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Effects of Top-Pruning Time on Fresh Flower Yield, Rose Oil Content and Compounds in Oil-Bearing Rose (*Rosa damascena* Mill.)

Sabri ERBAŞ[*1], **Soner KAZAZ**[2], **Hasan BAYDAR**[1]

[1]Isparta University of Applied Sciences, Faculty of Agriculture, Department of Field Crops, 32200, Isparta, Turkey
[2]Ankara University, Faculty of Agriculture, Department of Horticulture, 06100, Ankara, Turkey

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Oil-bearing rose,
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Flower yield,
Essential oil

Abstract: The aim of the research was to determine the effects of top-pruning time on flower yield, rose oil content and oil constituents in oil-bearing rose. Five-year old rose plants were top-pruned in three different dates starting from 15 March (early), 30 March (middle) and ending 15 April (late) before they started new growth in spring. In addition, non-pruned (untreated) parcels were used as controls. Top pruning time significantly affected the flower yield, oil content and its constituents. The highest flower yield (4302 kg/ha) was obtained from non-pruned plants. The fresh flower yield and rose oil content increased significantly from 3559 kg/ha to 3905 kg/ha and from 0.030 % to 0.045 %, respectively when the pruning time was delayed. GC-FID/MS analyses of rose oils revealed that monoterpenic constituents, such as geraniol and citronellol were higher in the rose oils distilled from late-pruned plants, paraffinic hydrocarbones (stearoptenes), such as nonadecane and heneicosane were higher in the rose oils distilled from early-pruned plants. The mid- pruning time (30 March) gave the highest citronellol and geraniol by 27.28 % and 30.64 %, respectively. A remarkable result was that methyl eugenol content, which is not desirable above a spesific quantity in rose oil due to allergic effects and mutagenic, decreased from early to late pruning.

Yağ gülü (*Rosa damascena* Mill.)'nde Çırpma Budama Zamanının Çiçek Verimi, Gül Yağ Oranı ve Bileşenleri Üzerine Etkisi

Anahtar Kelimeler
Yağ gülü,
Çırpma budama,
Çiçek verimi,
Uçucu yağ

Öz: Bu araştırmanın amacı, Isparta gülünde çırpma budama zamanının çiçek verimi, gül yağı oranı ve kompozisyonu üzerine olan etkilerini belirlemektir. Beş yaşındaki gül bitkileri ilkbaharda sürgünler patlamadan önce 15 Mart (erken), 30 Mart (orta) ve 15 Nisan (geç) olmak üzere üç farklı tarihte çırpma budama tekniğine göre budanmıştır. Kontrol parsellerinde budama yapılmamıştır. Çalışma sonucunda, budama zamanının çiçek verimi, gül yağ oranı ve bileşenlerini etkilediği bulunmuştur. En yüksek çiçek verimi (4302 kg/ha) kontrol bitkilerinden elde edilmiştir. Çiçek verimi ve uçucu yağ içeriği, budama zamanı ertelendiğinde sırasıyla 3559 kg/ha'dan 3905 kg/ha'a ve %0.030'dan %0.045'e çıkmıştır. Gül yağlarının GC-FID/MS analizlerine göre; geç budanan bitkilerden damıtılan gül yağlarında geraniol ve sitronelol gibi monoterpenik bileşenler daha yüksek iken, erken budanmış bitkilerde nonadekan ve heneikosan gibi parafinik hidrokarbonlar (stearoptenler) daha yüksek bulunmuştur. En yüksek sitronelol ve geraniol içeriği orta budama zamanında sırasıyla %27.28 ve %30.64 olarak tespit edilmiştir. Dikkat çekici bir sonuç, olumsuz toksik ve alerjik etkiler nedeniyle gül yağında belirli bir konsantrasyonun üzerinde istenmeyen metil öjenol içeriğinin erken budamadan geç budamaya kadar azalması olmuştur.

1. Introduction

The oil-bearing rose (*Rosa damascena* Mill) with pink colored and strongly scented flowers is a very valuable source of essential oil used in the fragrance, cosmetic, drug and food industries [1]. Rose oil and aromatic water is obtained through the distillation processes and rose absolute, rose concrete and phenylethyl alcohol is obtained through the extraction processes from oil-bearing rose flowers [2-5]. Especially rose oil,

*Corresponding author: sabrierbas@isparta.edu.tr

an important and expensive volatile oil, used to make the world's most exclusive and expensive perfume and cosmetic products. Bulgaria and Turkey are leading countries producing more than %90 of the world's rose oil, rose concrete and rose absolute. The area known as the Rose valley where Isparta province is located in the center covers all of the oil-bearing rose production in Turkey. Approximately 10 thousand tons of rose flowers from 4100 ha is produced every year, and over 2 tons of rose oil, 10 tons of concrete and 2 tons of absolute are obtained from 34 distillation and extraction plants in the Rose valley.

Since the oil-bearing rose is a perennial and bushy plant, pruning is one of the main agronomic practices for enhancing growth, shaping plant habitus, facilitating harvesting process, and finally increasing flower yield [6]. There are two main pruning techniques, namely rejuvenating or sub-pruning (once every 10 years for rejuvenation) and whipping or top-pruning (once a year in March). After 8-10 years of planting, the roses begin to age leading to reduced productivity. To prevent the aging process, old rose bushes are periodically rejuvenated by pruning just above the ground [7].

Oil-bearing rose is traditionally propagated by the laying of the shoot cuttings remaining from rejuvenating pruning into the furrows in autumn [8]. In top-pruning, which is held regularly every year in the early spring months, the top parts of the rose bushes are pruned away at 45-65 cm from the ground level with pruning shears, loppers or saws [9]. In general, light to medium top pruning increased flower yield and essential oil content of flowers, while heavy pruning decreased both. This practice is beneficial for removing old twigs and regenerating new healthy twigs, thereby increasing flower yield [10]. Un-pruning oil-bearing rose is not sustainable because it complicates agricultural practices, such as tilling, weeding, watering, fertilizing, spraying and harvesting. However, there are many different applications of top-pruning in the rose valley region [11]. Therefore, the aim of the present research was to examine effects of top-pruning time on flower yield, rose oil content and composition in oil-bearing rose.

2. Material and Method

The research was conducted at Isparta Applied Sciences University in Isparta province of Turkey in 2009. Five-year old *R. damascena* plants (Figure 1) at the experimental field (longitude 30°33'E, latitude 37°45'N, altitude 997 m) of Rose and Aromatic Plants Implementation and Research Center and Application Center (GULAB) were used. Rose plants were pruned by applying traditional top-pruning method (about 60 cm from the ground level) three different dates starting from 15 March (early), 30 March (mid) and 15 April (late) before they started new growth in spring. In addition, non-pruned (untreated) parcels were used as controls. Each treatment contained 4 replications and each replication consisted of 20 m long bush rows extending parallel to each other at distances of 3 meters. The total precipitation, mean humidity and mean temperatures for the experimental area were given in Table 1.

Figure 1. The flower of oil-bearing rose (*Rosa damascene* Mill.)

Table 1. The climate data of Isparta for 2009 (Anonymous, 2020)

Month	Total Precipitation (L/m)	Mean temperature (ºC)	Mean humidity (%)
January	3.4	124.7	75.1
February	4.0	70.3	78.7
March	5.5	55.2	68.8
April	11.0	40.4	60.7
May	15.0	66.6	58.8
June	20.9	26.8	44.8
July	23.6	18.0	44.8
August	23.1	0.2	38.5
September	18.0	15.7	55.3
October	15.1	77.6	61.6
November	7.5	13.6	71.3
December	5.7	84.2	80.2

The soil was alkaline and loamy-clay with a pH 7.9. The flowers at full blooming stage were handpicked daily in the early hours of day during the flowering season between May 15-June 10, 2009. The picked flowers from pruned and non-pruned plants were weighed, and the total fresh flower yield was recorded as kg per hectare (ha) at the end of the harvest season. To determine the essential oil content, fresh rose flowers (0.5 kg) and water (1.5 L) were placed in a Clevenger-type hydrodistillation flask (5 L) according to the standard method recommended in the European Pharmacopoeia [12]. After distilling for 3 hours, the amount of rose oil was measured as an average percentage (%, v/w). Afterwards, the rose oils were dried with anhydrous sodium sulfate and stored at 4 °C until the analysis of fragrance components. The components of rose oils were determined by GC-FID/GC-MS device [13]. Rose oil (25 µL) dissolved in

2.5 mL of n-hexane and injected in to the split mode (1/100). GC-MS (Gas Chromatography/Mass Spectrometry) analysis of rose oil was carried out on Shimadzu 2010 Plus (a Quadrapole (QP-5050) detector). GC-MS conditions are given below; capillary column, (50 m × 0.32 mm, film thickness 0.25 µm; CP-Wax 52 CB); detector and injector temperatures, 240 °C; oven temperature program, it was waited at 60 °C for 10 minutes, then it reached 90 °C with an increase of 4 °C per minute and 240 °C with an increase of 15 °C per minute, and waited at 240 °C for 11.5 minutes; carrier gas, helium (2 ml/min); 70 eV; ionization type, EI; 1 µl of sample injected. Identification of the components was carried out with the help of the data given in the Nist, Wiley and Tutor library, the composition of the mass spectra and the retention times of the standard substances. Quantitative analysis was conducted using Shimadzu Model Thermo Ultra Trace, GC-FID (Gas Chromatography/Flame Ionization Detector), operating under similar conditions to GC-MS.

All data for flower yield and rose oil content were analyzed by Analysis of Variance (ANOVA) procedures using Statistical Program (SAS 9.1). Differences between means were separated by LSD (Least Significant Difference) test at $p \leq 0.05$ significance level.

3. Result and Discussion

The effects of pruning time on flower yield, rose oil content and compositions in oil-bearing rose was given in Table 2. Analysis of variance revealed that top-pruning times including no pruning practice had significant effects on fresh flower yield ($p \leq 0.05$). Early-pruned plants started to bloom a few days earlier than the control and late-pruned plants. Although the highest flower yield (4302 kg/ha) was produced by the non-pruned (control) plants, the lowest flower yield (3559 kg/ha) was obtained from the early-pruned roses. The rose oil content was also affected significantly by the pruning time. Maximum essential oil yield (0.045 %) was obtained by the late-pruned roses in April and minimum oil yield (0.030 %) was obtained from early-pruned roses in March (Table 2). Saffari et al. [14] reported that pruning in the first week of March (approximately 75 days before flowering) results in higher flower yield per plant than pruning in the first week of April and control (no pruning). In the same study, it was determined that pruning times did not affect the rose oil content. Previous reports found that non-pruned rose plants had higher flower yield and rose oil content in oil-bearing rose [11].

In oil-bearing rose flowers, essential oils are mostly found in the epidermis cells of the petals, and essential oils contain complex organic odor molecules, such as esters, aromatic alcohols, oxides, ethers, aldehydes, monoterpenes and sesquiterpenes, [15]. Quantities and relative contents of fragrance components are the most important factors affecting the quality of extraction products of rose [16]. Monoterpene alcohols (geraniol, citronellol, linalool and nerol), hydrocarbons (nonadecene, heneicosane,

Table 2. The effects of pruning time on flower yield, rose oil content and composition in oil-bearing rose

Essential oil constituents	Rt (min.)	Top-pruning dates			
		Control	15 March (early)	30 March (mid)	15 April (late)
Tetradecane	35.1	0.46	0.68	t	0.44
Linalool	38.6	0.39	0.38	t	t
α-Guaiene (azulene)	42.1	1.50	3.20	1.29	1.63
t–caryophyllene	42.8	1.15	2.45	1.02	1.30
Citrenellol acetate	46.1	0.93	1.24	0.90	1.11
α–Humulene	47.4	0.76	1.56	t	0.71
Hexadecane	47.8	2.80	2.32	2.15	2.10
Germacrene-D	49.8	2.22	4.49	1.97	2.21
Germacrene-B	50.0	0.97	1.62	t	0.92
Geranyl acetate	51.8	6.06	7.21	6.96	6.77
Citronellol	52.3	22.72	20.29	27.28	26.85
Nerol	54.4	7.17	5.47	7.49	8.24
Garaniol	57.0	27.11	26.29	30.64	28.46
Nonadecane	59.5	14.69	12.46	10.09	9.68
9-Nonadecene	60.6	3.58	2.68	2.49	2.37
Phenylethyl alcohol	61.1	0.51	0.40	0.83	0.70
Eicosane	65.0	0.81	0.91	0.49	0.67
Methyl eugenol	66.4	0.97	1.35	1.42	1.06
Heneicosane	70.3	3.90	4.38	2.71	2.68
Eugenol	74.6	1.30	0.42	1.47	1.74
Essential oil yield (%)		0.040 b*	0.030 d	0.035 c	0.045 a
Flower yield (kg/ha)		4302 a	3559 d	3750 c	3905 b

Rt: Retention time (min), t: trace amount, <0.1 %,

*Means followed by the same letter within the same row are not significantly different from each other ($p \leq 0.05$)

heptadecane, nonadecane, tricosane, sesquiterpene and octadecane) sesquiterpenes (δ-guaiene, λ-muurolene, humulene and α-guaiene), esters and aldehydes (geranial and geranyl acetate), oxides and ethers (methyl eugenol), phenols (eugenol) are among the significant rose oil constituents identified in Turkish rose oil [2, 3, 17]. A total of 20 constituents with a ratio higher than 0.1 % were detected by the GC-FID/MS analyses of rose oils (Table 2). Top pruning treatments had a specific effect on rose oil components that were within the normal range of change intervals for international standard (ISO 9842-2003) [18]. According to our results, the essential oil distilled from non-pruned plants had a similar composition with the oils from early top-pruned plants. On the other hand, the oils distilled from the mid and late pruned plants had higher levels of monoterpene alcohols like geraniol and citronellol, which are the main fragrance compounds of rose oil, and had lower the percentages of stearoptenes like nonadecane and heneicosane (Table 2). Based on these findings, it can be said that the rose oils distilled from the late top-pruned plants were slightly higher quality than the oils distilled from the early top-pruned and non-pruned plants. The mid-pruning gave the highest citronellol (27.28 %), geraniol (30.64 %) and phenylethyl alcohol (0.83 %) (Table 2). The amount of methyl eugenol of rose oil can increase up to 5% with long-term fermentation of the harvested flowers [19] or with the late harvesting of the flowers [20]. A remarkable result was that methyl eugenol content, which is not desirable above a specific quantity in rose oil due to allergic and mutagenic effects [21], decreased in oils distilled from late pruning's from 1.35 % to 1.06 % (Table 2).

4. Conclusion

Top-pruning is a very important practice in the cultivation of oil-bearing rose. In this pruning method, the upper parts of the rose bushes in the early spring months are pruned away from a certain height above the ground level to improve flower yield and quality, and also to facilitate agronomic practices and harvesting process. To obtain expected benefits, it is important to prune at the right time to avoid damaging the plants. Oil-bearing roses should not be top-pruned too early to avoid winter and spring frosts. On the other side, when pruned too late, plants may loss excessive amount of twigs and buds. Thus, the climatic conditions of the growing area should be followed closely, and rose plants should be pruned at the proper time. Top pruning of oil rose plants had a significant effect on flower yield and quality. It had a specific effect on rose oil compounds within the change intervals for international standard (ISO 9842). The flower yield and essential oil content increased significantly when the pruning time was delayed from 15 March to 15 April. The highest flower yield was obtained from non-pruned bushes of the plants. Top-pruning can be interrupted for few years to reduce pruning cost and increase flower yield.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

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