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Full Length Research Paper

Effect of irrigation disruption and biological nitrogen on growth and flower yield in *Calendula officinalis* L.

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To evaluate the effect of irrigation disruption and biological nitrogen on growth and yield of in *Calendula officinalis* L., an experiment was conducted as split plot at the research farm of Faculty of Agriculture of Urmia University (latitude 37.53°N, 45.08°E, and 1320 m above sea level), Urmia-Iran in 2010. Treatments including irrigation (irrigation disruption at first, second, third harvest and without disruption as control) as main plot and amount of biological nitrogen (0, 3, 6 and 9 L/ha of nitroxin) as sub plots were arranged in randomized complete block design, with three replications. Results show the significant effect of irrigation disruption on the stem weight and of biological nitrogen on the stem weight and capitulate diameter. Interaction effect between irrigation and biological nitrogen was significant on the leaf weight, stem length, the number of sub stem, biomass, and seed yield, and harvest index of seed. The highest biomass (1298.5 g/m^2) and seed yield (68.83 g/m^2) were obtained from irrigation disruption at second and third harvest with 6 and 9 L/ha of nitroxin application, respectively. In addition, the maximum (4.29 %) harvest index was obtained from irrigation disruption at third and second harvest with 9 L/ha of nitroxin application. In conclusion, lower amounts of nitrogen was needed to produced the optimal yield of seed in water deficit situation compared with non stress condition, while the nitrogen needed to produce the biomass was in higher amounts.

Key words: *Calendula officinalis*, capitulate, nitroxin, water stress, yield.

INTRODUCTION

Marigold (*Calendula officinalis* L.) belonging to the Asteraceae family and native to Mediterranean region, is an annual herb with pinnately divided leaves and flower that are used as a decorative plant in horticultural industry (Duke et al., 2002). However, it is considered as a medicinal plant (Beerentrup and Robbelen, 1987) and its flower is normally used in food industry to confer both color and flavor to foods (Blumenthal et al., 2000; Hamburger et al., 2003). Pot marigold is well known for its pharmacological effects such as anti-inflammatory, antiviral, anti-HIV, anti-tumor, anti-mutagenic and cytotoxic properties (Boucaud-Maitre et al., 1988; Amirghofran et al., 2000) and it is used mainly for treating cutaneous and internal disease (Re et al., 2009). The main chemical constituents of *C. officinalis* include terpenoids, phenolic acids, flavonoids, isorhamnetin, carotenoids, glycosides, vitamin C and sterols (Re et al., 2009; Andersen, 2001), which have antioxidant activities and play important role in human health (Meda et al., 2005).

In agriculture, the use of artificial fertilizers ensures better yields, but soils and the environment become more polluted and depleted of important nutrients (Kumar et al., 2001, 2008). Biofertilizers are inputs containing micro-organisms that are capable to mobilizing nutritive elements from non-usable form to usable form through biological processes (Tien et al., 1979). Symbiotic nitrogen fixation provides 80% of the biologically fixed nitrogen on land. Nitrogen-fixing bacteria are very selective in choosing roots of particular legumes to infect, invade and form root nodules (Subba, 1993). Drought is the most significant factor restricting plant growth and

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Table 1. Effect of irrigation disruption and biological nitroxin application on growth and flower yield in *Calendula officinalis* L.

Source of variation	df	Mean square (MS)				
		Stem weight	Leaf weight	Stem length	Stem diameter	Numbers of sub stem
Rep	2	25.71	0.3	17.97	0.007	0.35
irrigation disruption (A)	3	226.84**	17.38**	38.73*	0.01	20.44**
Error	6	9.01	1.54	25.65	0.007	0.83
Nitroxin (B)	3	59.88*	6.89**	38.98*	0.0023	30.90**
Irrigation × nitroxin	9	31.05	6**	86.64**	0.0067	9.92**
Error	24	15.8	1.05	13.16	0.0061	1.78
Coefficient of variance (%)		25.34	20.32	10.23	19.8	16.31

*, ** Significant at $P≤0.05$, $P≤0.01$, respectively; df, degree of freedom.

crop productivity in the majority of agricultural fields of the world (Tas and Tas, 2007; Abedi and Pakniyat, 2010). Stress imposed during the course of plant life time drastically affects their growth, ultimately leading to a massive loss in yield and quality (Govindarajan et al., 1996; Hudak and Patterson, 1996; Moreshet et al., 1996).

To the best of our knowledge, information about the response of *C. officinalis* to irrigation disruption and nitrogen supply, especially from biological source is scarce. Therefore, the main objective of the present study was to evaluate the response of *C. officinalis* to nitrogen fixing bacteria (*Azotobacter* and *Azospirillum*) under different water deficit stress.

MATERIALS AND METHODS

A field experiment was carried out as split plot arrangement based on randomized complete block design with three replications. Experiment was conducted at the Research Farm of Urmia University with latitude of 37.53°N, 45.08°E and 1320 m above sea level in 2010. Experimental units in each replication comprised of 10 line of 2 m long. Row to row and plant to plant spacing was 0.3 and 0.05 m, respectively. Treatments were irrigation disruption after first, second and third harvest as well as control in main plots, and amount of biological nitrogen in four levels including 0, 3, 6 and 9 L/ha of nitroxin (included *Azotobacter* and *Azospirillum*) in sub plots.

The following measurements were recorded at the flowering stage on five repetitive plants in each treatment per replication: stem length (cm), stem diameter (cm), number of sub stem, capitulate diameter (cm), stem weight (g), leaf weight (g) and capitulate weight (g). The dry weight was recorded after drying of samples in an oven at 70°C for 72 h. Seeds were separated from leaves and stems before all plant parts were oven-dried at 70°C. Harvest index was calculated by dividing the total dried seed weight to total above-ground plant dry weight. The calculation was carried out for each treatment in replicate at each harvest time.

Statistical analysis

Analysis of variance (ANOVA) on data was performed using the general linear model (GLM) procedure in the SAS software (SAS Institute Inc.). The Student-Neuman Keul's test (SNK) was applied to compare treatment means using the MSTATC software package.

RESULTS

Irrigation disruption showed significant effect on the stem weight ($P≤0.01$). Biological nitrogen showed significant effect on the stem weight and capitulate diameter ($P≤0.01$). Irrigation × biological nitrogen interaction effect was significant on leaf weight, stem length, numbers of sub stem, biological and seed yield ($P≤0.01$), and harvest index ($P≤0.05$) (Table1). Means comparison revealed that the maximum stem weight (19.35 g/p) was obtained from plants grown under irrigation disruption at second harvest. Mean value of stem weight of plants in irrigation disruption after second harvest treatment was in same group with the mean value of control plants. The minimum shoot weight (10.70 g/p) was observed in irrigation disruption after third harvest treatment (Figure 1). The maximum stem weight (18.25 g/p) was observed from 3 L/ha nitroxin application, and the minimum one (12.85 g/p) was obtained from control treatment, that is 0 kg/ha of nitroxin application (Figure 2A).

The maximum capitulate diameter (4.88 cm) was observed in 9 L/ha of nitroxin application and the minimum (3.74 cm) was observed in control plants without nitroxin application (Figure 2B). The highest weighed leaves (8.43 g/p) was observed from well-watered plants without irrigation disruption and 9 L/ha nitroxin application, while the lowest weighed leaves (2.23 p) was observed from plants with irrigation disruption after third harvest and 6 L/ha of nitroxin application (Figure 3). Moreover, the longest stem (41.10 cm) was observed by irrigation disruption after third harvest and 6 L/ha nitroxin application and the shortest stem (22.66 cm) was obtained from control plants that is without irrigation disruption and 9 L/ha of nitroxin application (Figure 4). Furthermore, the maximum numbers of sub stem (11.93) was shown on plants with irrigation disruption after third harvest and 6 L/ha nitroxin application treatments, while the minimum (3.33) was shown on plants without irrigation disruption and 9 L/ha nitroxin application (Figure 5).

The highest biomass yield (1298.5 g/m^2) was obtained from irrigation disruption at second harvest and 6 L/ha of

Table 1. Contd.

Source of variation	df	Mean square (MS)				
		The latest sub stem number	Capitulate diameter	Yield of biomass	Yield of seed	Harvest index
Rep	2	0.27	0.047	14.79	8.09	0.2
irrigation disruption(A)	3	0.4	0.26	9681.35**	447.93**	1.26*
Error	6	0.159	0.077	234451.7	66.17	0.25
Nitroxin (B)	3	0.243	3.90**	7411.62	530.27**	5.37**
Irrigation × nitroxin	9	0.222	0.056	262507.30**	417.38**	0.91*
Error	24	0.354	0.065	417.38**	85.52	0.38
Coefficient of Variance (%)		15.44	5.96	16.7	14.79	17.61

*, ** Significant at $P \leq 0.05$, $P \leq 0.01$, respectively; df, degree of freedom.

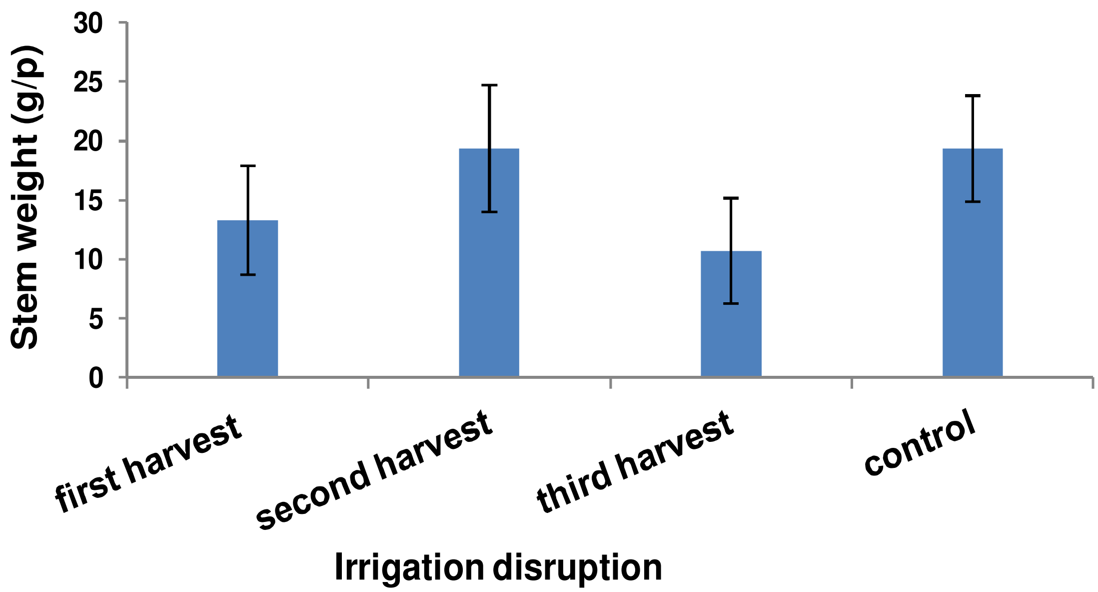

Figure 1. Means comparison of stem weight in *Calendula officinalis* L. under different irrigation disruption. Error bars show the standard deviation (SD).

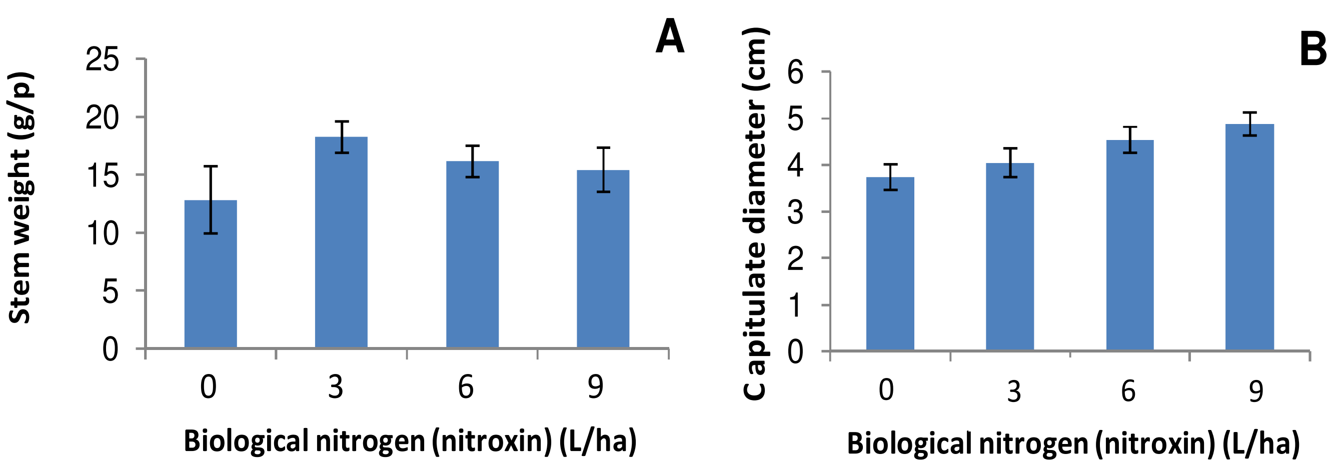

Figure 2. Means comparison of stem weight (A), capitulate diameter (B) in *Calendula officinalis* L. under different amounts of nitroxin application (biological nitrogen). Error bars show the standard deviation (SD).

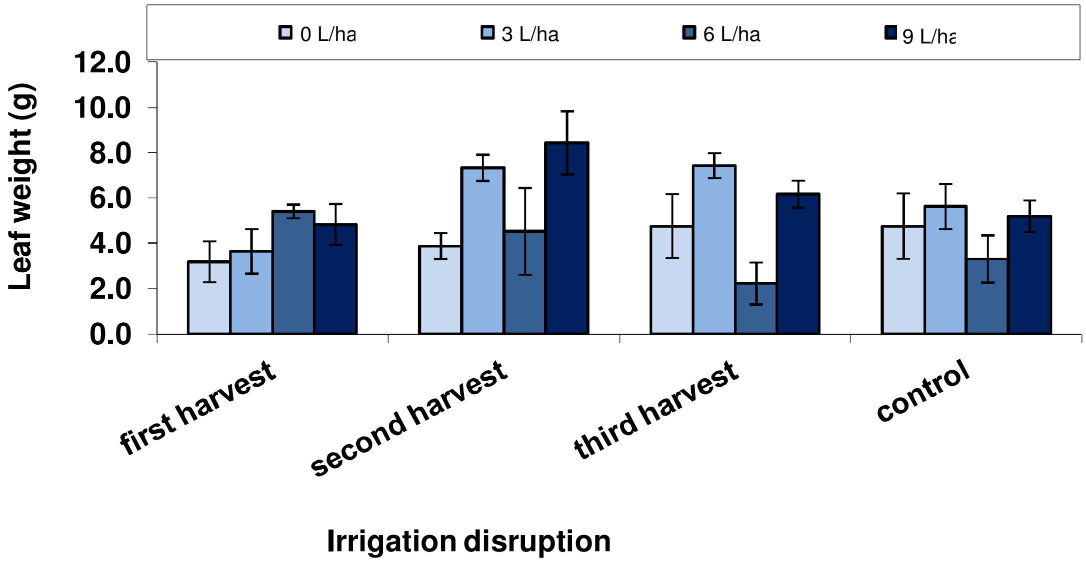

Figure 3. Means comparison of leaf weight in *Calendula officinalis* L. under different irrigation disruption and varying amounts of nitroxin application (biological nitrogen). Error bars show the standard deviation (SD).

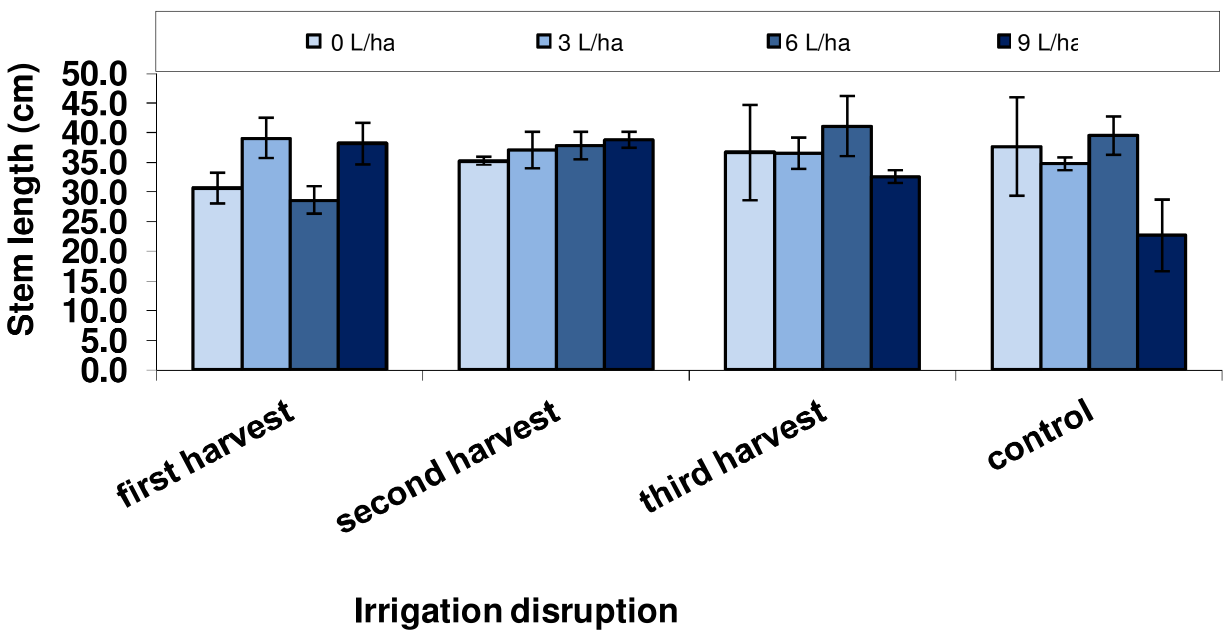

Figure 4. Means comparison of stem length in *Calendula officinalis* L. under different irrigation disruption and varying amounts of nitroxin application (biological nitrogen). Error bars show the standard deviation (SD).

nitroxin application, and the lowest biomass yield (442.80 g/m^2) was obtained from irrigation disruption at first harvest and 0 L/ha nitroxin application (Figure 6). The maximum seed yield (68.83 g/m^2) was obtained from irrigation disruption at the third harvest and 9 L/ha of nitroxin application that was in the same group with the control treatment (without irrigation disruption and 0 and 6 L/ha of nitroxin application). The lowest seed yield (31.18 g/m^2) was obtained from irrigation disruption at second harvest and 6 L/ha of nitroxin application (Figure 7). In addition, the maximum harvest index (4.29 %) was obtained from irrigation disruption at third harvest and 9 L/ha of nitroxin application, while the lowest harvest index (1.30 %) was obtained from irrigation disruption at second harvest and 3 L/ha of nitroxin application. Harvest index obtained in all 3, 6 and 9 L/ha of nitroxin applica-

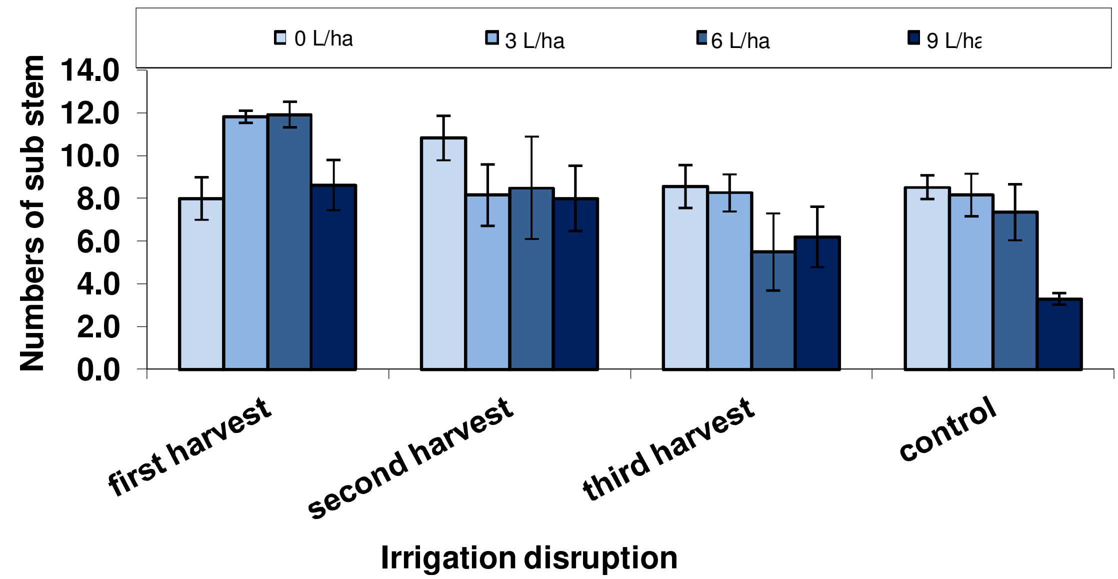

Figure 5. Means comparison of numbers of sub stem in *Calendula officinalis* L. under different irrigation disruption and varying amounts of nitroxin application (biological nitrogen). Error bars show the standard deviation (SD).

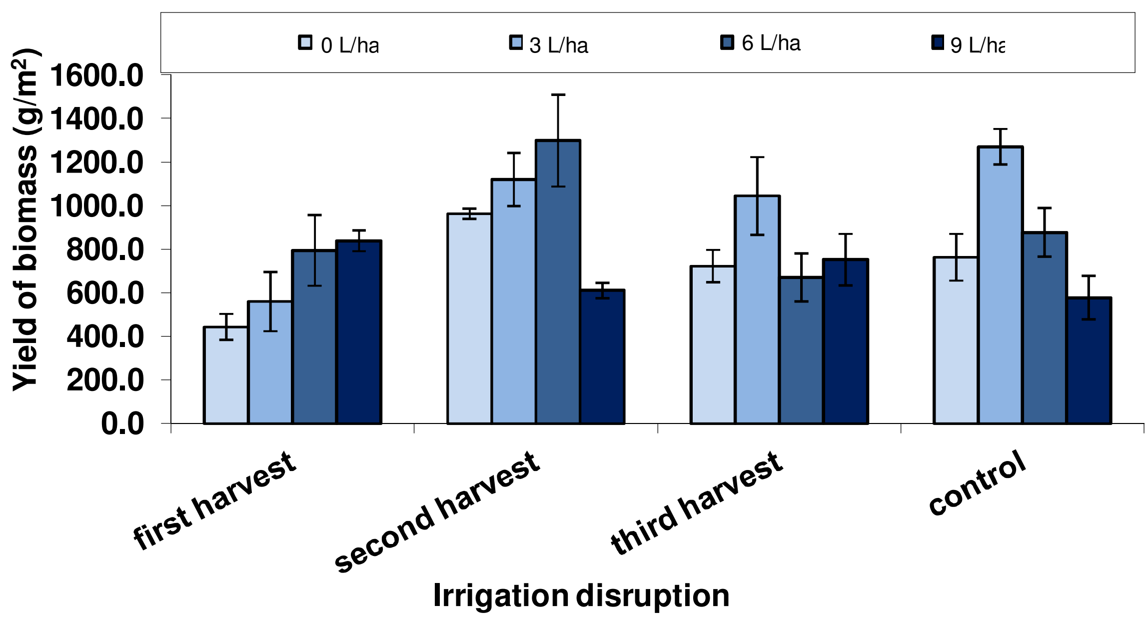

Figure 6. Means comparison the yield of biomass in *Calendula officinalis* L. under different irrigation disruption and varying amounts of nitroxin application (biological nitrogen). Error bars show the standard deviation (SD).

tions and irrigation disruption at second harvest result to the same and lowest amounts (Figure 8).

DISCUSSION

The stimulatory effect of biofertilizers on growth parameters of plants were reported by Mahmoud and Amara (2000) and Shehata and El-khawas (2003). Maize and wheat plants inoculated by *Azospirillum* along with synthetic fertilizers mostly showed increased shoots and roots length (Rousta et al., 1998), and our results on stem weight and the yield of biomass are in accordance with their reports (Figures 1A and B). According to Das and Saha (2007), combined utilization of *Azotobacter* and *Azospirillum* in the presence of partial application of

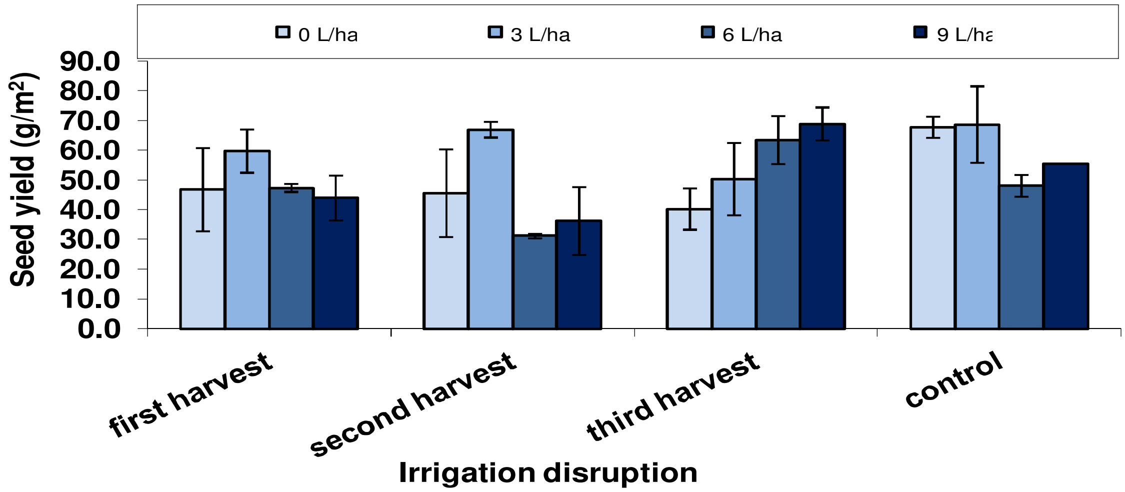

Figure 7. Means comparison of seed yield in *Calendula officinalis* L. under different irrigation disruption and varying amounts of nitroxin application (biological nitrogen). Error bars show the standard deviation (SD).

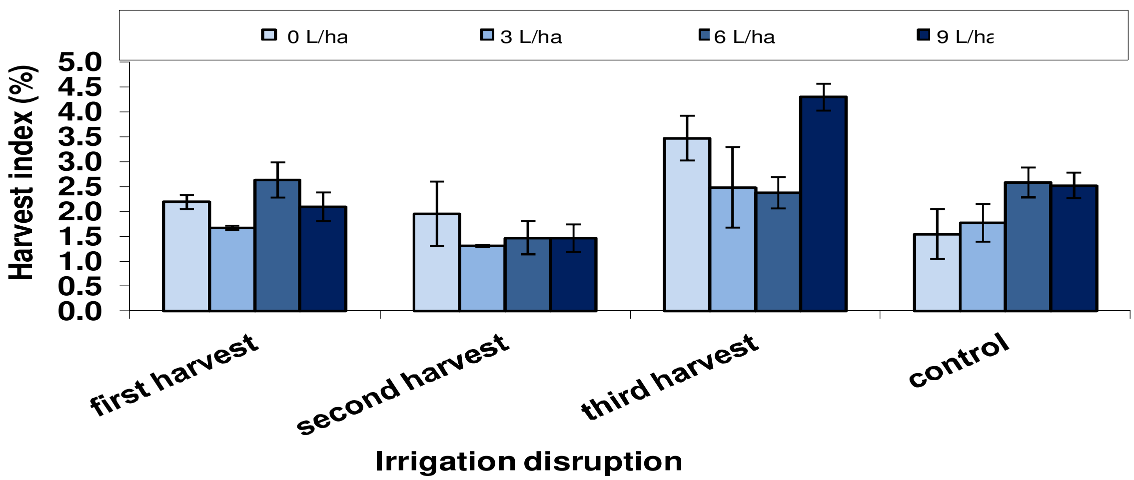

Figure 8. Means comparison of harvest index in *Calendula officinalis* L. under different irrigation disruption and varying amounts of nitroxin application (biological nitrogen). Error bars show the standard deviation (SD).

farmyard manure increases crop productivity. The microbial inoculants such as *Azotobacter, Rhizobium* and *Trichoderma*, are responsible for plant growth and yield under field inoculation (Rouzbeh et al., 2009). Inoculation of seeds with *Azotobacter* and *Azospirillum* in the presence of chemical fertilizers resulted in improving both growth and yield of anise (Gomaa and Abou-Aly, 2001).

Furthermore, the inoculation of seeds with *Azotobacter* and *Azospirillum* promoted the growth and increased the sepal yield of rosette plants compared to the chemical fertilization alone and decreased the production cost and obtaining high quality products (Abo-baker and Mostafa, 2011). It is evident that the biofertilizers had a beneficial effect on growth attributes namely plant height, by contributing nitrogen. Similar results were reported in maize and wheat plants where the inoculation with *Azospirillum* along with synthetic fertilizers mostly increased the height of shoots and roots (Rousta et al., 1998). Treatment with plant growth promoting *rhizobacteria* might also increase length after inoculation (LAI), chlorophyll content (Amujoyegbe et al., 2007; Chandrasekar et al., 2005), germination percentage, seedling vigor, seedling emergence, root and shoot growth, total biomass of plants, seed weight, early flowering, grains fodder and fruit yields (Berova and Karanatsidis, 2008).

The inhibition of flower growth characters under water deficit treatments would almost certainly be due to exposure to injurious levels of drought during both harvests (Figure 1), causing a decrease of turgor which would result in a decrease of growth and development of cells (Ozturk et al., 2004; Scalia et al., 2009). The reduction in plant height seems to be the result of disturbed plant water relations in particular turgor potential (Hussain et al., 2009). This means that reduced water uptake results in a decrease in tissue water contents and turgor. As a consequence, impaired mitosis, cell elongation and expansion resulted in reduced plant height and growth (Farooq et al., 2009). Razmjoo et al. (2008) and Baghalian et al. (2011) reported that drought stress caused a significant reduction in plant height, shoot weight and flower yield of *Matricaria chamomilla*. Moreover, Drought stress significantly reduces the plant height, shoot dry weight, flower diameter, flower fresh and dry weight in marigold based on reports of Abdul-Wasea and Khalid (2010), which is in accordance with our results (Figures 1, 3 and 4).

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