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Full Length Research Paper

Evaluation of nitrogenous fertilizer influence on oil variations of Calendula (*Calendula officinalis* L.) under drought stress conditions

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To evaluate the beneficial impact of nitrogen fertilizer on droughted calendula (*Calendula officinalis* L. var. 'Qazvin') some yield characters were investigated. In this experiment, the interactive effects of different N fertilizer and water restriction on oil variations and percentage in Iran during 2009 were studied. The experimental unit had designed by achieved treatment in factorial on the basis completely randomized block design with four replications. Certain factors were including N fertilizer (0, 30, 60 and 90 kg urea/ha) and water stress. In this study, calendula water supply was determined by indicated irrigation conditions by water evaporation from evaporation pan (40, 80 and 120 mm water evaporation). The results showed that however application of N fertilizer significantly increased the yield compounds such as: oil content, seed yield, oil percentage, head diameter, thousand seed weight and number of seeds in head in $p \leq 0.01$ of plants. Although, the non-drought stress treatment significantly increased yield compounds which were achieved under irrigation conditions (non-drought). The findings indicated that application of N fertilizer persist less damaging of drought stress result and it enabled plant to significantly increased its photosynthesis by root development and chlorophyll production under the drought conditions.

Key words: Nitrogenous fertilizer, drought stress, oil variations, seed yield, calendula.

INTRODUCTION

Drought resistance refers to a plant's ability to grow and reproduce satisfactorily under drought conditions, and drought acclimation refers to a plant's ability to slowly modify its structure and function so that it can better tolerate drought (Turner, 1986). Drought stress is one of the most important environmental stresses affecting agricultural productivity around the world and may result in considerable yield reductions (Boyer, 1982; Ludlow and Muchow, 1990). Calendula is an annual flower that prefers cool growing weather but nevertheless is tender and killed by frosts. Depending on variety and culture, the plants grow 12 to 30 in (30.5 to 76.2 cm) in height and about as wide. The leaves are bright green and typically about 4 in (10.2 cm) long. The lower leaves are oval with a rounded tip (spatulate) and upper leaves are lance shaped with pointed tips. The flowers are typically 2 to 3 in (5.1 to 7.6 cm) in diameter and held on thick sturdy stems. Calendulas are single or double flowered and come in a range of colors from cream to light yellow to electric yellow to orange. Some have dark brown centers and all are beautiful. *Calendula officinalis*, the pot marigold, is native to Southern Europe around the Mediterranean Sea.

C. officinalis is easy to grow in average soil and is bothered by few pests or cultural problems providing the soil is well-drained. Cut back plants when hot weather arrives. If you can keep them alive through the heat of summer they will recover and bloom again in fall. Calendula

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has been used medicinally for centuries and still finds use in this regard. Creams and ointments containing calendula are used to soothe skin and sprained muscles. The florets and extracts prepared from them are incorporated into soothing teas, lotions, and other formulations. Other preparations are used for antisepsis, spring tonics, chapped skin, tooth aches, and insect repellent among others (Zargari, 1986). Apart from the effect of drying soil on the transport of nutrients to plant roots, the morphological and physiological mechanisms involved in cellular and whole plant responses to water stress are of considerable interest and are frequently examined (Hsiao, 1973; Levitt, 1980; Blum, 1988; Davies and Zhang, 1991; Smith and Griffiths, 1993; Close and Bray, 1993; Kramer and Boyer, 1995; Neumann, 1995). The effect of water stress on essential oil content was studied in excised leaves of palmarosa (*Cymbopogon martinii* var. motia) and citronella java (*Cymbopogon winterianus*). The essential oil percentage increased under water stress and essential oil content decreased under this condition (Fatima et al., 2006).

Also, Khalid (2006) evaluated the influence of water stress on essential oil yield of two species of an herb plant that is, *Ocimum basilicum* L. (sweet basil) and *Ocimum americanum* L. (american basil). For both species under water stress, essential oil percentage and the main constituents of essential oil increased. Seventy five percent field water capacities resulted in the highest yield of herb and essential oil for both species. Also, water stress had significant effect on flowering shoot yield, essential oil yield and essential oil percentage in coriander and highest, these characteristics were achieved under without stress conditions and also, highest oil percentage was achieved under water stress conditions (Aliabadi Farahani et al., 2008). In this study, the results showed that drought stress significantly increased the essential oil percentage and reduced kaempferol content in chicory.

Although, the non-drought stress treatment significantly increased essential oil content of plants (Taheri et al., 2008). Nitrogen is the major nutrient that influences plants yield and protein concentration. When the amount of available soil N limits yield potential, additions of N fertilizers can substantially increase plants yield. However, plants protein concentration can decrease if the amount of added N is not adequate for potential yield (Olson and Swallow, 1984; Grant et al., 1985). Abbaszadeh et al. (2006) 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 (2003) investigated the effect of N fertilizer on essential oil yield and composition of fennel aerial parts and N application increased essential oil yield sorely. Therefore, the objective of this study was to evaluate the influences of N fertilizer and drought stress on oil variations and yield compounds in *C. officinalis* L.

MATERIALS AND METHODS

The experimental unit had designed by achieved treatment in factorial on the basis completely randomized block design with four replications. Certain factors were including N fertilizer (0, 30, 60 and 90 kg urea/ha) and water stress. In this study, calendula water supply was determined by indicated irrigation conditions by water evaporation from evaporation pan (40, 80 and 120 mm water evaporation). For this approach, experimental field was prepared in the dimension of 15 m^2 per plot (5 m × 3 m), totally 48 plots and calendula (*C. officinalis* L. var. 'Qazvin') was used in this experiment. Initially, plant nutrient request of phosphorus and potassium supply were added by apply 100 kg/ha ammonium phosphate, 200 kg/ha K_2O and nitrogen treatments in cultivation time, respectively.

Soxhlet method

In order of determination of oil percentage of calendula, at the end of growth stage, 2 g powder of dry seed matter from each plot placed in Soxhlet extractor and added diethyl ether to the samples. After 4 h, calendula oil was accumulated in the Erlen of Soxhlet extractor. In order of evaporation of diethyl ether, Erlen 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. Finally, oil content was determined by the following formula (Leal et al., 2009).

Oil yield = Oil percentage × Seed yield.

To determine seed yield, head diameter, thousand seed weight and number of seeds in head, 10 plants was assembled by chance from each plot at the maturity. Finally, obtained data were subjected to analysis of variance (ANOVA) using Statistical Analysis System and terms were considered significant at $p < 0.05$ (SAS institute Cary, USA 1988).

RESULTS

The final results of plants characters showed that drought stress significantly decreased the oil yield, seed yield, head diameter, thousand seed weight and number of seeds in heat ($p \leq 0.01$) as compared to non-drought conditions which was higher in control plants, while, highest oil percentage was achieved under the drought stress (Tables 1 and 2) (Figures 1 and 3). These findings are in agreement with the observations of Aliabadi Farahani et al. (2008) and Fatima et al. (2006). In addition, application of N fertilizer had significant effect on oil yield, seed yield, oil percentage, head diameter, thousand seed weight and number of seeds in head ($p \leq 0.01$). Therefore, the findings indicated a significant improved of plant morphological characteristics under application of N fertilizer (Tables 1 and 2) (Figures 2 and 4).

The results were similar with the findings of Abbaszadeh et al. (2006); Alizadeh et al. (2006); Sharifi

Table 1. Analysis of variance.

Value sources	df	Means square					
		Seed yield	Oil percentage	Oil yield	Thousand seed weight	Number of seeds in head	Head diameter
Replication	3	48266.33	6.625	1805.672	11.103	12.389	9.845
Drought stress	2	17998632.75 **	225.13 **	291282.498 **	186.444 **	193 **	83.871 **
Error a	6	12261.11	1.38	1434.917	10.337	4.22	6.435
N fertilizer	3	155808.13 **	25.708 **	11771.432 **	8.02 **	51.5 **	7.71 **
Drought stress × N fertilizer	6	24528.17	18.344 **	4064.798 **	0.495	10.5	5.312 **
Error b	27	13877.88	1.304	839.844	1.044	6.07	1.47
CV (%)		6.4	5.58	8.25	8.58	9.13	5.23

* and ** : Significant at 5 and 1% levels, respectively.

Table 2. Means comparison of simple effects of treatments on determined characteristics.

	Treatments	Seed yield (kg/ha)	Oil percentage (%)	Oil yield (kg/ha)	Thousand seed weight (gr)	Number of seeds in head	Head diameter (mm)
Drought stress	40 mm evaporation	3044 a	16.65 c	505.25 a	15.18 a	31 a	25.66 a
	80 mm evaporation	1437 b	20.56 b	296.2 b	12.16 b	25.25 b	22.66 b
	120 mm evaporation	1042 c	24.15 a	252.9 c	8.37 c	24.75 b	21.17 b
N fertilizer	Non-application	1734 c	18.79 d	311.53 c	11.22 b	24.25 c	22.13 b
	30 kg/ha	1789 c	19.83 c	341.5 b	11.17 b	24.5 ab	22.98 ab
	60 kg/ha	1842 b	22.16 a	375.46 a	12.56 a	24 b	23.6 a
	90 kg/ha	1998 a	21.04 b	377.4 a	12.66 a	29.25 a	23.95 a

Means within the same column and factors, followed by the same letter are not significantly difference.

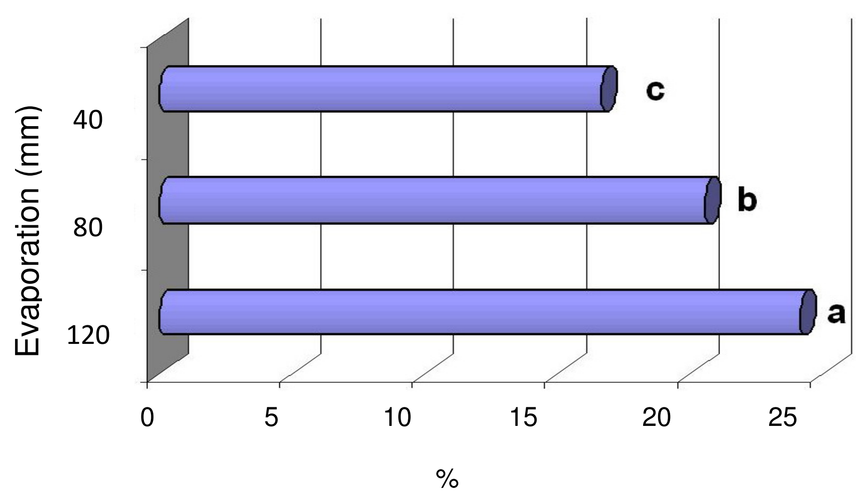

Figure 1. Oil percentage of calendula under different levels of drought stress.

and Abbaszadeh (2003). Data of the interactive effect between application of N fertilizer and drought stress has been demonstrated in Table 3. Significant different between plants treated with application of N fertilizer

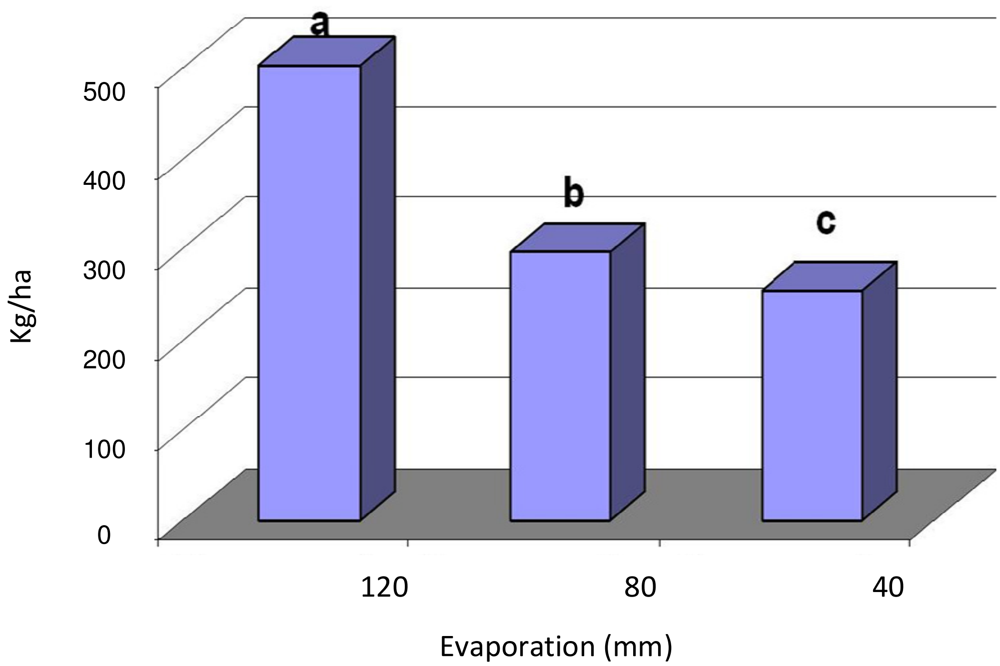

Figure 3. Oil yield of calendula under different levels of drought stress.

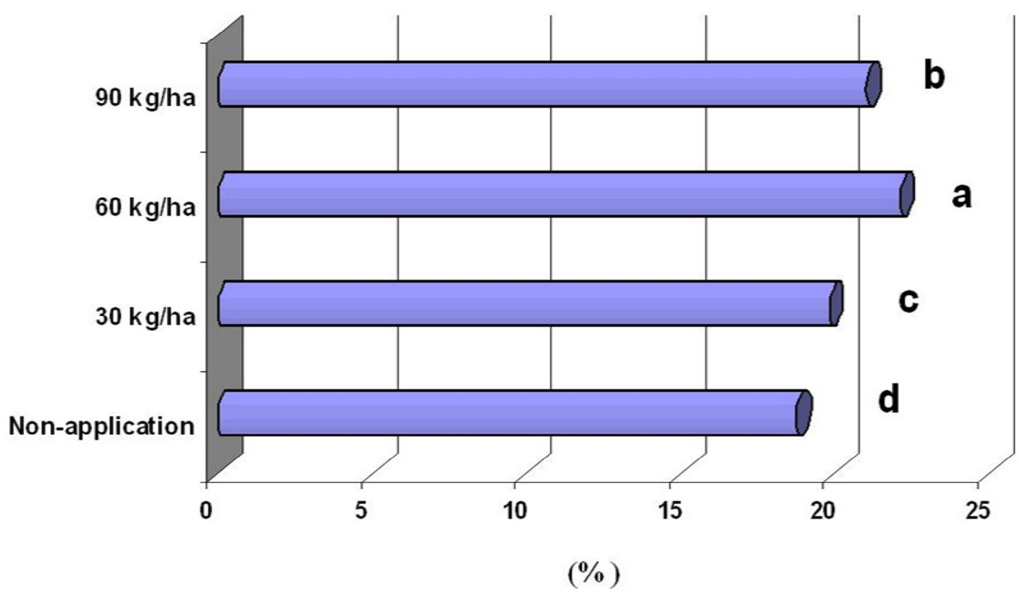

Figure 2. Oil percentage of calendula under different levels of N fertilizer.

under water restriction was highlighted with compare to non- application of N fertilizer under drought conditions. Treatment was indicated in oil yield, oil percentage and head diameter ($p \leq 0.01$) and seed yield, thousand seed weight and number of seeds in head were not significantly affected due to the interactive effect between application of N fertilizer and drought stress (Table 1). However, highest oil yield was indicated in the 30 kg/ha application of N fertilizer at no-stress conditions. Surprisingly, 90 kg/ha application of N fertilizer contributed plants to approach highest oil percentage as well as the condition of drought stress (Table 3). Also, highest seed yield, head diameter, thousand seed weight and number of seeds in head were achieved under 90 kg/ha

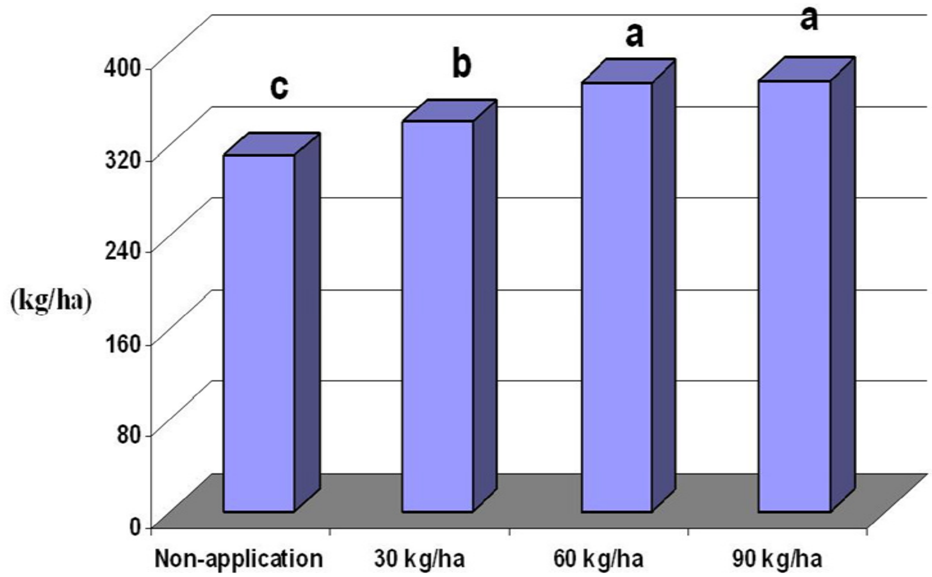

Figure 4. Oil yield of calendula under different levels of N fertilizer.

Table 3. Means comparison of interaction of treatments on determined characteristics.

Evaporation (mm)	Survey instance qualification	Seed yield (kg/ha)	Oil percentage (%)	Oil yield (kg/ha)	Thousand seed weight (gr)	Number of seeds in head	Head diameter (mm)
40	Non application (kg/ha)	2901 b	16.62 fg	482.3 b	14.7 bc	27 bcd	24.15 bc
	30	2965 b	18 ef	533.46 a	13.92 cd	33.75 a	24.99 b
	60	2980 b	16.61 fg	493.9 ab	15.8 ab	29.75 b	25.24 b
	90	3332 a	15.37 g	511.33 ab	16.27 a	33.5 a	28.3 a
80	Non-application (kg/ha)	1337 d	18.62 e	248.84 f	11.27 f	22.5 e	21.69 de
	30	1395 cd	18.61 e	259.81 ef	11.6 ef	25.5 cde	23.13 cd
	60	1459 cd	24.37 bc	355.78 c	12.85 de	26.25 bcde	23.05 cd
	90	1556 c	20.62 b	320.64 cd	12 de	26.75 bcd	22.79 cd
120	Non-application (kg/ha)	937 e	21.12 b	203.45 g	7.69 g	23.25 de	20.56 f
	30	1008 e	22.87 c	231.22 fg	7.99 g	23.26 de	20.82 ef
	60	1087 e	25.5 ab	276.71 def	8.99 g	25 cde	22.54 cde
	90	1108 e	27.12 a	300.2 de	8.8 g	27.5 bc	20.87 ef

Means within the same column and factors, followed by the same letter are not significantly difference

application of N fertilizer and non-drought stress (Table 3). Our results of treatments interaction were similar to the results of Diaz et al. (2005).

DISCUSSION

As it was shown in our results, drought stress had a negative effect on most of the emphasized growth compounds. In contrary, reducing water supply in soil achieved a situation for plant to pursue root growth though soil depth. This shows that in order to resist drought stress, the plant employed different strategies throughout individual survival straggle by drought conditions. In terms of reduce in evaporation plants showed an extreme reduce of leaf length and width (reduction in evaporation area). Although, significantly reduction in plant height and tiller number might be due to decreasing of the evaporation area of leaves and it eventually caused of low dry matter at the end of growth period under drought conditions. Those might be correspond to the fact that under drought stress stomata's become blocked or half-blocked and this leads to a decrease in absorbing CO_2 and on the other hand, the plants consume a lot of energy to absorb water, these cause a reduction in producing photosynthetic matters. Our observation indicated that with rising increase of drought stress, its biological yield and grain yield decreased with rising of drying in soil. Further reducing of shoot dry weight might be due to the reduction of photosynthesis area in leaf, drop in producing chlorophyll, the rise of the energy consumed by the plant in order to take in water and to increase the density of the protoplasm and to change respiratory paths and the activation of the path of phosphate pentose, or the reduction of the root deploy, etc.

Also, the results showed that applications of N fertilizer increased oil content of calendula, because nitrogen, which is a primary constituent of proteins, is extremely susceptible to loss when considering that average recovery rates fall in the range of 20 to 50% for dry matter production systems in plants. Nitrogenous fertilizers generally cause deficiency of potassium, increased carbohydrate storage and reduced proteins, alteration in amino acid balance and consequently change in the quality of proteins and are a main element in chlorophyll production. Toxic concentrations of nitrogen fertilizers cause characteristic symptoms of nitrite or nitrate toxicity in plants, particularly in the leaves. Although, pre plant fertilizer applications decrease the potential for nutrient deficiencies in early stages of growth, presence of residual soil NO_3–N (plant-available mineral N from the previous season) may pose a risk to the environment. The water of soil being salt by inordinate N application and increase its potential. Finally, the plant use high energy for absorption of salt water that causes dry matter reduces in this condition. Therefore, the oil content of calendula appeared under 60 kg/ha application of N fertilizer and was decreased to 90 kg/ha application of N fertilizer.

Conclusion

This study showed that under drought condition oil yield of calendula was reduced, while the application of N fertilizer was contributed to protect root against damaging effects of drought stress by root development and chlorophyll production. Currently, the control of drought stress has been paying attentions due to the most important environmental factor in arid and semi –arid regions. It may be useful to consider on screen of certain local calendula and fertilizer strategies to yield higher predictability oil under scope of limited available water recourse in these regions. Practically, these findings may suggest farmers and agricultural researchers to consider carefully on limiting or control the huge among of application of N fertilizer in suffered soils by water restriction as current challenge of scientist in global changes.

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