Leaf and Essential Oil Yield Response of Rosemary
(*Rosmarinus officinalis* L., Syn *Salvia rosmarinus* Spenn.)
Varieties under Varying Harvesting Ages at Wondo Genet, Southern Ethiopia

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**Abstract:** Rosemary (*Rosmarinus officinalis* L., syn *Salvia rosmarinus* Spenn.) is well known aromatic, medicinal and condiment plant that belongs to the Family Labiatae. In Ethiopia, there are a total of three released varieties of Rosemary. However, there was a gap on at what age these varieties harvested after transplanting. Thus, the field experiment was carried out during 2014-2016 to determine optimum harvesting age and variety for maximum leaf and essential oil yield of rosemary. The field experiment consisted of three varieties (WG-Rosemary-I, II and III) and five levels of harvesting age (7, 8, 9, 10 and 11 months after transplanting (MAT)). The combinations of these treatments were arranged in factorial Randomized Complete Block Design (RCBD) with three replications on a plot size of 3.6 cm length and 3.6 cm width. SAS (version 9.3) software was used to compute the analysis of variance. LSD test at 5% probability level was used for comparison of the characters measured. The result showed that, the main effect of harvesting age and variety had a significant (p ≤ 0.05 or p ≤ 0.01 or p ≤ 0.001) effect on all the quantitative traits studied. The highest number of branches plant⁻¹, fresh leaf yield plant⁻¹, fresh leaf yield ha⁻¹, essential oil content and essential oil yield ha⁻¹ were obtained by variety WG-Rosemary-I, with the respective mean value of 161, 1337.2 g, 15, 125.6 kg, 1.63% and 308.42 kg. This could be due to the vigorous nature of the variety with the highest leaf-bearing and essential oil accumulation as compared to the remaining two varieties tested. The longest plant height and the least fresh leaf yield plant⁻¹, fresh leaf yield ha⁻¹, essential oil content and essential oil yield ha⁻¹ were obtained by variety WG-Rosemary-II. This might be due to the vertical growth nature of the variety without spreading resulted the longest plant height with inferior fresh leaf biomass yield. Harvesting rosemary at 7 MAT resulted the highest essential oil content (1.57%) and essential oil yield ha⁻¹ (259.68 kg) followed by 11 MAT. This would be the best age of rosemary at which the highest accumulation of essential oil obtained. The highest fresh leaf yield plant⁻¹ (1284.4 g) and fresh leaf yield ha⁻¹ (16, 467 kg) was obtained when rosemary was harvested at 11 MAT followed by 10 MAT. This would be the best age of rosemary at which the highest leaf biomass obtained. Therefore, planting variety WG-Rosemary-I and harvesting at 7 MAT and 11 MAT is highly recommended to get the highest essential oil yield ha⁻¹ and fresh leaf yield ha⁻¹, respectively.

**Key words:** Rosemary • Labiatae • Aromatic • Medicinal • Condiment • Quantitative Traits

**INTRODUCTION**

Rosemary (*Rosmarinus officinalis* L., syn *Salvia rosmarinus* Spenn.) is an evergreen, shrubby herb that belongs to the family Lamiaceae and grows to a height of 1 to 2 m, with a unique aromatic odour and its stems are erect and divided into numerous long, slender branches that have ash-coloured and scaly bark [1, 2]. Rosemary is a dense evergreen undershrub with lavender-like leaves and a characteristic aroma. The leaves and essential oil are the economic products of rosemary. Its essential oil is used almost wholly in the perfumery industry in the production of soaps, detergents, household sprays and other such products. It is an excellent fixative material and...
contributes a strong, fresh odour, which blends well with various other oil odours and also serves to mask unpleasant smells of certain other ingredients. It is used in shampoo, toilet soaps and medicine, as well as its oil is known to have antimicrobial activity [3].

The production of essential oils not only depends upon the metabolic state and present developmental differentiation program of the synthesizing tissue, but also is highly integrated with the physiology of the whole plant [4]. The growth, yield and yield components of plants are determined by a series of factors including plant genetics [5], climate, edaphic, elevation, topography and also an interaction of various factors [6]. Among these factors harvesting age and variety are the most important factors that can affect the production of biomass, essential oil content and yield of rosemary. As many authors reported, harvesting age had a great impact on Lavender (Lavandula angustifolia L.) [7], Java Citronella (Cymbopogon Winterianus Jowitt) [8], Lemongrass [9], Rose-scented geranium [10], Rosemary (Rosmarinus officinalis L.) [11], Peppermint (Mentha piperita L.) [12], Artemisia (Artemisia annua L.) [13], Sage (Salvia officinalis L.) [14] and Sweet basil (Ocimum basilicum L.) [15]. Similarly, the effect of variety on agronomic traits was reported on Roselle (Hibiscus sabdariffa L.) [16], Lemongrass [9] and Sweet basil (Ocimum basilicum L.) [15]. Therefore, optimization of these factors has its own contribution towards the maximization of biomass and essential oil production.

Even though, rosemary has diverse uses a limited effort has been made to optimize of harvesting age in Ethiopia. This indicates as there is a lack of information on how to produce this miracle plant. This makes the producers less benefited from this crop. Therefore, the objective of this paper was to determine the optimum harvesting age and variety for maximum leaf and essential oil yield of rosemary.

MATERIALS AND METHODS

Description of the Study Area: The experiment was conducted at Wondo Genet Agricultural Research Center (WGARC) experimental field with a supplement of irrigation for two consecutive years from August, 2014...
to June, 2016. WGARC has located between 7°5′38.98″ N latitude and 38°37′19.536″ E longitude; it is found at an altitude of 1766 m.a.s.l (meter above sea level). The site map of the experimental area was made by ArcGIS Desktop Advanced version 10.8 [17].

It receives mean annual rainfall of 1128 mm with minimum and maximum temperatures of 11°C and 26°C, respectively. The soil textural class of the experimental area is sandy loam with pH of 6.4 [18].

Plant Materials, Experimental Design and Field Management: Seedlings were raised at nursery site by taking cuttings from the top portion of healthy mother plants and planted on the polyethylene tubes filled with 1:2:1 ratio of topsoil, forest soil and sand. Seedlings were transplanted to the main field after two months of the establishment at row and plant spacing of 60 cm. The experiment consisted of three varieties (WG-Rosemary-I, II and III) and five levels of harvesting age (7, 8, 9, 10 and 11 months after transplanting (MAT)) arranged in factorial randomized complete block design with three replications. The size of the plot was 3.6 m length and 3.6 m width. Healthy and uniform seedlings were transplanted from the nursery to the main field after two months of the establishment at the commencement of the main rainy season after the land was prepared well. Proper field management practices like hoeing, watering and weeding were carried out as needed. Chemical or fertilizer was not applied during its growth cycle. The whole plant was cut 15 cm above the ground while harvesting.

Data Collection: During the experiment data on plant height, number of branches plant⁻¹, fresh leaf yield plant⁻¹, fresh leaf yield ha⁻¹, essential oil content and essential oil yield ha⁻¹ were recorded critically based on five randomly selected plants.

Essential Oil Content (%): Essential oil content was obtained by hydro-distillation, according to the procedure described by Daniel Bisrat et al. [19]. The harvested plants were separated into leaf and stem, fresh leaves having biomass of 300 g composite sample was charged in the Clevenger apparatus along with 700 ml of water poured into the flask until the plant part submerged completely. Then, the round bottom flask with the sample and water inside was placed on the heating mantle. It was boiled for 3 hours and the essential oil was collected and measured by using pipette and reading was taken place. The percentage of essential oil content was determined by the following formula.

\[
\text{Essential oil content (\%) } = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100
\]

Essential Oil Yield (kg/ha): The volume of essential oil collected in the collecting tube of the apparatus dehydrated, measured and expressed on weight by weight (%/w/w) fresh basis. Then the essential oil yield/ha was determined by the following formula.

\[
\text{Essential oil yield (kg/ha) } = \frac{\text{Fresh leaves yield (kg/ha)}}{\text{Essential oil content (\%)}} \times \text{Essential oil content (\%)}
\]

Data Analysis: Data on quantitative traits were subjected for statistical analysis using SAS computer software program (SAS 9.4) [20] following the procedures for the general linear model ANOVA. Comparisons between means were done using LSD at a 5% probability level.

RESULTS AND DISCUSSION

The result revealed the main effects of variety and harvesting age had a significant (p ≤ 0.05 or p ≤ 0.01 or p ≤ 0.001) effect on all traits studied. Whereas, the interaction of variety and harvesting age did not significantly (p > 0.05) affect all of the traits studied (Table 1). This might be due to the consistency of the value of each trait at all levels of interaction.

Plant Height (cm): Plant height was significantly (p ≤ 0.01) affected by harvesting age (Table 1). Similar results were reported by Jimayu and Gebre [14] on sage, Abewoy et al. [15] on sweet basil, Haileslassie and Kebede [10] on rose-scented geranium, Zewdinesh et al. [11, 13] on rosemary and Artemisia. The highest plant height (165.7 cm) was recorded when rosemary was harvested at 10 MAT followed by 11 MAT (156.8 cm); whereas, the least value (139.8 cm) was recorded when it was harvested at 9 MAT (Table 2).

Likewise, variety had a significant (p ≤ 0.001) effect on plant height (Table 1). These results are in line with the finding of Abewoy et al. [15] on sweet basil. The highest plant height (183.13 cm) was recorded at variety WG-Rosemary-II; whereas, the least value (136.08 cm) was recorded at variety WG-Rosemary-I. (Table 2). This could be due to the typical vertical growth nature of variety WG-Rosemary-II as compared to others.

Number of Branches Plant⁻¹: Number of branches plant⁻¹ was significantly (p ≤ 0.05) affected by harvesting
Table 1: Effects of variety (Var) and Harvesting Age (HA) on Agronomic and Chemical traits of Rosemary (*Rosmarinus officinalis* L., *syn* *Salvia rosmarinus* Spenn.) at Wondo Genet during 2014 to 2016

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Replication (DF=2)</th>
<th>Var (DF=2)</th>
<th>HA (DF=4)</th>
<th>Var*HA (DF=8)</th>
<th>Error (DF=28)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>735.4</td>
<td>10148.74***</td>
<td>826.61**</td>
<td>49.98ns</td>
<td>160.35</td>
<td>8.3</td>
</tr>
<tr>
<td>NBP</td>
<td>206.62</td>
<td>1042.5**</td>
<td>439.24*</td>
<td>67.12ns</td>
<td>120.74</td>
<td>7.1</td>
</tr>
<tr>
<td>FLYP</td>
<td>205174.39</td>
<td>621498.95***</td>
<td>65350*</td>
<td>26167.27ns</td>
<td>23067</td>
<td>13.2</td>
</tr>
<tr>
<td>FLYH</td>
<td>11194090.5</td>
<td>86052315.1***</td>
<td>47270380.1***</td>
<td>4041082.8ns</td>
<td>6369948</td>
<td>19.1</td>
</tr>
<tr>
<td>EOC</td>
<td>0.14</td>
<td>1.26***</td>
<td>0.25**</td>
<td>0.05ns</td>
<td>0.04</td>
<td>15.0</td>
</tr>
<tr>
<td>EOYH</td>
<td>13172.28</td>
<td>114489.28***</td>
<td>10766.5***</td>
<td>689.41ns</td>
<td>1576.54</td>
<td>18.2</td>
</tr>
</tbody>
</table>

where, DF= Degree of freedom, PH= Plant height (cm), NBP= Number of branches plant−1, FLYP= Fresh leaf yield plant−1 (g), FLYH= Fresh leaf yield ha−1 (kg), EOC= Essential oil content (%), EOYH= Essential oil yield ha−1 (kg), and CV= Coefficient of variance.

* *, ** and *** = Statistically significant at 5, 1 and 0.1% probability level, respectively; and ns= Not significant at 5% probability level.

Table 2: Mean performance of Agronomic and Chemical traits of Rosemary (*Rosmarinus officinalis* L., *syn* *Salvia rosmarinus* Spenn.) varieties at each harvesting age at Wondo Genet from 2014 to 2016

<table>
<thead>
<tr>
<th>Treatment and statistics</th>
<th>PH (cm)</th>
<th>NBP</th>
<th>FLYP (g)</th>
<th>FLYH (kg)</th>
<th>EOCFB (%)</th>
<th>EOYH (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WG-Rosemary-I</td>
<td>136.1†</td>
<td>161.0†</td>
<td>1337.2†</td>
<td>15125.6†</td>
<td>1.63†</td>
<td>308.42b</td>
</tr>
<tr>
<td>WG-Rosemary-II</td>
<td>183.1†</td>
<td>144.7†</td>
<td>933.5†</td>
<td>10525b†</td>
<td>1.06†</td>
<td>134.1b</td>
</tr>
<tr>
<td>WG-Rosemary-III</td>
<td>140.4†</td>
<td>155.7†</td>
<td>1180.4†</td>
<td>13981.3†</td>
<td>1.26†</td>
<td>210.82b</td>
</tr>
<tr>
<td>LSD@0.05</td>
<td>9.47</td>
<td>8.22</td>
<td>113.6</td>
<td>1887.8</td>
<td>0.14</td>
<td>29.7</td>
</tr>
<tr>
<td>Harvesting age (MAT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>149.2w</td>
<td>151.5w</td>
<td>1161.4w</td>
<td>13178w</td>
<td>1.57w</td>
<td>259.68w</td>
</tr>
<tr>
<td>8</td>
<td>154.4w</td>
<td>149.3w</td>
<td>1059.2w</td>
<td>10933w</td>
<td>1.29w</td>
<td>196.48w</td>
</tr>
<tr>
<td>9</td>
<td>139.8w</td>
<td>163.2w</td>
<td>1099.0w</td>
<td>11193w</td>
<td>1.17w</td>
<td>190.89w</td>
</tr>
<tr>
<td>10</td>
<td>165.7w</td>
<td>146.3w</td>
<td>1147.8w</td>
<td>14282w</td>
<td>1.17w</td>
<td>190.63w</td>
</tr>
<tr>
<td>11</td>
<td>156.8w*</td>
<td>158.7w*</td>
<td>1284.4w*</td>
<td>16467w*</td>
<td>1.39w*</td>
<td>251.24w*</td>
</tr>
<tr>
<td>LSD@0.05</td>
<td>12.23</td>
<td>10.61</td>
<td>146.66</td>
<td>2437.1</td>
<td>0.19</td>
<td>38.34</td>
</tr>
</tbody>
</table>

where, DF= Degree of freedom, HA= Harvesting age (MAT), PH= Plant height (cm), NBP= Number of branches plant−1, FLYP= Fresh leaf yield plant−1 (g), FLYH= Fresh leaf yield ha−1 (kg), EOC= Essential oil content (% w/w fresh base), EOYH= Essential oil yield (kg/ha), CV= Coefficient of variance, ns= Statistically non-significant at 0.05 probability level.

age (Table 1). A similar result was reported by Abewoy *et al.* [15] on sweet basil. The highest number of branches plant−1 was recorded when rosemary was harvested at 9 MAT (163) followed by 11 MAT (159); whereas, the least (146) value was recorded when it was harvested at 10 MAT (Table 2).

Similarly, variety had a significant (p ≤ 0.01) effect on number of branches plant−1 (Table 1). This result is in line with the finding of Abewoy *et al.* [15] on sweet basil. The highest number of branches plant−1 (161) was recorded at variety WG-Rosemary-I followed by variety WG-Rosemary-III (156); whereas, the least value (145) was recorded at variety WG-Rosemary-II (Table 2). This could be due to the highest branching habit of variety WG-Rosemary-I as compared to the remaining two varieties.

**Fresh Leaf Yield Plant−1 (g):** Fresh leaf yield plant−1 was significantly (p ≤ 0.05) affected by harvesting age (Table 1). Supporting result was reported by Jimayu and Gebre [14] on sage. The highest fresh leaf yield plant−1 (1284.4 g) was recorded when rosemary was harvested at 11 MAT followed by 7 MAT (1161.4 g); whereas, the least value (1059.2 g) was recorded when it was harvested at 8 MAT (Table 2). This indicates that harvesting rosemary at 11 months after transplanting is very important to get the highest fresh leaf yield plant−1.

Likewise, variety had a significant (p ≤ 0.001) effect on fresh leaf yield plant−1 (Table 1). The highest fresh leaf yield plant−1 (1337.2 g) was recorded at variety WG-Rosemary-I; whereas, the least value (933.5 g) was recorded at variety WG-Rosemary-II (Table 2). This could be due to the highest nature of fresh leaf biomass bearing plant−1 of rosemary variety WG-Rosemary-I.

**Fresh Leaf Yield ha−1 (kg):** Fresh leaf yield ha−1 was significantly (p ≤ 0.001) affected by harvesting age (Table 1). Supporting results were reported by Jimayu and Gebre [14] on sage, Jimayu and Gebre [9] on lemongrass.
and Haileslassie and Kebede [10] on rose-scented geranium. The highest fresh leaf yield ha\(^{-1}\) (16, 467 kg) was recorded when rosemary was harvested at 11 MAT; whereas, the least value (10, 933 kg) was recorded when it was harvested at 8 MAT (Table 2). This indicates that harvesting rosemary at 11 months after transplanting is very important to get the highest fresh leaf yield ha\(^{-1}\).

Similarly, variety had a significant (p ≤ 0.001) effect on fresh leaf yield ha\(^{-1}\) (Table 1). A similar result was reported by Jimayu and Gebre [9] on lemongrass. As to fresh leaf yield plant\(^{-1}\), the highest fresh leaf yield ha\(^{-1}\) (15, 125.6 kg) was recorded at variety WG-Rosemary-I; whereas, the least value (10, 525 kg) was recorded at variety WG-Rosemary-II (Table 2). This could be due to the highest fresh leaf biomass-bearing nature of the rosemary variety WG-Rosemary-I as compared to the rest variety.

**Essential Oil Content (\\% w/w Fresh Base):** Essential oil content was significantly (p ≤ 0.001) affected by harvesting age (Table 1). Similar results were reported by Basazinew and Sulti [7] on lavender, Jimayu and Gebre [14] on sage, Haileslassie and Kebede [10] on rose-scented geranium and Zewdinesh et al. [11, 13] on rosemary and Artemisia. The highest essential oil content (1.57%) was recorded when rosemary was harvested at 7 MAT; whereas, the least value (1.17%) was recorded equally when it was harvested at 9 and 10 MAT (Table 2). This indicates that as the harvesting age of rosemary increases the essential oil content decreases.

Likewise, variety had a significant (p ≤ 0.001) effect on essential oil content (Table 1). This result is in line with the finding of Jimayu and Gebre [9] on lemongrass. The highest essential oil content (1.63%) was recorded at variety WG-Rosemary-I followed by variety WG-Rosemary-III (1.26%); whereas, the least value (1.06%) was recorded at variety WG-Rosemary-II (Table 2). This indicates variety WG-Rosemary-I had the highest content of essential oil as compared to the rest two varieties.

**Essential Oil Yield ha\(^{-1}\) (kg):** Essential oil yield ha\(^{-1}\) was significantly (p ≤ 0.001) affected by harvesting age (Table 1). Similar results were reported by Basazinew and Sulti [7] on lavender, Jimayu and Gebre [14] on sage, Jimayu and Gebre [9] on lemongrass, Haileslassie and Kebede [10] on rose-scented geranium and Zewdinesh et al. [11, 13] on rosemary and Artemisia. The highest essential oil yield ha\(^{-1}\) (259.68 kg) was recorded when rosemary was harvested at 7 MAT followed by 11 MAT (251.24 kg); whereas, the least value (190.63 kg) was recorded when it was harvested at 10 MAT (Table 2).

Similarly, variety had a significant (p ≤ 0.001) effect on essential oil yield ha\(^{-1}\) (Table 1). This result is supported by the finding of Jimayu and Gebre [9] on lemongrass. The highest essential oil yield ha\(^{-1}\) (308.42 kg) was recorded at variety WG-Rosemary-I followed by variety WG-Rosemary-III (210.82 kg); whereas, the least value (134.1 kg) was recorded at variety WG-Rosemary-II (Table 2). This could be due to the vigorous nature of the variety with the highest leaf-bearing and essential oil accumulation as compared to the remaining two varieties tested.

**CONCLUSION AND RECOMMENDATION**

The result revealed the main effect harvesting age and variety had a significant effect on all the quantitative traits studied. The highest number of branches plant\(^{-1}\), fresh leaf yield plant\(^{-1}\), fresh leaf yield ha\(^{-1}\), essential oil content and essential oil yield ha\(^{-1}\) were obtained by variety WG-Rosemary-I. This might be due to the vigorous nature of the variety with the highest leaf-bearing and essential oil accumulation as compared to the remaining two varieties tested. The longest plant height and the least fresh leaf yield plant\(^{-1}\), fresh leaf yield ha\(^{-1}\), essential oil content and essential oil yield ha\(^{-1}\) were obtained by variety WG-Rosemary-II. This could be due to the vertical growth nature of the variety without spreading resulting in the longest plant height with inferior fresh leaf biomass yield. Harvesting rosemary at 7 MAT resulted in the highest essential oil content and essential oil yield ha\(^{-1}\) followed by 11 MAT. This would be the best age for rosemary at which the highest accumulation of essential oil is obtained. The highest fresh leaf yield plant\(^{-1}\) and fresh leaf yield ha\(^{-1}\) were obtained when rosemary was harvested at 11 MAT followed by 10 MAT. This would be the best age for rosemary at which the highest leaf biomass is obtained. Therefore, planting a variety of WG-Rosemary-I and harvesting at 7 MAT and 11 MAT is highly recommended to get the highest essential oil yield ha\(^{-1}\) and fresh leaf yield ha\(^{-1}\), respectively.

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