International Journal of Plant Breeding and Crop Science
Vol. 4(3), pp. 300-307, November, 2017. © www.premierpublishers.org. **ISSN**: XXXX-XXXX

IJPBCS

Research Article

Characterisation of some Lamiaceae Species Distributed in the Rize Province, Turkey

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The Black Sea region belongs to one of the richest regions of Turkey regarding biological diversity. In total 2239 species are present in the East Black Sea region, 514 of them are endemic and the endemism ratio is ca. 23 %. More than half of the plants distributed at the East Black Sea region are present in the Rize province and almost 70 % of the plants are of medicinal and aromatic value. Four Mentha species, three Origanum species, three Thymus species, two Salvia species, two Stachys species and one Calamintha species were collected from 19 different localities in Rize during 2015. A field nursery was established using collected material. A field nursery was established using collected material. Further, Principal Component Analysis was used to distinguish present genetic diversity based on essential oil composition. The obtained data valued that large diversity could be determined and collected materials can be used as genetic resources in further investigations.

Keywords: *Mentha*, *Thymus*, *Origanum*, Sage, *Stachys*, *Calamintha, essential oil*

INTRODUCTION

Between Mediterranean countriesTurkey is one of the countries displaying rich plant diversity (Davis 1965-1986; Davis et al., 1988; Güner et al., 2001). A number of human races and tribes have settled here during different periods bringing in different cultures and customs. As a result of this we come across a great accumulation of knowledge of traditional medicine in our country. A recent survey of traditional and folk medicine in Turkey has revealed that most of these plants are still in use by the local inhabitants (Yesilada and Sezik, 2003).

The Anatolian, Mediterranean, the Black Sea Region, and their transition zones are the biogeographical regions where Turkey is located. Turkey is a bridge between components, therefore its climatic and geographical features change within short intervals of space due to the country's position. Thanks to its location, Turkey's biological diversity can be compared to that of a small continent: the country's territory consists of forests, mountains, steppe, wetlands, coastal and marine ecosystems and different forms and combinations of these systems. This extraordinary ecosystem and habitat diversity has produced considerable species diversity (Kahraman et al., 2011; Kahraman et al., 2012).

The Black Sea region belongs to one of the richest regions of Turkey regarding biodiversity. Totally 2239 species are present in the East Black Sea region, 514 of them are endemic and the endemism ratio is ca. 23 %. More than half of the plants distributed at the East Black Sea region are present in the Rize province. 70 % of the plants are of medicinal and aromatic value.

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Table 1: Collected species and their localities

Species	Collected locality	Altitude	Species	Collected locality	Altitude
Mentha aquatica	Handüzi route	455 m	***Thymus longicaulicus***	Anzer	1534 m
	Ormanlı	160 m		Anzer	2106m
Mentha puligeum	Hamidiye	33 m		Cimil	2145 m
	Hamidiye	41 m		Cimil	2154 m
	Orta Mahalle	350 m	***Thymus praecox* subsp. *caucasicus***	Çağırankaya	2038 m
	Subaşı	300 m		Çağırankaya	2122 m
	Şehitlik	500 m	***Thymus praecox* subsp. *caucasicus* var *caucasicus***	Anzer	1534 m
Mentha x piperita	Şehitlik	450 m		Anzer	1750 m
Mentha longifolia* subsp. *longifolia	Anzer	1533 m		Anzer	2106 m
	Anzer	1800 m		Palovit	2400 m
	Anzer	**2075 m**	***Thymus praecox* subsp. *grosheimii* var. *grosheimii***	Cimil	2145 m
	Anzer	2139 m		Cimil	2154 m
	Amlakıt	1901 m		Çağırankaya	2122 m
	Amlakıt	1998 m		Handüzi	1833 m
	Anzer	2106 m	***Thymus praecox* subsp. *grosheimii* var. *medwedewii***	Başyayla	2558 m
	Cimil	1819 m	***Salvia glutinosa***	Çat	1295 m
	Cimil	2145 m	***Salvia verticillata*** subsp. ***verticillata***	Anzer	1533 m
	Çamlıhemşin	1838 m		Anzer	1533 m
	Çat	1299 m		Anzer	1800 m
Origanum vulgare* subs. *hirtum	Çat	1209 m		Anzer	1850 m
Origanum vulgare* subsp. *viride	İkizdere	314 m		Anzer	1850 m
	İkizdere	787 m		Cimil	2099
	İkizdere	1533 m		Çat	1321
	Uğrak	496 m		Çat	1412 m
Calamintha nepata subsp. **glandulosa**	Amlakıt	1900 m			
Stachys anuua subsp. ***annua*** var. annua	Anzer	1533 m			

Healing with plants dates back probably to the evolution of mankind, therefore natural environment has been a source of medicinal agents for thousand of years. As well known about 80 % of human in the World rely mainly on traditional medicines for their primary health care, while medicinal plants continue to play an important role in the health care system of the remaining 20 % (Mathe, 2015). Partly based on their use in traditional medicine, an impressive number of modern drugs have also been isolated from natural plant species. Remarkably, even today there is no real definition to this special group of plants that has been accompanying mankind through history. Medicinal and aromatic plants form a numerically large group of economically important plants which provide basic raw materials for medicines, perfumes, flavours and cosmetics. These plants and their products not only serve as valuable source of income for small landholders farmers and entrepreneurs but also earn valuable foreign exchange by way of export facilities.

During the present study four *Mentha* species, three *Origanum* species, three *Thymus* species, two *Salvia* species, two *Stachys* species and one *Calamintha* species were collected from 19 different localities in Rize during 2015. Different traits were determined, but only the most common essential oils detected in the samples were used to distinguish the investigated plant material.

MATERIALS AND METHODS

The research material was collected during 2015 in the Rize province (Turkey). The collected species, the localities and the altitudes are given in Table 1 and Fig. 1.

Isolation of the essential oils

100 gr were taken from dried samples. These samples were water distillated for 5 hours using Clevenger apparatus. The obtained essentail oil was transferred to vials for reading using GC-MS.

Gas chromatographic (GC) analysis

SPME (Solid Phase Microextraction) method was used to determine the essential oil components of investigated material. 0.2 from each sample was weighted and inverted to vials. Further, it was maintained for 15 min in a fiber heater at 50 ^{o}C. Thereafter the fiber was transferrered to the instrument via manuel injection and, after 5 min duration the analysis of samples was done. The essential oil was analyzed using GC/MS-QP 2010 device with EST Flex Autosampler. The GC-MS system has been equipped with a capillary column with length 30 m, 0.25 mm inner diameter and 0.25 μm film thickness.

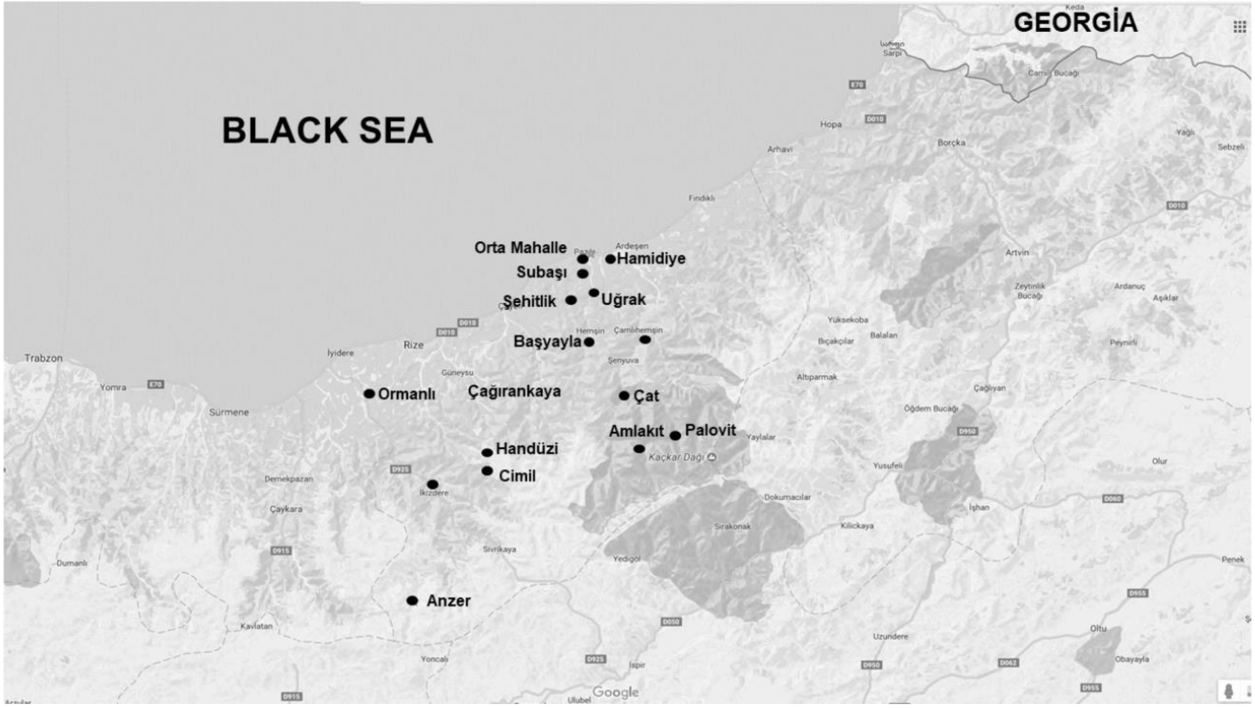

Fig. 1. Map of collection sites

Gas chromatography / Mass spectrometry (GC-MS) analysis

Injector temperature was 250ºC, split flow was 1 ml / min adn split ratio 10:1. . The GC oven temperature was kept at 40ºC for 2 min. and programmed to 240ºC at a rate of 4ºC / min. The carrier gas was helium, a RXİ-5MS (30m, 0.25 mm ID, 0.25 µl df) column was used. The determination of essentail oil components was done using the Wiley, Nist Mass Spektral and aroma method databases.

Statistical analysis

Principal component and Biplot analysis were used to distinguish the collected samples regarding analysed characteristics (Backhaus et al., 1989). Principal component and Biplot analysis was performed using XLSTAT2016 Trial Version. The principal components represent the axes which are the orthogonal projections for the values representing the highest possible variances in the case of PC1 and PC2. The obtained data were used to create scatter plot diagrams. Therefore, a factor analysis was performed, whereby each variable was used to calculate relationships between sample and investigated trait. Based on the obtained data, the Biplot diagram was created showing the relationship of investigated samples regarding to their chemical composition.

RESULTS AND DISCUSSION

Mentha species

Fig.2 shows the distribution of investigated Mentha species based on most detected essential oils.

Plant-derived natural products are extensively used as biologically active compounds.

Among them, essential oils were the first preservatives used by man (Thompson, 1989). Many of these crude mixtures have been found to have antifungal, antimicrobial, cytostatic and insecticidal activities (Sivropoulu et al., 1995). The essential oils extracted from the mint species (*M. pulegium* and *M. spicata*), containing mainly, pulegone, menthone, and carvone, were tested for insecticidal and genotoxic activities on Drosophila melanogaster. The essential oil of both these aromatic plants showed strong insecticidal activity, while only the oil of M. spicata exhibited a mutagenic one. Among the constituents studied, the most effective insecticide was found to be pulegone, whereas the most effective for genotoxic activity was menthone. The strong toxicity of pulegone is suppressed in the presence of menthone (Franzios et al., 1997).

As can be seen in Fig. 2 *Mentha* species collected from different localities and altitudes showed different essential compositions. Specially in *M. longifolia* subsp. *longifolia* large varaition was detected. The same can be said for *M. puligeum* collected from different localities and altitudes.
The calculated principal components PC1 and PC2 corresponded to 93,12 % of the present variation in the investigated material, very useful for the separation of the study material. The essential oil menthone was detected specially in M. *longifolia* subsp. *longifolia*. Carvone, myristic alcohol and menthalactone was useful in separating the other collected samples.

Commercially, the most important mint species are peppermint (M. x piperita), spearmint (*M. spicata*), and corn mint (*M. canadensis*.). From these species, corn mint

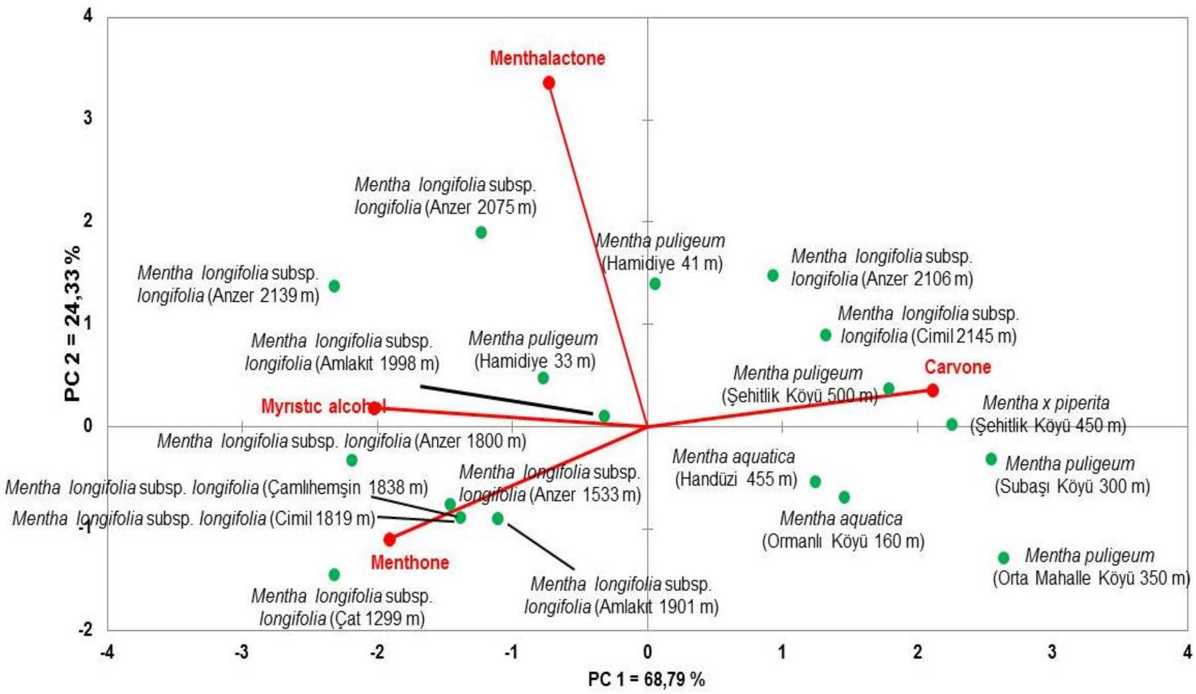

Fig. 2: Biplot analysis of Mentha species based on differentiating essential oils

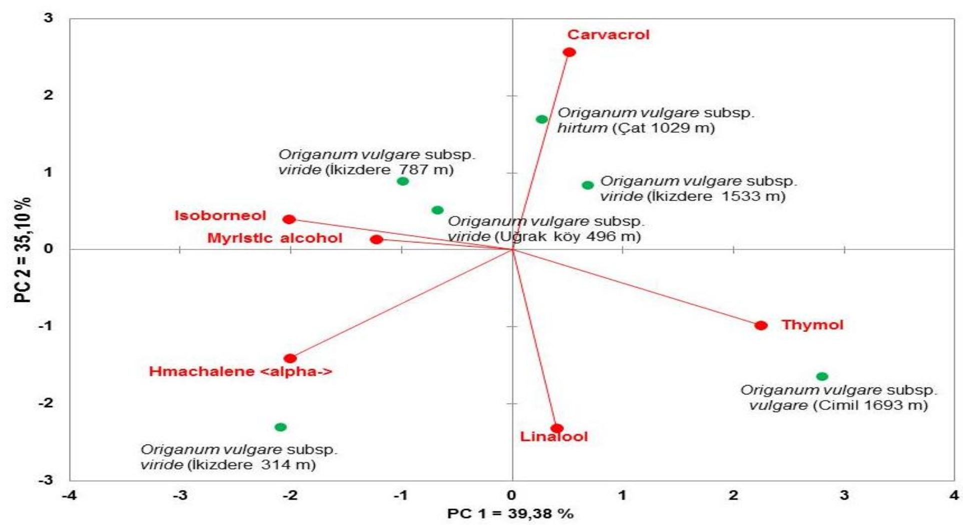

Fig. 3: Biplot analysis of Origanum species based on differentiating essential oils

is cultivated only because of oil production (Small, 1997; Oudhia, 2003). Peppermint (M. x piperita) oil is one of the most popular and widely used essential oils, mostly because of its main components menthol and menthone (Gul, 1994). Cornmint is the richest source of natural menthol (Sharma and Tyagi, 1991; Shasany et al., 2000). Carvone-scented mint plants, such as spearmint (*M. spicata*), are rich in carvone and are widely used as spices and cultivated in several countries (Kokkini et al., 1995).

Peppermint oil is used for flavouring pharmaceuticals and oral preparations, such as toothpastes, dental creams, and mouth washes. It is also used as a flavouring agent in cough drops, chewing gums, confectionery and alcoholic liqueurs. It is used in medicines for internal use. Its pleasant taste makes it an excellent gastric stimulant (Budavari et al., 1989; Gupta, 1991).

Origanum species

The essential oil of oregano is composed of carvacrol and thymol as dominant components, followed by; terpinene, p-cymene, linalool, terpinen-4-ol and sabinene hydrate (Azizi et al., 2009).

Fig. 3 shows the distribution of investigated Origanum species based on most detected essential oils. *Origanum vulgare* subsp. *viride* samples collected from different altitudes of İkizdere and from Uğrak showed different essential oil composition compared with the other Origanum species. Specially *Origanum vulgare* subs. *vulgare* collected from Cimil and *Origanum vulgare* subsp. *hirtum* collected from Çat showed different essential oil compositions. *Origanum vulgare* subsp. *hirtum* collected from Çat and *Origanum vulgare* subsp. *viride* collected

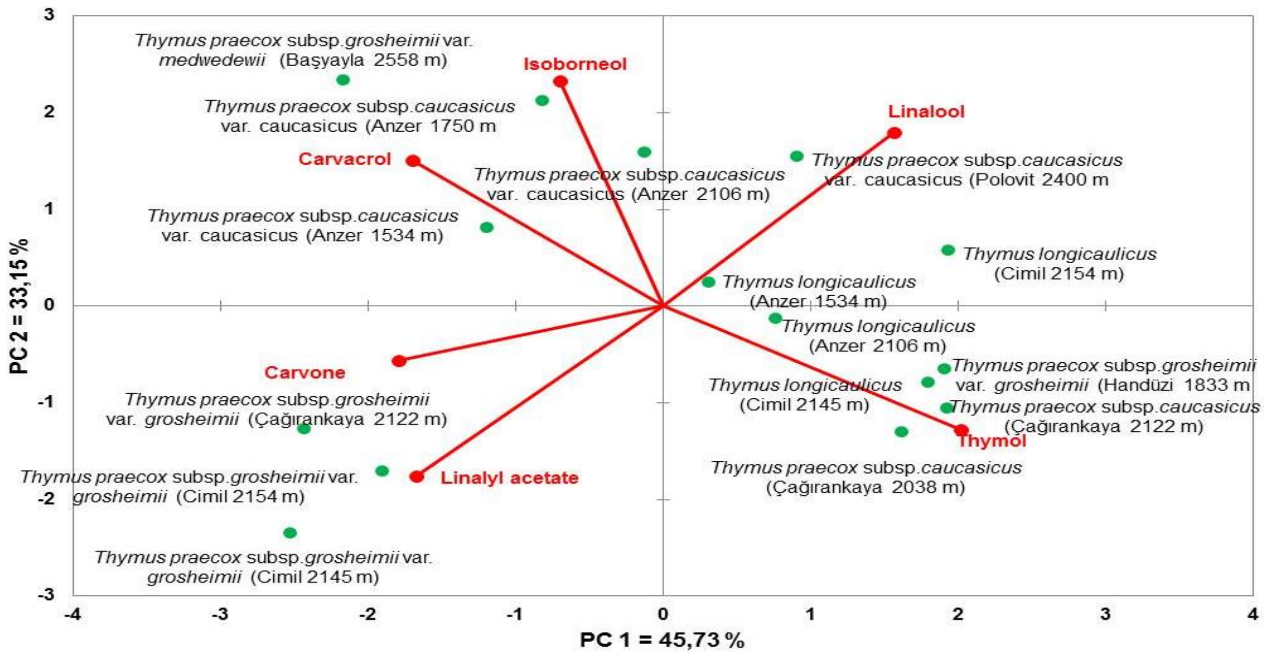

Fig. 4: Biplot analysis of Mentha species based on differentiating essential oils

from İkizdere at 1533 altitude could be differentiated from other samples regarding carvacrol. *Origanum vulgare* subsp. *viride* collected from İkizdere at 787 altitude and from Uğrak could be separated from other collected samples regarding the compounds İsoborneol and myristic.

Origanum vulgare subsp. *vulgare* collected from Cimil stand out regarding thymol from the other samples; *Origanum vulgare* subsp. *viride* collected from İkizdere at 314 m altitude showed differences regarding Hmachalene alpha. The compund linaool was useful to separate all investigated samples. PC1 (39,38 %) and PC 2 (35,10 %) corresponded to 74,48 % of the total variation regarding the chosen chemical components and these two principal components were very useful in distinguishing the investigated material.

Thymus species

Fig. 4 shows the Biplot analysis of collected Thymus samples based on their main essential oil composition. Thyme oil contains 46% phenols of which 44% thymol and 3.6% carvacrol and also important components It is confirmed by various studies confirmed that thyme oil contains polyphenolic acid (oleanic acid, rosmarinic acid, triterpene and caffeic acid). Thyme oil also contains other components such as thymol, borneol, gerniol, pinen, linalool, cineol, sabinen, myrcen limonene and cymene (Rizk, 1986).

The present variation regarding used essential oil components could be displayed using the calculated principal components PC1 (45,73 %) and PC2 (33,15 %), which corresponds to 78,87 % useful for the separation of investigated material.

T. praecox subsp. *grosheimii* var. g*rosheimi* samples collected from Cimil and Çağırankaya showed similar essentail oil compositions. Only the same species collected from Handüzi displayed similar composition with the species *T. longicaulicus* and *T. praecox* subsp. *caucasicus*. Further, *T. praecox* subsp. *grosheimii* var. *medwedewii* showed similar essentail oil composition with *T. praecox* subsp. *caucasicus* var. *caucasicus* samples collected from Palovit and different altitudes of Anzer.

T. praecox subsp. *grosheimii* var. *medwedewii* collected from Baş Yayla, *T. praecox* subsp. *caucasicus* var. *caucasicus* collected from Anzer and Polovit and T. *longicaulicus* collected from Cimil and Anzer 1534 showed differentiation from the other samples regarding the essetial oil compounds Linalool, İsoborneol and Carvacrol. Carvone and linalyl acetate were helpful to seperate *T. praecox* subsp. *grosheimii* var. *grosheimii* collected from Cimil and Çağırankaya from the other species. *T. longicaulicus* collected from Cimil, *T. praecox* subsp. *caucasicus* collected from Çağırankaya and *T. praecox* subsp. *grosheimii* var. *grosheimii* collected from Handüzi were different from the other samples regarding thymol.

Salvia, Stachys and Calamintha species

Fig. 5 shows the Biplot analysis of collected Salvia, Stachys and Calamintha samples based on their essential oil composition.

Salvia glutinosa, *Calamintha nepeta* subsp. *glandulosa* and *Stachys annua* subsp. *annua* var. *annua* differed from the other species regarding their essential oil composition. Samples of the species *Salvia verticillata* subsp. *verticillata* showed nearly similar essentail oil compositions.

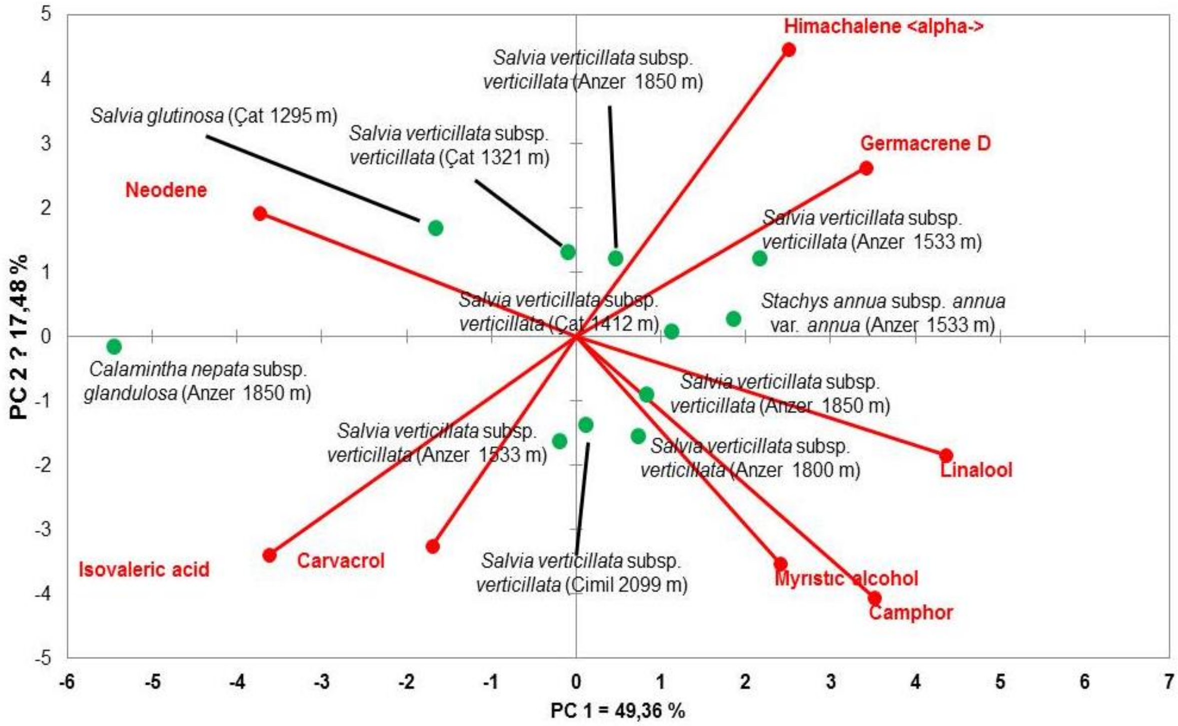

Fig. 5: Biplot analysis of Salvia, Stachys and Calamintha species based on differentiating essential oils

Calamintha nepeta subsp. *glandulosa* differed from the other samples regarding neoden, carvacrol and isovaleric acid. Further, *Salvia glutinosa* displayed different neodene composition. *Stachys annua* subsp. *annua* var. *annua* on the other side could be diffentiated based on germacrene D and linaool.

The calculated principal components PC1 (49,36 %) and PC2 (17,48 %) corresponded totally to 66,84 % of the present variation and was very useful in distinguishing the analysed material.

Sage leaves and essential oils are stated to possess carminative, antispasmodic, antiseptic, and astringent properties (Lawrence, 2005). The biological properties of essential oil of S. officinalis are attributed mainly to α- and β-thujone, camphor and 1,8-cineole (Raal et al., 2007).

The main components of the essential oil of the Stachys pecies were observed to be germacrene D, caryophyllenes, cadinene, spathuleneol and caryophyllene. The moderate antibacterial activity of β-caryophyllene and germacrene D were also reported. Germacrenes were produced as antimicrobial and insecticidal agents from Stachys species (Gören et al., 2011; Omuda et al., 2006). Moreover, the monoterpenes such as α-pinene, β-pinene, phellandrene and carvacrol were also extracted from Stachys species.

The presence, yield and composition of secondary metabolites in plants, viz. the volatile components and those occurring in essential oils, can be affected in a number of ways, from their formation in the plant to their final isolation (Figueiredo et al., 2008). Several of the factors of influence have been studied, in particular for commercially important crops, to optimize the cultivation conditions and time of harvest and to obtain higher yields of high-quality essential oils that meet market requirements. Knowledge of the factors that determine the chemical variability and yield for each species are thus very important. These include: (a) physiological variations; (b) environmental conditions; (c) geographic variations; (d) genetic factors and evolution; (e) political/social conditions; and also (f) amount of plant material/space and manual labour needs. İn our case it could be stated that different species collected from different localities and altitudes showed variation regarding their essential oil composition due to environmental conditions, geographic variations and genetic factors, which of course needs further investigation.

CONCLUSION

Lamiaceae species are very popular in folk medicine to treat various health problems such as throat infections, stomach disorders, ulcer, spasm, cold, hemorrhages and skin problems. Species of this botanical family contain a wide variety of bioactive substances that make them very important from pharmacological point of view. A large group of chemical compounds, such as mono-, di- and triterpenoids, iridoids, flavonoids, steroids, phenolic compounds, saponins, coumarins, alkaloids, tannins have been reported from the members of this family

(Richardson, 1992). The family is also famous for the presence of essential oils. Their constituents have been found to be anti-inflammatory, hemostatic, cicatrizing, stomachic, sedative, spasmolytic, diuretic, expectorant, cardiac, hypotensive (Nieto 2017; Saad et al., 2013; Okach et al., 2013).

Four Mentha species, three Origanum species, 3hree Thymus species, two Salvia species, two Stachys species and one Calamintha species were collected from 19 different localities in Rize during this study. The comparison of this species regarding their chosen main essential oil components showed that large variation exists inside the collected material. İt can be foresaid that the investigated material will serve as genetic material for further breeding purposes.

ACKNOWLEDGEMENT

This work was supported by Research Fund of the Recep Tayyip Erdogan University. Project Number 2014.112.02.02.

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Accepted 11 November 2017

Citation: Yurteri E, Özcan A, Seyis F, Kevseroğlu K. (2017). Characterisation of some Lamiaceae Species Distributed in the Rize Province, Turkey. International Journal of Plant Breeding and Crop Science 4(3): 300-307.