Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231– 6345 (Online)
An Open Access, Online International Journal Available at www.cibtech.org/sp.ed/jls/2015/04/jls.htm
2015 Vol. 5 (S4), pp. 660-668/Otaghvari et al.
Research Article

THE MORPHOMETRICAL STUDY ON A NUMBER OF *LAMIUM* (LAMIACEAS) SPECIES IN THE NORTH OF IRAN

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ABSTRACT
There are 9 species of *Lamium* in Iran and 5 ones in the north of Iran; and this research studies the morphologies of 3 species, namely, *L. album subsp. album, L. album sub sp.crinitum L. amplexicaule,* and *L. galeobdolon*. Due to the importance of this plant in identifying and distinguish the species and subspecies and since there is no conducted morphological studies on this genus in Iran and on these species in the world so far, this research aims at investigating the morphological characteristics of this species and determining the applied value of these traits in separation of species. The results of morphological studies in dendrogram of cluster analysis by Ward method indicate that the cluster is classified into two main clusters, A and B, and the main cluster B separates all populations of *L. amplexicaule* species from the population of other species according to the main traits including the existence of basal leaf, the shape of stem leaf, the shape of inflorescence leaf, the lack of Petiole in inflorescence leaf, the length of calyx dent, corolla color, the lack of flap in the lower lobe of flower, the coverage inside the corolla (without the fluff ring) and the absence of bracts. The main cluster A is classified into two sub-clusters A-1 and A-2. The sub-cluster A-1 has separated all populations of *L.galeobdolon* species from other populations of *L.album* according to the main traits namely the base of stem leaf, the length of calyx, the length of calyx, the corolla color, the shape of bottom edge of corolla, and the lack of fluff on the anther. The sub-cluster A-2 is in turn classified into two sub-clusters (A-2,1 and A-2,2) which properly separate two subspecies, *album* and *crinitum*, according to the size of bract and length of calyx dent, and length of corolla.

Keywords: *Lamium, Lamiaceae, Morphometry, North of Iran*

INTRODUCTION
Lamium genus from Lamiaceae family (mint) approximately has 40 species of annual or perennial herbaceous plants (Flora Iranica, Vol. 76, Lamiacea family, p. 354) which are the native to Europe, Asia and North Africa. This genus is distributed particularly in Iran- Turan and Mediterranean geographical area (Yalcin & Kayal 2006). *Lamium* genus has 9 species in Iran and 5 ones in the north of Iran (Mennema, 1989; Mabberly, 1997). This research morphologically investigates three species namely *L.album subsp.album, and L.album subsp.crinitum L.amplexicaule* and *L.galeobdolon*. Since the medieval times, *Lamium* has been taken into account as a medicinal plant. Its head branches and white nettle have gently the blood purifier astringent, cholagogue, narcotic, exhilarating and healer effects, and thus this plant is consumed in treating different diseases such as simple diarrhea, bleeding, Hemoptysis, treating the skin pimples in children, anemia, tissue edema, diseases of respiratory tract and spleen, female non-disposal and gynecological infections (Medicinal plants, Dr. Ali Zargari, Vol.4, pp. 93-96).
A laboratory research is conducted on the anti-cancer effect of herbal extract from *Lamium*; the results indicate the anti-cancer effect of *Lamium album* extract (Topouzova-Hristova *et al.,* 2012). The breeding ordination of *Lamium* genus was done by Mennema in 1989 and then by Jamzad in 2012. There are a few conducted studies on the morphology of this genus throughout the world such as the study of morphology, anatomy and cytology in *L. cymbalarifolium* and *L. pisidicum* species by Canna Ozdemir and Pelin Baran in 2012 and 2013. M Ángeles Martín Mosquero and Julio Pastor y Rocío Juan (2006) studied the anatomical and cytological morphology of akene in *L. flexuosum* Ten, *L. purpureum* L.y and L *L.amplexicaule* species in the South West of Spain. Shamila *et al.,* (2015) conducted a morphological

study on the pollen in two species, *L.album* and *L.amplexicaule*. Ferhat *et al.,* (2011) conducted a morphological and anatomical study on the fluff in *L. truncatum* (Boiss). Wink *et al.,* (1996) investigated the phylogenetic relationships in some members of Lamiaceae sub-family according to the nucleotide sequence by rbcl gene. Fernandez *et al.,* (2005) studied the morphology of pollen in Lamium (Lamiaceae) in the southwestern Spain. Funda *et al.,* (2007) determined the Iridoid glycosides from *Lamium garganicum*. Funda *et al.,* (2008) determined the Iridoid glycosides from 4 *Lamium* species. Onder *et al.,* (2010) determined the antioxidant activity of different extracts from *Lamium amplexicaule* in laboratory conditions. Oznur *et al.,* (2011) studied the anatomy of petiole in taxa from Lamiaceae family *(Lamium purpureum / Var: purpureum)*. Olivia *et al.,* (2012) extracted the phenolic compounds from *Lamium album.* Since there is no conducted morphometrical study on this species in Iran and throughout the world and also due to the importance of mint medicinal plants and species of *Lamium* genus, the morphometry of *L. album, L.ampiexicaule and L.galeobdolon* species is investigated in order to identify and differentiate the species and sub-species.

MATERIALS AND METHODS

This study has utilized the samples collected from the natural habitats and samples in the Sari Payame Noor University herbarium (SPNH) and in the herbarium of Medical Plants Research Center of Iran.

Table 1: Studied populations of Lamium species

Sr. NO.	Name of the Species	Locality	Herbarium Number
1	*L.album subsp:album*	Mazandaran-Sari - Sangedeh	3892
2	*L.album subsp:album*	Mazandaran-Aghamashad-kashid area	3969
3	*L.album subsp:album*	Mazandaran-Sari -Sangedeh village	3998
4	*L.album subsp:album*	Mazandaran-Bola protected area	2700
5	*L.album subsp:album*	Mazandaran-Savadkouh- Berenjestanak	1854
6	*L.album subsp:album*	Mazandaran-Savadkouh - Berenjestanak	1853
7	*L.album subsp:album*	Mazandaran-Ghaem shahr- Vostakola	3378
8	*L.album subsp:album*	Mazandaran- Sari- Panbesarkati Village	2810
9	*L.album subsp:album*	Mazandaran-Sari -Vezmela Village	2735
10	*L.album subsp:album*	Mazandaran-Aghamashad	1908
11	L.album subsp:album	Mazandaran- Reineh- Damavand peak	1855
12	*L.album subsp:album*	Mazandaran- Amol tob nour road	2969
13	*L.album subsp:album*	Gorgan- Kord-Kuy	23096
14	*L.album subsp:album*	Gilan- Fouman- Masoule- 5km to Masouleh	55964
15	*L.album subsp:crinitum*	Mazandaran- Larigan- Rineh- khommeh	23130
16	*L.album subsp:crinitum*	Gorgan- Kord-Kuy- region of jahan-nama	23139
17	*L.album subsp:crinitum*	Gilan- Asalem-Khalkhal 12 km asalem	23142
18	*L.album subsp:crinitum*	Mazandaran- Pole- Sefid- - Bola	23128
19	*L.album subsp:crinitum*	Mazandaran- Larigan- Rineh- kuhe- damavand	23137
20	*L.album subsp:crinitum*	Mazandaran- Larigan-Gousefand- sara	23129
21	*L.album subsp:crinitum*	Mazandaran- Kelardasht Vers Abbas- abad	23132
22	*L.album subsp:crinitum*	Mazandaran- 29km.E.Gorgan Jaafar- abad	23127
23	*L.album subsp:crinitum*	Gilan- Asalem-Khalkhal 30-40 km khalkhal	23093
24	*L.galeobdolon*	Gilan- Asalem-Khalkhal 12 km asalem	23178
25	*L.galeobdolon*	Mazandaran- Wayssar	23182
26	*L.galeobdolon*	Mazandaran- Kelardasht	23181
27	*L.galeobdolon*	Gilan- Asalem-Khalkhal 30 km asalem	23180
28	*L.galeobdolon*	Gilan- Asalem-gileh- dareh	23179
29	*L.galeobdolon*	Gilan- Talesh-Assalem	23176
30	*L.galeobdolon*	Gilan- asalem of a khalkhal Shvndvl	4739
31	*L.galeobdolon*	Gilan- Asalem area Naysay8	4740
32	*L.amplexicaule var:amplexicaule*	Gilan- Deylaman	23169
33	*L.amplexicaule var:amplexicaule*	Mazandaran- Village of Chalous	23166
34	*L.amplexicaule var:amplexicaule*	Gorgan- Parke- Mellie Golestan	55953
35	*L.amplexicaule var:amplexicaule*	Gilan- Astara- Lavaudvil Lattom forest	55956
36	*L.amplexicaule var:amplexicaule*	Mazandaran- Sari- Vezmella Village	2736

After drying, the samples are put on the sheet by herbarium technique, and then the measurements are done after determining the characteristics, and the data analyzed statistically by SPSS software.

RESULTS AND DISCUSSION

Results

This study applies the multivariate statistical methods to determine the relationships between the studied species of cluster analysis by Ward method, and thus the Scree plot is drawn.

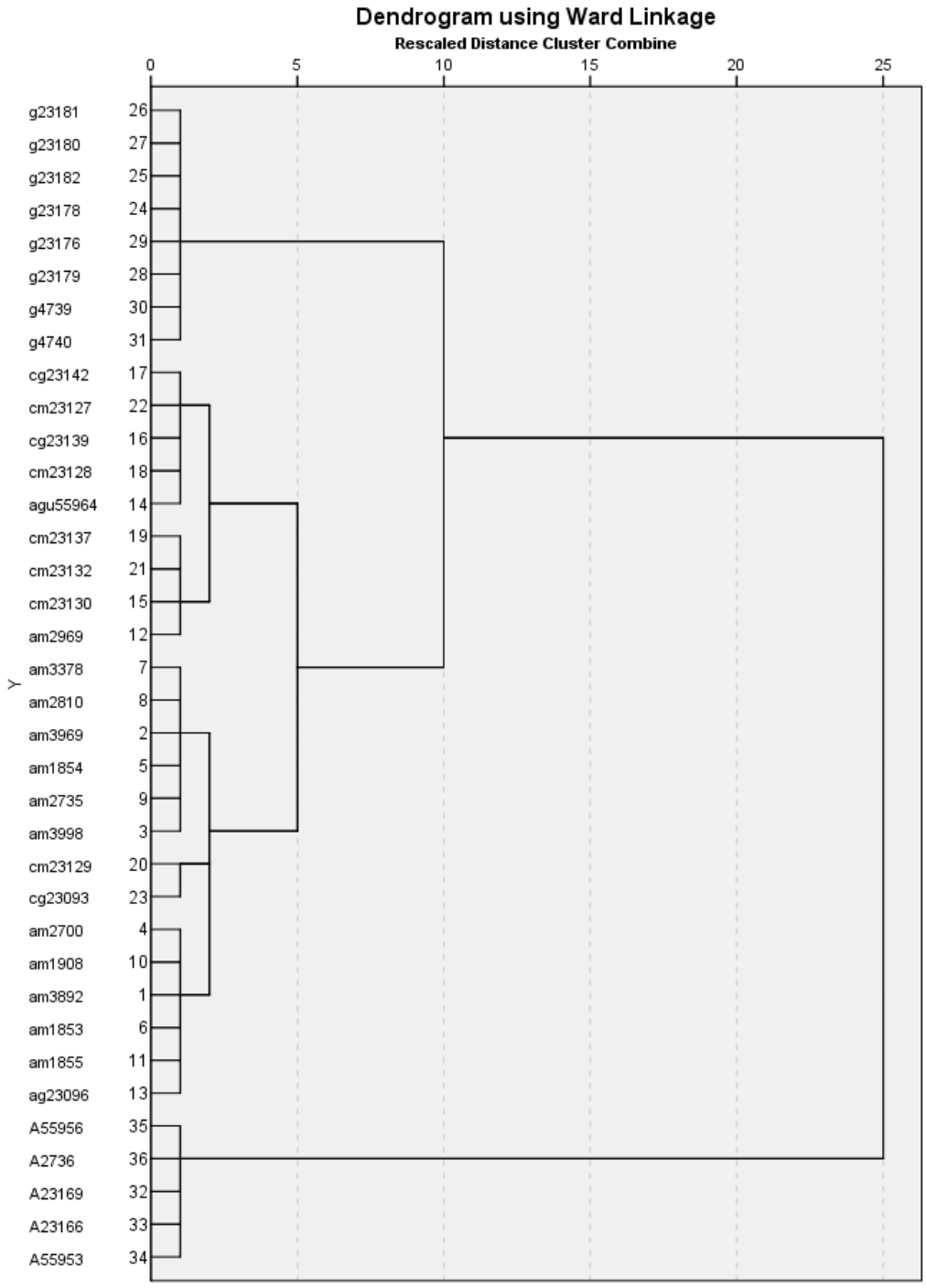

Figure 1: Ward cluster diagram

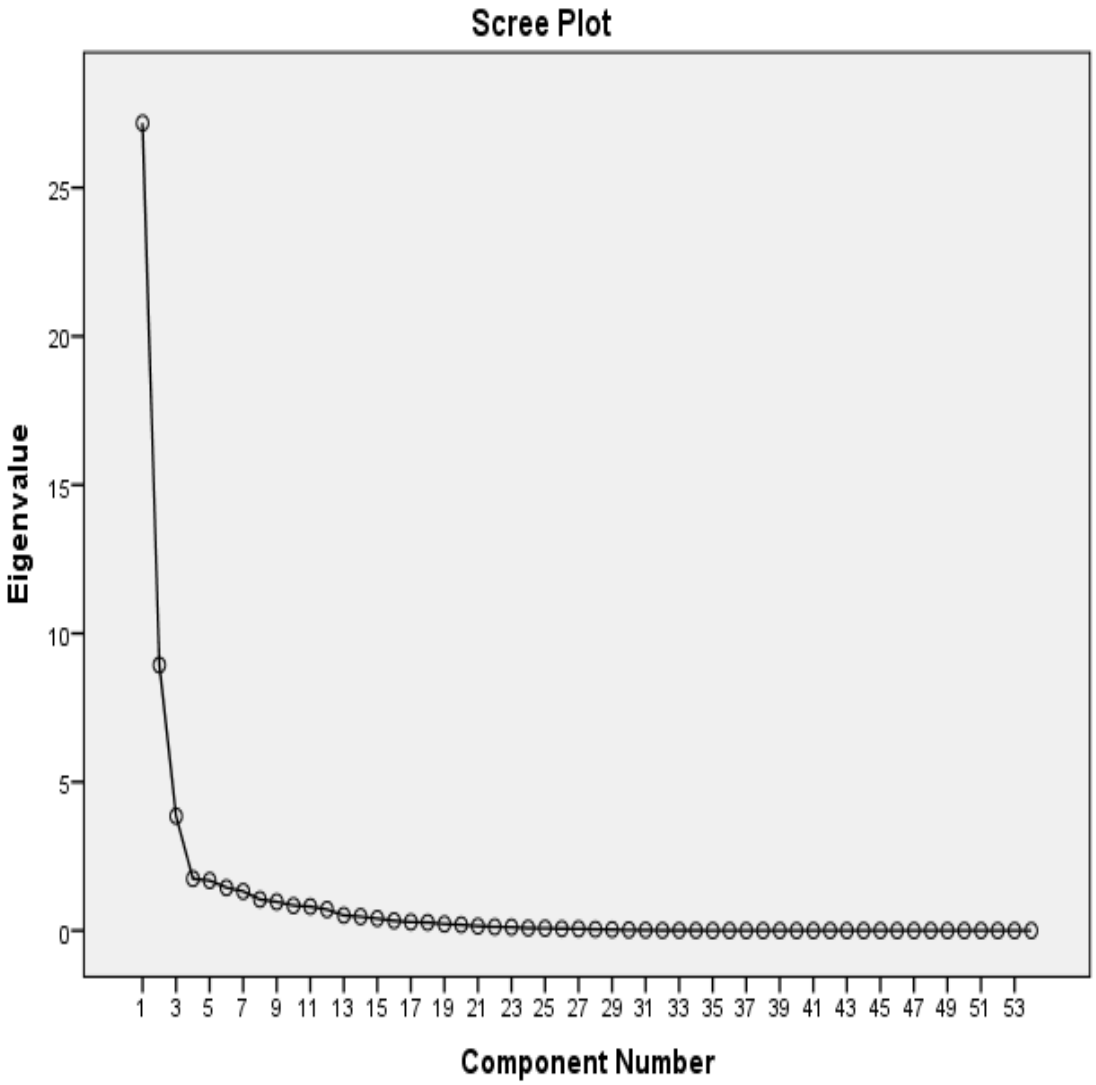

Figure 2: Scree plot

Table 2: Total Variance Explained

Component	Initial Eigen values			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	27.175	50.324	50.324	27.175	50.324	50.324	25.763	47.709	47.709
2	8.937	16.549	66.874	8.937	16.549	66.874	7.876	14.585	62.295
3	3.851	7.131	74.005	3.851	7.131	74.005	4.104	7.600	69.894
4	1.745	3.232	77.236	1.745	3.232	77.236	2.609	4.831	74.725
5	1.683	3.117	80.353	1.683	3.117	80.353	2.131	3.945	78.670
6	1.443	2.673	83.026	1.443	2.673	83.026	1.746	3.234	81.904
7	1.319	2.442	85.468	1.319	2.442	85.468	1.514	2.805	84.709
8	1.046	1.937	87.405	1.046	1.937	87.405	1.456	2.696	87.405
9	.966	1.789	89.194						
10	.840	1.556	90.751						
11	.807	1.494	92.245						
12	.708	1.311	93.555						

Table 3: Rotated Component Matrixa

	Component							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
VAR00009	.985	.124	-.019	.077	-.062	-.025	-.011	.019
VAR00031	-.985	-.124	.019	-.077	.062	.025	.011	-.019
VAR00056	-.985	-.124	.019	-.077	.062	.025	.011	-.019
VAR00081	-.985	-.124	.019	-.077	.062	.025	.011	-.019
VAR00010	.985	.124	-.019	.077	-.062	-.025	-.011	.019
VAR00013	.985	.124	-.019	.077	-.062	-.025	-.011	.019
VAR00024	-.985	-.124	.019	-.077	.062	.025	.011	-.019
VAR00049	.985	.124	-.019	.077	-.062	-.025	-.011	.019
VAR00061	.985	.124	-.019	.077	-.062	-.025	-.011	.019
VAR00066	.985	.124	-.019	.077	-.062	-.025	-.011	.019
VAR00015	.985	.124	-.019	.077	-.062	-.025	-.011	.019
VAR00016	.985	.124	-.019	.077	-.062	-.025	-.011	.019
VAR00059	-.985	-.124	.019	-.077	.062	.025	.011	-.019
VAR00014	.981	.123	-.016	.071	-.058	-.022	-.004	-.003
VAR00062	-.974	-.077	-.025	-.013	.134	-.018	.052	-.065
VAR00083	-.972	-.054	-.118	-.040	.118	-.003	.018	-.005
VAR00011	.961	.118	-.025	.088	-.052	.023	-.015	.051
VAR00012	.961	.118	-.025	.088	-.052	.023	-.015	.051
VAR00082	-.955	.107	.028	-.074	.061	.106	.121	.058
VAR00032	-.945	-.262	.045	.016	.046	.032	-.034	-.024
VAR00033	-.926	-.160	-.210	-.116	.037	-.032	-.008	-.066
VAR00029	.794	.003	-.172	.204	-.286	-.049	.156	.124
VAR00008	.788	-.032	-.259	.242	.047	-.176	-.015	-.173
VAR00034	-.736	.378	.223	-.018	.145	.117	-.104	-.055
	Component							
	1	2	3	4	5	6	7	8
VAR00004	.556	.144	.066	.451	-.148	.054	.246	.161
VAR00042	.526	-.437	.056	-.208	.094	.016	-.364	.262
VAR00007	.496	-.319	-.046	.264	-.168	-.406	-.255	-.330
VAR00075	.289	.843	.332	.088	.093	.099	.164	.030
VAR00047	.243	.829	-.103	.129	-.203	-.029	-.041	.143
VAR00045	.265	.819	-.160	-.016	-.031	-.225	.048	-.002
VAR00074	.307	-.806	-.391	-.057	-.111	-.123	-.183	-.026
VAR00043	.372	.785	.290	.094	-.067	-.033	.054	.196
VAR00073	.446	.725	-.213	.241	.182	-.099	.014	-.022
VAR00052	-.443	.718	.162	-.159	.095	.218	-.001	-.030
VAR00039	.439	.712	.033	.012	.174	.049	.287	-.019
VAR00028	.542	.656	-.041	.189	-.224	.022	.027	-.123
VAR00060	-.616	.649	.344	.021	.118	.115	.161	.016
VAR00057	-.308	-.627	-.461	-.363	.148	.010	.106	.103
VAR00058	.258	.011	.839	-.020	.176	-.018	.020	-.109
VAR00019	-.382	-.122	.755	.007	-.057	.048	.106	.355
VAR00035	.079	.394	.630	-.198	.162	.435	.075	-.048
VAR00048	.144	.437	.589	-.016	.090	.037	-.057	.103
VAR00005	-.445	.272	.585	-.328	-.014	-.029	.194	-.067
VAR00006	.008	.190	-.120	.860	.122	-.095	-.063	-.050

VAR00023	.374	.207	-.053	.573	-.156	-.046	.277	.173
VAR00030	.428	.080	-.359	.484	-.160	.252	.082	-.078
VAR00079	-.373	-.038	.086	.201	.800	.029	.185	.206
VAR00021	.365	.031	-.423	.200	-.621	.035	.322	.045
VAR00020	.385	-.317	-.391	.242	-.604	-.084	.278	.011
VAR00046	.218	.137	-.038	.001	.063	-.803	.030	.071
VAR00026	.068	.248	.477	-.293	.313	.504	.157	-.160
VAR00053	.307	.390	.030	.325	.089	.417	-.062	.113
VAR00025	.097	-.248	-.125	-.038	.014	-.012	-.778	.109
VAR00080	.188	.116	.035	.052	.094	-.082	-.127	.894

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 11 iterations.

The results of morphological studies on the dendrogram of cluster analysis by Ward method indicate that the cluster is classified into two main clusters, A and B, and the main cluster B has separated all populations of *L. amplexicaule* species from populations of other species according to the main traits, namely, the existence of basal leaf, the shape of stem leaf, the shape of inflorescence leaf, the lack of Petiole in inflorescence leaf, the length of calyx dent, corolla color, the lack of flap in the lower lobe of flower, the coverage inside the corolla (without the fluff ring) and the absence of bracts. The main cluster A is classified into two sub-clusters A-1 and A-2. The sub-cluster A-1 has separated all populations of *L.galeobdolon* species from other populations of *L.album* according to the main traits namely the base of stem leaf, the length of calyx, the length of calyx, the corolla color, the shape of bottom edge of corolla, and the lack of fluff on the anther. The sub-cluster A-2 is in turn classified into two sub-clusters (A-2, 1 and A-2,2) which properly separate two subspecies, *album* and *crinitum*, according to the size of bract and length of calyx dent, and length of corolla.

The Scree plot is also drawn based on the morphological data and it is found that 8 main factors cause the greatest differences with a total of 87.40% variance and have separated the species from each other in this plot. According to the variance table (Table 3), it is shown that the first factor has about 47.70%, the second factor 14.58% and third one 7.6% and highest portion of the total variance.

According to the rotated component matrix table (Table 4), it is found that the traits including the basal leaf, the layout of calyx vein, flap in the middle lobe, coverage inside the corolla, the plant size, distribution of fluff on anther, the fluff on anther, the shape of calyx dent, the length of calyx, the coverage of inflorescence leaf, the total length of corolla, and the distance between the bundles of flowers are the most important first component traits; and the traits including the basal leaf, the layout of calyx veins, the coverage inside the corolla, bract coverage, the length of stem leaf, the width of basal leaf, the length of inflorescence leaf, the inflorescence leaf base, the plant size, the distribution of fluff on anther, tip of calyx dent, length of calyx dent, margin of lower lobe, corolla color, stem shape, and the overall length of Corolla are the most important traits of second component; and the corolla color, the length of calyx, margin of lower lobe, petiole in inflorescence leaf, corolla shape, the existence of bract, the tip of stem leaf, margin of upper lobe, coverage of bract, petiole length in the inflorescence leaf, the plant size, distribution of fluff on anther, and the shape of calyx dent are the most effective traits in creating the variance of third component.

Conclusion

The morphological findings of *L.amplexicaule, L.galeobdolon, L,album subsp album* and *L.album subsp crinitum* species are generally consistent with the morphological description of these species in Flora Iranica (Jamzad, 2012). Some of the observed differences in numerical data can be related to the collected plant samples as well as the changes due to the seasonal conditions in each year.

Jamzad described *subsp. album L. album* species in 2012 as follows: It has the stem with the height of 20 to 80 cm, the petioles with the length of 1 to 7 cm, leaf length of 2 to 10 cm, leaf width of 1.5 to 7.5 cm,

the calyx length of 10 mm, the dents as long as the calyx or shorter, the bract as the line with the length of 2 to 3 mm, white corolla with the length of 2 to 3 cm. The obtained results of morphometrical study in the north of Iran describe this species *(L.album subsp. album)* as follows: The stem height of 16 to 55 cm, petiole in the stem leaf with the length of 0.9 to 4.2 cm, the inflorescence leaf with the length of 0.1 to 1.9 cm, the leaf lamina in stem leaf with a length of 1.4 to 7 cm and a width of 1.2 to 4.3 cm, the lamina in leaf inflorescence with a length of 2.2 to 5.9 cm and a width of 1.2 to 3.2 cm, the length of calyx from 9 to 14 mm, the length of calyx dents from 4 to 9 mm, the bract as a line with a length of 1.5 to 5 mm, the white corolla in some seasons with purple spots on the upper lobe and with a length of 1.6 to 2.8 cm.

Jamzad described *subsp. crinitum L. album* species in 2012 as follows: The stem with a height of 20 to 80 cm, petiole with a length of 1 to 7 cm, the leaf length of 2 to 10 cm, the leaf width of 1.5 to 7.5 cm, the calyx length of 15 mm, the dents longer than calyx tube, the bract as a line with a length of 4 to 6 mm, and the white corolla with a length of 2 to 3 cm. The results of morphometrical study in the north of Iran describe this species *(L.album subsp. crinitum)* as follows: The stem height of 20 to 76 cm, the petiole in stem leaf with a length of 1 to 4.6 cm, the petiole in leaf inflorescence with the length of 0.1 to 1.4 cm, the lamina in stem leaf with the length of 1.2 to 5 cm and a width of 1.1 to 4.7, the inflorescence leaf lamina with a length of 1.9 to 8 cm and a width of 1.1 to 4 cm, the calyx length of 10 to 20 mm, the calyx dent length of 4 to 11 mm, the bract as a line with a length of 2.5 to 7 mm, and the white corolla with a length of 1.9 to 3 cm.

Jamzad described *L. galeobdolon* species in 2012 as follows: The stem with a height of 20 to 25 or rarely 60 cm, the petiole with a length of 1 to 2 or hardly 6 cm, the leaf length of 1.5 to 7.5 cm, the leaf width of 1.5 to 4 cm, the calyx length of 8 to 10 mm, the calyx dent length of 2 to 2.5 mm, and the corolla with a length of 2 to 2.5 cm.

The results of morphometrical study in the north of Iran describes this species *(L.galeobdolon)* as follows: The stem height from 19 to 42 cm, the petiole in stem leaf with a length of 1.3 to 3.5 cm, the lamina in stem leaf with a length of 1.9 to 8.7 cm and a width of 1.3 to 4.2 cm, the lamina in inflorescence leaf with a length of 2.3 to 6.7 cm, and a width of 1.7 to 3.8 cm, the calyx length of 6 to 10 mm, the triangular dents with lengths of 2 to 3.5 mm, the yellow corolla with a length of 1.8 to 2.3 cm.

Jamzad described *L. amplexicaule* species in 2012 as follows: The stem with a height of 5 to 35 cm, the petiole with a length of 3 to 35 mm, the leaf length of 4 to 20 mm, the leaf width of 4 to 15 mm, the inflorescence leaf with a length of 6 to 20 mm and a width of 10 to 30 mm, the calyx length of 5 mm, and the white corolla length of 15 to 22 mm.

The results of morphometrical study in the north of Iran describe this species *(L.amplexicaule)* as follows: The stem height of 5.5 to 30 cm, the lamina at the leaf base with a length of 3 to 16 mm and a width of 3 to 16 mm, the lamina in the stem leaf with a length of 5 to 11 mm and a width of 5 to 12 mm, the lamina in the leaf inflorescence with a length of 5 to 13 mm and a width of 9 to 21 mm, the petiole in basal leaf with a length of 0.9 to 2.2 cm, the petiole in stem leaf with a length of 0.5 to 1.6 cm, the calyx length of 5 to 7 mm, and the purple corolla with a length of 1.9 to 2 cm.

This study initially describes the morphological quantitative traits such as the petiole length in the inflorescence leaf, the length and width of lamina in leaf inflorescence, the size of flap in the middle lobe of corolla, the calyx dent length, the distance between the bundles f flowers, the number of calyx veins, bract size, and the quantitative morphological traits such as the shape of inflorescence leaf, inflorescence leaf base, inflorescence leaf tip, inflorescence leaf coverage, the layout of calyx veins, the flap in the lower lobe of Corolla, the bract coverage in *L.album subsp. album, L,album subsp. crinitum, L.amplexicaule,* and *L.galeobdolon* species which have played very effective roles in separating the species of *Lamiun* genus.

According to this study, which is conducted for the first time in Iran and the world on *L.amplexicaule, L.galeobdolon, L,album subsp. album* and *L.album subsp. crinitum* species, it is found based on the morphological data that 8 main factors have caused the greatest differences with a total variance of 87.40% and they well separated the species. According to the table of variance (Table 3), the main traits of first factor with the main role in creating the variance are as follows with a variance of 47.70%: The

basal leaf, the layout of calyx vein, flap in the middle lobe, the coverage inside the corolla, the plant size, distribution of fluff on anther, the fluff on anther, the shape of calyx dent, the length of calyx, the coverage of inflorescence leaf, the total length of corolla, and the distance between the bundles of flowers. In the second factor with a variance of 14.58%, the most important traits are as follows: The basal leaf, the layout of calyx veins, the coverage inside the corolla, bract coverage, the length of stem leaf, the width of basal leaf, the length of inflorescence leaf, the inflorescence leaf base, the plant size, the distribution of fluff on anther, tip of calyx dent, length of calyx dent, margin of lower lobe, corolla color, stem shape, and the overall length of corolla. Furthermore, for the second factor with a variance of 7.6%, the most important traits are as follows: the corolla color, the length of calyx, margin of lower lobe, petiole in inflorescence leaf, corolla shape, the existence of bract, the tip of stem leaf, margin of upper lobe, coverage of bract, petiole length in the inflorescence leaf, the plant size, distribution of fluff on anther, and the shape of calyx dent which play the most important role in creating the variance and differentiation of mentioned species (Figure 1).

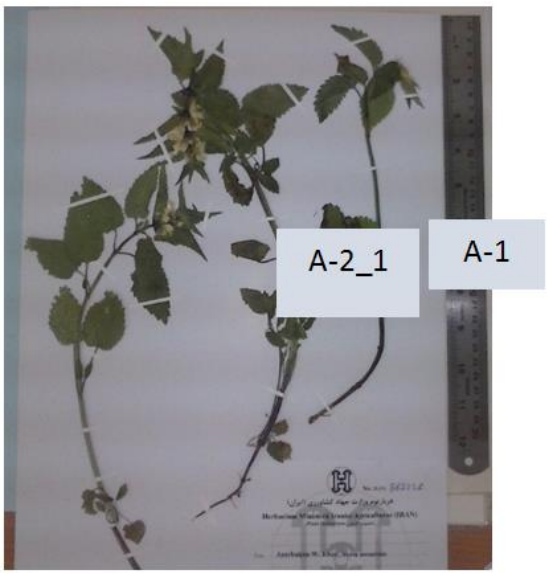

Figure 3: *L.album subsp.crinitum*

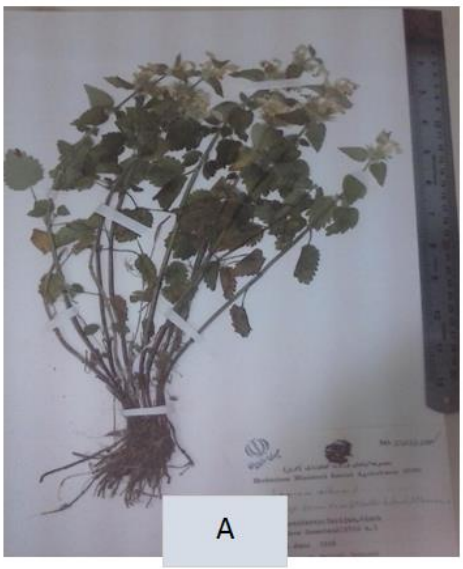

Figure 4: *L.album subsp.album*

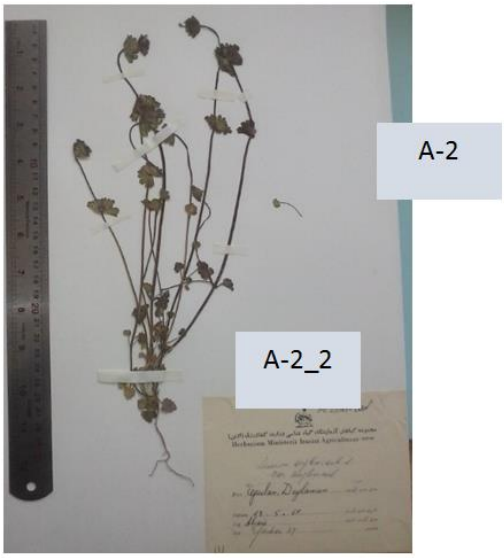

Figure 5*: Galeobdolon*

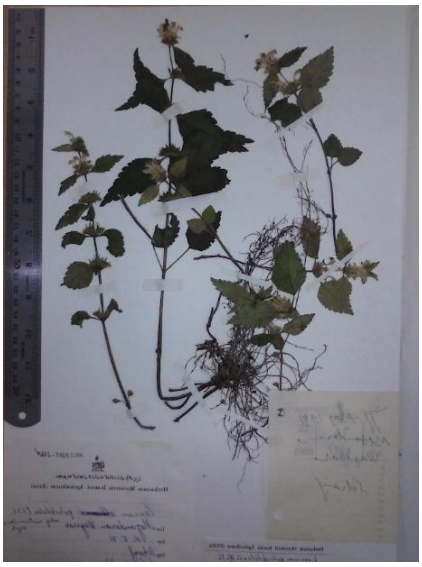

Figure 6: *L. amplexicaule*


Herbarium Number:2736


Herbarium Number:23129


Herbarium Number:4739

Based on the morphometrical study on three species, *L.amplexicaule, L.galeobdolon, L.album subsp. album* and *L. album subsp. crinitum* in three northern provinces of Iran (Mazandaran, Gilan and Golestan), it can be concluded that the morphological traits can properly separate these species from each other.

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