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Research article

Evaluation of Yield, Yield Component, and Essential Properties of Pot Marigold (*Calendula officinalis* L.) under Water Stress and Urea

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Abstract

To investigate the effect of irrigation and urea levels on some agronomic traits and the essential oil content of pot marigold, a study was conducted in a split-plot based on a completely randomized block design with three replications. The treatments included four levels of irrigation (irrigation after 5, 10, 15, and 20 days) and four levels of nitrogen (0, 80, 160, and 240 kg ha^{-1} of urea). The plant height, flower number, flower dry weight, number of seeds/head, thousand kernel weight, biological yield, flower essence percentage, and essence yield were measured in pot marigold under different irrigation and nitrogen levels. The results revealed that water stress, nitrogen, and their interaction had a significant effect on all investigated traits. The highest number of flowers/m^2, number of seeds/head, thousand kernel weight, biological yield, and grain yield, were observed under simultaneous application of 5-day irrigation interval and 80 kg ha^{-1} of urea. Furthermore, the maximum flower essence percentage was obtained under the combined application of a 20-day irrigation interval and 80 kg ha^{-1} of urea. Nonetheless, the highest essence yield was obtained at 5, 15, and 15-day irrigation intervals and 80 kg ha^{-1} of urea. It was concluded that water stress and nitrogen can affect the yield and secondary metabolite production in pot marigold. Nonetheless, the highest essence yield can be produced under a 15-day irrigation interval and 80 kg ha^{-1} of urea. However, the highest biological yield and grain yield can be obtained at 5-day irrigation interval and 80 kg ha^{-1} of urea in pot marigold.

Keywords

flower dry weight;

essence;

nitrogen;

pot marigold;

water deficit

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1. Introduction

Pot marigold (*Calendula officinalis* L.), which belongs to the family of Asteraceae, is an annual perennial plant. Its propagation is done by sowing seeds in spring. Pot marigold or Calendula is known as a medicinal plant, and its main essential oil compound is α-cadinol, which has antioxidant, antibacterial, anti-inflammatory, antiviral, anti-HIV, anti-tumor, anti-mutagenic, and cytotoxic properties [1]. Oxygenated sesquiterpenes are the most represented natural compounds contributing to the chemical composition in its oil [2].

Nonetheless, terpenoids, phenolic acids, flavonoids, isorhamnetin, carotenoids, glycosides, vitamins, and sterols are the most important constituents of pot marigold [3]. Through the growing period, it requires great solar radiation and can well tolerate drought, but it is sensitive to high soil moisture [4]. However, its growth and essential oil production can be affected by abiotic and biotic factors in plants [5-9]. Nitrogen deficiency is very common in arid and semiarid regions due to the lack of organic matter in the soils, which is the main source of soil nitrogen.

Fertilizers play a significant role in enhancing the biomass yield of wild pot marigold, with a percentage increase of even more than 50% [10]. It has been reported that higher or lower levels of optimum nitrogen can improve plant growth and development [11]. The maximum biomass and grain yield of rosemary were recorded with irrigation disruption at the second and third harvest stages [12]. Nitrogen application significantly increased the yield and secondary metabolites in fenugreek [13, 14]. It was reported that higher yield and essential oil production in Greek oregano were found for the fertilization with higher nitrogen amount (150 kg ha^{-1}) [10].

The highest petal yield in pot marigold was observed under normal irrigation with an application of 90 kg ha^{-1} urea [15]. Furthermore, the highest and the lowest number of flowers/m^2 and single flower weight were reported under irrigation after 60 mm and 180 mm accumulative evaporation treatments in bergamot mint, respectively [16]. They also found that the number of flowers/m^2, and fresh and dry weight of flowers were significantly raised by increasing the level of nitrogen fertilizer from 0 to 180 kg ha^{-1} of urea. Therefore, the purpose of this study was to evaluate the response of yield, yield components and essential properties of pot marigold under different levels of nitrogen fertilizer and irrigation regimes.

2. Materials and Methods

To investigate the effect of drought stress and nitrogen on some agronomic traits and essential oil content of pot marigold (Calendula), an experiment was conducted at Agricultural Research Station of Saat Lo -Urmia, Iran (37.53°N, 45.08°E, and 1320m above sea level) in 2016-2018.

A field experiment was carried out in a split-plot based on a completely randomized block design with three replications. Treatments included four levels of irrigation regimes (irrigation after 5, 10, 15, and 20 days) assigned as the main plots, while nitrogen levels (0, 80, 160, and 240 kg ha^{-1} from urea) were assigned as the subplots. The soil physical and chemical properties of the experimental site are shown in Table 1. According to the results of soil analysis, 150 kg ha^{-1} triple superphosphate and 100 kg ha^{-1} potassium sulfate were added to soil at the soil preparation stage. Seeds were planted in 6 rows of 6 m at a distance of 50 cm from each other and with a planting depth of 2 to 3 cm. Planting was done manually on March 16th, 2016, 2017, and 2018. To guarantee uniformity and facilitate seed germination, the first irrigation was done immediately after planting. At the 4-6 leaves stage, the plants were thinned to reach the desired density (50 plants per square meter) (distance between plants was 10 cm). Manual weeding was done in three stages; after emergence, at the 4-6 leaves stage, and at the same time as canopy closure. Also, nitrogen fertilizer was used at two stages (half after thinning and another half before the start of flowering). It should be noted that the amount of water given in the whole growth period under the irrigation treatment after 5, 10, 15, and 20 days was equal to 12500, 10300, 7500, and 4400 m3 ha^{-1}, respectively. A drip irrigation system was implemented. Drought stress was applied after plant establishment (stage 4 or 5 leaves).

The plant height, number of seeds/ head, thousand- grain weight, the number of flowers/ m^2, flower dry yield, biological yield, and grain yield were measured. For this purpose, the samples were collected from the two middle rows of each trial plot from an area of 3 m^2 during the growth period.

Table 1. Soil physical and chemical characteristics at the experimental site

Salinity dS m^{-1}	Saturation%	Lime%	Clay%	Sand%	Organic matter (%)	Available potassium (ppm)	Available phosphorous (ppm)	Total nitrogen (%)
1.3	49	17	31	25	1.1	335	4.8	0.11

To extract essential oils, flowers were gathered from each treatment at the flowering stage, air-dried and weighed, and the ground samples (25 g) were distilled using 300 ml distilled water by Clevenger standard method for 3 h [12]. Essential oil yield was calculated as per the following formulae:

$$\text{Essential oil yield (kg ha}^{-1}\text{)} = \text{Essential oil percentage} \times \text{Flower yield (kg ha}^{-1}\text{)}$$

Statistical analysis: The performed statistical analyses included the Shapiro-Wilk normality test, and analysis of variance, carried out with SAS 9.2 and SPSS19 software. A comparison of mean treatments was conducted with the SNK test at 5% probability level.

3. Result and Discussion

3.1 Water stress and urea treatment significantly affected the plant height in pot marigold

According to the results of the analysis of variance, plant height was significantly influenced by irrigation regimes, nitrogen levels, and their interaction (Table 2). Simultaneous application of nitrogen and water deficit had a significant effect on plant height. In this respect, applying 160 kg ha^{-1} of urea with a 5-day irrigation interval had the highest significant effect on the plant height compared to control (Table 3). The lowest plant height was observed under a 20-day irrigation interval with an application of 240 kg ha^{-1} of urea. The influence of diminished irrigation on the growth of the plant can be associated with the lower availability of adequate moisture in the rhizosphere, lower nutrient uptake, reduction in cell vitality and cellular extension, and the leading in a decline of photosynthesis limitation and plant growth [17]. Based on the results, the application of nitrogen fertilizer had a positive effect on plant height. Higher nitrogen fertilizer levels caused a liner extension of the stem by increasing the vegetative growth phase and providing the required assimilates. Furthermore, it has been stated that nitrogen insufficiency can limit plant height by hindering parenchyma and sclerenchyma development, but nitrogen sufficiency can enhance plant height by expanding the meristem cells division and turgidity. In agreement with our results, it was demonstrated that salt stress, water deficit and nitrogen deficiency decreased plant height in pot marigold, pepper, lettuce and bergamot mint [15, 16, 18-20].

Table 2. Analysis of variance of irrigation regime and nitrogen levels on studied traits in medicinal Calendula plant

		MS								
	DF	**Plant height (cm)**	**Flower number per m^2**	**Flower dry weight ($g\ m^{-1}$)**	**Number of seeds per head**	**Thousand kernel weight (g)**	**Bio-logical yield ($kg\ ha^{-1}$)**	**Grain yield ($kg\ ha^{-1}$)**	**Flower essence percentage**	**Essence yield ($kg\ ha^{-1}$)**
Year	1	216.08^{ns}	1797^{ns}	1.74^{ns}	16.66^{ns}	0.01^{ns}	2604.17^{ns}	65612^{ns}	0.0009^{ns}	6.77^{ns}
E1	4	76.81	6636.12	1.28	226.08	0.086	5066.67	433844	0.018	22.99
Irriga-tion	3	162.11**	134653**	18.30**	174.11**	59.81**	484600**	15510**	0.0045**	33.56**
Y×IR	3	15.68^{ns}	2.56^{ns}	1.56^{ns}	8.11^{ns}	0.01^{ns}	16250^{ns}	8090^{ns}	0.0008	0.08
Nitro gen	3	30.47*	4465.11**	9.02**	36.28**	11.45**	173400**	1138886**	0.009**	119.13**
Y×N	3	46.91**	32.11^{ns}	1.8^{ns}	3.29^{ns}	0.01^{ns}	46805^{ns}	2033^{ns}	0.0009^{ns}	0.02^{ns}
I×N	9	28.06*	1343.52**	0.52^{ns}	16.85**	11.45**	372745**	251894**	0.001**	25.69**
IR×N IT×Y	9	17.20^{ns}	32.25^{ns}	1.11^{ns}	3.03^{ns}	0.01^{ns}	7453.70^{ns}	5500^{ns}	0.0002^{ns}	0.05^{ns}
E2	60	10.03	552.11	1.18	3.50	1.71	7157.78	67922.3	0.0005	5.19
CV	-	13.18	9.55	20.42	17.88	9.90	17.58	11.40	9.47	19.57

ns, *, and ** were no significant, significant at level 1, and 5%, respectively.

Table 3. The effect of irrigation regimes and nitrogen levels on studied traits in medicinal Calendula plant

Treatment	**Plant height (cm)**	**Flower number per m^2**	**Flower dry weight ($g\ m^{-1}$)**	**Number of seeds per head**	**Thousand kernels weight (g)**	**biological yield ($kg\ ha^{-1}$)**	**grain yield ($kg\ ha^{-1}$)**	**Flower Essence percentage**	**Essence yield ($kg\ ha^{-1}$)**
Irrigation regimes									
5 Days	34.53^{a}	313.09^{a}	6.06^{a}	27.08^{a}	14.56^{a}	12638^{a}	2972.2^{a}	0.20^{d}	12.40^{a}
10 Days	34.30^{a}	269.14^{b}	6.09^{a}	27.91^{b}	13.76^{b}	12067^{b}	2656.4^{b}	0.21^{c}	12.88^{a}
15 Days	30.47^{b}	263.37^{b}	4.78^{b}	21.75^{c}	13.52^{b}	10336^{c}	2382.0^{c}	0.23^{a}	10.91^{b}
20 Days	29.47^{b}	138.79^{c}	4.40^{b}	21.33^{c}	10.97^{c}	9621^{d}	1136.3^{d}	0.22^{b}	10.39^{b}
Urea levels									
0	30.84^{c}	230.13^{c}	4.60^{b}	22.08^{c}	13.98^{a}	10204^{c}	2053.8^{b}	0.20^{d}	9.18^{b}
80 $kg\ ha^{-1}$	31.92^{bc}	263.11^{a}	5.78^{a}	24.75^{a}	13.99^{a}	11725^{a}	2502.4^{a}	0.24^{a}	13.98^{a}
160 $kg\ ha^{-1}$	33.55^{a}	248.14^{b}	5.89^{a}	24.58^{ab}	13.23^{a}	12017^{b}	2439.3^{a}	0.22^{b}	13.00^{a}
240 $kg\ ha^{-1}$	32.46^{ab}	243.02^{bc}	5.07^{b}	23.58^{b}	12.30^{b}	10713^{b}	2151.5^{b}	0.20^{c}	10.41^{b}

ns, *, and ** were no significant, significant at level 1, and 5%, respectively.

3.2 Water stress and urea treatment significantly affected flower number/m^2 in pot marigold

The results gained from variance analysis showed that the irrigation regimes and nitrogen levels had a significant effect on flower number/m^2 (Table 2). Based on the results, water deficit significantly reduced flower number/m^2, but nitrogen application up to 80 $kg\ ha^{-1}$ of urea increased it and after that, it decreased (Table 3). The highest flower number/m^2 was observed under the simultaneous application of 80 $kg\ ha^{-1}$ of urea with 5-day irrigation interval. The lowest flower number/m^2 was recorded under 20-day irrigation interval without nitrogen application, while the simultaneous application of nitrogen fertilizer with 20-day irrigation interval increased it (Table 3). The decrease in the number of flowers/m^2 under drought stress can be associated with a decline in leaf area, and subsequently a loss in the assimilates. Therefore, it can be concluded that nitrogen application under water deficit can increase flower number/m^{-2} in pot marigold. In harmony with our findings, it was found that nitrogen application increased flower number/m^{-2} in pot marigold [4].

3.3 Water stress and urea treatment significantly affected flower dry weight in pot marigold

The results of the variance analysis indicated that water deficit and nitrogen levels had a significant effect on flower dry weight (Table 2). The result showed water deficit significantly reduced flower dry weight. The highest flower dry weight was found under irrigation levels after 5 and 10 days with an average of 6.06 and 6.09 g m^{-2}, while the lowest one was recorded from irrigation levels after 15 and 20 days with 4.78 and 4.40 g m^{-2} (Table 3). This can be related to a reduction in the flowering phase, the unfavorable effect of water deficit on photosynthesis, and a decline in assimilating translocation to flowers. In agreement with our results, it was demonstrated that water deficit markedly decreased flower dry weight in pot marigold [16].

Nitrogen application (80 and 160 kg ha^{-1} of urea) under water deficit conditions significantly increased flower dry weight compared to control. However, higher doses of nitrogen (240 kg ha^{-1} of urea) did not have a significant effect on flower dry weight compared to control. Similarly, it was found that nitrogen application significantly increased flower dry weight in pot marigold [21].

3.4 Water stress and urea treatment significantly affected number of seeds/ head in pot marigold

As presented in Table 2, it was found that water deficit and nitrogen application had a significant effect on the seeds/head. Water deficit significantly decreased the number of seeds/head in pot marigold, but nitrogen application increased the number of seeds/head. Furthermore, the simultaneous application of 80 kg ha^{-1} nitrogen fertilizer with all irrigation regimes had a positive effect on grain number (Table 4).

The results revealed that the highest grain number (30.66) was observed under 80 kg ha^{-1} nitrogen level and irrigation after 5 days, and the lowest amount was observed under irrigation after 20 days without nitrogen application (Table 4). It had been stated that the number of seeds/head in *Nigella Sativa* increased with a reduction in irrigation intervals [22]. In harmony with our results, the maximum number of seeds/head in Calendula was found under irrigation after 40 mm evaporation (31seeds/head) and application of 90 kg ha^{-1} of urea [23].

3.5 Water stress and urea treatment significantly affected thousand-grain weight in pot marigold

Based on the results of variance analysis, it was indicated that water deficit, nitrogen levels, and their interaction had a significant effect on the thousand-grain weight (Table 2). In this respect, water deficit significantly, and higher doses of nitrogen, decreased the thousand-grain weight (Table 3). The highest thousand-grain weight of 16.53 g was gained from the application of 80 kg ha^{-1} nitrogen fertilizer under irrigation after 5 days; however, the lowest amount of thousand-grain weight was observed in plants treated with 240 kg ha^{-1} nitrogen fertilizer and irrigation after 20 days (Table 4). In the present study, under water deficit treatment, the application of 80 kg ha^{-1} nitrogen fertilizer had the most positive effect on grain weight. Similarly, it was found that the maximum thousand seed weight was achieved under irrigation after 40 mm evaporation and application of 90 kg ha^{-1} nitrogen fertilizer in bergamot mint [23].

Table 4. Mean comparison of the interactive effects of irrigation and nitrogen levels on studied traits of Calendula

Irrigation regimes	Urea levels	Plant height (cm)	Flower number per m^2	Flower dry weight ($g\ m^2$)	Number of seeds per head	Thousand kernels weight (g)	Biological yield ($kg\ ha^{-1}$)	Grain yield ($kg\ ha^{-1}$)	Flower Essence percentage	Essence yield ($kg\ ha^{-1}$)
5 Days	0	28.78^{gh}	283.97^{b-e}	5.53^{a}	24.66^{cd}	12.46^{d}	11283^{bcd}	2596.4^{cde}	0.18^{h}	10.61^{def}
	80 kg ha^{-1}	35.23^{bcd}	351.45^{a}	6.46^{a}	30.66^{a}	16.53^{a}	13800^{a}	3271.1^{a}	0.22^{c}	14.41^{ab}
	160 kg ha^{-1}	37.88^{a}	309.06^{b}	6.84^{a}	27^{b}	15.37^{ab}	14067^{a}	3277.3^{a}	0.20^{ef}	13.75^{abc}
	240 kg ha^{-1}	36.23^{b}	307.89^{bc}	5.43^{a}	25.66^{bc}	14.23^{b}	11400^{bc}	2743.9^{cd}	0.20^{fg}	10.82^{de}
10 Days	0	33.41^{de}	253.35^{f}	5.21^{a}	21.66^{efg}	12.53^{cd}	10733c-f	2273.5^{f}	0.20^{fg}	10.44^{def}
	80 kg ha^{-1}	33.75^{cd}	290.27^{bcd}	6.60^{a}	26.36^{bc}	15.10^{ab}	11800^{b}	3076.1^{ab}	0.24^{b}	16.09^{a}
	160 kg ha^{-1}	35.78^{abc}	268.60^{def}	6.88^{a}	23.00^{de}	14.36^{b}	14500^{a}	2892.2^{bc}	0.19^{fgh}	13.65^{abc}
	240 kg ha^{-1}	34.25^{bcd}	264.34^{def}	5.69^{a}	22.36^{def}	14.00^{bc}	11733^{b}	2383.4^{ef}	0.20^{fgh}	11.34^{cde}
15 Days	0	30.63^{fg}	261.50^{ef}	3.81^{a}	22.33^{ef}	12.10^{d}	9933^{fg}	2355.0^{ef}	0.19h	7.57g
	80 kg ha^{-1}	28.98^{gh}	264.34^{def}	5.47^{a}	25.66^{bc}	14.26^{b}	10933^{b-e}	2582.2^{ef}	0.26a	13.57^{abc}
	160 kg ha^{-1}	31.00^{fg}	280.89^{cde}	5.02^{a}	19.66^{g}	11.93^{d}	10943^{b-e}	2383.4^{ef}	0.24b	12.37^{bcd}
	240 kg ha^{-1}	31.30^{ef}	246.75^{f}	4.83^{a}	25.63^{bc}	14.69^{b}	10133^{efg}	2207.5^{f}	0.21de	10.15^{d-g}
	0	30.53^{fg}	121.68^{h}	3.85^{a}	19.36^{g}	10.03^{e}	8867^{h}	990.1^{g}	0.19^{h}	8.11^{fg}
20 Days	80 kg ha^{-1}	29.78^{fgh}	146.37^{gh}	4.61^{a}	23.31^{de}	12.13^{d}	10367^{def}	1080.3^{g}	0.25^{ab}	11.87^{b-e}
	160 kg ha^{-1}	29.53^{fgh}	134.03^{gh}	4.83^{a}	22.01^{ef}	11.36^{de}	9867^{fg}	1203.7^{g}	0.24^{b}	12.25^{bcd}
	240 kg ha^{-1}	28.06^{h}	153.10^{g}	4.32^{a}	20.66^{fg}	10.36^{e}	9383^{gh}	1271.0^{g}	0.21^{de}	9.34^{efg}

In each column, averages with same superscript letters do not have a significant difference at the 5% level.

3.6 Water stress and urea treatment significantly affected biological yield in pot marigold

As seen in Table 2, the water deficit, nitrogen levels, and their interaction significantly affected biological yield. The maximum biological yield (Table 3) was recorded under 160 kg ha^{-1} nitrogen fertilizer and irrigation after 5 and 10 days, and 80 kg ha^{-1} nitrogen fertilizer under irrigation after 5 days. However, the minimum one was gained from the plants subjected to irrigation after 20 days. The results also showed that the use of 80 and 160 kg ha^{-1} nitrogen fertilizer was able to moderate the adverse impact of drought stress on biological yield compared to control treatment. The decline in biomass production by water deficiency can be connected with a decline in plant height and leaf area, and the rise in the partitioning of assimilates to roots rather than shoots. Ordinarily, it can be described that water deficit reduced the grain and biological yield of pot marigold by reducing the growth phase, and it resulted in a decrease in photosynthesis rate, the assimilation phase, and the mobilization of assimilates. Similarly, it was demonstrated that the biological yield under irrigation after 120 mm accumulative evaporation mm was limited by 11.5% compared to irrigation after 60 mm accumulative evaporation in pot marigold [16]. The increase in the dry matter by nitrogen fertilizer treatments might be due to an increase in uptake of all the nutrients which have a crucial role in better plant growth.

3.7 Water stress and urea treatment significantly affected grain yield in pot marigold

Based on the results, water deficit, nitrogen levels, and their interaction had a significant impact on the grain yield (Table 2). Water deficit significantly decreased grain yield in absence of nitrogen treatment, but in absence of water stress, application of nitrogen up to 160 kg ha^{-1} of urea increased it and after that, it decreased.

The maximum grain yield was gained from the use of 80 and 160 kg ha^{-1} nitrogen fertilizer under irrigation after 5 days by 3271.1 and 3277.3 kg ha^{-1}, respectively (Table 4). In the present research with increasing irrigation intervals, grain yield reduced significantly, but under irrigation after 10 days, the application of 80 and 160 kg ha^{-1} was able to moderate the effects of water deficit and was able to increase grain yield by 30.35 and 21.27 percent compared to the plants treated with irrigation after 10 days. It should be noted that the application of nitrogen fertilizer at irrigation intervals after 15 and 20 days did not have a significant effect on grain yield increase. The increase in grain yield can be assigned to the effect of nitrogen on vegetative growth, increase in the canopy, and consequently, better use of solar radiation and higher photosynthesis in excellent irrigation situations [24].

3.8 Water stress and urea treatment significantly affected flower essence percentage in pot marigold

The results of this study indicated that drought stress, nitrogen levels, and their interaction had a significant effect on the flower essence percentage (Table 2). The result showed that drought stress significantly increased flower essence percentage (Table 3). The highest flower essence percentage was observed with the application of 80 kg ha^{-1} of urea, and 15-day irrigation interval. The lowest one was recorded from the plants treated with 5-day irrigation interval, and control (Table 4). Similarly, it was found that the essential oil content of dill increased significantly when exposed to water deficit during the vegetative growth stage [25]. This can be associated with the role of secondary metabolites that play a protective role in plants against water stress. Furthermore, it was demonstrated that the nitrogen fertilizer had a positive effect on the improvement and increase of essential oil percent in *Origanum syriacum* and *Ocimum americanum* [26, 27].

3.9 Water stress and urea treatment significantly affected essence yield in pot marigold

Based on the results of variance analysis, irrigation levels, fertilizers, and their interaction had a significant effect on essence yield (Table 2). As presented in Table 4, the maximum essence yield was gained with the utilization of 80 kg ha^{-1} of urea and 10-day irrigation interval. However, the lowest essence yield was observed in the plants treated with 15-day irrigation interval, without nitrogen application.

Nonetheless, water stress decreased essence yields significantly. However, in the presence of water stress, the application of nitrogen fertilizer (80 and 160 kg ha^{-1} of urea) moderated the adverse effects of water deficit on essence yield. Water stress in plants may cause physiological disturbances such as a decrease in photosynthesis and transpiration that can decrease plant performance [28]. In harmony with our results, it has been reported that the water deficit decreased the oil yield of rosemary, and this can be related to the positive effect of the application of nitrogen fertilizer on growth characteristics, photosynthetic pigment content, and flower yield [29].

4. Conclusions

According to the results of this study, it can be stated that nitrogen fertilizer application is effective when the plant has access to sufficient water. However, an increase in the application of nitrogen levels under water shortage conditions can minimize plant growth. Considering the limitations of water in the semi-dry area, the 10-day irrigation interval and application of 80 kg ha^{-1} of urea can lead to maximum plant performance in pot marigold (Calendula).

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