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Effect of water deficit stress and nitrogen fertilizer on flower yield and yield components of marigold (*Calendula officinalis* L.)

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Abstract

In order to study the effect of water deficit stress and different nitrogen levels on flower yield and yield components of *Calendula officinalis* L., an experiment was conducted as split plot design based on randomized complete blocks with three replications, at research field of Islamic Azad University of Birjand branch in 2009. 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}). The number of flowers per m^2 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. Also 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. Means comparison showed that the increase in N fertilization rate from 0 to 180 kg N.ha^{-1} significantly increased the number of flowers per m^2, flower fresh and dry yield by 33.7, 36.8 and 35.4% respectively. Finally, 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.

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Introduction

Marigold (*Calendula officinalis* L.), is an annual plant belong to Asteraceae family, aromatic, medicinal and ornamental herb with yelow and orange flowers, a native to Mediterranean region (Anderson, 2013; Gazim *et al.*, 2008). The species have been reported to contain a variety of phytochemicals, including carbohydrates, lipids, phenolic compounds, steroids, terpenoids, tocopherols, carotenoids and quinones (Kishimoto *et al.*, 2005; Re *et al.*, 2009; Shahrbabaki *et al.*, 2013; Wojciak-Kosior *et al.*, 2003) with potential health benefits [Miliauskas *et al.*, 2004; Muley *et al.*, 2009; Vodnar, 2012). Calendulas produced for ornamental use include both cut flowers and potted flowering plants. While cut flowers and herbs may be grown either in the field (Berimavandi *et al.*, 2011; Gazim *et al.*, 2008; Mohammad and Kashani, 2012) or in the greenhouse (Hamrick, 2003), potted flowering plants are grown almost exclusively in the greenhouse in a soilless substrate (Fornes *et al.*, 2007; Hamrick, 2003).

In study on marigold, Shubhra *et al.* (2004) found that drought stress considerably decreased the number of flowers per plant. The study of the effect of the treatments of irrigation after 25, 50, 75 and 100 mm accumulative evaporation on chamomile indicated that the highest capitulum yield per plant and per area unit and the highest number of capitulum per plant was obtained from the treatment of irrigation after 50 mm accumulative evaporation and they were significantly lower in the treatments of irrigation after 25 and 100 mm accumulative evaporation than in treatment of irrigation after 50 mm accumulative evaporation. Also, significant increase in chamomile capitulum and seed harvest index was reported with the increase in water deficit stress (Pirzad, 2007). A significant reduction in fresh and dry biomass was observed with increasing water stress level in *Jatropha curcas* (Tiwari *et al.*, 2013). Rahmani *et al.* (2012) stated that the maximum petals yield of marigold was achieved by normal irrigation (irrigation according to 40 mm evaporation from class A pan) along with maximum use of nitrogen fertilizer (90 kg N. ha^{-1}).

In study of Shahrbabaki *et al.* (2004) on marigold reported that nitrogen fertilizer had a significant effect on flower harvest index, flower fresh weight per plant, flower fresh and dry yield per hectare, such that maximum flower harvest index (27/90%), flower fresh weight per plant (183 g), flower fresh yield (36723 kg.ha^{-1}) and flower dry yield (4800 kg.ha^{-1}) in marigold were achieved under application of 150 kg N. ha^{-1}. 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 the impact of four N levels of 0, 75, 150 and 225 kg.ha^{-1} on the yield of German chamomile, different N levels were shown to have significant differences in the number of flowers per plant and flower fresh and dry yield per plant and per area unit and that the increase in N level significantly affected the increase in these traits (Hamzeie *et al.*, 2004).

Given the importance of water and N and their numerous functions in living processes of a plant, they are regarded as some of the most important environmental parameters affecting the cultivation and production of the crops. Therefore, the current study was carried out to study the effect of irrigation and N fertilization levels on flower yield and yield components of marigold in Birjand, Iran.

Materials and methods

Study side

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.

Experimental design and treatments

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).

Cultivation

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 April 20 at the depth of 2-3 cm.

In this research, the studied traits included the number of flowers per m^2, flower fresh and dry yield, biological yield, harvest index, single-plant weight. 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 m^2 twenty times during growth period. Then, they were counted to have the number of flowers per m^2 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.

Statistical analysis

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 m^2 and flower fresh and dry weight at 1% level, but single-flower dry weight was affected only by irrigation levels (Table 1). The number of flowers per m^2 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 m^2 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.

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

		Means of squares					
Sources of variation	df	Flower No. per m^2	Single-flower dry weight	Flower fresh yield	Flower dry yield	Biological yield	Harvest index of flower
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.

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.

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

Irrigation (mm accumulative evaporation)	Flower Number per m2	Single-flower dry weight (gr)	Flower fresh yield (kg. ha-1)	Flower dry yield (kg. ha-1)	Biological yield (kg. ha-1)	Harvest index of flower (%)
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

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

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 m^2 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 (Saleem, 2009) and chamomile (Arazmjo *et al.*, 2009), too.

Table 3. Means comparison for yield and yield components of marigold as affected by different levels of nitrogen.

Nitrogen rate (kg N. ha-1)	Flower number per m2	Single-flower dry weight (gr)	Flower fresh yield (kg. ha-1)	Flower dry yield (kg. ha-1)	Biological yield (kg. ha-1)	Harvest index of flower (%)
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 ($P<0.05$).

Means comparison showed that the increase in N fertilization rate from 0 to 180 kg $N.ha^{-1}$ increased the number of flowers per m^2 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 m^2. Also, some researchers have revealed that higher N fertilization levels increase shoot growth and the number of flowers in chamomile (Hamzeie *et al.*, 2004; Zeinali *et al.*, 2008).

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 of flower

Irrigation and N rate significantly affected biological yield and harvest index of flower (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 photosynthesis 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.

Conclusion

Based on the results of the current experiment, 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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Reference

Aboomardani Y, Faraghi A, AsadiAtrakchali MA, Mhafi R. 2010. Study of different nitrogen and plant density levels on yield and harvest index of canola. Proceedings of 3rd International Conference on Oilseeds and Edible Seeds, Tehran, Iran, Dec. 22-23.

Al-Badawy AA, Abdalla NM, El-Sayed AA. 1995. Response of *Calendula officinalis* L. plants to different nitrogenous fertilizers. Horticulture Science **30,** 195-914.

Alizade Sahzabi A, Sharifi Ashorabadi A, Shiranirad AH, Abaszadeh B. 2007. Effect of rates and application methods of nitrogen fertilizer on some quantitative and qualitative traits of *Satureja hortensis* L. Iranian Journal of Medicinal and Aromatic Plants **23(3),** 416-431.

Ameri AA, Nasiriemahalati M. 2008. Effects of different nitrogen and plant density levels on flower and essential oil yield and light use efficiency in marigold (*Calendula officinalis* L.). Journal of Pajoohesh and Sazandegi **81,** 133-144.

Anderson VM. 2013. *Calendula officinalis* and production of secondary compounds in greenhouse and soil-based herbal organic production systems. Theses and Dissertations-Plant and Soil Sciences. Paper 26.
http://uknowledge.ukyedu/pss_etds/26.

Ansarinia E. 2010. Effect of irrigation and nitrogen levels on agronomic traits and quantitative and qualitative yield of sunflower. M.Sc. Thesis, Department of Agriculture, Islamic Azad University, Birjand Branch, Birjand, Iran.

Arazmjo AM, Heydari H, Ahmadian A. 2009. Effect drought stress on quantitative traits and essential oil yield of chamomile. Proceeding of congress of water crisis in agriculture and natural resources, November, Islamic Azad University, Share-rey Branch, Share-rey, Iran.

Arganosa GC, Sosulski FW, Slikard AE. 1998. Effect of nitrogen levels and harvesting management on quality of oil in *Calendula officinalis*. Indian

Perfumer **33(3),** 82-195.

Berimavandi AR, Hashemabadi D, Ghaziani MVF, Kaviani B. 2011. Effects of plant density and sowing date on the growth, flowering and quantity of essential oil of *Calendula officinalis* L. Journal Medicinal Plants Research **5(20),** 5110-5115.

Black CR, Squire GR. 1979. Effects of atmospheric saturation defficit on the stomatal conductance of pearl millet (*Pennisetum typhoides)* and groundnut (*Arachis hypogea* L.). Journal Exprimental Botany **30,** 935-945.
http://dx.doi.org/10.1093/jxb/30.5.935

Fornes, F, Belda RM, Carrion C, Noguera V, Garcia-Agustin P, Abad M. 2007. Pre-conditioning ornamental plants to drought by means of saline water irrigation as related to salinity tolerance. Scientia Horticulturae **113(1),** 52-59.
http://dx.doi.org/10.1016/j.scienta.2007.01.008

Gazim ZC, Rezende CM, Fraga SR, Svidzinski TIE. 2008. Antifungal activity of the essential oil from *Calendula officinalis* L. (Asteraceae) growing in Brazil. Brazilian Journal Microboilogy **39,** 61-63.
http://dx.doi.org/10.1590/S151783822011000300002 0

Hamrick D. 2003. Calendula. In: Hamrick, D. (ed) Ball Redbook crop production, vol 2. 17 edn. Ball Publishing, Batavia, IL, 277-278 p.

Hamzeie R, Majnonhoseyni N, Sharifi Ashorabadi A, Tavakolafshari R. 2004. Study of plant density and nitrogen levels on quantitative and qualitative yield of German chamomile. Proceeding of 8th Iranian crop science congress.

Kishimoto S, Maoka T, Sumitomo K, Ohmiya A. 2005. Analysis of carotenoid composition in petals of Calendula (*Calendula officinalis* L.). Bioscience Biotechnology Biochemistry **69,** 2122-2128.

Miliauskas G, Venskutonis PR, Van Beek TA. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry **85,** 231-237.
http://dx.doi.org/10.1016/j.foodchem.2003.05.007

Mohammad SM, Kashani HH. 2012. Pot marigold (*Calendula officinalis*) medicinal usage and cultivation. Scientific Research and Essays. **7(14),** 1468-1472.
http://dx.doi.org/10.5897/SRE11.630

Mohamadkhani N, Heydari R. 2007. Effects of water stress on respiration, photosynthetic pigments and water content in two maize cultivar. Pakistan Journal of Biological Science **10(22),** 4022-4028.

Muley BP, Khadabadi SS, Banarase NB. 2009. Phytochemical constituents and pharmacological activities of *Calendula officinalis* L. (Asteraceae). Tropical Journal Pharmaceutical Research **8,** 455-465.

Pirzad A. 2007. Effects of irrigation and plant density on some physiological traits and essence of *Matricaria chamomile* L. Ph.D. Thesis, Department of Agriculture, University of Tabriz, Tabriz, Iran.

Pop G, Pirsan P, Mateoc-sirb N, Mateo T. 2007. Influence of technological elements on yield quantity and quality in marigold (*Calendula officinalis* L.) cultivated in cultural conditions of Timisoara. 1th international scientific conference on Medicinal, Aromatic and Spice plant: Nitra, 20-23.

Rahemi M. 2004. Pollination and fruit formation. Publication of Shiraz University.

Rahmani N, Valadabadi SA, Daneshian J, Bigdeli M. 2008. The effects of water deficit stress and nitrogen on oil yield of *Calendula officinalis* L. Iranian Journal of Medicinal and Aromatic Plants **24(1),** 101-108.

Rahmani N, Taherkhani T, Zandi P, Moradi Aghdam A. 2012. Effect of regulated deficit irrigation and nitrogen levels on flavonoid content and extract performance of marigold (*Calendula officinalis* L.). Annals of Biological Research **3(6),** 2624-2630.

Rahmati M, Azizi M, Hasanzadeh Khayyat M, Neamati H. 2009. The effects of different level of nitrogen and plant density on the agro-morphological characters, yield and essential oils content of improved chamomile (*Matricaria chamomilla*) cultivar "Bodegold". Journal of Horticulture Science **23,** 27-35.

Re TA, D Mooney, E Antignac, E Dufour, I Bark, V Srinivasan, G Nohynek. 2009. Application of the threshold of toxicological concern approach for the safety evaluation of calendula flower (*Calendula officinalis*) petals and extracts used in cosmetic and personal care products. Food Chemistry Toxicology **47,** 1246-1254.
http://dx.doi.org/10.1016/j.fct.2009.02.016

Shahrbabaki SMAK, Zoalhasani S, Kodory M. 2013. Effects of sowing date and nitrogen fertilizer on seed and flower yield of pot marigold (*Calendula officinalis* L.) in the Kerman. Advances in Environmental Biology **7(13),** 3925-3929.

Shubhra K, J Dayal, CL Goswami, R Munjal. 2004. Effects of water deficit on oil of *Calendula* aerial parts. Biologia Plantarum **48(3),** 445-448.

Saleem M. 2009. Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. Cancer Lett. **285,** 109-115.

Tiwari N, Purohit M, Sharma G, Nautiyal AR. 2013. Changes in Morpho-Physiology of Jatropha curcas grown under different water regimes. Nature and Science of Sleep **11(9),** 76-83.

Vodnar DC. 2012. Inhibition of Listeria monocytogenes ATCC 19115 on ham steak by tea bioactive compounds incorporated into chitosan-coated plastic films. Chemistry Central Journal **6,** 74-81.
http://dx.doi.org/10.1186/1752-153X-6-74

Wojciak-Kosior M, Matysik G, Soczewinski E. 2003. Investigations of phenolic acids occurring in plant components of Naran N by HPLC and HPTLC densitometric methods. Herba Polonica **49,** 194-201.

Zeinali H, Bagheriekholenjani M, GolparvarJafarpoor MR, Shiranirad AH. 2008. Effect sowing date and nitrogen rates on flower yield and it components in chamomile. Iranian Journal of Crop Science **3(10),** 220-230.