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Characterization of aroma compounds of Daffodil (*Narcissus tazetta* L.) ecotypes from Turkey

Mozhgan Zarifikhosroshahi[1]*, Şevket Alp[3] and Ebru Kafkas[1]

[1]*Çukurova University, Agriculture Faculty, Department of Horticulture, 01330, Balcalı, Adana, TURKEY.*
[2]*Yüzüncü Yıl University Agriculture Faculty Department of Landscape Architecture Van-Turkey.*

**Corresponding author: e-mail: mn_zarifi@yahoo.com*

Abstract

Daffodil (*Narcissus tazetta* L.) is used for indoor and outdoor ornamental plants as well as a cut flower. It is widely preferred in the perfume and cosmetic sector due to its aromatic characteristics. It is believed that Prophet Noah sowed Daffodil seeds in Şırnak/Cizre regions and it spread out to the entire world after Noah flood. It is asserted that the Daffodil grown-up Cizre region has a more severe smell than the others. In this study, Headspace solid-phase microextraction (HS-SPME) was used for the analysis of volatile compounds of flower samples. A total of 26 compounds of natural Daffodil growing up in Şırnak/Cizre region were identified followed by 21 compounds from Izmir and 16 compounds from Siirt locations. The main aroma compounds found to be β-ocimene and benzyl acetate with the rates next to each other.

Key words: *Narcissus tazetta* L., ecotypes, HS-SPME/GC/MS, volatile compounds.

INTRODUCTION

Daffodil (*Narcissus tazetta* L.) is one of the ornamental plants with high consideration both in cosmetics and folk medicine. The genus Narcissus belongs to the monocotyledonous family Amaryllidaceae well-known for its application in folk medicine since the time of Hippocrates. Narcissus consists of about 62 species, classified into 12 sections (Kington, 1989). Daffodils are spread out in the Mediterranean basin (Rivera et al., 2003). Although a few species are cultivated (Wylie, 1952), 40-60 wild species of daffodils are applied based on their usage (Jimenez et al., 2009). Cultivation of daffodils has been done in southern Anatolia (Izmir, Antalya) in Roman times, later, many horticultural forms were cultivated in Istanbul during the Ottoman Empire. *Narcissus orientalis, N. byzantinus and N. constantinopolitanus* were ancestors of *N. tazetta* which distributed from Istanbul to Europe (Baytop and Mathew, 1984). However, *N. tazetta* and *N. serotinus* are spread out in different regions of Turkey. *N. tazetta* is the most preferred *Narcissus* species in Turkey due to its attractive flower clusters and its special unique aroma (Zeybekoglu et al., 2019). Daffodils are applied in the form of garden perennial, cut flowers, and also as the flowering potted plant. Native *Narcissus* (*Narcissus tazetta* L.) has 3–20 fragrant flowers in a stem. *Narcissus* flowers are used in different fields such as medicine, landscape architecture, and cosmetics industries (Kebeli and Celikel, 2013). Different parts of *Narcissus tazetta* are used in the treatment of different diseases in many parts of the world (Hanks, 2002). Bulbs are used for abscesses, wounds, joint pains, sores, sedatives, hypertension, and boils, roots are used for the treatment of skin problems; flowers are used for aromatherapy and cancer (Katoch and Sharma, 2019).

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ORCID and Mail:
M. Zarifikhosroshahi: 0000-0001-5491-1430 (mn_zarifi@yahoo.com);
Ş. Alp: 0000-0002-9552-4848 (alpsevket@yyu.edu.tr);
N.E. KAFKAS: 0000-0003-3412-5971 (ebruyasakafkas@gmail.com)
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Studies showed that *N. tazetta* is rich in bioactive compounds and alkaloids (Katoch and Sharma, 2019, Karakoyun and Unver, 2019). *Narcissus* contains more than 20 alkaloids with pharmaceutical properties such as anticholinesterase, antineoplastic, antitumor, antiviral, antifungal, anti-inflammatory, antimalarial, and antibacterial and cytotoxic activities (Abou-Donia et al., 1989; Lubbe et al., 2013; Khalafalla et al., 2019). Among the alkaloids of *Narcissus*, the main one is Galanthamine in the bulbs followed by haemanthamine, lycorine, lycoramine, and O-methyllycorenine (Lubbe et al., 2013, Torras- Claveria et al., 2013). Galanthamine is Pharmacollogically produced for treating Alzheimer's disease (Karakoyun and Unver, 2019). However, the main use of *Narcissus* flowers as ornamental reveals the importance of flower quality researches especially the aroma. Studies on flower aroma indicate a significant aspect of modern biological research leads to biological recognition (Barták et al., 2003). The color and the aroma of a flower may be the main signal in attracting insects to ensure pollination (Jurgens et al., 2000). The evaluation of a flower's aroma needs the combination of extremely efficient extraction with high-performance separation methods. Because of the important role of floral fragrances in modern life and industry, including, for example, cosmetics, foods, aromatherapy, household products are considerable (Kim et al., 2000). To determine the total content of fragrance compound(s), the relatively new technique of Solid Phase Microextraction (SPME), which has been originally introduced by Pawliszyn for water analysis was promptly optimized for the headspace analysis of volatile compounds (Barták et al., 2003). However, the quantitative composition of flower aroma may differ considerably depending on many factors such as the geographical environment and/or the season of harvesting the flowers (Edris et al., 2008). Hence, this study aimed to compare volatile compounds of Daffodil accounting for its unique scent in different regions of Turkey.

MATERIALS AND METHODS

Narcissus tazetta L. flowers from Izmir (altitude: 30m) ecotype in the Aegean Region and Cizre/Şırnak (altitude: 1400m) and Siirt (altitude: 670m) provinces in the Southeastern Anatolia region were used as the study material. The Aegean Region climate is the Mediterranean at the coast, with hot, dry summers and mild to cool, wet winters and a semi-arid continental climate in the interior with hot, dry summers and cold, snowy winters. However, Southeastern Anatolia Region has a completely different semi-arid continental climate and is known for very hot and dry summers and cold and snowy winters.

Daffodil flowers were collected in April after flowering and were brought to Cukurova University Faculty of Agriculture, Horticulture Laboratory, and odor analyses were immediately carried out. Two grams of flowers were weighed and extracted with 5 molar calcium chloride on a magnetic stirrer at 40 °C for 20 minutes in the standard Headspace glass bottle (Supelco 75 mm x 23 mm). Analyzes were made with 3 replications. Absorption of volatiles was done by polydimethylsiloxane (PDMS) SPME needle (Supelco, Bellefonte, PA). Perkin Almer GC (Clarus 600) with splitless mode equipped with HP-5 MS (30 m × 0.25 mm × 0.25 μm), fused-silica capillary column was used. The carrier gas was Helium (1 ml/min). The injection temperature was 250 °C. The oven temperature was set at 50 °C and increased with the rate of 4 °C per minute and reached 200 °C. Compounds were determined by taking mass spectra and using NIST, Wiley, and Flavor libraries according to their retention time.

RESULTS AND DISCUSSION

SPME is a fast and solventless technique based on the partitioning of volatiles between the headspace above the sample and a stationary phase coated on a fused-silica fiber. The results showed that the number of detected compounds were different in studied locations. In Şırnak, a total of 26 components were identified including seven monoterpenes, two aromatic esters, six aliphatic esters, two aromatic and two aliphatic aldehydes, four aliphatic and one aromatic alcohol, one phenol, and one other compound. In Izmir, a total of 21 components were identified including six monoterpenes, two aromatic esters, four aliphatic esters, one aromatic aldehyde, one aliphatic aldehyde, three aliphatic and one aromatic alcohol, one acid, one phenol, and one other compound. In Siirt, a total of 16 components were identified including six monoterpenes, one aromatic and three aliphatic esters, one aliphatic aldehyde, four aliphatics, and one aromatic alcohol. The main fragrances of the Daffodil flowers were (β-ocimene (30.9%–37.53%), benzyl acetate (30.12%–34.11%), 3-Hexenyl acetate (8.51%–12.95%), Linalol (1.05%–6.91%), and (Z)-3-Hexen-1-ol (1.37%–5.38%). Although the numbers of detected monoterpenes were higher in Şırnak, the rate of these volatiles was higher in Siirt location. However, Şırnak had a higher rate of aromatic esters (38.3%) including benzyl acetate and phenyl propyl acetate compared to other locations. The rate of aliphatic alcohols especially (Z)-3-Hexen-1-ol in Siirt location with the least overall number of detected volatiles was found to be higher than in other locations and was the lowest in Şırnak location (Table 1). These results are in agreement with several studies. Ruíz-Ramón et al., study (2014), and Melliou et al., (2007) reported β-ocimene and benzyl acetate as the major scent compounds in narcissus flowers. Surburg (1993) also reported that β-ocimene was the main aroma in *Narcissus pseudonarcissus* in both the headspace method (75%) and the vacuum headspace method (30.1%). Chen et al., (2013) also reported that β-ocimene contributed to 62.73 and 66.06% of aroma in *Narcissus tazetta* var. *chinensis* detected by HS-SPME technique. Although Sakai (1979) did not detect β-ocimene in *N. tazetta* two cultivars by hydrodistillation method and found the main compounds as benzyl acetate, benzyl alcohol, linalool, and indole by hydrodistillation method, formerly in another study Sakai (1961) also detected β-ocimene. Zhu and Ding (1991) reported that *Narcissus tazetta* L. var. essential oil contains benzyl acetate (28%), Linalool (7%), 1,8-cineole (14%), 3-methylbutenyl acetate (7%), Linalool oxide (3%) and phenylethyl acetate (3%) by hydrodistillation method. In both Sakai (1979) and Zhu and Ding (1991), β-ocimene was not detected although in many studies was determined as the main aroma. Chen et al., (2013) stated that heating during distillation may decrease the content of β-ocimene and lead to be not detected. Contrary to Chen et al., (2013) we did not detect isoamyl acetate and prenyl acetate. However, benzyl acetate was the second major compound found in this study similar to what Chen et al., (2013) reported. But, benzyl acetate was reported as the first major volatile component in several studies such as Zhu and Ding (1991), Mookherjee et al., (1989). Contrary to our study, Bruno et al., (1994), found the major aroma of *Narcissus tazetta* subsp.

Table 1. Fragrance compounds of *Narcissus* flower from different locations in Turkey (%)

R.T	Compound	IZMIR	SIIRT	ŞIRNAK
	Monoterpenes			
3.956	D-Limonene	0.10±0.01	1.3±0.03	0.14±0.007
4.172	Eucalyptol	1.05±0.01	0.77±0.08	1.1±0.13
5.432	β-Cimene	10.9±0.68	10.32±0.10	33.61±1.20
5.765	4-Carene	0.84±0.02	1.22±0.04	0.12±0.04
7.1	a-pinene	ND	ND	0.3±0.007
	Σ Monoterpens	**32.9**	**40.82**	**35.26**
	Monoterpene Alcohols			
12.896	Linalool	6.91±0.21	6.69±0.56	1.05±0.08
15.848	α-terpineol	2.33±0.07	0.8±0.06	0.22±0.03
	Σ Monoterpene Alcohols	**9.24**	**7.49**	**1.27**
	Aromatic Esters			
17.61	Phenylpropyl acetate	7.13±0.27	ND	4.19±0.20
16788	Benzyl acetate	30.4±0.01	30.12±0.64	34.11±0.45
	Σ Aromatic Esters	**37.53**	**30.12**	**38.3**
	Aliphatic Esters			
1.77	Methyl acetate	ND	ND	0.72±0.05
2.21	Ethyl acetate	ND	ND	0.48±0.071
2.800	Isopentyl acetate	0.42±0.03	ND	1.34±0.014
6.039	Hexyl acetate	0.72±0.06	0.93±0.049	0.59±0.035
7.323	3-Hexenyl acetate	8.51±0.24	37.53±0.94	12.92±1.30
10.742	3Z-hexenyl-d3 butanoate	0.19±0.01	0.78±0.08	0.45±0.02
	Σ Aliphatic Esters	**9.84**	**12.03**	**16.5**
	Aliphatic Aldehyde			
2.38	Hexanal	ND	ND	0.09±0.007
3.150	(Z)-3-Hexenal	0.31±0.02	0.21±0.007	0.17±0.01
	Σ Aliphatic Aldehyde	**0.31**	**0.21**	**0.26**
	Aromatic Aldehyde			
12.178	Benzaldehyde	0.24±0.03	ND	0.82±0.03
19.57	Hydrocinnamaldehyde	ND	ND	1.52±0.03
	Σ Aromatic Aldehyde	**0.24**	**ND**	**2.36**
	Aliphatic Alcohol			
3.419	1-Penten-3-ol	ND	0.74±0.01	ND
4.376	2-Methylbutyl alcohol	ND	0.76±0.00	0.13±0.007
8.332	1-Hexanol	0.66±0.12	0.76±0.07	0.26±0.064
9.126	(Z)-3-Hexen-1-ol	3.69±0.29	5.38±0.41	1.37±0.014
11.571	2-ethyl-1-Hexanol	0.12±0.03	ND	0.14±0.03
	Σ Aliphatic Alcohol	**4.47**	**7.64**	**1.9**
	Aromatic Alcohol			
22.798	Phenylethyl Alcohol	1.89±0.16	0.75±0.07	0.2±0.04
	Acid			
14.652	Butanoic acid	0.27±0.08	ND	ND
	Other			
18.25	phenol	0.41±0.007	ND	0.08±0.007
34.060	Indole	1.92±0.04	ND	2.93±0.23

Tazetta essential oils as y-terpinene, methyl cinnamate, carene and benzyl alcohol by gas chromatography equipped with flame ionization detector. The profile of volatile compounds was different based on different methods of extraction including headspace and distillation and also using different detectors. The comparison of results from different studies proved that the use of headspace analysis gives a more natural profile than studies using hydrodistillation of plant volatiles (Chen et al., 2013). Hassan et al., (2006) reported that α-pinene, Ethyl cinnamate, α-terpineol, and Linalool were important components in essential oils of *Narcissus tazetta* under different habitats with the GC-MS technique. They also reported that *Narcissus* essential oil from three different regions of Egypt contained the same main components, but with different percentages. Regarding the monoterpene alcohols, α-terpineol and linalool were detected with an almost high rate. Similarly, Terpineol was reported as a major aromatic component of narcissus flowers in studies by Joulain (1986), Loo and Richard (1988), and Ehret et al., (1992). Similar to Sakai (1961) or Sakai (1979), aliphatic hydrocarbons were not detected in this study. Indol is also detected in flowers from Şırnak and Izmir locations in rates higher than one. α- Indole is known as being very important in floral odors (Van et al., 1993). Briefly, although the same major volatile compounds were found in all three locations, the rate of these compounds was different besides the overall number of detected compounds. This is a natural consequence because various external and internal factors, in addition to changes in the amount of essential oil produced by plants, also affect the type and amount of their compounds. External factors such as temperature, humidity, light, geographic time, soil, etc. are important. Genetic factors that may themselves be affected by the environment are also important (Nakhaei, 2008). However, factors such as flower age, daytime, and flower architecture should be considered using narcissus flowers for the production of absolute fragrance extracts.

CONCLUSION

Different numbers of volatile compounds were detected in Daffodil flowers from different locations. β-ocimene, benzyl acetate, 3-Hexenyl acetate, Linalol, and (Z)-3-Hexen-1-ol were the main aroma components for Daffodil flowers in all studied locations. Indole known as floral odor was detected only in Izmir and Şırnak locations. Results obtained from this study proved that the main fragrances accounting for Daffodil flowers may not be affected much by geographic location. However, the more number of aromas and high rate of important aroma in Şırnak location may support the belief of severe smell of Daffodil from Şırnak/Cizre.

AUTHOR CONTRIBUTIONS

Ş. ALP conceived and designed the research and provided the material. M. ZARIFIKHOSROSHAHI conducted experiments and analyzed the data contributed with reagents and materials and wrote the manuscript. E. KAFKAS provided the chemicals and allocated her laboratory for analyses and conducted the research. All authors read and approved the manuscript. The authors declare that they have no conflict of interest.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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