Full Length Research Paper

Effect of foliar application of α-tocopherol and pyridoxine on vegetative growth, flowering, and some biochemical constituents of *Calendula officinalis* L. plants

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A field experiment was conducted during 2010 at the Experimental Farm of Fajr, SB University of Kerman, Iran. The aim was to study the effects of foliar spray of α-tocopherol (0, 50, 100 part per million (ppm)) and pyridoxine (0, 50, 100 ppm) on vegetative growth, flowering parameters, and some chemical constituents of *Calendula officinalis* L. plants. Most parameters were significantly affected by application of two vitamins which were used in this study. The obtained results could be summarized as follows; pyridoxine at 100 ppm recorded the best value of leaf area, stem height, chlorophyll b, reducing sugars and hyperoside content; no significant difference was found between plants treated with vitamins in terms of chlorophyll a, root length, fresh weight and dry weight of root; the treatment with α-tocopherol at 100 ppm resulted in the highest yield of seed, fresh and dry aerial parts, as compared to control plants; maximum values of carotenoid were obtained by the application of pyridoxine at 50 ppm; and application of both vitamins led to the reduction of flower diameter.

Keys words: *Calendula officinalis* L., α-tocopherol, pyridoxine.

INTRODUCTION

Marigold (*Calendula officinalis* L. (Asteraceae)) is a medicinal plant that is widely cultivated for obtaining extracts used in phytotherapy and ornamental purposes. It has been used as a medicinal plant in Iran for a long time. *C. officinalis* is an annual herb native to Southern Europe, which is used in traditional system of medicine to treat various diseases. Main constituents in the marigold extract are flavonoids, flavonol, glycosides, and saponin (Re et al., 2009). This plant is used because of its varied sources of biological activities like anti-inflammatory, anti-

mutagenic, diuretic, and antispasmodic and is also used in gastro-intestinal, gynecological, eye diseases, skin injuries and in some types of skin burning (Chakraborthy, 2008). Phytopharmacological studies of different marigold extracts have shown anti-tumoral (Jiménez-Medina et al., 2006), anti-inflammatory, wound healing (Zitterl-Eglseer et al., 1997) and antioxidant activities (Katalinic et al., 2006).

Vitamins could be considered as bio-regulator compounds which in relatively low concentrations exerted profound influences on plant growth regulating factors that influence many physiological processes, such as synthesis of enzymes, act as co-enzymes and affects plant growth (Nahed et al., 2009; Reda et al., 2005). α-Tocopherol (vitamin E) is a small molecule that is synthesized in plants (Fryer, 1992). Tocopherols appear to be universal constituents of all higher plants (Bafeel

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Abbreviations: ROS, Reactive oxygen species; **CRBD,** completely randomized block design.

and Ibrahim, 2008). Tolerance to salt stress, chilling stress, ultraviolet (UV)-B stress and pollutant stress in plants are partially correlated with tocopherol content (Munné-Bosch and Alegre, 2002). α-Tocopherol is a strong antioxidant that assists the transport of electrons in photosystem-II protein complex (Muhammad, 2011). El-Bassiouny et al. (2005) reported that foliar spray with α-tocopherol on faba bean plants induced increase in growth parameters, yield components, chlorophyll a, b and carotenoids content. Tocopherols play a role in a range of different physiological phenomena including plant growth and development, senescence, preventing lipid peroxidation and to interact with the signal cascade that convey abiotic and biotic signals (Sattler et al., 2004; Baffel and Ibrahim, 2008).

Hendawy and Ezz EL-Din (2010) reported that vitamin B complex act as co-enzymes in the enzymatic reactions by which carbohydrates, fats and proteins are meta-bolized and involved in photosynthesis and respiration. Pyridoxine (Vitamin B_6) is an essential metabolite in all organisms. It can act as a coenzyme for numerous metabolic enzymes and has recently been shown to be a potent antioxidant. It is an essential cofactor for nume-rous metabolic enzymes including amino acid metabolism and antibiotic biosynthesis and is a requirement for growth and differentiation of some plant species (Dolatabadian and Sanavy, 2008). It has been established that pyridoxine enhances the growth of root system (Samullah, 1991; Shimasaki and Fukumoto, 1998) and lead to better nutrient uptake and higher economic yield (Lone et al., 1999). Treatment with pyridoxine has been shown to improve growth and yield parameters in corn plants (Farrokhi and Paykarestan, 2010).

Hyperoside (quercetin-3-O-galactoside) is a flavonoid compound, which has been shown to possess various biological functions against reactive oxygen species (ROS) induced damage, such as anti-viral activity, anti-inflammatory, antidepressant, hepatoprotective and gastric mucosal-protective effects (Qin et al., 2010; Xing et al., 2011).

In this experiment, the effects of two vitamins on hyperoside biosynthesis were studied. The aim was to determine the best concentration of pyridoxine and α-tocopherol which could improve growth and flower characters, chemical constituents and hyperoside biosynthesis in marigold plants.

MATERIALS AND METHODS

Field experiment was conducted at the Experimental Farm of Fajr, SB University of Kerman, Iran. One month old seedlings of marigold (*C. officinalis* L.) were prepared in the nursery. Uniform seedlings were then transplanted to the field plots of loamy soil on 20th May, and sprayed twice with freshly prepared solutions of α-tocopherol at 0, 50, and 100 ppm, and pyridoxine at 0, 50, and 100 ppm. The first time of application was on 10th June, and the second was on 30th June, both early in the morning. Control plants were sprayed with distilled water. All groups received normal fertilization of this plant.

The experiment was arranged in a completely randomized block design (CRBD) with three replicates per each treatment with sampling. Each plot contained 5 rows, 50 cm apart and the distance between plants was 30 cm. Treatments means were compared using Duncan's multiple range test at 5% level of significance.
Four samples were taken randomly from each plot. Plant height (cm), root length (cm), leaf area (cm^2), plant fresh weight (aerial and root parts) g plant^{-1}, plant dry weight (aerial and root parts) g plant^{-1}, number of flowering stem, flower diameter, seed weight per plant, chlorophyll content, reducing sugar and hyperoside content were measured.

More parameters were recorded two weeks after second stage of foliar spray and some parameters such as seed yield, stem height, root length, and fresh and dry weight of plant organs were measured at final vegetative growth. Shoot and roots of the plants were weighed and kept in an electric oven at 70°C for 72 h to measure dry weight.

Samples from fresh leaves of each treatment were used to determine photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids). These pigments were estimated by the spectrophotometric method recommended by Lichtenthder (1987). In this method, the extraction was measured at 3 wavelengths of 646.8, 663.2 and 470 nm using spectrophotometric SPUV-26 SCO-TECH. The concentration of the pigment fraction (chlorophyll a, chlorophyll b, and carotenoids) was calculated as mg ml^{-1} using the following equations:

Chlorophyll a (mg ml^{-1}) = (12.25 A663.2 – 2.79 A646.8)

Chlorophyll b (mg ml^{-1}) = (21.21 A646.8 – 5.1 A663.2)

Car (mg ml^{-1}) = (1000 A470- 1.8 Chlorophyll a – 85.02 Chlorophyll b)/198

Measurement of reducing sugars was performed by the spectrophotometric method at wavelength of 600 nm recommended by Somogy-Nelson (1952) and glucose concentration (mg L^{-1}) was calculated using the following equation:

Y= 0.0216 X - 0.0097

Where, X is the reading obtained from spectrophotometer.
Hyperoside content in this study was obtained from dry petal powder. In this method, data obtained from spectrophotometer at wavelength of 425 nm were used to calculate the hyperoside concentration using the following equation:

Hyp % = (1.25 × E)/b

Where, E is the spectrophotometer data and B is the plant sample weight (g)

RESULTS AND DISCUSSION

Growth and flower characters

Data presented in Table 2 shows that foliar application of α-tocopherol at 100 ppm and concentration of pyridoxine at 50 and 100 ppm significantly increased leaf area compared with application of 50 ppm α-tocopherol and untreated plants. Maximum leaf area obtained with application of pyridoxine at 100 ppm and α-tocopherol at 100 ppm lead to increasing (11.9 and 9.48%) leaf area, respectively.

Table 1. Mean values of some characteristics of the marigold plants treated with α-tocopherol and pyridoxine.

Parameter	Flowering stem (Num)	Flower diameter (mm)	Chl a (mg. ml^{-1})	Chl b (mg. ml^{-1})	Carotenoid (mg. ml^{-1})	Reducing sugar (mg/L)	Hyperoside (%)
Control	10.75^c	31.16^a	8.02^a	3.98^c	2.75^b	72.62^d	1.04^b
α-tocopherol 50 ppm	11.83^{bc}	30.7^{ab}	9.41^a	4.76^b	2.97^b	77.91^c	1.03^b
α-tocopherol 100 ppm	11.75^{bc}	30.5^{ab}	9.28^a	4.16^c	3.09^b	77.75^c	1.06^b
Pyridoxine 50 ppm	12.33^{ab}	29.25^b	9.96^a	4.18^c	3.12^a	84.54^b	1.02^b
Pyridoxine 100 ppm	12.33^a	29.8^{ab}	9.64^a	4.93^a	2.81^b	98.31^a	1.21^a

Means in the same column followed by the same letter are not significantly from each other at P < 0.05.

Table 2. Mean values of vegetative growth parameters of marigold plants treated with α-tocopherol and pyridoxine.

Parameter	Leaf area (cm^2)	Stem height (cm)	Root length (cm)	Fresh aerial parts (g/plant)	Dry aerial part (g/plant)	Fresh root (g/plant)	Dry root (g/plant)	Seed yield (g/plant)
Control	111.7^b	36.58^{bc}	25.37^a	120^b	13.26^b	40.79^{ab}	8.45^b	10.18^c
α-Tocopherol 50 ppm	112.1^b	35.41^c	26.3^a	119.7^b	13.35^b	39.99^b	8.38^b	10.83^b
α-Tocopherol 100 ppm	122.3^a	38.08^b	26.7^a	143.5^a	16.21^a	41.77^{ab}	8.77^b	13.38^a
Pyridoxine 50 ppm	121.8^a	42.95^a	26.4^a	140.2^a	15.93^a	43.26^{ab}	9.03^{ab}	10.55^b
Pyridoxine 100 ppm	125.1^a	44.62^a	26.9^a	140.2^a	15.85^a	45.26^a	9.51^a	10.56^b

Means in the same column followed by the same letter are not significantly from each other at P < 0.05.

Generally, application of all treatments significantly increased fresh and dry weight of aerial parts, except the treatment with α-tocopherol at 50 ppm. The highest weights were recorded with spraying α-tocopherol at 100 ppm concentration. The increase effect on fresh and dry weight of aerial parts by application of α-tocopherol at 100 ppm was 19.58 and 22.24%, respectively (Table 2). By application of pyridoxine at 50 and 100 ppm, fresh and dry weight of roots significantly increased compared to untreated plants. Fresh and dry weight of root increased by application of all treatments, however, the increase was not significant (Table 4). El-Quesni et al. (2009) reported that application of α-tocopherol increased fresh weight of shoots and roots in *Hibiscus rosa sineses* L. plants. Studies conducted at Aligarh, India, have indicated that the soaking of seeds in pyridoxine solution resulted in enhanced growth of the juvenile root system of black gram (Khan and Ansari, 1984), lentil (Ansari et al., 1990), and mustard (Samiullah et al., 1991).

Data presented in Table 2 shows that foliar spray of pyridoxine at concentrations of 50 and 100 ppm significantly increased means of plants height while effect of α-tocopherol on plants height was not significant. Regarding the average length of roots, the data showed that this parameter was not affected by application of any treatments. However, all treatments increased number of flowering stems compared to the control plants, and maximum number of flowering stem obtained with application of pyridoxine at 100 ppm. Flower diameter was reduced by application of all treatments as compared to flower diameter of control plants though the difference

was only significant in the case of application of 50 ppm pyridoxine (Table 1). Results of this study showed that pyridoxine and α-tocopherol have adverse effect on flower diameter which are not in agreement with the finding of El-Quesni et al. (2009) on hibiscus plant, who mentioned that number of flowers/plant, leaf area, and fresh and dry weight of plant organs were significantly affected by the application of α-tocopherol. Application of α-tocopherol has been shown to improve growth in two cultivars of sunflower plants (Sadak et al., 2010). On the other hand, Barakat (2003) reported that treatment of wheat (*Triticum aestivum* L.) plants with pyridoxine considerably increased the cell division.

Data presented in Table 2 shows that the highest increase in seed weight (g/plant) belongs to the plants treated with 100 ppm α-tocopherol as compared with the control and other treatments. The increased effect on weight of seed by application of 100 ppm α-tocopherol was 34.69% compared to the control plants. Sadak et al. (2009) demonstrated that in sunflower plants, application of α-tocopherol led to increased seed yield. The seed yield increase of sunflower plant in response to α-tocopherol is mainly due to the effect of vitamins on protein synthesis and delaying senescence or might be related to increase in photosynthetic products.

El-Bassiouny et al. (2005) reported that foliar spray with α-tocopherol on faba bean plants increased growth parameters and seed yield. Samiullah et al. (1984) reported that pyridoxine, applied to seeds before sowing or by spraying of a standing crop of moong (*Vigna radiata)* significantly enhanced leaf nitrate reductase

Table 3. Mean square for flowering and biochemical characters.

Parameter	df	Flowering stem (Num)	Flower diameter (mm)	Chlorophyll a (mg. ml^{-1})	Chlorophyll b (mg. ml^{-1})	Carotenoid (mg. ml^{-1})	Reducing sugar (mg/L)	Hyperoside (%)
Replication	2	0.35ns	24.9*	2.81 ns	1.33 ns	4.23 ns	61.22ns	0.006ns
Treatment	4	10.2**	6.94*	8.61 ns	4.65 *	0.49 *	1183**	0.07**
Sampling error	45	2.37	3.18	1.15	1.29	0.14	23.03	0.01
Experimental error	8	1.22	1.67	6.23	4.05	1.64	32.02	0.003
CV %		12.5	5.8	7.37	4.41	5.12	5.83	9.38

NS,** and * are respectively non significant and significant at the P 0.01 and 0.05 levels.

Table 4. Mean square for vegetative growth characters and yield.

Parameter	df	Leaf area (cm^2)	Stem height (cm)	Root length (cm)	Fresh aerial parts (g/plant)	Dry aerial parts (g/plant)	Fresh root (g/plant)	Dry root (g/plant)	Seed yield (g/plant)
Replication	2	150.4 *	89.4 ns	13.07 ns	521.4 ns	14.6 ns	160 *	8.57 *	0.43 ns
Treatment	4	468 **	196 *	4.54 ns	1678 *	26.3 *	56.4 ns	2.54 ns	20.1 **
Sampling error	45	52.4	6.98	2.29	185.3	2.9	12.84	0.69	0.8
Experimental error	8	38.9	59.05	4.96	351.7	5.01	31.2	1.2	1.54
CV %		6.1	6.68	5.76	7.25	7.42	8.48	9.41	8.09

NS,** and * are respectively non significant and significant at the P 0.01 and 0.05 levels.

activity and seed yield.

Chemical constituents

None of the treatments led to significant changes in the amount of chlorophyll a (chl a). Meanwhile, chlorophyll b (chl b) content was affected by some treatments such as α-tocopherol at 50 ppm and pyridoxine at 100 ppm compared to the other treatments and control (Table 1). The highest concentration of chlorophyll b was obtained with pyridoxine at 100 ppm. Highest concentration of the carotenoid was obtained by foliar spray of pyridoxine at 50 ppm which was 13.45% higher than the control plants. In plants, tocopherols are believed to protect chloroplast membranes from photooxidation and help to provide an optimal environment for the photosynthetic machinery (Munne-Bosch and Algere, 2002). El-Quesni et al. (2009) reported that foliar application of α-tocopherol increased chl a, chl b and carotenoids content compared to untreated plants. El-Bassiouny et al. (2005) reported that foliar spray of α-tocopherol on faba bean plants also increased chl a, b and carotenoids content.

In this study, all treatments increased the amount of reducing-sugars compared to the untreated plants. Also, both concentration of pyridoxine 50 and 100 ppm increased reducing-sugars compared to application of α-tocopherol. Highest content of reducing-sugars obtained with application of pyridoxine at 100 ppm was 34.96% higher compared to the control plants (Table 1). El-

Quesni et al., (2009) reported that by application of α-tocopherol, the amount of soluble sugars in hibiscus plants significantly increases. Sadak et al. (2010) found that application of α-tocopherol stimulated the accumulation of total carbohydrates in sunflower plants.

Data presented in Table 1 shows that the only treatment which increases the amount of hyperoside, was pyridoxine at 100 ppm and effect of other treatments was not significant. The hyperoside content was increased to 16.34% which was higher as compared to the control.

Conclusion

Based on the results of this study, we recommend the application of pyridoxine at 100 ppm to obtain the highest values of root weight, leaf area, chl b, carotenoid, reducing sugars and hyperoside content, while α-tocopherol at 100 ppm can be used to obtain the highest seed yield and highest growth of shoot.

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