Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 1, p. 31-37, 2015
http://www.innspub.net

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RESEARCH PAPER
OPEN ACCESS

Essential oil yield stability of 20 populations of thyme (*Thymus kotschyanus*) across 11 environments of Iran

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Article published on July 4, 2015

Key words: Thyme (*Thymus kotschyanus*), oil yield, stability analysis.

Abstract

Thyme is an important medicinal plant of Iran that in order to have Thymol and Carvacrol using as antimicrobial and antibacterial agent. The present study was carried out to determine the oil yield performances of 20 Thyme (*Thymus kotschyanus*) genotypes across eleven environments of Iran over 2 years (2013 and 2014). The experimental layout was randomized complete block design. Stability parameters were estimated as Eberhart-Russel stability, Lin-bin cultivar superiority, Ecovalence Wricke and Shukla stability variance analysis methods. Significant differences were observed for genotypes, Environment, and genotype × environment interaction (GE). According to Eberhart and Russel method, the high yielding genotypes 5, 56 and 70 had general stability with regression line (b =1), and thus considered adapted to all of environments. The results of Lin and Binns cultivar superiority (Pi) showed that, the genotypes of 5, 54, 56 and 50 with the lowest (Pi) values couple with higher oil yield were considered the most stable. Ecovalence (Wi) proposed by Wricke, showed that genotypes of 5, 56, 54 and 70 had lower Wi values couple with higher oil yield were considered more stable. The same genotypes in terms of Shukla stability variance also were introduced. In comparison between stability statistics, the genotypes stabilities in various methods were more and less similar. The genotypes of G5 (Ghazvin 2), G56 (Zarand) and G70 (Oromiea2) with average values of 1.66 to 1.70 Kg h^{-1} had higher general salability over all of environments. The genotypes G54 (Nagade) and G58 (Sanandaj2) with average values of 1.685 and 1.499 Kg h^{-1} had specific stability for poor environments. The genotypes G22 (Ghazvin3) and G50 (Zanjan4) with average values of 1.78 and 1.74 respectively had specific stability for rich environments.

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Introduction

Thymus kotschyanus Boiss. & Hohen is one of the Thyme species that has wide applications in health care, pharmaceutical and food industries. Thyme Phenolic essential oil is one of the 10 important essences which had antibacterial, antifungal, antioxidant, preservative food and delay the aging mammals (Seidler *et al*, 2008). Essential oil percentage and yield are the main goal in Thyme breeding programs. High levels of variability among such crop populations had been reported for oil yield. Babalar *et al.* (2014) found considerable variation between different populations thyme, especially for oil yields here In Iran. Kaveh *et al.* (2013) in comparison of morphological and phytochemical traits in populations of *Thymus kotschyanus* and *Th. vulgaris* found the lower dry matter production of *Th. kotschyanus* with average values of 20.66 to 82 g/plant than that for *Th. vulgaris* ranged from 56.66 to 110.67 g/plant. Essential oil percentage in *Thymus kotschyanus* was from 0.42 to 2.17% and in *Thymus vulgaris* from 0.42 to 1.75%.

Essential oil yield is a complex trait which is depended on yield components and is highly influenced by many genetic as well as environmental factors. Therefore, evaluating genotypic potential in different environments is the important step in breeding programs of *Thymus kotschyanus* before selecting desirable ones to commercial cultivation. Analyzing Genotype and environment interaction (GEI) for varieties can reduce errors in the breeding process for proper selection by multiple locational conditions. (Gauch *et al.,* 1988).

Several stability parameters have been proposed in two main groups (Lin *et al.*, 1986). The first group includes environmental variance, which it is independent to tested genotypes. The second group measure genotypic stability relative to the mean of the tested genotypes. The latter one has two sub groups, as: a) linear and non linear components of stability (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966) and b) measurement of bulk stability without reference to linear and non linear components (Plaisted, 1960; Shukla, 1972). Eberhart and Russell (1966) considered a stable genotype to have a slope (*b* value) equal to unity and deviation from regression (S^2_d) equal to zero. The stable genotypes will be those having mean yield higher than the average yield of all the genotypes under test. This method has been widely used for evaluating of yield stability in both annual and perennial plants.

According to the joint linear regression model which was developed by Finlay and Wilkinson (1963) and modified by Eberhart and Russell (1966), a stable variety is one with a high mean yield, regression coefficient equals to one (bi=1) and deviation from regression equals to zero (S^2_{di}=0). In this method, the sum of squares due to environments and genotype x environment are partitioned into environments (linear), genotype x environment (linear) and the pooled deviations from the regression model. If the variation among the genotypes and for G x E interaction were significant, it means that genotypes exhibited different performance in different locations /environments which is due to their different genetic structure or the variation due to the environments or both.

Lin and Binns cultivar superiority (Pi) is estimated by the square of differences between a genotype's and the maximum genotype mean at location, summed and divided by twice the number of locations (Lin and Binns, 1988). The genotypes with the lowest values are considered the most stable.

Wricke (1962) proposed using the contribution of each genotype to the G x E interaction sum of squares as a stability measure and defined this concept or statistics as ecovalence (Wi). Genotypes with a low Wi value have smaller deviations from the overall mean across environments and are thus more stable.

Shukla's stability variance (Shukla, 1972) is a modified version of the ecovalence in order to give unbiased estimate of the G x E variance for every genotype using the stability variance. A genotype is called stable if its stability variance is equal to the environmental variance which means that stability

variance equal to zero. A relatively large value of stability variance will thus indicate greater instability of genotype.

The objectives of this study were to evaluate oil yield performance and stability of 20 genotypes of *Th. kotschyanus* across eleven environments in Iran by determine the magnitude of genotype by environment interaction for oil yield, of *Th. kotschyanus* genotypes under irrigation in Iran and determine oil yield stability for promising *Th. kotschyanus* populations and to identify populations that are widely adapted (stable) and specifically adapted (with narrow adaptation) for oil yield. Materials and Methods

The study was conducted in 11 locations in Iran consist of Damavand, Hamedan and Markazi (Cold and semiarid), Qom, Yazd and Esfahan (warm and arid), Tabriz, and Zanjan (old Sub- steppe zone with annual precipitation between 230-450 mm), Tehran and Khorasan (warm and semiarid) and finally Golestan (humid)(Table 1).

Table 1. Mean of Oil yield average over 20 genotypes and some meteorological characteristics of the research locations.

Locations name	Locations code	Oil Yield Kg h^{-1}	Longitude (E)	Latitude (N)	Altitude (m)	Average annual temperature (°C)	Annual rainfall (mm)
Damavand	L1	2.578	52.05	35.70	2050	9.2	530
Esfahan	L2	2.578	51.67	32.65	1570	16.3	123
Golestan	L3	1.327	54.44	36.86	174	17.8	600
Hamedan	L4	0.358	48.51	34.80	1741	11.4	317
Khorasan	L5	1.074	59.36	36.17	1065	14.1	258
Markazi	L6	0.536	49.67	34.08	1708	13.7	341
Qom	L7	1.892	50.89	34.64	932	18.2	141
Tabriz	L8	1.438	46.28	38.05	1345	12.3	289
Tehran	L9	0.449	51.20	35.41	1370	17.4	233
Yazd	L10	2.366	54.36	31.89	1230	19.4	61
Zanjan	L11	0.361	48.48	36.67	1638	11.1	313

The 20 *Th. kotschyanus* genotypes (originated from different parts of Iran) were provided from natural resources gene bank (Research Institute of Forests and Rangelands, Iran). Seeds were From each genotype, required seedlings were established in compost in March 2010. After growing in the glasshouse, the seedlings were transplanted to the field in spring 2011. An experiment was established using a randomized complete block design with three replications. In each plot, three 5m lines with 1m distance between each spaced plants were allocated. Non-experimental spaced plants were planted in two border rows surrounding the experimental area. Irrigation was made according to the plant requirement. Weeds were control mechanically. Each unit of experiment consists of three rows with 1 m distance between rows and plants within rows. Data were collected for aural dry weight, essential oil percentage and essential oil yield. The essential oil was produced by hydro distillation using a Clevenger instruments for 2 hours on the base of Hungarian plant pharmacopoeia letter (Anonymous. 1984).

For calculation of essential oil percent, 10 g of each sample was dried in oven 50°C for 24 h then reweighed and moisture % was calculated. The essential oil was calculated by following formula as Siddiqui *et al.* (2006):

$$\text{Essential content oil \%} = \frac{\text{Essential oil weight g}}{\text{Shoot dry matter g}} \times 100$$

Yield of essential oil were calculated by essential oil% x Shoot dry weight.

Combined analysis of variance over eleven environments was used to estimate mean square of genotypes, locations and genotypes × locations interactions. Genotype stability was evaluated on the bases of genotypes × location interactions.

Based on Eberhart/Russell stability regression model, the regression coefficient values (bi) and deviation from regression (S^2_{di}) were calculated for each of the 20 genotypes. A stable genotypes with a high mean oil yield, regression coefficient equals to one (bi=1) and deviation from regression equals to zero ($S^2_{di}=0$) were identified (Eberhart and Russell, 1966).

Lin and Binns cultivar superiority (Pi) was estimated by the square of differences between a genotype's and the maximum genotype mean at location, summed and divided by twice the number of locations (Lin and Binns, 1988). Genotypes with the smallest values tend to have larger oil yield and also be more stable.

Ecovalence (Wi), were calculated for each of the 20 genotypes using (Wricke, 1962) method. Genotypes with a low Wi value have smaller deviations from the overall mean across environments and are thus more stable. Shukla's stability variance were estimates of an entry's variance across environments using (Shukla, 1972) method. Stable genotypes have smaller estimates.

The stability parameters as Eberhart/Russell stability regression model, Lin and Binns cultivar superiority (Pi), Ecovalence (Wi) and Shukla's stability variance were performed using Agrobase (Agronomix, 2000), and MINTAB16 was used to illustrate the relationships among genotypes, environments.

Results and Discussion

Result of combined analysis of variance for essential oil yield showed significance effect of location ($P<0.01$) genotypes ($P<0.05$), and genotype × Location interaction ($P<0.01$) indicating that the response of genotypes to different locations were no similar. By significant of this effect the genotype stability analysis can be done (Table 2).

Table 2. Combined analysis of variance oil yield for 20 genotypes in 11 locations.

Source	df	SS	MS	F-value	Pr> F
Locations	10	129.4	12.94	27.74	0.00
Reps within locs.	33	15.393	0.466		
Entry	19	8.229	0.434	1.57	0.049
Entry x location	190	52.633	0.277	9.45	0.00
Residual	627	18.371	0.029		
Total	879	223.985			
C.V.	15.54%				

Eberhart/Russell Regression

According to Eberhart and Russel (1966) method the genotypes 5, 56 and 70 had higher oil yield than the average and (b=1) were near the unity, therefore they were stable for all environments. Deviation of regression in some other genotypes was low.

Genotypes 23, 47, 29 and 7 had the lowest deviation from regression, indicating the stability oil yield of this parameter. According to Finlay and Wilkinson (1963) and Eberhart and Russell (1966) a stable variety is one with a higher mean oil yield, regression coefficient equals to one (bi=1) and deviation from regression equals to zero ($S^2di=0$). The genotypes with (bi) value lower than 1.0 couple with higher production had good stability for low-performing environments. Therefore the genotypes 58 and 54 had ($bi<1$) and higher oil yield for poor environment. The genotypes 10, 22 and 50 with ($bi>1$) coupled with high yield performance had above average stability for high performing environments. The higher deviation from regression indicate sensitivity to environmental changes for oil yield (Table 3).

The relationship between the regression coefficients (bi) and mean oil yield for 20 genotypes (Table 2) were plotted (Fig. 1). The stable genotypes would therefore be those whose slope was 1.0 and the deviation from the regression (S^2di) close to zero.

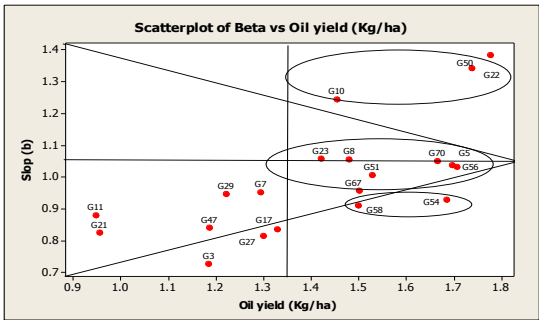

Fig. 1. The regression coefficients plotted against genotypic mean, adapted from Finlay and Wilkinson (1963).

Lin and Binns cultivar superiority

Lin and Binns cultivar superiority (Pi) was estimated by the square of differences between a genotype's and the maximum genotype mean at location, summed and divided by twice the number of locations (Lin and Binns, 1988). From this analysis, the most stable genotype for Lin and Binns Pi coupled with oil yield was 5, 54, 56, 22 and 50. The ranks of the Pi and mean oil yields were nearly similar and indicate that the Pi is a good indicator of stability (Table 3). The cultivar performance of oil yield with pi values of the 20 genotypes tested at eleven locations were plotted in Fig 2. The genotypes with the lowest Pi values were considered the most stable. In contrast, the weak genotypes with lower production according to this Pi values were 11 and 21 (Fig. 2).

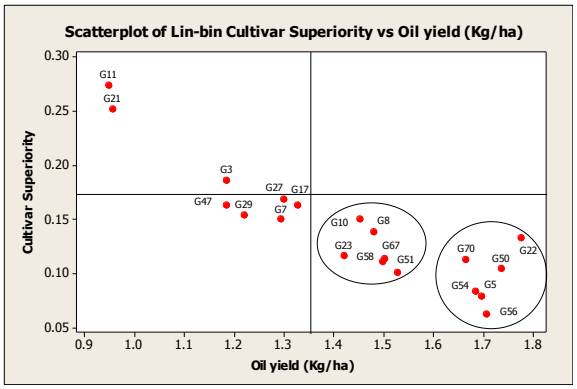

Fig. 2. Distribution of the genotypes on based the superiority index Lin-bin vs oil yield.

Ecovalence (Wi), Wricke

Ecovalence (Wi), was calculated for each of the 20 genotypes at eleven locations using (Wricke, 1962) method (Table 3). According to Becker and Léon (1988) ecovalence measures the contribution of a genotype to the G x E interaction; a genotype with zero ecovalence is regarded as stable. The results indicated that the most stable genotypes with higher production were 5, 56, 54 and 70. These genotypes were rank as highest oil yield production and had smaller deviations from the overall mean across environments and are thus more stable; in contrast, the unstable genotypes coupled with higher oil yield were 50 and 22 (Fig. 3).

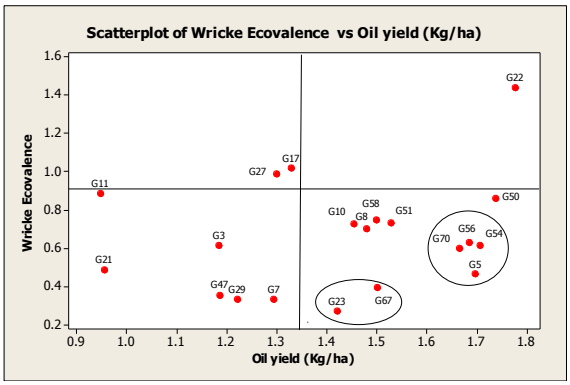

Fig. 3. Distribution of the genotypes on based the Wricke Ecovalence vs oil yield*Shukla stability variance.*

According to Shukla (1972) stability variance a genotype is called stable if its stability variance is equal to the environmental variance which means that stability variance equal to zero. A relatively large value of stability variance will thus indicate greater instability of genotype. The result of Shukla's stability variance analysis method is presented in Table 3. The results of calculating the Shukla stability variance had similar with Wi coefficient, So that genotypes 5, 56, 54 and 70 were more stable. The unstable but high yielding genotypes were 50 and 22 (Fig. 4).

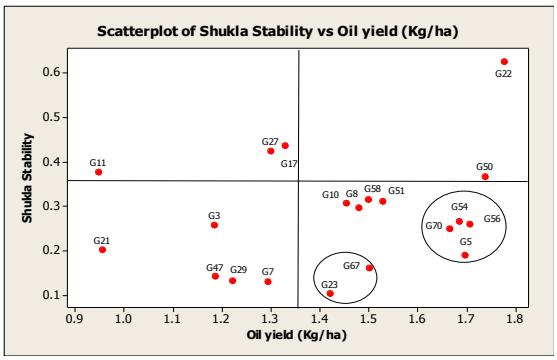

Fig. 4. Distribution of the genotypes on based the Shukla Stability vs oil yield.

Table 3. Mean of Oil yield, Eberhart/Russell Regression indices (*b*), deviation from regression (S^2_d) and Lin-bin Cultivar Superiority, Wricke Ecovalence and Shukla Stability Variance for 20 genotypes on 11 environments.

Genotype names	Genotypes code	Oil Yield Kg h^{-1}	Eberhart/Russell Regression		Lin-bin Cultivar Superiority	Wricke Ecovalence	Shukla Stability Variance
			b ($H_0 : b = 1$)	$S^2_d (H_0 : S^2_i = 0)$			
Ghazvin 1	G3	1.185	0.727	0.042	0.186	0.610	0.256
Ghazvin 2	G5	1.698	1.037	0.038	0.079	0.461	0.190
Zanjan 1	G7	1.295	0.951	0.023	0.150	0.328	0.130
Zanjan 2	G8	1.481	1.055	0.064	0.139	0.700	0.296
Az. Gharbi 1	G10	1.454	1.244	0.057	0.150	0.726	0.307
Zanjan 3	G11	0.948	0.878	0.083	0.274	0.883	0.377
Az. Gharbi 2	G17	1.329	0.834	0.095	0.163	1.017	0.437
Sanandaj 1	G21	0.957	0.825	0.036	0.252	0.486	0.201
Ghazvin 3	G22	1.776	1.582	0.086	0.133	1.440	0.625
Divandare	G23	1.421	1.056	0.016	0.116	0.267	0.103
Unknown 1	G27	1.300	0.813	0.091	0.168	0.987	0.423
Unknown 2	G29	1.221	0.945	0.023	0.154	0.331	0.132
Lorestan	G47	1.186	0.840	0.022	0.163	0.353	0.142
Zanjan 4	G50	1.737	1.341	0.062	0.104	0.859	0.367
Tehran	G51	1.528	1.005	0.069	0.101	0.732	0.310
Nagade	G54	1.685	0.927	0.056	0.083	0.630	0.265
Zarand	G56	1.707	1.030	0.055	0.062	0.615	0.258
Sanandaj 2	G58	1.499	0.908	0.068	0.111	0.744	0.315
Oromiea 1	G67	1.502	0.956	0.031	0.114	0.394	0.160
Oromiea 2	G70	1.666	1.049	0.053	0.113	0.596	0.250

Conclusion

In comparison between stability statistics, the overall ranking of the 20 genotypes for stability parameter of Eberhart and Russell's (1966) deviation from regression, Wricke's (1962) ecovalence and Shukla's (1972) stability variance indicating that the genotypes stability in various methods of stability analysis were more and less the similar. The genotypes of G5 (Ghazvin 2), G56 (Zarand) and G70 (Oromiea2) with average values of 1.66 to 1.70 Kg h^{-1} had higher general salability over all of environments. The genotypes G54 (Nagade) and G58 (Sanandaj2) with average values of 1.685 and 1.499 Kg h^{-1} had specific stability for poor environments (Hamedan(L4), Markazi(L6), Tehran(L9) and Zanjan(L11)). The genotypes G22 (Ghazvin3) and G50 (Zanjan4) with average values of 1.776 and 1.737, respectively had specific stability for rich areas of (Damavand(L1), Esfahan(L2), Qom(L7) and Yazd(L10)). All of these genotypes were suggested for breeding improved synthetic varieties of *T.kotschyanus*.

Acknowledgements

The Author thanks Iranian research institute forests and rangelands (RIFR) for providing genetic resources samples and financial support.

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