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Evaluation of Biological Nitrogen Influence on Oil Variations and Nutrient Content of *Calendula officinalis* L. Under Drought Stress Conditions

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Abstract: In order to study the effect of *Azotobacter* and *Azospirillum* as a biofertilizer on oil variations and nutrient content of calendula (*Calendula officinalis* L.) under drought stress conditions, a field experiment was carried out as split plot based on Randomized Complete Block Design with three replications. 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). Analysis of variance showed the significant interaction effect between irrigation and biological nitrogen on percentage and yield of oil in first and second harvest and Leaf Nitrogen Content. Means comparison indicated the highest yield of oil in first harvest (134.3 kg/ha) and second harvest (121.6 kg/ha) belonged to control treatment of irrigation disruption and 9 liter per hectare of biological nitrogen. The highest Leaf Nitrogen Content (3.39 %) belonged to control treatment of irrigation disruption and 9 liter per hectare of biological nitrogen.

Key words: *Azospirillum* · *Azotobacter* · Irrigation disruption · Oil · Pot marigold

INTRODUCTION

Pot marigold, *Calendula officinalis* L. is an aromatic herb that belongs to the Asteraceae family, is an annual herb. While the biennial form grows wild in the Southern, Eastern and Central Europe [1], the annual form is more widely cultivated [2]. *Calendula* can be broadly applied as an antiseptic, anti-inflammatory and cicatrizing [3]. The main chemical constituents of *C. officinalis* included terpenoids, phenolic acids, flavonoids, isorhamnetin, carotenoids, glycosides, C vitamin and sterols [4]. Iran's climate is mostly arid and semi-arid, where water availability is a major problem [5]. Water deficiency is increasingly becoming a serious problem in agriculture in Iran whereas the national average annual precipitation is less than 249 mm. In such a situation drought management is an urgent necessity [6]. Drought is the most significant factor restricting plant growth and crop productivity in the majority of agricultural fields of the world [7]. James *et al*. [8] suggested that the change of finite nutrient availability would have the largest impact in altering community and ecosystem properties rather than changes in water availability or efficiency of water utilization. The highest oil percentage was achieved under water stress conditions [9]. Biofertilizers are products containing living cells of different types of microorganisms, that have an ability to convert nutritionally important elements from unavailable to available form through biological processes [10]. *Azotobacter* is Gram negative bacteria [11]. *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. They also increase germination and vigour in young plants, leading to improved crop stands [12]. Abbaszadeh *et al*. [13] indicated N fertilizer had significant effect on oil yield and oil percentage of balm. Rahmani *et al*. [14] indicated that application of N fertilizer significantly increased the yield compounds such as: oil percentage and oil yield. Therefore, the objective of this study was to evaluate the effects of biological nitrogen and water stress on oil variations and nutrient content of calendula (*Calendula officinalis* L.).

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MATERIALS AND METHODS

In order to study the effect of *Azotobacter* and *Azospirillum* as a biofertilizer on oil variations and nutrient content of calendula (*Calendula officinalis* L.) under drought stress conditions, a field experiment was carried out as split plot based on Randomized Complete Block Design 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), Urmia, Iran in 2010. Experimental units comprised of 10 lines of 2 meters long. Row to row and plant to plant spacing was 30 and 5 cm, 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. To determine the yield of seed, plants were harvested from 2.5 m^2 of the two middle rows of each plot and threshed manually. The harvested crop consisted of typical freshly gathered heads. After harvesting, seed heads remained under dark to drying and to determining of dry seed yield.

In order of determination of oil percentage of calendula, at the end of growth stage, 2 g of powdered seed from each plot placed in Soxhlet extractor and added diethyl ether to the samples. After 6 h, calendula oil was accumulated in the erlenmeyer flask of Soxhlet extractor. In order of evaporation of diethyl ether, erlenmeyer flask was placed under 70 °C in electrical oven for 24 h and then cooling of samples, weighed by electrical scale carefully and determined oil percentage of calendula.

The yield of seed oil was calculated by the following formula [15]:

$$\text{Oil yield} = \text{Oil percentage} \times \text{Seed yield}$$

Leaf Nitrogen Content was determined with Kjeldahl method, leaf phosphorus was determined by spectrophotometer using red filter at 470 nm and Leaf Potassium Content was determined by flame photometer (Elico, made in India) [16, 17].

Analysis of variance (ANOVA) on data was performed using the general linear model (GLM) procedure in the SAS software [18]. The Student-Neuman Keul's test (SNK) was applied to compare treatment means using the MSTATC software package.

RESULTS

Analysis of variance showed the significant interaction effect between irrigation and biological nitrogen on percentage and yield of oil in first and second harvest (P=0.01) (Table 1). However, there was significant interaction effect between irrigation and biological nitrogen on the leaf nitrogen content (P=0.01) (Table 2).

Means comparison indicated that the highest percentage of oil in first harvest (23.83 %) belonged to irrigation disruption after first harvest and 9 liter per hectare of biological nitrogen and lowest percentage (11 %) was obtained from plants grown under control treatment (without irrigation disruption and without biological nitrogen). In each irrigation level, biological nitrogen caused in higher oil content compared to control treatment, without nitrogen fixing bacteria. Despite of increasing trend in oil content of all irrigation treatments along with raising of nitrogen, this increase

Table 1: Evaluation of biological nitrogen influence on oil variations of Calendula (*Calendula officinalis* L.) under drought stress conditions

		Oil percentage		Oil yield	
Source of Variation	df	First harvest	Second harvest	First harvest	Secondharvest
Replication	2	1.96	0.60	19.13	69.12
irrigation disruption(A)	3	30.21**	24.13**	3199.41**	2513.91**
Error	6	2.01	1.51	64.62	271.58
Biological nitrogen(B)	3	64.88**	48.50**	2650.26**	194665**
A×B	9	17.45**	18.30**	1092.25**	1449.77**
Error	24	2.12	0.66	157.15	77.29
Coefficient of Variance (%)		7.47	4.42	11.98	9.75

* and ** Significant at P=0.05, P=0.01, respectively; df, degree of freedom

Table 2: Evaluation of biological nitrogen influence on NPK variations of *Calendula officinalis* L. under drought stress conditions

		Means Squares (MS)		
Source of Variation	df	Leaf Phosphorus Content	Leaf Potassium Content	Leaf Nitrogen Content
Replication	2	0.006	0.68	0.020
irrigation disruption(A)	3	0.071	0.49	0.35**
Error	6	0.46	0.79	0.17
Biological nitrogen(B)	3	0.092	0.39	0.058
A×B	9	0.17	0.57	0.37**
Error	24	0.23	0.66	0.065
Coefficient of Variance (%)		16.68	17.55	8.92

* and ** Significant at *P*=0.05, *P*=0.01, respectively; df, degree of freedom

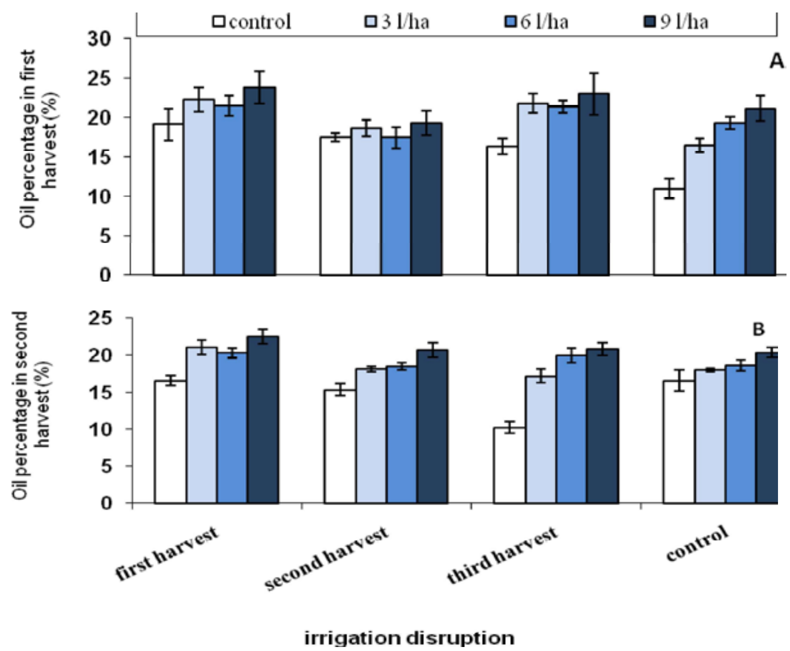

Fig. 1: Means comparison biological nitrogen influence on oil percentage in first harvest and second harvest of Calendula (*Calendula officinalis* L.) under drought stress conditions. Error bars show the Standard deviation (SD).

was great in plants irrigated during growth season without any disruption (Figure 1-A).

The highest percentage of oil in second harvest (22.5 %) belonged to irrigation disruption after third harvest and control treatment of biological nitrogen (0 liter per hectare) and lowest percentage of oil (10.26 %) belonged to irrigation disruption after third harvest and control treatment of biological nitrogen (0 liter per hectare). Here we obtained ascending trend in oil percent along with biological nitrogen increase, in all irrigation regimes. Application of 9 liter of biological nitrogen per hectare produced the highest percentage of seed oil of marigold in all irrigation levels (Figure 1-B).

Because of oil yield formula (Oil yield = Oil percentage × Seed yield), changes of percentage and yield of seed oil showed the same trends along with irrigation and nitrogen treatments. So, the highest yield of oil in first harvest (134.3 kg/ha) belonged to control treatment of irrigation disruption and 9 liter per hectare of biological nitrogen and lowest yield of oil (63.27 kg/ha) belonged to irrigation disruption after third harvest and control treatment of biological nitrogen, had no difference with irrigation disruption after third harvest and without biological nitrogen. Despite of increasing oil yield in first harvest along with higher amounts of nitrogen, this increase was greater in 6 and 9 liter per hectare in

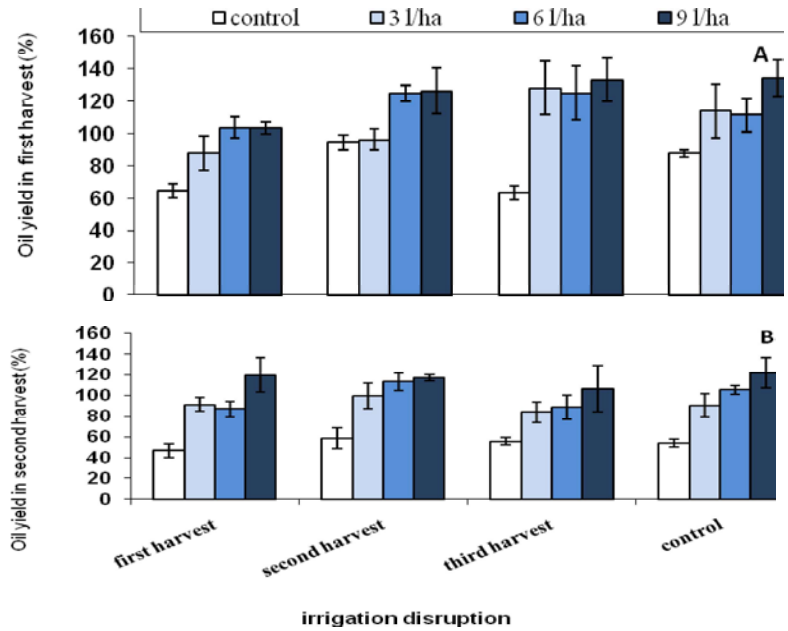

Fig. 2: Means comparison biological nitrogen influence on oil yield in first harvest and second harvest of Calendula (*Calendula officinalis* L.) under drought stress conditions. Error bars show the Standard deviation (SD).

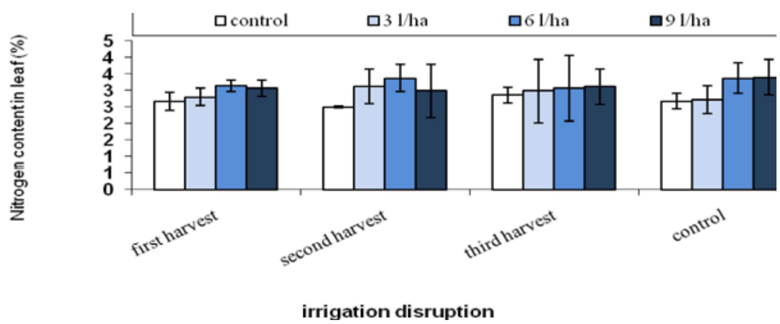

Fig. 3: Means comparison biological nitrogen influence on nitrogen contain of leaf of Calendula (*Calendula officinalis* L.) under drought stress conditions. Error bars show the Standard deviation (SD).

comparison with 3 liter per hectare. However, the sever stress (irrigation disruption at first harvest) caused to reduce the yield of seed oil more than mild water deficit stress (control and irrigation disruption after third harvest) (Figure 2-A).

The same trends of oil yield in first and second harvest indicated that the highest yield of oil in second harvest (121.6 kg/ha) belonged to control treatment of irrigation disruption and 9 liter per hectare of biological nitrogen and lowest yield of oil (46.6 kg/ha) belonged to irrigation disruption after first harvest and control treatment of biological nitrogen. In this harvest, reduction of oil yield was greater in control treatment of biological nitrogen than the first harvest. However, 9 liter per hectare biological nitrogen maintains the yield of oil in highest amounts as well as first harvest. But, the oil yield in 3 and 6 liter per hectare nitrogen was decline rather than first harvest (Figure 2-B).

Means comparison indicated the highest content of leaf nitrogen (3.39 %) belonged to control treatment of irrigation disruption and 9 liter per hectare of biological nitrogen. The lowest content of leaf nitrogen (2.46 %) was obtained from irrigation disruption after first harvest and control treatment of biological nitrogen, no difference with irrigation disruption after second harvest and control treatment of biological nitrogen. A considerable increase of leaf nitrogen was occurred in 6 and 9 liter per hectare biological nitrogen application for control treatment. But, the difference in nitrogen content along with biological nitrogen use was small for stress condition (irrigation disruption) (Figure 3). Under our experimental condition, Leaf Phosphorus Content and Leaf Potassium Content had no changes in range of our treatments.

DISCUSSION

In this study, we found that the end season water deficit (irrigation disruption) caused to reduce the percentage and yield of seed oil in *Calendula officinalis*. And sever stress (irrigation disruption at first > second > third harvest) led to a greater reduction in oil production. However, this reduction was rather in second harvest of seed than the first harvest, because of long time stress exposure.

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].

Although, the non-drought stress treatment significantly increased essential oil content of plants [19]. Nitrogen is the major nutrient that influences plants yield and protein concentration. Abbaszadeh *et al*. [13] indicated N fertilizer had significant effect on oil yield and oil percentage of balm. Their results showed that highest oil yield was achieved under 100 kg/ha N application and highest oil percentage was achieved under 50 kg/ha N application. Also, Sharifi and Abbaszadeh [20] investigated the effect of N fertilizer on essential oil yield and composition of fennel aerial parts and N application increased essential oil yield sorely. Water stress resulted in significant reduction of fresh and dry matter and nutrient content of Japanese mint (*Mentha Arvensis*) plants [21].

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