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Effects of Foliar Application of Gibberellic Acid on Chlorophyll and Carotenoids of Marigold (Calendula officinalis L.)

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ABSTRACT

Effect of gibberellic acid on marigold (Calendula Officinalis L.) was evaluated in a pot culture experiment. A factorial experiment based on completely randomized design including 12 treatments and four replications was carried out. Main factor was foliar application stages (first, second and third) and sub factor included different concentrations of GA_3 (0, 50, 150 and 250 mg L^{-1}). Results showed that foliar application of GA_3 had positive effect on photosynthetic pigments. Effect of different concentrations of GA_3 on chlorophyll a was significant ($p<0.01$). Chlorophyll a content was enhanced by increase in GA_3 concentration up to 250 mg L^{-1} treatment of 250 mg L^{-1} resulted in production of 7.78 µg/L^{-1} chlorophyll a, the index which was to some extent dropped in other concentrations. Different concentrations of GA_3 had significant effect on chlorophyll b ($p<0.01$). Chlorophyll b was increased by increase in GA_3 concentration up to 250 mg L^{-1}. the highest rate of total chlorophyll content and total pigment in three times of application and one application of 250 mg L^{-1} was 14.6 and 15.4 µg/L^{-1} respectively; whereas the lowest chlorophyll and pigment content was observed in one foliar application of control treatment with mean value as 4.67 and 5.5 µg/L^{-1}.

Keywords: Benzyladenine, Calendula officinalis, Chlorophyll, Carotenoids, Gibberellic Acid.

INTRODUCTION

Calendula officinalis (pot marigold, common marigold, garden marigold, English marigold, or Scottish marigold) is a plant in the genus Calendula of the family Asteraceae. It is probably native to southern Europe, though its long history of cultivation makes its precise origin unknown, and it may possibly be of garden origin (Gazim et al., 2008). This plant is an annual plant with yellow to orange flowers and includes a high number of carotenoids such as flavoxanthin, lutein, rubixanthin, b-carotene, g-carotene, and lycopene (Pintea et al., 2003). These carotenoids have been found to have antioxidant, antimicrobial and antiproliferative properties. Research suggests that it can be very protective against prostate cancer (Dahan, et al., 2008) Plant growth regulators (PGRs), either produced naturally by the plant or synthetically by a chemist, are small organic molecules that act inside the plant cells and alter the growth and development of plants. PGRs can be broadly divided into two groups: plant growth promoters (auxins, gibberellins and cytokinins) and bio inhibitors (ABA, methyljasmonate). GA_3, increases stem

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length, the number of flower per plant and induces fruit setting (Azuma et al, 1997). Recent research has shown that GA_3 treatment leads to higher polyphenol and anthocyanin content (Teszl´ak et al, 2005). It has been known that growth regulators among in the agriculture practices which is most favourable for promoting and improving plant-growth of different plants. The beneficial effect of gibberellic acid on different plants were recorded by Shedeed et al. [1991] on croton plant, Eraki [1994] on Quen Elizabeth rose plants, Bedour et al. [1994] on Ocimum basillicum, they concluded that gibberellic acid is used to regulating plant growth through increasing cell division and cell elongation. The results of this test indicated this problem that regulators of BA and GA_3 were effective on photosynthesis pigments. The highest value of chlorophyll of a, b was total and sum of pigments in level of 400 mg l^{-1} BA+100 mg l^{-1} GA_3 with average of 18/48, 10/74, 28/73 and 33/87 mg l^{-1}. By increasing concentration of GA_3, value of chlorophyll a is increased [Rahbarian et al, 2014]. Application of 100 mg l^{-1} GA_3 +200 mg l^{-1} BA, 200 mg l^{-1} GA_3 and 100 mg l^{-1} GA_3 +100 mg l^{-1} BA with averages of 19.59, 18.8 and 18.66 µg l^{-1} followed highest value of sum of pigments and its minimum was obtained in100 mg l^{-1} GA_3 and control application with average of 11.1 and 11.82 µg l^{-1} [salehi sardoei et al, 2014]. Since no data exists on the impact of GA_3 Marigold treatment on chlorophyll and Carotenoids, present study was conducted in order evaluation of the chlorophyll and Carotenoids of C. officinalis at three spraying stages and different GA_3 levels.

MATERIALS AND METHODS

This experiment was conducted to investigate effect of vermicompost on growth, flowering and photosynthetic pigments of medical plant calendula variety yellow. Marigold seeds were cultured in nursery and transplanted in to culture media three parts of soil, one part of sand and one part of cow rotten manure. one irrigation per day was performed during the experiment and was increased to two times per day by increase in air temperature during spring. Results regarding soil analysis are presented in table (1). the experiment was carried out as factorial based completely randomized design with four replications and in each replication, four pot were investigated.

Tab 1- Results of the analysis of soil used in experimental pots. Data are means for four replications

Depth (cm)	pH	EC (ds/m^{-1})	SP (%)	Total N (%)	A W P (ppm)	A W K (ppm)	Texture
0 – 30	8.1	0.89	25	0.03	12	220	Loamy sand

Material Chemicals

methanol were purchased from Merck (Darmstadt, Germany). Gibberellic acid (GA_3), Tween 20 were from Fluka (Buchs, Switzerland), all chemicals were of reagent grade.

Treatment

Airial parts of marigold were sprayed at three stages (first, second and third) with an aqueous GA_3 solution (containing 0.2% Tween 20). GA_3 was dissolved in deionised water directly on site before use to ensure constant application doses of 0, 50, 150 and 250 mg L^{-1}.

Estimation of Chlorophyll and Carotenoids

Photosynthetic pigments were measured using Lichtentaller method (1987). 0.2 g of fresh leaf tissue was weight by laboratory balance with accuracy of 0.0001gr and pulverized with mortar in the presence of 10ml of 80% acetone. The resulted solution was filtered through wattman filter paper mounted in glass funnel. The solution volume was increased to 15ml by addition of 80% acetone. 3ml of the solution containing chlorophyll a and b and carotenoid was poured in cuvet and its absorbance was measured in wavelengths of 663.3 nm (chlorophyll a), 646.8 nm (chlorophyll b) and 470 nm (carotenoids) using spectrophotometer device; concentration of the pigments were calculated using.

Chl_a ($mg.ml^{-1}$) = (12.5 *A663.2)-(2.79* A646.8)

Chl_b ($mg.ml^{-1}$) = (21.51*A646.8)-(5.1*A663.2)

Chl T ($mg.ml^{-1}$) = Chl.a + Chl.b equation 3

Car ($mg.ml^{-1}$) = (1000* A470)-(1.8* Chl.a)-(85.02* Chl.b

Where chl.a, chl.b, ch1 total and car are concentration of chlorophyll a, chlorophyll b and carotenoids (carotene and xanthophyll); and A663.2, A646.8 and A470 stand for absorbance in 663.2 nm (chlorophyll a), 646.8 nm (chlorophyll b) and 470 nm (carotenoids), respectively.

Data Analysis

All these experiments were replicated three times, and the average values are reported. The effects of different GA_3 levels (Calendula officinalis) at three spraying stages on chlorophyll and carotenoids of marigold were determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at $P<0.05$ significant level using the SAS software (2001) program.

RESULTS AND DISCUSSION

Soil analysis results showed that the soil used in the experiment was of sand-loam texture, alkaline and had no limitation regarding salinity. The soil was poor in nitrogen, but concerning phosphorus and potassium it falls in a good range (Tab 1). Results showed that foliar application of GA_3 had significant effects on photosynthetic pigments. Effect of different concentrations of GA_3 on chlorophyll a was significant ($p<0.01$). Chlorophyll a content was enhanced by increase in GA_3 concentration up to 250 mg L^{-1} (salehi sardoei et al, 2014a, b, c; Rahbarian et al, 2014). treatment of 250 mg L^{-1} resulted in production of 7.78 $\mu g/L^{-1}$ chlorophyll a, the index was reduced in other concentrations (Tab 4).

Tab 2- Analysis of variance for the effect of Stage and GA_3 on Photosynthetic pigments

SOV	df	Chl. (a)	Chl. (b)	Total Chl. a+b	Carotenoids	sum pigments
Stage	2	9.05**	8.1**	32.97**	0.26*	34.29**
GA_3	3	18.93**	1.99**	31.86**	0.001^{ns}	31.97**
Stage × GA_3	6	5.77**	1.21**	9.61*	0.45**	9.55*
Error		0.91	0.2	2.31	0.005	2.5

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

According to Analysis of variance results (Tab 2), chlorophyll a contents were significantly different in various number of GA_3 applications ($p<0.01$) so that chlorophyll a was reduced by increase in the number of application times. The highest content of chlorophyll a as 5.8 µg L^{-1} was achieved by foliar applications of GA_3 foe third stage (Tab 4). Different concentrations of GA_3 had significant effect on chlorophyll b ($p<0.01$). Chlorophyll b was increased by increase in GA_3 concentration up to 250 mg L^{-1}. 250 mg L^{-1} treatment resulted in production of 3.53 µg/L^{-1} chlorophyll b, the index was partly reduced in other concentrations (Tab 4).

Tab 3- Mean comparison of different GA_3 levels on Photosynthetic pigments C. officinalis L.

	Chl. (a)	**Chl. (b)**	**Total Chl. a+b**	**Carotenoids**	**sum pigments**
Stage					
first	6.38c	2.27b	8.65b	0.86b	9.52b
second	7.45b	2.54b	9.99b	0.97a	10.96b
third	8.5a	4.13a	12.64a	0.96a	13.6a
Concentration					
0	4.86b	2.33b	7.2b	0.93a	8.13b
50	7.71a	2.67b	10.38a	0.91a	11.2a
150	8.42a	3.4a	11.82a	0.95a	12.77a
250	8.78a	3.53a	12.32a	0.93a	13.25a

Means followed by same letter are not significantly different at P< 0.05 probability using Duncan's test.

results analysis of variance (Tab 2), revealed that chlorophyll b content was significantly different in different times of GA_3 application ($p<0.01$) triat, so that chlorophyll b was gradually increased by increase in the number of application times. The highest content of chlorophyll b was 4.13 13 µg L^{-1}, achieved by foliar applications of GA_3 for third stage (Tab 3). Effect of different concentrations of GA_3 on carotenoids content was or non significant (Tab 3). Treatment of 150 mg L^{-1} resulted in production of 3.53 µg L^{-1} carotenoids; the index was to some extent reduced in other concentrations (Tab 4). According to analysis of variance (Tab 2), results for carotenoids, there is significant difference in different number of GA_3 application. ($p<0.05$). By increasing GA_3 application to second stage, carotenoids content was gradually increased. The highest carotenoids rate was 0.97 µg L^{-1} achieved by GA_3 application for second stage (Tab 3). There was or non difference between second and third stage application but they were significantly different from first stage application. Effect of different concentrations of GA_3 on total chlorophyll and pigments was significant ($p<0.01$). By increasing GA_3 concentration up to 250 mg L^{-1}, total chlorophyll and pigment content was increased. Treating the seedling with 250 mg L^{-1} GA_3 was accompanied by 12.32 and 13.25 µg L^{-1} of chlorophyll and pigment, but it was to some extent reduced in other concentrations. According to analysis of variance results (Tab 2) for total chlorophyll and pigment, there was significant difference between the numbers of GA_3 application ($p<0.01$). By increasing application times, total chlorophyll and pigment were gradually decreased. The highest rate of total chlorophyll and pigment were 12.64 and 13.6 µg L^{-1} achieved by three applications of GA_3 (Tab 3). Interaction of application times of GA_3 on chlorophyll a was significant ($p<0.01$), meaning that different application methods don't result in identical effects (Tab 4). By increasing application to second stage, chlorophyll a was increased but reduced when GA_3 was applied for third stage (Tab 4). Compared to control, chlorophyll a was increased when the plants were treated by 50 and 150 mg L^{-1} of GA_3, the value

was higher in second stage compared to application for either first or third stage applications. The highest and lowest rate of chlorophyll a as 9.29 and 3.01 µg L^{-1} was obtained by two applications of GA_3 at concentration of 50 and 0 (control) mg L^{-1} respectively, which show a significant difference. Chlorophyll a content in application of GA_3 for second stage was higher than first or third stage applications. The results of this test indicated this problem that plant growth regulators were effective on photosynthesis pigments. The highest value of chlorophyll of a, b was total and sum pigments in level of 200 mg l^{-1} GA_3+200 mg l^{-1} BA, with average of 12.19, 7.55, 19.74 and 21.88 mg ml^{-1}. By increasing concentration of GA_3 and BA, value of chlorophyll a is increased These results are consistent with the results of other investigators (salehi sardoei et al, 2014a, b, c; Rahbarian et al, 2014). Compared to control, chlorophyll b content was enhanced when the plants were treated by 150 and 150 mg L^{-1} of GA_3, the value was higher in third stage applications compared to application for first or second stage applications. The highest and the lowest amount of chlorophyll was 5.37 and 1.5 µg L^{-1} respectively, obtained by first and third stage applications of GA_3 at concentration of 250 and 0 mg L^{-1} showing significant difference. Results showed that chlorophyll b content in third stage applications of GA_3 was higher than those obtained by first or second stage applications.

Tab 4- Mean comparison interaction of different GA_3 levels on Photosynthetic pigments in stage C. Officinalis L.

stage	concentratioan GA_3	Chl. (a)	Chl. (b)	Total Chl. a+b	Carotenoids	sum pigments
first	0	3.16c	1.5f	4.67d	0.83def	5.5c
	50	5.74b	1.99ef	7.73cd	0.72f	8.45bc
	150	7.98a	2.91de	10.89abc	0.88cdef	11.77ab
	250	8.63a	2.69de	11.33abc	1.04abc	12.37a
second	0	3.01c	1.55f	4.57d	0.98abcd	5.55c
	50	9.29a	3.31cd	12.6ab	0.87cdef	13.47a
	150	9.01a	2.76de	11.77ab	1.06ab	12.84a
	250	8.49a	2.54def	13.03abc	0.97bcde	12ab
third	0	8.42a	3.94bc	12.36ab	0.99abcd	13.35a
	50	8.12a	2.7de	10.82bc	1.15a	11.97ab
	150	8.26a	4.52ab	12.79ab	0.9bcde	13.7a
	250	8.23a	5.37a	14.6a	0.8ef	15.4a

Means followed by same letter are not significantly different at P< 0.05 probability using Duncan's test.

Interaction of application times on carotenoids content was significant ($p<0.01$), suggesting that different application methods result in different effects (Tab 4). The highest and the lowest content of carotenoids was obtained by first and third stage application at concentration of 50 mg L^{-1} of GA_3 as 1.15 and 0.72 µg L^{-1} showing significant difference. Interaction of application times on total chlorophyll and pigment content was significant ($p<0.05$), indicating different effects of different application methods (Tab 4). Total chlorophyll and pigment content was increased by increase in application times to third stage, and was reduced in first or second stage applications (Tab 4). Compared to control, this item was increased when the plants were treated with 150 and 250 mg L^{-1} GA_3 and was higher in application for third stage compared to first or second stage applications. The highest total chlorophyll and pigment content as 14.6 and 15.4 µg L^{-1} was achieved by application of 250 mg L^{-1} GA_3 for first or third stage applications;

whereas the lowest values as 4.67 and 5.5 μg L^{-1} were obtained by first stage application of control treatment. Results showed that total chlorophyll and pigment content was higher in application of GA_3 for third stage compared to first or second stage applications, Use of plant growth regulators, the growth rate of indoors plants can be stimulated through increasing synthesis of photosynthetic pigments by applications of GA_3 and BA (Salehi Sardoei, 2014).

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