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Ornamental potential and freezing tolerance of six *Thymus* spp. species as ground-covering plants in the landscape

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Abstract

The current study looks at the possibility of using three native (*Thymus kotschyanus* Boiss. and Hohen, *T. fedtschenkoi* Ronniger and *T. pubescence* Boiss & Kotschy ex Celak), one endemic (*T. daenensis* Jalas) and two common (*T. serpyllum* L. and *T. vulgaris* L.) thyme (*Thymus* spp.) species as ground-covering plants in landscaping. The ornamental potential and freezing tolerance of the species were examined over a period of two years from 2014 to 2016. *T. kotschyanus* indicated some important ornamental characteristics, including early flowering habit, long flowering period, more inflorescences per plant, larger flowers, relatively high area coverage and a higher ranking in visual assessment. In contrast, *T. serpyllum* was observed to have more flowers per inflorescence, more and long inflorescences, high visual assessment ranking and area coverage. The survival rates of the plants put under freezing chamber conditions at −10°C and −30°C were 100% and 0%, respectively, while at −20°C the highest survival rates were observed only in *T. vulgaris* and *T. serpyllum*. Moreover, all species had 100% survival rate in harsh winter conditions of Ardabil Province, Iran. The results of electrolyte leakage measurements demonstrated that among all species, *T. serpyllum*, *T. kotschyanus* and *T. vulgaris* had the highest level of hardiness against the cold. The highest proline contents were also observed in *T. vulgaris* and *T. kotschyanus*, respectively. The activity of peroxidase antioxidant enzyme in *T. vulgaris* and *T. serpyllum* did not change in response to freezing stress. However, it decreased in *T. kotschyanus* by increasing the stress severity. No significant difference was observed in the superoxide dismutase and ascorbate peroxidase enzyme activities of the species. Overall, all the examined species showed valuable ornamental characteristics and freezing stress tolerance. However, only *T. kotschyanus*, *T. serpyllum* and *T. vulgaris* were suggested to be used for landscaping programs.

Key words: electrolyte leakage, flower number, flowering period, native plants, peroxidase, thyme.

Introduction

The planting and maintenance of plants in urban landscapes are very expensive. Therefore, having the policy to use plants that are cheaper to grow and more adaptable to local conditions, it is vital to decrease the expenses of large-scale practices. Plants are a source of human distress in urban areas if they are not selected well (Asgarzadeh et al., 2014). There are some factors influencing plant selection for landscaping programs, which include assessments of hardiness and aesthetics, ease of propagation and culture, naturalisation potential. Roloff et al. (2009) considered drought tolerance and winter robustness as decisive criteria for selecting tree species for urban habitats considering climate change. Freezing stress often limits the distribution of many plant species. It is well-documented that there is an intra-specific variation in the cold sensitivity of many plants (Cavender-Bares, 2007). During a period of low but non-freezing temperatures in a process called cold-acclimation plants can increase their ability to withstand freezing temperatures (Livingston et al., 2006). Cold acclimation is a complex process associated with physiological and biochemical changes in the plants, including modifications in membrane lipid composition, increases in soluble sugars and amino acids, synthesis and accumulation of antioxidants enzymes and changes in hormone levels (Hao et al, 2009). There are a lot of studies on screening for freezing tolerance of plant species in recent years: *Stenotaphrum secundatum*

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(Li et al., 2010), *Triticum aestivum* (Armonienė et al., 2013), blueberry (Rowland, Ogden, 2013), *Paspalum vaginatum* (Fabbri et al., 2015).

Besides having good stress tolerance, species introduced to landscape should have good ornamental characteristics. There is a growing demand for native plants in the landscape and the use of native plants by landscape designers has increased in recent years (Brzuszek et al., 2007). Moreover, these plants are the foundation for the local ecosystem, because they provide habitat and food for wildlife (Tallamy, 2007). Assessing the potential of native useful plants needs to be prioritized since they are still very poorly known and their potential is still insufficiently exploited (Mugge et al., 2016). There are lots of works on the possibility of using native and medicinal plants in landscaping, such as chamomile species (Ghani et al., 2011), *Sedum spurium* and *Thymus paraecox* (Acar, Var, 2001), *Ajuga reptans* (Foo et al., 2009). The performance of one turfgrass (*Poa pratensis* L. 'Apollo') and eight landscape species (*Achillea millifolium*, *Ajuga reptans* 'Bronze Beauty', *Liriope muscari* (Decne.), *Pachysandra terminalis* Siebold, *Sedum album*, *Thymus serpyllum*, *Vinca major* and *V. minor*) were studied during a severe drought and subsequent recovery. The results have shown that *S. album*, *L. muscari* and *P. terminalis* performed best (Domenghini, 2012).

The genus *Thymus* L. belongs to the Lamiaceae family, consisting of about 215 species of herbaceous perennials and small shrubs in the world. They are native to Mediterranean region, from which 14 species exist in Iran flora. These species include *T. daenensis* Jalas, *T. carmanicus* Jalas, *T. fallax* Fisch & C.A. Mey, *T. persicus*, *T. trautvetteri* Klokov & Desj-shost, *T. migricus* Klokov & Desj-shost., *T. kotschyanus* Boiss. & Hohem, *T. pubescence* Boiss & Kotschy ex Celak, *T. nummularius* M.B., *T. transcaspicus* Klokov, *T. eriocalyx* (Ronniger) Jalas, *T. causcasicus* wild ex Ronniger subsp. *grossheimii* (Ronniger) Jalas, *T. transcaucasicus* Klokov and *T. fedtschenkoi* Ronniger (Pirbaluti et al., 2015). The aim of these studies was to investigate the possibility of using three native, one endemic and two commonly used *Thymus* species in the landscape by evaluating their freezing tolerance and ornamental potential.

Materials and methods

Plant material. Six thyme (*Thymus* L.) species, including *T. kotschyanus* Boiss. and Hohen, *T. fedtschenkoi* Ronniger, *T. pubescence* Boiss & Kotschy ex Celak, *T. daenensis* Jalas, *T. serpyllum* L. and *T. vulgaris* L., were used in the present study. The seeds of the species were purchased from Research and Education Centre of Agriculture and Natural Resources Organization in East Azarbaijan Province, Iran.

Evaluation of ornamental potentials. This experiment was carried out at the experimental station of the College of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran over a period of two years (2014 to 2016). Annual rainfall in Ardabil Province is about 290 mm and precipitation falls in winter as snow and rainfall mainly occurs between November and May. The number of freezing days in winter months is often more than 15 days per month. The absolute and average minimum temperatures during the experiment are shown in Table 1.

Table 1. Meteorological data of cold months at Ardabil Weather Station, Iran

Month	Year	Freezing days No.	Absolute minimum temperature °C	Average minimum temperature °C
November	2014	18	−5.8	0.1
	2015	22	−10	−2.2
December	2014	18	−13.8	−2.4
	2015	21	−11	−1.6
January	2015	21	−8.8	−1.6
	2016	26	−16	−4.8
February	2015	26	−18.8	−5.1
	2016	14	−13.2	0.2

The field's soil is of sandy-loam type with a pH of 7.8. NPK fertilizer at rates (in pure elements) of 90-60-90 kg ha^{-1} were used before sowing. Weeds were controlled by hand and no pesticides were applied during the plant growing season. Several plots measuring 1.5 × 1.5 m were prepared for planting and then separated by 100 cm borders. Firstly, the seeds germinated in containers filled with sand/soil/decomposed cow manure substrate (1:1:1 v/v) in greenhouse conditions; the seedlings were then transferred from containers to the plots in early April. Morphological properties (leaf and flower size, inflorescence and flower number, diameter and length, plant height and area coverage), phenological properties (flowering period and the onset of flowering) and visual assessment (ranking) of the species were recorded during the growing seasons. This experiment was conducted in a completely randomized block design with 8 replications for each species (plots with six plants).

Laboratory and field experiment for evaluation of freezing stress tolerance. In order to evaluate the freezing tolerance, the seedlings germinated under greenhouse conditions were transferred to the outdoor conditions until late November to acclimatize naturally (the average mean temperature over the period of three years from 2014 to 2016 was 6.1–7.6°C). Twenty-four hours before the end of the acclimation period, the plants (in their containers) were irrigated and transferred to a freezing chamber with 3°C temperature for subsequent exposure to freezing temperatures (−10, −20 and −30 °C). The temperature was reduced at the rate of 2°C per h. After being exposed to the freezing temperature for 1 h, the plants were transferred to a growth chamber with 4°C temperature for 24 h to reduce the ice melting speed and then were returned to the glasshouse. There were 10 plants in each container as well as four containers for each species. Proline content, electrolyte leakage (EL), peroxidase (POD), superoxide dismutase (SOD) and ascorbate peroxidase (APX) enzymes activity of the species were measured immediately. Their survival rate, however, was evaluated after being kept under greenhouse conditions for 30 days. Moreover, winter survival rates of the species under field conditions were recorded and calculated in early April, when most of the plant species start to grow in the region.

Leaf size and area coverage. The area coverage and leaf size were measured by software *Image J* (Chaudhary et al., 2012). For this purpose, photos of the plants surrounded by a frame with a known area were taken by a digital camera from above. The photos were then penned in the software and the both the frame and plant areas were selected by selection tools and measured in pixels. Since the actual area of the frame was known, the area of the plant was calculated relative to the frame area.

Visual assessment. For visual assessment of species in both vegetative and reproductive stages over two years, the participants (about 20 PhD and MSc horticultural students) were asked to rank the beauty of species from 1 (the worst) to 6 (the best) (Ghani et al., 2011). The data were analysed based on non-parametric Freidman test. There were a total of 48 plants for each species (8 plots each containing 6 plants).

Morphological and phenological properties. The onset of the flowering was calculated from the time the seedlings were transplanted to the field until the time 50% of the plants in each plot produced flower. The flowering period was also calculated from the beginning of the flowers to the abscission of almost all flowers. Flower size and diameter, plant height, and inflorescence length were measured with a ruler. Moreover, two plants in each block were selected and inflorescences per plant and flower per inflorescence were counted precisely.

Determination of survival rate. The survival rate was determined by counting alive plants remaining within each container under freezing chamber conditions four weeks after the return of the plants to the greenhouse. Moreover, for calculating the survival rates under the outdoor conditions, the alive plants were counted in early spring each year. Survival rate was calculated based on the following formula:

$$\text{Survival rate } (\%) = \frac{N}{N_0} \times 100,$$

where N is the number of alive plants after freezing stress, N_0 – the number of alive plants before freezing stress.

Enzyme essay. Thyme leaves (0.25 g) were homogenized in 1 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM of ethylenediaminetetraacetic acid (EDTA) at the presence of polyvinyl pyrrolidone (PVP).

The homogenate was centrifuged at 15000 g for 15 min at 4°C. The supernatant was used to measure the activities of SOD, POD and APX. All assays were done at 25°C using a spectrophotometer Genway 6705 (Genway Biotech, USA). POD activity was determined at 470 nm by its ability to convert guaiacol to tetraguaiacol (ε = 26.6 mM^{-1} cm^{-1}) (In etal., 2007). The reaction mixture contained 1485 ml of guaiacol (112 µl guaiacol in 20 ml K-phosphate buffer), 1485 ml hydrogen peroxide (450 µl H_2O_2 in 20 ml K-phosphate buffer) and 30 ml enzyme extract. An increase in absorbance was noticed by adding H_2O_2 at 470 nm for 1 min. SOD activity was measured based on the method introduced by Giannopolitis and Ries (1977) in terms of the ability to inhibit the photochemical induction of nitroblue tetrazolium (NBT) to formazan at 560 nm. The amount of reduced NBT was calculated using the absorbance coefficient 100 mM^{-1} cm^{-1}. One unit of SOD, defined as the amount required to inhibit the reduction of NBT up to 50%, was recorded at 560 nm. In order to estimate the APX activity, 0.1 ml of enzyme extract was added to 2.8 ml reaction mixture composed of 0.5 m*M* ascorbic acid in 50 m*M* phosphate buffer (pH 7.0). H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm after the addition of 0.1 ml H_2O_2 (Nakano, Asada, 1984).

Estimation of proline. The proline was estimated based on the method of Bates et al. (1973). The absorbance level of chromospheres was taken at 520 nm. Proline concentration was estimated using the standard curve prepared from L-proline (0–100 µg ml^{-1}).

Electrolyte leakage. For this purpose, 20 mature leaves were thoroughly rinsed three times with deionized water to remove electrolytes adhering to the leaf surface. After the final rinsing, all the leaves-containing test tubes were filled with 10 ml of deionized water and capped with aluminium foil to prevent evaporation. They were kept at room temperature (25°C). After incubation, the electrical conductance (EC_1) of the samples was initially measured by an electrical conductivity meter at room temperature. The tubes were then autoclaved at 0.1 Mpa for 20 min to kill the tissues completely and release all the electrolytes (Al Busaidi, Farag, 2015). Finally, they were cooled to 25°C and the final conductance (EC_2) was measured. The electrolyte leakage (EL) was calculated as follows:

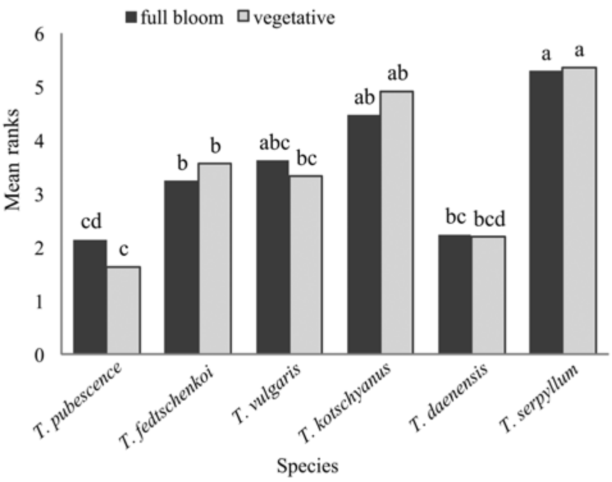
$$\text{EL } (\%) = (EC_1 / EC_2) \times 100.$$

Statistical analysis. The analysis of variance was performed using software *SAS 9.2* and *SPSS 16.0*. Mean values for different species were compared via Duncan multiple tests. The significance level was set as $P \leq 0.05$ in all analyses.

Results and discussion

Evaluation of ornamental potential. The analysis of variance of the data showed a significant difference among thyme species in all examined parameters. Moreover, such criteria as the plant height, flower number, onset of flowering, flowering period and plant area coverage both in vegetative and reproductive stages in two following years were statistically different ($P \leq 0.05$).

Visual assessment. The analysis of variance of Freidman's test with ranking showed that the acceptance level of *Thymus* species among the contributors was statistically different ($P \leq 0.05$) in both vegetative and reproductive stages. In full bloom stage, the acceptance rates of *T. serpyllum*, *T. kotschyanus*, *T. fedtschenkoi*, *T. vulgaris*, *T. daenensis* and *T. pubescence* were 5.36, 4.92, 3.56, 3.33, 2.19 and 1.64, respectively. In the vegetative stage, the highest scores were given to *T. serpyllum* and *T. kotschyanus* (5.29 and 4.47), while the lowest ranks were devoted to *T. pubescence* and *T. daenensis* (Figs 1 and 2).



Note. Different letters indicate statistically significant differences ($P < 0.05$) between the species.

Figure 1. Comparison of *Thymus* species ornamental potential in the full bloom and vegetative stages by Freidman's test with ranking

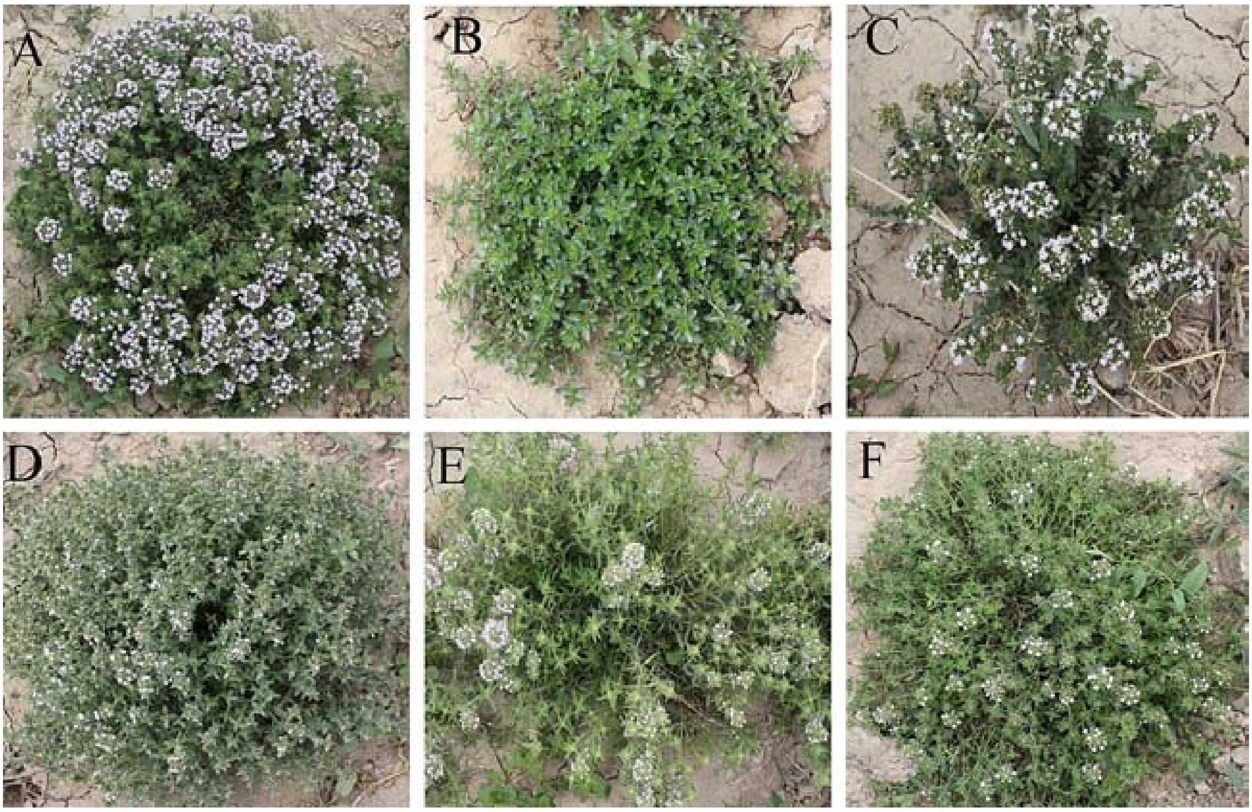
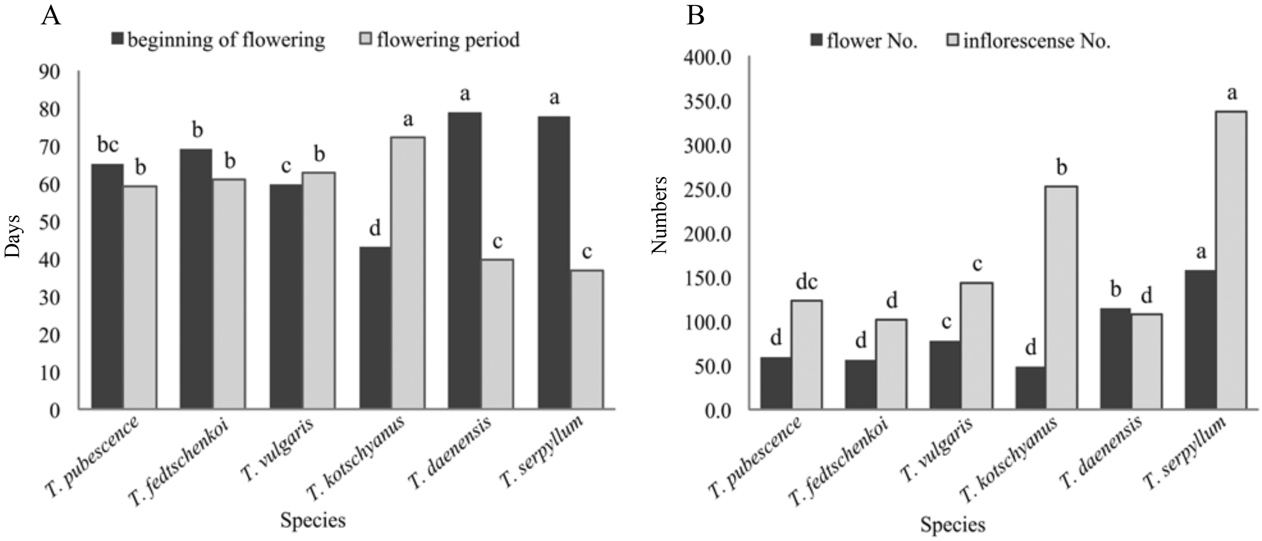


Figure 2. Species of *Thymus kotschyanus* (A), *T. serpyllum* (B), *T. fedtschenkoi* (C), *T. vulgaris* (D), *T. daenensis* (E) and *T. pubescence* (F)

Reproductive and vegetative parameters. The required time for the flowering of different species of *Thymus* varied between 40 to 80 days. *T. kotschyanus* started to flower about 40 days after being planted in outdoor conditions, whereas *T. serpyllum* needed about 80 days. Those species with early flowering habit due to favourable weather conditions in early spring also had the longest flowering period. In the second year, *T. kotschyanus* flowered twice in spring. The longest (72 days) and the shortest (37 days) flowering periods were recorded for *T. kotschyanus* and *T. daenensis*, respectively. However, all six species showed a long flowering period (Fig. 3A). *T. serpyllum* had the highest inflorescence number per plant (237) and flower number per inflorescence (158). There were more flowers per inflorescence in *T. serpyllum*, *T. daenensis* and *T. vulgaris*, compared to other species (Fig. 3B).



Explanation under Figure 1

Figure 3. Mean comparison of flowering period, beginning of flowering (A), inflorescence and flower numbers per inflorescence (B) in *Thymus* species

The longest inflorescences (92 and 90 mm, respectively) belonged to *T. vulgaris* and *T. serpyllum*, and the species with more flowers had smaller flowers, except for *T. daenensis*. In contrast, *T. serpyllum* and *T. vulgaris* with more flowers per inflorescence had the smallest flowers (2.86 and 3.56 mm, respectively). The flowers of *T. daenensis*, *T. kotschyanus* and *T. fedtschenkoi* were about twice as large as those of *T. serpyllum*. Due to larger flowers, the inflorescence diameter of *T. daenensis*, *T. fedtschenkoi* and *T. kotschyanus* was larger than that of other species which owned showy flowers (Table 2).

Table 2. Mean comparison of traits in different *Thymus* species during a two year experiment

Species	Inflorescence length mm	Inflorescence diameter mm	Flower size mm	Leaf size cm^2	Plant height cm	Area coverage cm^2
T. pubescence	36.8 b	26.6 a	5.38 c	0.28 c	10.2 c	875.5 c
T. fedtschenkoi	32 b	22.8 c	6.12 ab	0.37 b	13.8 b	647 c
T. vulgaris	92 a	14.42 e	3.56 d	0.05 e	20.6 a	1299 b
T .kotschyanus	22.9 c	23.97 bc	5.66 bc	0.25 d	10.6 c	1300 b
T. daenensis	35.5 b	24.8 ab	6.5 a	0.4 a	15 b	1188 b
T. serpyllum	90.5 a	20 d	2.87 d	0.22 d	13.8 b	2306 a

Note. Different letters in columns denote significant differences (Duncan test, $p < 0.05$).

The area coverage, which represents the growth habit and rate of the species, of *T. serpyllum* was about two times bigger than that of *T. pubescence* and *T. fedtschenkoi*, which is indicative of its high growth rate and creeping growth habit. The area coverages of *T. kotschyanus*, *T. vulgaris* and *T. daenensis* were not statistically different; meanwhile, they indicated relatively high growth rate and area coverage. Like the flower's size, size of the leaves in *T. daenensis* and *T. fedtschenkoi* were larger than other species. As shown in Table 2, the smallest leaves were measured in *T. vulgaris* (0.05 cm^2) which, nevertheless, were really persistent under hot and dry conditions. Additionally, colour of the leaves in *T. vulgaris* was different from other species, which might be a good combination with other species for planting design. Due to upright-growing habit, the plant height in *T. vulgaris* was higher than that of other species. Unlike that, the plant height in *T. daenensis* during the vegetative stage was relatively low because of the rosette growing habit. But during the reproductive stage, this species produced a long flowering stalk. In general, the plant height of all six species was really low, but different growth habits were observed among the species.

The performances of Thymus species in two following years. Onset of the flowering and flowering period were considerably affected by environmental conditions in two experimental years. Due to high adaptation to environmental conditions in the second year of the experiment, the species started to flower 30 days earlier than in the first year. This could also be due to late transplantation of the species from greenhouse to the outdoor conditions (because of extreme weather condition) in the first year (Table 3). As the result, the flowering time of the species coincided with hot summer weather that made a 12-day reduction in the flowering period. The area covered with the plants in the second year was much bigger than in the first year. These results can probably be attributed to higher efficiency of water

Table 3. The performance of *Thymus* species during the two experimental years

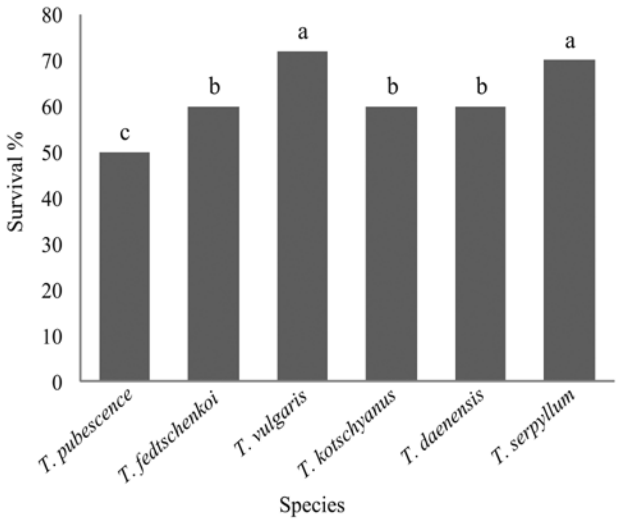
Year	Flowering period	Beginning of flowering	Flower number	Inflorescence number	Leaf size cm^2	Height cm	Area coverage cm^2
2015	49.5 b	51.2 b	70.5 b	158.7 a	0.25 a	12.1 b	517.5 b
2016	61 a	80.4 a	94 a	178.2 a	0.28 a	16 a	3614.7 a

Note. Different letters in columns denote significant differences (Duncan test, $p < 0.05$).

use in the second year as well as more developed root system and perennial growth habit of the species. It was reported that the onset of flowering and flowering period of *T. kotschyanus* were influenced by environmental conditions in different years (Moradi et al., 2014). Khazaie et al. (2008) also reported that *Thymus vulgaris* and *Hyssopus officinalis* produced higher biomass and essential oil yield in the second year, compared to the first year.

Regardless of being native or introduced, the plants' performance is influenced by the environment. Plants vary genetically in growth patterns, flowering and susceptibility to insects and diseases. Morphological and genetic variations in *Thymus* species and also thyme accessions have been reported in some studies (Ma et al., 2009; Javadi et al., 2012; Moradi et al., 2014). Species with more flowers per inflorescence, more and longer inflorescences, larger flowers, longer flowering period, higher area coverage and early flowering in spring could be valuable candidates to substitute other exotic and none-native species. *T. kotschyanus* showed an early flowering habit, a relatively long flowering period, more inflorescences per plant, large flowers, high area coverage, long inflorescence as well as ornamental potentials. Whereas, *T. serpyllum* covered larger area, obtained the highest ranking score and produced more flowers and inflorescences. Its higher acceptance rate could be attributed to the light-green colour of its leaves and its creeping growing habit. Moreover, *T. vulgaris* had small and persistent grey-green leaves, relatively high flower number per inflorescence, inflorescence length, high aesthetics ranking and upright-growth habit. However, *T. vulgaris* and *T. serpyllum* showed smaller flowers, compared to other species. The results also showed that *T. daenensis* had such valuable characteristics as large flowers, high flower number, relatively higher area coverage, large leaves and large inflorescence diameter. Nevertheless, *T. serpyllum* and *T. daenensis* flowered very late in spring, which could be a restricting factor in regions with hot and dry summers. The other two species (*T. fedtschenkoi* and *T. pubescence*) had quite large flowers, but their acceptance rate was relatively low. In spite of that, they can be used in breeding programs.

Evaluation of freezing stress. Survival rate. 100% survival rate was observed in all the examined species under harsh winter conditions of Ardabil, Iran during 2014 and 2016. In addition, 100% of the plants subjected to −10°C started to grow 30 days after the freezing treatment. However, all the plants were killed under −30°C (data are not shown). The survival rate among six *Thymus* species subjected to −20°C was statistically different ($P \leq 0.05$). The highest and the lowest survival rates were respectively recorded for *T. vulgaris* and *T. pubescence* (Fig. 4).



Explanation under Figure 1

Figure 4. Survival rates of different *Thymus* species under −20°C in the freezing chamber

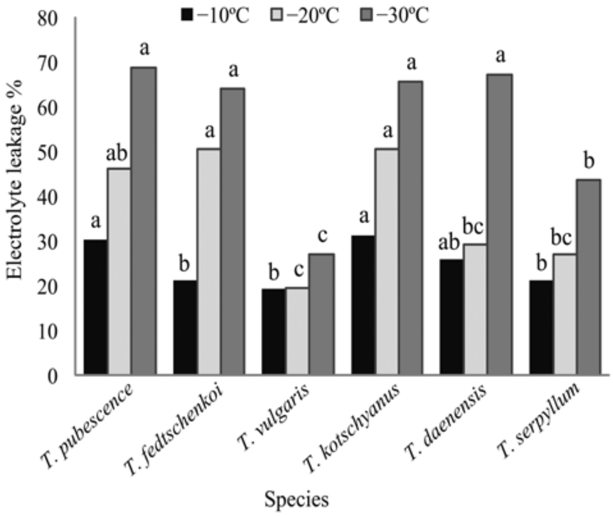
Beside, an average survival rate of 62% was observed in the species kept under −20°C at freezing chamber conditions, which indicates the *Thymus* species' tolerance against the freezing stress (Table 4).

Table 4. Mean comparison of the effects of freezing temperature on the studied traits in *Thymus* species

Temperature °C	Survival %	APX µmol g^{-1} FW min	SOD µmol g^{-1} FW min	POD µmol g^{-1} FW min	Proline µmol g^{-1} FW	EL %
−10	100 a	4.9 b	5.5 c	7.4 ab	0.86 c	25 c
−20	62 b	7.83 a	9 b	8.7 a	2 a	36 b
−30	0 c	7.22 a	20.2 a	6.15 b	1.72 b	54.7 a

Note. APX – ascorbate peroxidase, POD – peroxidase, SOD – superoxide dismutase, EL – electrolyte leakage; FW – fresh weight; different letters in columns denote significant differences between treatments (Duncan test, $p < 0.05$).

Electrolyte leakage. By increasing the freezing stress severity, electrolyte leakage from the leaf's cell membrane dramatically increased. Ion leakage also increased by about 29% when the temperature was lowered from −10°C to −30°C (Table 4). However, between −10 and −30°C, a small change was noticed in the electrolyte leakage of *T. vulgaris*. The lowest ion leakages were recorded in *T. vulgaris*, *T. serpyllum* and *T. daenensis*, respectively (Fig. 5).

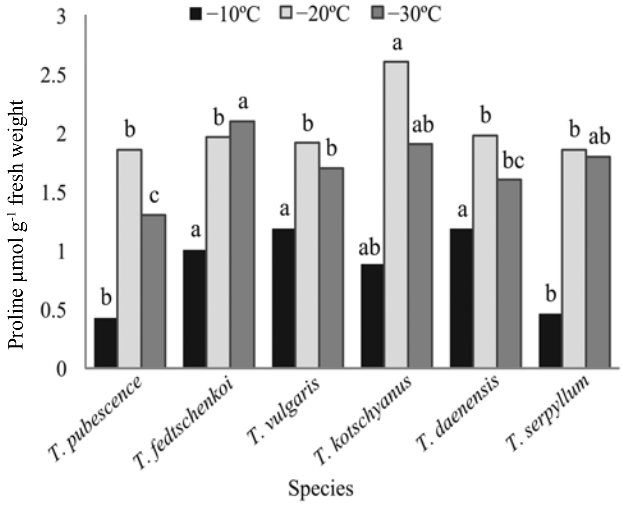


Explanation under Figure 1

Figure 5. Mean comparison of electrolyte leakage in *Thymus* species under freezing temperatures of −10, −20 and −30 °C

This means that these species might survive better than other species when exposed to freezing stress. There was also a significant negative correlation between the survival rate and electrolyte leakage. Species with high electrolyte leakage showed low survival rate. Cell membranes are one of the first targets of many plant stresses and it is generally accepted that the maintenance of their integrity and stability under stress conditions is a major component of stress tolerance in plants. The degree of cell membrane injury induced by an abiotic stress is easily estimated by measuring the cells' electrolyte leakage. Measurement of electrolyte leakage is one of the most frequently used methods to assess plant tolerance in response to drought and low temperature (Davik et al., 2013). Nunes and Ray Smith (2003) reported that the electrolyte leakage (EL) test performed on young leaves at −14°C temperature for 60 min proved to be the most efficient means for detecting both acclimation and cultivar differences in rose clover freezing tolerance. There are many studies in which the electrolyte leakage has been suggested to be used for ranking stress tolerance of such plant species as *Prunus*, *Fragaria* (Rugienius et al., 2016) and date palm (Al Busaidi, Farag, 2015).

Proline content. The proline content of leaves in all the species increased by lowering the temperature to −20°C; however, there was a decline in the proline content of the plants exposed to −30°C. The lowest proline contents in plants exposed to −10°C were respectively observed in *T. pubescence* and *T. serpyllum*, and other species showed similar values. Under moderate freezing stress (−20°C), the proline content of *T. kotschyanus* was higher than other species. Under severe freezing stress (−30°C), the lowest proline content was seen in *T. pubescence*, while *T. fedtschenkoi*, *T. kotschyanus* and *T. serpyllum* showed relatively higher proline contents, compared to other three species (Fig. 6). Plants generally respond to a variety of stresses by accumulating certain



Explanation under Figure 1

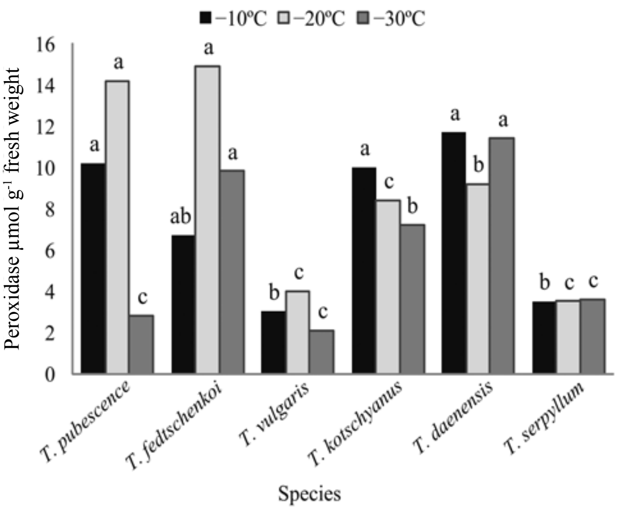
Figure 6. Mean comparison of leaf proline content in *Thymus* species under freezing temperatures of −10, −20 and −30 °C

specific metabolites, the most conspicuous of which is the proline content (Perveen et al., 2013).

Enzyme activities. According to the results, SOD activity increased by lowering the temperature from −10 to −30°C. The activity of POD and APX increased by increasing the stress severity (from −10 to −20°C) but decreased at −30°C (Fig. 7). Despite dramatic changes in SOD and APX activity, no significant difference was observed in the enzyme activities of thyme species. However, *T. serpyllum* and *T. vulgaris* showed low POD activity, compared to other species, and their POD activity did not change by increasing the stress severity.

Species with high survival rate and low electrolyte leakage showed a lower POD enzyme activity that undermines its capability as being a good index to test the freezing tolerance of the thyme species. The findings also showed that there is a significant negative correlation between POD and SOD enzyme activity and the survival rate (Table 5). The negative correlation might be due to increasing rate of reactive oxygen species (ROS), scavenged by other antioxidant enzymes as well as the increased H_2O_2 due to SOD inactivation (Azooz et al., 2012).

Plants have been evolved with a complex antioxidant system to mitigate and repair the damage caused by ROS. The activities of antioxidant enzymes such as SOD, POD and APX are considered as a general adaptation strategy to overcome oxidative stress (Foyer,



Explanation under Figure 1

Figure 7. Mean comparison of peroxidase activity in *Thymus* species under freezing temperatures of −10, −20 and −30 °C

Noctor, 2003). The results of the present experiment showed a significant enhanced SOD activity in seedlings exposed to freezing stress. Increase in SOD activity may be attributed to the increased production of active oxygen species as a substrate that lead to increased expression of genes encoding SOD. The present study shows a significant increase in POD activity of *Thymus* species under moderate freezing stress and a decline under severe freezing stress. The reduction of POD activity might be due to the inhibition of enzyme synthesis or change in the assembly of enzyme subunits under stress conditions (Abedi, Pakniyat, 2010). Decreased enzyme activity has been reported under low temperature (Asadi-Sanam et al., 2015), insecticide stress (Bashir et al., 2007) and drought (Abedi, Pakniyat, 2010). The higher activity of POD, SOD and APX under freezing conditions is a sound indication of *Thymus* species ability to cope with ROS. Therefore, it could be suggested that the increase noticed in the activities of antioxidant enzymes is attributed to the adaptive defence system of these species against the freezing stress.

Table 5. Pearson's correlation coefficient between variables under the freezing chamber condition

	EL	Proline	SOD	POD	APX	Survival
EL	1					
Proline	0.3**	1				
SOD	−0.63**	0.41**	1			
POD	0.35**	0.57**	0.41**	1		
APX	0.35**	0.11 ns	0.07 ns	0.13 ns	1	
Survival	−0.68**	0.5**	−0.9**	−0.47**	0.01	1

EL – electrolyte leakage, SOD – superoxide dismutase, POD – peroxidase, APX – ascorbate peroxidase; ** – significant at the 0.01 level, ns – not significant

Conclusion

The study showed that it is possible to use all of the investigated six thyme (*Thymus* L.) species as ground-covering plants because of their sufficiently high ornamental potential and tolerance to freezing stress. However, *T. kotschyanus*, *T. serpyllum* and *T. vulgaris* with good ornamental potential and also higher freezing stress tolerance are better candidates than *T. daenensis T. fedtschenkoi* and *T. pubescence* for being used in landscaping plans.

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Šešių rūšių čiobrelių, skirtų vietovėms apželdinti, dekoratyvumo potencialas ir atsparumas šalčiui

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Santrauka

Tyrimo metu analizuota galimybė vietovėms apželdinti panaudoti tris vietines (*Thymus kotschyanus* Boiss. Hohen, *T. fedtschenkoi* Ronniger bei *T. pubescence* Boiss & Kotschy ex Celak), vieną endeminę (*T. daenensis* Jalas) ir dvi dažnai aptinkamas (*T. serpyllum* L. bei *T. vulgaris* L.) čiobrelio (*Thymus* spp.) rūšis. Šių rūšių dekoratyvumo potencialas ir atsparumas šalčiui buvo vertinta dvejus (2014–2015 ir 2015–2016) metus. Lyginant su kitomis rūšimis, *T. kotschyanus* išsiskyrė šiomis dekoratyvumo savybėmis: ankstyvu ir ilgu žydėjimu, gausiu kiekiu žiedynų su dideliais žiedais, gana dideliu ploto dengimu ir aukštu vizualaus vertinimo balu. *T. serpyllum* pasižymėjo didesniu žiedynų ir žiedų skaičiumi žiedyne, ilgesniais žiedynais, dideliu ploto dengimu ir aukštu vizualaus vertinimo balu. Augalai, šaldymo kameroje veikiami −10 ir −30° C temperatūros, pademonstravo 100 ir 0 % išgyvenamumą, o esant −20° C temperatūrai daugiausia išgyveno *T. vulgaris* ir *T. serpyllum* rūšių augalų. Buvo nustatytas visų rūšių augalų šimtaprocentinis išgyvenamumas atšiaurios žiemos sąlygomis. Elektrolitų nuotėkio matavimas parodė didesnį *T. serpyllum*, *T. kotschyanus* ir *T. vulgaris* atsparumą šalčiui, palyginus su kitų tirtų rūšių čiobreliais. Be to, *T. vulgaris* ir *T. kotschyanus* rūšių augaluose buvo nustatyti didžiausi kiekiai prolino. Reaguojant į šalčio stresą oksidacinio fermento peroksidazės aktyvumas *T. vulgaris* ir *T. serpyllum* rūšių augaluose nepakito, o *T. kotschyanus* augaluose sumažėjo didinant streso lygį. Tarp tirtų rūšių nenustatyta superoksido dismutazės ir askorbato peroksidazės fermentų aktyvumo esminių skirtumų. Visos tirtosios rūšys pademonstravo vertingas dekoratyvumo savybes ir atsparumą šalčio stresui, tačiau kraštovaizdžio apželdinimo programoms buvo pasiūlytos tik *T. kotschyanus*, *T. serpyllum* ir *T. vulgaris*.

Reikšminiai žodžiai: čiobrelis, elektrolitų nuotėkis, peroksidazė, vietiniai augalai, žiedų skaičius, žydėjimo laikotarpis.