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Scientific Article

Physiological and morphological traits of tulip (*Tulipa* sp.) as affected by different concentrations of ethanol and methanol

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Abstract

Tulip (*Tulipa* sp.) is of the highest economic importance and cultivated area among all bulbous ornamental species. The spray of alcohol is regarded as a proper strategy to improve plant yields in sustainable agriculture systems. This study aimed to investigate the effect of different rates of ethanol and methanol on the traits of the tulip in a factorial experiment based on a Randomized Complete Block Design with two factors including ethanol at four levels (0, 10, 20, and 30 vol%) and methanol at four levels (0, 10, 20, and 30 vol%). The estimated traits included anthocyanin, carotenoid, chlorophyll *a* and *b*, total chlorophyll, stem and leaf Brix index, leaf length and width, leaf area, total and bulb fresh and dry weight, leaf number, and flowering stalk length. Analysis of variance showed that the simple and interactive effects of different treatments were statistically significant on most estimated traits. The highest anthocyanin content (3.92 mg 100 g^{-1} DM), leaf length (25.83 cm), leaf area (258.6 cm^2), and bulb fresh weight (25.81 g) were obtained from the plants treated with 30% ethanol, and the highest anthocyanin content (3.45 mg 100 g^{-1} DM) and leaf Brix index (10.15%) were related to 30% methanol. It can be concluded from the results that methanol and ethanol can be used as plant growth regulators.

Keywords: anthocyanin, carotenoid, ethyl alcohol, methyl alcohol.

Resumo

Influência de diferentes concentrações de etanol e metanol nas características fisiológicas e morfológicas de Tulipa (*Tulipa* sp.)

A tulipa (*Tulipa* sp.) é uma espécie ornamental bulbosa com alta relevância econômica e grande área cultivada. A aspersão de álcool é estimada como uma estratégia válida para aumentar a produtividade de plantas em sistemas sustentáveis de agricultura. Este estudo objetivou investigar o efeito da aplicação de diferentes proporções de etanol e methanol nas características de tulipas utilizando um esquema factorial baseado em um design de blocos completamente aleatório, com dois fatores incluindo etanol em quatro níveis (0, 10, 20 e 30 vol%) e metanol em quatro níveis (0, 10, 20 e 30 vol%). As características avaliadas incluíram antocianinas, carotenoids, clorofila *a* e *b*, clorofila total, índice Brix das folhas e hastes, comprimento e largura das folhas, área foliar, peso fresco e seco dos bulbos, número de folhas e comprimento da haste floral. A análise de variância mostrou que efeitos significativos simples e de interação entre os diferentes tratamentos na maioria das características avaliadas. O maior valor de antocianina (3.92 mg 100 g^{-1} DM), comprimento foliar (25.83 cm), área foliar (258.6 cm^2), e peso fresco de bulbo (25.81 g) foram obtidos em plantas tratadas com 30% de etanol, e o maior valor de antocianina (3.45 mg 100 g^{-1} DM) e índice Brix foliar (10.15%) foi relacionado com a aplicação de 30% de metanol. Conclui-se a partir dos resultados que methanol e etanol podem ser usados como reguladores do crescimento de plantas.

Palavras-chave: antocianina, carotenoide, álcool etílico, álcool metílico

Introduction

Given the fact that the overuse of chemical inputs and plant growth regulators in recent decades for higher crop production has damaged the environment and human health, the global community has been interested in the use of technologies for environmental conservation and sustainability. In this respect, the spray of alcohol, especially methanol and ethanol, is regarded as a strategy to improve plant yields (Valizadeh-Kamran et al., 2019). Benson and Nonomura (1992) reported that methanol can increase plant growth by reducing photorespiration. Tavassoli and Galavi (2011) indicated that the foliar spray of methanol improved the growth and yield of C_3 plants. They believe that methanol may act as a carbon source for the plant and a photorespiration inhibitor. Higher plants

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produce methanol as a result of pectin demethylation. This compound is produced especially during the primary phases of leaf growth. Plant tissue can also make use of methanol. Although there is no methanol oxidase in higher plants, they can convert methanol to CO_2. The assimilation of methanol by plants happens before its oxidation (Ramadan and Omran, 2005). The useful effects of the methanol spraying on growth and yield of different plants have been reported by (Ramadan and Omran, 2005) in grapevine and (Zheng et al., 2008) in wheat.

Khosravi et al. (2016b) observed the highest plant height, leaf dry weight, capitulum dry weight, and root dry weight of *Echinacea purpurea* in plants sprayed with 40% methanol, whereas the highest leaf area and chlorophyll content were obtained from the foliar application of 30% methanol. They concluded that the foliar application of methanol and ethanol would improve the biomass and yield of *E. purpurea* L. because they would act as a source of carbon and bio-stimulator. Sajedi Moghadam et al. (2012) found that the treatment with alcohol improved *Thymus vulgaris* growth and development, yield, and the formation of vegetative organs. The highest yield of thyme was obtained from 30% methanol and 20% ethanol. They can, also, increase the accumulation of carbohydrates and CO_2 concentration (Zbiec et al., 2003). Larzghadiri et al. (2013) obtained the highest leaf length and leaf chlorophyll of *Plantago psyllium* under 30% methanol application and also, the highest leaf fresh and dry weight and leaf width were obtained from 20% methanol treatment. However, the lowest ones were observed in the control samples. Methanol metabolism in plants is similar to CO_2 metabolism. The use of methanol as a carbon source for crops has been progressively advised because leaves can easily take up methanol and use it as a carbon source besides the atmospheric CO_2 (Moradi and Esfahani, 2016; Tavassoli and Galavi, 2011). The root application of methanol caused phytotoxic damage in *Arabidopsis*, tobacco, and tomato plants. But, foliar application improves fresh and dry weight in *Arabidopsis* and tobacco plants. The increase in the weight of *Arabidopsis* is not related to the increase in the sugars (Ramírez et al., 2006).

The central and western provinces of Iran have tremendous potential to produce bulbous plants including tulip because of their specific geographical conditions and ideal climate. The present study aimed at examining the effect of different rates of ethanol and methanol on some traits of tulip and comparing ethanol and methanol in terms of their impact on the yield of this flower. We hypothesized that alcohol can improve tulip growth parameters.

Materials and Methods

Plant material and experimental treatments

The impact of different rates of ethanol and methanol was studied on some traits of tulips (*Tulipa* 'Rosalie') in a factorial experimental design with two factors of ethanol at four levels (0, 10%, 20%, and 30%) and methanol at four levels (0, 10%, 20%, and 30%) with 3 replications (Figure 1).

Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1
Plot 101	Plot 102	Plot 103	Plot 104	Plot 105	Plot 106	Plot 107	Plot 108	Plot 109	Plot 110	Plot 111	Plot 112	Plot 113	Plot 114	Plot 115	Plot 116
A= 1	A= 4	A= 3	A= 1	A= 4	A= 2	A= 2	A= 4	A= 1	A= 2	A= 4	A= 3	A= 1	A= 3	A= 3	A= 2
B= 1	B= 4	B= 3	B= 3	B= 3	B= 3	B= 2	B= 2	B= 2	B= 1	B= 1	B= 2	B= 4	B= 1	B= 4	B= 4

Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2
Plot 201	Plot 202	Plot 203	Plot 204	Plot 205	Plot 206	Plot 207	Plot 208	Plot 209	Plot 210	Plot 211	Plot 212	Plot 213	Plot 214	Plot 215	Plot 216
A= 2	A= 3	A= 1	A= 2	A= 1	A= 1	A= 4	A= 1	A= 3	A= 2	A= 3	A= 3	A= 4	A= 4	A= 4	A= 2
B= 4	B= 1	B= 1	B= 3	B= 3	B= 4	B= 3	B= 2	B= 2	B= 2	B= 3	B= 4	B= 1	B= 2	B= 4	B= 1

Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3	Rep 3
Plot 301	Plot 302	Plot 303	Plot 304	Plot 305	Plot 306	Plot 307	Plot 308	Plot 309	Plot 310	Plot 311	Plot 312	Plot 313	Plot 314	Plot 315	Plot 316
A= 1	A= 4	A= 4	A= 2	A= 1	A= 1	A= 4	A= 2	A= 4	A= 1	A= 3	A= 2	A= 2	A= 3	A= 3	A= 3
B= 3	B= 2	B= 4	B= 4	B= 2	B= 1	B= 3	B= 3	B= 1	B= 4	B= 2	B= 1	B= 2	B= 1	B= 4	B= 3

Figure 1. Experimental map: randomized arrangement of plants in the experiment. A: four levels of ethanol (0, 10%, 20%, and 30%) and B: four levels of methanol (0, 10%, 20%, and 30%).

Tulipa 'Rosalie' is originated from the Netherlands. The planted leaves of bulbous plants were sprayed with ethanol and methanol applied in percentage by volume (0, 10%, 20%, and 30 vol%). Ethanol (96%) and methanol (96%) were supplied by Zagros Petrochemical Company (Bushehr, Iran). The first treatment was applied immediately after the emergence of the first leaf bud on February 26, 2016. The second to fifth foliar applications were applied weekly until March 25, 2016 (flower initiation). Both ethanol and methanol were applied at a rate of 10 mL/plant on the leaves. The trial was carried out in a greenhouse in the cocopeat (Asia Mines and Minerals Development Company) and garden soil medium. The pots were filled with a mixture of garden soil and cocopeat (1:1 v/v). There were 2 bulbs in each plot and a total of 96 bulbs were planted. Each plot included two 2 L pots (17 × 13 × 12 cm^3). Relative humidity and temperature in the plastic greenhouse were 75%-85% and 20-27 °C, respectively. Weed and pest control and fertilization (with NPK) were done when required (two times). Liquid NPK (Plant Feeder 8-8-8) was sprayed on all plants. This study has been tested in a research small scale, so the results in bigger scale might be different.

Measurements

The recorded traits included leaf number, flowering stalk length, leaf length and width, leaf area, total plant fresh and dry weight, and bulb fresh and dry weight.

Leaf area was estimated by the following equation (Palaniswamy and Gomez, 1974):

$$\text{Leaf area} = (\text{length} \times \text{width}) \times 0.75$$

To estimate plant and bulb fresh and dry weights, the harvested plants were weighed. Then, they were re-weighed after oven-drying (Pars Azma Co, model K.J 25, made in Iran) at 105 °C for 24-hours. All flowers were harvested at the wilting stage (petals wilting and abscission).

To measure the chlorophyll content of the leaves, different treatments were sampled 7 days after treating. Then, 0.5 g of the sample was weighed and grounded in a Chinese mortar containing 50 mL acetone 80% (80 mL acetone + 20 mL distilled water). The extract was infiltrated, reached 50 cc, and poured into small containers. The absorption maxima of chlorophyll *a* and chlorophyll *b* were recorded at 643 and 660 nm by a JASCO Model V-530 spectrophotometer. Then, the recorded figures (A) were put in the following equation to calculate total chlorophyll and chlorophyll *a* and *b* (Mazumdar and Majumder, 2017):

$$\text{Total chlorophyll (mg/ml)} = 7.12(A_{660}) + 16.8(A_{643})$$
$$\text{Chlorophyll a (mg/ml)} = 9.93(A_{660}) - 0.777(A_{643})$$
$$\text{Chlorophyll b (mg/ml)} = 17.6(A_{660}) - 2.81(A_{643})$$

To measure anthocyanin, 0.5 g of each sample (leaf) was weighed and ground in a mortar containing 50 mL ethanol hydrochloric acid (85% ethanol 95% + 15% hydrochloric acid). Then, the extract was infiltrated, reached 50 mL, and poured into small containers. The containers were placed in a refrigerator at 4 °C for 24-hours. After that, they were kept in darkness for 2-hours. The extracts were read at 535 nm with a JASCO Model V-530 spectrophotometer, and anthocyanin content was estimated by (Mazumdar and Majumder, 2017):

$$\text{Total absorption of sample} = \frac{e \times b \times c}{d \times a} \times 100$$

where,

a = the sample weight (0.5 g),
b = the volume taken for measurements (5 cc)
c = total volume (50 cc)
d = the fraction taken as sample 0.1
e = the read figure at 535 nm

$$\text{Total anthocyanin of sample} = \frac{\text{total absorption of sample}}{98.2}$$

Carotenoid content was measured on samples taken from all treatments. So, 0.5 g of the sample was ground in a Chinese mortar containing 50 mL acetone 80% (80 mL acetone + 20 mL distilled water). Then, the extract was infiltrated, adjusted to 50 cc, and placed in small containers. They were read at 645, 663, and 660 nm. Then, the figures (A) were put in the following equation to determine the carotenoid content of the treatments (Mazumdar and Majumder, 2017).

$$\text{Carotenoid content} = 4.69(A_{660}) - 0.268(A_{645}) + 8.02(A_{663})$$

To measure stem and leaf Brix index, a part of the stem and leaf tissue was cut. The stem and leaf tissue was placed in a simple squeezer and its sap was poured on the glass plate of a refractometer, Brix figure was estimated, and finally, the total soluble solids (TSS) content was specified according to the Brix table.

Data were analyzed by MSTATC statistical package for the experiment arranged in a Randomized Complete Block Design (with 48 treatments or samples). The data tested for independent and normal distribution before ANOVA analysis. Two-way ANOVA was used to assess the effect of each type of alcohol and the interactions. Means were compared by the LSD test after ANOVA analysis.

Results and Discussion

According to the analysis of variance (Table 1), the effect of experimental factors and the interactions between treatments were significant for the anthocyanin content of tulip flowers at the 1% level.

Table 1. Analysis of variance for the effect of ethanol and methanol on the physiological and morphological traits

Source of Variables	Means of squares												
	Antho-cyanin	Carote-noid	Chloro-phyll *a*	Total chloro-phyll	Leaf Brix	Stem Brix	Floral stalk length	Leaf width	Leaf length	Leaf area	Total fresh weight	Bulb fresh weight	Bulb dry weight
Replication	1.52ns	0.64ns	3.32*	1.89ns	3.30ns	0.54ns	227.70*	1.90ns	4.75ns	1161.57ns	114.87**	5.01ns	1.78ns
Ethanol	10.45**	0.51ns	0.71ns	1.57ns	2.46ns	0.33ns	309.57**	3.25ns	16.74*	4867.73*	48.47ns	40.93**	2.50ns
Methanol	4.97**	0.83ns	1.52ns	1.40ns	7.65**	0.94ns	257.99*	0.97ns	9.08ns	521.64ns	26.24ns	19.99ns	1.81ns
Ethanol × methanol	3.11**	3.91**	1.84*	6.66*	3.26*	1.62*	414.39**	2.44ns	10.85ns	3781.48**	42.18*	14.40ns	1.86ns
Error	0.82	0.40	0.78	2.71	1.17	0.66	58.83	1.19	5.71	1093.39	17.49	6.96	0.95
CV (%)	34.17	13.18	53.30	31.15	12.05	9.83	39.02	8.24	9.39	12.92	10.18	11.27	14.48

ns, * and ** show insignificant and significant differences at 1 and 5% level, respectively according to ANOVA. Ninety-six plants (samples) were used for the analysis.

The highest anthocyanin content was related to the treatment of 30% ethanol and the lowest to 20% ethanol (Table 2).

Means comparison to investigate the effect of methanol on anthocyanin content (Table 3) revealed that 30% methanol had the highest anthocyanin content, and 0% methanol (control) had the lowest one.

According to results for ethanol × methanol interaction, the highest anthocyanin content was produced under 30% ethanol × 30% methanol (Table 4).

Table 2. Means comparison for the effect of ethanol on the physiological and morphological traits (n=4)

Treatment	Anthocyanin (mg 100 g^{-1} DM)	Leaf length (cm)	Leaf area (cm^2)	Flowering stalk length (cm)	Bulb fresh weight (g)
0% ethanol	2.79 b	23.75 b	226.8 b	18.61 b	21.88 b
10% ethanol	1.97 c	25.67 ab	266.8 a	26.98 a	22.03 b
20% ethanol	1.92 c	26.50 a	271.5 a	15.27 b	23.89 ab
30% ethanol	3.92 a	25.83 a	258.6 a	17.77 b	25.81 a
SD	± 0.81	± 1.022	±17.43	± 4.40	± 1.59

Similar letter(s) in each column show insignificant differences at the 1 and 5% probability levels according to the LSD test. Twenty-four samples were used per treatment.

Table 3. Means comparison for the effect of methanol on the physiological and morphological traits (n=4)

Treatment	Anthocyanin (mg 100 g^{-1} DM)	Leaf Brix (%)	Flowering stalk length (cm)	Bulb fresh weight (g)
0% methanol	1.92 c	8.71 b	24.67 a	22.07 b
10% methanol	2.44 bc	8.57 b	16.48 bc	23.65 ab
20% methanol	2.78 ab	8.43 b	15.02 c	22.80 b
30% methanol	3.45 a	10.15 a	22.46 ab	25.08 a
SD	± 0.555	± 0.69	± 4.018	± 1.119

Similar letter(s) in each column show insignificant differences at the 1 and 5% probability levels according to the LSD test. Twenty-four samples were used per treatment.

Table 4. Means comparison for ethanol × methanol interaction for the physiological and morphological traits (n=16)

Treatment	Anthocyanin (mg 100 g^{-1} DW)	Carotenoid (g l^{-1})	Chlorophyll *a* (mg ml^{-1})	Total chlorophyll (mg ml^{-1})	Leaf Brix (%)	Stem Brix (%)	Floral stalk length (cm)	Leaf length (cm)	Leaf area (cm^2)	Total fresh weight (g)
a_1b_1	1.67 de	2.08 g	1.45 e	3.14 d	8.27 d	8.43 a-e	9.77 f	21.00 d	192.3 e	31.05 d
a_1b_2	3.14 bcd	2.76 fg	2.71 a-e	3.95 bcd	8.77 cd	8.75 a-d	17.33 c-f	25.00 bc	230.8 de	45.06 a
a_1b_3	3.08 bcd	2.78 fg	2.92 a-e	5.32 a..d	8.40 d	8.45a-e	17.33 c-f	24.33 bcd	231.0 de	37.50 cd
a_1b_4	3.26 bc	5.37 a	4.14 ab	6.56 ab	11.00 ab	8.25a-e	30.00 abc	24.67 bcd	253.0 bcd	41.64 abc
a_2b_1	0.55 e	2.67 fg	2.93 a-d	5.74 a..d	8.40 d	8.93 abc	23.90 b-e	27.33 ab	288.3 abc	41.07 abc
a_2b_2	0.90 e	5.05 ab	3.32 a-d	5.10 a..d	7.90 d	7.50 de	32.33 ab	27.33 ab	288.8abc	38.81 abc
a_2b_3	3.84 abc	2.91efg	2.61 cde	4.24 bcd	8.00 d	7.80 b..e	11.67 ef	23.33 cd	245.0 b..e	41.93 abc
a_2b_4	2.59 bcd	3.89 cde	3.35 a-d	6.53 ab	11.67 a	9.25 a	40.00 a	24.67 bcd	245.0 b..e	38.50 abc
a_3b_1	2.46 cd	3.95 c..f	2.30 acde	6.04 abc	7.77 d	7.50 de	25.33 bcd	26.33 abc	276.5 a..d	44.57 ab
a_3b_2	1.69 de	2.95 efg	3.55 a-d	5.36 a..d	9.07 cd	8.63 a-e	9.60 f	25.33 bc	241.3 cde	43.89 abc
a_3b_3	0.49 e	3.59 c..f	2.84 a-e	4.11 bcd	7.93 d	7.60 cde	17.67 c-f	24.33 bcd	237.2 cde	37.76 bcd
a_3b_4	3.05 bcd	3.23 def	2.69 b-e	6.32 ab	8.60 cd	9.10 ab	8.50 f	30.00 a	330.9 ab	42.48abc
a_4b_1	2.99 bcd	4.14 bcd	3.32 a-d	4.98 a..d	10.40 abc	7.37 e	39.67 a	25.00 bc	249.5 bcd	43.34abc
a_4b_2	4.05 ab	3.33 def	3.70 abc	7.27 a	8.53 d	7.77 b-e	6.67 f	26.33abc	251.5 bcd	43.07abc
a_4b_3	3.71 abc	4.60 abc	4.17 a	6.465 ab	9.40 bcd	9.10 ab	13.43 def	26.00 bc	299.5 ab	41.47abc
a_4b_4	4.91 a	2.55 fg	2.16 de	3.39 cd	9.33 bcd	8.17 a-e	11.33ef	26.00 bc	234.0 cde	45.17 a
SD	± 1.24	± 0.912	± 0.696	± 1.197	± 1.12	± 0.62	± 10.63	± 1.91	± 32.33	± 3.54

a_1: 0% ethanol; a_2: 10% ethanol; a_3: 20% ethanol; a_4: 30% ethanol; b_1: 0% methanol; b_2: 10% methanol; b_3: 20% methanol; b_4: 30% methanol

Similar letter(s) in each column show insignificant differences at the 1 and 5% probability levels according to the LSD test. Six samples were used per combined treatment.

Amini et al. (2014) reported the different concentrations of ethanol (4%, 6%, and 12%) and methanol (6%) could increase anthocyanin content of cut carnation (*Dianthus caryophyllus* cv. 'Sensi') petals. Anthocyanins are the largest and most important pigments of vacuolar in the plant species (Fernández-López et al., 2020). The flavonoid pigments are responsible for the red, blue, and purple colors of most fruits, vegetables, and flowers. These pigments, in particular, and all phenolic compounds, in general, protect plants against ultraviolet radiation and insects (Buchert et al., 2005). Natural pigments (including carotenoids, anthocyanins, and betacyanins), apart from color, provide extra properties and are, therefore, considered to be bioactive constituents (Khoo et al., 2017). Humans have always incorporated the natural pigments of fruits, vegetables, and ornamental plants in their life. Currently, natural pigments are used widely as additives or supplements in the food industry. Typical red carotenoid-pigmented fruits are tomato (*Solanum lycopersicum*), watermelon (*Citrullus lanatus*) and red pepper (*Capsicum annuum*) (Fernández-López et al., 2020). El Kereamy et al. (2002) reported that ethanol treatment triggers triggered gene expression leading to anthocyanin accumulation during grape ripening. The effect of ethanol spraying on anthocyanin accumulation in ripening berries seems to be due to its stimulatory effect on UFGT gene transcription.

According to Table 1, ethanol × methanol interaction was significant for the carotenoid content of tulip flowers ($p < 0.01$), but the simple effects of ethanol and methanol were not significant for this trait. Accordingly, 0% ethanol × 30% methanol was related to the highest carotenoid content and 0% ethanol × 0% methanol was related to the lowest one. Kiaseh and Yadegari (2016) stated that ethyl alcohol avoided the formation of anthocyanin in the petals of *Alstroemeria hybrida* flowers and provided the chance to preserve the natural color of flowers in the vase life. These results about anthocyanin are contrary to our results. Samadimatin and Hani (2017) found that the simple effect of ethanol on carotenoid content was not significant. Moreover, the interaction of humic acid and ethanol was significant on carotenoid content and 15% ethanol caused to increase in carotenoid content of *Dracocephalum moldavica* compared to control.

The analysis of variance revealed the significant impact of ethanol × methanol interaction on chlorophyll *a* of tulip flower ($p < 0.05$) (Table 1), but different rates of ethanol and methanol did not alone change this trait significantly. According to Table 4, 30% ethanol × 20% methanol resulted in the highest chlorophyll *a* content, and 0% ethanol × 0% methanol resulted in the lowest one. As revealed by the analysis of variance (Table 1), the simple effects of ethanol and methanol were insignificant on total chlorophyll content, but ethanol × methanol interaction was significant for this trait ($p < 0.05$). The highest total chlorophyll was observed in the plants treated with 30% ethanol × 10% methanol and the lowest one was obtained when no alcohol was applied (Figure 2).

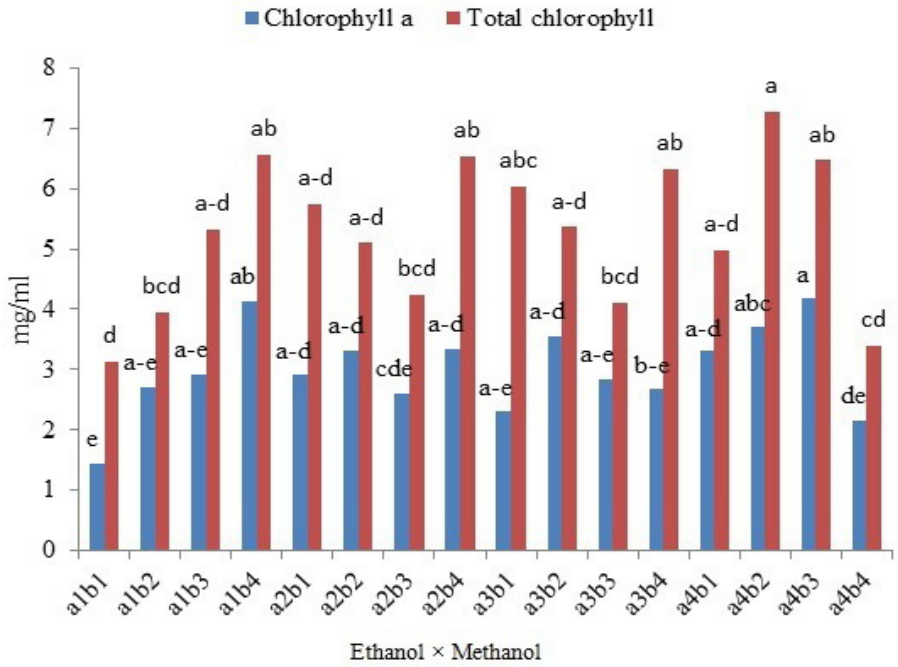

Figure 2. The comparison of means for ethanol × methanol interaction on chlorophyll *a* and total chlorophyll. a_1: 0% ethanol; a_2: 10% ethanol; a_3: 20% ethanol; a_4: 30% ethanol; b_1: 0% methanol; b_2: 10% methanol; b_3: 20% methanol; b_4: 30% methanol

Ethanol was converted into formaldehyde after penetrating plant tissue (lettuce) and finally oxidized to CO_2 (Morales and Santos, 1997). The interaction of humic acid and ethanol improved morphological characteristics, photosynthetic pigments, and yield of essential oil of *Dracocephalum moldavica* (Samadimatin and Hani, 2017). Mehrabani (2019) found that foliar application of methanol improved the chlorophyll content in leaves and the results indicated that the foliar application of methanol ameliorated the negative effects of salinity in *Pelargonium graveolens* by improving of photosynthesis pigments. Methanol spraying positively affected the chlorophyll content in *Calendula officinalis* (Vojodi et al., 2017).

The analysis of variance for leaf Brix index (Table 1) showed the significant effect of methanol ($p < 0.01$) and ethanol × methanol interaction ($p < 0.05$). The highest leaf Brix index was observed in the plants treated with 30% methanol (Table 3). Methanol can act as an alternative source of carbon, causing a considerable increase in their CO_2 fixation, growth, and yield, mainly due to inhibiting their photorespiration (Tavassoli and Galavi, 2011). The positive effect of methanol in plants is due to its fast uptake and quick metabolize to CO_2 in plant tissues (Tavassoli and Galavi, 2011). As well, the highest and lowest leaf Brix was observed in 10% ethanol × 30% methanol and 20% ethanol × 0% methanol, respectively (Table 4). The treatment with 10% ethanol × 30% methanol enhanced leaf and stem Brix. The analysis of variance (Table 1) revealed that ethanol × methanol interaction was significant for stem Brix index ($p < 0.05$), but the simple effect of the treatments was not significant. The highest stem Brix was observed in 10% ethanol × 30% methanol and the lowest in 30% ethanol × 0% methanol (Table 4). Methanol boosts sugar content and cell turgor in leaves, helping the growth and development of leaves and increasing chlorophyll and carotenoids (Zbiec et al., 2003). The present study also showed that methanol application resulted in a higher Brix (sugar) index. The application of methanol increased TSS and anthocyanin content in grapevine berry skins (Ramadan and Omran, 2005).

The analysis of variance (Table 1) indicated that the simple effect of ethanol, methanol, and their interaction were significant on floral stalk length (at least $p < 0.05$). According to the means comparison (Table 2), 10% ethanol resulted in the longest stalk of 26.98 cm. Data for the influence of methanol on floral stalk length (Table 3) showed that the longest stalk of 24.67 cm was obtained from 0% methanol. As the means comparison for interactions (Table 4) showed, the longest flowering stalk of 40 cm was obtained from 10% ethanol × 30% methanol and the shortest one from 30% ethanol × 10% methanol. A study on cotton (*Gossypium hirsutum* L.) showed that the application of 30% methanol hydro-alcohol increased plant height over control (Makhdum et al., 2002). In the present study, the simple effect of methanol on stalk length was negative, which is contrary to Makhdum et al.(2002) results. In a study on *Echinacea purpurea* L., plant height was increased in the treatment of 30% methanol hydro-alcohol, but the tallest plants were observed in the foliar application of 40% methanol hydro-alcohol, and higher methanol rate (i.e. 50%) resulted in the loss of plant height despite producing more flowers (Khosravi et al., 2016b). In another study on *Melissa officinalis* L., plant height was maximized by the foliar application of 30% methanol, but the application of 40% methanol decreased it (Khosravi et al., 2016a).

The analysis of variance (Table 1) indicated that leaf length was significantly influenced by ethanol ($p < 0.05$), but it was not significantly affected by ethanol × methanol interaction. Based on the means comparison (Table 2), the treatments of 30% and 20% ethanol produced the highest leaf length. There was no statistically significant difference between the effects of 20% and 30% ethanol on the leaf length. Although the analysis of variance showed that ethanol × methanol interaction was insignificant for this trait, 20% ethanol × 30% methanol was related to the highest leaf length of 30 cm and 0% ethanol × 0% methanol was related to the lowest leaf length of 21 cm. Khosravi et al. (2016b) observed the highest leaf length in the foliar application of 30% and 40% methanol. In an assessment of the impact of methanol and ethanol on *P. psyllium* by Larzghadiri et al. (2013), the highest leaf length was obtained from the treatment of 30% methanol. The application time, method, and leaf morphology play an important role in plants' responses to methanol and ethanol application (Mehrabani, 2019; Ramberg et al., 2002).

According to the analysis of variance, leaf area was significantly affected by ethanol ($p < 0.05$) and ethanol × methanol interaction ($p < 0.01$), whereas methanol did not change it significantly. The means comparison (Table 2) revealed insignificant differences among ethanol rates on leaf area, but the highest leaf area of 271.5 cm^2 was related to the treatment of 20% ethanol. Also, the means comparison (Table 4) revealed that the highest leaf area of 330 cm^2 was obtained from the treatment of 20% ethanol × 30% methanol. Tochaei et al. (2018) reported that the foliar treatment of groundnut shoot with methanol and ascorbic acid can increase grain yield and yield components. In a study on the effect of methanol spraying on soybeans, Mirakhoondi et al. (2010) concluded that the foliar application of methanol improved leaf area and total biomass. The plants treated with 40% methanol hydro-alcohol exhibited a higher leaf area, and an increase in methanol rate up to 50% resulted in the loss of leaf area (Khosravi et al., 2016b). In *Echinacea purpurea* L., the highest leaf area was related to the treatment with 30% methanol and it was decreased at methanol rates of 40% and 50%. Different responses of the leaves of these two plants to alcohol treatments with similar concentrations can be ascribed to the different leaf structures and different metabolisms induced by genetic properties (Khosravi et al., 2016b).

Ethanol × methanol interaction was significant for plant total fresh weight ($p < 0.05$), but ethanol and methanol could not alone change total fresh weight significantly. According to Table 4, the highest plant fresh weight of 45.17 g was related to the treatment of 30% ethanol × 30% methanol and the lowest fresh weight of 31.05 g was related to the treatment of 0% ethanol × 0% methanol. According to Samadimatin and Hani (2017), 10% ethanol treatment increased the biological yield of aromatic plant *Dracocephalum*. Both alcohol types, i.e., ethanol and methanol (20%), increased leaf and stem fresh and dry weights of tomato plants. Methanol leads to a greater increase in stem length and stem weight than ethanol (Rowe et al., 1994).

The analysis of variance (Table 1) indicated that among studied factors and their interactions, the simple effect of ethanol was significant on tulip bulb fresh weight ($p<0.01$), but the simple effect of methanol and ethanol × methanol interaction were not significant. It was found that 30% ethanol was related to the highest bulb fresh weight of 25.81 g (Table 2). Despite the insignificant influence of methanol and ethanol × methanol on bulb fresh weight, the highest bulb fresh weights of 25.08 and 28.29 g were obtained from 30% ethanol and 30% ethanol × 30% methanol, respectively. Some reports on the positive effect of the foliar application of methanol on plant growth and yields show that methanol improves biomass in plants suffering from water deficit but results in biomass loss in those possessing adequate water (Valizadeh-Kamran et al., 2019; Nourafcan and Kalantari, 2017; Ramberg et al., 2002; Zbiec et al., 2003). Methanol had a positive effect on morphological traits of peppermint including leaf number and leaf size. Also, ethanol increased essential oil contents, and shoot fresh and dry weight (Nourafcan and Kalantari, 2017). Low concentrations of exogenous methanol can improve the biomass of some microalgal species. In addition, methanol increases growth, photosynthesis. and respiration of *Chlamydomonas reinhardtii*, which is a unicellular green alga (Stepanov et al., 2020).

Conclusions

The present study revealed that methanol and ethanol had favorable impacts on physiological and morphological traits of tulips and that 30% methanol, both by itself and in interactions, had the strongest effect on the estimated traits. Therefore, it can be proposed as the best concentration. Since methanol and ethanol can improve important traits of tulips, such as anthocyanin, TSS, and bulb fresh weight, it can be concluded that they can be used as plant growth regulators.

Author Contribution

SR: performed the experiments. **SS:** conceived the study, planned the experiments and analyzed the data, manuscript write and review.

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