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Ar-Rayhan (Sweet basil, *Ocimumbasilicum* L.) Chemo Types from Different Ecological Zones in Saudi Arabia

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Abstract:- Five Saudi ARrAyhan (*O. basilicum* L.) accessions were studied to establish a possible relationship between them through their morphological characteristics and essential oil composition. The morphological parameters were recorded at the beginning of the flowering stage and the essential oils, obtained by hydrodistillation, were analyzed by gas chromatography (GC) and GC/mass spectrometry (GC/MS). Among the ARrAyhan (*O. basilicum* L.) accessions , three phenotypes were distinguished on the basis of leaf shape, area, and plant height, weight and branching. The Saudi accession varied in their proximal content as well as their mineral content. As for the composition of the essential oils, Linalool (30.8±0.0%-59.3±0.0%),Eugenol(16.9±0.0%-21.3±0.0%), 1,8-cineole (3.86±0.0%-11.6±0.0%), Limonene (3.50±0.0%-6.42±0.0%), Tau-cadinol (2.91±0.0%-4.42±0.0%), α-Phellendrene (1.19±0.0%-3.27±0.0%), E-Caryophyllene (1.57±0.0%-1.95±0.0%) and Isobornyl acetate(1.68±0.0%-1.74±0.0%)were recorded as the main constituents of the essential oils of the tested chemotypes.

***Keywords:-** ARrAyhan,Ocimumbasilicum L., Kingdom of Saudi Arabia, chemotypes,morphological characteristics, essential oilcomposition.*

I. INTRODUCTION

The Genus Ocimum belongs to the Lamiaceae family which includes approximately 50-150 species of herbs and shrubs that are found in the tropical regions of Asia, Africa and Central and South America (Daniel et al.,2011). The species of this genus differ in their characteristics, leaf size, flower colour, phenotypic characteristics and flavour.These species include ArRayhan (sweet basil Ocimumbasilicum),O. viride, O. gratissimum, O. americanus, and represent an important source of essential oils used in food and perfume industry, cosmetics and some types of Ocimum are used in many cases, in folk medicine, especially in Asia and Africa (Marwat et al.,2011).Ar-Rayhan(*O.basilicum*L.) comprises 65 species (Paton et al., 1999).It is anannual herb, 20–60 cm stem height, with white-purple flowers and is native to India and the southwestern regionsof Asia.

Medicinal and aromatic plants are, at the moment, receiving considerable attention because of their growing economic potential especially for their natural products to be used in herbal medicines, whether in local or foreign markets and ArRayhan is one of the most important of these plants. Over 80 per cent of the world's population relies on traditional medicines, largely plant-based, for primary health care (WHO,2002).Its leaves contain essential oils of distinctivearoma.The main aroma compounds from the volatile extracts of *O. basilicum* have antioxidant activity (Samson et al., J.,2007)and the aerial parts especially leavescan be usedin foodseasoning both in fresh and dried forms.

In addition to its culinary use, ArRayhan is used as a medical plant for the treatment of headache and ear pain, cough, diarrhea, constipation, worms, warts and as a folk medicine for the treatment of indigestion, nausea, abdominal cramps, inflammation of the stomach and intestine, insomnia and relieving fever and anti-malaria and to reduce inflammation, itching, bronchitis, common cold, fatigue, nervous depression, fear and as a treatment for high fat concentration in the blood(Politeo et al., 2007).ArRayhan is used against insect stings and snakes bites and its oil is placed directly on the skin to treat acne. The natural compounds in ArRayhan are used for food flavour, such as spices and sausage meat, tomato paste, vinegar, pickles and drinks as well as used in the mouthwash industry and pastes for shaving (Lawrence,1988).

ArRayhan extract is an effective antimicrobial against the yeast, Candida albicans, and some pathogenic bacteria as well as against the fungi , Aspergillus flavus, and Geotrichum species (Samson et al.,2007). Acetone and ethanol extracts of ArRayhan plant are effective against several types of pathological bacteria included Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa as well as Proteus sp. The plant extracts are effective anti-oxidant (Politeo et al.,2007).

The genotype of theplant ,the agricultural practices and the environmental conditions under which the plantgrows, greatly influencemorphological characteristics,chemical composition of the different plant parts as well as their essential oils contents and composition (Jirovetzet al.,2003).The recorded morphological differences between Ar-Rayhanecotypes include plant height, leaf shape, leaf color, leaf dimensions, and smoothness (Marwat et al.,2011).

Significant differences were noted inAr-Rayhan oil content and composition between the different plant species, and a number ofchemotypes from different geographical regions have been identified (Daniel et al.,2011).The essential oil*O. basilicum*is slightly yellowish in colourwith a characteristic smell. The essential oilyield from the different plant parts ranges between 0.15–1.59% depending on the environmental conditions that prevail in the region in which it grows (Özcan and Chalchat,2002). 1,8-cineole,methyl cinnamate, methyl chavicol, and linaloolare generally considered as the maincomponentsofAr-Rayhanessential oils.essentialoils that are found in the different plant parts and their composition determine thespecific aroma of plants 'parts and the flavour of the seasonings.*O. basilicum* L. wasclassified into four majoressential oil chemotypes including methyl chavicol-rich,linalool-rich, methyleugenol-rich and methyl cinnamate-rich as well asmany subtypes (Lawrence,1988).

Due the prevalence of the use of ArRayhan plant in the Kingdom of Saudi Arabia as spices for food cooking as well as its use in folk medicine for treating many diseases, the aim this research is to study the chemical composition and determine the nutritional value of 5 ArRayhanchemotypes that grow naturally in different regions of the Kingdom of Saudi Arabia in order to enrich the knowledge of our local natural resources and stimulate a more extensive use of Ar-Rayhan in Saudi Arabia as well as the qualitative determination of the effective chemical compounds in the plant extracts and to quantify the concentration of these compounds in the leaves.

II. MATERIAL AND METHODS

➢ *The Plant Materials*

Seeds offive accessions of Ar-Rayhan (*O. basilicum* L.) were collected from different Governate in the Kingdom [Hassa (16°17′25″N, 34°4′15″E), Hail (27°31'N), Kharaj (24°8′54″N, 47°18′ 18″E.), Medina (24°28′06″ N,39°36′51″ E) and Najran (7° 29' 32"N, 44° 7' 39" E)]. The seeds were grown in pots containing loamy sand soil.Seedlings of each accession were transplanted 3 rows each in 2×3 m plotswhich were arranged in a randomized complete block design with three replications, in a greenhouse at the Agriculture Research Farm of King Faisal University,Hafouf, Al-Hassa in Oct, 2014 for multiplication and evaluation. The accessions received the same culture practices. For each accession, 24 plants were grown in three rows, 1m apart. The soil was a well-drained sandy loam. Drip fertigation lines were placed among the rows throughout their length. Weeds were manually eliminated.They were evaluated for various morphological and essential oil characteristics.

➢ *Measurements*

Plant height, branches / plant, leaf shape, leaf length, leaf width, leaf colour, shoot fresh weight, days to 50% flowering, inflorescences / plant , inflorescence length and flower colourwere determinedbefore harvesting on three randomly selectedAr-Rayhan plants. Plant samples were harvested before flowering.The aerial parts were separated and dried using an electric oven at a temperature of 40 ° C and then milled in an electric grinder and the plant powder was stored in a glass sealed flasks at a temperature of 4 ° m to ensure no chemical degradation or damage till the time for extraction and analysis.

➢ *Proximate Analysis*

The proximate analysis of Ar-Rayhanaerial parts samples for moisture,dry matter, crude protein, crude fiber, ash and fat wasconducted in accordance to the recommended methods of (AOAC,1990).Moisture (method 14:004), ash (method 14:006),crude fiber (method 14.020), fat (method7.056) andcrude protein (method 2.057).The nitrogen was determined by micro-Kjeldahl method as described by(Pearson,1976),the percentage Nitrogen was then converted to crude protein by multiplying with 6.25. The carbohydrate content was obtained by difference. All determinations were in triplicates.

➢ *Phytochemical Screening*

The phytochemical (cardiac glycosides, tannins, saponins, flavonoids, alkaloids and resins)contentof Ar-Rayhan leaf samples was conducted onhexane, ethyl acetate and ethanolic extracts by the standard method as described by Trease and Evans(1989).

➢ *Extraction of the essential oil*

The essential oil was extracted by subjectedthe leaves of Ar-Rayhan plants to hydro-distillation for 2 hours using a Clevenger-type apparatusand thus their oil yield was determined.

➢ *Essential oil composition*

The constituents of Ar-Rayhan essential oil were determined by gaschromatography coupled to mass spectrometry(Özcan and Chalchat,2002).

➢ *Mineral composition*

In the leaves ofAr-Rayhan samples,themineral composition were determined on aliquots of the ash solutionsby established atomicabsorption/emissionspectrophotometer model 200-Aproduced by Buck Scientific. Phosphorus wasdetermined by the colorimetric method with ammonium vanado-molybdate(AOAC, 1990), chlorine was determined colorimetrically with silver nitrate.

➢ *Statistical analysis*

Statistical analysis was performed by one way ANOVA using SPSS software.The probability at $p \leqslant 0.05$ was considered for statistical significance. Data are presented as means ± standard deviations.

III. RESULTS AND DISCUSSION

➢ *Morphological characteristics*

The examined ArRayhan(*Ocimumbasilicum* L.) accessions showed significant differences in the recorded phenotypic characteristics including leaf shape, leaf length, leaf width, leaf colour and flower color (Table 1). The same trend was observed for plant height, No. ofbranches/plant, Shoot fresh weight (g), No. of inflorescences/plant and inflorescence length (Table 1). Morphological variability was recognized by several authors reporting on *O. basilicum* growing in several geographical zones of the world (e.g. Grayer et al., 1996). The morphological variability within ArRayhan species has been accentuated by the effects for centuries of environmental conditions and is characterized by great diversity in pigmentation and leaf area and shape (Simon et al., 1990).Also, there are ecotypes within these species that show insignificant morphological differences (Simon et al.,1999). The ease of cross-pollination in *O. basilicum* has resulted in a large number of species and forms(Mondello etal.,2002.). *O. basilicum* had been divided into groups based on their chemical composition to their morphological characteristics (Suchorska andOsinska, 2001).

Based on the results of this study, it was possible to consider the studied Saudi ecotypes that had similar phenotypic traits as a one group. Thus, three groups were identified: group 1 that included Hassa ecotype, which was characterized by its tallest plants as compared to those of the other ecotypes (94.6 ±3.4cm), no. of branches /plant (17.1±0.7),large green leaves (Leaf length 6.4±0.4 cm , Leaf width 3.8±0.3cm)with ovate – lanceolate shape and highest no. of Inflor./plant (23.8±2.8a) and the greatest Inflor. length (22.6 ±0.9 cm) with their white flowers (Table1), group 2 that included Hail and Medina ecotypes which had medium purplish-green leaves (Leaf length 5.6±0.2 cm , Leaf width 2.9±0.1 cm and Leaf length 5.4±0.5 cm, Leaf width 2.8±0.2 cm for Hail and Medina ecotypes , respectively) with Lanceolate shape and representing the second tallest plants when compared to those of the other ecotypes (91.3±5.6 cm,90.7±2.4 cm for Hail and Medina ecotypes , respectively), and has the second highest no. of branches/plant (15.3±0.8,15.6± 0.6 for Hail and Medina ecotypes , respectively) and this group has also the second highest no. of Inflor./plant (19.7±2.5 and 20.2 ±1.0 for Hail and Medina ecotypes , respectively) and the second greatest Inflor. length (0.4 ±0.7cm and 20.2 ±1.0cm for Hail and Medina ecotypes , respectively) with their pink flowers(Table1), and group 3 that included Kharaj and Najran ecotypes, which had small green leaves (Leaf length 4.4±0.5 cm , Leaf width 2.3±0.3 cm and Leaf length 4.2± 0.3 cm, Leaf width 2.2±0.2 cm for Kharaj and Najran ecotypes , respectively) with Ovate shape, representing the shortest plants when compared to those of the other ecotypes (84.6±2.5 cm and 83.7±1.9 cm for Kharaj and Najran ecotypes , respectively), with the lowest number of branches/plant (14.0±0.6 and 13.9± 0.5for Kharaj and Najran ecotypes, respectively) and also this group has the lowest No. of Inflor./plant (17.3 ±2.3 and 17.2±1.9 for Kharaj and Najran ecotypes , respectively) and the least Inflor. length (18.4 ±1.7 and 18.2±0.9 cm for Kharaj and Najran ecotypes, respectively) with their white flowers(Table 1). Differences in *O. basilicum* morphology are amply discussed in the literature(Massimo et al.,2004, Mondello et al.,2002, Suchorska, K. and E. Osinska. 2001 and Marotti et al.,1996). Interspecific hybridization and polyploidy which are common in the genus Ocimum were claimed to be responsible for the taxonomic confusion within the genus (Simon et al.,1999).

Table 1. Morphological traits of five ArRayhan (*O. basilicum* L.)accessions collected from different regions in KSA.

Traits	Hassa	Hail	Kharaj	Medina	Najran
Plant height (cm)	94.6 ±3.4a	91.3±5.6b	84.6±2. 5c	90.7±2.4b	83.7±1.9c
No. of branches /plant	17.1±0.7a	15.3±0.8b	14.0±0.6c	15.6± 0.6b	13.9± 0.5c
Leaf shape	Ovate - Lanceolate	Lanceolate	Ovate	Lanceolate	Ovate
Leaf length (cm)	6.4±0.4a	5.6±0.2b	4.4±0.5c	5.4±0.5b	4.2± 0.3c
Leaf width (cm)	3.8±0.3a	2.9±0.1b	2.3±0.3c	2.8±0.2b	2.2±0.2c
Leaf colour	green	Purplish green	green	Purplish green	green
Shoot fresh weight (g)	96.7±0.4a	94.4 ±0.5b	84.3±0.3c	95.1 ±0.3b	83.8 ±0.4c
Days to 50% flowering	47.5 ±2.1a	46.6 ±1.7a	42.9 ±3.3b	46.6 ±4.1a	42.4 ±2.2b
Inflor./plant	23.8±2.8a	19.7±2.5b	17.3 ±2.3c	20.2 ±1.0b	17.2±1.9c
Inflor. length (cm)	22.6 ±0.9a	20.4 ±0.7b	18.4 ±1.7c	20.2 ±1.0b	18.2±0.9c
Flower colour	white	pink	white	pink	white

Means within a row followed by the same letter are not significantly different at $P \leq 0.05$

➢ *Proximate Analysis*

Table shows (2) the percentages for some of the basic chemical ingredients in the shoots of the five ArRayhan (*O. basilicum* L) chemotypes accessions which consisted of moisture, carbohydrates, crude fiber, ash, total fat and crude protein on a dry weight basis. The ANOVA analysis showed significant differences for all the proximate variables analyzed (Table 2). This means that measurable differences in nutritional composition were evident among the five ArRayhanchemotypes accessions. The proximate composition of ArRayhan (*O. basilicum* L) chemotypes were comparable to those values reported in the literature ((Murillo-Amador et al., 2013,Khalid, 2006, Labra et al.,2004, Javanmardi et al.,2002 and Nacar and Tansi,2000).

The mean moisture content of the different ArRayhan (*O. basilicum* L) chemotypes was significantly different (Table 2). The chemotype that showed the highest value of moisture content was Hassa accession(3.67±0.1%),while the chemotype from Najran had the lowest value (2.78±0.0%)(Table 2). Ifesan et al.(2006) reported moisture content of O. gratissimum(8.11%) and *O. basilicum*(9.35%) shoots which were relatively higher to those recorded in this study. The differences in moisture content between the studied Saudi chemotypes may be due to genotypic differences since these accessions were planted under similar environmental conditions.

ArRayhan (*O. basilicum* L) chemotypes plants had high carbohydrates content which were 74.5±0.0%,72.4±0.0%, 72.1±0.0%, 71.5±0.1% and 69.5±0.1% for Najran, Medina, Kharaj, Hail and Hassachemotypes, respectively. Carbohydrates content ranged between (66.24 - 75.87%) for the three types of basil (Edeoga et al.,2006). Carbohydrate content can significantly vary between plant chemotypes and is affected by both the plant genotype and the environment (Ehdaie et al., 2008) as well as the interaction between the genotype and the environmental conditions during the growing period(Buxton and Fales,1994).Since these plants were grown in the greenhouse under the same environmental conditions, the differences were attributed mainly to genotypic differences between ArRayhan (*O. basilicum* L) chemotypes. Carbohydrates constitute a major class of natural products that are considered an essential energy source for plants, animals and human and also provide raw materials for many industries (Ebun-Oluwa and Alade,2007).

Crude fiber content showed significant differences between the tested ArRayhan (*O. basilicum* L) chemotypes (Table 2). Hassa(13.9±0.0%) and Hail(13.4±0.0 %) chemotypes had accumulated the highest crude fiber content. Medina and Najranchemotypes means for crude fiber content were significantly lower(10.2±0.0% and10.0 ±0.0%, respectively) than the other Saudi tested chemotypes (Table 2). The tested ArRayhanchemotypes had values of crude fiber lower than previously reported for O. gratissimum(Idris et al.,2011) for *O. basilicum* L (Ifesan, et al.,2006). A recent study reported values in the range of 4.79%- 10.89% under shade and 4.07%- 10.48% under Open-field conditions for twenty-four basil varieties (Murillo-Amador et al., 2013). Our data fall within these reported ranges. High values of crude fiber content could help a lot in digestion (Ifesan et al.,2006).The tested Saudi ArRayhan (*O. basilicum* L) chemotypes would represent a good source of dietary fiber. The consumption of an appropriate amount of dietary fiber can reduce cholesterol level in the blood serum and the risk of coronary heart disease, hypertension, constipation, diabetes and colon and breast cancer (Murillo-Amador et al., 2013, Emeka and Chimaobi, 2012 and Sarfraz et al.,2011).

Table 2. Proximate analysis (% dry matter) of the shoots of five ArRayhan (O. basilicum L) chemotypes accessions collected from different regions in Saudi Arabia.

Composition (%)	Hassa	Hail	Kharaj	Medina	Najran
Moisture	3.67±0.1a	3.03±0.1b	3.00±0.2b	2.97±0.1b	2.78±0.0c
Carbohydrates	69.5±0.1c	71.5±0.1b	72.1±0.0b	72.4±0.0b	74.5±0.0a
Crude fiber	13.9±0.0a	13.4±0.0a	11.9±0.0b	10.2±0.0c	10.0 ±0.0c
Ash	12.7±0.2a	9.67±0.0b	9.64±0.0b	9.60 ±0.1b	7.61 ±0.2c
Total fat	3.19 ±0.0a	3.00±0.0b	2.77±0.0c	2.97±0.0b	2.73±0.0c
Crude protein	12.0 ±0.2a	10.9 ±0.1b	10.8±0.0b	10.8±0.0b	8.3±0.0c

Means within a row followed by the same letter are not significantly different at P≤0.05.

The ash content showed significant differences between the tested ArRayhanchemotypes (Table 2).The Saudi chemotype that accumulated the highest ash content was Hassachemotype (12.7±0.2%),followed by Hail, Kharaj and Medina(Table 2). Najranchemotype had the least amount of ash(7.61 ±0.2%)(Table 2). Since ash gives a rough estimate to the mineral concentration in a plant sample (Oduntan and Olaleye 2012 and Aliero and Abdullahi 2009),the higher ash contents in the plant sample can imply high minerals contents in the sample. So, the result of this study indicates clearly that ArRayhan(*O. basilicum*) shoots constitute a rich source of mineral elements.

ArRayhan aerial parts were found to be significantly different in their total fat content (Table 2). The highest total fat content, on dry weight basis, was for Hassachemotype (3.19 ±0.0%), followed by Hail and Medina chemotypes (3.00±0.0% and 2.97±0.0%, respectively), while Kharaj and Najranchemotype had the lowest percentage (2.77±0.0% and 2.73±0.0%, respectively). These values are less than the total fat content in sweet basil which is reported by Ifesan et al.(2006).

The crude protein contents were in the range of 12.0 ±0.2%- 8.3±0.0% and there were significant differences between the tested ArRayhanchemotypes in crude protein(Table 2). These percentages are low when compared with those in other basil plants (17.94%) as reported by Edeoga(2005) as well as those reported for sweet and pink basil (20.15and 20.18%, respectively, Ifesan, et al.,2006). The result revealed that the aerial parts of ArRayhan plant are to some extent poor sources of protein.

➢ *Mineral content*

The results of the analysis of mineral composition clearly indicate that ArRayhan (*O. basilicum* L.) chemotypes accessions were found to contain significant concentrations of a number of essential elements and thus, constitute rich sources of minerals(Table 3). It reveals that the Saudi chemotypes significantly differ in their macro and micro nutrients. The results show that the aerial parts of the five ArRayhan (*O. basilicum* L.) chemotypes accessions serve as source for the mineral supplements. The highest among all the minerals contents that was obtained in the aerial parts of the five ArRayhanchemotypes tested was for K followed by Ca and then Na (Table3). Potassium content was the highest in HassaArRayhan (*O. basilicum* L.) chemotypes (4.99±0.0%) and lowest in Najran (4.66±0.0%)(Table 3). No significant differences were observed in K content between Hail, Kharaj, Medina and Najran (Table3).Potassium has a big role in protein synthesis, enzymes functions, movement of the intestinal tract stimulation, all living cells functions of and so it is found in all plant and animal tissues(Ozawa et al.,2012). Diets high in potassium can reduce the risk of hypertension(Ozawa et al.,2012).

Calcium values of the plants ranged from 3.55±0.0% in Hail ArRayhan (*O. basilicum* L.) chemotype to 2.87±0.0% in Hassachemotype(Table 3). The differences between Hail, Kharaj, Najran and Medina , Hassachemotypes in Ca content were significant. Calcium is essential for conduction of nerve impulse and activation of some enzymes, and important for building strong and healthy bones and teeth(Ettinger et al.,2007).

Sodium content varied significantly among ArRayhan (*O. basilicum* L.) chemotypes. The lowest Na content was determined in Hail chemotype (2.59±0.0%) and the highest in Najranchemotype (3.22±0.0%)(Table 3). Although Na is often considered as a cause of high blood pressure, it also has a number of essential roles in human body. Na helps to control blood pressure and in regulating muscles and nerves functioning(Nagaya et al.,2002).

Phosphorus content among the various ArRayhan (*O. basilicum* L.) chemotypes was fairly variable. Kharajchemotype had the highest P content (2.79±0.0%) and Hail chemotype had the lowest (2.24±0.0%)(Table 3). P the second most common mineral, after Ca in human tissues. 85 % of P is present in the bones and teeth. In bones, P is present in the form of phosphate. Phosphorus is involved in many functions besides forming bones and teeth. Like calcium, it is found in all cells and is involved in some way in most biochemical reactions. P is vital to energy production in the body , it provides the phosphate in adenosine triphosphate ,ATP, which is the high-energy carrier molecule for all metabolic cycles. P is essential for the use of carbohydrates and fats for energy production and in protein synthesis and in the formation of nucleic acids(Takeda et al., 2012).

Table 3. Mineral content (%) of the aerial parts of five ArRayhan (*O. basilicum* L.) chemotypes accessions collected from different regions in Saudi Arabia.

Mineral composition	Hassa	Hail	Kharaj	Medina	Najran
P	2.37±0.0b	2.24±0.0c	2.79±0.0a	2.77±0.0a	2.70±0.0a
K	4.99±0.0a	4.72±0.0b	4.68±0.0b	4.65±0.0b	4.66±0.0b
Mg	2.46±0.0b	2.36±0.0c	2.86±0.0a	2.84±0.0a	2.81±0.0a
Ca	2.87±0.0b	3.55±0.0a	3.53±0.0a	3.07±0.0b	3.54±0.0a
Na	2.68±0.0b	2.59±0.0c	2.93±0.0c	2.91±0.0c	3.22±0.0a
Fe	2.26±0.0a	2.25±0.0a	2.18±0.0b	2.17±0.0b	1.59±0.0c
Mn	1.97±0.0b	1.92±0.0b	2.01±0.0b	2.20±0.0a	1.95±0.0b
Zn	2.16±0.0b	2.21±0.0a	1.79 ±0.0c	1.78±0.0c	1.76±0.0c
Cu	1.75 ± 0.0a	1.49 ± 0.0b	1.44 ±0.0bc	1.50±0.0b	1.19±0.0c

Means within a row followed by the same letter are not significantly different at P≤0.05.

The highest content of magnesium was found in KharajArRayhan (*O. basilicum* L.) chemotype (2.86±0.0%) followed by that of Medina chemotype (2.84±0.0%) and then Najranchemotype(2.81±0.0%) which were not significantly different and lowest in Hail chemotype (2.36±0.0%) which differed significantly from all the others(Table 3). Mg is a critical co-factor in enzymatic reactions in the human body. $MgSO_4$ is extensively used in the treatment of high blood pressure and pre-eclampsia and eclampsia of pregnancy(Ozawa et al.,2012).

The highest contents of Fe and Mn obtained in ArRayhan (*O. basilicum* L.). chemotypes were 2.26±0.0% and 2.20±0.0% in Hassa and Medina chemotypes, respectively(Table 3),While the highest contents of Zn (2.21±0.0%) and Cu (1.75 ± 0.0%) were recorded in Hail and Hassachemotypes, respectively(Table 3). The lowest Fe and Mn contents of wild plants were determined as 1.59±0.0% and 1.92±0.0% in Najran and Hail chemotypes, respectively. The lowest Mn and Cu contents in plants were observed as 1.76±0.0% and1.19±0.0%, respectively in Najran(Table 3). In general micro nutrient contents of the studied ArRayhan (*O. basilicum* L.) chemotypes differed significantly between the chemotypes (Table 3). Iron is required for heamoglobin formation. Anaemia, due to iron deficiency, is widespread (Organic Information Services Private Ltd. 2013 and Akhter et al.,2005). Mn is found in all human tissues, and is required for the metabolism of carbohydrate, protein and lipid (Erikson et al.,2007). Mn is important for bone and cartilage development and healing of wounds (Stipanuk and Caudill,2012).Similarly, zinc is especially important for the normal functioning of the immune system(Zheng et al.,2007 and Prasad,2008). Cu is an essential mineral for human health and may be toxic, depending on its concentration in the diet ingested and Cu is linked to bone health, immune system and cardiovascular problems (Arya et al., 2007).

➢ *Essential oil content*

Chemical composition of the essential oils of the five ArRayhan (*O. basilicum* L.) chemotypes accessions are given in Table 4. Twenty constituents were identified in the tested chemotypes (Table 4). Linalool (27.7±0.0%-42.5±0.0%), Eugenol(7.0±0.0%-10.3±0.0%), 1,8-cineole (3.86±0.0%-5.6±0.0%), Limonene (3.50±0.0%-5.42±0.0%), Tau-cadinol (2.91±0.0%-4.53±0.0%), α-Phellendrene (1.19±0.0%-3.27±0.0%), E-Caryophyllene (1.57±0.0%-1.95±0.0%) and Isobornyl acetate(1.68±0.0%-1.74±0.0%)were found as the main constituents of the essential oils of the tested chemotypes, while. α-guaiene was found to be in Hassa, Hail and Medina chemotypes but in low or traces concentrations and it was not detected in the other two (Table 4).

Statistical analysis of the essential oil data revealed significant differences in quantitative composition. All chemotypes were characterized by high contents of linalool, followed by Eugenol , 1,8-cineole, and Limonene(Table 4). The chemotypes also differed in the presence or absence of α-guaiene and α-Terpineol(Table 4). These compounds were used for grouping ArRayhan (*O. basilicum* L.) accessions. The distribution of the main compounds of the essential oils from the three chemotype groups are shown in Table5.

The first group was formed by one accession namely Hassachemotype, which could be identified by having the highest content of the six compounds specially linalool, thus, it is the linalool chemotype. The second group included Hail and Medina chemotypes which have moderate contents of linalool, Eugenol,1,8-cineole and Limonene (Table5). This group has traces of α-Guaiene, while α-Terpineol was not detected (Table5). Within this group, Hail ArRayhan accession has a lower amount of linalool and higher contents of Limonene,1,8-cineole and Eugenol than Medina accession(Table 4). The last group was composed of two chemotypes, namely, Kharaj and NajranArRayhan accessions and was characterized by its lower contents of Linalool, Eugenol,1,8-cineole and Limonene as compared with the other groups(Table5).The members of this group showed traces of α-Terpineol , while α-Guaiene was not detected.

Table 4. Chemical Composition of tested essential oils of the aerial parts of five ArRayhan (*O. basilicum*L.) chemotypes accessions collected from different regions in Saudi Arabia.

Component	Hassa	Hail	Kharaj	Medina	Najran
Essential oil	3.19 ±0.0a	3.00±0.0b	2.77±0.0c	2.97±0.0b	2.73±0.0c
α-guaiene	0.06±0.0	tr[a]	nd[b]	tr	nd
Nerol	0.85±0.0a	0.54±0.0b	0.45±0.0c	0.54±0.0b	0.43±0.0c
Limonene	5.42±0.0a	4.43±0.0b	3.50±0.0c	4.40±0.0b	3.63±0.0c
Geraniol	0.44±0.0a	0.31±0.0b	0.17±0.0c	0.30±0.0b	0.20±0.0c
1,8-cineole	5.60±0.0a	5.10±0.0b	3.86±0.0c	4.89±0.0b	3.94±0.0c
Linalool	42.5±0.0a	35.4±0.0b	27.7±0.0c	37.3±0.0b	23.5±0.0c
Germacrene-D	0.40±0.0	0.37±0.0	0.30±0.0	0.36±0.0	0.34±0.0
Isobornyl acetate	1.68±0.0	1.72±0.0	1.70±0.0	1.74±0.0	1.69±0.0
Eugenol	10.3±0.0a	9.9 ±0.0b	7.3±0.0c	9.2±0.0b	7.0±0.0c
Tau-cadinol	3.38±0.0b	4.42±0.0a	2.91±0.0c	4.53±0.0a	2.96±0.0c
Carvacrol	0.28±0.0	0.28±0.0	0.29±0.0	0.27±0.0	0.28±0.0
Cis-γ-cadinene	1.32±0.0	1.34±0.0	1.35±0.0	1.33±0.0	1.32±0.0b
α-Phellendrene	3.27±0.0a	2.08±0.0b	1.37±0.0c	2.28±0.0b	1.19±0.0c
β-Myrcene	0.72±0.0a	0.73±0.0a	0.49±0.0b	0.68±0.0a	0.46±0.0b
α-Terpineol	0.04±0.0	nd	tr	nd	tr
E-Caryophyllene	1.72±0.0b	1.60±0.0c	1.95±0.0a	1.57±0.0c	1.90±0.0a
α-humulene	0.07±0.0	0.06±0.0	0.04±0.0	0.05±0.0	0.04±0.0
α-Pinene	0.62±0.0a	0.52±0.0b	0.40±0.0c	0.51±0.0b	0.38±0.0c
β-Pinene	0.08±0.0	0.06±0.0	0.04±0.0	0.07±0.0	0.05±0.0
Sabinene	0.09±0.0	0.10±0.0	0.07±0.0	0.09±0.0	0.08 ±0.0
Total compounds 20	78.83	68.96	53.86	70.08	49.32

a = Traces. b = Not detected Means within a row followed by the same letter are not significantly different at P≤0.05.

Table 5. The main essential oil components of the testedSaudi ArRayhan (*O. basilicum* L.) chemotypes.

Group / Compound	1	2	3
Linalool	42.5±0.0	36.4±0.0	25.6±0.0
Eugenol	10.3±0.0	9.6±0.0	7.2±0.0
1,8-cineole	5.60±0.0	5.00±0.0	3.90±0.0
Limonene	5.42±0.0	4.42±0.0	3.57±0.0
α-Guaiene	0.06±0.0	tr	nd
α-Terpineol	0.04±0.0	nd	tr

a = Traces. b = Not detected

The differences in essential oil content and composition of the collected Saudi ArRayhan (*O. basilicum* L.) chemotypes accessions may be due to environmental and genotypic factors, but since these accessions were grown under the greenhouse conditions, the differences might be due to genetic factors . In conclusion, our study has shown that the chemical composition of the essential oil obtained from the five ArRayhan (*O. basilicum* L.) chemotypes collected from different regions of KSA have different qualitative and quantitative properties.

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