobacter and Azospirillum had the highest plant height, number of rows per ear, number of grains per row, 1000-grain weight, grain yield, biological yield and protein content compare with other treatments. The interaction effect of deficit irrigation× bio-fertilizer on grain yield, biological yield and protein content was significant. The highest and lowest grain yield obtained from 80 mm and Dual inoculation with Azotobacter and Azospirillum and 120 mm and non inoculation treatment, respectively. It is concluded that bio-fertilizer can positively have effect on the increase of plant in maize plant, leading to plant tolerance improving under drought stress conditions.
***Key word**: Bio-fertilizer; Drought stress; Maize; Yield and yield components*
***Abbreviations:** PGPR-Plant growth promoting rhizobacteria; Bio-fertilizer-Biological fertilizer; P-Phosphorus, N-Nitrogen; S.C.704 - Single Cross 704*

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INTRODUCTION

Population and economic increasing rate resulted in broad demand for food at last two decades. To meet this demand might be difficult, because current fields are not accountable and yield loss clearly appears. Under these conditions, agriculture could not supply growing global requirement to food. Corn is one of the high yielding cereals that ranked as third cereal crop after wheat and rice to supply global population consumption [1]. Current estimates indicate that 25% of the world agricultural lands are affected by water stress. Iran, with an annual 240 mm of rainfall, is classified as a dry region of the word [2]. Water stress is considered principal environmental factor limiting growth and yield [3]. Thomas *et al.*, [4] found that mungbean plants in the rain shelter and rain fed treatments attained maturity earlier than the well-watered treatment. It has been showed that water deficit during the critical period of silking to early grain filling inhibited photosynthesis and consequently lowered the carbohydrate reserves to levels that were insufficient to support optimum reproductive development [5]. It was observed that shoot fresh and dry weight of two leguminous plants, *Phaseolus vulgaris* and *Sesbania aculeata,* decreased significantly due to water deficit. Also reduction in vegetative growth of plants under drought, in particular shoot growth, reduced cyclin-dependent kinase activity results in slower cell division as well as inhibition of growth [6-4-7-8].

PGPRs are microorganisms living in the rhizosphere of cultivated crops with known plant growth promotion effects. Their introduction into cropping systems could contribute to better crop productivity.

The effects of PGPRs on plant growth and productivity are either direct (e.g. biological N fixation, S oxidation or P solubilization, increasing nutrient availability) or indirect ''catalytic'' actions [9]. There are some evidence that plant growth and yield increase may be stimulated by plant growth promoting bacteria due to their ability of N2-fixing, phosphate solubilizing and production of plant growth hormones [10]. Bi-fertilizer with %50 of chemical fertilizers (N and P) led to an increase in plant growth, plant height, branch number, fresh and dry weight of safflower in comparison to applying chemical fertilizers alone, also utilization of *Azotobacter* bio-fertilizer, bio-phosphate fertilizer, organic fertilizers, with half rate of chemical fertilizer, increased grain yield of safflower [11]. Asghar *et al*., [12] indicated that Azospirillum, *Pseudomonas* and *Azotobacter* strains could affect seed germination and seedling growth. Yield responses of cereal to inoculation may also depend on plant genotype, bacterial strains and soil type as well as environmental conditions [13]. The positive effects of PGPRs on the yield and growth of crops such as wheat, maize, soybean and sugar beet were explained by N_2 fixation ability, P solubilizing capacity and phytohormons production [14-15-16-18]. Based on this the main objective the present study was assess effects of bio-fertilizer application that containing *Azotobacter*, *Azosprillum* on reduction of drought stress induced loss in maize S.C.704 in Agricultural Research Center, Ilam, Iran in 2010-2011 growing season.

MATERIALS AND METHOD

In order to study effect of deficit irrigation and PGPR on yield and yield components of maize (Sc704), an experiment was conducted in Agriculture Research Station (longitude 46 and 7° of north, latitude 33 and 7° of east with height of 155 m above sea level), Ilam, Iran in 2010-2011 cropping season. Three levels of irrigation (80, 100 and 120 evaporation (mm) from pan (Class A)) were assigned as main plots and bio-fertilizer (non-inoculation, inoculation with *Azotobacter Chroococcum*, *Azospirillum brasilense* and double-inoculation of *Azotobacter* and *Azospirillum*) were randomize in subplots. Experiment plots were seeded with S.C.704 cultivar with 75 cm row to row distance and 2 cm between plants. Maize was planted manually in August 2011. Seeds were sown 5 cm deep. Weeds were removed by hand. Before planting 7g inoculation with 1g had 10^7 active and live bacteria, were used inoculating seeds. Seeds which must mix with *Azotobacter*+ *Azospirillum*, soaked with sugar water with concentration 2% and with ratio 2kg inoculation 100kg seeds. After planting, irrigation was applied as required during the growing season. The maize was harvested in October 2011. For measurement of plant traits, two edge rows were eliminated as margin effects and one square meter of each plot was used for sampling.

Plots consisted of sex plant lines with row spacing 25cm and 4m long. N and P fertilizer used based on soil test and plants need and phosphorous fertilizer also used based on soil test (Table 1). Total N fertilizer was split equally at planting the beginning of tillerig stage and stem stage**.** After emergence and establishment of plants in 10-15 cm height stage, thinning was done to maintain a stronger plant and desirable density.

Table 1. Soil physical and chemical properties of experimental area

Soil texture	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Total N (%)	Organic Carbon (%)	E.C(dS/m)	pH
clay loam	1.09	220	0.08	0.66	1.09	7.05

Crop sampling and calculation

Agronomic traits including Number of row per ear, Number of grain per row, 1000-grain weight, grain yield, biological yield, plant height that were measured after of physiology maturity by selected 10 plants of each experimental plot randomly. Protein content was measured using Micro Kejeldahl digestion.

Statistical analysis

Data analysis was done by using SAS and MSTATC software. The ANOVA test was used to determine significant ($p \leq 0.05$) treatment effect and Duncan Multiple Range Test to determine significant difference between individual means.

RESULTS AND DISCUSSION

Plant height

According to date variance analysis, deficit irrigation had significant difference on plant height (Table 2). As Table 3 showed the highest and the lowest plant height was obtained by 80 and 120 evaporation, respectively. According to analysis of variance, bio-fertilizer had significant difference on plant height (Table 2). It is observed in Table 3 that dual inoculation with *Azotobacter* and *Azospirillum* caused to 21% increasing in plant height. main cause was increasing nutrients up taking by plant, improving soil

properties such as organic content and increasing available N in general, plant height is influenced by water and nutrients availability through increasing number of nodes middle nodes length. It has been reported that inoculation chickpea of with PGPR enhances stem height [19]. Mekki and Amel [20] also claimed that application of bio-fertilizer increases plant height and dry weight of soybean. Interaction effect between deficit and bio-fertilizer was not affect on plant height, but the highest and lowest plant height was obtained from 80 mm and inoculation with *Azotobacter* and *Azospirillum* and 120 mm and non-inoculation treatment, respectively (Fig 1).

Table 2 Analysis of variance for grain yield, yield components in deficit irrigation and bio-fertilizer

S.O.V.	df	Plant height	Number of row per ear	Number of grain per row	1000-grain weight	Grain yield	Biological yield	Protein content
Replication	2	2565	85.6	83.6	1743.6	1684713	6187669	0.1
Cultivar	2	*1214.1	* 29.5	*246.3	**4110.1	**3122396	**55212269	*6.9
Error a	4	112.2	9.2	30.1	115.1	1136169	1999546	0.9
Bio-fertilizer	3	**3045.3	*13.5	**239.5	**4010.2	**7161226	**20169812	**0.8
Interaction	6	^{ns} 112.5	^{ns} 4.3	^{ns} 9.1	^{ns} 99.9	**414441	**4111212	*0.01
Error b	18	55.3	3.3	8.4	79.1	41598	1556912	0.01
CV%	-	8.6	11.6	9.5	10.3	21.1	23.6	7.6

*: Significant at 0.05 level, **: Significant at 0.01 level

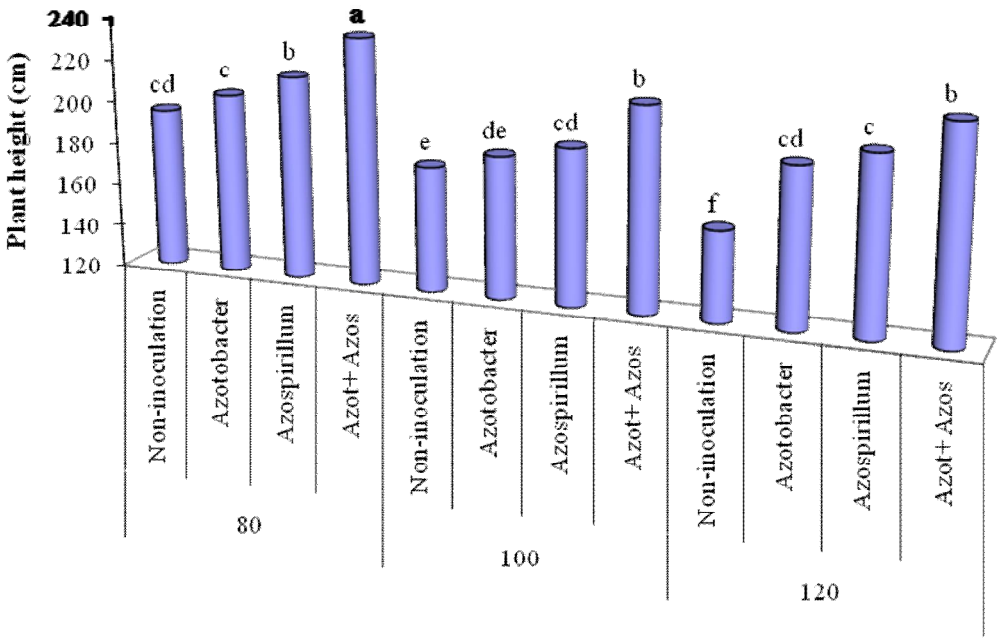

Fig. 1. Interaction effect of deficit irrigation and bio-fertilizer on Plant height

Number of row per ear

Number of row per ear was significant affected with deficit irrigation (Table 2). Mean comparisons indicated that the 80 and 120 mm evaporation had the highest and the lowest Number of row per ear, respectively (Table 3). Analysis of variance showed that Number of row per ear was affected by using bio-fertilizer on number of row per ear (Table 2). Mean comparisons indicated that the Dual inoculation with *Azotobacter+ Azospirillum* and non inoculation evaporation had the highest and the lowest number of row per ear, respectively (Table 3). Yasari and Patwardhan [21] also indicated positive effect of bio-fertilizers on number of pod per plant in rapeseed. Interaction effect between deficit and bio-fertilizer was not affect on number of row per ear, but the highest and lowest number of row per ear was obtained from 80 mm and inoculation with *Azotobacter* and *Azospirillum* and 120 mm and non-inoculation treatment, respectively (Fig 2).

Table 3. Mean comparisons of deficit irrigation and bio-fertilizer on grain yield and yield components

Treatment	Plant height (cm)	Number of row per ear	Number of grain per row	1000-grain weight (g)	Grain yield (kg.ha^{-1})	Biological yield (kg.ha^{-1})	Protein content (%)
Deficit irrigation (mm)							
80	a 211.9	a 19.6	a 50.5	b 282	11289.1 a	a 25123	b 10. 1
100	b 194.9	ab 18.6	ab 43.6	a 310	b 10925.3	b 22269	ab 11.09
120	186.1c	ab 17.7	b 40.1	a 314.3	b 96509	b 19998	a 11.9
Bio-fertilizer							
Non-inoculation	c 179.1	b 16.1	c 38.2	c 279.6	d 9100	b 19413	c 10
Azotobacter	b 195.9	ab 18.8	b 44.1	b 303.9	9750 b	ab 22360	b 10.5
Azospirillum	b 203.1	ab 18.9	b 43.1	b 302.9	c 9689	b 22326	b 10.6
Azot+ Azos	a 213.1	a 20.9	a 49.1	a 326.7	a 10960	a 24123	a 10.9

Means, in each column, followed by similar letter are not significantly different at the 5% probability level- using Duncan's Multiple Range Test.

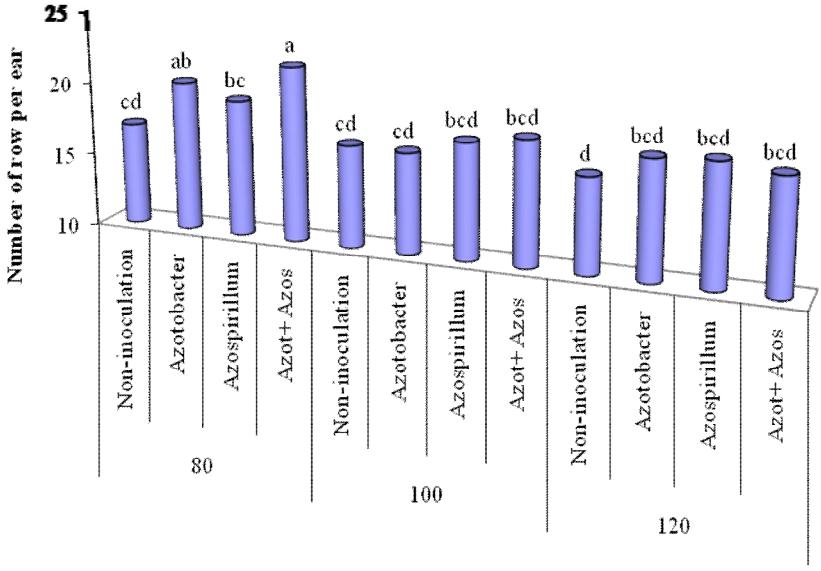

Fig. 2. Interaction effect of deficit irrigation and bio-fertilizer on Number of row per ear

Number of grain per row

Number of grain per row is on of the important components of maize. Deficit irrigation affected significantly number of grain per row (Table 2). Mean comparisons indicated that the highest and the lowest number of grain per row by 80 and 120 evaporation, respectively (Table 3). Monneveux *et al.*, [22] indicated that number of grains per row affected more reducing yielded in compared with drought stress. Zenselmeier *et al.,* [23] showed that, drought stress in flowering stage delayed tassels emergence. So tassels emerge when the pollination has done and no longer pollen has exists or reduced. Hence, no ovule, have fertilized and consequently no grain forms and this resulted in few grains formation at whole ear.

Results of this study showed that there is a significant difference by deficit irrigation on number of grain per row (Table 2). According to the results, Dual inoculation with *Azotobacter*+ *Azospirillum* and non inoculation had the highest and the lowest number of grain per row, respectively. Thus it can said that double- inoculation of *Azotobacter* and *Azospirillum* caused to increasing sink portion and as a result increasing maize yield by increasing number of grain per row. Bacteria, living within the root zone promote plant growth, it's reproductively and nutrient uptake by releasing auxins and gibberellins [24]. Interaction effect between deficit and bio-fertilizer was not affect on number of grain per row, but the highest and lowest number of grain per row was obtained from 80 mm and inoculation with *Azotobacter* and *Azospirillum* and 120 mm and non-inoculation treatment, respectively (Fig 3).

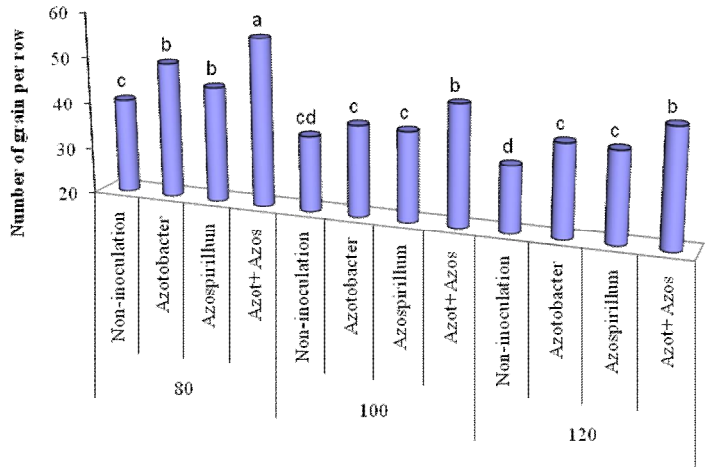

Fig. 3. Interaction effect of deficit irrigation and bio-fertilizer on Number of grain per row

1000-grain weight

Results of analysis of variance indicated that there was a significant difference by deficit irrigation weight on 1000-grain weight (Table 2). Mean comparisons indicated that the highest and the lowest harvest index by 80 and 120 evaporation, respectively (Table 3).

Thus it is seemed that water deficit would cause to reducing photosynthetic matters and decreasing leaf duration and producing less dry matter and as a result cause to fading grain and its weight. Campos *et al*., [25] reports showed that water deficit cause to decreasing stalk storage due to reducing photosynthesis content in maize and finally cause to reducing 1000-grains weight. Babaogli *et al*., [1] Also suggested that, drought stress raising could led to dehydration stages faced with grain filling and resulted in less transmission of photosynthetic compounds to grains, that it decreased 100 grain weight.

Analysis of variance showed that there was a significant difference by bio-fertilizer on 1000-grain weight (Table2). 1000 grain weight also increased by using application bio-fertilizer. The highest and the lowest grain yield was obtained by dual inculcation with *Azotobacter*+ *Azospirillum* and non-inoculation treatment, respectively (Table 3). This is indicated the in fact that dual inculcation with *Azotobacter*+ *Azospirillum* was more effective than alone inoculation of them. On of the increasing 1000 grain weight under double- inoculation treatment was due to improving attributes such as leave number, ear length and height that finally caused to increasing assimilates production. Also due attention to longer period of ripening maize due to double- inoculation of *Azotobacter* and *Azospirillum* it is possible to transforming more photosynthetic matter from source to and as aresult increasing 1000 grain weight. Combined application of deficit irrigation and bio-fertilizer highly influenced deficit irrigation under water deficit stress. Meshram and Shende [26] suggested that *Azospirillum* increases root surface area and thus promotes intake of N, P, K, other nutrients, and water and consequently above ground weight of plants. Interaction effect between deficit and bio-fertilizer was not affect on 1000-grain weight, but the highest and lowest 1000-grain weight was obtained from 80 mm and inoculation with *Azotobacter* and *Azospirillum* and 120 mm and non-inoculation treatment, respectively (Fig 4).

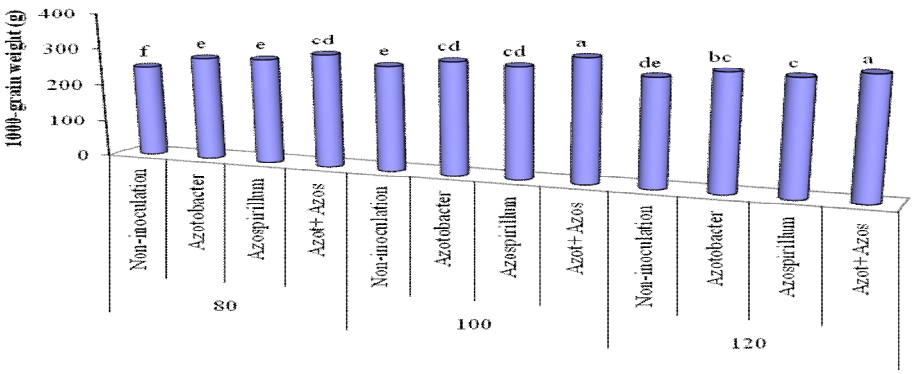

Fig. 4. Interaction effect of deficit irrigation and bio-fertilizer on 1000-grain weight

Grain yield

Analysis of variance showed that there was significant difference by deficit irrigation on grain yield (Table 2). As Table 3 showed the highest grain yield was obtained by 80 evaporation. Drought stress reduces plant growth and yield through reduced leaf surface area and rate of photosynthesis. Tolk *et al*., [27] concluded in a study on various soil type and different irrigation levels effect on maize that grain yield will decrease due to limiting receivable water from below soil layers. In general most attributes showed negative respond to water stress and grain yield affected by water stress more then other attributes due to sever decreasing number of grains per row ear length and 1000-grains weight. The present results agree with Thomas *et al.,* [4] who recorded that the yield was reduced by 25% when water was excluded during vegetative growth, whereas yield was reduced by 59% when water stress was imposed at flowering stage. Maleki *et al*., [28] also indicated that drought stress can reduce grain and yield components of soybean. Results obtained from analysis of variance indicated that there are significant differences by using bio-fertilizer on grain yield (Table 2). Mean comparison indicated that dual inoculation with *Azotobacter* and *Azospirillum* had the highest grain yield and non inoculation treatment had the lowest grain yield. Also it is showed in Table 3 that *Azotobacter* is more effective than *Azospirillum* on grain yield due to more role of *Azotobacter* in up taking N produced by biological fixing by this. Bacteria that finally will cause to more grain yield of plant. Interaction effect of deficit irrigation and bio-fertilizer was significant effect on grain yield (Table 2). As Fig 4 showed, the highest ant the lowest grain yield was obtained by 80 mm and dual inoculation with *Azotobacter* and *Azospirillum* and 120 mm *and* non inoculation respectively Fig 5). These observations indicated that addition of bio-fertilizer mitigated the harmful effect of water stress. Kloepper *et al*., [29] has been shown that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with Bacillus inoculation. Strains of *Pseudomonas* putida and *Pseudomonas* fluorescens could increase root and shoot elongation in rapeseed [30]. Also This finding is agreement with [31-32-33] they reported that bio-fertilizer caused increasing of grain yield due to improve of yield components in safflower, rapeseed and barley plant, respectively . It was also evident that bio-fertilizer are able to enhance plant growth of maize plants, leading to improved plant tolerance under drought stress.

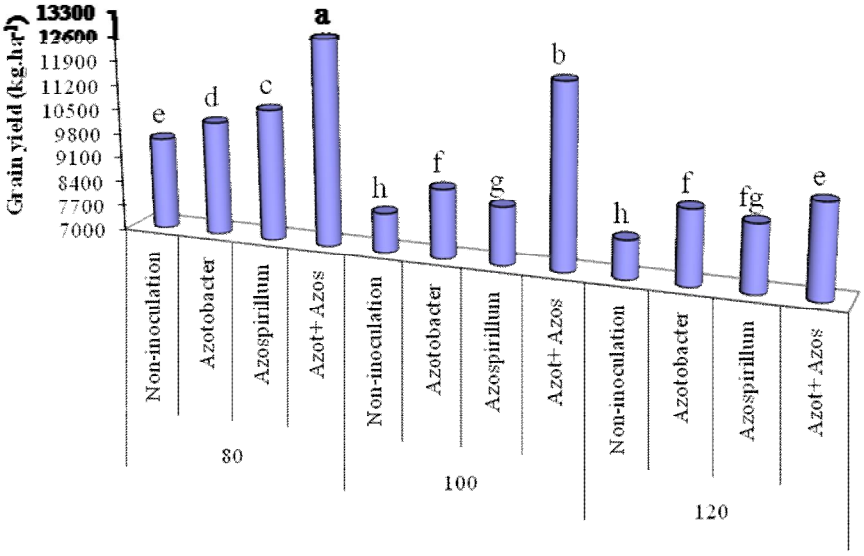

Fig. 5. Interaction effect of deficit irrigation and bio-fertilizer on Grain yield

Biological yield

Biological yield was significant affected by deficit irrigation (Table 2). Results related to biological yield of Table 3 showed that 80 and 100 mm had the highest and the lowest biological yield, respectively (Table 3). Cosculleola and Fact [34] observed that leaf water potential reduced increasingly and dry matter yield would reduce due to increasing water stress. Results of analyzing data variance also showed a significant different by application bio-fertilizer on biological yield. Bio-fertilizer also had a positive affection biological yield. Using bio-fertilizers resulted in improving this trait, but the highest and lowest biological yield was obtained by dual inoculation with *Azotobacter+ Azospirillum* and non inoculation, respectively (Table 3). It is seem that growth prompting bacteria caused to increasing biological yield

through affecting plant dry weight and allocating more dry matter to vegetative organs. Nanda *et al*., [35] reported that inoculating maize seed with *Azotobacter* and *Azospirillum* caused to increasing biological yield. This is explaining due attention to giberlines cause to increasing cells growth specially middle nodes and oxines cause to more cell division and as a result plant height, stem diameter and leave number per ear increase. This study has shown that use of bio-fertilizer could improve plant growth and biological yield of corn crops, and thus a reduction in the use of chemical fertilizer and the corresponding production cost of the crop. Interaction effect between deficit and bio-fertilizer was affect on biological yield, the highest and lowest biological yield was obtained from 80 mm and inoculation with *Azotobacter* and *Azospirillum* and 120 mm and non-inoculation treatment, respectively (Fig 6).

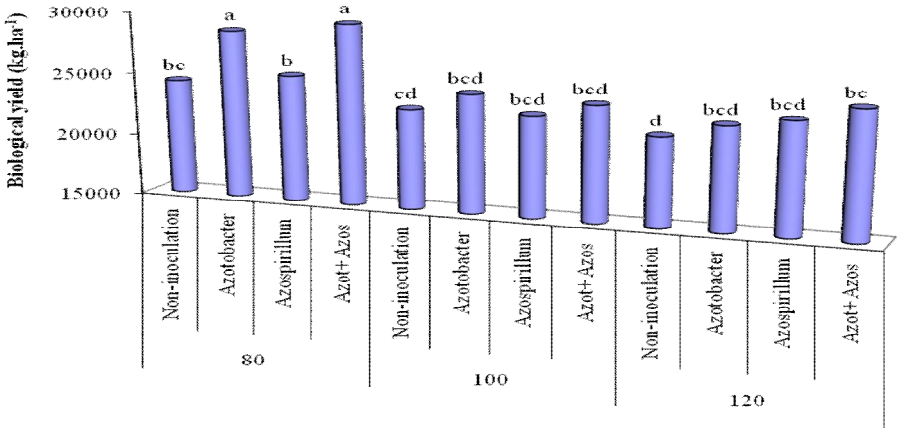

Fig. 6. Interaction effect of deficit irrigation and bio-fertilizer on Biological yield

Protein content

Protein content was affected by deficit irrigation (Table 2). Mean comparisons indicated that the highest and the lowest protein content was obtained by 120 and 80 mm respectively (Table 3). Protein content was affected by bio-fertilizer (Table 2). Mean comparisons indicated that the highest and the lowest protein content was obtained by Dual inoculation with *Azotobacter*+ *Azospirillum* and t non inoculation, respectively (Table 3). Since *Azotobacter* and *Azospirillum* are N fixing bacteria and N is basic matter to forming protein treatment.

Shehata and EL-Khawas [36] studied bio-fertilizers effect on growth parameter yield and yield components of sunflower and they found that usingbio-fertilizers including growth prompting bacteria improved the sunflower yield and qualitative parameters in compared with control (non inoculation) treatment and as a result caused to increasing protein content.

Interaction effect between deficit and bio-fertilizer was affect on protein content, the highest and lowest protein content was obtained from 80 mm and inoculation with *Azotobacter* and *Azospirillum* and 120 mm and non-inoculation treatment, respectively (Fig 7).

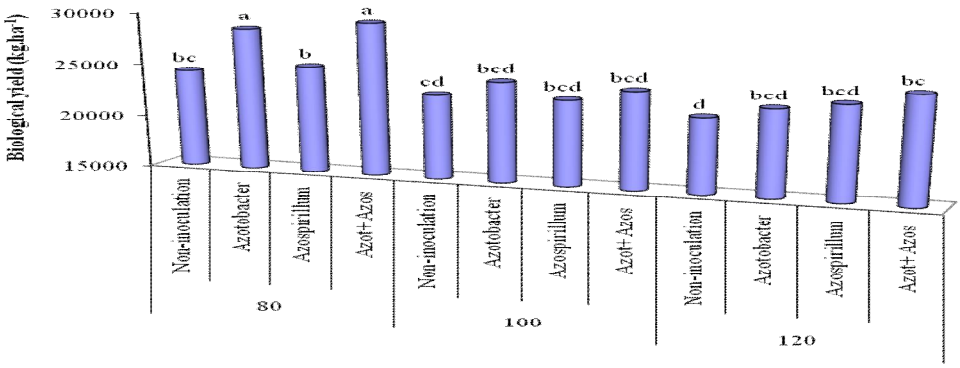

Fig. 6. Interaction effect of deficit irrigation and bio-fertilizer on Biological yield

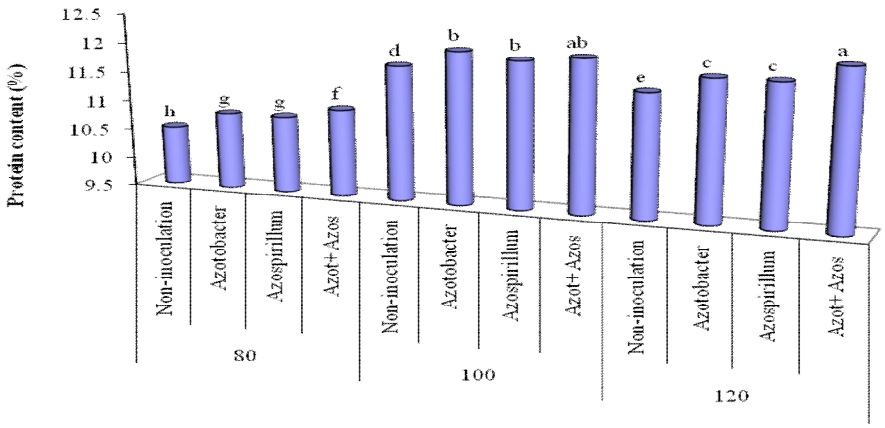

Fig. 7. Interaction effect of deficit irrigation and bio-fertilizer on protein content

CONCLUSION

Results of this study showed that drought stress caused decreases of yield and its component and measured indices such as number of row per ear, number of grain per row and 1000-grain weight, biological yield, plant height and protein content. Drought stress is a major abiotic constraint responsible for heavy production losses. Results of this study also showed that dual inoculation with *Azotobacter* and *Azospirillum* was most effective treatment in all deficit irrigation, caused to increasing yield and yield components of maize. This is indicated that using bio-fertilizer as a dual inoculation caused to increasing maize yield via synergistic effects by improving growth prompting hormones, controlling pathogenesis and growth reducing agents due to producing fungicide antibiotics and compounds (antagonistic effect) and also air mollocular N fixing and also producing growth prompting hormones such as oxine, cytokenine and giberlines and solving mineral compound. Also our study showed that chemical fertilizer combined with bio-fertilizer was beneficial to the environment because with decreasing the use of chemical fertilizer and use of organic inputs we can side with sustainable agriculture.

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