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Original Article

Effects of Mycorrhizal Symbiosis and Drying Methods on Physiological Traits of *Carthamus tinctorius* Flowers

Running title: Mycorrhization and Drying of *Carthamus* Flowers

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Abstract

Safflower (*Carthamus tinctorius* L.) flowers have widespread uses in food and medicine industries due to high content of coloring pigments and antioxidant ingredients. Effects of mycorrhization with a mixture of *Funneliformis mosseae*, *F. caledonius*, *Rhizophagus intraradices*, and *R. iregularis* (seed inoculation, soil inoculation and control), four drying methods (room (25 °C), sun (40 °C), oven (70 °C), and split system air conditioning (19 °C)) and their interactions were investigated on safflower petaloids in 2019, in Rafsanjan, Iran. Soil inoculation and oven drying provided the highest content of phenolics and vitamin C, while sun drying showed the lowest content of phenolics. Oven was the best tool regarding antioxidant activity but was not statistically different from other methods. Soil inoculation showed higher antioxidant activity than seed inoculation. Total soluble solids, anthocyanin content, and pH were not altered by mycorrhization and drying. Mycorrhization did not have a significant effect on carotenoid content, but sun drying reduced the carotenoids significantly. Soil-inoculated and oven-dried samples had significantly higher chlorophyll content. Oven displayed the lowest L*, a*, and b* values meaning that oven-dried samples were darker and had more green pigments while the other methods produced redder and yellower color. Room drying led to the highest chroma fallowed by split system and sun, but oven drying resulted in the lowest chroma. Sun drying caused a reduction in hue angle showing a small change in basic color. The results suggest soil inoculation and oven drying for biochemical preservation, but oven had adverse effects on color.

Keywords: Antioxidant, Dyeing, Food quality, Medicinal plant

Introduction

Carthamus tinctorius L. commonly known as safflower or false saffron is an herbaceous, annual crop belonging to Asteraceae family that reaches a height of about 60 cm with broad, toothed leaves without petioles [1]. The plant produces capitulum inflorescences which have yellow to red flowers, resembling petals (petaloids). The petaloids contain carthamin and carthamidin pigments that are responsible for coloring characteristics of the plant [2]. The fruits are white achenes with a seed inside. Safflower is cultivated mainly for its seed, which is used for edible oil production but is also grown for its flowers, used for coloring and flavoring foods and making dyes [1]. The flowers of safflower are also important medicinal material effective in treating numerous diseases. The plant possesses high content of secondary metabolites contributing to its antioxidant and anti-inflammatory properties; hence, its economic importance is growing [2]. Safflower probably originated from Southern Asia and is known to have been cultivated in China, India, Iran and Egypt almost from prehistoric times. This plant is compatible with harsh and dry climates and grows in some provinces of Iran mainly Sistan and Baloochestan, Kerman and Khorasan. It is also cultivated in several provinces especially Isfahan and Fars [1]. According to Jehad and Agriculture ministry (www.maj.ir), 22851 hectares were under safflower cultivation in Iran in 1397-98 with 19782 tones production.

Drying helps the preservation of plant material because it prevents microbial corruption and enzymatic degradation of beneficial ingredients. Drying at room temperature is the traditional method for preservation of medicinal plant material, but it is a slow way and metabolic activities

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continue for a relatively long time that may result in loss of quality, and loss of active ingredients [3]. Sun drying is another traditional and a faster way of plant drying, but it is not commonly preferred because the sun rays are thought to destroy the organic molecules. For example, the crocin content of saffron samples dried in a freeze dryer was about 40% higher than that of the samples dried naturally under the sun [4]. Nowadays, several other methods such as oven, freeze, microwave and some other drying technics are used to overcome the disadvantages of traditional ways [3].

Arbuscular mycorrhizal fungi are symbiotically associated to the roots of the majority of land plants, including the main crop species. They play ecologically important roles such as increasing water and nutrient uptake, and soil aggregation, therefore, leading to plant growth improvement [5]. These fungi may influence the physiological processes of the host plants and enhance the plant tolerance to biotic and abiotic stresses through increment of secondary metabolites synthesis. For instance, root colonization by *Glomus intraradices* resulted in higher amounts of most of the phenolic compounds in purple coneflower [6]. Seed coating of chickpea seeds with multiple arbuscular mycorrhizal fungal isolates had better performance than those inoculated with single isolate under greenhouse and field conditions [7].

Since both the mycorrhization and drying methods can alter the active ingredients of plants and because safflower is a plant with various benefits such as coloring and medicinal properties, the aim of this study was to determine the effect of mycorrhizal symbiosis and four drying technics on the color, antioxidant activity and the content of some critical metabolites of safflower petaloids.

Material and Methods

Plant Material, Mycorrhizal Inoculation, and Drying Methods

The seeds (actually the achene fruits) of safflower (*C. tinctorius* cv. Goldasht Isfahan) were prepared and surface sterilized with bleach solution (10% commercial bleach + 0.02% Triton X-100) for 15 min and washed 3 times with sterile water prior to usage. A mixed inoculum of 4 arbuscular mycorrhizal fungi including: *Funneliformis mosseae*, *Funneliformis caledonius*, *Rhizophagus intraradices*, and *Rhizophagus iregularis* was obtained from the collection of mycorrhizal fungi, Department of Plant Protection, Vali-e-Asr University, Rafsanjan, Iran. A field area of 30 m^2 was used for plant cultivation.

Mycorrhization was done in three levels. (a) 50 g of seeds was mixed by 20 g of fungi powder which was previously kneaded through addition of 20 ml of distilled water. The seeds were then planted at a depth of 4 cm in autoclaved bed soil in a field. (b) 20 g of fungi powder which was previously kneaded through addition of 20 ml of distilled water was added to 100 g of autoclaved soil. The bed soil of field was replaced by mycorrhizal soil in some areas. Then, the seeds were planted at a depth of 4 cm of mycorrhizal areas. (c) the seeds were planted in the autoclaved bed soil without mycorrhization as control. The plants were irrigated twice a week. Mycorrhization was confirmed through microscopy method and observation of fungi hyphae around roots. No hyphae were observed around the roots of control plants [6, 7].

Completely opened flowers were harvested and the petaloids were separated and dried in 4 ways including room drying (average temperature of 25 °C), sun drying (average temperature of 40 °C), oven drying (70 °C), and in a room with split system air conditioning (19 °C). All the samples were dried to equilibrium moisture content. Color determination was performed immediately after drying. The dried petaloids were kept in -20 °C until biochemical analyses [3,4].

Extraction and Biochemical Assays

Extraction: Each sample (repeat) consisted of the petaloids of 5 flowers from 5 different plants. One g of each sample was ground in 80% methanol by a mortar and pestle. The mixture was centrifuged at 10000 rpm for 10 min. Extraction was repeated three times. The supernatants were collected, brought to 10 ml volume by 80% methanol and kept at -20 °C until usage [8].

Phenolic content: Total phenolic concentration was determined according to the Folin–Ciocalteu procedure, using gallic acid for preparing the standard curve. Gallic acid was diluted as 20, 40, 60, 80, 100, 120, and 140 mg ml^{-1}. An amount of 5 ml of Folin agent (1:10 in water) and 4 ml sodium carbonate (7.5%) were added to 0.5 ml of plant extracts or standard solutions. Absorbance was read at 760 nm by a UV-visible spectrophotometer (Lambda-Elmer Perkin, American). The results were expressed as mg of gallic acid in 100 g of dry weight [9].

Antioxidant activity: Antioxidant activity was determined by the 2,2-diphenyl-1- picryl-hidrazil (DPPH) radical-scavenging method. An amount of 900 µl of DPPH solution (500 µM) was added to 100 µl of plant extract. For correction factor, 900 ml of distilled water was added to 100 µl of plant extract. The absorption of these was read at 517 nm. A mixture of 100 µl of distilled water and 900 µl of DPPH solution was used as blank. The absorbance was read at 517 nm, using a UV-visible spectrophotometer. The results were concluded by the fallowing formula and expressed as the inhibition percentage of the DPPH radical [10].

$$\text{Antioxidant activity (\%)} = 1 - \frac{\text{Sample absorbance} - \text{Correction factor}}{\text{Blank}} \times 100$$

Vitamin C: The vitamin C content was determined by titration with iodine solution using oxidation-reduction reaction. To 10 ml of extract, 20 ml of distilled water and 2 ml of 1% starch solution were added. Titration was performed by a solution containing 1.6 g iodin 16 g potassium iodide in 1 L of water, and the number was noted as soon as the first color change was observed. The results were obtained by the fallowing formula and expressed as mg of vitamin C in 100 ml of extract [11].

mg of vitamin C in 100 ml = (0.88 × obtained number)/2 × 100

Total soluble solids (TSS): Total soluble solids were measured at room temperature by a digital refractometer (PAL-1 Atago, Japan). First, the refractometer was calibrated with distilled water and then a few drops of filtered extract were poured on the prism of the device and the amount of soluble solids was determined in terms of percentage [12].

Anthocyanin: One ml of extract was brought to 25 ml by the first solution (0.2 N KCl, 0.2 N HCl, pH=1). Ten ml of the same extract was brought to 25 ml by the second solution (1N CH_3CO_2Na, 1N HCl, pH=4.5). Their absorbance was read at 510 nm using a UV-visible spectrophotometer and anthocyanin content was determined by the fallowing formula and expressed as mg L^{-1} of extract [13].

Anthocyanin content (mg L^{-1}) = (Abs $_{pH1}$ − Abs $_{pH4.5}$) 482.82 × 1000/24825 × DF

In this formula, 482.82 is the molecular mass of cyanidin-3-glucoside, 24825 is the molecular adsorption of cyanidin-3-glucoside at pH = 1 and DF is the dilution factor.

pH: The pH of extract was measured with pH meter (Germany inolab720, WTW82362).

Photosynthetic Pigments

Each sample consisted of the petaloids of 5 flowers from 5 different plants. One g of each sample was ground in 80% acetone (in water) by a mortar and pestle. The mixture was centrifuged at 5000 rpm for 20 min. Extraction was repeated three times. The supernatants were collected and brought to 10 ml volume by 80% acetone according to Lichtenthaler's method. The absorbance was read at 470, 646.8 and 663.2 nm using a UV-visible spectrophotometer. The chlorophyll and carotenoid concentrations were calculated using the following formula and expressed as mg g^{-1} of dry weight [14].

Chl.a = (12.25 A663.2 - 2.79 A646.8)
Chl.b = (21.21 A646.8 - 5.1 A663.2)
Chl.T = Chl.a + Chl.b
Car = [(1000A470 - 1.8 chl.a - 85.02chl.b)/198]

Color Indicators

Color values were directly measured with a color meter (Minolta Chroma Meter Model CR-400, Minolta, Japan). The color was measured as the lightness (L*), red-green (a*) and blue-yellow (b*). The chroma and hue angle were calculated by the fallowing formulas [8].

Chroma = $\sqrt{(a*)^2 + (b*)^2}$ Hue angle = $\tan^{-1}(\frac{b*}{a*})$

Statistical Analysis

The study was a factorial experiment based on a completely randomized design with four replications. Sources of variation were mycorrhization (seed inoculation, soil inoculation and control), drying methods (room, sun, oven and in a room with split system air conditioning) and their interaction. Mean values were calculated and reported as the mean ± standard error of means. Data were analyzed by SPSS 25 statistical software in Vali-e-Asr university of Rafsanjan, and Duncan multiple range test at P = 0.05 significance level was used to compare the means. The charts were plotted in MS-Excel software package.

Results and Discussion

Phenolic Content

Seed and soil inoculation resulted in 6% and 35% higher phenolic content than control, however, these differences were not statistically significant (Table 1). Among the drying methods, oven- and sun-dried samples revealed the highest and the lowest phenolic content (Table 2). Therefore, soil inoculation plus oven drying was the best treatment regarding the preservation of phenolic compounds (Fig. 1).

Higher phenolic content in oven-dried samples could be due to the less time of drying in this method. Vacuum-oven drying resulted in higher total phenolics in rosemary and thymus than freeze drying [15]. Higher levels of total phenols were also found in oven-dried green tea leaves than sun-, shade-, microwave-, and freeze-dried samples [16]. Similar results were obtained from pigeon pea seeds [17]. However, these results are in contrary with some other research in with, oven drying resulted in lower phenolics than other methods [3, 18]. Similar to our results, sun exposure resulted in lower phenolic content in *Kappaphycus alvarezii* algae than room drying [19].

Arbuscular mycorrhizal fungi can induce the production of defence-related compounds in plants, including the phenolics. Phenolic compounds, such as flavonoids and tannins, are the active ingredients in many medicinal plants [20]. Purple coneflower plants colonized by *Glomus intraradices* through soil inoculation had higher amounts of most of the phenolics including cichoric, caftaric, chlorogenic acids and cynarin in roots [6]. However, some research indicated that mycorrhization did not change the composition of phenolic ingredients. For example, mycorrhizal fungus, *G. intraradices*, failed

to alter the polyphenolic profile of leaves and stems of basil [21].

Antioxidant Activity

Lower antioxidant activity was observed in seed-inoculated (89.23%) samples than control (94.85%). The difference between soil treatment and control was not significant (Table 1).

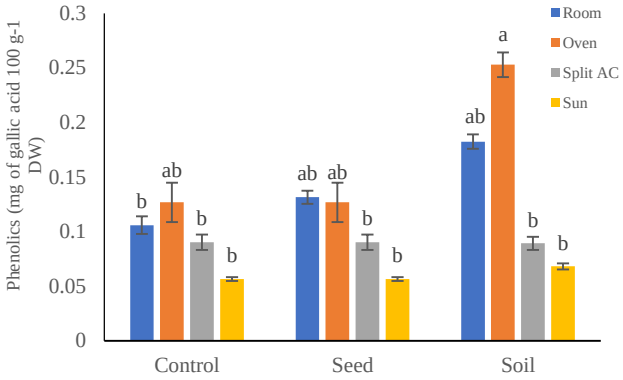

Fig. 1 The interaction effect of drying methods and mycorrhization on phenolic content of *C. tinctorius* L. flowers

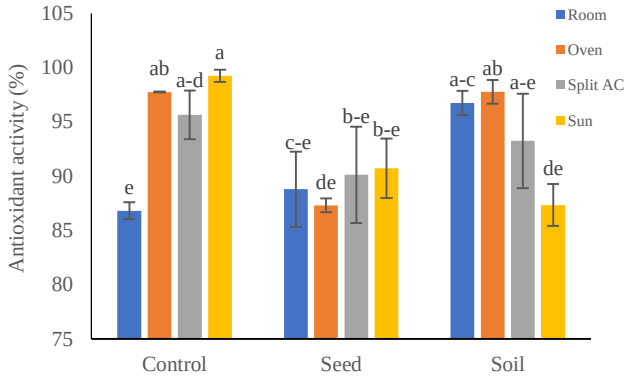

Fig. 2 The interaction effect of drying methods and mycorrhization on antioxidant activity of *C. tinctorius* L. flowers

Oven drying showed the highest antioxidant activity (94.27%) fallowed by split system AC, sun, and room drying. But the differences were not statistically significant (Table 2). The results of interactions are presented in (Fig. 2).

There is an increasing interest in antioxidants usage in food and pharmaceutical industries.

The highest antioxidant activity in oven drying is consistent with the highest phenolic content in this treatment.

Table 1 Effect of mycorrhizal symbiosis on biochemical compounds and color of *C. tinctorius* L. flowers

	Phenolics (mg gallic acid 100 g^{-1} DW)	Antioxidant activity (%)	Vitamin C (mg 100 ml^{-1})	TSS (%)	Anthocyanin (mg L^{-1})	pH
Control	0.094±0.008 a	94.85±1.54 a	12.17±0.75 ab	0.241±0.048 a	3.08±0.32 a	6.10±0.11 a
Seed inoculation	0.101±0.010 a	89.23±1.40 b	10.81±0.58 b	0.241±0.051 a	3.96±0.31 a	6.26±0.05 a
Soil inoculation	0.148±0.036 a	93.77±1.62 a	13.06±1.26 a	0.241±0.054 a	3.47±0.42 a	6.20±0.04 a

Continue Table 1

	Carotenoid (mg g^{-1} DW)	Chlorophyll (mg g^{-1} DW)	L*	a*	b*	Chroma	Hue
Control	152.41±0.75 a	5.30±0.96 a	33.24±1.22 a	21.84±1.55 a	19.30±1.14 b	29.18±1.87 a	0.729±0.015 a
Seed inoculation	151.62±2.66 a	2.84±0.73 a	31.64±1.45 a	22.05±1.23 a	20.39±0.41 a	30.13±1.07 a	0.756±0.026 a
Soil inoculation	141.96±9.89 a	5.46±2.34 a	31.28±1.89 a	20.98±1.45 a	19.13±1.04 b	28.43±1.73 a	0.743±0.011 a

Table 2 Effect of drying methods on biochemical compounds and color of *C. tinctorius* L. flowers

	Phenolics (mg gallic acid 100 g^{-1} DW)	Antioxidant activity (%)	Vitamin C (mg 100 ml^{-1})	TSS (%)	Anthocyanin (mg L^{-1})	pH
Room	0.140±0.022 ab	90.78±1.85 a	9.95±0.69 b	0.255±0.052 a	3.32±0.18 a	6.13±0.07 a
Oven	0.168±0.039 a	94.27±1.78 a	13.99±1.63 a	0.222±0.064 a	3.58±0.53 a	6.27±0.03 a
Split AC	0.090±0.003 bc	92.99±2.06 a	10.87±0.25 b	0.255±0.072 a	2.92±0.46 a	6.17±0.15 a
Sun	0.060±0.008 c	92.43±2.02 a	13.26±0.67 a	0.233±0.047 a	4. 18±0.35 a	6.18±0.04 a

Continue Table 2

	Carotenoid (mg g^{-1} DW)	Chlorophyll (mg g^{-1} DW)	L*	a*	b*	Chroma	Hue
Room	158.74±3.87 a	3.33±0.73 a	35.02±1.58 a	24.10±0.92 ab	22.02±0.31 a	32.68±0.83 a	0.743±0.015 a
Oven	151.26±1.34 a	7.59±2.99 a	25.39±1.44 b	15.30±1.50 c	15.31±1.12 c	21.70±1.79 c	0.792±0.026 a
Split AC	152.65±2.18 a	2.49±0.58 a	33.02±0.92 a	21.74±0.49 b	21.00±0.27 ab	30.25±0.41 b	0.768±0.013 a
Sun	132.01±11.53 b	4.73±1.32 a	34.78±0.60 a	25.34±0.58 a	20.09±0.31 b	32.35±0.59 ab	0.671±0.009 b

It is reported that vacuum-oven drying resulted in higher total phenolics and antioxidant activity (ferric reducing antioxidant property) than freeze drying in rosemary and thymus [15]. The oven-dried (40 and 80 °C) and shade-dried *K. alvarezii* algae also displayed stronger scavenging activity and reducing ability as compared to sun-, hang- sauna-, and freeze-dried samples [19].

Mycorrhization has the potential to alter the antioxidant capacity of plants because it is able to change the secondary metabolite profiles. However, in our study mycorrhizal symbiosis was not effective in enhancing antioxidant activity. In the same way, antioxidant activity of stigmata and tepals of saffron were not significantly influence by *Glomus mosseae* inoculation [22]. Mycorrhizal colonization by *G. intraradices* improved dry biomass and increased the content of antioxidant metabolites such as ascorbate and reduced glutathione content of sage plants. Applied treatments lowered the activities of the antioxidant enzymes catalase, ascorbate peroxidase and superoxide dismutase, while guaiacol peroxidase increased [23]. The activities of antioxidant enzymes, peroxidase, catalase, and superoxide dismutase, were found to be increased in arbuscular mycorrhiza inoculated garlic plants [24].

Vitamin C

Higher vitamin C content was observed in soil- than seed-inoculated and control samples (Table 1). Oven was the most efficient drying method for vitamin C preservation, followed by sun drying (Table 2). Among all the interactions, soil inoculation plus oven drying was the best treatment for vitamin C preservation (Fig. 3).

Vitamin C is an important nutrient in plant tissues. It is usually considered as a nutritional value because of its labile nature compared to other nutrients in foods. The degree of vitamin C loss during drying depends on the physical properties of the product and the type of process [25]. Higher preservation of vitamin C in oven and sun drying is probably related to the less time needed for these methods than room and split system dryings. It is assumed that vitamin C oxidizes and disappears in high temperatures and long drying times [26]. In green tea, oven drying at 80 and 100 °C had similar effects with shade drying regarding vitamin C content [16]. Similarly, sun drying of Amaranth leaves showed higher (31.6 mg/g FW) vitamin C preservation than shade drying (24.7 mg/g FW), but opposite effects were observed in savoy beet and fenugreek leaves [27].

Mycorrhizal colonization of sage plants by *G. intraradices* at seeding stage by layering method increased ascorbate content of leaves [23]. Likewise, a mixture of arbuscular mycorrhizal fungi positively affected the concentration of vitamin C in the tomato fruits [28].

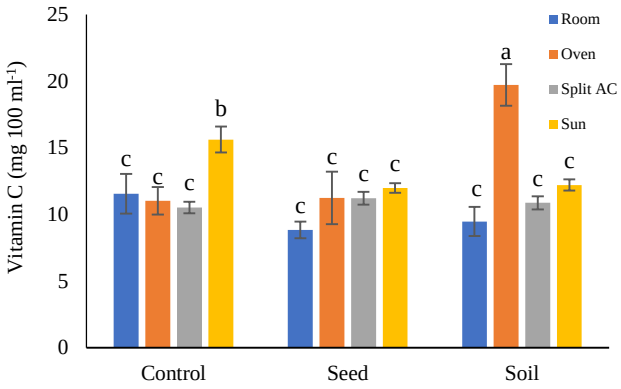

Fig. 3 The interaction effect of drying methods and mycorrhization on vitamin C content of *C. tinctorius* L.flowers

Total Soluble Solids

Mycorrhizal inoculations did not significantly affect the TSS content (Table 1). Room and oven dryings showed the highest and lowest TSS content respectively, but these differences were not statistically significant (Table 2). The interaction of two factors was not significant as well (Table 3).

TSS of plant tissues is usually reduced by drying due to the non-specific hydrolysis of macromolecules, interconversions of sugars and aggregation of monomers [29]. Different drying methods may show different TSS contents resulting from variable temperatures and durations. Similar TSS content among the drying methods tested in this study may be attributed to the low sugar content in *Carthamus* petaloids. Consistently, microwave and oven dryings at most of the tested powers and temperatures displayed similar contents of soluble sugars in *chrysanthemum* flower heads [30]. In roasted coffee beans, heat pump drying and hot air drying had a similar effect on TSS, although other drying methods (room, solar, and freeze dryings) showed significant differences [31]. The TSS content of sun-dried pumpkin fruit slices was lower than oven-dried samples [32]. Oven-dried tomato slices experienced a reduction in total soluble solids with increasing drying temperature [33].

TSS content was not significantly affected by mycorrhizal inoculation. The reason may be that the petaloids are not a main sink for sugars. Correspondingly, the content of soluble sugars in roots of maize plants was higher in *Glomus etunicatum* mycorrhiza inoculated plants than control, but soluble sugars of leaves were not influenced by mycorrhization [34]. Sugar content of tomato fruits was increased in mycorrhiza inoculated plants than control [28].

Anthocyanin

The mycorrhiza and drying methods did not affect the anthocyanin content significantly. The highest (3.96 mg L^{-1}) and lowest (3.08 mg L^{-1}) anthocyanin contents were seen in seed-inoculated and control samples, respectively.

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Table 3 The interaction effect of drying methods and mycorrhization on biochemical compounds of *C. tinctorius* L. flowers

	TSS (%)	Anthocyanin (mg L^{-1})	pH
Control×Room	0.300±0.115 a	3.29±0.22 a	6.12±0.22 a
Control×Oven	0.166±0.066 a	2.90±0.27 a	6.19±0.05 a
Control×Split AC	0.366±0.120 a	2.14±0.56 a	5.93±0.45 a
Control×Sun	0.133±0.033 a	4.00±0.95 a	6.14±0.03 a
Seed×Room	0.133±0.033 a	3.62±0.34 a	6.16±0.14 a
Seed×Oven	0.200±0.100 a	3.67±0.83 a	6.30±0.04 a
Seed×Split AC	0.333±0.145 a	4.16±1.02 a	6.42±0.05 a
Seed×Sun	0.300±0.115 a	4.40±0.29 a	6.16±0.07 a
Soil×Room	0.333±0.088 a	3.05±0.38 a	6.10±0.06 a
Soil×Oven	0.300±0.173 a	4.18±1.51 a	6.33±0.08 a
Soil×Split AC	0.066±0.033 a	2.47±0.22 a	6.15±0.08 a
Soil×Sun	0.266±0.066 a	4.15±0.67 a	6.23±0.12 a

These values were 4.18 mg L^{-1} for sun and 2.92 mg L^{-1} for split system regarding drying methods (Tables 1, 2). The interaction of two factors was not significant as well (Table 3).

Anthocyanins are a group of common phenolic compounds mainly detected in flowers and fruits, and are believed to play important roles such as coloring and antioxidant response in tissues. Drying the plant tissues usually results in a reduction in anthocyanin content which is highly dependent on the conditions of drying. The temperature is a major factor affecting the content of anthocyanins where it is higher at increased air drying temperatures, especially those above 77 °C [35]. Total anthocyanin of cabinet-dried blueberries was significantly reduced, in contrast, the frozen samples did not show any significant decrease in anthocyanin level [36]. However, in our study the anthocyanin content was similar among the tested drying methods. Similarly, anthocyanin content of *K. alvarezii* algae was similar between oven (80 °C) and shade dryings [19]. In blueberries, microwave vacuum drying and microwave vacuum drying plus hot air convective drying (90 °C) showed similar anthocyanin contents [37]. Oven drying and sun drying revealed equal anthocyanin content in black grape as well [38].

It is known that arbuscular mycorrhizal fungi can influence the plant secondary metabolic pathways. For example, mycorrhizal inoculation increases anthocyanin concentration in the fruits of strawberry [39]. But similar to our results, mycorrhizal inoculation of barely influenced the total amount and the profiles of anthocyanins in grape berries [40]. Likewise, soil inoculation with *R. intraradices* did not alter the anthocyanin content of tomato leaves [41].

PH

The samples showed a pH range of 6-6.5, which was not statistically different between the samples. It means that neither the mycorrhiza nor the drying methods affected the pH value significantly (Tables 1, 2, 3).

The pH value, controls many chemical and microbiological reactions [42]. PH of plant tissues may undergo some changes by drying depending on the drying conditions, the amount of total acids and total sugars, and also the plant species [32]. Similar to our study, the pH values for fresh, shade-, sun-, vacuum-, and freeze-dried leaves of Jute plant were not significantly different [43]. Oven- and sun-dried pumpkin fruit slices displayed similar pH values when they had not any pretreatments. But pre-drying treatment significantly affected the pH value of dried fruit, so that salting had the highest value followed by blanching, and untreated control slices had the lowest pH value [32].

Moreover, it can be an advantage that the pH value was not affected by mycorrhiza, although there exists some research that report an alteration in pH caused by mycorrhizal inoculation. For example, tomato plants colonized by arbuscular mycorrhiza fungi produced fruits having a lower pH value and a higher titratable acidity compared to control [28]. In contrary, different arbuscular mycorrhiza (*F. mosseae, Septoglomus viscosum, and R. irregularis*) that were used to inoculate strawberry plantlets, did not change the pH value of fruits [5].

Photosynthetic Pigments

Carotenoid: mycorrhizal treatments did not have a significant effect on carotenoid content (Table 1), but among drying methods, sun drying reduced the carotenoid content of flowers significantly. Three other methods were not significantly different (Table 2). Among the interactions, soil-inoculated sun-dried samples displayed the lowest carotenoid content, while the others were similar to each other (Fig. 4a).

Carotenoids have the function of light absorbing, electron transmitting, and protecting the chlorophylls, chloroplasts and cells [30]. Lower carotenoid content in sun-dried

samples of this study can be explained by the mentioned functions. Comparably, sun-dried *K. alvarezii* algae samples exhibited lower carotenoid content than oven- (40 and 80 °C), shade-, and freeze-dried samples [19]. The highest carotenoid retention of dried sweet potato was observed in oven drying followed by boiling and frying methods. The lowest retention of total carotenoids was recorded in the sun drying method [44].

Carotenoid content was not influenced by mycorrhization. Similar to our results, carotenoid contents in leaves of nonmycorrhizal and mycorrhizal pepper plants were not significantly different when the plants were grown in soils with 2 or 4 mM of copper [45]. The amount of carotenoid in tomato fruits did not change following plant inoculation with a mixed mycorrhizal inoculum [28]. In like manner, no significant differences were recorded between mycorrhizal and control strawberry plants for leaf carotenoid content [5].

Chlorophyll: the simple effects of mycorrhiza and drying were not significant on chlorophyll content. The highest chlorophyll contents were related to soil inoculation and oven drying (Tables 1, 2). Among the interactions, soil-inoculated samples that were dried in oven had significantly higher chlorophyll content (Fig. 4b).

It is believed that chlorophyll is sensitive to heat and its retention is dependent on temperature and duration of heat treatment. Similar to our results, oven-dried (80 °C) green tea leaves displayed higher contents of chlorophylls a and b than shade-dried samples [16]. Amaranthus and fenugreek leaves that were dried using cabinet drier had higher chlorophyll content than samples that were dried by sun, solar, and shade drying methods [27]. Roselle leaves that were dried by oven (65 °C) showed higher chlorophyll content than microwave dried samples, although sun, room, and freeze dryings were more effective in chlorophyll retention in this plant [46].

It is well-known that arbuscular mycorrhizal fungi stimulate the growth of plants which in turn requires increased photosynthesis. Moreover, the application of mycorrhizal fungi helps the plants to confront the photoinhibition and photodestruction and to increase chlorophyll synthesis [47]. In this study, soil inoculation by mycorrhiza had a positive effect on chlorophyll content. Comparably, colonization of pepper roots by *G. mosseae* improved chlorophyll content of leaves in control plants and also in plants under low and high copper treatments [45]. A significant increase in terms of biomass, root length, shoot length, and chlorophyll content was observed in tomato plants inoculated with arbuscular mycorrhizal fungi [48]. Positive effect of arbuscular mycorrhizal inoculation on chlorophyll content were reported in leaves of strawberry [5] and tomato [41].

Color Indicators

None of the color indicators were affected by mycorrhizal treatments (Table 1). But drying methods influenced all the indicators significantly (Table 2). The interactions were also significant (Fig. 5). Oven-dried samples had a lower L* value that means they were darker than others (Table 2, Fig. 5a).

Oven-dried samples had the lowest a* and b* values among all the treatments. Other three methods were relatively similar to each other regarding a* and b* values (Table 2, Fig. 5b, c). These results mean that the oven-dried samples had more green pigments than others; while the samples that were dried by the other three methods had higher content of red and yellow pigments but lower green pigments. This is in agreement with the results of chlorophyll content. Room-dried samples had the highest chroma which refers to the color intensity. Split system- and sun-dried samples were statistically similar to room-dried ones, but oven drying resulted in the lowest chroma (Table 2, Fig. 5d). Sun-drying caused a small reduction in hue angle comparing other methods that shows a small change in basic color. Other methods did not affect the hue angle (Table 2, Fig. 5e).

Color is an important quality factor determining the market value of the product [42]. More darkness in oven-dried samples is probably due to higher phenolic compounds in this treatment (Table 2).

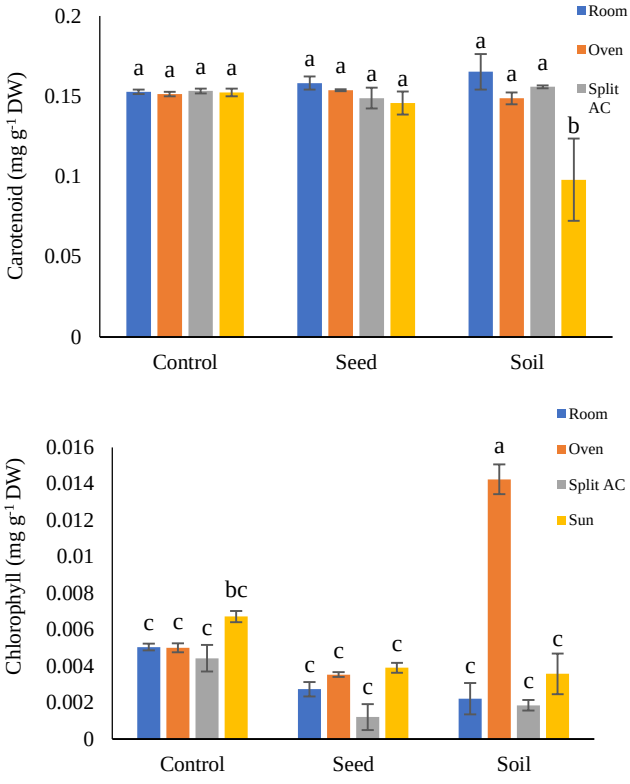

Fig. 4 The interaction effect of drying methods and mycorrhization on (a) carotenoid and (b) chlorophyll content of *C. tinctorius* L. flowers

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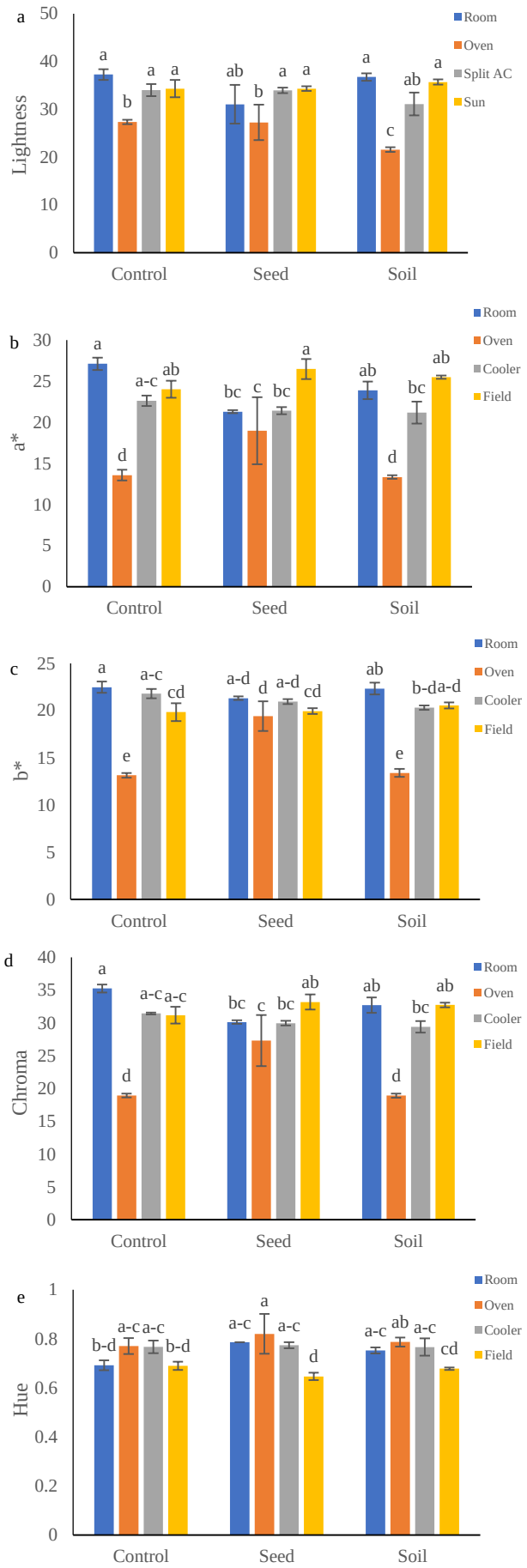

Fig. 5 The interaction effect of drying methods and mycorrhization on (a) lightness, (b) a*, (c) b*, (d) chroma and (e) hue angle of *C. tinctorius* L. flowers

Consistently, the barks of willow stems that were dried in oven at 70 °C were significantly darker than air-dried samples [3]. More darkness due to higher phenolic content has also been reported in meadowsweet [3] and honeybush tea [49]. The redder and yellower color of room-, split system- and sun- dried samples than oven-dried ones means that the red and yellow pigments were somehow destroyed in high temperatures, 70 °C in oven, while the green pigments (chlorophylls) were more stable in this treatment. In saffron, freeze drying was found as the best drying method. Oven drying decreased L*, a*, and b* values comparing freeze drying, but was still much better than vacuum and microwave dryings [50]. In our study, a reduction in chroma was observed in oven drying, while sun-drying resulted in a reduction in hue angle. Some other studies have reported that high drying temperatures can change the color of plant material. In meadowsweet, oven drying at 70 °C caused a significantly lower chroma than air- and freeze-drying [3]. Drying chamomile flowers at high temperatures (80 °C) resulted in a significant decrease in both hue angle and chroma comparing lower temperatures [51]. Similarly, increasing the drying temperature from 45 to 60 °C decreased the chroma, hue angle and lightness of tarragon [52].

Mycorrhization did not affect the color of flowers. This is in agreement with the results obtained from phenolics, anthocyanin and carotenoid in which mycorrhization did not show a significant effect individually.

In saffron, mycorrhization with *R. intraradices* and *F. mosseae* did not change the content of crocin I which is a metabolite responsible for saffron color [53]. Similarly, *Glomus fasciculatum* inoculation did not alter the color properties of marigold flowers [54].

Conclusion

It can be inferred from the literature that each drying method has its own advantages and disadvantages. In this study, oven drying was a better method regarding the contents of phenolic compounds, vitamin C and chlorophyll, and also antioxidant activity. But at the same time, it changed the color of petaloids in an adverse way. Sun drying was not a suitable way because it resulted in the lowest phenolic content and also changed the basic color of petaloids. Room drying caused the lowest content of vitamin C but produced brighter petaloids with higher color intensity and the highest carotenoid content. Split system air conditioning was similar to room drying regarding phenolic, vitamin C, carotenoid and chlorophyll content.

Therefore, if the medicinal properties of the flowers are considered, oven drying is suggested, while room and split AC are better choices if the coloring properties are

considered. Mycorrhizal symbiosis through soil inoculation resulted in higher content of vitamin C, but in most cases was not statistically different from control. Therefore, symbiosis with the four mycorrhiza species that were used in this study may not be cost effective and is not seriously suggested. Mycorrhization by other species or genera of arbuscular mycorrhiza is suggested for further investigations.

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