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Assessment of Relationship between Effective Traits on Yield and Compounds of Essential Oil and Morphological Traits of Lemon Balm (*Melissa officinalis L.)* Accessions Using Path Analysis and Canonical Correlation

Behzad Talle[1], Farokh Darvish[1], Abdollah Mohammadi[2]*, Bohloul Abbaszadeh[3], Mahyar Rohami[4]

[1]Department of Plant Breeding, Research and Science Branch, Islamic Azad University, Tehran, Iran
[2]Department of plant breeding, Karaj Branch, Islamic Azad University, Karaj, Iran
[3]Research institute of forests and rangelands, Karaj, Iran
[4]Young Researchers Club, Science and Research Branch, Islamic Azad University, Tehran, Iran

ABSTRACT

In order to assess the relationship between the effective traits on yield and compounds of essential oil and morphological traits in six lemon balm accessions which gathered from different provinces of Iran, an experiment was conducted in field conditions. For this study, a Randomized Complete Block Design (RCBD) with four replications was used. At the beginning of the flowering stage, shoot parts of plants were harvested and dried in shade, and essential oil of them was extracted by water distillation method; at this stage plant morphological traits was measured. Correlation between the measured traits showed that leaf length, leaf width, essential oil yield, dried leaf weight, dried stem weight and shoot yield had positive significant correlation with essential oil yield. The results from path analysis showed that shoot yield had the highest direct effect on essential oil yield in the assessed accessions. Furthermore, leaf length, leaf width and dried stem weight had the lowest direct effect on essential oil yield. Study of the first canonical equation showed the high relationship between essential oil compounds and morphologic traits which is related to correlation between Geranial and Citronellal in essential oil compounds, dried leaf width and aerial part yield among morphologic traits. These results showed Geranial and Citronellal which have medicinal characteristics can be increased indirectly by these morphological traits. By using these results, breeders can collect plants with high shoot yield, in order to select plants with high essential oil.
KEYWORDS: Lemon balm, Canonical correlation, Path analysis, essential oil, morphological traits.

INTRODUCTION

Lemon balm (*Melissa officinalis L.)* is a member of the Labiatae family. It's perennial, with four-angled and fluff less stem. Roots of this plant are cylinder shape, woody and have large number of lateral roots. Leaves are oval shape, serrated and fluffy. Flowers are complete and hermaphrodite, with yellow, violet or white colors. Fruits are achene and brown. The main parts that are used for essential oil are leaf and young shoots [4]. This plant has important roles in pharmaceutics such as remedy application in child's spasm [12], breath tightness, cold and fever [1], some fungi diseases [9], as painkiller [10], improving memory [11] and positive effects on Alzheimer therapy [3]. Anicic *et al* (2005) had shown the high antimicrobial effect of lemon balm essential oil on some bacteria. Asgari and Sefidkon (2004) reported the essential oil percentage of three accessions of this plant which gathered from Fars, Tehran and Semnan regions in Iran, 0.14, 0.25 and 0.26 percent respectively.

Path analysis method was suggested by Sewall wright (1921). This correlation can be formed by complex reciprocal effects, uncontrolled effects or unknown factors [6]. Besides, there is possibility to separate correlation coefficient to its direct and indirect components [6]. Mirzaie-Nodoushan *et al.* (2001), assessed the relationship between effective traits on producing essential oil in Menta and reported that percentage of leaf essential oil and leaf length have great direct effect on the amount of flower's essential oil. Furthermore, stem thickness and total flower's essential oil have positive and equal effects on leaf's essential oil. Also, Mirzaie-Nodoushan *et al.* (2006) in a study of effective traits in increasing essential oil in three species of Thyme (*Thymus kotschyanus ,T. pubscense and T. persicus*) by using means of path analysis method showed the number of stomata and leaf length had the highest direct effects on increasing essential oil.

Hotelling (1936) explained canonical correlation analysis method for studying the relationship between several independent variants and several dependent variants [5]. Certainly, multivariate regression is a special mode of canonical correlation; it means when there is only one independent variant, canonical correlation is reduced into multivariate regression [5].

*Corresponding Author: Abdollah Mohammadi, Department of plant breeding, Karaj Branch, Islamic Azad University, Karaj, Iran Email: a-mohamadi@kiau.ac.ir

MATERIAL AND METHOD

In order to assess the genetic diversity of six lemon balm accessions, Genotypes gathered from different areas of Iran, this research was conducted in field conditions in medicinal plants section of Islamic Azad University-Karaj branch farm. The purpose of this research was recognition of those morphological traits which affect essential oil yield, determining the direct and indirect linkage of these traits in essential oil yields and assessment of the relationship between essential oil components and morphologic traits. This experiment was conducted based on randomized complete block design with four replications. Treatments were six accessions of lemon balm gathered from Ardebil, Estahban, Alamoot, Qazvin, Karaj and Gilan. Plants had 40 cm space between each other and 50 cm between rows; therefore, there were 10 plants in a row. Seeds were planted in pot in greenhouse on February. Young seedlings were transplanted to farm on May. At the end of September and beginning of the flowering stage, morphologic traits of plants in each plot were measured. Shoots of plants were harvested at this stage. The harvested parts were dried under shade. Essential oil of dried leaves was extracted by water distillation (Clevenger) method, using 100 g of dried leaves in four replications for each accession. The percentage of essential oil for each accession was determined and after that samples were hold in glass containers with plastic lid in refrigerator. The compounds of essential oil of samples were determined by gas chromatography method (GC, GC/MS) and from each sample 12 compounds were detected. For analysis of variation and canonical correlation, SAS software was used. For mean comparison, Duncan multiple range test was used. Correlation was calculated by SPSS (version 13) software and Path coefficient was calculated by Path 2 software.

RESULTS AND DISCUSSION

Analysis of variances showed significant difference between studied accessions for stem dried weight, shoot yield, essential oil percentage, essential oil yield ($P \leq 0.01$) and leaf dried weight ($P \leq 0.05$; Table 1). Analysis of correlation between traits showed positive correlation between lateral stem and plant height, leaf length and shoot diameter and finally essential oil yield and essential oil percentage. Furthermore significant correlation observed between shoot yield with plant height, leaf length, leaf width, shoot diameter, essential oil yield, essential oil percentage, leaf dried weight and stem dried weight (Table 2).

Table1- Variance Analysis of six lemon balm (*Melissa officinalis L.*) accessions

		Mean squares (MS)											
	df	Plant height	Tiller no.	Leaf length	Leaf width	Internodes length	Shoot diameter	Lateral stems	Stem dry weight	Leaf dry weight	Shoot yield	Essential oil percentage	Essential oil yield
Treatment	5	52.97 n.s	12.10 n.s	0.99 n.s	0.33n.s	0.83n.s	64.56n.s	17.45n.s	129052.02**	1869837.28*	2932511.38*	0.0225**	71728.08**
Error	18	19.61	7.91	0.52	0.42	0.91	45.60	17.1	4259.28	565012.47	610813.91	0.00063	16795.82
(CV%)		12.68	32.09	15.73	19.53	22.10	15.43	33.55	9.1	30.64	24.62	13.61	24.61

Ns, nonsignificant; *, Significant at p≤0.05; **, Significant at p≤0.01

Table-2 correlation coefficients of assessed traits on lemon balm's accessions

	Plant height	Tiller no.	Leaf length	Leaf width	Internodes length	Shoot diameter	Essential oil yield	Essential oil percent age	Lateral stems	Dried stem weight	Dried leaf weight	Shoot yield
Plant height	1											
Tiller no.	0.577**	1										
Leaf length	0.330	0.075	1									
Leaf width	0.183	-0.064	0.854**	1								
Internodes length	0.083	-0.08	-0.108	-0.149	1							
Shoot diameter	0.765**	0.418*	0.459*	0.387	0.109	1						
Essential oil yield	0.330	0.204	0.466*	0.506*	0.002	0.342	1					
Essential oil percentage	-0.318	0.108	-0.191	-0.065	-0.069	-0.382	0.447*	1				
Lateral stems	0.075	0.397	0.505*	-0.445*	0.105	0.019	-0.203	0.15	1			
Dried stem weight	0.672**	0.321	0.421*	0.329	0.268	0.788**	0.463*	-0.414*	0.078	1		
Dried leaf weight	0.682**	0.297	0.545**	0.440*	0.241	0.814**	0.434*	-0.503*	-0.089	0.957**	1	
Shoot yield	**0.685****	**0.305**	**0.521****	**0.418***	**0.249**	**0.815****	**0.444***	**-0.486***	**-0.051**	**0.974****	**0.998****	**1**

*, Significant at p≤0.05; **, Significant at p≤0.01

Path analysis identified that shoot yield had the highest direct effect (P= 1.697) on essential oil yield (Table 3). Also leaf dried weight (- 0.847) and essential oil percentage (0.818) had the highest direct effect on essential oil yield after shoot yield. Also, shoot yield had the highest indirect effect on all traits. Leaf length (0.11), leaf width (0.175) and stem dried weight (- 0.147) had the lowest direct effect on essential oil yield (Table 3 and Figure 1).

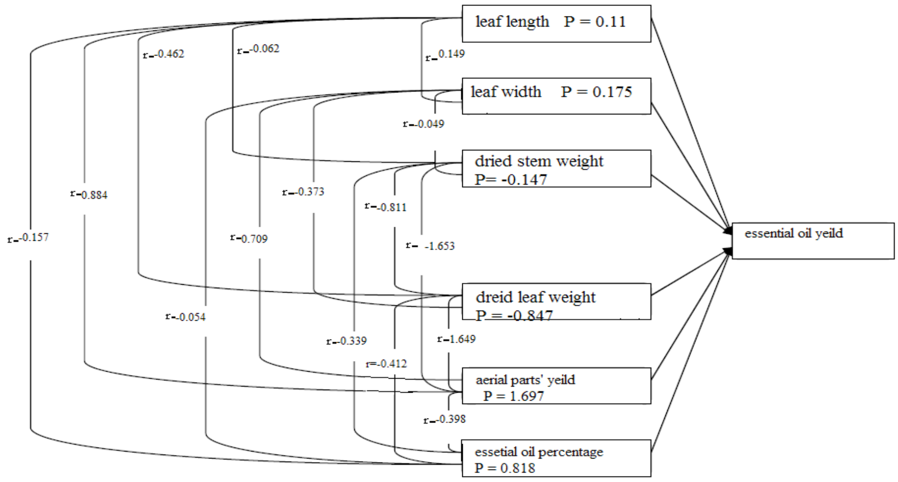

Figure 1- Path analysis diagram and manner of relationship between traits affecting essential oil yield on lemon balm (*Melissa officinalis L.)* accessions

Table-3 correlation coefficients of assessed traits on lemon balm's accessions

Traits	X1	X2	X3	X4	X5	X6	Correlation with essential oil yield
Plant height (X1)	0.110	0.149	-0.062	-0.462	0.884	-0.157	**0.466***
Leaf width (X2)	0.094	0.175	-0.049	-0.373	0.709	-0.054	**0.506***
Dried stem weight (X3)	0.046	0.057	-0.147	-0.811	1.653	-0.339	**0.463***
Dried leaf weight (X4)	0.060	0.077	-0.14	-0.847	1.649	-0.412	**0.434***
Shoot yield (X5)	0.057	0.073	-0.143	-0.845	1.697	-0.398	**0.444***
Essential oil percentage (X6)	-0.022	-0.012	0.06	0.425	-0.826	0.818	**0.447***
Residual (R) =0.418							

*, Significant at p≤0.05; **, Significant at p≤0.01

Table 4- direct and indirect effects of traits on essential oil yield

Canonical equation	Canonical correlation (rc)	Estimated standard variation	Square canonical correlation (r2)
1	0.997**	0.001	**0.993**
2	0.982*	0.007	**0.965**
3	0.959	0.016	**0.921**
4	0.952	0.019	**0.906**
5	0.799	0.075	**0.639**
6	0.742	0.093	**0.551**
7	0.685	0.11	**0.469**
8	0.515	0.153	**0.265**
9	0.389	0.177	**0.152**
10	0.289	0.191	**0.084**
11	**0.173**	**0.202**	**0.029**

*, Significant at p≤0.05; **, Significant at p≤0.01

Results showed that the first and second canonical correlations (0.997 and 0.982) were significant (Table 4). Eleven pairs of canonical correlations were resulted from canonical analyses that are shown in Tables 5 and 6.

Table 5- Correlations Between chemical values and Canonical Variables of phenotype values

Traits of Group X		U1	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11
Octane-3-ol	X1	0.3633	-0.1084	0.4479	0.2087	-0.0459	-0.3074	-0.0593	0.0528	0.1539	-0.1225	0.042
Hepten-2-one	X 2	0.4215	-0.209	0.4925	0.0757	0.0964	-0.2979	-0.1139	0.0958	0.1557	-0.0874	0.0207
3-octanol	X 3	0.675	-0.4697	0.168	-0.2064	0.0249	-0.2197	-0.0964	0.1121	0.1088	-0.0052	-0.0044
Trans rose oxide	X 4	0.5153	-0.3874	0.2001	-0.0006	-0.2004	-0.2899	0.0516	0.0683	0.1343	-0.0893	0.0447
Neo-isopulegol	X 5	0.1524	0.0478	-0.4271	0.3796	-0.3027	-0.0848	0.1686	-0.0585	0.0962	-0.1231	0.0645
Citronellal	X 6	-0.4957	0.5082	-0.054	0.3025	0.0552	0.3074	0.0893	-0.1352	-0.1182	0.0322	-0.0202
Cis-chrysanthenol	X 7	0.5427	-0.413	-0.2418	-0.0714	-0.205	-0.2491	0.0205	0.0718	0.1357	-0.0744	0.0453
Neral	X 8	0.4183	-0.5386	0.0902	-0.4572	0.0046	-0.2584	-0.1344	0.1453	0.0804	0.0109	0.0021
Methyl citronellate	X 9	-0.7029	0.3406	0.0836	-0.0214	0.1263	0.214	-0.0001	-0.0657	-0.1464	0.0761	-0.0324
Geranial	X 10	0.4205	-0.5148	0.1535	-0.3952	0.0078	-0.3001	-0.1281	0.1498	0.0981	-0.0088	0.0079
Geranial acetate	X 11	0.2628	0.4932	0.2179	-0.5367	0.0889	-0.2249	-0.154	0.1609	0.0197	0.054	-0.0229
Caryophyllene oxide	X 12	-2825	0.049	0.1724	-0.3334	0.2191	0.2068	-0.09	0.0179	-0.1543	0.148	-0.0706

Table 6- Correlations Between phenotype values and Canonical Variables of chemical values

Traits of group Y		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
Plant height	Y1	-0.0572	0.3455	-0.4053	-0.4927	-0.3096	0.5526	0.0862	0.0512	-0.2275	0.0825	**-0.0237**
Tiller no.	Y2	0.24	0.186	-0.3095	-0.094	-0.5884	0.1923	0.299	0.3945	-0.0479	0.2201	**0.3525**
Leaf length	Y3	-0.0542	0.5044	-0.5214	0.1152	0.4459	0.1974	0.2535	-0.2854	-0.0218	0.2234	**0.1538**
Leaf width	Y4	0.0779	0.443	-0.5393	0.0829	0.424	-0.1053	-0.0667	-0.3395	-0.1583	0.4053	**0.0084**
Internodes length	Y5	0.1013	0.2812	0.4839	-0.6519	0.0081	-0.2308	0.3681	-0.2074	0.0966	-0.0588	**-0.083**
Aerial part' diameter	Y6	-0.1149	0.7305	-0.4761	-0.3712	-0.1304	0.1555	-0.0727	0.1864	-0.024	-0.0673	**-0.0489**
Essential oil yield	Y7	0.5435	0.5062	-0.192	-0.2165	-0.1208	0.3305	-0.196	-0.0517	0.2728	0.352	**0.0363**
Lateral stems	Y8	0.1729	-0.0616	0.3288	-0.2204	-0.1367	-0.0617	0.0006	0.8189	-0.3203	0.1069	**0.0497**
Dried stem weight	Y9	-0.1792	0.6061	-0.1626	-0.53	0.0494	0.3034	-0.1138	0.1737	0.2223	0.3155	**0.081**
Dried leaf weight	Y10	-0.261	0.7113	-0.1882	-0.4232	0.0058	0.2842	-0.0992	-0.0148	0.1234	0.279	**0.1657**
Aerial parts yield	**Y11**	**-0.244**	**0.6925**	**-0.1837**	**-0.4512**	**0.0159**	**0.2909**	**-0.1033**	**0.0289**	**0.1472**	**0.2898**	**0.147**

Study of the first canonical equation (V_1 and U_1) with regard to the high correlation between V_1 and U_1 (r = 0.997), recognized high degree of numerical coefficients for Methylcitronellate (-0.7053) and 3-Octanol (0.6772) in U_1 equation (table 5) and essential oil yield (0.5435) in V_1 equation. This result showed that the high connection between essential oil compounds and the studied morphologic traits is the result of high correlation between essential oil compounds and essential oil trait. It means that essential oil yield have effect on 3-Octanol and Methylcitronellate which have medicinal characteristics. Thus, in order to breed plants, the high content of these two compounds and morphological traits can be used.

Study of the second pair of canonical equation (V_2 and U_2) identified high correlation (r = 0.982) between these two equations which is because of high coefficients of Neral (0.5483), Geranial (-0.524) and Citronellal (0.5174) from essential oil compounds (Table 5) and shoot diameter (0.7305), dried leaf weight (0.7113) and aerial part yield (0.6925) from morphologic traits (Table 6). Love (1968) and Miller (1969) by using the concept of the ratio of extracted variance by a variable, proposed a prediction index [5], which is shown for canonical variable of essential oil compounds in Table 7. This table shows that 21.72% of total variance of U_1 is predictable by the first canonical variable (V_1) and 15.32% of total variance of U_2 is predictable by the second canonical variable (V_2). Thus 11 extracted canonical variable from morphological traits presented 65.93% of variation of the essential oil compound canonical variables.

Table 7- Analysis of prediction index for essential oil traits

Justification of standardized variance of essential oil traits by					
Canonical variant	**Canonical variants of essential oil group**			**Canonical variants of morphologic trait group**	
	Ratio	**Additive ratio**		**Ratio**	**Additive ratio**
1	0.2172	0.2172		0.2157	**0.2157**
2	0.1532	0.3704		0.1478	**0.3636**
3	0.0788	0.4492		0.0726	**0.4362**
4	0.101	0.5502		0.0915	**0.5276**
5	0.034	0.5842		0.0217	**0.5494**
6	0.1173	0.7015		0.0646	**0.614**
7	0.0234	0.7249		0.011	**0.6249**
8	0.0409	0.7657		0.0108	**0.6358**
9	0.0995	0.8652		0.0151	**0.6509**
10	0.083	0.9482		0.0069	**0.6578**
11	**0.0486**	**0.9967**		**0.0015**	**0.6593**

CONCLUSION

The results of this study indicated that Geranial and Citronellal which can be increased indirectly by morphological traits. So in order to select plants with high essential oil, breeders can collect plants with high shoot yield.

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