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Soil Cover Effects on Yield and Some Physiological Characteristics of Marigold (*Calendula officinalis* L.) under Methanol Foliar Application

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Abstract

This study was conducted to assay the effects of methanol foliar application (0, 10, 20 and 30% v/v) and bed cover (control, white and black covers) on yield and some physiological traits of marigold as a factorial based on RCBD. The results revealed the significant interaction effects of bed cover and methanol foliar application on chlorophyll a content and plant height. The highest plant height was belonged to black mulch × methanol (except for 30% methanol). The highest chlorophyll a content (2.3 mg g^{-1} FW) was attained by black mulch × 30% methanol. Chlorophyll b content was influenced by soil cover and the highest chlorophyll b content (1.1 mg g^{-1} FW) was recorded with black mulch. The flowers and leaves dry weight as well as relative water content and total soluble solids content were affected by the treatments and, the highest data for flower dry weight (116 g) was in 30% foliar spray and black mulch (103.6 g). The highest data for total soluble solids content (120°Brix) and relative water content (49.7%) were recorded with 30% methanol foliar application and by black soil cover. For root-dry weight (15.3 g) and total phenolics content (95.05 mg GA g^{-1} Dwt), black mulch was the treatment of choice.

Keywords: *Calendula officinalis* L., Chlorophyll, Flower, Phenolics compounds.

INTRODUCTION

Marigold (*Calendula officinalis* L.) is an annual plant, belongs to the Asteraceae family. Its flowers are containing essential oils, saponin, organic acids, calendoline, and albumin (Yazdi Far *et al*., 2015).The plant and its preparations are used in industry (production of color and nylon), pharmacy (production of creams and lotions) (Nejad Ali Rezaei *et al.*, 2011), as an ornamental plant and even, the flowers are in use as cut flower. Considering, marigold is a candidate for landscape designing and also is a cut flower due to its long-term flowering time-course.

Using methanol and ethanol have been verified as a safe and easy way to stimulate the growth potential in plants. During the 1990, there were several reports showing that foliar application of these alcohols increase the yield, enhances ripeness and strengthens the drought tolerance and reduces the water demands with many agronomic crops (Nonomura and Benson, 1992; Rajala *et al.*, 1998). The exogenous application of methanol directly affects metabolic pathways related to plant growth and development (*e.g*. the content of amino acids). In addition, pathways related to plant defense mechanisms such as the genes involved in the jasmonic acid biosynthesis were affected (Gout *et al.*, 2000). Methanol in trace amounts is produced during leaf growth and the demethylation of cell wall pectins (Haston and Roje, 2001). It is known that methanol increases the stomatal conductance, has effects on leaf area index, leaf durability, growth responses and cell turgidity by increasing glucose metabolism in the leaves as well as decreases leaf temperature and transpiration rate (Downie *et al.*, 2004; Rajala *et al.,* 1998). After production, some of the alcohol emits to the atmosphere and the remained part is converted to formaldehyde, formic acid and finally is converted to CO_2. The produced CO_2 affects the CO_2 assimilation rate in plants (Galbal and Kristine, 2002). Methanol is easily metabolized by the plants and can be used in photosynthesis and for the production of sugar and some amino acids (Nadali *et al*., 2010). Yazdi Far *et al*. (2015) reported that methanol foliar application easily and in a short time increased chlorophyll content of *Caliendula officinalis* plant. Using soil covers has gained a great interest in organic agriculture for controlling weeds, to preserved soil moisture and for soil conservation against erosions. Soil cover prevents the sudden water streams in the fields and by trapping the nutrients amends the soil fertility.

Considering the semi-arid climatic condition and low precipitation rates and un-even rain distribution patterns as well as the deteriorated soil structure in northwest Iranian region, it seems that using soil covers would be a suitable easy way to overcome these production restraints. Marigold is an excellent ornamental medicinal plant thoroughly adapted to the NorthWest of Iran. Furthermore, it seems that its commercial production could be an alternative for high water demanding crops at the region. The present experiment was conducted to assay the growth and yield responses of marigold to the foliar application of methanol besides using soil covers.

MATERIALS AND METHODS

In order to evaluate the effect of soil cover and methanol foliar application on yield and some physiological traits of *Caliendula officinalis*, a field experiment was carried out during 2015 in Azarbaijan Shahid Madani University Research Farm, as factorial experiment based on Randomized Complete Block Design (RCBD) with three replications. After preparing the field, white and black polyethylene plastic covers were spread on the beds. The seeds (provided by Pakan Seed Company) were directly planted in the field during late April. Each block was composed of six rows spacing 30 Cm, within the row. Plants were spaced 15 cm in between. Three seeds were planted in each hole and later, they were tinned to one. Common irrigation regime was followed. Treatments were foliar application of methanol [0 (control), 10, 20 and 30 % (v/v)], and soil covers (control, black and white polyetelene). Methanol spray was applied 2 times during growth season. The first spray applied 40 days after germination and the other was applied 70 days after germination. The plants were digged from each block and the dry weight of the plants were measured behind drying in an oven at 35 °C for 72 h.

Traits Measurment

Total chlorophyll content was calculated according to Prochazkova *et al.* (2001). TSS was assessed with the use of a refractometer and expressed in °Brix. Relative water content was estimated based on Karakas *et al.* (1997), according to this formula:

Leaf RWC (%) = ((FW-DW) / (TW-DW)) × 100

Where: FW = fresh weight; TW = turgid weight; DW = dry weight.

Determination of total phenolics content

Total phenolics content (TPC) was determined using the Folin-Ciocalteu method according to Kim *et al*. (2006). The phenolics content was expressed as mg of gallic acid equivalent per gram of dry sample (mg GAE/g) using the linear equation based on the calibration curve.

Essential oil extraction

The essential oil extraction was done from 80 g dry plant material by water distillation during 5 h is using a Clevenger-type apparatus and, the oils were dried over anhydrous sodium sulphate.

Data analysis

The data obtained were subjected to standard analysis of variance by SPSS and MSTATC (version 9.2) software. The values of LSD were calculated at 1 and 5% levels.

RESULTS AND DISCUSSION

Plant height

Analysis of variance (Table 1) revealed that there were interaction effects between foliar application of methanol and soil cover on plant height. Plant height was affected by black soil cover and methanol foliar applications in all treatments except black soil cover × 30% methanol foliar application (Table 2). The results obtained from Yazdi Far *et al*. (2015) showed that methanol had significant effect ($P<0.01$) on plant height, chlorophyll, essential oil and total phenolic compounds content. Our results are in line with the findings of Abbasdokh *et al.* (2015) in *Portulaca oleracea.* They suggested that soil plastic covers establish warm environment in the plant growing bed and at the same time improve the water availability for the plants and so enhance plant growth and height.

Flower dry weight

The result showed that methanol foliar application (Table 3) and also soil cover (Table 4) had significant effects ($P<1\%$) on flower dry weight. Koukourikou-Petridou and Koukounara (2002) noted that foliar application of methanol had positive effects on tomato and pepper growth. Mathanol foliar application may influence cytokinin production and carbon conversion efficiency and hence stimulates plant growth and flowering (Zbiec *et al.*, 1999). Methanol foliar application increased the chlorophyll content, leaf area and the number of stomata per unit leaf area as well as the yield of grapevine cv. 'Flame Seedless' (Ramadant and Omran, 2005). It is known that methanol, in small quantities is a plant common metabolism product and is a source of carbon for the biosynthesis of several metabolites necessary for plants normal growth and development (McGiffen and Manthey, 1996). Methanol is a small biomolecule can be easily metabolized by C_3 crops and has the potential to ameliorate the photosynthesis rate. Therefore, the accelerated photo-assimilation and sugars production go to the increased water potential of the plant and hence raised plants growth.

Leaf and root dry weight

Leaf dry weight was influenced by soil covers and also by methanol foliar application (Table 1). The highest amount of leaf dry weight was recorded in 20 and 30% methanol foliar application (Table 3) and in black soil cover (Table 4). Soil cover had significant effect ($P<1\%$) on root dry weight (Table 1) and the highest root dry weight was recorded in black soil cover (15.23 g) (Table

Table 1. Analysis of variance for the effects of methanol foliar application and soil cover on some physiological characteristics and yield of *Calendula officinalis* L.

S.o.V	df	Plant hieght	Flower diameter	Root dry weight	Leaf dry weight	Flower dry weight	RWC	Phenolic content	Chl.a	Chl.b	TSS	Essential oil content
Replication	2	56.77^{ns}	5.3^{**}	83.2^{**}	0.69^{*}	12.81^{ns}	260.2^{*}	4763.87^{**}	0.65^{*}	0.29^{ns}	11546.1^{**}	0.0004^{ns}
Soil cover (A)	2	36.13^{ns}	0.75^{ns}	56.5^{**}	4.4^{**}	2241.5^{**}	690.8^{**}	3470.8^{**}	1.64^{**}	0.35^{*}	11778.4^{**}	0.0001^{ns}
Metanol (B)	3	147.4^{**}	0.21^{ns}	15.5^{ns}	0.75^{**}	4223.5^{**}	138.22^{**}	440.38^{ns}	0.28^{ns}	0.135^{ns}	1240.4^{**}	0.0009^{ns}
A×B	6	86.98^{*}	0.41^{ns}	10.5^{ns}	0.15^{ns}	47.53^{ns}	1.98^{ns}	211.43^{ns}	0.32^{*}	0.22^{ns}	128.7^{ns}	0.0007^{ns}
Error	22	26.47	0.40	8.69	0.15	72.8	9.13	404.1	0.12	0.096	57.1	0.0001
CV%		9.49	14.8	23.19	10.77	9.28	6.7	26.51	24.9	31.6	7.3	29.4

ns, * and ** show non-significant and significant at P<0.05 and P<0.01, respectively.

Table 2. Mean comparison for the interaction effects of soil cover and methanol foliar application on yield and some physiological traits of *Calendula officinalis* L.

Soil cover	Methanol concentration (v/v)	Plant height (cm)	Chl. a (mg g^{-1} FW)
Control	0	36.0^{c}	0.83^{d}
Control	10	37.3^{bc}	1.02^{d}
Control	20	45.5^{b}	1.43^{bcd}
Control	30	47.5^{b}	1.09^{cd}
Black cover	0	50.8^{a}	1.1^{cd}
Black cover	10	58.5^{a}	1.33^{bcd}
Black cover	20	59.1^{a}	1.3^{bcd}
Black cover	30	47.3^{b}	2.36^{a}
White cover	0	42.1^{c}	1.2^{bc}
White cover	10	39.7^{c}	0.53^{d}
White cover	20	41.2^{bc}	1.83^{bc}
White cover	30	44.1^{bc}	1.36^{bc}
LSD 1%		1.35	0.39

*Similar letter(s) in each column show non-significant differences at 1 and 5% probability level according to LSD test.

Table 3. Mean comparison for the main effect of methanol foliar application on yield and some physiological traits of *Calendula officinalis* L.

Methanol foliar application (%)	Flower dry weight (g)	Leaf dry weight (g)	RWC (%)	TSS (°Brix)
0	65.4^{d}	2.86^{b}	40.4^{c}	93.6^{b}
10	85.2^{c}	2.9^{b}	44.01^{bc}	102.8^{b}
20	101.1^{b}	3.8^{a}	46.4^{ab}	113.4^{a}
30	116^{a}	3.7^{a}	49.7^{a}	120.4^{a}
LSD%	11.34	0.5	0.16	10.05

*Similar letter(s) in each column show non-significant differences at the 1 and 5% probability level according to LSD test.

Table 4. Mean comparison for the main effects of soil cover on yield and some physiological traits of *Calendula officinalis* L.

Soil cover	Flower dry weight (g)	Root dry weight (g)	Leaf dry weight (g)	Chl. b (mg g^{-1}FW)	Total phenolic (mg GA g^{-1} Dwt)	RWC (%)	TSS (°Brix)
Control	76.8^{b}	11.7^{b}	2.9^{c}	0.88^{b}	62.7^{b}	37.53^{c}	78.7^{c}
White cover	95.4^{a}	11.21^{b}	3.61^{b}	0.87^{b}	69.6^{b}	45.1^{b}	103^{b}
Black cover	103.6^{a}	15.23^{a}	4.19^{a}	1.1^{a}	95.05^{a}	52.7$_{a}$	148.9$_{a}$
LSD %	9.81	3.39	0.44	0.26	23.13	3.47	8.7

*Similar letter(s) in each column show non-significant differences at the 1 and 5% probability level according to LSD test.
GA: Gallic Acid.

4). Farajpour *et al*. (2011) reported that the fresh and dry yield of tobacco plants were increased when the plants were treated with methanol (30%) compared to control plants. These results support the hypothesis that methanol may actively influence physiological processes for example photosynthesis. Increased crop growth rate following methanol foliar application is in main part due to elevated CO_2 concentrations in the leaves boundary and even is because of the use of methanol as a direct biomolecule source for the biosynthesis of serin amino acid (Nonomura and Benson, 1992). Moreover, methanol foliar application increases the availability of substrates for auxin and cytokinins metabolism and delays the leaf senescence by the limitations has on ethylene production. The high rates of plants growth regulators biosynthesis drastically affect the plants growth, yield and quality attributes (Khosrawi *et al.*, 2011).

Flower diameter and essential oil content

Treatments used in this experiment had no significant effects on the mentioned factors (Table 1).

Chlorophyll content

Chlorophyll a content was effected by interaction effects of soil cover and methanol foliar application (Table 1). Maximum chlorophyll a content was recorded in black soil cover × 30% methanol foliar applications (Table 2). Chlorophyll b content was affected by soil cover (Table 1) and the highest amounts (1.1 mg g^{-1} FW) was recorded in black soil cover (Table 2). Jabbrzadeh *et al.* (2011) reported that methanol foliar application increased chlorophyll content in tobacco. Zheng *et al.* (2008) reported that methanol foliar application on wheat plants increased leaf chlorophyll content and photochemical efficiency of photosystems that led to the increased stomatal conductance and photosynthesis rate and had significant effect on grain yield. Nadali *et al*. (2010) reported that methanol foliar application increased the plants tolerance to abiotic stresses and with the enhanced light protection, compensates the damages on photosynthetic apparatus. Rajala *et al.* (1998) noted that the major reason for losses in light respiration under stressful conditions was the accelerated methanol oxidation to CO_2. Carbon dioxide is promptly reacts with rubisco carboxylase to enhance the photo-assimilation and gross photosynthesis rate in plants and later goes to enhanced growth and yield components.

Total phenolics content

The result obtained from Table 1 and 4 showed that black soil cover had significant effects (P<1%) on phenolic content. Low water availability (water shortage) is one of the predominant limiting factors affects growth and productivity of agricultural crops under arid environments. Using soil covers is an easy agronomic practice affects crops productivity by increasing water availability and by thermal adequacy in the root medium. Heiska *et al.* (2005) reported that soil covers slowed transpiration rate and limited weeds growth and development. It is quite obvious that black soil covers by the great light absorption and heat accumulation beneath them warm the soil environment efficiently and hence promote plant growth potential.

Relative water content (RWC)

Relative water content was influenced by methanol levels and soil cover (Table 1). The results obtained from Table 3 revealed that with increasing methanol concentration (30%), RWC content was increased. The results also showed that the highest amount of RWC (52.7%) was recorded in black soil cover (Table 4). Nadali *et al*. (2010) also demonstrated that in sugar beet, drought stress limited the photosynthesis potential and yield of the plants by a great reduction in RWC. Jabbarzadeh *et al*. (2010), reported that methanol foliar application improved RWC of tobacco plants.

Total soluble solid content (TSS)

The results documented in Table 1 and mean comparisons showed that there were significant effects of black cover (Table 4) and 30% methanol foliar application (Table 3) on TSS content. Ramadan and Omran (2005) mentioned that application of methanol (30 %) increased total soluble solids (TSS), the TSS/acid ratio and total anthocyanins in berry skins of grapevine cv. 'Flame Seedless'.

CONCLUSION

Methanol can be easily used as a carbon source for the improvement of growth parameters. The overall results revealed the positive effects of methanol foliar application and bed covers on growth, yield and some growth characteristics of marigold. It seems that besides the beneficial effects of soil covers and the feasibility of their utilization, methanol foliar application would be promising agricultural practice to enhance the growth potential, tolerance and durability of agricultural products under semi-arid salinity faced environment of Northwest Iranian climatic conditions. Moreover, would be a priority to evaluate the effects of methanol foliar application on urban horticulture and landscape management.

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