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Influence of Harvesting Season, Leaf Position and Wilting Period on Essential Oil Content of *Eucalyptus citriodora* 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 citriodora* leaves was evaluated as independent factors that are harvesting season (Long Rain season and Dry Season), the leaves 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 hydro distillation 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. citriodora* 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. citriodora* is significant ($p<0.001$) in higher yielding of the EOC_0 and EOC_w were found at wilting period 24 h (W_1) and 72 h (W_3) with dry season according to the respective value of EOC_0 and EOC_w (1.95% and 3.59%), respectively. The smallest essential oil content EOC_0 (1.08%) and EOC_w (1.09%) was obtained for samples harvested during long rain season from top part distilled without wilting (W_0). In view of the results, it can be concluded that the day three and the day four are the most advantageous for a maximum of essential oil recovered (3.59%, 3.37%) when the *E.citriodora* leaves was distilled for dry harvesting season.

Key words: *Eucalyptus Citriodora* · Hydro Distillation · Distillation · Leaf Type · And Wilting Day

INTRODUCTION

The genus *Eucalyptus*, family Myrtaceae*,* is a large genus comprising more than 800 species [1]. The plant, is native in Australia [2] and widely grown in many parts of the world, of which produces at least 500 type of essential oil [3]. The genus *Eucalyptus* consists of tall, magnificent and evergreen trees with aromatic foliage rich in oil glands and is an excellent source of commercially important oil [4]. Currently, growing Eucalyptus trees at a farm level have become very popular among some farmers [5]. The planting rate is increasing due to the high demand for the trees especially for fuel, poles, construction materials and other domestic consumptions. *Eucalyptus globules* and *Eucalyptus citriodora* as the most important *Eucalyptus* oil sources [6]. 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 [7]. According to Silva [8] 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 [9].

Essential oil of the *E. citriodora* leaves is a powerful antiseptic and is used all over the world as a respiratory decongestant, for relieving colds, coughs, bronchitis, flu, pneumonia, headache and sore throats [10, 11]. Essential oil production of *Eucalyptus* influenced by different factors such as the type of species/genotypes leaves the position, leafage, harvesting season and tree age [12]. Moisture content, wilting time and chopping size also affect essential oil production of the plant [7, 13-15].

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Eucalyptus species are widely planted in Ethiopia for firewood, construction poles and 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. citriodora* 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. [16]. 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 [17]. 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 [18].

Sample Collection and Preparation: *Eucalyptus citriodora* trees were randomly selected from Wondo Genet Agricultural Research Center experimental site and the leaf samples were harvested during the long rain season from September, 2016 to October, 2016 and the dry season from March, 2017 to May, 2017. 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 Wight 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 hydro distillation for 3 h using Clevenger type apparatus [19, 20]. 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 (EOC_0) [21].
- 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) [21, 22].

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 (EOC) of *Eucalyptus citriodora*: The EOC_0 and EOC_w of *E. citriodora* were highly significantly ($P < 0.001$) affected by harvesting season, leaf position and wilting period (Table 1). These results have shown similar tendency with previous study by Fikremariam *et al*. [23, 24], which has shown that the effect of harvesting season, wilting period and leaf position on the yield of the resulting essential oil extracted from the *E. globules* and *E. camaldulensis* leaves were indicated that the post-harvest wilting period before distillation of the studies species of *Eucalyptus* leaves up to 96 h (W_4) 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 (0.21%, 29%) and top and bottom (0.53%, 0.38%) for EOC_w, respectively. Interaction effect of harvesting season, leaf position and the wilting period was also highly significant on EOC_0 and EOC_w of *E. citriodora* (Table 1).

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 citriodora*: As shown in Table 1, the interaction effect of harvesting season, leaf position and

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

Source of Variation	Df	EOC0 (%)	EOCw (%)
Harvesting season (HS)	1	4.34***	13.95***
Leaf Position (LP)	2	0.18***	0.227***
Wilting Period (WP)	4	0.13***	6.80***
HS x LP x WP	2	0.017**	0.04*
Error	60	0.0054	0.01
CV(%)		5.00	0.039

***= Significant at $P < 0.001$; **= Significant at $P < 0.01$; 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 citriodora*

		Means			
		Harvesting Season			
		Dry season		Long rain season	
Leaf Position	Wilting Period	EOC_0 (%)	EOC_w (%)	EOC_0 (%)	EOC_w (%)
Top	W0 (0 h)	1.43^{ghi}	1.43^{ij}	1.08^{l}	1.08^{k}
	W1 (24 h)	1.95^{a}	2.54^{e}	1.33^{l}	1.43^{ij}
	W2 (48 h)	1.85^{ab}	3.01^{c}	1.67^{kl}	1.95^{g}
	W3 (72 h)	1.49^{fgh}	3.02^{c}	1.18^{kl}	2.36^{ef}
	W4 (96 h)	1.49^{fgh}	3.16^{c}	1.33^{l}	2.49^{def}
Middle	W0 (0 h)	1.61^{def}	1.61^{hi}	1.27^{jk}	1.27^{jk}
	W1 (24 h)	1.88^{ab}	2.44^{def}	1.38^{hij}	1.6^{h}
	W2 (48 h)	1.76^{bc}	3.13^{c}	1.35^{ij}	2.04^{g}
	W3 (72 h)	1.84^{ab}	3.59^{a}	1.37^{ij}	2.4^{ef}
	W4 (96 h)	1.61^{def}	3.37^{b}	1.35^{ij}	2.64^{d}
Bottom	W0 (0 hr)	1.69^{dc}	1.69^{h}	1.27^{jk}	1.27^{jk}
	W1 (24 h)	1.85^{ab}	2.52^{dci}	1.27^{jk}	1.58^{hi}
	W2 (48 h)	1.81^{bc}	3.05^{c}	1.33^{ij}	2.01^{g}
	W3 (72 h)	1.63^{de}	3.13^{c}	1.3^{ij}	2.3^{f}
	W4 (96 h)	1.55^{gef}	3.06^{c}	1.27^{jk}	2.44^{def}

Means followed by the same letter at the same column are statistical non-significant at $p < 0.05$ of probability

wilting period on EOC_o and EOC_w *E. citriodora* is significantly maximum yielding of EOC_o and EOC_w were found at wilting period 24 h (W1) and 72 h (W3) with dry season in top leaf part and long rain season in middle leaf part according to the respect value of EOC_o and EOC_w (1.95% and 3.59%), respectively. These results found to be comparable with the reported by [25] which were reported 2% fresh leaves yield on Physico-Chemical Profile and Antioxidant Activities of *E. globulus Labill* and *E.citriodora* Essential Oils in Ethiopia. There are many literature reported for *E.citriodora* where the essential oil yield was 0.5–2% (w/w, based on the air dried weight of the leaves) in Pakistan [26]; 1.6-3.3% (w/w, dry weight) on Determination of yield and chemical composition of eucalyptus oil from different species and locations in Indonesia [27].

The next higher value of EOC_o were observed middle leaf part at the wilted period for 24 h (W_1) and top leaf part at the wilted period for 48 h (W_2) with the value of 1.88% and 1.85% in the dry harvesting season. EOC_w also was recorded at middle leaf part at the wilted period for 96 h (W_4) with the value of 3.37% in the same harvesting season (Table 2). These results are comparable with other studies reported by Drew James King *et al.* [28], which was in the range 1.2% to 3% W/W (DB) on Chemical Composition and antibacterial activities of seven Eucalyptus species essential oils leaves. The smallest values of EOC_0 (1.08%) and EOC_w (1.08%) were obtained for samples harvested during long rain season from top leaf part and distilled without wilting (W_0) and after wilting, respectively (Table 2). In general, from the experimental data it has been observed that the percentage of EOC_0 and EOC_w come across wilting period from 0 h (W_0) to 96 h (W_4) in all positions of leaves (i.e. top, middle and bottom) of *E.citriodora* for both harvesting seasons, the sign of irregular increment was noted; These results have shown the same tendency with the research work corporate by Fikremariam *et.al* [23].

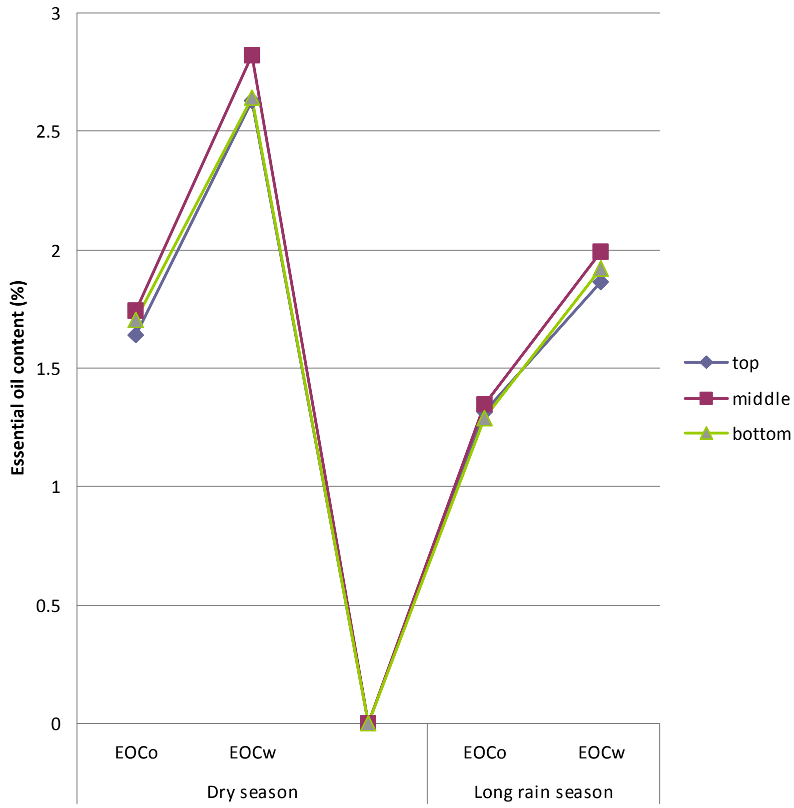

Fig. 1: Interaction between harvesting season, leaf position and wilting period on essential oil content of *E. citriodora*.

The significant variation between different wilting period of *E. citriodora* 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 [28] and the loss of moisture with subsequent wilting of the *E. citriodora* leaves which contributed to the increase in the percent composition of essential oil as was described by Solomon and Fikremiram [15]. While computing along with leaf type maximum yield of EOC_o (1.74%, 2.82%) and EOC_w (1.34%, 1.99%) (Figure 1) were observed on average in leaf middle part than top and bottom leaf parts at dry season and long rain season, respectively. 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. citriodora* leaves [29-31] are found extra growth in middle leaf part than bottom and top leaf parts.

CONCLUSION

This study has clearly shown the impact of harvesting season, leaf position and wilting period on EOC_o and EOC_w of the *E. citriodora* leaves. When, wilted *E. citriodora* leaves up to 96 h (W_4) gave significantly high essential oil contents than early distillations in each leaf type for EOC_w in both harvesting seasons (i.e. dry season and long-rain season). The amount of increase in variations between 0.034 (middle and bottom) and 0.098 (middle and top) for EOC_0 and 0.138 (middle and bottom) and 1.88 (middle and top) for EOC_w were observed during the dry season harvesting time. The amount of increase in variations between 0.056 (middle and bottom) and 0.026 (middle and top) for EOC_0 and 0.07 (middle and bottom) and 0.128 (middle and top) for EOC_w were observed during the long-rain harvesting season. Therefore, it can be concluded that the present study has shown us the significance of can excellent model for handling mechanism of *E. citriodora* leaves before distillation. Wilting middle leaf position at day three and day four are the most advantageous for a maximum of essential oil recovered (3.59%, 3.37%) when the *E. citriodora* leaves was distilled for dry harvesting season. This result has significant importance to save time management and minimize running cost/maximize the cost of production into the distillation process for cost of production into the distillation process for commercially produce essential oil content from the leaves studied.

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