OPEN ACCESS

Scholars International Journal of Biochemistry
Abbreviated Key Title: Sch Int J Biochem
ISSN 2616-8650 (Print) | ISSN 2617-3476 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Performance of Lemon balm (*Melissa officinalis* L.) for Morphological and Economic Traits under Different Ecologies of Ethiopia

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DOI: 10.36348/sijb.2024.v07i01.002 | **Received:** 05.11.2023 | **Accepted:** 16.12.2023 | **Published:** 27.01.2024

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Abstract

The experiment was conducted to assess the adaptability of Lemon balm in different ecologies of Ethiopia during 2017/2018 and also during the 2022/2023 cropping seasons. The RCBD design in four replications was used and two harvesting cycles were considered for evaluation of the performance of the lemon balm for agronomic and economic traits. Data on plant height, fresh leaf weight per plant, fresh stem weight per plant, fresh leaf-to-stem ratio, fresh leaf yield per hectare, and dry leaf yield per hectare were recorded and the recorded experimental data were statistically analyzed by analysis of variance (ANOVA) using SAS PROC GLM (2002) at $P < 0.05$. Differences between means were assessed using the least significance difference (LSD) test at $P < 0.05$. During the 2017/18 cropping season, ANOVA showed lemon balm performance varied significantly due to the harvest cycle and location. The first harvest cycle was superior in all traits considered for evaluation compared to the second harvest cycle. At the Debrezeit testing location there were very highly significant ($p<0.001$) results for fresh leaf weight per plant, fresh stem weight per plant, fresh leaf yield per hectare, and dry leaf yield per hectare except fresh to stem ratio. Similarly, the harvest cycle and location exerted significant variation during the 2022/2023 cropping season. The first harvest cycle was significantly higher in all traits again, while the Manche experimental site was significantly higher in dry leaf yield per hectare (2.83 tons per hectare). Therefore, the results of the current experiment showed that lemon balm can be produced in Ethiopia at various locations similar to the current testing site and we recommend the production of the existing lemon balm for similar agro-ecologies.

Keywords: Harvest cycle, herbal yield, lemon balm, location.

1. INTRODUCTION

1.1 Origin and Taxonomy of Lemon balm

Lemon balm (*Melissa officinalis* L.) is one of the important medicinal and aromatic plants that belong to the Lamiaceae (mint) family. It is native to the Mediterranean basin and cultivated in many countries globally (Bonacina *et al.,* 2017). Genus Melissa belongs to the Kingdom: Plantae, Family: Lamiaceae; and Subfamily: Nepetoideae, with numerous species. The most common species include Melissa axillaris, Melissa flava Benth, and Melissa officinalis L. (Taiwo *et al.,* 2012). These species are found in the Mediterranean region, including central Asia, western Asia, southern Europe and northern Africa. The most common species under cultivation and which has commercial value and the characteristic lemony odor is Melissa officinalis L. and it is commonly referred to as 'lemon balm' because of its lemon-like flavor and fragrance (Tucker and Baggio, 2000).

1.2 Botanical Description of Lemon balm

Lemon balm (Melissa officinalis L) is a perennial bushy and upright plant that reaches about 1 meter in height (Moradkhani *et al.,* 2010). The leaves of the lemon balm are soft, hairy, 2-8cm long, veined, and the edges either toothed or scalloped (Turhan, 2006). The stem of the plant is erect, with branches; it is quadrangular and frequently glabrous, while the leaves are ovate in shape and they are petiolated. Flowers are white or pale pink, consisting of small clusters of 4 to 12 blossoms. Lemon balm is a cross-pollinating species and has completely perfect flowers with petals (Virchea *et al.,* 2021).

1.3 Ecology

The suitable agro-ecology for lemon balm production includes mid to highland areas with well-distributed rainfall of 500-600 mm throughout the growing season, a temperature range of 15-35oc, sandy loam fertile soil with pH 4.5-7.6, and well-drained

(Mihajlov *et al.*, 2013; Moradkhani *et al.*, 2010). Lemon balm grows in full sun, but also in partial shade (Janina, 2003) and the plant is sensitive to drought and requires irrigation during the dry season.

1.4 Propagation

The possible propagation methods of the lemon balm (*Melissa officinalis* L.) include using seeds, cutting with roots, stem cuttings, layering, and micropropagation methods (Meftahizade *et al.*, 2010; Mészáros *et al.*, 1999; Saglam *et al.*, 2004). If the seeds of the lemon balm are used for production, care should be taken not to mix the true-to-type varieties. For direct field sowing seed, 200-250 grams of seed per hectare is required. However, the easiest and the best propagation method for Melissa officinalis L. is by using stem cuttings from insect and disease-free and uniform mother plants. After 45-60 days, the cut will be ready for transplanting. On the other hand, the advantages of Melissa officinalis L. seedling preparation and transplanting over direct sowing seeds on the field were reported. In their study, Kleitz *et al.*, (2008) reported that transplanting lemon balm rather than direct seeding shortened the time for first harvest by 14-97 days, allowed more harvests per year, and crop stand establishment. Besides, the authors also reported transplanting increased yields substantially over direct seeding. Lemon balm seedling preparation from cuttings can be done by using various growing media. For instance, vermicompost + soil + FYM in ratio of 1:1:1 had recorded highest survival of cutting (85 %), number of branches (6), plant height (36 cm), number of leaves/plant (38), fresh weight/plant (1.95 g), dry weight/plant (0.98 g), number of root/plant (6) and root length (11.2 cm) (Verma, 2018).

1.5 Lemon Balm Production Management

Lemon balm production requires appropriate agronomic practices such as quality planting material, site selection, land preparation, planting time, spacing, fertilizer, pest control, and harvesting (Chizzola *et al.*, 2018; Renata *et al.*, 2023). Plant density, way of harvesting, and environmental conditions such as light, temperature, and precipitation determine the yield and quality (Nematian *et al.*, 2014; Saki *et al.*, 2019). Healthy and vigorous seedlings were planted at a spacing of 60cm*40cm* between rows and plants respectively, which resulted in 41,666 seedlings being recommended (Massoud, *et al.*, 2012). The authors indicated that wider spacing resulted in higher herbal and essential oil yield. This might be due to less competition in the case of wider spacing as compared to narrow spacing. But, their findings showed no significant difference in essential oil content. However, spacing can vary depending on agroecologies and agronomic practices and a single recommendation may not work for all places. For instance, Renata *et al.*, (2023) recommended a spacing of 40cm*40cm for lemon balm growth and herbal and essential oil yield.

For direct field sowing, a hectare of land requires 150-200 grams of lemon balm seed. Organic fertilizer for lemon balm production is preferable as compared to production using inorganic fertilizer. Hence, fertilizer type, rate and time of application affect the yield and quality of lemon balm (Abbaszadeh *et al.*, 209). According to the authors, the highest tiller number (32.6 tillers per plant), essential oil percentage (0.2577%), and essential oil content (16.05 kg per ha) were obtained under the application of 60 kg N per hectare. Proper harvesting hours and way of harvesting (twice or one harvesting) and harvesting stage exert lemon balm yield. Scholars reported that harvesting at the flower bud formation phase and flowering stages were found to be the best stages to harvest the plant to obtain the highest lemon balm herbal and essential oil yield (Németh-Zámboriné *et al.*, 2019).

1.6 Lemon Balm Economic Parts and Their Uses

The most economical part of lemon balm is leaf and young twigs. The herbal yield is utilized in the form of fresh, dry, and essential oils. Both herbal and essential oils of lemon balm are used in various sectors such as medicine, food, perfume, and the cosmetic industry. Scholars showed that lemon balm is used traditionally as medicine because of its memory-enhancing properties, to treat sleep disorders, and as a mild sedative, spasmolytic, and antibacterial agent (Bagdat and Cosge, 2005; Pineau *et al.*, 2016). Moreover, Blumenthal *et al.*, (2000) also reported that lemon balm has been traditionally used for different medical purposes as a tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedative-hypnotic strengthening the memory, and relief of stress-induced headache.

The essential oil of lemon balm has pharmaceutical uses because of its antimicrobial properties. According to study reports, the essential oils of lemon balm are used as an anti-tumoral agent with the potential for cancer remedy or prevention and also indicated as being used as an anti-virus agent and contain antiherpes simplex virus type 2 (HSV-2) substances (Turhan, 2006), antidiabetic (Chung *et al.*, 2010). On the other hand, lemon balm has many culinary uses as it can be used to flavor many different types of dishes, from beverages to appetizers, main courses, and desserts (Tramte, 2007).

Globally, lemon balm is cultivated for its herbal and essential oil products. However, in Ethiopia, lemon balm is a new plant for a few commercial growers that grow it for export purposes on their farms. Lemon balm is one of the high-value crops that can contribute to the social and economic sectors of the country. Lemon balm production expands livelihood opportunities concerning income generation for smallholder growers, investors, and the country in general. Lemon balm can be one of the important medicinal plants for the pharmaceutical industry of the country. No registered/released variety of lemon balm is available in Ethiopia. Moreover, the

production possibility for lemon balm was not studied before this national variety trial initiated at Wondogenet agricultural research center and conducted at various regional states in the country. In addition, enhancing lemon balm production and safe utilization will contribute to health benefits, and social and economic benefits, particularly for the low-income community. Hence, the objective of the current work is to assess the adaptability of the lemon balm in Ethiopia for agronomic and economic traits.

2. MATERIAL AND METHODS

2.1 Site Description

The study was conducted in different regional positions of Ethiopia during the 2017/18 and also during the 2022/23 cropping season. During the 2017/18 cropping season, the trial was conducted at Wondo Genet, Chencha, Debrezeit, and Holeta, and the testing locations as described below (Table 1).

Table 1: Site description summary during 2017/18 cropping season

Location	Altitude (mal)	Rainfall (mm)	Temperature(ºC)		Soil type		Latitude	Longitude
			Min.	Max.	Type	pH		
Wondo Genet	1876	1000	12	26	Sandy loam	6.4	07°19' N	38°38' E
Chencha	2500	1172	13	22	Clay loam	-	-	-
Holeta	2400	1114	6	22	Clay loam	-	09°00' N	38°30' E
Debrezeit	1900	851	8	28	Clay loam	-	08°44' N	38°58' E

During 202/23 cropping season, the study was conducted at five (Wondo Genet, Wonsho, and Manche, Anshabeso and Berehan kitkita) locations described below (Table 2).

Table 2: Site description summary during the 2022/23 cropping season

Location	Altitude (mal)	Rainfall (mm)	Temperature (ºC)		Soil type		Latitude	Longitude
			Min.	Max.	Type	pH		
Wondo Genet	1876	1000	12	26	Sandy loam	6.4	07°19' N	38°38' E
Wonsho	1964	1245	16	27	Clay loam	-	06°44' N	38°29' E
Manche	1839	1314	18	27	Sandy loam	-	06°41' N	38°27' E
Anshabeso	2341	1299	12	26	Clay loam	-	07°55' N	38°13' E
Berehan Kitikita	2020	1406	16	26	Clay loam	-	07°47' N	039°05' E

Initially, planting material of the Lemon balm for the study was collected from Green Path P.L.C Ethiopia and propagated using stem cuttings. The cutting was allowed to have 4-6 nodes during preparation and stayed for 80-85 days in the nursery site of the Wondo Genet Agricultural Research Center before transplanting on the main experimental plots. The seedlings were raised using polyethylene pots. No fertilizer and pesticides were applied at the nursery. Appropriate nursery management practices such as watering, guarding, and weeding were applied during seedling-raising undertaking. Planting on the main field was done in a 3.6mx 2.4m plot area at the commencement of the main rainy season. The trial at all locations was conducted using RCBD design in four replications. A spacing of 60cm * 60 cm was used for the experimental sites. The distance between blocks was 2 meter and the distance between plots was 1.5m. During the trial data on plant height, fresh leaf weight per plant, fresh stem weight per plant, fresh leaf-to-stem ratio, fresh leaf yield per hectare, and dry leaf yield per hectare were recorded carefully.

2.2 Data Analysis

The recorded experimental data were statistically analyzed by the analysis of variance (ANOVA) using SAS PROC GLM (2002) at $P < 0.05$ and the differences between means were assessed using the least significance difference (LSD) test at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Experimental results for the 2017/2018 cropping season

Mean squares of the combined analysis of growth and yield traits of lemon balm during the 2017/18 cropping season at the four testing locations are presented in Table 3 for two harvest cycles. The performances of lemon balm were found statistically different ($P < 0.05$) in plant height, fresh leaf weight per plant, fresh stem weight per plant, fresh leaf-to-stem ratio, fresh leaf yield per hectare, and dry leaf yield per hectare. Testing location exerted a very highly significant ($P < 0.001$) influence on plant height, fresh leaf weight/plant, fresh stem weight/plant, fresh leaf to stem ratio, fresh leaf yield per hectare, and dry leaf yield per hectare. The differences observed at different locations might be due to variations in environments. Besides, the harvest cycle significantly influenced plant height, fresh leaf weight/plant, fresh stem weight/plant, fresh leaf to stem ratio, fresh leaf yield per hectare, and dry leaf yield per hectare which were also significantly influenced by the interaction effects of harvest cycle and location (Table 3). The variations might be due to the difference in environmental variability as there were

more rainy months between transplanting and the first cycle harvest than the periods between the first harvest and the second harvest.

Table 3: Mean squares from combined analysis of variance for agronomic characters and lemon balm yield tested over four locations and two harvesting cycles during 2017/18 cropping season

Source of variation	DF	PH	FLWPP	FSTWPP	FLTSR	FLYPH	DLYPH
Replication	3	8.6^{ns}	295.51^{ns}	10.29^{ns}	0.06^{ns}	0.23^{ns}	0.05^{ns}
Harvest Cycle (HC)	1	1057.42***	148396.71***	4711.05***	67.16***	114.53***	4.98***
Location	3	381.51***	48442.09***	3084.28***	107.13***	37.40***	2.12***
HC*location	3	472.14***	19753.06***	4751.37***	49.10***	15.24***	0.74***
Error	21	3.89	1014.20	50.29	1.88	0.78	0.04
$LSD_{0.05}$		1.97	31.85	7.09	1.37	0.88	0.21
CV (%)		7.27	12.81	21.07	20.75	12.81	14.32

*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. PH=plant height, FLWPP=Fresh leaf weight per plant, FSWPP=fresh stem weight per plant, FSTSR=Fresh leaf to stem ratio, FLYPH=Fresh leaf yield per hectare, DLYPH=Dry leaf yield per hectare, ns=non significance, ***=very highly significance.*

The higher significant values of plant height, fresh leaf weight per plant, fresh stem weight per plant, fresh leaf to stem ratio, fresh leaf yield per hectare, and dry leaf yield per hectare were obtained from harvesting cycle I (Table 4).

Table 4: Mean performances of lemon balm during harvest cycle 1 and harvest cycle 2 over the testing locations tested during the 2017/18 cropping season

Harvest cycles	PH(cm)	FLWPP(g)	FSTWPP(g)	FLTSR	FLYPH(t)	DLYPH(t)
Cycle I	32.87	316.77	45.79	8.05	8.80	1.85
Cycle II	21.37	180.57	21.53	5.16	5.02	1.06
$LSD_{0.05}$	1.45	23.41	5.21	1.00	0.65	0.15
CV(%)	7.27	12.81	21.07	20.75	12.81	14.32

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. PH=plant height, FLWPP=Fresh leaf weight per plant, FSWPP=fresh stem weight per plant, FSTSR=Fresh leaf to stem ratio, FLYPH=Fresh leaf yield per hectare, DLYPH=Dry leaf yield per hectare.

Very highly significant (p<0.001) plant height was observed at Debrezeit (35.35cm) followed by Chencha (28.65cm) testing location (Table 5). At the Debrezeit testing location, there were also very highly significant (p<0.001) results for fresh leaf weight per plant, fresh stem weight per plant, fresh leaf yield per hectare, and dry leaf yield per hectare except fresh to stem ratio. The least significant fresh leaf yield per hectare (4.73 tons) and dry leaf yield per hectare (0.97 tons) were obtained at Wondo Genet. Hence, testing location effects might be due to the variability among the locations in terms of soil and weather conditions.

Table 5: Mean performances of lemon balm for its traits tested at Debrezeit, Holeta, Chencha and Wondo Genet during the 2017/18 cropping season

Location	PH(cm)	FLWPP(g)	FSTWPP(g)	FLTSR	FLYPH(t)	DLYPH(t)
Debrezeit	35.35	356.11	60.14	3.33	8.89	2.18
Holeta	25.79	238.4	12.98	11.80	6.62	1.34
Chencha	28.65	230.04	28.42	6.22	6.39	1.33
Wondo Genet	18.68	170.12	33.11	5.07	4.73	0.97
LSD 0.05	2.05	33.11	7.37	1.42	0.92	0.22
CV (%)	7.27	12.81	21.07	20.75	12.81	14.32

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. PH=plant height, FLWPP=Fresh leaf weight per plant, FSWPP=fresh stem weight per plant, FSTSR=Fresh leaf to stem ratio, FLYPH=Fresh leaf yield per hectare, DLYPH=Dry leaf yield per hectare

3.2 Experimental results for the 2022/2023 cropping season

Mean squares of the combined analysis of growth and yield traits of lemon balm during the 2017/18 cropping season trial at the five testing locations are presented below (Table 6) for two harvest cycles. Similar to the results of the first-year data, the performances of lemon balm during the second-year trial were found statistically different (P < 0.05) in plant height, fresh leaf weight per plant, fresh stem weight per plant, fresh leaf

to stem ratio, fresh leaf yield per hectare, dry leaf yield per hectare. Both the harvest cycle and testing location and their interaction exerted very highly significant ($P < 0.001$) influence on plant height, fresh leaf weight/plant, fresh stem weight/plant, fresh leaf to stem ratio, fresh leaf yield per hectare, and dry leaf yield per hectare. The harvest cycle significantly influenced plant height, fresh leaf weight/plant, fresh stem weight/plant, fresh leaf to stem ratio, fresh leaf yield per hectare, and dry leaf yield per hectare (Table 6).

Table 6: Mean squares from combined analysis of variance for agronomic characters and lemon balm yield tested over five locations and two harvesting cycles during the 2022/23 cropping season

Source of variation	DF	PH	FLWPP	FSTWPP	FLTSR	FLYPH	DLYPH
Replication	3	10.7^{ns}	321.41^{ns}	11.20^{ns}	0.04^{ns}	0.30^{ns}	0.04^{ns}
Harvest Cycle (HC)	1	1204.23***	128218.25***	3978.40***	56.88***	98.84***	4.56***
Location	4	258.45***	46216.10***	2962.55***	91.53***	38.15***	2.01***
HC*location	4	406.65***	20426.53***	4831.62***	45.28***	16.45***	0.89***
Error	27	3.14	886.32	62.68	2.75	0.86	0.03
$LSD_{0.05}$		1.08	21.64	14.04	9.21	0.62	0.42
CV (%)		20.78	18.84	22.78	26.72	23.34	28.91

*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. PH=plant height, FLWPP=Fresh leaf weight per plant, FSWPP=fresh stem weight per plant, FSTSR=Fresh leaf to stem ratio, FLYPH=Fresh leaf yield per hectare, DLYPH=Dry leaf yield per hectare, ns=non significance, ***=very highly significance.*

At the first harvest cycle, there were higher significant values of plant height, fresh leaf weight per plant, fresh stem weight per plant, fresh leaf to stem ratio, fresh leaf yield per hectare, and dry leaf yield per hectare as compared to the second harvest cycle (Table 7).

Table 7: Mean performances of lemon balm during harvest cycle 1 and harvest cycle 2 over the testing locations tested during the 2022/23

Harvest cycles	PH(cm)	FLWPP(g)	FSTWPP(g)	FLTSR	FLYPH(t)	DLYPH(t)
H/Cycle I	28.85	286.22	40.65	10.00	6.88	1.46
H/Cycle II	18.21	160.45	18.33	6.21	5.84	0.98
$LSD_{0.05}$	2.64	60.44	12.51	3.51	0.94	0.38
CV(%)	20.78	18.84	22.78	26.72	23.34	28.91

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. PH=plant height, FLWPP=Fresh leaf weight per plant, FSWPP=fresh stem weight per plant, FSTSR=Fresh leaf to stem ratio, FLYPH=Fresh leaf yield per hectare, DLYPH=Dry leaf yield per hectare, H/cycle=harvest cycle.

For all traits evaluated, very highly significant ($p<0.001$) results were observed at the Manche testing site followed by Wondo Genet and Wonsho sites, respectively (Table 8). The least significant plant height, fresh stem weight per plant, fresh stem-to-stem ratio, fresh leaf yield per hectare, and dry leaf yield per hectare were obtained from both Berehan Kitikita and Anshabeso sites.

Table 8: Mean performances of lemon balm for its traits tested at Wondo Genet, Wonsho, Manche, Anshabeso and Berehan Kitkita sites

Location	PH(cm)	FLWPP(g)	FSTWPP(g)	FLTSR	FLYPH(t)	DLYPH(t)
Wondo Genet	28.26	316.44	40.42	8.34	8.25	1.56
Wonsho	26.84	325.41	23.87	8.54	8.52	1.80
Manche	32.60	420.04	52.40	10.24	10.20	2.83
Anshabeso	18.24	289.84	27.45	6.24	6.92	0.83
Berehan Kitkita	18.20	188.11	18.54	5.07	6.26	0.67
LSD 0.05	2.04	53.45	8.02	1.01	0.84	0.42
CV (%)	20.78	18.84	22.78	26.72	23.34	28.91

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. PH=plant height, FLWPP=Fresh leaf weight per plant, FSWPP=fresh stem weight per plant, FSTSR=Fresh leaf to stem ratio, FLYPH=Fresh leaf yield per hectare, DLYPH=Dry leaf yield per hectare

Therefore, in spite of the variations in lemon balm growth and yield performance at different harvest cycles and also at different testing locations, lemon balm (*Melissa officinalis* L.) can be grown and give considerable yield in Ethiopia. For instance, the higher dry leaf yield per hectare obtained during the present trial

was similar and comparable with the yield obtained in Mexico (Kleitz *et al*., 2008).

4. SUMMARY AND CONCLUSION

The current experimental findings showed that lemon balm can be grown for herbal yield in different agro-ecologies of Ethiopia. Lemon balm can be harvested twice per year and the yields obtained from the experiment were comparable with that of other countries. Lemon balm has wide adaptability; hence, it is possible to use the existing lemon balm cultivar for the production of both fresh and/or dried leaves as spice and medicinal leaves in Ethiopia.

Acknowledgements

We acknowledge the Ethiopian Institute of Agricultural Research for the financial support and Wondo Genet agricultural research center for facilitation and logistic support during the field work.

Conflict of Interest: The authors declare that they have no competing interests

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