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Foliar Application Gibberellic acid and ethanol on flower yield and phenolic compositions in marigold (*Callendula officinalis* L.)

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Abstract

Marigold (*Calendula officinalis* L.) is an annual aromatic herb belongs to the Asteraceae family. In order to study the effects of of gibberellic acid and ethanol on flower yield and phenolic compositions marigold, an experiment was conducted at Agriculture Research Center & Natural Resources of Hamedan Province in 2013.

The first factor consisted gibberellic acid in 4 levels (0, 250, 500, 750 ppm) and the second factor consisted of ethanol in 3 levels (0, 3, 15%) in factorial based on randomized complete block design with three replications.

The obtained results from this study on the number of flowers in bush indicate significant effect of gibberellic acid, ethanol and their interaction effects in 1% probability level, also number of flowers in surface unit and function of wet flower in the surface unit was significant effect of gibberellic acid, ethanol and their interaction effects in 1% level, but function of wet flower in bush and function of wet flower in bush and phenolic compositions was significant in 5% probability level. gibberelic acid causes to increase the active substance. Comparison of mean interaction effects for gibberelin and ethanol indicated that the level of gibberelin 500 ppm and 30% ethanol had the highest amount of phenolic compositions with the average of 0.92 and the level of gibberelin 500 ppm and 30 % ethanol, and the level of gibberelin 500 ppm and witness ethanol had the least amount of phenolic compositions with the average of 0.55.

Keywords: Gibberellic acid; Ethanol; *Callendula Officinalis*; Chlorophyll; Carotenoid.

1. Introduction

Calendula officinalis L is an annual aromatic herb belongs to the Asteraceae family. It grows wild in the southern, eastern and central Europe [1]. The plants belonging to this family are mostly herbal, annual, bi- annual or multi- annual and rarely have wooden stems. (They are not real tree plants). *Callendula Officinalis* is a poly-ploid plant and 2n=28 and 32.The active substances of this plant are made in the flowers and reserved. Their most important ones are flavonoids dissolved in water (0.04 to 0.1%) [2].

The flowers of *Callendula Officinalis* with sepal or with no sepal have been introduced as drug in some of the pharmacies [3]. This plant is used for gastro-intestinal diseases. Also from the extracted materials of this plant's flowers some creams are provided for treating skin wounds. The extracted colored materials from *Callendula Officinalis* are used in coloring the food stuff as well as in coloring some types of fats [4]. Since producing secondary metabolites in the plants are changed by the environmental factors, so presenting some methods that can produce a plant with more active substance seems essential. One of these strategies is using plant growth substances [5].

Plant Growth regulators play an active role in improving plant vegetative growth. Gibberelic acid is one of these substances which promote the vegetative growth of a wide range of ornamental or medicinal plants [6]. The mechanism by which gibberellins might stimulate cell elongation is that the hydrolysis of starch resulting from the production of GA3 increased mobilization of starch in cotyledons by increasing amylase activity [7].

Studies [8] showed that the spraying micronutrients mixtures at 200 or 400 ppm combined with GA3 at 400 ppm was remarked in this respect. Anatomical properties of main stem were investigated and the highest increase in stem section diameter was recorded at the interaction of 400 ppm GA3 and 200 ppm of mixture of micronutrients.

In a research for the effect of Ethanol and Methanol on the clove it was concluded that alcoholic style treatments (ethanol 3 % and methanol 3 % and 6 %) caused to a content the plants growth, increase the number of flowering branches and number of flowers. It means that low concentrations of alcohol particularly ethanol 3 % cause to shorten the growing period and preparation of the plant for entering into the generative phase

which its result is acceleration in flowering [9]. The aim of this study was the effect of gibberellic acid and ethanol on the number of flowers and the content of phenolic compositions in marigold (*Callendula officinalis* L.).

2. Materials and Methods

Plant materials

This research was evaluated for studying the effect of foliar gibberelic acid and ethanol alcohol on quality and quantity treatment of *Callendula Officinalis* during 2013 in Hamedan Province Natural Resources & Agriculture Research Center. Research Center is situated in Hamedan Province with the altitude of (34° 48 ′N and 48° 31 ′E with the height of 1671 m from the sea level), Hamadan province lies in a temperate mountainous region to the east of Zagros. Hamadan is in the vicinity of the Alvand mountains and has a dry summer continental climate, in transition with a cold semi-arid climate (Koppen climate), with snowy winters. In fact, it is one of the coldest cities in Iran. The temperature may drop below −30 °C (−22 °F) on the coldest days. The experiment was performed in the form of factorial within the framework of fully randomized block design with 3 repetitions and two factors of gibberelic acid and ethanol. The first factor consisted gibberelic acid in 4 levels (0, 250, 500, 750 ppm) and the second factor consisted of Ethanol in 3 levels (0, 3, 15%). The soil of the used place was ploughed in Aprill 2011 for cultivation and the leveling action was performed. Gibberelin foliar treatment at first was performed in the stage of rapid growth and beginning of June, and then Ethanol foliar treatment was performed. Gibberelin in 4 levels (0, 250, 500, 750 ppm) and ethanol in 3 levels (0, 3, 15%) were performed. The final harvest was performed in the peak flowering phase and then the amount of phenolic compositions, number of flowers, flower function in the surface unit and in the bush were measured.

Measuring content of phenolic compositions

Measuring the content of phenolic compositions was performed by the method of [10]. According to this method the amount of 0.01 g from fresh sepal was pulverized and it was kept in darkness for a period of 24 hours. Then 1 mL of ethanol 95% was added to 1 mL of solution and with distilled water twice distillation of the solution volume reached to 5 mL; afterward 0.5 mL of 50% Folin Reagent and 1mL of $NaCO_3$ was added to it. The obtained mixture was kept in darkness for a period of 1 hour and then the attraction percent for each sample was read in the wavelength of 725 nm by the spectrophotometer Model uv 2100 unico. Statistical analysis of the experiment data was performed based on the factorial test within the framework of complete randomized blocks by the Software SAS and all the graphs were drawn by Excel Software and comparison of the means related to the levels of gibberelic acid and ethanol as well as their interaction effect was performed in 5% level by Danken Test.

3. Results

3.1 Number of flowers in bush

The obtained results from analysis of variance for this feature indicate significant effect of gibberelin, ethanol and their interaction effects in 1 % probability level (Table 1).

Table 1. Analysis of variance for different measured traits.

S.V	df	PC	NFS	NFB	FDFB	FWF	FDF	FFS
Block	2	0.05^{ns}	135.12^{ns}	3.34^{ns}	0.53^{ns}	53.17^{ns}	30.04^{ns}	1726.79^{ns}
Gibberellin (A)	3	0.02^{ns}	2674.49^{**}	10.54^{**}	14.45^{*}	199.30^{*}	1347.35^{**}	43854.51^{**}
Ethanol (B)	2	0.01^{ns}	146978^{**}	9.36^{**}	1.21^{ns}	72.94^{ns}	1076.91^{**}	29656.96^{**}
(A × B)	6	0.04^{ns}	1300.12^{**}	8.84^{**}	9.53^{*}	171.73^{*}	690.72^{**}	17860.04^{**}
Error	22	0.01	45.64	1.52	3.21	51.66	36.86	1155.31
C.V(%)	-	18.64	17.95	16.82	34.22	26.14	21.45	22.83

*,**: Significant at 0.05 and 0.01 probability levels, respectively, PC, (Phenolic compositions), NFS, (Number of flower in the surface unit), NFB, (Number of flower in the bush), FDFB, (function of dry flower in the bush), FWF, (function of wet flower in the bush), FDF, (function of dry flower in the surface unit), FFS, (function of wet flower in the surface unit).

The (Table 2) for comparison of mean gibberelin effect indicates that the highest number of flower in bush (8.68) is related to gibberelin 500 ppm and its least number is related to gibberelin 750 ppm (6.10). With regard to the comparison of mean ethanol effect on the feature of number of flower in bush it was specified that highest rate of this feature (8.13) is related to 15% ethanol, and its least rate (6.23) is related to 30% ethanol and there is no significant difference statistically between 15% ethanol and witness (Table 3). Comparison of mean interaction effects of ethanol and gibberelin indicated that the range of changes for the flowers, number in the bush varies between (3.07) in the level of gibberelin 750 ppm and 30% ethanol and (10.87) related to gibberelin 500 ppm and 15% ethanol (Table 4).

Table 2. Comparison of the mean of gibbberelin on different measured traits in *Callendula Officinalis.*

Gibberellin (ppm)	NFS	NFB	FDFB	FWF	FDF	FFS
0	62.20_{a}	7.52_{ab}	5.25_{ab}	28.64_{a}	42.76_{a}	242.51_{a}
250	36.64_{b}	6.97_{bc}	5.13_{ab}	25.17_{ab}	26.32_{b}	135.80_{b}
500	27.34_{c}	8.68_{a}	6.67_{a}	31.67_{a}	21.07_{b}	104.50_{bc}
750	24.26_{c}	6.10_{c}	3.56_{b}	20.72_{b}	14.01_{c}	88.50_{c}

NFS, (Number of flower in the surface unit), NFB, (Number of flower in the bush), FDFB, (function of dry flower in the bush), FWF, (function of wet flower in the bush), FDF, (function of dry flower in the surface unit), FFS, (function of wet flower in the surface unit).

Table 3. Comparison of the mean of ethnaol on different measured traits in *Callendula Officinalis.*

Ethanol(%)	NFS	NFB	FDFB	FWF	FDF	FFS
0	24.73_{b}	7.50_{a}	4.89_{a}	26.79_{a}	17.02_{c}	85.40_{b}
15	43.93_{a}	8.13_{a}	5.25_{a}	30.38_{a}	28.24_{b}	165.30_{a}
30	44.19_{a}	6.23_{b}	5.51_{a}	25.58_{a}	35.85_{a}	177.08_{a}

NFS, (Number of flower in the surface unit), NFB, (Number of flower in the bush), FDFB, (function of dry flower in the bush), FWF, (function of wet flower in the bush), FDF, (function of dry flower in the surface unit), FFS, (function of wet flower in the surface unit).

Table 4.: comparison of the mean interaction of gibbberelin and ethnaol on the reviewed traits in *Callendula Officinalis.*

Gibberellin	Ethanol	PC	NFS	NFB	FDFB	FWF	FDF	FFS
0	0	0.70_{bcde}	18.05_{de}	7.15_{b}	4.87_{bcd}	26.95_{b}	12.24_{fg}	66.67_{de}
15	0	0.66_{cde}	81.38_{a}	7.01_{b}	4.62_{bcd}	28.64_{b}	53.29_{b}	321.05_{a}
30	0	0.67_{cde}	87.32_{a}	8.42_{b}	6.48_{abc}	33.39_{ab}	65.79_{a}	432.96_{a}
0	250	0.65_{de}	49.31_{b}	7.71_{b}	5.78_{bc}	26.09_{bc}	36.58_{c}	163.09_{b}
15	250	0.83_{abc}	26.68_{cde}	7_{b}	5.19_{bcd}	28.89_{b}	19.59_{ef}	109.35_{cbd}
30	250	0.72_{bcde}	34.07_{c}	6.3_{b}	4.74_{bcd}	23.54_{bc}	25.85_{de}	128.99_{cbd}
0	500	0.55_{e}	14.04_{e}	7.54_{b}	4.08_{bcd}	23.35_{bc}	7.44^{g}	42.09_{e}
15	500	0.73_{abcde}	37.32_{bc}	10.87_{a}	7.13_{ab}	42.76_{a}	24.75_{de}	147.18_{bc}
30	500	0.92_{a}	30.62_{cd}	7.62_{b}	9.09_{a}	31.97_{ab}	34.03_{cd}	124.29_{cbd}
0	750	0.87_{ab}	18.03_{de}	8.03_{b}	5.25_{bcd}	31.24_{ab}	11.59_{fg}	70.27_{de}
15	750	0.76_{abcd}	30.04_{cd}	7.26_{b}	3.59_{cd}	20.85_{bc}	15.44_{efg}	87.78_{cde}
30	750	0.77_{abcd}	24.65_{cde}	3.07_{c}	2.14_{d}	13.08_{c}	17.72_{efg}	110.09_{cbd}

PC, (Phenolic compositions), NFS, (Number of flower in the surface unit), NFB, (Number of flower in the bush), FDFB, (function of dry flower in the bush), FWF, (function of wet flower in the bush), FDF, (function of dry flower in the surface unit), FFS, (function of wet flower in the surface unit).

3.2 Number of flowers in surface unit

The obtained results from variance analysis indicated that gibberelin has significant effect on the number of flowers per surface unit in 1 % probability level. Also with regard to the results from table for analysis of variance it was determined that ethanol has significant effect on this feature in 1% probability level. The results from table for analysis of variance indicated that interaction effects of ethanol and gibberelin has significant effect on the number of flowers per surface unit in 1% probability level (Table 1). The results from comparison of mean gibberelin effect on feature of number of flowers per surface unit indicated that the highest number of flowers (62.20) is related to the witness and the least number of flowers (24.26) is related to the level of gibberelin 750 ppm. With increasing gibberelin the number of flowers per surface unit is reduced (Table 2). Comparison of mean ethanol effect on this feature indicated that the highest number of flowers (44.19) is related to 30 % ethanol and is the least number (24.73) is related to the witness (Table 3). Also the results from comparison of mean interaction effect of ethanol and gibberelin on this feature indicated that the highest number of flowers (87.32) belonged to the level of the witness gibberelin and 15% ethanol and the level of gibberelin 500 ppm and zero ethanol had the least number of flowers (14.04) (Table 4).

3.3 Function of wet flower in the surface unit

The results obtained from (Table 1) for analysis of variance indicated that gibberelin has significant effect on function of wet flower in the surface unit in 1 % probability level. Also with regard to the results of table for analysis of variance it was determined that ethanol has significant effect on this feature in 1 % probability level. The results obtained for analysis of variance indicated that interaction effects

of ethanol and gibberelin has significant effect on function of wet flower in the surface unit in 1 % probability level. The results for comparison of mean effect of gibberelin on the function of wet flower in the surface unit indicated that the highest function of the wet flowers (242.51) is related to the witness level and the least number (88.50) is related to the level of gibberelin 750 ppm (Table 2). Comparison of mean ethanol effect on this feature indicated that the highest function of the wet flowers in the surface unit (177.08) is related to 30% ethanol and its least function (85.40) is related to the witness (Table 3). Also the results for comparison of interaction effect of ethanol and gibberelin on this feature indicated that the level of the witness gibberlin and 15% ethanol had the highest function of the wet flowers (432.96) and the least function of the wet flowers (42.09) is related to 500 ppm gibberelin and witness zero ethanol (Table 4).

3.4 Function of dry flower in the surface unit

The results obtained from (Table 1) for analysis of variance indicated that gibberelin has significant effect on this feature in 1 % probability level. Also with regard to the results of table for analysis of variance it was determined that ethanol has significant effect on function of dry flower in 1 % probability level. The results analysis of variance indicated that interaction effects of ethanol and gibberelin has significant difference on function of dry flower in the surface unit in 1 % probability level (Table 1). The results for comparison of mean gibberelin effect on the feature of dry flower in the surface unit indicated that the highest function of the dry flowers in the surface unit (42.76) is related to the witness level and its least function (14.01) is related to 750 ppm gibberelin, and with increasing gibberelin level the function of dry flower in the surface unit is reduced (Table 2). Comparison of mean ethanol effect on this feature indicated that the highest function of the dry flowers in the surface unit (35.85) is related to 30% ethanol and its least function (17.02) is related to the witness. And with increasing ethanol level the function of dry flower in the surface unit increases (Table 3). Also the results for comparison of interaction ethanol and gibberelin effect on this feature indicated that the highest function of the dry flowers (65.79) is related to gibberelin in the level of witness and 30% ethanol, and its least function of dry flower (7.44) is related to the level of 500 ppm gibberelin and witness ethanol (Table 4).

3.5 Function of wet flower in bush

The results obtained from (Table 1) for analysis of variance indicated that gibberelin has significant effect on the function of wet flower in the bush in 5% probability level. Also with regard to the results of analysis of variance it was determined that ethanol. The results analysis of variance indicated that interaction effects of ethanol and gibberelin has significant difference on function of wet flower in bush in 5 % probability level. The results for comparison of mean gibberelin effect on the feature of function of wet flower in bush indicated that the highest function of the wet flower in the bush (31.67) is related to 500 ppm gibberelin, and its least function (20.72) is related to gibberelin 750 ppm (Table 2). Comparison of mean ethanol effect on this feature indicated that the highest function of the wet flower in the bush (30.38) is related to 15% ethanol and its least function (25.58) is related to 30 % ethanol (Table 3). Also the results for comparison of interaction ethanol and gibberelin effect on this feature indicated that the highest function of the wet flower (42.76) is related to the level of gibberelin 500 ppm and 15% ethanol, and the least function of wet flower in bush (13.08) is related to the level of 750 ppm gibberelin and 30% ethanol (Table 4).

3.6 Function of dry flower in bush

The results obtained from (Table 1) for analysis of variance indicated that gibberelin has significant effect on the function of dry flower in the bush in 5% probability level. Also with regard to the results of table for analysis of variance it was determined that ethanol. The results analysis of variance indicated that interaction effects of ethanol and gibberelin has significant difference on function of dry flower in the bush in 5% probability. The results for comparison of mean gibberelin effect on the feature of function of dry flower in the bush indicated that the highest function of the dry flower in the bush (6.67) is related to the level of 500 ppm gibberelin, and its least function (3.56) is related to gibberelin 750 ppm (Table 2). Comparison of mean ethanol effect on this feature indicated that the highest function of the dry flower in the bush (5.51) is related to 30 % ethanol and its least function (4.89) is related to the witness (Table 3). Also the results for comparison of interaction ethanol and gibberelin effect on this feature indicated that the highest function of the dry flower in the bush (9.09) is related to the level of gibberelin 500 ppm and 30% ethanol, and the least function of the dry flower in the bush (2.14) is related to the level of 750 ppm gibberelin and 30% ethanol (Table 4).

3.7 Phenolic compositions

According to the obtained results from the (Table 1) for analysis of variance, gibberelin, ethanol and their interaction has no significant effect on Phenolic compositions. The results which obtained from table for comparison of mean gibberelin effect indicated that the amount of phenolic compositions increases with increasing gibberelin level and the least amount (0.68) is related to the witness and its highest function (0.80) is related to gibberelin 750 ppm but statistically there is no significant difference between various levels (Figure 1). Comparison of mean

ethanol effect on this feature indicated that the highest amount of phenolic compositions (0.77) is related to 30% ethanol and its least amount of phenolic compositions (0.70) is related to the witness. But there is no significant difference between various levels of ethanol (Figure 2). Comparison of mean interaction effects of ethanol and gibberelin indicated that the level of gibberelin 500 ppm and 30 % ethanol had the highest amount of Phenolic compositions (0.91) and the level of gibberelin 500 ppm and witness ethanol had the least amount of phenolic compositions (0.56) (Table 4).

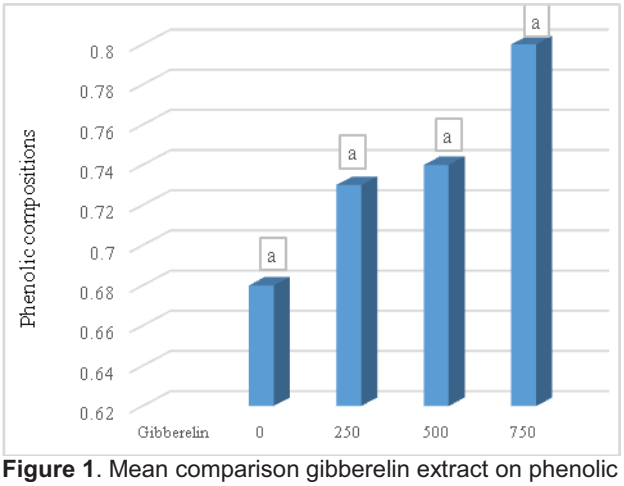

Figure 1. Mean comparison gibberelin extract on phenolic compositions.

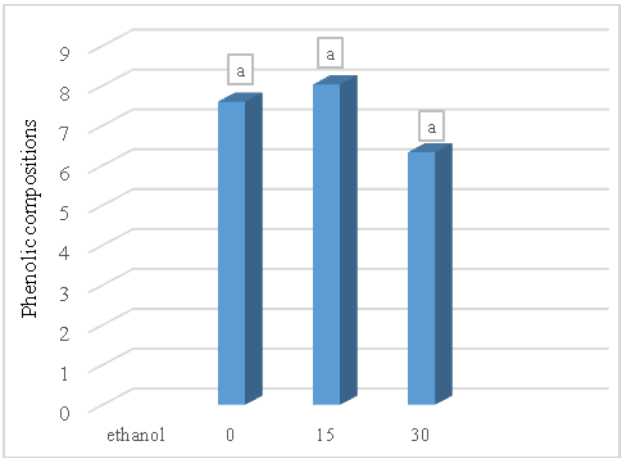

Figure 2. Mean comparison ethanol extract on phenolic compositions.

4 Discussions and Conclusions

The obtained observations from this research indicated that gibberelin had significant effect on number of flowers per square meter, function of wet flower in the surface unit, function of dry flower in the surface unit, and also gibberelin was significant on function of wet flower in the bush and function of dry flower in the bush in the level of 5 %. Number of flowers in the surface unit, function of wet flower in the surface unit, function of dry flower in the surface unit has reduced with increasing the gibberelin level and function of wet and dry flower in the bush had the highest amount in the level of 500 ppm.

Phytohormones and plant growth regulators (PGRS) have been defined as one of the main factors influence plants growth and their primary and secondary metabolites pool. Gibberellins are PGRS with stimulating effects on the majority of plants internal and visible responses with many detectable demonstrations such as increased shoot length due to accelerated cell division and enlargement and their unique effects on flowering behavior of plants [11, 12]. As a result it is effective in increasing the amount of the plant's Carbohydartes and each factor that can be effective on increasing, attraction or making of sugars can cause to increase the amount of total anthocyanin in the petals. Increasing other pigments may be due to this reason as well. Also according to the Theory of [13], GA_3 changes the form of plastids structure which contain pigments and causes to increase plastids and as a result increasing pigments.

At the same time, [14] noted that GA3 foliar spray enhanced the height and ornamental wealth of *Araucaria heterophylla* plants. Same results for the positive effects of GA3 on plant growth and development have been reported by [15] in *Ocimum* spp. Furthermore, [16] in their work on carnation cv. "Red sim" demonstrated that gibberellic acid foliar application surprisingly affected plant growth and its subsequent flower production potential also [16] wrote that GA3 foliar implementation had stimulating effects on flower induction of *Dianthus caryophyllus* L. and hence led to the increased inflorescence biomass and essential oil production.

In *Salvia officinalis* L. the chemical composition changed with gibberellic acid (100 mg L^{-1}) application, with a significant reduction of β-tujone and α-humulene in relation to the control plants [17]. Several investigators studied these effects. For instance, [18] on *Asparagus sprengeri*, [19] on croton plants, [20] on black cumin, [21] on *Calendula officinalis*, [22] on *Hemerocallis auantiaca*, [23] on *Solidags hybrida* and [24] on *Palanites aegyptiaca* plants, revealed that GA3 treatments enhanced vegetative, flowering growth and chemical composition.

The results for the effect of interaction gibberelin and ethanol on the number of flowers per surface unit indicated that the level of witness gibberelin and 15% ethanol had the highest number of flowers per surface unit and the level of gibberelin 500 ppm and zero ethanol had the least number of flowers per surface unit. Comparison of mean interaction effects of gibberelin and ethanol indicated that the range of changes in number of flowers per bush varies between 3.07 in the level of gibberelin 750 ppm and 30 % ethanol and 10.87 gibberelin 500 ppm and 15% ethanol. With regard to the obtained results from this research we can conclude that gibberelic acid causes to increase the function of wet and dry flower in bush d increasing the active substance and

ethanol also causes to increase the function of flower in the surface unit and for increasing the function of flower in the surface unit their interaction effects has no effect and we can use 30% ethanol. For increasing the function of flower in the bush the best level is gibberelin 500 ppm. Also gibberelic acid causes to increase the active substance. Comparison of mean interaction effects for gibberelin and ethanol indicated that the level of gibberelin 500 ppm and 30 % ethanol had the highest amount of phenolic compositions with the average of 0.92 and the level of gibberlin 500 ppm and 30 % ethanol, and the level of gibberelin 500 ppm and witness ethanol had the least amount of phenolic compositions with the average of 0.55. But statistically interaction effects of gibberelin and ethanol have no significant effect on this feature.

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