INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 1, p. 242-249, 2014

RESEARCH PAPER OPEN ACCESS

Effects of different fertilizer sources on vegetative characteristics of pot marigold plant (*Calendula officinalis* L.)

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Key words: Flower yield, manure fertilizer, plant height.

http://dx.doi.org/10.12692/ijb/5.1.242-249 Article published on July 11, 2014

Abstract

Medicinal plants are one of very valuable resources in wide range of natural resources of Iran that it can have an important role in health. In order to investigate the effect of nitrogen fertilizer and zinc sulfate on vegetative characteristics of *Calendula Officinalis*, as factorial experiment based on randomized complete block design was carried out with three replications in Takestan, Iran during 2010-2011 cropping season. The experimental factors were consisted of nitrogen fertilizer (manure and urea) with 5 levels (0 tons of manure + 120 kg.ha^{-1}urea, 10 tons of manure + 90 kg.ha^{-1}urea, 20 tons of manure + 60 kg.ha^{-1}urea, 30 tons of manure + 90 kg.ha^{-1}urea and 40 tons of manure + 0 kg.ha^{-1}urea) and zinc sulfate of 3 levels (no spraying, once spraying and twice spraying). The results showed that the effect of nitrogen fertilizer (manure and urea) and zinc sulfate was significant on number of stem sub, leaf dry weight, stem dry weight, capitulum dry weight and flower yield. The highest flower yield was obtained from 20 tons manure + 60 kg.ha^{-1} urea with the amount of 77.96 g.m^{-2}. In the case of zinc Sulfate, was obtained the maximum yield flower with twice spraying, the amount of 78.05 g g.m^{-2}, interaction between treatments had no significant effect on shoot weight and flower yield. The maximum flower yield was obtained from combination treatment of 20 tons of manure + 60 kg.ha^{-1} urea and twice spraying zinc to amount of 90.2 g.m^{-2}.

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Introduction

The use of medicinal plants for treat diseases is concurrence with human history. The purpose of cultivating this plants is create drugs of effective ingredients in flowers and especially in the petals of flowers such as glucoside, mucilage, to copherol, carotenoids which are effective in the treatment of various diseases. Plants need to various nutrients for optimal growth and development, that the lack of these elements in soil is reduced plant growth and production, hence the nitrogen is most effective element in improving the quantitative and qualitative yield of plants (Maleki *et al.,* 2014). Namakha *et al.* (2008) also found that it may be due to optimum and regular supply of nitrogen nutrient to plant from soil during growth period by more assimilation rate and it is integral part of protein the building blocks of plant. Due to environmental conditions, geographical location and growth of medicinal plants, Iran has been considered as one of the best region in the world. Due to the side effects of chemical drugs and with emphasis on one of the agriculture ministry's goals for developing herbs, cultivation of medicinal plants is not only a desirable case, but also it is necessary for our county. *Calendula Officinalis* belongs to Asteraceae family is an annual plant with flowers by coloring yellow to orange, native to Mediterranean region which are use as the active substance in the pharmaceutical industries. The purpose of growing this plant is to produce active substances such as flavonoids, carotenoids, essential oils, triterpene saponins, calenduloside, phytoncids, tannins, resins, slime, glycosides, C vitamin and organic acids (Martin and Deo, 1993). Its medicinal properties include anti-bloating stomach and intestine, stimulating the kidneys, anti-viral, anti-HTV, anti-rnutagentic, anti-inflammatory, anti-spasmotic, and antioxidant. It increases blood flow and cardiac activities. Also used locally in the treatment of skin inflammation, eczema and dry skin. Among these, nitrogen is one of the most effective elements in improving the yield and quality of plants. In between these elements, nitrogen is one of the most effective elements in the improvement of crops quantitative and qualitative yield plants. The numerous results of the impact of this element on different medicinal plants reported such as pot marigold (*Calendula officinalis*) by Ameri *et al.,* (2007), German chamomile (*Chamaemelum nobile*) by Rahmati *et al.,* (2009), that all of them indicate significant effects of nitrogen on the yield and other traits. Organic matters due to improvement of the physical, chemical and biological properties of the soil, so minerals are obtain in the form of soluble, as a result of wear and wash it reduced at least (Manna *et al.,* 2008).

Farm Yard Manure (FYM) is the decomposition of dung and urine of farm animals along with litter and left over material from roughages or fodder fed to the cattle, on an average it contains 0.5% N, 0.2% P and 0.5% K (Verma *et al.*, 2012). Plant growth is dependent on micronutrients in addition to its macronutrient. Zinc is micronutrients that are essential for normal growth and reproduction of plants and also it use in building of proteins and plant hormones such as auxin. Rahimi *et al.,* (2009) in a study on coriander (*Coriandrums ativum*) stated that using of zinc sulfate fertilizer increased the yield of the branches and leaves as compared to control sample. Regarding the numerous importance of pot marigold plant because of its applications such as pharmaceutical, medical and industrial, the presented study have been conducted in order to investigate the effects of spraying with zinc sulfate and for comparison of nitrogen sources on the vegetative growth and yield of pot marigold *(Calendula officinalis*).

Materials and methods

Site description

The present experiment was done as a factorial in randomized complete blocks design with three replications in Takestan, Iran during 2010-2011 cropping season. The experiment field was located in geographical longitude of and 51° 38′ and geographical latitude of 35° 21′ and 927 m sea level.

Experimental details

The experimental factors including nitrogen fertilizer

sources (manure and urea) with 4 levels (control (zero tons manure) +120 kg.ha^{-1} urea, 10 tons manure + 90 kg.ha^{-1} urea, 20 tons manure + 60 kg.ha^{-1} urea, 30 tons manure + 90 kg.ha^{-1} urea and 40 tons manure + without application of urea fertilizer) and zinc sulfate with 3 levels including no spraying, once spraying and twice spraying. Plant density was obtained about 6 plants.m^{-2}. Some of the relevant soil properties of the research field and the climatic properties of region are presented in Table 1 and 2.

Considering the analysis of manure, the content of nitrogen in the fertilizer was about 2.87%. Zinc sulfate fertilizer with purity (35%) was used at a rate of 5 kg.ha^{-1} in 1000 liters water. Manure and half of urea after the first irrigation and before the second irrigation was added to the ground. Spraying of zinc sulfate was performed after two weeks from the first fertilization (02/20/2011). After the second sampling (4/20/2011), the residual urea fertilizer was added to farm, then spraying of zinc sulfate was done at third level (twice sprayings).

Crop sampling and calculation

Sampling was performed from 2 rows in the middle of each plot. After drying samples, operation weighing was performed by using a detailed micro-balance instrument. Then average of dry weight of the leaves, stems, capitulum and flower yield g.m^{-2} was calculated.

Statistical analysis

Ultimately analysis of variance (ANOVA) was done using SAS 9.1 statistical software (Naseri *et al.,* 2013). Mean comparison was also conducted with Duncan's Multiple Range Test (DMRT).

Results and discussion

The results of the analysis variance show that the application of different treatments (nitrogen fertilizer sources (urea +manure) and zinc sulfate) have significant effects on the different vegetative growth characteristics of pot marigold (*Calendula Officinalis*) which consist of the number of lateral branches, weight of leaf, weight of stem, weight of capitulum and flower yield but the interaction between nitrogen fertilizer sources and zinc sulfate have significant effect only on the dry weight of stem and flower yield (Table 3).

Table 1. Chemical and physical characteristics: from zero to 30cm soil depth of field.

Sand (%)	Silt (%)	Clay (%)	Zinc (ppm)	K (ppm)	P (ppm)	Total N (%)	O.C (%)	T.N.V (%)	pH	EC (ds/m)
52	30	18	0.7	380	9	0.06	0.64	3	7.7	0.913

Table 2. The climatic properties of region during performance of study in 2010-2011 cropping season.

Months	April	may	June	July	August
Monthly mean of minimum temperature (°C)	14	17.4	25.1	25.1	26.1
Monthly mean of maximum temperature (°C)	21	25	28	29.1	30.1
Monthly mean of temperature (°C)	14.1	19.2	20.2	22.3	26
Monthly mean of precipitation (mm)	36.1	25.3	2.3	0	0

In most cases, application of the different fertilization treatments improves vegetative growth and resulted in significant increases in the values recorded for the different growth parameters, compared to the unfertilized control plants. The favorable effect of the nitrogen fertilization treatments on the vegetative growth characteristics (compared to the control) can be explained by the important role of N in the different physiological processes and also the presence of nitrogen in the structure of protein molecules within the plants (Hussein *et al.,* 2011).

Plant height

The results of this study show that none of the

experimental treatments has significant effect on the final height of pot marigold plants (Table 4). Similar results were observed by Rahmati *et al.*, (2009) in pot marigold.

Dadkhah *et al.*, (2012) expressed that consumption of fertilizers by stimulate plant growth and better establishment of plants, leads to the increase in morphological characters such as plant height of matricaria (*Matricaria chamomilla*).

Table 3. Analysis of variance for studied traits in pot marigold plant.

		MS					
S.O.V	d.f	Plant height	Number of sub stem	Stem dry Weight	Leaf dry weight	Capitulum dry weight	Flower yield
Replication	2	ns0.31	34.8 ns	533 ns	ns433.2	ns1014	76.06*
Nitrogen source	4	ns7.58	108.1*	1974**	4725**	2026 **	138 **
Zinc	2	ns3.59	105.4*	7018**	1820**	1926*	435**
Nitrogen *Zinc	8	ns4	ns25.7	931 *	ns213	ns135	45.9 *
Residual	28	6.4	9.28	399	121.5	424	19.5
CV (%)	-	10	13.84	7.06	4.69	13.29	6.14

*: Significant at 0.05 level, **: Significant at 0.01 level.

Table 4. Simple and interaction effects for plant height, number of sub stem and weight dry leaf in pot marigold plant.

Nitrogen source					
Urea fertilizer ($kg.ha^{-1}$)	Manure ($kg.ha^{-1}$)	Zinc sulfate	Plant height (cm)	Number of sub stem	Leaf dry weight
120	0		25.1 a	34.1 b	205.05 b
90	10		24.9 a	37.2 b	215.59 b
60	20		27.08 a	43.5 a	248.82 a
30	30		24.9 a	39.4 ab	257.51 a
0	40		25.3 a	39.8 ab	246.36 a
		non-use	24.9 a	36.3 b	223.2 c
		1 time spray	25.9 a	38.6 ab	235.62 b
		2 time spray	25.6 a	41.6 a	245.17 a
120	0	non-use	23.8	33.6	191.40
90	10		24.2	33.8	208.60
60	20		27.9	37.7	230.03
30	30		23.9	37.4	244.42
0	40		24.9	39.3	241.59
120	0	1 time spray	27.0	33.5	207.85
90	10		26.3	36.0	211.75
60	20		26.4	42.1	243.34
30	30		24.5	39.9	263.09
0	40		25.4	41.6	252.08
120	0	2 time spray	24.6	35.5	215.90
90	10		24.3	41.8	226.44
60	20		27.0	50.9	273.10
30	30		26.4	41.1	265.03
0	40		25.9	38.8	245.41

Means, in each column, followed by similar letter are not significantly different at the 5% probability level- using Duncan's Multiple Range Test.

Number of sub stem and capitulum dry weight

The comparison of means showed that highest values for The capitulum dry weight and number of lateral branches (182.1 $g.m^{-2}$ and 43.5 stem/plant) were obtained from plants that received 20 ton/ha of manure fertilizer + 60 $kg.ha^{-1}$ of urea fertilizer and the plants were sprayed twice with zinc (41.65stem/plant, 179.7 $g.m^{-2}$) compared with control plants. Zinc has

effective roles to synthesize proteins and increases pollination, which finally can increase weight of the reproductive organs of plant. According to our results, also zinc sulfate increases reproductive organs of the plant such as capitulum dry weight. Pirzad *et al.,* (2013) reported that spraying of iron and zinc has significant effect on the number of sub branches of anise (*Pimpinella anisum*).

Table 5. Simple and interaction effects for weight dry stem and capitulum and yield flower in pot marigold plant.

			Nitrogen source		
Urea fertilizer (kg.ha^{-1})	Manure (kg.ha^{-1})	Zinc sulfate	Stem dry weight (g.m^{-2})	Capitulum dry weight (g.m^{-2})	Flower yield (g.m^{-2})
120	0		266 c	145.9 c	67.41 c
90	10		276.62 bc	158.6 bc	70.33 bc
60	20		304.63 a	182.2 a	77.96 a
30	30		289.45ab	176.5 ab	73.14 b
0	40		276.77bc	175.0 ab	70.97 bc
		non-use	264.93 b	157.2 b	67.79 b
		1 time spray	276.37 b	166.0 ab	70.05 b
		2 time spray	306.78 a	179.7 a	78.05 a
120	0	non-use	244.33 d	135.4	64.90 d
90	10		268.08 b-d	141.5	64.26 d
60	20		266.69 b-d	166.8	70.16 b-d
30	30		282.00 b-d	168.9	72.40 b-d
0	40		263.56 cd	173.6	67.22 cd
120	0	1 time spray	268.33 b-d	145.3	63.82 d
90	10		258.34 cd	158.0	68.64 cd
60	20		287.57 bc	177.3	73.53 bc
30	30		289.76 bc	177.5	72.15 b-d
0	40		277.89 b-d	171.7	72.11 b-d
120	0	2 time spray	285.33 bc	156.9	73.50 bc
90	10		303.46 b	176.2	78.09 b
60	20		359.66 a	202.6	90.20 a
30	30		296.61 bc	183.1	74.86 bc
0	40		288.88 bc	179.8	73.59 bc

Means, in each column, followed by similar letter are not significantly different at the 5% probability level- using Duncan's Multiple Range Test.

Weight dry leaf

As shown in Table 4, comparison of the data average as compared with control plants show that highest values for leaf dry weight (257.5 g.m^{-2}) was obtained from plants that received 30 ton/ha of manure fertilizer + 30 kg.ha^{-1}of urea fertilizer and plants that were spraying twice with zinc (245.1 g.m^{-2}). Mehr-Afarin *et al.,* (2011) stated the fertilizer treatments have the significant impact on the leaf fresh and dry weight in peppermint (*Mentha piperita*). In study the coriander (*Coriandrum Sativum*), Rahimi *et al.,* (2009) observed significant increase in dry matter of leaf by using the fertilizer containing nitrogen and zinc as compared to the control samples. Probably the high values of the leaf dry matter in the control samples might be due to improvement in

specifications ob physical, chemical and biological soil and also the easier absorption of water and the minerals nutrient by the plants with the use of manure. Naz-Ebrahimian *et al.,* (2011) stated that due to the zinc deficiency in the plants, the leaf chlorophyll concentration and the photosynthetic rates is decreased and consequently the leaf production is reduced. Also interaction effect of treatments was significant on stem dry weight.

Stem dry Weight and Flower yield

The treatments samples that received 20 ton manure + 60 kg.ha^{-1} urea + Twice Spraying with zinc sulfate show the highest of stem dry weight (359.6 g.m^{-2}) and flower yield (90.1 g.m^{-2}).The significant interaction effect of treatments indicates that the application of twice spraying of zinc obtain required zinc on *sorghum bicolor*, the effect of applying the same quantities of the nitrogen sources disappeared so that the highest values of this traits obtained in 20 ton manure + 60 kg.ha^{-1} urea, therefore in case of the supply of the requirement zinc, the deficiency of the manure can not caused a significant reduction effect on these two traits and thus can prevent it from the more consuming of the nitrogen sources. Shivay and Singh (2000) reported that highest plant height, LAI and dry matter accumulation were recorded with 120 kg N h. Economic yield of medicinal plant "pot marigold" is the dry flower rate per unit area, thus, for management the use of fertilizer must be such that obtained the maximum economic yield. Similar findings reported by Ameri *et al.,* (2007) in German chamomile (*Chamaemelum nobile*) and pot marigold *(Calendula officinalis*), respectively. The important point in this case is the maintaining of appropriate balance between the vegetative and the reproductive growth related to pot marigold due to its indeterminate growth. It seems that reduction of the flowering shoot yield through excessive consumption of the nitrogen could be due to the decreasing of the minerals stress. Dadkhah *et al.,* (2012) reported that increasing the flower yield of chamomile due to use of fertilizers (nitrogen and phosphorus) is the resulting effect of the increasing morphological traits such as the plant height, the number of main stem, the lateral branches and subsequently increasing of the number of flowers per plant and reducing competition between plants due to the presence of nutrients. Saleem *et al.* (2009) found that the maximum dry matter accumulation per plant was the positive effect of growth characters; significantly more value was recorded with 150 N2O kg ha^{-1}.

Conclusion

Overall, results of this study show the positive effects on vegetative properties and also increasing the flower yield of pot marigold is related to the use of moderate values of nitrogen sources (the combination of manure fertilizer and urea fertilizer) and the use of twice spraying of zinc sulfate.

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