ABSTRACT: To evaluate effect of irrigation disruption on the harvest index of flower and essential oil of *Calendula officinalis* L., an experiment was conducted as split plot at the Research Farm of Faculty of Agriculture, Urmia University (latitude 37.53 °N, 45.08 °E, and 1320 m above sea level) in 2010. Treatments included end season water deficit (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 liter per hectare of biological nitrogen included *Azotobacter* and *Azospirillum* sp.) as sub plot were arranged in randomized complete block design with three replications. Data analysis of variance showed the significant interaction effect between irrigation disruption and biological nitrogen on essential oil and flower harvest index in first harvest, second harvest, third harvest, fourth harvest, fifth harvest and sum of total harvest. The highest harvest index of essential oil (0.38 %) in sum of total harvest belonged to irrigation disruption after first harvest and 9 liter per hectare of biological nitrogen. The maximum flower harvest index in sum of total harvest (11.8 %) was obtained from control treatment of irrigation (without irrigation disruption) and 9 liter per hectare of biological nitrogen.

Keywords: *Azospirillum, Azotobacter*, Harvest index, Irrigation disruption, Pot marigold.

Introduction

Marigold (*Calendula officinalis* L.) belonged to Asteraceae family and native to Mediterranean region, is an annual herb which are used as a decorative plant in horticultural industry (Duke et al., 2002). The essential oils of this herb are highly medicinal (Janke, 2004) with several therapeutic activities, such as anti-inflammatory, anti-tumorogenic (Jimenez-Medina et al., 2006) and cicatrizing (Hamburger et al., 2003). In addition, the in vitro antimicrobial activities of its oils have been documented (Sarrell et al., 2003).

Water stress tolerance is seen in all plant species, but its extent varies from species to species. The general effects of drought on plant growth in crop plants are fairly well known (Sankar et al., 2007; Manivannan et al., 2007). The primary effects of water deficit on medicinal plants are not well understood (Tan et al., 2006). Speath and Sinclair (1985) found in soybean that seed growth might be better defined by determining the change in harvest index (HI) with time during the seed growth period. The HI can be very low on soils with decreasing water supply (Bolanos and Edmeades, 1993). Therefore, transpiration efficiency (TE) and HI are three important avenues for the importance of agricultural productivity. Additionally, the aerial environment plays a role in determining the carbon-gain-to-water-use ratio, because the vapour pressure deficits between the leaf and the air determine the transpiration rate (Tanner and Sinclair, 1983).
Chemical fertilizers have contributed significantly toward the pollution of water, air and soil. So the current trend is to explore the possibility of supplementing chemical fertilizers with organic ones that are eco-friendly and cost-effective. The use of biological nitrogen fixation by living nitrogen fixers will help minimize use of chemical nitrogen fertilizer and to improve plant growth to decrease the production cost and environmental risk (El-Hawary et al., 2002). *Azotobacter* and *Azospirillum* are free-living N$_2$-fixing bacteria that in the rhizospheric zone have the ability to synthesize and secret some biologically active substances that enhance root growth (Chen, 2006). 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 (Abobaker and Mostafa, 2011). A positive effect of *Azotobacter* on yield and yield components has been reported in *Apium graveolense* (Migahed et al., 2004).

On the basis of our knowledge, information regarding the effect of water stress, especially end season water deficit on the production of biomass, flower and essential oil is scarce available. These effects on the relations among biomass and economic yield (flower and essential oil) under nitrogen fixing by *Azotobacter* and *Azospirillum* species were not studied. Then, evaluate the effect of varying amounts of biofertilizer (*Azotobacter* and *Azospirillum*) under end season water deficit condition on the allocation of biomass to flower and essential oil production is the main objective of this study.

**Materials and methods**

In order to study the effect of *Azotobacter* and *Azospirillum* as a biofertilizer on harvest index of flower and essential oil of pot marigold (*Calendula officinalis* L.) under drought stress conditions, a field experiment was carried out as split plot with three replications. Experiment was conducted at the Research Farm of Urmia University (latitude 37.53 °N, 45.08 °E, and 1320 m above sea level) in 2010. Experimental units comprised of 10 lines of 2 meters long. Row to row and plant to plant spacing was 0.3 and 0.05 meters, respectively. Treatments were end season water stress (irrigation disruption after first, second and third harvest and control as main plots) and amount of biological nitrogen (0, 3, 6 and 9 liter per hectare of biological nitrogen as sub plots). The seeds were inoculated with biological nitrogen included mixed of *Azotobacter* sp. and *Azospirillum* sp., and then were sown. Hand control of weeds was carried out during the whole experimental period.

**Harvest Index (HI)**

Harvest index was calculated as total dried flower, essential oil and seed weight divided by total above-ground plant dry weight for harvest index of dried flower and essential oil production.

To determine the yield of flower, plants were harvested from 2.5 m$^2$ of the three middle rows of each plot, and threshed manually. The harvested crop consisted of typical freshly gathered flower heads. Fresh flower heads were collected from each experimental unit five times at flowering stages in both seasons; air dried and weighed dry flower heads. Distilled water (250 CC) was added to 25 gram of gathered flowers obtained from each plot, and was hydrodistilled for 3 hours using a Clevenger-type apparatus (Clevenger, 1928).

- Flower harvest index= (yield of flower/ biomass yield)$\times$100
- Essential oil harvest index= (essential oil yield / biomass yield)$\times$100

Analysis of variance (ANOVA) on data was performed using the general linear model (GLM) procedure in the SAS software (SAS Institute, 2000). The Student-Neuman Keul's test (SNK) was applied to compare treatment means using the MSTATC software package.

**Result**

Result of analysis of variance (ANOVA) showed that interaction effect between irrigation and biological nitrogen on the harvest index of essential oil (Table 1) and flower harvest index (Table 2).

The highest ratio of essential oil harvest index in first (0.070 %), second (0.079%), third (0.074%), fourth (0.073%), fifth (0.098%) harvest, and however yearly total of essential oil harvest index (0.38%) belonged to irrigation disruption after first harvest and 9 liter per hectare of biological nitrogen. The lowest ratio of essential oil harvest index in first (0.010%) was obtained to control treatment (without irrigation disruption and without biological nitrogen). The lowest ratio of essential oil harvest index in second (0.022 %) belonged to irrigation disruption after third harvest and control treatment (without biological nitrogen). The minimum essential oil harvest index in third (0.019 %), fourth (0.014 %) and fifth (0.019 %) were observed to irrigation disruption after second harvest and control treatment (without biological nitrogen) (Figure 1-A, 1-B, 1-C, 1-D, 1-E and 1-F).
Table 1: Effect of irrigation disruption and biological nitrogen on essential oil harvest index in *Calendula officinalis* L.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>First harvest</th>
<th>Second harvest</th>
<th>Third harvest</th>
<th>Fourth harvest</th>
<th>Fifth harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.00007</td>
<td>0.0001</td>
<td>0.00004</td>
<td>0.0001</td>
<td>0.00002</td>
</tr>
<tr>
<td>Irrigation disruption</td>
<td>3</td>
<td>0.0029**</td>
<td>0.0013**</td>
<td>0.0010**</td>
<td>0.0007**</td>
<td>0.0015**</td>
</tr>
<tr>
<td>Replication×irrigation</td>
<td>6</td>
<td>0.0000</td>
<td>0.0001</td>
<td>0.00008</td>
<td>0.0001</td>
<td>0.00007</td>
</tr>
<tr>
<td>Biological nitrogen</td>
<td>3</td>
<td>0.0008**</td>
<td>0.0011**</td>
<td>0.0019**</td>
<td>0.0016**</td>
<td>0.0015**</td>
</tr>
<tr>
<td>Irrigation×nitrogen</td>
<td>9</td>
<td>0.0009**</td>
<td>0.0014**</td>
<td>0.0012**</td>
<td>0.0014**</td>
<td>0.00062**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.0001</td>
<td>0.00012</td>
<td>0.00009</td>
<td>0.00009</td>
<td>0.00005</td>
</tr>
</tbody>
</table>

Coefficient of Variance (%) 28.21 23.78 21.83 23.57 17.77

* and ** Significant at \( P \leq 0.05, P \leq 0.01 \), respectively; df, degree of freedom

Table 2: Effect of irrigation disruption and biological nitrogen on flower harvest index in *Calendula officinalis* L.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>First harvest</th>
<th>Second harvest</th>
<th>Third harvest</th>
<th>Fourth harvest</th>
<th>Fifth harvest</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.79</td>
<td>2.39</td>
<td>1.88</td>
<td>0.71</td>
<td>0.96</td>
<td>1.27</td>
</tr>
<tr>
<td>Irrigation disruption</td>
<td>3</td>
<td>22.15**</td>
<td>24.73**</td>
<td>16.76**</td>
<td>18.36**</td>
<td>16.15</td>
<td>438.98**</td>
</tr>
<tr>
<td>Replication×irrigation</td>
<td>6</td>
<td>0.65</td>
<td>0.82</td>
<td>0.77</td>
<td>1.30</td>
<td>0.93</td>
<td>26.84</td>
</tr>
<tr>
<td>Biological nitrogen</td>
<td>3</td>
<td>17.51**</td>
<td>2.84**</td>
<td>3.81**</td>
<td>7.28**</td>
<td>9.30</td>
<td>316.51**</td>
</tr>
<tr>
<td>Irrigation×nitrogen</td>
<td>9</td>
<td>12.24**</td>
<td>7.92**</td>
<td>8.90**</td>
<td>11.58**</td>
<td>7.16**</td>
<td>422.67**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>2.35</td>
<td>0.51</td>
<td>1.08</td>
<td>0.71</td>
<td>1.49</td>
<td>27.99</td>
</tr>
</tbody>
</table>

Coefficient of Variance (%) 28.21 21.72 8.26 12.71 10.25 18.85

* and ** Significant at \( P \leq 0.05, P \leq 0.01 \), respectively; df, degree of freedom

In all irrigation regimes, the lowest essential oil harvest index belonged to control treatment of biological nitrogen, and it increased along with higher nitrogen amounts. So that ascending trends were sharper in severe stress than non stress or mild stress condition (Figure 1-A, 1-B, 1-C, 1-D, 1-E and 1-F).

The maximum flower harvest index in first harvest (10.18%) was obtained from control treatment of irrigation (without irrigation disruption) and 9 liter per hectare of biological nitrogen. The minimum one (4.16 %) belonged to irrigation disruption after first harvest and control treatment of biological nitrogen. This lowest harvest index of flower had no significant difference with irrigation disruption at first harvest and 3 liter per hectare of biological nitrogen (Fig 2-B, 2-C, 2-D, 2-E and 2-F). In all irrigation regimes, the lowest
flower harvest index belonged to control treatment of biological nitrogen, and it increased along with higher nitrogen amounts. So, the ascending trend was sharper in severe stress than non stress and mild stress condition (Figure 2-B, 2-C, 2-D, 2-E and 2-F).
Figure 1: Effect of irrigation disruption and biological nitrogen on essential oil harvest index of first (A), second (B), third (C), fourth (D), fifth (E) and yearly total (F) harvest in *Calendula officinalis* L.
Figure 2: Effect of irrigation disruption and biological nitrogen on flower harvest index of first (A), second (B), third (C), fourth (D), fifth (E) and yearly total (F) harvest in *Calendula officinalis* L.
Discussion

Our results showed the slight increase in essential oil harvest index along with 0 to 3 liter per hectare of biological nitrogen. Then we observed a sharp increase in essential oil harvest index along with 6 to 9 liter per hectare of biological nitrogen. While, this ascending trend was higher at sever stress (irrigation disruption treatments) than control (without irrigation disruption). These results indicated that the stress condition need to a great amounts of biological nitrogen to root inoculation and nitrogen fixing.

We obtained the ascending in harvest index of dried flower at all harvest along with increasing of biological nitrogen, too. These rising levels of flower harvest index were more in biological nitrogen application at 6-9 liter per hectare than 3 liter per hectare. The all treatments of bacteria inoculation had the higher ratio of harvest index in comparison with control treatment (without biological nitrogen). Reduction of harvest index of flower was observed along with server stress of end season water deficit.

High water availability promoted vegetative growth and decreased the harvest index in faba bean. The decreases in harvest index were more pronounced in the indeterminate plants, with reductions up to 48%, compared to 25% in the determinate plants (De Costa et al., 1997). The harvest index may be reduced on soils with decreasing water supply (Bolaños and Edmeads, 1993). Water deficit reduces plant photosynthesis by closing stomata, decreasing leaf area, stomata gravity, chloroplast and protoplast hydration, and protein and chlorophyll synthesis. However, reducing of photosyntate transport accumulates the products in leaves results in diminution in photosynthesis, limiting growth and crop yield (Hornok, 1992; Levitt, 1980). Foster et al. (1995) observed that, under moderate moisture stress, water use efficiency and harvest index (HI) were reduced in common bean. Razmjoo et al. (2008) and Baghalian et al. (2011) reported that drought stress caused a significant reduction in flower yield of *Matricaria chamomilla*. Narula et al (2002) detected a significant interaction between the inoculants and N fertilizer rate on N uptake and yield of wheat. The effect of *Azotobacter* and *Azospirillum* on grain yield and N uptake was most pronounced without fertilizer application (+57% and +94%, respectively). Jayathilake et al. (2006) detected application of *Azospirillum* in combination with VC and chemical fertilizers significantly highest harvest index (67.3%).

The microbial inoculants such as *Azotobacter*, *Rhizobium* and *Trichoderma*, are responsible to plant growth and yield under field inoculation (Rouzbeh et al., 2009). Inoculation of seeds with *Azotobacter* and *Azospirillum* in the present of chemical fertilizers resulted in improving both growth and yield of anise (Gomaa and Abou-Aly, 2001). While N rate had no effect on the HI, a water deficit before tasseling increased the HI in all the years (Moser et al., 2006).

CONCLUSIONS

The significant interaction effect between irrigation and activity of these two biofertilizer (*Azotobacter* and *Azospirillum*) indicated different response of nitrogen fixing to end season water deficit severity (irrigation disruption). In general, the harvest index of essential oil increased along with higher amounts of biological nitrogen in comparison with control as well as the harvest index of flower. However, the strength water stress (irrigation disruption at first and second harvest) caused in to lower ratio of flower to biomass than mild stress (irrigation disruption at third harvest) and control treatment (without irrigation disruption). But there was a inverse results in the harvest index of essential oil, so the higher ratio of essential oil harvest index was observed at stress condition compared to control because of growing up the percent of essential oil at stress condition.

References


Treatments were end season water stress (irrigation disruption after first, second and third harvest and control as main plots) and amount of biological nitrogen (0, 3, 6 and 9 liter per hectare of biological nitrogen as sub plots). 3: Means comparison biological nitrogen influence on nitrogen contain of leaf of Calendula (Calendula officinalis L.) under drought stress conditions. Error bars show the Standard deviation (SD). comparison with 3 liter per hectare. Water stress had significant effect on essential oil yield and essential oil percentage in Coriander (Coriandrum sativum) and highest, these characteristics were achieved under without stress conditions and also, highest oil percentage was achieved under water stress conditions [9]. To evaluate the effects of iron application on growth characters of Calendula officinalis L. under end season water deficit condition, an experiment was conducted as split plot at the Research Farm of Faculty of Agriculture, Urmia University (latitude 37.53°N, 45.08°E and 1320m above sea level), Urmia, Iran in 2010. Treatments were irrigation (irrigation disruption at first, second, third harvest and without disruption as control) as main plot and amount of iron fertilizer (0, 0.5, 1 and 1.5 l/ha iron as sub plot were arranged in randomized complete block design with three replications. Data a Investigation into the biological activities and chemical composition of Calendula officinalis L. growing in Tunisia. 1,2*Rigane, G., 3Ben Younes, S., 3Ghazghazi, H. and 1Ben Salem, R. Article history. 1. Calendula officinalis L. (English marigold, pot marigold) belongs to the Asteraceae (Compositae) family; is an annual herbaceous plant, native of Mediterranean countries (Danielski et al., 2007). C. officinalis can be broadly applied as an antiseptic, anti-inflammatory and cicatrizing as well as a light antibacterial and antiviral agent (Khalid et al., 2010). 2. Antioxidant activity The flower and leaf extracts of Calendula. officinalis L. were subjected to in vitro tests to evaluate their antioxidant activities. Calendula officinalis, the pot marigold, ruddles, common marigold or Scotch marigold, is a flowering plant in the daisy family Asteraceae. It is probably native to southern Europe, though its long history of cultivation makes its precise origin unknown, and it may possibly be of garden origin. It is also widely naturalised farther north in Europe (as far as southern England) and elsewhere in warm temperate regions of the world.