World Journal of Agricultural Sciences 14 (4): 123-127, 2018
ISSN 1817-3047

DOI: 10.5829/idosi.wjas.2018.123.127

Influence of Harvesting Season, Leaf Position and Wilting Period on Essential Oil Content of *Eucalyptus globulus* Leaves

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Abstract: The aim of this study is to determine the essential oil content variation at harvesting day (EOC_0) and at wilting day (EOC_w) of *Eucalyptus globulus* leaves was evaluated as independent factors that are harvesting season (Long Rain season and Dry Season), the leafs type (Top, middle and bottom) and wilting period from 0 h (W_0) to 96 h (W_4) were selected in to the experiment. A completely randomized design with three replications was used in the experiment. The oil was obtained by hydrodistillation of *Eucalyptus* leaves using Clevenger apparatus for 3 h. The distillate oil was measured and the essential oil content was expressed in percent based on harvesting day and wilting day calculations. EOC_0 and EOC_w of *E. globules* were highly significantly ($p<0.001$) affected by harvesting season, leaf position and wilting period. Interaction effect of harvesting season, leaf position and wilting period on essential oil content at harvesting day and wilting period of *E.globulus* is highly significant ($p<0.0001$) in higher yielding of the EOC_0 and EOC_w were found at wilting period 72 h (W3) and 96 h (W4) with long rain season and dry season according to the respective value of EOC_0 and EOC_w (1.58% and 3.13%), respectively. The smallest essential oil content EOC_0 (0.93%) and EOC_w (0.94%) was obtained for samples harvested during long rain season from bottom part distilled without wilting (W_0). In view of the results, it can be concluded that the day four wilting period and top leaf part are the most advantageous for a maximum of essential oil recovered when the *globulus* leaves were distilled.

Key words: *Eucalyptus globulus* · Hydro distillation · Leaf position · Leaf type · and Wilting day

INTRODUCTION

The genus *Eucalyptus*, family Myrtaceae, is a large genus comprising more than 800 species [1]. It is native to Australia and widely grown in many parts of the world [2]. Of which at least 500 species produce essential oil [3]. Tasmanian blue gum (*Eucalyptus globulus* Labill) is one of the species of the genus and a native tree of south-eastern Australia and neighboring countries [4]. It is a medium to a large evergreen broadleaf tree, growing up to 60 m [5]. It is cultivated worldwide for its oil, gum, pulp, timber, medicine and aesthetic value. Among the various wood and non-wood products essential oil (EO) is the most important one [6]. According to Silva [7] the plant produces one of the most important *Eucalyptus* oil. The oils are found in the leaves, fruits, buds, and bark. However, the most important commercial oil is isolated from the leaves by hydro and steam distillation [8].

Essential oils from *E. globulus* have extensive use in food, perfumery and pharmaceutical industry [6]. The oil possesses a wide spectrum of biological activity including anti-microbial, anti-fungicidal, anti-insecticidal/insect repellent, herbicidal, acaricidal and nematicidal [9]. In addition, the oils can be used as folk medicine and are anesthetic, anodyne, antiperiodic, antiphlogistic, antiseptic, astringent, deodorant, diaphoretic, disinfectant, expectorant, febrifuge, fumigant, hemostat, inhalant, insect repellant, preventative, rubefacient, sedative stimulant, suppurative, tonic, and vermifuge [4]. Essential oil production of *Eucalyptus* influenced by

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different factors such as the type of species/genotypes leaves the position, leafage, harvesting season and tree age [10]. Moisture content, wilting time and chopping size also affect essential oil production of the plant [6, 11-13].

Eucalyptus species are widely planted in Ethiopia for firewood, construction poles, windbreak and erosion control and as a border tree. Even though the plants are grown in different part of Ethiopia and are potentially a good source of high-quality essential oils, Research effort was not made to evaluate the influence of different factors on essential oil quality and quantity of the plant in the country.

Therefore, the aim of this study was to investigate most advantageous harvesting season, the leave position and optimum wilting period of *E. globulus* leaves for highest EO content.

MATERIALS AND METHODS

Site Descriptions: The experimental materials were taken from Wondo Genet Agricultural Research Center experimental site. Geographically, Wondo Genet is located at 07° 03' 19.1" to 07° 04' 00.2" North latitude and from 38° 30' 08.4" to 38° 31' 01.8" East longitude. It covers a wide altitudinal range of 1600–2580 m.a.s.l. [14]. The rainfall of Wondo Genet area is characterized by a bimodal distribution, with the main rainy season between July and October, which accounts for 50% of the total rainfall, and a short rainy season between March and May. The mean annual rainfall is 1247 mm, and the mean monthly temperature is 19.5 °C, with mean monthly maximum and minimum temperatures of 26.3 °C and 12.4 °C, respectively [15]. The main parent material of the soil of the study area is developed on volcanic deposits of ignimbrite, ash, lava, and tuff, which have formed gentle and undulating terrain [16].

Sample Collection and Preparation: *Eucalyptus globulus* trees were randomly selected from Wondo Genet Agricultural Research Center experimental site and the leaf samples were harvested during the long rain season from June, 2014 to October, 2014 and the dry season from November, 2014 to February, 2015. A total of 30 treatment combinations comprising 5 levels of wilting periods (0, 24, 48, 72 and 96 h after harvest), 3 levels of leaf positions (Top, Medium and Bottom) and 2 levels of harvesting season (Long Rain Season and Dry Season) were used for the experiment. The design of the experiment was completely randomized design with three replications. For each treatment constant weight of leaf, samples were taken and subjected to open air for wilting purposes under shade having an average temperature of 25°C.

Extraction of Essential Oils: The essential oil extraction was performed by hydrodistillation for 3 h using Clevenger type apparatus [17, 18]. The distillate oil of each sample was measured and the percentage of essential oil content was calculated by using the following mathematical methods:

- The weight of distilled oil was divided by fresh leaf weight and multiplied by hundred, it was given essential oil content at harvested day (EO C_0) [4].
- The weight of distilled oil was divided by wilted leaf weight and multiplied by hundred, it was given essential oil content at wilting day (EOC_w) [4,19].

Data Analysis: The statistical analysis was done with SAS software version 9.0 and SAS Studio (Which is free university license and very good for assumption checking). The classical general linear model with two-way ANOVA fits the data very well as shown in the results. Mean separation was carried out using LSD at ($P<0.001$).

RESULTS AND DISCUSSION

Variation in Essential Oil Content of *Eucalyptus globulus*: EOC_0 and EOC_w of *E. globulus* were highly significantly ($P<0.001$) affected by harvesting season, leaf position and wilting period (Table 1). This result of significant variation with wilting treatment was corroborated by the works of [13], the previous finding has shown that the effect of wilting on the yield of the resulting essential oil produced from the grass species (i.e. Lemongrass, Palmarosa and citronella) being wilted up to day three gave significantly higher amount of EOC_w than that of early distillation and the amount of the corresponding EOC_w increases within the range 0.21- 0.85%, 0.27 - 0.82% and 0.83 - 1.53% for Lemongrass, Palmarosa and citronella, respectively. Interaction effect of harvesting season, leaf position and the wilting period was also highly significant on EOC_0 and EOC_w of *E. globulus* (Table 1).

Table 1: Analysis of Variance for Essential oil content of *Eucalyptus globulus*.

Source of Variation	Df	$EOC_{0\,(\%)}$	$EOC_{w\,(\%)}$
Harvesting season (HS)	1	0.54***	5.8***
Leaf Position (LP)	2	0.78***	2.17***
Wilting Period (WP)	4	0.022***	3.71***
HS xLP x WP	2	0.35***	0.12***
Error	60	0.0035	0.01
CV(%)		4.85	5.44

***= Significant at $P < 0.001$; **= Significant at $P < 0.01$; *= Significant at $P < 0.05$; ns= Non significant at $P < 0.05$, EOC_0 = Essential oil content at harvest, EOC_w = Essential oil content at wilting day

Table 2: Interaction effects of harvest time, leaf position and wilting period on essential oil content at harvest EOC_0 (%) and essential oil content on the witting day of *Eucalyptus globulus*.

		Means			
		Harvesting Season			
		Dry season		Long rain season	
Leaf Position	Wilting Period	EOC_0 (%)	EOC_w (%)	EOC_0 (%)	EOC_w (%)
Top	W0 (0 h)	$1.38b^{cde}$	1.38^{hi}	$1.25g^{hij}$	1.25^{ijk}
	W1 (24 h)	1.35^{cdef}	1.65^{f}	$1.28f^{ghi}$	1.43^{gh}
	W2 (48 h)	$1.33d^{efg}$	2.19^{d}	1.3^{efgh}	1.64^{f}
	W3 (72 h)	1.23^{hijk}	2.63^{bc}	1.58^{a}	2.21^{d}
	W4 (96 h)	1.4^{bcd}	3.13^{a}	$1.4b^{cd}$	2.21^{d}
MiddleMiddle	W0 (0 h)	$1.32d^{efgh}$	1.32^{hij}	1.14^{lm}	1.14^{kl}
	W1 (24 h)	1.4^{bcd}	1.67^{f}	1.06^{mn}	1.18^{jk}
	W2 (48 h)	1.43^{bc}	2.2^{d}	1.04^{no}	1.35^{hi}
	W3 (72 h)	1.4^{bcd}	2.53^{c}	1.17^{jkl}	1.65^{f}
	W4 (96 h)	1.45^{b}	2.7^{b}	1.17^{jkl}	1.91^{e}
Bottom	W0 (0 hr)	1.00^{nop}	1.00^{lm}	0.93^{p}	0.94^{lm}
	W1 (24 h)	1.15^{klm}	1.39^{hi}	1.00^{nop}	1.13^{kl}
	W2 (48 h)	$1.15k^{lm}$	1.68^{f}	0.96^{op}	1.25^{ijk}
	W3 (72 h)	1.2^{ijkl}	2.11^{d}	0.83^{q}	1.24^{ijk}
	W4 (96 h)	1.2^{ijkl}	2.12^{d}	0.94^{p}	1.56^{fg}

Means followed the same letters under the same column are statistical non-significant at 0.05 leavel of probability

Interaction Effect of Harvesting Season, Leaf Position and Wilting Period on Essential Oil Content at Harvest (Eoc_0) and Essential Oil Content at Wilting Day (Eoc_w) of *Eucalyptus globulus*: As shown in Table 1, the interaction effect of harvesting season, leaf position and wilting period on EOC_0 and EOC_w of *E. globulus* is significant. Maximum yielding of the EOC_0 and EOC_w were found at wilting period 72 h (W3) and 96 h (W4) with long rain season and dry season in top leaf part according to the respect value of EOC_0 and EOC_w (1.58% and 3.13%), respectively. These results found to be higher than the previous reported by [20] which was reported 1.05% w/w (DB) on the determine the oil content variation and antimicrobial activity of three different *Eucalyptus* species. There are many literatures reported for *E.globulus* where the essential oil yield was 1.9-2.7% (w/w, based on the fresh weight of the youg leaves) in Morocco [21]; 2.68% (w/w, based on the fresh weight of the adult leaves) in Argentina [22].

The next higher value of EOC_0 and EOC_w 1.45% and 2.7% were recorded in middle leaf part and the wilted period for 96 h (W_4) (Table 2). These results are comparable with other studies reported by [23], which was in the range between 0.8 to 2.% W/W (DB) on the Yield, Contents and Chemical Composition Variations in the essential oils of different *E. globulus* trees from Tigray, Northern Ethiopia. EOC_0 during the dry season from middle part with wilted period 24 h (W_1) and 72 h (W_3) were yielded statistically similar essential oil content (1.4%) with samples top part and wilted period 96 h (W_4). EOC_w during long rain season from top part wilted period 72 h (W_3) and 96 h (W_4) were yielded statistically similar essential oil content with sample harvested during the dry season from the top, middle and bottom part and wilted for 48 h (W_2),72 h (W_3) and 96 h (W_4). The smallest EOC_0 (0.93%) and EOC_w (0.94%) was obtained for samples harvested during long rain season from bottom part distilled without wilting (W0) (Table 2). In general from

the experimental data's observed that the percentage of EOC_0 and EOC_w are maximum recorded at W_4 in each leaf type and the significant increment with increase of wilting period from 0 h (W_0) to 96 h (W_4) in all leaf parts (Top, middle and bottom) of *E. globulus* at both harassing season. consequently, computing along with leaf type maximum yielded EOC_0 and EOC_w are observed in leaf tip part at all wilting period than medium and bottom parts. The significant variation between different wilting period of *E. globulus* leaves might be the synthesis of essential oil is more concentrated at wilting day four than others wilting periods payable the leaf characters such as leaf area, leaf thickness and leaf mass per area [24] and the loss of moisture with subsequent wilting of the *E. globulus* leaves which contributed to the increase in the percent composition of essential oil as was described by Solomon and Fikremariam [13]. The significant variation between leaf types (Top, middle and bottom) is probably because of the glandular trichomes or specific oil cells that are present in parenchymal tissues of *E. globulus* leaves [25-27] are found extra growth in top leaf part than middle and bottom leaf parts.

CONCLUSION

This experiment has clearly shown the impact of harvesting season, leaf position and wilting period on EOC_0 and EOC_w of the *Eucalyptus globulus* leaves. The post-harvest wilting period before distillation of the *E. globulus* leaves up to 96 h (W4) gave significantly high essential oil contents than early distillations in each leaf type during the harvesting season. An average of essential oil content on wilting period for both harvesting season, increases variably between top and middle (0.11%) and top and bottom (0.31%) for EOC_0, and top and middle (0.21%) and top and bottom (0.53%) for EOC_w. Therefore, it can be concluded that the present study has shown us the significance of an excellent model for handling mechanism of *E.globulus* leaves before distillation. The wilting period at day four and the leaf position at the top are the most advantageous for a maximum of essential oil recovered when the *E. globulus* leaves was distilled. This result has significant importance to save time management and minimize running cost/maximize the cost of production into the distillation process for commercially produce essential oil content from the leaves studied.

ACKNOWLEDGMENT

Authors would like to acknowledge Dr.Tesfaye Bekele, the director of Essential Oil Research Center for all rounded supported and some involvement in the project. In addition, the authors are grateful and thankful to Mr. Birara Tilahun for his valuable contribution during the collection of *Eucalyptus globulus* leaves and also to Mr. Zerihun Jomba, for his active participation for laboratory work analysis throughout the period of this study.

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