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Effects of Harvesting Age and Spacing on Leaf Yield and Essential Oil Yield of Rosemary (*Rosmarinus officinalis* L.)

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ABSTRACT

A study was conducted to evaluate the effect of harvesting age and spacing on agronomic and chemical traits of rosemary (*Rosmarinus officinalis* L.). Data on plant height, branch number/plant, fresh and dry leaf weight/plant, fresh and dry leaf yield/ha, essential oil (EO) content and EO yield/ha were collected and analyzed. Harvesting age exerted a very highly significant ($P < 0.001$) influence on plant height and dry leaf weight/plant, and a highly significant ($P < 0.01$) influence on dry leaf to stem ratio. Spacing affected fresh and dry leaf weight/plant very highly significantly ($P < 0.001$) and highly significantly ($P < 0.01$) plant height. The interaction effect of harvesting age and spacing was significant ($P < 0.05$) on dry leaf yield/ha, EO content and yield, and highly significant ($P < 0.01$) on fresh leaf yield/ha. Maximum plant height and dry leaf weight/plant were obtained 11 months after transplanting (MAT) and maximum dry leaf to stem ratio at 9 MAT. Higher plant height was attained at 60 × 60 cm while higher fresh and dry leaf weight/plant at 90 × 90 cm. Higher fresh leaf yield/ha (38.43 t) and dry leaf yield/ha (10.83 t) were obtained at 60 × 60 cm at 9 MAT. Maximum EO content (2.77 %) and EO yield (271.43 kg) were recorded at 10 MAT at a spacing of 90 × 120 cm and 60 × 60 cm, respectively.

Keywords: agronomic traits, chemical traits, plant age

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is an aromatic, evergreen and highly branched shrubby herb that belongs to the family Labiatae (Republic of South African Directorate of Plant Production 2009). The long slender branches bear many sessile opposite leaves, smooth and green, woolly whitish and glandular beneath, 2-4 cm long, almost cylindrical and folded inwards; flowers are situated in small clusters towards the ends of the branches; the calyx is 2-lipped, with an upper single broad oval lobe and a lower two segmented triangular lobe; the corolla is two-lipped with two violet stamens and a long style projecting from it; fruit is an oval 4-sectioned cremocarp (Joy *et al*. 2001).

Rosemary is indigenous to South Europe, Asia Minor and North Africa (Mishra *et al*. 2009). It grows wild on the Mediterranean shores and in Spain, Portugal, Morocco and Algeria, and it is cultivated in Spain, Tunisia, Yugoslavia, France, Italy, North Africa and India (Joy *et al*. 2001). The plant requires light dry soil, preferably lying over chalk with neutral to alkaline pH.

The leaves and essential oils (EOs) are the economic products of rosemary. Dried and fresh leaves are directly used as a culinary herb and EOs extracted from leaves are extensively used in food flavoring and fragrance industries (Beemnet *et al*. 2009; Mishra *et al*. 2009). The EO of rosemary is also used in the perfumery industry for the production of soaps, detergents, household sprays, shampoo, toilet soaps and medicine (Republic of South African Directorate of Plant Production 2009). It is an excellent fixative material and contributes a strong, fresh odor, which blends well with various other EO odours and serves to mask the unpleasant smells of certain other ingredients (Joy *et al*. 2001). Moreover, rosemary EO is known to have antimicrobial activity, carminative and mildly irritant (Directorate of Plant Production 2009). It is also used in formulations of compounded EOs for flavoring meat, sauces, condiment and distilled water obtained from flowers and is used as an eyewash (Joy *et al*. 2001).

The production of herbage biomass and EOs depends upon not only the metabolic state and present developmental differentiation program of the synthesizing tissue, but also is highly integrated with the physiology of the whole plant (Khorshidi *et al*. 2009). Plant spacing and age of the plant are important factors in determining the microenvironment and physiology of the plant in a rosemary field. The optimization of these factors can lead to a higher yield of the crop by favorably affecting the absorption of nutrients and exposure of the plant to light. According to Zewdinesh (2010), Beemnet *et al*. (2011) and Solomon and Beemnet (2011), row spacing and harvesting age significantly affected herbage biomass yield and EO yield in artemisia (*Artemisia annua* L.), peppermint (*Mentha piperiata* L.) and spearmint (*Mentha spicata* L.), respectively. Likewise the effect of spacing on agronomic and chemical traits were studied for fennel (*Foeniculum vulgare* Mill Var. Soroksary) (Masood *et al*. 2004; Khorshidi *et al*. 2009), coriander (*Coriandrum sativum* L.) (Akbariani *et al*. 2006) sweet basil (*Ocimum basilicum* L.) (Arabasi and Bayran 2004) and rosemary (*Rosmarinus officinalis* L.) (Mishra *et al*. 2009).

Despite the diverse uses of this crop, and despite the influence of harvesting age and plant spacing on yield and quality of this plant, no research have been undertaken so far to optimize cultural requirements in Ethiopia. This lack of information is the major hindrance allowing benefit from the plant. The present study was thus designed to determine the effect of spacing and harvesting age on agronomic and chemical traits of this aromatic plant.

MATERIALS AND METHODS

This investigation was carried out from January, 2010 to December, 2010 at the Wondo Genet Agricultural Research Center experimental site. The experimental site is located at 7° 192' N latitude and 38° 382' E longitude at an altitude of 1780 m.a.s.l. The site

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Table 1 Mean squares of agronomic and chemical traits of rosemary as affected by harvesting age and spacing.

Source of variation	Df	PH	BN	FLSR	DLSR	FLWP	FLYH	DLWP	DLYH	EOC	EOY
Replication (RP)	2	7.887778	0.05	0.00	0.04	0.02	1.74	0.01	1.33	0.06	1594.05
Harvesting age (HG)	2	687.74***	0.38 ns	0.48 ns	0.93**	0.18 ns	15.18 ns	0.10***	16.84***	0.88***	17939.89***
Spacing (SP)	3	69.69**	0.42 ns	0.01 ns	0.02 ns	0.87***	451.49***	0.07***	45.27***	0.14 ns	23987.12***
HG × SP	6	11.66 ns	0.23 ns	0.14 ns	0.05 ns	0.13 ns	47.76**	0.02 ns	4.57*	0.22*	2642.65*
Error	22	15.17	0.23	0.16	0.12	0.09	11.79	0.01	1.43	0.07	764.13

*** = Significant at $P < 0.001$; ** = Significant at $P < 0.01$; * = Significant at $P < 0.05$; ns = Non significant at $P < 0.05$, PH = plat height, BN = branch number, FLSR = fresh leaf to stem ration, DLSR = dry leaf to stem ratio, FLWP = fresh leaf weight/plant, FLYH = fresh leaf yield/ha, DLWP = dry leaf weight/plant, DLYH = dry leaf yield/ha, EOC = essential oil content, EOY = essential oil yield

Table 2 Performance of agronomic and chemical traits of rosemary as affected by harvesting age and spacing.

Treatments	Plant height (cm)	Number of branches/plant	Fresh leaf weight/plant (kg)	Fresh leaf to stem ratio	Dry leaf to stem ratio	Dry leaf weight/plant (kg)
Harvesting age (Months after transplanting)						
9	87.550 c	3.2167	1.4611	2.2162	1.7071 a	0.39543 c
10	97.400 b	2.8667	1.6055	2.0155	1.4150 b	0.49561 b
11	102.433 a	3.1000	1.7042	1.8166	1.1499 b	0.57900 a
LSD 0.05	3.2974	ns	ns	ns	0.2888	0.0794
Spacing (cm)						
60 × 60	98.978 a	3.2222	1.2315 b	1.9728	1.4470	0.37489 c
60 × 90	97.200 ab	2.7556	1.4315 b	2.0629	1.3711	0.46666 b
90 × 90	94.044 bc	3.2000	1.8092 a	2.0349	1.4640	0.55438 ab
90 × 120	92.956 c	3.0667	1.8889 a	1.9938	1.4140	0.56414 a
LSD0.05	3.8075	ns	0.2985	ns	ns	0.0917
CV %	4.07	15.69	19.20	19.62	23.95	19.15

Means followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to least significant difference (LSD) test; ns = Non significant at $P < 0.05$

receives a mean annual rainfall of 1128 mm with minimum and maximum temperatures of 11.47 and 26.51°C, respectively. The soil textural class of the experimental site is sandy loam with a pH of 7.2 (Abayneh *et al.* 2006). The experiment consisted of three levels of harvesting ages (9, 10 and 11 months after transplanting (MAT)) and four levels of spacing (60 × 60 cm, 60 × 90 cm, 90 × 90 cm and 90 × 120 cm) in a randomized complete block design with three replications. A spacing of 2 and 1.5 m were maintained between replications and plots, respectively.

Cuttings were taken from a one-year-old disease-free mother plant and planted in a polyethylene tube. All the necessary cultural practices were carried out when seedlings were raised in a nursery. Uniformly grown seedlings were selected, hardened and transplanted to the experimental fields after 60 days of planting in polyethylene tubes. All cultural practices were uniform for all plots of the main experimental field.

Data collection for the field experiment was carried out by taking 10 random samples from central rows of each plot. Data on plant height, number of branches/plant, fresh leaf yield/plant, fresh leaf yield/ha, dry leaf yield/plant, dry leaf yield/ha, EO content (%) and EO yield/ha were recorded and analyzed. EO content was determined from 500 g of fresh leaves of composite samples using hydro-distillation in a Clevenger apparatus according to Guenther (1972). All data were analyzed using SAS PROC GLM (2002) version 9 computer software and significant means were compared by list significant difference (LSD) at the 0.05 probability level.

RESULTS AND DISCUSSION

Effects of harvesting age on agronomic and chemical traits of rosemary

There was a very highly significant ($P < 0.001$) influence of harvesting age on plant height and dry leaf weight/plant and a highly significant ($P < 0.01$) influence on the dry leaf to stem ratio. Harvesting age did not influence branch number, fresh leaf weight/plant, fresh leaf to stem ratio and fresh leaf yield/ha (**Table 1**).

Plant height and dry leaf weight/plant increased from 87.55 to 102.45 cm and 0.39 to 0.58 kg, respectively with increasing age from 9 to 11 MAT (**Table 2**). This result agreed with the report of Belay (2007) and Zewdinesh (2010) that showed an increment of plant height with increasing age in *Artemisia*. An increment of plant height with age was also reported by Mastiholi (2008) in *Coleus forskohlii* BRIQ. An increase of dry leaf weight/plant with increasing age indicated the accumulation of more dry matter over time. The result reported by Zheljazkov and Cerven (2009) shows higher biomass yield of peppermint at the flowering stage than at the bud formation stage, also supporting our finding.

The dry leaf to stem ratio was maximum (1.71) at 9 MAT and declined with increasing age and reached a minimum value of 1.15 at 11 MAT (**Table 2**). The decrease in dry leaf to stem ratio when harvesting age was prolonged from 9 to 11 MAT was 32.75%. In agreement with this finding, a decreasing trend of leaf to stem ratio with increasing age was reported by Solomon and Beemnet (2011) in Japanese mint and by Beemnet *et al.* (2011) in peppermint. As indicated above, dry leaf weight increased with increasing age but the ratio of dry leaf to stem decreased with age. This indicated a higher proportion of dry stem increment than dry leaf at a later age.

Effects of spacing on agronomic and chemical traits of rosemary

The effect of spacing was not significant ($P > 0.05$) for branch number/plant, fresh leaf to stem ratio, dry leaf to stem ratio and EO content. However, spacing influence very highly significantly ($P < 0.001$) fresh leaf weight/plant and dry leaf weight/plant, and highly significantly ($P < 0.01$) influenced plant height (**Table 1**). Plant height was minimum (92.96 cm) at a wider spacing, increasing to a maximum value of 98.98 cm with decreasing plant spacing (**Table 2**). An increase in plant height with decreasing plant spacing was also reported by Simon *et al*. (1990) in artemisia, Khorshidi *et al*. (2009) in fennel, Gopisichand *et al*. (2006) in *Curcuma aromatic*, Hussein *et al*. (2006) in *Dracocephalum moldavica*, Mastiholi (2008) in *Coleus forskohlii* BRIQ and by Ghosh and Pal (2008) in *Tagetes erecta*. At a higher density, there is greater inter plant competition for available light. As a result, plants responded by diverting their resources to vertical growth to capture the available light.

Fresh leaf weight/plant and dry leaf weight/plant ranged from 1.23 to 1.89 kg and 0.37 to 0.56 kg, respectively when plant spacing was increased from 60 × 60 cm to 90 × 120 cm (**Table 2**). This increment was 53.66 and 51.35% for fresh leaf weight/plant and dry leaf weight/plant, respectively. Maximum herbage yield/plant at wider spacing was

Table 3 fresh leaf yield and dry leaf yield of rosemary as affected by harvesting age and spacing interaction.

	Fresh leaf yield (t/ha)			Dry leaf yield (t/ha)		
	9 months after transplanting	10 months after transplanting	11 months after transplanting	9 months after transplanting	10 months after transplanting	11 months after transplanting
60 × 60	38.43 a	34.88 ab	29.32 bc	10.85 a	10.37 ab	10.02 ab
60 × 90	21.91 de	28.50 c	29.11 bc	5.99 de	9.38 ab	10.55 ab
90 × 90	18.86 e	21.95 de	26.20 cd	5.09 de	6.72 cd	8.73 bc
90 × 120	16.20 e	17.13 e	19.14 0 e	4.22 e	5.17 de	6.28 d
CV %		13.67			15.37	

Means followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to least significant difference (LSD) test

Table 4 Essential oil content and essential oil yield of rosemary as affected by harvesting age and spacing interaction.

	Essential oil content (%)			Essential oil yield (t/ha)		
	9 months after transplanting	10 months after transplanting	11 months after transplanting	9 months after transplanting	10 months after transplanting	11 months after transplanting
60 × 60 cm	1.93 cd	2.64 ab	2.27 bc	207.79 bc	271.43 a	227.49abc
60 × 90 cm	2.07 cd	2.37 abc	2.29 bc	124.51 ef	217.20 bc	241.64ab
90 × 90 cm	2.23 bc	2.59 ab	2.54 ab	114.03 ef	182.75 cd	222.07bc
90 × 120 cm	2.07 cd	2.77 a	1.63 d	90.20 f	142.65 de	102.44ef
CV %		11.73				15.47

Means followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to least significant difference (LSD) test

reported by Mishra *et al*. (2009) for the same crop. The finding was also consistent with the data reported by Simon *et al*. (1990) and Zewdinesh (2010) in artemisia, and by Hussein *et al*. (2006) in *Dracocephalum moldavica*. At a wider spacing, there is less inter-plant competition for available resources. This allowed plants to spread and grow and may be a possible reason for higher fresh and dry leaf weight/plant at wider spacing.

Interaction effects of spacing and harvesting age on agronomic and chemical traits of rosemary

Analysis of variance showed the existence of a highly significant ($P < 0.01$) influence of harvesting age and spacing interaction on fresh leaf yield/ha and a significant ($P < 0.05$) influence on dry leaf yield/ha, EO content and EO yield. Maximum fresh leaf yield/ha (38.43 t) and dry leaf yield/ha (10.85 t) were obtained for harvests made at 9 MAT with a 60×60 cm plant spacing. At this age, respective minimum value of 16.2 and 4.22 t were recorded for fresh leaf yield/ha and dry leaf/ha when rosemary was planted at 90 × 120 cm plant spacing (**Table 3**). At 10 and 11 MAT, higher fresh leaf yield/ha and dry leaf yield/ha were recorded at a narrower spacing and decreased with increasing plant spacing. Generally, maximum values were obtained with narrower spacing and decreased with increasing plant spacing at all harvesting ages for both parameters. The decrease in fresh leaf yield/ha and dry leaf yield/ha due to an increase in plant spacing from 60 × 60 cm to 90 × 120 cm was 57.85 and 61.11%, respectively. Lower herbage yield/ha at a wider spacing compared to narrow spacing was also reported by Mishra *et al*. (2009) in rosemary. The decrease in fresh and dry leaf yield/ha with increasing plant spacing was also reported by Solomon and Beemnet (2011) in Japanese mint, Beemnet *et al*. (2011) and Aflatuni (2005) in peppermint, Rao (2002) in *Pelargonium* species, and by Zewdinesh (2010) and Laughlin (1993) in artemisia. Patra *et al.* (2004) showed a reduction of herbage yield/ha with increasing plant spacing in palmarosa (*Cymbopogon martini*), also supporting our finding. According to Mishra *et al*. (2009), herbage yield/ha is lower in wider spacing due to the accommodation of the least number of plants in one hectare of land.

EO content at all tested spacings was lower at a younger age, but with increasing age, highest values were reached at 10 MAT. The highest EO content (2.77%) in this study was obtained for harvests made at 10 MAT with the wider spacing (90 × 120 cm). The EO content was decreased by 41.15% after 10 MAT to a minimum value of 1.63% at 11 MAT at the same spacing (**Table 4**). In agreement with this finding, higher EO content at a wider spacing and at an older age was reported by Zewdinesh (2010) in artemisia. Similarly, an increment of EO content at lower plant density was reported by Khorshidi *et al*. (2009) and El-Gandi *et al*. (2001) in fennel and sweet basil, respectively.

EO yield/ha ranged from 90.2 kg to 271.43 kg with minimum value at 90 × 120 cm for plants harvested at 9 MAT and a maximum value at 60 × 60 cm for plants harvested at 10 MAT (**Table 4**). Therefore, closer spacing yields higher EO/ha than wider spacing in rosemary. A similar finding was reported by Mishra *et al*. (2009) for rosemary. An increase of EO yield/ha with increasing plant density was also reported for lemongrass (*Cymbopogon citratus* Stapf) (Linares *et al*. 2005), palmarosa (Patra *et al*. 2004), peppermint (Beemnet *et al*. 2011), Japanese mint (Solomon and Beemnet 2011), artemisia (Zewdinesh 2010) and sweet basil (El-Gandi *et al*. 2001). As explained by Linares *et al*. (2005), with increasing plant density the arial part of the plant increases, which increases the yield of secondary metabolites. The increment of EO yield at a higher density was also due to higher leaf yield/ha at higher density.

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