

Effect of Cycocel and Daminozide on Vegetative Growth, Flowering and the Content of Essence of Pot Marigold (*Calendula officinalis*)

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Pot marigold (*Calendula officinalis* L.) is a medicinal and ornamental plant. The effect of different concentrations of chlormequat (cycocel), and daminozide, two plant growth retardant, on plant height, flowering, the content of essence and some other traits in pot marigold (*Calendula officinalis*) was assessed. Plant growth retardants are commonly applied to limit stem elongation and produce a more compact plant. The experiment was done as a factorial in randomized completely blocks design (R.C.B.D.) with 16 treatments and 3 replications in Rasht. Cycocel at 4 concentrations (0, 500, 1000 and 1500 mg/L) and daminozide at 4 concentrations (0, 1500, 3000 and 4500 mg/L) were used. Investigated characteristics were plant height, leaf number, flower number, flowering time, fresh weight, dry matter, the content of essence and carotenoid in flowers. Based on analysis of variance (ANOVA), the effect of different treatments and their interaction on most traits was significant at 0.05 level of probability. The minimum height (24 cm/plant) in treatment of 500 mg/L cycocel + 3000 mg/L daminozide, the largest number of flowers (4.66 flowers/plant) in treatment of 1000 mg/L cycocel + 4500 mg/L daminozide and most essence content (0.154 mg/100 g) in treatment of 4500 mg/L daminozide without cycocel were obtained.

Abstract

Keywords: Chlormequat, Drench, Plant height, Ornamental plants.

INTRODUCTION

Calendula officinalis (pot marigold) from the family Asteraceae is probably native to southern Europe. It is a short-lived aromatic perennial plant, growing to 80 cm tall, with sparsely branched lax or erect stems. The inflorescences are yellow, comprising a thick capitulum or flower head 4-7 cm diameter surrounded by two rows of hairy bracts. *Calendula* are considered by many gardening experts as one of the most versatile flowers to grow in a garden, especially since they are easy to grow, and tolerate most soils. *Calendula officinalis* L. is an annual, aromatic, medicinal and ornamental herb (Gazim *et al.*, 2008). The composite flowers blossom in the spring-summer seasons for 3 times per year (Gilman and Howe, 1999; Omidbaighi, 2005). The leaves and flowers of marigold are applied in horticulture, medicine, cosmetics, perfume, pharmaceutical preparation, food and other industries (Van Wyk and Wink, 2004; Gazim *et al.*, 2008). One of the main components of active ingredient in *Calendula officinalis* L. are essential oils which the most of them synthesize in its orange petals (Omidbaighi, 2005). Flower essence is using for food and medicine (Hamburger *et al.*, 2003; Janke, 2004; Jimenez-Medina *et al.*, 2006). Plant growth retardants are commonly applied to limit stem elongation and produce a more compact plant. Production of high quality, compact pot plants may be achieved through the use of plant growth retardants including cycocel (Tayama *et al.*, 1992). Effectiveness of plant growth retardants depends on time and method of application, concentration, type of species and cultivar, and type of target organ as well physiological and environmental conditions (Pobudkiewicz and Nowak, 1994; James *et al.*, 1999). The most common methods of application of growth retardants are foliar sprays and media drenches (Al-Khassawneh *et al.*, 2006). Plant growth retardants can delay cell division and elongation of plant aerial parts as well restrict gibberellins biosynthesis, resulted in reduces internodes length and vegetative growth (Magnitskiy *et al.*, 2006). Adding cycocel has also proven to be effective in controlling growth of some other plants (Al-Khassawneh *et al.*, 2006; Rossini Pinto *et al.*, 2005; Leclerc *et al.*, 2006). Proper doses of cycocel and daminozide foliar spray and drench rate need to be assessed because they can either inhibit or promote growth and development. Therefore, the objective of the present study was to evaluate the effect of different concentrations of cycocel and daminozide on some growth characters especially plant height and the content of essence in *Calendula officinalis* L.

MATERIALS AND METHODS

Seeds of *Calendula officinalis* L. were obtained from Bazr va Nahal Company, Tehran, Iran. Investigation was carried out in an experimental field in Rasht city located in the northern part of Iran. Seeds were sown in pots filled with 60% sand, 20% cocopeat and 20% animal fertilizer on November 2011. Plants were treated with a drench application at the rate of 0, 500, 1000 and 1500 mg/L of cycocel and 0, 1500, 3000 and 4500 mg/L of daminozide 5-6 weeks after potting. Control plants were drenched with water. Investigated characteristics were the plant height, leaf number, flower number, flowering time, fresh weight, dry matter, the content of essence (essential oil) and carotenoid in flowers. Data were calculated at 60-70 days after transplanting. Plant height was measured by a ruler. Leaf number was obtained by counting leaves from center of each plot and their mean was calculated. Fresh weight of plants was weighted by a digital balance. To obtain the plant dry matter, they were cut from crown and dried at 105°C for 24 h. For determination of the essential oil, the plant materials (flowers) were dried in 45°C. The essential oil was obtained in a Clevenger apparatus by steam distillation. Thus, the 50 g of dried plant materials was extracted with 1000 ml of water. The water collected was re-extracted with 0.5 ml hexane. The essence and hexane was separated from water physically and weighted until the plant essence obtained. Leaf carotenoids were determined using acetone as extracting solvent and the absorbance was measured at 440, 645 and 663 nm. Sample extract was prepared as follows: 0.5 g of dry sample was thoroughly crushed and homogenized in a mortar with a pestle using 20 ml of 80% acetone. Filtered

extract was reached to 50 ml by adding of 80% acetone. Concentration of carotenoids was calculated by following formula after spectrophotometry:

$$\text{The amount of carotenoids} = 4.69 \times A_{440} - 0.268 \times (20.2) A_{645} + (8.02) A_{663}$$

Where; A is wave length. Data processing of the results was carried out by an EXCEL. Analysis of variance (ANOVA) was done using SPSS statistical software and means were compared using Duncan's test.

RESULTS

The overall results of the effects of different concentrations of cycocel and daminozide on the plant height, leaf number, flower number, flowering time, fresh weight, dry matter, the content of essence and carotenoid in flowers are summarized in Tables 2 and 3.

Plant height

Based on analysis of variance (Table 2), the effect of different treatments and their interaction on the plant height was significant at 0.05 level of probability. Plant height did not decrease linearly with increasing the cycocel concentration (Table 3). *Calendula officinalis* L. plants treated with all concentrations of cycocel and daminozide were shorter than the control plants (Table 3). 500 mg/L cycocel and 4500 mg/L daminozide produced the shortest plants, individually (25.43 and 24.16 cm/plant, respectively). Among all treatments, plants treated with 500 mg/L cycocel along with 1500 mg/L daminozide (C₂D₂) had the least plant height (23.33 cm/plant) (Table 3). C₂D₃ treatment was good for control of plant height (24.00 cm/plant), too. The highest plant height (38.33 cm/plant) was obtained in C₁D₁ treatment (control). Comparison between different concentrations of cycocel and daminozide shows that daminozide is more suitable plant retardant than cycocel for reduction of plant height (Table 3).

Leaf number

Analysis of variance (Table 2) showed that the effect of different treatments and their interaction had no significant effect on leaf number. Table 3 shows that the largest number of leaf (5.00/plant) was obtained in C₁D₂ and C₂D₃. The smallest number of leaf (2.75 and 2.83/plant) was calculated in C₁ and D₁.

Flower number

Mean comparison obtained from the data showed that the maximum (4.66/plant) and minimum (1.41/plant) number of flower were obtained from plants treated with 1000 mg/L cycocel along with 4500 mg/L daminozide (C₃D₄) and C₁ (control), respectively (Table 3). Plants treated with highest concentration of cycocel (C₄) had 1.58 flower/plant. Treatments of C₃D₁, C₂D₃ and C₂D₂ by induction of 4.33 flowers/plant were proper, too. Analysis of variance presented in Table 2 showed that the effect of cycocel and interaction effect of cycocel and daminozide on the number

Table 1. Treatments used in the present study and their symbols.

Treatments (mg/L)	Treatments Symbol
Cycocel 0	C ₁
Cycocel 500	C ₂
Cycocel 1000	C ₃
Cycocel 1500	C ₄
Daminozide 0	D ₁
Daminozide 1500	D ₂
Daminozide 3000	D ₃
Daminozide 4500	D ₄

Table 2. Analysis of variance (ANOVA) for the effect of different concentrations of cycocel and daminozide on plant height, leaf number, fresh weight, dry matter, flower number, time to flowering, essence and carotenoid contents of *Calendula officinalis* L.

Source of variations	df	Plant height	Leaf number	Fresh weight	Dry matter	Flower number	Time to flowering	Essence content	Carotenoid content
C	3	8.13*	2.02ns	33.05*	0.27ns	0.07*	155.96*	45.91*	12.59*
D	3	3.90*	0.58ns	9.38*	1.03ns	0.18ns	14.85*	5.32*	6.11*
C × D	9	4.37*	0.52ns	7.88*	0.05ns	0.50*	39.55*	2.55*	52.55*
Error	32	6.50	0.29	69.27	0.08	1.33	85.16	32.12	9.12
CV (%)	-	7.36	28.11	26.21	18.92	45.8	7.85	28.61	35.91

ns: Non significant, *: Significant at 5%

of flower per plant were significant at 0.05 probability. The effect of daminozide on the number of flower per plant was no significant.

Time to flowering

Variance analysis of data (Table 2) showed that the impact of cycocel, daminozide and their interaction effect was significant at 0.05 probability on time to flowering. The mean comparison of data in different treatments (Table 3) showed that the lowest time of flowering is related to C₄D₂ treatment (98.00 days). The longest time of flowering was obtained in treatments of C₁D₁ (122.60

Table 3. Mean comparison of the effect of different concentrations of cycocel and daminozide on plant height, leaf number, fresh weight, dry matter, flower number, time to flowering, essence and carotenoid contents of *Calendula officinalis* L.

Treatments (mg/L)	Plant height	Leaf number	Fresh weight	Dry matter	Flower number	Time to flowering	Essence content	Carotenoid content
C ₁	35.83 ^a	2.75 ^a	24.41 ^b	4.33 ^a	1.41 ^b	119.66 ^a	0.12 ^c	5.21 ^b
C ₂	25.43 ^b	4.25 ^a	32.00 ^a	5.66 ^a	2.58 ^a	114.16 ^a	0.13 ^b	5.87 ^b
C ₃	34.25 ^a	4.33 ^a	32.25 ^a	5.58 ^a	2.50 ^a	104.58 ^b	0.14 ^a	6.51 ^a
C ₄	34.08 ^a	3.83 ^a	33.33 ^a	5.41 ^a	1.58 ^b	121.33 ^a	0.14 ^a	6.91 ^a
D ₁	35.41 ^a	2.83 ^a	25.50 ^b	4.41 ^a	2.58 ^a	118.00 ^a	0.12 ^c	5.11 ^b
D ₂	24.58 ^b	4.08 ^a	30.91 ^a	5.41 ^a	2.66 ^a	121.33 ^a	0.13 ^b	6.14 ^a
D ₃	24.25 ^b	4.33 ^a	31.58 ^a	5.91 ^a	2.41 ^a	106.91 ^b	0.14 ^a	6.53 ^a
D ₄	24.16 ^b	3.91 ^a	33.00 ^a	5.41 ^a	2.41 ^a	118.66 ^a	0.14 ^a	6.64 ^a
C ₁ D ₁	38.33 ^a	4.33 ^a	21.33 ^c	5.33 ^a	2.00 ^d	122.60 ^a	0.12 ^c	5.11 ^c
C ₁ D ₂	35.00 ^{ab}	5.00 ^a	23.33 ^c	5.00 ^a	3.33 ^b	112.66 ^b	0.13 ^b	5.16 ^{bc}
C ₁ D ₃	32.23 ^{bc}	4.66 ^a	29.66 ^b	6.00 ^a	3.00 ^c	115.00 ^b	0.12 ^c	6.68 ^b
C ₁ D ₄	35.66 ^{ab}	3.33 ^a	26.33 ^b	5.00 ^a	2.33 ^{cd}	115.33 ^{ab}	0.15 ^a	7.02 ^{ab}
C ₂ D ₁	24.66 ^{cd}	3.33 ^a	25.66 ^b	6.00 ^a	2.33 ^{cd}	101.66 ^c	0.14 ^{ab}	6.23 ^b
C ₂ D ₂	23.33 ^{cd}	4.33 ^a	33.00 ^a	5.66 ^a	4.33 ^a	116.33 ^{ab}	0.14 ^a	6.14 ^{bc}
C ₂ D ₃	24.00 ^{cd}	5.00 ^a	31.66 ^{ab}	6.33 ^a	4.33 ^a	113.33 ^b	0.13 ^b	6.58 ^b
C ₂ D ₄	34.00 ^b	4.33 ^a	32.66 ^{ab}	4.66 ^a	2.33 ^{cd}	115.33 ^{ab}	0.14 ^a	7.11 ^{ab}
C ₃ D ₁	34.33 ^{ab}	4.66 ^a	26.00 ^b	4.66 ^a	4.33 ^a	108.66 ^c	0.13 ^b	6.12 ^{bc}
C ₃ D ₂	25.33 ^c	3.00 ^a	31.33 ^{ab}	6.00 ^a	2.66 ^{cd}	120.66 ^a	0.14 ^{ab}	6.89 ^b
C ₃ D ₃	25.00 ^{cd}	3.00 ^a	31.00 ^{ab}	5.66 ^a	3.66 ^b	120.00 ^{ab}	0.14 ^{ab}	7.01 ^{ab}
C ₃ D ₄	32.33 ^{bc}	3.00 ^a	34.66 ^a	6.00 ^a	4.66 ^a	117.33 ^{ab}	0.14 ^a	8.23 ^a
C ₄ D ₁	34.33 ^{ab}	3.33 ^a	27.00 ^b	5.00 ^a	2.33 ^{cd}	111.33 ^b	0.12 ^b	6.35 ^b
C ₄ D ₂	24.66 ^{cd}	4.00 ^a	33.00 ^a	5.00 ^a	3.00 ^c	98.33 ^d	0.14 ^{ab}	6.95 ^{ab}
C ₄ D ₃	26.66 ^c	3.63 ^a	34.00 ^a	5.00 ^a	3.33 ^b	121.66 ^a	0.14 ^{ab}	7.21 ^{ab}
C ₄ D ₄	26.66 ^c	4.33 ^a	34.33 ^a	6.00 ^a	3.66 ^b	119.66 ^{ab}	0.14 ^a	7.69 ^a

In each column means followed by the same letters are not significantly different at 5 % level of probability using Duncan's test.

days). Time to flowering in treatments of C₃ (104.58 days) and D₂ (106.91 days) were also good (Table 3). On the other hand, time to flowering in treatments of C₃D₂, C₃D₃ and C₄ (about 120.00 days for all) was not suitable (Table 3).

Fresh weight

Based on Table 2, the effect of different concentrations cycocel, daminozide and the interaction effect was significant at 0.05 probability on plant fresh weight. Among concentrations of cycocel, C₄ treatment had maximum effect on fresh weight (33.33 g/plant), also, C₁ (control) treatments with 24.41 g fresh weight was minimum (Table 3). Plant fresh weight increased as the concentration of cycocel and daminozide increased. Among concentrations of daminozide, D₄ treatment had maximum effect on fresh weight (33.00 g/plant), also, D₁ (control) treatments with 25.50 g fresh weight was minimum (Table 3). Among all treatments, maximum (34.66 and 34.33 g/plant) and minimum (21.33 g/plant) of plant fresh weight were obtained in C₃D₄, C₄D₄ and C₁D₁ (control), respectively (Table 3).

Dry matter

Table 2 shows that the effects of cycocel, daminozide and the reciprocal effect were no significant effect on plant dry matter. Table 3 shows that dry matter in plants treated with 500 mg/L cycocel along with 3000 mg/L daminozide (6.33 g/plant) was higher than that of other treatments. The 6.00 g dry matter was obtained in 5 treatments including C₁D₃, C₂D₁, C₃D₂, C₃D₄ and C₄D₄ (Table 3). Mean comparison obtained from the data showed that the least dry matter (4.33 and 4.41 g/plant) was calculated from C¹ and D₁ treatments.

Essential oil contents

The effect of cycocel, daminozide and interaction effect of these two factors were significant ($p \leq 0.05$) on the content of petal essential oil (Table 2). Maximum essential oil (0.15 ml/100 g FW) was obtained when pot marigold was planted in treatment of C₁D₄ (Table 3). Minimum essential oil (0.12 ml/100 g FW) was obtained when pot marigold was planted in C₁, D₁, C₁D₁, C₁D₃ and C₄D₁ (Table 3).

Carotenoid content

ANOVA showed that the effect of cycocel, daminozide and interaction effect of these two factors were significant ($p \leq 0.05$) on the content of petal carotenoid (Table 2). Maximum carotenoid (8.22 mg/L) was obtained when pot marigold plants were planted in treatment of C₃D₄ (Table 3). Minimum essential oil (5.11 mg/L) was obtained when pot marigold plants were planted in D₁, C₁D₁ (Table 3).

DISCUSSION

One of the most important applications of plant growth retardant is elevation of plant quality, especially ornamental plants by reduction of vegetative growth. Plant growth retardants decrease the internodes length and eliminate the apical dominance (Lee *et al.*, 1999). Plant growth retardants increase cytokinins which enhance the amount of leaf chlorophyll (Rossini Pinto *et al.*, 2005). Some of the most important factors concerning plant growth retardants are type, time, number, application method and concentration of growth retardant (Cramer and Bridgen, 1998). Cycocel and daminozide are two important plant growth retardant. Several studies revealed effectiveness of cycocel in decreasing plant height (Rossini Pinto *et al.*, 2005; Olivera and Browing, 1993; Garner, 2004; Karlovic *et al.*, 2004; Hashemabadi and Zarchini, 2010). Studies of Al-Khasawneh *et al.* (2006) on growth and flowering of *Iris nigricans* showed that cycocel reduced plant height only at the highest drench concentration. These researchers revealed that cycocel spray at

the higher concentrations (1000-1500 mg/L) reduced plant height. In current study, cycocel caused decreasing of plant height in *Calendula officinalis* L. Karlovic *et al.* (2004) reported decreasing height in *Chrysanthemum* by 2000, 3000 and 4000 mg/L cycocel. Hashemabadi and Zarchini (2010) showed that the least stem length (29.93 cm) was obtained by using 1500 mg/L cycocel in rose. Saffari *et al.* (2004) sprayed *Rosa damascena* with cycocel and found that 3000 mg/L cycocel decreased stem length about 5 cm relative to the control. Increasing application rates did not positively influence plant development when compared to the lower rates used in the study. Cycocel (1000 and 2000 mg/L) decreased *Zinnia* plant height (Hojjati *et al.*, 2009). Current study confirms these studies. Cycocel, also, reduced plant height in *Euphorbia* and *Bougainvillea* (Shekari *et al.*, 2004), *Rosa* (Saffari *et al.*, 2004) and *Pelargonium* (Latimer and Beden, 1994). Other plant growth retardants such as prohexadione-Ca, uniconazole, paclobutrazol and bayleton are applied for decreasing the plants growth as spray or drench (Gibson and Whipker, 2000; Bazzocchi and Giorgioni, 2003). Gholampour *et al.* (2012) showed that the 1500 mg/L of cycocel resulted in about 50 and 20% shorter *Brassica oleracea* cultivar 'Kamome White' and 'Nagoya Red' plants than the control plants, 60 and 90 days after transplant, respectively. The growth of these cultivars decreased with increasing the concentration of cycocel. Some other researches demonstrated the positive effect of cycocel and daminozide on reduction of stem length in some species such as *Euphorbia*, *Rosa*, *Pelargonium* and *Bougainvillea* (Saffari *et al.*, 2004; Joyce *et al.*, 2004; Shekari, 2006).

Studies of Al-Khassawneh *et al.* (2006) on growth and flowering of *Iris nigricans* showed that maximum number of leaves (average of 12.2-13.6) was obtained when the plants were untreated with cycocel and paclobutrazol, sprayed with 250 mg/L paclobutrazol, or drenched with 0.25 mg/L. Our finding is consistent with these results. Study of Agrawal and Dikshit (2008) on *Achras sapota* L. demonstrated positive effect of cycocel on leaf number. Current study showed that the highest dry matter percentage was obtained from untreated plants with cycocel. In agreement with our finding, Al-Khassawneh *et al.* (2006) also showed that untreated plants had the highest leaf dry weight. Study of Garib Sahi (2009) on *Zinnia elegans* revealed that spraying plants with 2000 mg/L cycocel and 1 mg/L CaCl₂ increased leaves and roots dry weight. Our study showed the positive effect of plant growth retardants on increasing the content of essential oil and carotenoid in pot marigold petals. Some studies confirm these findings (Khalid and El-Ghorab, 2006; Gliozzeris, 2007). Optimum concentrations of cycocel in these studies were 500 and 1000 mg/L. Plant growth retardants increase cytokinins synthesis and this hormones enhances the content of pigments in plants (Fletcher *et al.*, 2000).

In conclusion, some concentrations of cycocel and daminozide increased morphological and physiological characteristics in *Calendula officinalis* L. The 500 mg/L of cycocel along with 1500 mg/L daminozide resulted in shorter plants than the other concentrations and control plants. Maximum flower number, fresh weight, dry matter and carotenoid content were obtained in plants treated with 1000 mg/L cycocel along with 4500 mg/L daminozide. The 4500 mg/L caused the highest amount of essential oil.

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