

Response of Marigold Flower Yield and Yield Components to Water Deficit Stress and Nitrogen Fertilizer

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In order to study the effect of water deficit stress and different nitrogen levels on flower yield, yield components and water use efficiency of *Calendula officinalis* L., an experiment was conducted as split plot based on randomized complete block design with three replications, at research field of Islamic Azad University, Birjand branch in 2009. In this experiment, irrigation treatments (irrigation after 60, 120 and 180 mm cumulative evaporation from pan class A) set as main plots and nitrogen rates (0, 60, 120 and 180 kg N ha⁻¹) set as sub plots. The results showed that increasing irrigation interval from 60 to 180 mm cumulative evaporation reduced flower number per m⁻², biomass yield and plant height 65.6, 69.3 and 8.3%, respectively. Also in comparison with control, irrigation after 120 and 180 mm evaporation reduced flower dry yield 16.2 and 72%, respectively. However, the highest WUE was related to irrigation after 120 mm evaporation (0.161 and 0.788 kg m⁻³ for dry flower and biomass, respectively). Nitrogen fertilizer utilization significantly increased flower yield, flower number, biological yield, WUE and plant height, but there was not any significant difference between 120 and 180 kg N ha⁻¹ treatments. Interaction of irrigation and nitrogen on all traits was not significant. Totally, the results indicated that treatment of irrigation after 120 mm evaporation with 120 kg N ha⁻¹ application is suitable for marigold cultivation in Birjand.

Abstract

Keywords: *Calendula officinalis* L., Irrigation, Nitrogen, WUE, Yield.

INTRODUCTION

Iran is considered as an arid and semi-arid region in the world. Therefore, efficient water use and understanding the influential factors such as N fertilization, and identifying drought-tolerant plants are crucially important. The diverse climate with a great temperature difference (over 50°C) of Iran and coastal, mountainous and desert lands (Javadzadeh, 1997) provides favorable conditions for the cultivation of most drought-tolerant medicinal herbs.

Marigold (*Calendula officinalis* L.) is an annual to perennial plant belonging to the family of Asteraceae. It needs high solar radiation during growing period and is able to well tolerate drought. It is, however, susceptible to high soil moisture. Hence, it can be considered for cultivation in such regions as Southern Khorasan, Iran. Marigold is known as blood purifier, energizer and anti-convulsion. It heals nausea, liver disorders, peptic ulcer disease, skin wounds, burns and blood cholesterol. It acts as skin softener, too. Marigold is used in production of toothpaste, shampoo and infant lotions (Omidbeigi, 2005., Zargari, 1982). The results of some studies show that essential oil of marigold counteracts HIV (Kalvatchev *et al.*, 1997).

In their study on marigold, Shubhra *et al.* (2004) found that drought stress considerably decreased the number of flowers per plant. Raesi *et al.* (2010) in a study on the effect of different manure levels and drought stress on roselle, stated that the delay in irrigation from 50 to 200 mm accumulative evaporation from evaporation pan significantly decreased sepal yield and the number of fruits per area unit. Arazmjo *et al.* (2009) studied the effect of drought stress on German chamomile and reported reduction in dry flower yield, single-plant biomass, plant height and number of flowers under drought stress conditions. Moosavi *et al.* (1988) indicated that both high and low irrigation levels decreased water use efficiency (WUE) of soybean compared with moderate irrigation level. Also, in a study on the effect of irrigation levels on WUE for seed and biological yield, Khajoenejad *et al.* (2005) revealed that the highest WUE was obtained under moderate water deficit stress.

In another experiment on chamomile, the effect of irrigation treatments (irrigation after 25, 50, 75 and 100 mm accumulative evaporation) on the plant was evaluated. The results showed that the highest capitulum yield per plant and per area unit and the highest number of capitulum per plant was obtained under the treatment of irrigation after 50 mm accumulative evaporation. On the other hand, significant increase in chamomile capitulum and seed harvest index was reported with the increase in water deficit stress. Also, the treatments of irrigation after 50 and 75 mm accumulative evaporation had significantly higher WUE for capitulum and seed production as compared with other treatments (Pirzad, 2007).

In a study on the effect of different N fertilization levels on flower yield of marigold, it was reported that the highest dry flower yield (102.86 g m⁻²) was obtained by the application of 150 kg N ha⁻¹ (Ameri and Nasiriemahalati, 2008). Arganosa *et al.* (1998) reported the highest biological yield of marigold at N fertilization level of 80 kg ha⁻¹.

In other study on German chamomile, different N levels were shown to have significant effects on the number of flowers per plant and flower fresh and dry yield per plant and per ha. In the experiment increase in N level significantly affected these traits (Hamzeie *et al.*, 2004).

This experiment was carried out to study the effect of irrigation and N fertilization levels on yield and yield components of marigold in Birjand, Iran.

MATERIALS AND METHODS

This experiment was conducted at the Agricultural Research Station of Islamic Azad University, Birjand branch, Iran (latitude: 32°52'; longitude: 59°13' and 1400 m above sea level) in 2009. The soil texture was loam with pH 8.21, organic matter 0.29%, total nitrogen 0.015% and EC 4.33 ms/cm.

The average long-time minimum and maximum temperature in Birjand are 4.6 and 27.5°C

with average annual precipitation of 169 mm and average minimum and maximum relative humidity of 23.5 and 59.6%, respectively. The regional climate is warm and arid.

Given the results of soil analysis, the field was fertilized with 150 kg triple super phosphate per ha and 100 kg potassium sulfate per ha. All phosphorus and potash fertilizer were applied at field surface at planting time. However, N fertilizer was applied at two phases (half after thinning and other half before start of flowering) with irrigation water in closed furrows. The seeds were planted in 20 April at the depth of 2-3 cm.

In this research, water deficit stress set as main factor with three levels (irrigation after 60, 120 and 180 mm cumulative evaporation from pan class A) and nitrogen set as sub factor with four levels (0, 60, 120 and 180 kg N ha⁻¹ from urea source).

The studied traits included the number of flowers per m², flower fresh and dry yield, biological yield, harvest index, single-plant weight, WUE for flower and biomass production, plant height and flower diameter. For this purpose given the unsimultaneous ripening of flowers, the ripened flowers were harvested from two middle rows of each experimental plot from an area of 3 m² twenty times during growth period. Then, they were counted to have the number of flowers per m² and flower yield which was the total flower weight harvested at different stages. The mean single-flower weight was calculated by dividing flower dry yield by the number of flowers per area unit. The division of flower yield by biological yield multiplied by 100 resulted flower harvest index. In addition, WUE for flower and biomass production (in terms of kg m⁻³) was measured through dividing flower dry yield by the amount of applied water and through dividing biological yield (biomass) by the amount of applied water, respectively. In order to measure plant height, 10 plants were randomly selected from two middle rows of experimental plots and their means were recorded as plant height. The flower diameter was measured out of the diameter of 20 flowers at each flower harvesting step.

The data were analyzed by software MSTAT-C and the means were compared by Multiple Range Duncan Test at 5% probability level.

RESULTS AND DISCUSSION

Yield and yield components of flower

Analysis of variance showed that irrigation and N fertilization significantly affected the number of flowers per m² and flower fresh and dry weight ($p < 0.01$), but single-flower dry weight was affected only by irrigation levels (Table 1). The number of flowers per m² was 2.58 times greater in the treatment of irrigation after 120 mm accumulative evaporation than in the treatment of irrigation after 180 mm, but it showed an 11.2% loss compared with the treatment of irrigation after 60 mm accumulative evaporation (Table 2). Probably the loss of the number of flowers per

Table 1. Results of analysis of variance for yield and yield components of marigold as affected by different levels of irrigation and nitrogen.

C.V.	df	Means of squares					
		Flower number per m ²	Single-flower dry weight	Flower fresh yield	Flower dry yield	Biological yield	Harvest index
Replication	2	101963.14*	0.0001 ^{ns}	6234955.45 ^{ns}	207474.35 ^{ns}	6323723.22*	2.12 ^{ns}
Irrigation (A)	2	139611**	0.003**	104713138.8**	3353845**	69896139.9**	9.612*
Error a	4	12545.82	0.0001	922421.57	26023.59	796955.25	1.142
Nitrogen rate (B)	3	68415.48**	0.0001 ^{ns}	5386648.11**	173498.09**	2778626.88**	1.1**
A × B	6	12891.48 ^{ns}	0.0001 ^{ns}	1107872.43 ^{ns}	34024.26 ^{ns}	535532.52 ^{ns}	0.256 ^{ns}
Error b	18	5291.81	0.0001	454253.76	14027.19	314431.17	0.149
CV (%)	-	11.13	1.72	12.61	11.99	11.81	1.88

^{ns} Non Significant at 0.05 probability level and *, ** Significant at 0.05 and 0.01 probability levels, respectively.

Table 2. Means comparison for yield and yield components of marigold as affected by different levels of irrigation and nitrogen

Treatment	Flower number per m ²	Single-flower dry weight (gr)	Flower fresh yield (kg ha ⁻¹)	Flower dry yield (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)	Harvest index (%)
Irrigation (mm accumulative evaporation)						
60	878.27 ^a	0.159 ^a	7719.78 ^a	1399.12 ^a	6499.53 ^a	21.47 ^a
120	779.91 ^a	0.150 ^a	6274.49 ^b	1172.26 ^b	5752.28 ^a	20.44 ^{ab}
180	302.17 ^b	0.130 ^b	2036.12 ^c	391.34 ^c	1996.1 ^b	19.68 ^b
Nitrogen rate (kg N ha ⁻¹)						
0	562.19 ^b	0.146 ^a	4581.34 ^b	850.40 ^b	4180.36 ^b	20.12 ^b
60	600.98 ^b	0.146 ^a	4846.83 ^b	897.30 ^b	4408.75 ^b	20.27 ^b
120	698.54 ^a	0.146 ^a	5676.83 ^a	1051.31 ^a	5012.97 ^a	20.71 ^a
180	751.98 ^a	0.148 ^a	6269.06 ^a	1151.30 ^a	5395.12 ^a	20.93 ^a

Means followed by the same letters in each column-according to Duncan's multiple range test are not significantly ($p < 0.05$)

m² with the increase in water deficit stress can be related to the loss of leaf area and their shedding and the resulting loss of assimilates, the decrease in the activities of photosynthesis-affecting enzymes and the disruption of pollination.

Means comparison revealed that although there was no significant difference in single-flower dry weight between the treatments of irrigation after 60 and 120 mm accumulative evaporation at 5% level, the increase in irrigation interval and drought stress up to the treatment of irrigation after 180 mm accumulative evaporation decrease of single flower weight by 13.3 and 18.2% at the treatments of irrigation after 120 and 60 mm accumulative evaporation, respectively (Table 2). Seemingly, shortened flowering period and the adverse effect of water deficit stress on the existing photosynthesis and the loss of assimilates translocation into flowers were the main causes of the significant decrease in single-flower dry weight under severe water deficit stress.

The treatments of irrigation after 120 and 180 mm accumulative evaporation resulted in 18.7 and 73.6% loss of flower fresh yield and 16.2 and 72% loss of flower dry yield compared with the treatment of irrigation after 60 mm accumulative evaporation, respectively (Table 2). To be able to bear flowers, plants need suitable vegetative growth and must produce constituting parts of the flowers at different vegetative and reproductive growth stages. The effect of water deficit stress on every yield component can finally change the number of flowers. Therefore, it can be said that the loss of current photosynthesis as well as the coincidence of flowering with high temperatures and the increase in embryo abortion under water deficit conditions can lead to the loss of flower fresh and dry yield through reducing the number of flowers per m² and single-flower weight. Also, Mohamadkhani and Heydari (2007) stated that the loss of leaf area resulted in the loss of light interception and the resulting loss of total photosynthesis capacity and obviously, the limitation of assimilate production under water deficit conditions led to the stunted growth of the plants and finally decreased their yield. The decrease in flower yield with the increase in water deficit stress has been reported for marigold (Shubhra *et al.*, 2004) and chamomile (Arazmjo *et al.*, 2009), too.

Means comparison showed that the increase in N fertilization rate from 0 to 180 kg N ha⁻¹ increased the number of flowers per m² by 33.7% (Table 2). Soil fertility deeply influences the flowering. Higher levels of N fertilization induces vegetative growth, increases leaf area index and duration and increases assimilate availability and flowering potential per area unit through increasing photosynthesis duration. In addition, N increases flower formation percentage by supplying the protein needed by pollens to move through stigma and reach to ovule, by increasing effective pollination time and helping the formation of stronger embryo sac (Rahemi, 2004). Therefore, the increase in N rate can justifiably increase the number of flowers per m². Also, some researchers

Table 3. Results of analysis of variance for plant height, flower diameter and water use efficiency for flower and biomass production of marigold as affected by different levels of irrigation and nitrogen

Sources of variation	df	Means of squares			
		Plant height	Flower diameter	WUE for flower	WUE for biomass
Replication	2	20.514 ^{ns}	7.83 ^{ns}	0.004 ^{ns}	0.121 ^{ns}
Irrigation (A)	2	211.286**	163.99**	0.016**	0.386**
Error a	4	6.444	1.577	0.001	0.021
Nitrogen rate (B)	3	14.911**	0.314 ^{ns}	0.002**	0.038**
A × B	6	1.359 ^{ns}	0.09 ^{ns}	0.0001 ^{ns}	0.007 ^{ns}
Error b	18	1.56	0.394	0.0001	0.003
CV (%)	-	6.41	2.21	10.18	10.02

^{ns} Non Significant at 0.05 probability level and *, ** Significant at 0.05 and 0.01 probability levels, respectively.

have revealed that higher N fertilization levels increase shoot growth and the number of flowers in chamomile (Zeinali *et al.*, 2008, Hamzeie *et al.*, 2004, Letchmo, 1993).

Means comparison for flower fresh and dry yield of marigold at various N rates indicated that although the increase in N rate from 0 to 180 kg N ha⁻¹ significantly increased flower fresh and dry yield by 36.8 and 35.4%, respectively, no significant differences in these traits were observed between N rates of 0 and 60 kg N ha⁻¹ and between N rates of 120 and 180 kg N ha⁻¹ (Table 2). Since N application increased leaf area index and green area duration through which it positively influenced photosynthesis, light use efficiency, plant growth period duration, dry matter accumulation in shoots and flower bearing potential per area unit, it expectedly increased flower fresh and dry yield, too. In addition, given statistically non-significant difference in single-flower dry weight means of marigold (Table 2) and significant difference in flower yield at different N fertilization levels, it can be concluded that N fertilization enhanced flower yield mainly through increasing the number of flowers per area unit. Ameri and Nasiriemahalati (2008) reported the increase in light use efficiency for flower bearing in marigold with the increase in N rate from 0 to 150 kg N ha⁻¹ and Al-Badavi *et al.* (1995) reported the positive impact of various nitrogenous fertilizers on vegetative growth, the concentration of photosynthesizing pigments and the flowering of marigold compared with no-N fertilization treatment which could be the possible reasons for higher flower yield under abundant N levels. Higher flower yield at higher N fertilization levels has been reported by Ameri and Nasiriemahalati (2008) and Pop *et al.* (2007) for marigold and Rahmati *et al.* (2009) and Hamzeie *et al.* (2004) for chamomile as well which is in agreement with our findings.

Biological yield and harvest index

Irrigation and N rate significantly affected biological yield and harvest (Table 1). Biological yield at the treatment of irrigation after 120 mm accumulative evaporation was decreased by 11.5% as compared with that at the treatment of irrigation after 60 mm accumulative evaporation, while the increase in irrigation interval up to 180 mm accumulative evaporation reduced biological yield by 69.3%. The loss of biomass production by drought stress can be associated with the loss of plant height, the loss of leaf area and the increase in the partitioning of assimilates to roots vs. shoots. In total, it can be drawn that drought stress significantly decreased economical and biological yield of marigold by shortening growth period and consequent loss of photosynthesis rate, shortening assimilation period and decreasing the mobilization of assimilates (Black and Squire, 1979).

N application increased dry matter production of marigold, so that biological yield was increased by 5.5, 19.9 and 29.1% with the application of 60, 120 and 180 kg N ha⁻¹ compared with no-N application, respectively and the application of 180 kg N ha⁻¹ gave rise to the highest biological yield with mean dry matter of 5395.12 kg ha⁻¹ (Table 2). It seems that N deficiency decreased leaf area and duration which resulted in lower light interception rate, light use efficiency and pho-

tosynthesis rate of canopy. Consequently, N deficiency led to the loss of biological yield. The studies conducted by Rahmani *et al.* (2008) on marigold and by Alizadeh Sahzabi *et al.* (2007) on savory showed significantly higher biological yield at higher N fertilization rate, too.

The results revealed that water deficit stress negatively affected harvest index of marigold, so that this index in treatments of irrigation after 180 and 60 mm accumulative evaporation was 19.7 and 21.5%, respectively. Moreover, means comparison showed that the increase in N fertilization rate from 0 to 180 kg N ha⁻¹ increased flower harvest index from 20.12 to 20.92% (Table 2).

In other words, water and nitrogen deficit stress disrupted the mobilization of assimilates to reproductive organs. Thus, it decreased potential flower yield more than biological yield. The results of the study of Ansarinia (2010) indicated that water deficit stress significantly decreased harvest index of sunflower. Also, Aboomardani *et al.* (2010) on canola and Ansarinia (2010) on sunflower reported that harvest index increased with the increase in rate of N application.

Plant height and flower diameter

Considering the results of analysis of variance, plant height and flower diameter were significantly affected by irrigation treatment ($p < 0.01$), but the effect of N rate was significant only on plant height (Table 3). The non-significant effect of N fertilization on flower diameter has been reported in chamomile, too (Rahmati *et al.*, 2009). As irrigation interval was increased from 60 to 180 mm accumulative evaporation, plant height and flower diameter were significantly decreased by 39.4 and 22.5%, respectively. Means comparison for plant height and flower diameter indicated classification of irrigation levels in distinct groups (Table 4). Some likely causes of plant height and flower diameter loss under water deficit conditions are the decrease in cell vigor and cellular growth and the resulting loss of leaf area, stomatal closure (Safarnejad, 2003) and photosynthesis limitation (Hassani and Omidbeigi, 2002). The loss of plant height with the increase in water deficit stress has been reported in basil (Hassani and Omidbeigi, 2002), chamomile (Arazmjo *et al.*, 2009) and isabgol (Najafi and Rezvanimoghadam, 2002), too.

Means comparison for plant height and flower diameter showed that although different rates of N application had no significant effect on increasing flower diameter, it significantly affected plant height, so that 180 kg N ha⁻¹ application had 6.3, 8.3 and 17.5% higher plant height than N rates of 120, 60 and 0 kg N ha⁻¹, respectively (Table 4). It is likely that higher N fertilization levels paved the way for longitudinal growth of stem by extending vegetative growth period and supplying the required assimilates. Moreover, it has been reported that N deficiency decreased plant height by inhibiting the formation of parenchyma and sclerenchyma and N application improved plant height by increasing the division of meristem cells and the turgidity of these cells (Mengel, 1988). Also, Najafpoorenavaei (2002) found the application of N fertilizer important in

Table 4. Means comparison for plant height, flower diameter and water use efficiency for flower and biomass production of marigold as affected by different levels of irrigation and nitrogen.

Treatment	Plant height (cm)	Flower diameter (mm)	WUE for flower (kg m ⁻²)	WUE for biomass (kg m ⁻²)
Irrigation (mm accumulative evaporation)				
60	23.80 ^a	31.26 ^a	0.108 ^b	0.501 ^b
120	19.21 ^b	29.74 ^b	0.161 ^a	0.788 ^a
180	14.42 ^c	24.23 ^c	0.090 ^b	0.459 ^b
Nitrogen rate (kg N ha ⁻¹)				
0	17.84 ^c	28.21 ^a	0.102 ^b	0.508 ^b
60	19.37 ^b	28.43 ^a	0.111 ^b	0.555 ^b
120	19.72 ^b	28.34 ^a	0.128 ^a	0.618 ^a
180	20.97 ^a	28.66 ^a	0.137 ^a	0.651 ^a

Means followed by the same letters in each column-according to Duncan's multiple range test are not significantly ($p < 0.05$)

improving the growth of borage. The increasing effect of N fertilization on plant height has been reported in savory (Alizadeh Sahzabi *et al.*, 2007) and *Tanacetum parathenium* (Hassani Malayer *et al.*, 2004), too. The results of the current study regarding the effect of water and N deficiency on plant height are consistent with the reports of Ram *et al.* (1995) and Mishra and Srivastava (2000) about mint, Mirshekari *et al.* (2007) about chamomile and Hassani and Omidbeigi (2002) about basil.

WUE for flower and biomass production

The results of analysis of variance showed that the effects of irrigation and N were significant on WUE for flower and biomass production at 1% level (Table 3). Means comparison indicated that the delay in irrigation until reaching to 120 mm accumulative evaporation significantly increased these traits as compared with two other irrigation levels; that is, at this irrigation treatment (moderate stress) more flower and biomass yield per each m^{-3} applied water were produced. The highest WUE for flower and biomass production (on average, 0.161 and 0.788 kg m^{-3} , respectively) was obtained at the treatment of irrigation after 120 mm accumulative evaporation which was 78.9 and 71.7% higher than those obtained at the treatment of irrigation after 180 mm accumulative evaporation (Table 4).

Higher WUE for flower and biomass production under moderate water deficit stress can be related to greater loss of water by evapotranspiration and deeper penetration at optimum irrigation treatment. On the other hand, the disruption of photosynthesis due to stomatal closure, the loss of leaf area and finally, the loss of biomass and flower yield at severe water deficit stress treatment. Nissanka *et al.* (1997) stated that the loss of WUE at severe moisture stress was caused by greater loss of photosynthesis vs. respiration. They related it to the injuries to leaf mesophyll under moisture stress. In addition, it can be said that increase in mesophyll and stomatal resistance under severe water stress decreased the entrance of CO_2 into plants which in turn, reduced net photosynthesis rate. Therefore, biomass was decreased under water stress. Higher WUE under moderate water deficit stress than under severe or no stress had been reported for chamomile (Pirzad, 2007), soybean (Moosavi *et al.*, 1998) and rape (Vafabaksh *et al.*, 2009), too which is in agreement with the results of the current study.

As N fertilization rate was increased from 0 to 180 kg N ha^{-1} , WUE for flower and dry matter production improved. Means comparison for these traits showed that although N application rate of 180 kg N ha^{-1} by producing 0.137 kg flower and 0.651 $\text{kg biomass per m}^3$ applied water had the highest WUE, the treatments of 120 and 180 kg N ha^{-1} were ranked in the same statistical group for these traits (Table 4). Given that the same amount of water was used at all fertilization rates, higher WUE for flower and biomass production at higher N rates can be related to the increase in flower and biomass yield. The increase in N application rate enhanced biomass weight by increasing net photosynthesis. Under the conditions of the current study, although higher N rates probably increased transpiration, they finally resulted in higher WUE due to higher flower yield. The increase in WUE with the increase in N fertilization rate has been reported in spinach (Sadegipoor Marvi, 2010), too.

CONCLUSION

Generally it can be concluded that given the prevailing water deficit in Southern Khorasan, Iran, the treatment of irrigation after 120 mm accumulative evaporation was superior over the treatment of irrigation after 60 mm accumulative evaporation because of its 51% higher WUE in spite of its 16% lower dry flower yield. Also, it is recommended to use N rate of 120 kg N ha^{-1} owing to its statistically non-significant difference with N rate of 180 kg N ha^{-1} , preventing environmental problems, avoiding redundant costs of fertilization and realizing optimum yield of marigold.

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