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Evaluation of dry matter yield and essential oil production in 84 accessions of *Tanacetum polycephalum* Sch.Bip. through multivariate analyses

Evaluación del rendimiento de materia seca y producción de aceites esenciales en 84 accesiones de *Tanacetum polycephalum* Sch.Bip. a través de análisis multivariado

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Abstract

In order to investigate diversity and explain the relationships of essential oil yield with morphological traits, 84 populations *T. polycephalum*, were sown in the field using randomized complete blocks design (RCBD) with three replicates in Research Institute of Forest and Rangeland, Tehran, Iran in 2013-2014. Data were collected for flowering date, growth degree days (GDD), stem number, plant height, dry matter (DM) yield, essential oil percentage, and essential oil yield. The relationships among traits were determined using correlation, regression and factor analysis. The genetic distance and genotype classification were determined using cluster analysis. DM yield was positively correlated with plant height, canopy cover, stem number and oil yield ($\rho<0.01$), whereas oil% was negatively correlated with plant height and stem number ($\rho<0.05$). Result of stepwise regression in which essential oil yield were considered as dependent variable showed that essential oil%, and DM yield accounted for 39.95 and 47.75%, of the total variations in essential oil production, respectively. In factor analysis, the Eigen values obtained from 1 to 3 factors were higher than one with 88.9% of total variations. In the first factor, traits of DM yield, plant height, canopy cover, stems number, and in the second factor flowering date, GDD and oil yield and in the third factor oil% had higher Eigen vector coefficients for genotype classification. In cluster analysis, genotypes were categorized in four groups. According to the obtained results, cluster 1 for early maturity, clusters 3 for plant height canopy cover, stems number, DM yield and essential oil production and cluster4, for essential oil%, had significantly higher mean values than other groups. There was a good agreement between the results obtained from cluster and factor analyses in scatter diagram representation of 84 genotypes based on the first and second factors. It was concluded that population of cluster 3 are capable to breeding improved new cultivars and should be focused on selection higher aerial biomass yield, plant height, flower number, essential oil%, coupled with early flowering date.

Key words: Early flowering date, genetic variation, phenotypic correlations, morphological traits, plant genetic resources.

Resumen

Para investigar la diversidad y explicar las relaciones del rendimiento de aceite esencial con rasgos morfológicos, 84 poblaciones de *T. polycephalum* fueron sembradas en el campo utilizando diseño de bloques completos al azar (BCA) con tres réplicas en el Instituto de Investigación de Bosques y Pastizales, Teherán, Irán en 2013-2014. Los datos fueron colectados para la fecha de floración, días de grado de crecimiento (DGC), número de tallos, altura de planta, rendimiento de materia seca (MS), porcentaje de aceite esencial y rendimiento de aceite esencial. Las relaciones entre las características agronómicas se determinaron mediante correlación, regresión y análisis factorial. La distancia genética y la clasificación del genotipo, se determinaron mediante análisis de conglomerados. El rendimiento de MS se correlacionó positivamente con la altura de la planta, cobertura del dosel, número de tallos y rendimiento de aceite ($\rho <0.01$), mientras que el% de aceite esencial se correlacionó negativamente con la altura de la planta y el número de tallos ($\rho <0.05$). El resultado de la regresión gradual en la cual el rendimiento de aceite esencial se consideró como variable dependiente mostró que el% de aceite esencial y el rendimiento de

MS representaron 39.95 y 47.75% de las variaciones totales en la producción de aceite esencial, respectivamente. En el análisis de factores, los valores de Eigen obtenidos de 1 a 3 factores fueron mayores que uno con 88.9% de las variaciones totales. En el primer factor, los rasgos de rendimiento de MS, altura de la planta, cobertura del dosel, número de tallos y en la segunda fecha de floración del factor, DGC y producción de aceite esencial y en el tercer factor, % de aceite esencial, tuvieron coeficientes de vectores Eigen más altos para la clasificación del genotipo. En el análisis de conglomerados, los genotipos se categorizaron en cuatro grupos. De acuerdo con los resultados obtenidos, el grupo 1 para madurez temprana, los grupos 3 para cobertura vegetal del dosel, el número de tallos, el rendimiento de MS y la producción de aceite esencial y el grupo 4, para% de aceite esencial, tuvieron valores promedio significativamente más altos que otros grupos. Hubo una buena concordancia entre los resultados obtenidos de los análisis de grupos y factores en la representación del diagrama de dispersión de 84 genotipos en función del primer y el segundo factor. Se concluyó que la población del grupo 3 es capaz de producir nuevos cultivares mejorados y debe centrarse en la selección de mayor rendimiento de biomasa aérea, altura de la planta, número de flores, % de aceite esencial, junto con la fecha de floración temprana.

Palabras clave: Características agronómicas, fecha de floración temprana, variación genética, correlaciones fenotípicas, recursos fitogenèticos.

Introduction

Genus of *Tanacetum* with name of tansy are perennial, herbaceous plants and all of its species smell like mint. This genus belongs to family Asteraceae and tribe of Anthemideae. This genus has 160 species and they are native to many regions of the Northern Hemisphere as Europe, temperate Asia, North America, and North Africa (Oberprieler, Himmelreich & Vogt, 2007). The origin of Tanacetum is southwest of Asia and Caucasus (Heywood & Humphries, 1977). Its distribution pattern in Iran, in the rangelands of Zagros and Alborz Mountains and also in North Khorasan in the northeast of Iran (Mozaffarian, 2005). The species of *Tanacetum* genus is a valuable medicinal plant. The flora of Iran comprises 26 species of *Tanacetum*, of which 12 are endemic (Mozaffarian, 2005; 2008).

The genus is rich in essential oils, bitter substances and sesquiterpene lactones (Akpulat, Tepe, Sokmen, Daferera & Polissiou, 2005). It traditionally been used as a spicy additive for food. In cosmetics and as herbal remedies, due to existence of biologically active compounds, this species is known as a good treatment for infectious disease (Nezhadali, Soleymani & Akbarpour, 2009). Some members of the genus *Tanacetum* has traditionally been used in balsams, cosmetics, dyes, insecticides, medicines and have been found to act as a preservatives and are used in herbal remedies. According some published study, the oils and extracts of members of the Tanacetum genus exhibit anti-inflammatory, antibacterial and antifungal (Hethelyi, Tetenyi, Danos & Koczka, 1991), and insecticidal effects (Hough & Hahn, 1992). The terpenes in the essential oil are thought to associate with the biological activity of *Tanacetum.* With respect to *T. parthenium*, many studies were related to the sesquiterpene lactone parthenolide and flavonoids, to the strong biological activity (Long, Sauleau, David, Lavaud, Cassabois, Ausseil & Massiot, 2003).

Tancetumt polycephalum is perennial or biennial species with multiple fertile and sterile stems, Covered with short hairs gray felt, 20 to 40 cm height. The time of flowering and fruiting is summer. This species belongs to Iranian Turonian and Caspian region. It is distributed in north-west of Iran, Turkey, Caucasus and north of Iraq. The achenes is cylindrical, 2 to 2.5 mm height (Mozzaffarian, 2008).

Currently, there is no comprehensive information about field evaluation of *T. polycephalum* genetic resources in Iran. According to the importance of this plant in Iran medicinal industry and also high potential, is necessary this plant to be domesticated as a crop plant. The aim of this study was to evaluate the genetic variation and relationship between DM yield and essential oil production in 84 populations of *T. polycephalum* through a multivariate analysis.

Material and methods

Plant material

In this research, 84 populations *Tanacetum polycephalum* (Table 1), were evaluated in the field. Seeds were provided from the Natural resource gene bank of research Institute of Forest and Rangeland, Tehran, Iran. Initially, seeds were sown in Jiffy pots in 2012. Subsequently, seedlings were transferred in the field in Alborz research station in Karaj, Iran in March 2013. Experiment was carried out in a randomized complete block design (RCBD) with three replicates. Each unit of experiment consisted of six rows with 50 cm apart each other with distance of 40 cm and length of 3 m. Data were collected for flowering date, growth degree days (GDD), stem number, plant height, dry matter (DM) yield, essential oil percentage, and essential oil yield.

Table 1. Accessions codes and origins in natural resource gene bank and their classification using cluster analysis Ward method.

Row	Accessions code	Origin	1000 seeds weight	Cluster No	Row	Accessions code	Origin	1000 seeds weight	Cluster No
1	498	Zanjan	0.40	1	4	1419	Foreign	0.40	3
3	1268	Zanjan	0.43	1	18	17639	Qom	0.39	3
6	2787	Zanjan	0.31	1	32	22615	Bijar	0.35	3
8	6505	Shiraz	0.29	1	33	22786	Khoy	0.28	3
11	12766	Arak	0.23	1	40	25991	Qorvah	0.30	3
13	17475	Ilam	0.37	1	43	26543	Asadabad	0.50	3
14	17493	Ilam	0.36	1	47	27511	Khoy	0.14	3
34	22824	Salmas	0.85	1	53	29855	Qorvah	1.21	3
36	23863	Isfahan	0.15	1	54	29865	Qorvah	0.31	3
37	25949	Divandarah	0.40	1	55	29878	Qorvah	0.33	3
38	25957	Dehgolan	0.40	1	59	30352	Dorood	0.45	3
42	26535	Hamadan	0.33	1	72	33170	Asadabad	0.80	3
44	27255	Kashan	0.36	1	73	33260	Qazvin	0.60	3
45	27452	Zanjan	0.38	1	75	33321	Zanjan	0.50	3
48	29698	Rodbar	0.43	1	78	35028	Golpaygan	0.20	3
50	29814	Dena	0.38	1	80	35073	Faridnoshar	0.20	3
56	29892	Qorvah	0.45	1	83	35186	Khoy	0.40	3
57	29893	Qorvah	0.51	1	86	35635	Amol	0.20	3
58	29899	Qorvah	0.52	1	5	1459	Malayer		4
60	30393	Azna	0.52	1	7	6357	Shiraz	0.29	4
61	31200	Yasoj	0.39	1	15	17500	Ilam	0.35	4
65	31928	Yasoj	0.30	1	17	17600	Qom	0.31	4
70	32819	Tarom	0.35	1	19	17650	Qom	0.26	4
74	33314	Zanjan	0.40	1	22	18189	Isfahan	0.41	4
84	35187	Shahindezh	0.50	1	25	21306	Ilam	0.35	4
85	35579	Savadkoh	0.45	1	27	21310	Ilam	0.35	4
87	35813	Mahneshan	0.19	1	30	22576	Sanandaj	0.38	4
					31	22613	Banah	0.35	4
2	506	Hamadan	0.40	2	35	22837	Salmas	0.21	4
9	10760	Chardavol	0.44	2	39	25987	Sanandaj	0.40	4
10	11184	Kashan	0.43	2	41	26034	Qorvah	0.35	4
12	14340	Hamadan	0.31	2	46	27502	Sardasht	0.58	4
16	17521	Ilam	0.44	2	49	29707	Rodbar	0.35	4
20	17752	Qom	0.35	2	51	29815	Yasoj	0.35	4
21	17790	Boovanat	0.23	2	52	29816	Yasoj	0.63	4
23	21303	Ilam	0.30	2	62	31204	Dena	0.31	4
24	21304	Ilam	0.38	2	64	31380	Dahdaz	0.15	4
26	21307	Ilam	0.50	2	66	31929	Yasoj	0.30	4
28	21373	Ilam	0.47	2	67	31930	Yasoj	0.40	4
29	21374	Ilam	0.43	2	68	31931	Yasoj	0.40	4
63	31211	Dena	0.31	2	69	32540	Qom	0.35	4
76	33365	Zanjan	0.31	2	71	33165	Malayer	0.30	4
					77	33367	Ghaidar	0.24	4
					79	35030	Samirom	0.70	4
					81	35184	Tekab	0.40	4
					82	35185	Ormia	0.28	4
					88	35914	Malayer	0.30	4

Flowering date was recorded as number of days from date 21 March till first flowering emergence date per plant (Siddiqui, Oad & Jmaro, 2006). GDD were recorded using Equation 1, as follows:

$$GD\boldsymbol{D} = \frac{T_{\min} + T_{\max}}{2} \square T_b \quad \text{Equation 1}$$

Where:

GDD= Growth degree days

T_{max} = Maximum daily temperature

T_{min} = Minimum daily temperature

T_b = Base temperature (Physiological zero for germination). Base temperature for those species was considered as 6°C.

For extraction and measuring of essential oil, herb collected at full flowering stage, subsequently, 80 g of dried grinded material were taken and mixed with 500 ml of tap water in flask and the water was distilled for 2 h to determine essential oil as follows (Equation 2):

$$\text{Essential oil content } \% = \frac{\text{Essential oil weight g}}{\text{Aerial biomass yield g}} \times 100$$

Equation 2

Essential oil yield was calculated by essential oil% x aerial biomass yield. Kg.h^{-1}.

Statistic analysis

Collected data were analyzed for normality test. Means of 84 populations over replicate, were used for multivariate analysis. Descriptive statistics as means, standard deviation, maximum and minimum from all morphological traits, were determined. Relationships among traits were determined using correlation analysis. A stepwise regression equation was developed for essential oil yield as dependent variable and other traits as independent variables. Data were also subjected to factor analysis after varimax rotation. Genetic distance and genotype classification were determined using cluster analysis (Solouki, Mehdikhani, Zeinali & Emamjomah, 2008). All statistical analyses were conducted through MINITAB™ ver16 software. To identify significant difference among treatments and statistical significance for all comparisons was made at $p<0.05$. Tukey's multiple range test was used to compare the mean values of treatments.

Results

Result of analysis of variance showed significance differences among populations for all morphological traits ($P<0.01$) (ANOVA and mean comparisons are not shown).

Descriptive statistics

Result of descriptive statistics including mean, standard deviation, standard error, maximum and minimum from all morphological traits is presented in Table 2.

Table 2. Mean, standard deviation, maximum and minimum of DM yield and other traits in populations of *T. polycephalum*

Traits	DM yield (kg/h)	Flowering date	GDD	Plant height (cm)	canopy cover (cm²)	Stem No.	Oil%	Oil yield (kg/h)
Maximum	1366.0	106.70	1187.60	46.00	909.00	19.00	0.96	6.58
Minimum	190.0	65.03	416.60	13.00	150.30	5.00	0.14	0.43
Mean	590.3	74.69	573.10	28.64	493.50	10.16	0.45	2.50
St Deviation	288.3	9.49	167.00	6.87	181.60	3.22	0.23	1.49
SE Mean	30.7	1.01	17.80	0.73	19.40	0.34	0.02	0.16
CV%	48.8	12.71	29.15	24.00	36.80	31.67	51.38	59.75

Results showed higher coefficient of variation for DM yield, essential oil% and oil yield, which indicates that should be possible plant breeding could improve new cultivars for both, oil% and oil yield using mass selection method in the study populations.

Correlation among morphological traits

Result of simple correlation analysis showed that there was significant positive correlation between flowering date and GDD ($\rho<0.01$). Both, flowering date and GDD, had achieved a negatively correlation with plant height, canopy cover ($\rho<0.05$) and stem number $\rho<0.01$). DM yield was positively correlated with plant height, canopy cover, stem number and oil yield ($\rho<0.01$), whereas essential oil% was negatively correlated with plant height and stem number ($\rho<0.05$). There were also positive correlations among plant height, canopy cover, stems number and essential oil yield ($\rho<0.01$) (Table 3).

Table 3. Phenotypic correlations between DM yield and other traits in populations of *T. polycephalum*

Traits	DM yield (kg.h⁻¹)	flowering date	GDD	Plant height (cm)	Canopy cover (cm²)	Stem No.	Oil%
Flowering date	-0.13						
GDD	-0.15	0.98**					
Plant height	0.67**	-0.31*	-0.32*				
Canopy cover (cm²)	0.77**	-0.33*	-0.34*	0.72**			
Stem No.	0.52**	-0.39**	-0.39**	0.69**	0.66**		
Oil%	-0.12	0.11	0.09	-0.29*	-0.15	-0.30*	
Oil yield (kg/h)	0.62**	0.01	-0.03	0.36**	0.45**	0.33*	0.64**

*, ** significant at 5% and 1% level, respectively

Regression analysis

Results of stepwise regression in which essential oil yield were considered as dependent variable and other traits as independent variables.

Table 4, showed that essential oil%, DM yield and stems numbers, were entered in final equation with coefficient of determination (R^2=88.86). These three variables accounted for

39.95, 47.75 and 1.15%, of the total variations for essential oil production, respectively (Table 4).

Table 4. Results of stepwise regression analysis for oil yield as dependent variables and other morphological traits as independent variables

Step	Step1	Step 2	Step 3
Constant	0.65	-1.73	-1.95**
Oil%	4.15	4.67	4.73**
DM yield (kg.h^{-1})		0.04	0.03**
Stem No.			0.03*
R-Sq(adj)	39.95	87.71	88.86

*, ** significant at 5% and 1% level, respectively.

Factor analysis

Results of factor analysis (Table 5), showed three factors with Eigen values higher than one with 88.9% from total variations. The amount of variance explained by each factor reflects its importance in explaining the total variance of the traits under study. The first three factors accounting for 56%, 18%, and 12% variation (Table 5).

Table 5. Factor matrix after varimax rotation and total variance explained for each factor on 84 productions in *T. polycephalum*.

Variable	Factor1	Factor2	Factor3
DM yield (kg.h^{-1})	**0.80**	-0.37	0.20
Plant height (cm)	**0.84**	-0.09	0.24
Canopy cover (cm^2)	**0.87**	-0.17	0.18
Stem no.	**0.81**	0.08	0.16
Flowering date	-0.55	**-0.68**	0.48
GDD	-0.57	**-0.65**	0.48
Oil yield (kg.h^{-1})	0.50	**-0.72**	-0.43
Oil%	-0.13	-0.57	**-0.77**
Eigen values	3.67	1.92	1.40
% of variance	46.5	24.1	18.3
Cumulative % of variance	46.5	70.6	88.9

The bold and underlined data had higher Eigen values in the relevant factors.

In the first factor, traits of DM yield, plant height, canopy cover and stems number had positive loading and the first factor was regarded as productivity factor. In the second factor flowering date, GDD and oil yield had higher eigin vectors and trends of these traits were in the same direction. Therefore, this factor was named as phenology and oil yield factor. In the third factor oil% had higher Eigen vector coefficients and was named as oil content factor (Table 5). The results of this study indicated that selection of variables for productivity factor (factor1) could enable plant breeding programs to release the desirable increasing in DM production in *T. polycephalum*.

Cluster analysis

According to Dendrogram of cluster analysis, (Figure 1), the 84 populations of *T.polycephalum* were divided into four groups by genetic distance of 15.56. According to the obtained results, cluster 1 to 4 had 27, 14, 18 and 29 accessions, respectively (Figure 1, Tables 1, 6). Results of means comparison of total average values of four clusters from all morphological traits.

Table 6, showed that cluster 1 with lower flowering date and GDD recognized as early maturity group, clusters 3 for plant height, canopy cover, stems number, DM yield and essential oil production and cluster 4, for essential oil%, had significantly higher mean values than the other groups (Table 6).

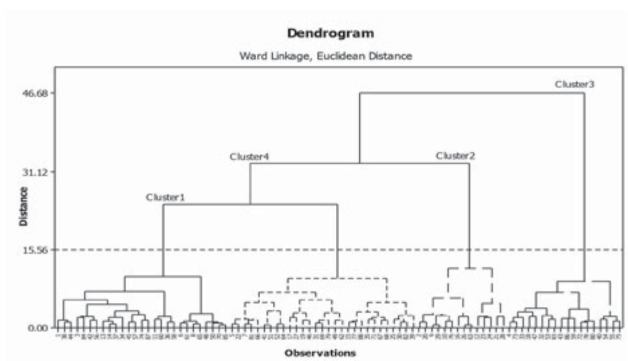

Figure 1. Dendrogram of 84 populations of T. polycephalum using ward cluster analysis method.

Table 6. Means comparison of traits used in classification of the 4 clusters

Clusters No.	Acc. No.	DM yield (kg.h^{-1})	Flowering date	GDD	Plant height (cm)	Canopy cover (cm^2)	Stem number	Oil%	Oil yield (kg.h^{-1})
1	27	566.2 b	68.91 c	476.70 b	30.25 b	518.86 b	10.45 b	0.25 c	1.42 c
2	14	358.6 c	90.34 a	849.98 a	20.96 d	305.12 d	7.09 c	0.39 bc	1.46 c
3	18	1005.8 a	72.91 bc	538.51 b	36.08 a	735.15 a	13.86 a	0.49 b	4.38 a
4	29	466.8 bc	73.64 b	550.56 b	26.23 c	410.91 c	9.08 bc	0.63 a	2.83 b

Means of each column with the same letters is not significantly different based on Duncan method (P<0.05).

Based on the first two factor scores, we scattered the genotypes in biplot (Figure 2). The first factor was considered as productivity factor since it had higher positive Eigen vectors coefficients for DM yield, plant height, canopy cover and stems number, so genotypes in the left and right hand side of Figure 2 (cluster 2 and 3), which had achieved lower and higher productivity, respectively. As we found significant negative correlation of flowering date, GDD and oil yield with the second factor, in addition, genotypes in upper part of Figure 2 (cluster 1), had achieved lower values of these traits indicating the early maturity genotypes are coupled with lower essential oil production.

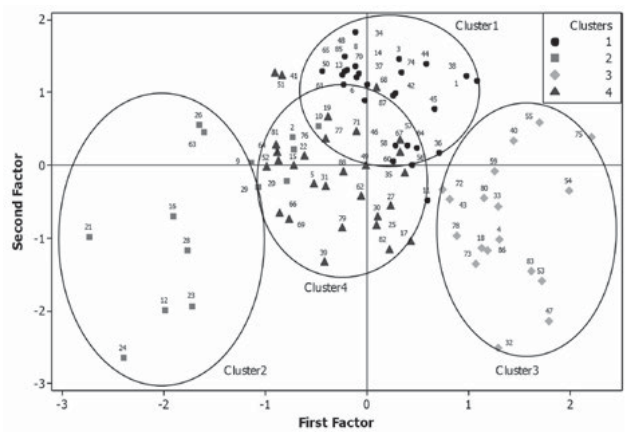

Figure 2. Biplot Scatter plot of 84 populations of *T. polycephalum* for the first two factors.

There was a good agreement among results obtained from cluster and factor analyses in scatter diagram representation of 84 genotypes based on the first and second factors.

Discussion

Result showed that there were significant positive correlation between DM yield and plant height, canopy cover, stem number and oil yield (ρ <0.01). These results are comparable in variability to the report by Alizadeh, Adeli & Jafari (2015), in evaluation of chamomile populations (*Anthemis triumfettii* (L.) DC.). Time required for flowering and GDD had the same trend in relationships with other traits. Both traits GDD had negatively correlated with plant height, canopy cover and stem number, indicating that selection of early flowering accessions led to increasing morphological traits.

Our result indicted oil% was negatively correlated with, plant height and stem number, in contrast, Adeli, Alizadeh & Jafari (2013), in two species: *Matricaria recutita* L. and *Matricaria aurea* (Loefl.) Sch.Bip., found that essential oil% was positively correlated with plant height, stem number and DM yield. In our result there was no correlation between essential oil% and maturity date, however, Adeli, Alizadeh & Jafari (2016), found negative relationships and suggested that early maturity plants had higher essential oil content. There was strong correlation between essential oil yield and essential oil%. This result was in concordance to previous results obtained by Soluki *et al.* (2008), in *Matricaria chamomilla* L., and Hamisy, Sefidkon, Nasri & Lebaschi (2012), in *Tanacetum parthenium* (L.) Sch.Bip.

Results of stepwise regression in which essential oil yield were considered as dependent variable showed that essential oil%, DM yield and stems number were entered in final equation. Similar to our result, Fulton, Clark & Fulton (2001), found higher pyrethrins (one of essential oil components) yield of *Tanacetum cinerariifolium* (Trevir.) Sch.Bip. They also found that DM yield was positively correlated with stem number. They found flowering stem number is one of the yield component in *T. polycephalum,* which increased with plant density. Our results also confirmed the result of Golparvar, Ghasemi & Karimi (2011), in determination of the effective traits on essential oil% in German chamomile (*Matricaria chamomilla* L.) populations. They found positive and significant relation of dry flower yield and essential Oil%.

Results of multivariate statistical analysis in 8 traits in 84 accessions of *T. polycephalum* using PCA and Ward cluster, indicating that DM yield, plant height, canopy cover and stems number accounted for 56% of total variation and had positive loading and the first factor was regarded as productivity factor. In the second factor flowering date, GDD and oil yield had higher were accounted for 18% of total variation. Similar to our result, Kazemi, Sonboli, Zare & Kazempour (2014), using cluster analysis in *Tanacetum aureum, T. oligocephalum and T. heimerlii* well differentiated the accessions into separate species. Similarly, Alizadeh & Jafari (2016), in cluster analysis for grouping of the accession of *Anthemis triumfettii* (L.) DC., *Anthemis tinctoria* L., *Anthemis haussknechtii* Boiss. & Reut. and *Anthemis pseudocotula* Boiss. well separated accession based on aerial biomass yield, morphological traits and maturity.

Conclusion

There was strong correlation between essential oil yield and essential oil%. Similarly, essential oil yield had positive and significant correlations with all of traits except phenological traits. Analysis of correlation and regression indicates that selection should be focused on high aerial biomass yield and essential oil%, plant height and high flower number. Regarding to cluster analysis, it was cleared that lower value of flowering time and GDD were observed in genotypes of cluster1. Therefore, populations of this cluster were considered as early maturity population compare with other populations. Populations in cluster 3 had higher DM yield, stem number, canopy cover and essential oil production than the other clusters. It was concluded that population of cluster 3 are capable to breeding improved new cultivars and should be focused on selection higher aerial biomass yield, plant height, flower number, essential oil%, coupled with early flowering date.

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