GENETIC DIVERSITY, CONSERVATION, AND POTENTIAL CULTIVATION OF *CORIDOTHYMUS CAPITATUS* (L.) REICHENB. FIL. IN JORDAN

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تعتمد كلية الدراسات العليا
هذه النسخة من الرسالة
التوقيع.....التاريخ.....٥/٨/٢٠٠٩

DEDICATION

TO THE SOUL OF MY FATHER,

TO MY MOTHER,

AND

TO MY SMALL FAMILY

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First, thanks to God for helping me and providing me with health, strength and patience to complete this study.

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ABBREVIATION	WORD OR SENTENCE
AFLP	Amplified Fragment Length Polymorphism
alt.	Alteration
APS	Ammonium Per Sulphate
ANOVA	Analysis of variance
asl	Above sea level
bp	Base pair
BSA	Acetylated Bovine Serum Albumin
°C	Degree Celsius
CBD	Convention of Biological Diversity
CTAB	Cetytrimethylammonium bromide
cm	Centimeter
C.V.	Coefficient of variation
D_p	Nei standard genetic distance
DNA	Deoxyribonucleic acid
dun	Dunum
dH_2O	Distilled water
EDETA	Ethylene Diamine Tetra Acetic Acid
et al.	Abbreviation for the Latin phrase et alii meaning and others
FAO	Food and Agriculture Organization
g	Gram
GC/MS	Gas chromatography/ mass spectrometry
ha.	Hectare
hr	Hour
i.e	That is
i.g	For example
IPGRI	International Plant Genetic Resources Institute
Kg	Kilogram
L	Liter
LSD	Least significant difference
µg	Microgram
µl	Microliter

mg	Millegram
min	Minute
NCARE	National Center for Agricultural Research and Extension
ng	Nanogram
PCR	Polymerase Chain Reaction
pH	Unit measure much free or active acid in a substance
ppm	Part per million
rpm	Round per minute
sec.	Second
syn	Synonym
Taq	*Thermus aquaticus*
TBE	Tris- Boric Acid-EDETA
TE	Tris- EDETA
TEMED	Tetra Ethel Methyl Ethylene Diamine
UNDP	United Nation Development Program
UNEP	United Nation Environment Program
UPGMA	Un weighed Pair-Group Method using Arithmatic Averages
V	Volt
WHO	World Health Organization
w/w	Weight per weight
WRI	World Resources Institute
≈	Around
$	Dollar, United state of American currency
%	Percentage
±	Plus minus
√	Squre root
□	Summation

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ABSTRACT

Coridothymus capitatus (L.) Reichenb. fil., is an aromatic plant growing wild in Jordan and locally known as Za'tar Farisi. The genetic diversity, conservation, and potential cultivation study comprised fifteen wild populations of *Coridothymus capitatus,* one wild population of *Thymbra spicata* and two *Thymbra. spicata* landraces.

The investigated wild populations of *Coridothymus capitatus* showed various degrees of phenotypic variation based on the characters under investigation. Significant variations were obtained for quantitative characters, the coefficient of variation percentage (C.V %) ranged from 12.60 %. to 39.20 %. The average estimate of Shannon's diversity index (*H`*) was 0.58. The genetic distance among pairs of populations was low.

Essential oils were quantitatively analyzed using gas chromatography and gas chromatography-mass spectrometry techniques. Significant diversity was obtained among wild *Coridothymus capitatus* populations. Thymol percentage ranged from 0.03 to 0.57 %, and carvacrol percentage ranged from 0.10 to 0.90 %. Populations showed average dissimilarity of 10.68. The Unweighted Pair Group Method with Arithmatic Mean (UPGMA) cluster analysis revealed thymol and carvacrol chemotypes. Monthly quantitative changes in thymol and carvacrol were obtained.

Coridothymus capitatus populations introduced for cultivation showed a good stand and potential toward producing dry herbage yield (3046 kg/ha). Cultivated populations showed phenotypic variation in the investigated traits, and also variation in relation to their essential oil content. Thymol percentage ranged from 0.01 to 0.90 % and carvacrol percentage ranged between 0.10 and 0.87 %. The genetic diversity among cultivated population was estimated at the molecular level using Amplified Fragment Length Polymorphism (AFLP) analysis. A total of 235 bands were scored using ten selective primer combinations. Five groups of *Coridothymus capitatus* were identified by the UPGMA clustering indicating genetic variation among populations.

The results of this study indicate that a broad range of genetic variation exist among populations of *Coridothymus capitatus* collected from wild habitats in Jordan, and among *Thymbra spicata* populations. Seeds of *Coridothymus capitatus* and *Thymbra spicata* were conserved (*ex situ*) in seed bank and in the field bank. The results obtained pave the road for a potential commercial and large-scale cultivation and essential oil production from *Coridothymus capitatus* species.

1. INTRODUCTION

Jordan is located about 80 km east of the Mediterranean Sea with an area of approximately 88,878 km² (DOS, 2003). Altitude ranges from -400 m at the surface of the Dead Sea up to the 1750 m at Jebel Rum. The climate varies from semi-humid Mediterranean conditions with rainfall more than 500 mm to arid conditions with less than 200 mm, this over only 100 km distance. Four main eco-geographical regions have been recognized in Jordan (Jordan Meteorological Department, 2008); the highland mountains region, the eastern desert, the Jordan valley, and the Aqaba gulf. The variations in topography and climate revealed a wide diversity in ecological habitat and even in microhabitat, which in turn reflected a tremendous diversity in flora of the country. The richness of plant species in Jordan is estimated to be 9.1 species/100 km² is considered to be high (Danin, 2001).

Approximately 0.01% of total world flora is represented in Jordan, which includes important medicinal, herbal and aromatic species. More than 485 species from 330 genera and 99 families were listed (GCEP, 1998; Oran and Al-Eisawi, 1998; Afifi and Abu-Ermaileh, 2000). People of Jordan have been almost entirely dependent on these plants and utilized them in folk medicine and also to generate income. In addition, people grow some medicinal and aromatic plant species in their home gardens for their aroma and medicinal properties. Some of the most popular wild medicinal and aromatic plants in Jordan are *Achillea fragrantissima* (Forssk.) Sch. Bip. (Kaisoum قيصوم), *Artemisia sieberi* Asso. (Sheh شيح), *Teucrium polium* L. (Jadeh جعده), *Salvia fruticusa* Mill. (Mairameih ميرميه), *Origanum majorana* L. (مردقوش), and *Thymus* species (Za'tar زعتر) (Al Khalil, 1995).

Medicinal plants in Jordan are subjected to ecosystem and habitat degradation caused by many factors including urbanization, over exploitation and over grazing. A

number of aromatic plants were extinct like indeginous sage and origano (Haddad and Turk, 2002; Khairallah, 2005; NCSA, 2006).

In- situ conservation is one approach needed to protect and sustain medicinal plant productivity. *Ex situ* conservation is needed as a back up for the genetic material of medicinal and aromatic plant species growing in wild, and also to facilitate a direct utilization of these species in different fields. Cultivation is another approach suggested as possible mitigation to the unsustainable wild harvest of medicinal plants. This takes the pressure off the wild stock while boosting commerce in term of producing regular, uniform, and large quantities of medicinal and aromatic plants as raw material and at same time generate additional income for local communities.

The available information about Jordanian medicinal plants in aspects like phenotypic and genotypic diversity, ecology, distribution and usage is fragmentary and scattered. Most of the published materials are about listing wild species distributed in the country (El-Oqlah and Lahham, 1985; Abu- Irmaileh, 1988; Al-Eisawi and Takrori, 1989; Al-Khalil, *et al*., 1990; Oran, 1994) while few studies addressed the pharmaceutical properties of some species (Abu-Zarqa, *et al*., 1987; Alali, *et al*., 2007).

Recognizing the commerce potential of medicinal plant species native to Jordan, production of medicinal plants as raw material for export purpose is relatively weak, as most of plant species have not been utilized by the national pharmaceutical industry. Therefore, investigating the medicinal and economic value of native species is highly important. Also comprehensive agricultual and pharmaceutical studies are needed.

Among the plant species listed for their pharmaceutical and aromatic value, *Coridothymus capitatus* (L.) Reichenb. fil., is an important member of the family Labiatae which originated in Mediterranean region and is growing wild in Jordan. The *C. capitatus* is among the most valuable medicinal, aromatic, and honey bee plant. It is

considered one of the best sources for volatile oils, mainly carvacrol and thymol (Goren, *et al*., 2003; Miceli, *et al*., 2006; Rodrigues, *et al*., 2006). Globally, *C. capitatus* considered a valuable species for pharmaceutical industry and volatile oil extraction as outlined in the World Trade Organization (WTO) agreement. This plant is a well known among local population in Jordan and is currently used as culinary herb, hot drink, food preservative and additive, aromatizing and medicinal purposes. The habitat of *C. capitatus* in Jordan is highly subjected to degradation mainly caused by over exploitation and urbanization.

So far, information about *C.capitatus* in term of distribution, genetic diversity, conservation, and production potentiality is limited and almost absent in Jordan. Thus, the objectives of the present research are:

1. To explore and identify sites of distribution of *C. capitatus* in Jordan.
2. To collect seeds and herbarium specimens from sites of distribution.
3. To study the genetic diversity and the variation among *C. capitatus* populations at morphological and molecular levels.
4. To quantitatively determine the major essential oils (thymol and carvacrol) in collected populations.
5. To investigate cultivation potential of *C. capitaus* populations under field conditions and estimate the biomass yield.
6. And to conserve (*Ex situ*) the collected material at the National Centre for Agriculture Research and Extension (NCARE) gene bank and herbarium.

2. LITERATURE REVIEW

2.1. Medicinal and Aromatic Plants

Medicinal and aromatic plants represent a substantial part of the natural biodiversity. Medicinal plants are plants containing inherent active ingredients tending or used to cure or prevent diseases. Aromatic plants on the other hand, are plants which have strong characteristic smell or fragrance (King, 1992). Aromatic plants could also be medicinal plants. Plants represent a huge reservoir of secondary metabolites as they produce more than 100,000 different compounds to protect themselves and serving, at the same time, as a potential new drugs (King, 1992; Izuakor, 2005). Newman and Cragg, 2007 reported that almost 60% of all small organic drugs introduced into the market during 1982-2006 are either a natural product or derived from or inspired by a natural product.

Historically, plant medicines were discovered by trial and error. Just as people learnt to exploit plants for food, they learnt to use plants as medicine (UNESCO, 1994). Finding healing powers in plants is an ancient idea and people in all continents have long used indigenous plants for treatment of various ailments (Cowan, 1999).

The traditional knowledge of using medicinal plants for therapeutic use in many countries is acknowledged over the world (Cunningham, 1993). In Asia, India is considered the largest producer of medicinal herbs with over 3000 plants were recognized for their medicinal value and over 6000 plants are in use in traditional medicine (Dubey, *et al*, 2004). On the other hand, the successful Chinese experience in developing cultivated plantations of formerly wild-harvested medicinal plants provides a model for other countries and regions (Foster, 1993). In Africa, the socio-cultural heritage including traditional medicine has been in existence for hundreds of years (Elujoba, *et al*., 2005), an increasing reliance on the use of medicinal plants in industry

has been traced to the extraction and development of several drugs and chemotherapeutics from these plants (UNESCO, 1994). In Brazil, high attention was given toward conservation of medicinal plants distributed in tropical forest (Luiz, 2005).

The indigenous medicine of the Middle Eastern regions is similar to the traditional medicine of several other countries with an adaptation to the locally available medicinal species. The results of an ethno-pharmacological survey was conducted by Said *et al*. (2002) revealed that there are 129 plant species (including *C. capitatus*) still in use in traditional Arabic medicine for treatment of various diseases like diabetes, liver, respiratory, skin, urinary and nervous system. However, more than 30% of these herbs are rare or endangered in the region. On the other hand, Azizeh, *et al*. (2003) studied the current status knowledge of traditional Arabic medicine of local Arab practitioners in the Middle Eastern region, and found that knowledge in identification of plants was good among Bedouins and shepherds who utilize 22 major herbs for treating patients and those plants collected only from the wild. This indicates that medicinal plant species require preservation as well as ethno-botanical and ethno-pharmacological knowledge.

In Jordan, ethno-pharmacological survey of traditional drugs consumed showed that there are 236 plant species used in traditional drugs for treatment of different diseases (Lev and Amar, 2002). Moreover, Many studies investigated the antimicrobial (Mahasneh and El-Oqleh, 1999), antidiabetic (Hamdan and Afifi, 2004; Al- Mustafa and Al-Thunibat, 2008), anticancer (Abuharfeil, *et al*., 2000), antioxidant (Alali *et al*. 2007; Tawaha, *et al*., 2007) and antiulcer (Alkofahi and Atta, 1999) activities of different extracts from local plants.

Indigenous medicine is now recognized in the world as a health care resource, the World Health Organization (WHO, 1993) pointed out that traditional medicine is an important contributor to organization health goals. A bout 75-80% of the world people depend on traditional medicine because of better cultural acceptability, lesser cost, better compatibility with human body and lesser side effects (Prajapati, *et al.*, 2003).

2.2. Species *Coridothymus capitatus*

2.2.1. Taxonomy and Nomenclature

Coridothymus capitatus (L.) Reichenb. fil., is an important species of the family Lamiacea (alt. Labiatae). A dilemma has been occured in taxonomy of this species (Stahl-Biskup and Sáez, 2002). The researchers differ about the scientific name; some (Feinbrun, 1978; Greuter *et al*., 1986) placed it under genera *Coridothymus* and species capitatus in family *Labiatae*, while others (Al-Eisawi, 1982; Boulos, 1999) placed it in family *Labiatae* under genera *Thymus* and species *capitatus* and used the name *Thymus capitatus* (L.) Hoffmanns & Link to describe the plant, and consider *Coridothymus capitatus* as synonym. Other synonym used is *Satureja capitata* L. (Feinbrun, 1978).

This species was recently classified as *Thymbra capitata* (L) Cav., based on findings from investigations made by Stahl-Biskup and Sáez (2002) on *Thymus capitatus* taxon and upon evaluation of the phytochemical studies along with chemotaxonomy. Skoula, *et al*. (2004) mentioned the work of Barberan, *et al*. (1986) who found that this taxon externally accumulated the same unusual 5,6-dihydroxy-7-methoxy-flavonoid previously isolated from *Thymbra spicata* (Miski, *et al*., 1983) and occasionally reported in the genus *Thymus*. On the other hand, the 6-hydroxyflavone glycosides which is universally present in *Thymus* species was absent from *T. capitatus* (Tomas-Barberan, *et al.,* 1988)) and this supports its inclusion in the genus *Thymbra*. The allocation of this species to the genus *Thymbra* has emphasized the chemotaxonomic

value of thyme flavonoids composition (Martínez, *et al*. 2002; Stahl-Biskup and Sáez, 2002; Skoula, *et al*., 2004).

There are several common and English names of *C. capitatus* like: Conehead thyme, Persian hyssop, and Headed savory. It is known in trade as "Spanish oregano". In Jordan local people named it Za'tar Farisi (زعتر فارسي) which means Persian Thyme.

2.2.2. Botanical Description

C. capitatus is a compact shrub with grey stems, linear fleshy leaves and pink flowers, with red-tinged bracts. It is an evergreen shrub growing to 0.25 m by 0.25 m and it is in flower from July to September. The flowers are hermaphrodite and are pollinated by Bees, flies, and Lepidoptera (Davis, 1985).

Polunin (1980) described *C. capitatus* plant as a dwarf-shrub with 20-40 cm height. The branches are woody, growing in high density to form a tangled dome. Leaves are narrow, hardened and pointed, growing densely on the branches. The leaf is covered with pits, containing glandular hairs giving off a characteristic smell. The pinkish-purple flowers are arranged in a dense egg like head and the fruit is composed of four nutlets. The species name capitata comes from the Latin for 'headed', referring to the flowers grouped at the end of the stems. This species is collected throughout the year.

Feinbrun (1978) described *C. capitatus* plant growing in Jordan as a perennial shrub of 20-40 cm height. Stems are white-tomentose, erect or ascending. Leaves are rigid and linear, keeled and boat-shaped. Heads are ovoid. Floral leaves densely imbricate, flat and thinner in texture. Corolla purplish-pink and the flowering time from May to October.

2.2.3. Habitat and Geographical Distribution

C. capitatus is originally from Mediterranean basin. Danin, 2004 described the habitat of *C. capitatus* as bathas and semisteppe bathas with soils derived from

calcareous sandstone. Batha is a technical term derived from the Bible and best illustrated by a landscape dominated by *Sarcopoterium spinosum* semishrub, semi-steppe batha developed at the margins of the Mediterranean territory (Danin, 2004). Zohary and Feinbrun, 1966 defined the habitat batha as "a Mediterranean dwarf-shrub formation", they mentioned that the low arid and often spiny shrub lands in the eastern Mediterranean Basin are termed *phrygana* in Greece and batha in Israel.

C. capitatus is native and common in Mediterranean countries like Spain, Portugal, Greece, Cyprus and Tunisia (Neffati, *et al*., 2006). It is one of the phrygana vegetation of Cyprus which growing from sea level to 2,900 ft on dry rocky slopes (Della, 1995).

In Jordan, Al-Eisawi, (1996) considered *Thymus capitatus* as one of the leading species to the Mediterranean non-forest vegetation. Populations of *C. capitatus* found in Jordan were occupied habitats of Batha on rocky ground, calcareous hills and compact soil (Feinbrun, 1978, Boulos, 1999; Fragman, *et al*., 2001). Feinbrun, 1978 reported that *C. capitatus* is distributed in Jordan in Gilead (Salt), Ammon (Amman) and Moav (Madaba and Naor).

2.2.4. Ecological Value

The species *C. capitatus* has an important role in ecological studies particularly those related to pollination, palynology, demography and ecosystems. It is one of species belonging to family Labiatae, where its members were by far the most nectar rewarding species in the phrygana, both in volume and in sugar content (Petanidou, 1996). At community level, this is very important because Labiatae consist of unique group that bees rely on for energy, and most importantly for water supply in an area characterized by water shortage.

On the other hand, Petanidou (1996) cited that populations of species *C. capitatus* have the ability to support an exceptionally high number of flower visitors (123

species) and 50% of monotropous visitors (i.e. those that are exclusive to one plant species). Among these 15 species of solitary bees were recognized, the importance of *C. capitatus* for specialist bee was due to the fact that this species was the main host plant at the end of the flowering season during the hot and rainless summer.

In relation to palynological studies, Tsigouri, *et al*. (2004) mentioned that *C. capitatus* is considered an important source for production unifloral honeys. The physiochemical parameters measurements of *C. capitatus* honey revealed that it contains pollen ranging from 18.3 to 69.3% and more than 30 pollen types.

C. capitatus populations are affected by air pollution stress. Studies of plant demography in lightly, mildly and highly polluted sites indicated that reproductive deficiency in polluted plants is an indirect effect of resources limitation rather than a direct effect of air pollutants on the reproductive organs (Troumbis, 1990).

2.2.5. Economic Value

C. capitatus is known in trade as "Spanish origano". Oregano essential oils obtained from the genera *Origanum, Thymus, Coridothymus, Thymbra, Satureja and Lippia* are rich in carvacrol and thymol (Can Baser, 2008). These species have commercial importance in Mediterranean countries as well as in the world, the overall production reached 8000 ton of fresh herb per year (Goren, *et al*., 2003). Turkey is the biggest exporter of oregano herb and oil to the world markets (Can Baser, 2008).

Among the oregano oils, the essential oil obtained from *C. capitatus* is being one of the most expensive. It has been used since ancient times in pharmaceutical, food flavoring, cosmetic and perfumery (Rodrigues, *et al*., 2006). The carvacrol essential oil extracted from *C. capitatus* is considered a general tonic and immune stimulant with value of 10 ml reach $11.75 in U.S.A market (Foster, 2006).

In Tunisia, the Herbal and Medicinal Plants Project (HMPP, 2003) list *C. capitatus* as one of species of high priority, due to its ability to grow in arid areas, economic value and for export. The project economical studies showed that among 72 plant species collected and used, the sale of the medicinal plant was limited to 4 main species (*Rosmarinus officinalis*, *Thymus capitatus*, *Juniperis phoenicea*, *and Artemisia herba-alba*). The disposed mean quantity on the market was 750 kg/year. Selling prices and the sold mean quantities of the HMAP were marked by a big variability in relation to the species, the period of sale, the climatic conditions of the year and the quality of the product. The frequency of sales was more important for Thymus species than others (Neffati and Belgacem, 2006).

In Jordan, local communities of Ajloun produce "Za'tar Farisi" in their home garden. The price for fresh herb is about 2.5 $JDkg^{-1}$ and for dried leaves is 5-6 $JDkg^{-1}$. The price is not fixed as it is mostly depend on season of the year, climatic conditions, production quantity and market demand. However, the cost of 250 g of dried leaves is about 2.5 JD in local markets where the plant material is mainly imported from abroad.

2.3. Value of Genetic Diversity of Medicinal and Aromatic Plants

Biodiversity is the total variations found within all living organisms along with ecological complexis they inhabit (Wilson, 1992). It encompasses diversity at all levels of biological organizations: communities, species, individuals, and genes (Wilson, 1992, Frankel, *et al.*, 1995). Hence, genetic diversity represents the heritable variation within and between populations of organisms (WCMC, 1992) and this diversity is important to be maintained at all levels (Hawkes, *et al*., 2000).

Since ancient history, diversity among and within plant species have been recognized by farmers, herbalists and shepherds as a tangible resource for them, nowadays, people expand exploitation of the variation exist in plant as a valuable

genetic resources they can use for improving their products (WCMC, 1992). IPGRI (1993), define these genetic resources as the "Genetic material of plants which is of value as a resource for the present and future generations".

Markets for genetic resources products are large. Tenkate and Laird (1999) reviewed the annual global market price for various plant genetic resources products and suggested a figure between US $ 500-800 billion. The contribution of pharmaceutical and botanical medicine products in this figure was US $ 95-190 billion. Around 119 pure chemical substances extracted from 90 plant species are used in medicines throughout the world (WCMC, 1992). The World Health Organization listed over 21,000 plant species including *C. capitatus* with medical uses (WHO, 1993) and about 5,000 species have been investigated as potential source of new drugs (Farnsworth, 1988; Farnsworth and Soyjarto, 1991). Based on FAO statistics, the average value of world trade in medicinal plants for the period 1987-1991 was about US$ 853 million for imports and about US $ 591 million for export (Iqbal, 1993). Essential oils of thyme, mint, eucalyptus, cinnamon leaf, and camphor considered medicines which contribute in the world trade (Lewington, 1993). China is the biggest producer of medicinal plants as it accounts for 30% of total world trade. Singapore and Hong Kong are the main re-exporters while Japan, USA, Germany, France, Italy, Malaysia, Spain and USA are the major markets for medicinal and herbal plants.

In Jordan, high diversity of medicinal and aromatic plants are recognized, and some hot spots for medicinal species (e.g. *Ornethogalum* sp., *Crocus* sp., *Colchicum* sp, *Biarum* sp, *Origanum* sp., *Thymus* sp., *Salvia* sp.) have been identified (Haddad and Turk, 2002; Khairallah, 2005). However, exploiting this diversity and utilizing these species is still limited in traditional medicine and only common among local peoples and Bedouins (Haddad and Turk, 2002). Some research studies were conducted on

some Jordanian species, most of them concerned with investigation for the major active component (Mahasneh and El-Oqleh, 1999; Hamdan and Afifi, 2004; Alali, *et al.*, 2005; Alali, *et al.*, 2008; Al-Mustafa and Al-Thunibat, 2008). Thus, production of herbal drugs at commercial level is still under development.

2.4. Conservation of Medicinal and Aromatic Plants

2.4.1. The Needs for Conservation

Preservation of medicinal plants in the wild and their cultivation outside their natural habitat has assumed significance. Diversity of medicinal and aromatic species is mainly threatend by over exploitation, which decline the genetic variation and lead to genetic erosion (WCMC, 1992; Walter and Gillett, 1998). Other factors also lead to loss of genetic diversity of medicinal and aromatic plants including destruction and fragmentation of natural ecosystem, human socio-economic changes, overgrazing, and environmental degradation (Diamond, 1989; WCMC, 1992; WRI *et al*., 1992; Bhadula *et al*., 1996).

In Jordan, medicinal, aromatic and herbal plants are endangered and some are threatened with extinction. The continuous and accelerating over-exploitation of these plants in their natural habitats, combined with the increasing demands for their use, have led to destruction of the natural stocks in the wild (Haddad and Turk, 2002). The inventory study conducted by RSCN in 2002 listed around 28 medicinal and herbal plant species that are under threats and required immediate action for their conservation. Among the listed species, 6 taxa like *Artimisia sieberi* and *Teucrium polium* were found in high demand, and 9 taxa like *Artimisia judaica* and *Majorana syriaca* were found at risk because of limited abundance or limited distribution. The study indicated that it is important to focus on those of high demand for their medicinal and aromatic properties but at the same time all plant species whether rare or

endangered should be considered in a national effort for conservation. Therefore, sustainable management and conservation of these endangered species are important not only because of their value as potential therapeutics, but also due to their value in preserving ecological systems.

2.4.2. Conservation Techniques

Article 2 of the Convention of Biological Diversity (CBD) defined both *ex situ* and *in situ* conservation as follows: "*ex situ* conservation means the conservation of components of biological diversity outside their natural habitats", and "*in situ* conservation means the conservation of ecosystems and natural habitats and maintenance and recovery of viable populations of species in their natural surroundings".

Genetic conservation focuses explicitly on conserving the full range of genetic (allelic) variation within taxa (Hawkes, *et al.*, 2000). Conservation acts as a link between the genetic diversity of a species and its utilization, this was illustrated in a model developed by Maxted *et al*. (1997). Within this model, two basic conservation strategies are recommended; *in situ* and *ex situ*, each composed of various techniques.

Over continents, serious efforts afforded to protect medicinal plants facing threat. Africa experience in saving medicinal and aromatic plant resources from extinction showed that effective conservation strategies should take place within four main areas: *in-situ* conservation, *ex-situ* conservation, education and research (Okigbo, *et al*., 2008). In Egypt, a national strategy on conservation of medicinal and aromatic plants (MAP) was adopted to eliminate the continuous threats of medicinal plant growing in wild habitat (MPCP, 2006). Five main activities were integrated to preserve and sustain the natural heritage of the Egyptian wild flora: conservation *in-situ* and *ex-situ*, strengthen and sustain markets of MAP products, reduce pressure on wild genetic

resources of MAP, increase awareness and evaluate value of MAP, and develop legislation concerned with MAP protection.

Jordan ratified the CBD and was committed to achieve targets of the Global Strategy for Plant Conservation (GSPC) which aims to halt the current and continuing decline of world plant diversity by 2010. The target 8 of GSPC states that 60% of threatened plant species should be held in accessible *ex situ* collection, as well as *in situ*, preferably in their country of origin. In an attempt to match CBD objectives and GSPC targets with the national strategy for plant diversity conservation in Jordan, the NCARE, through its gene bank, contributed effectively in conservation of medicinal and aromatic plant species as well as other Jordanian species.

One of the mega project implemented in Jordan is the "Conservation of Medicinal and Herbal Plants of Jordan" which was emerged out of the crucial needs to conserve local medicinal and herbal plant resources (MoPIC, 2005). The project aiming to design and test models to promote the conservation (*in situ* and *ex situ*) of medicinal and herbal plants, and to improve the livelihood of rural communities through the management and sustainable use of medicinal and herbal plants for human and livestock needs in specific areas of Jordan (the central upper slopes of the rift valley and the Mujib nature reserve) while ensuring effective *in-situ* protection of threatened habitats and ecosystems in these areas. The project consisted of four components: public awareness and education, income generation activities, institutional strengthening, *in-situ* conservation and sustainable use of medicinal and herbal plants in cooperation with local communities, and *ex-situ* conservation through cultivation. (MoPIC, 2005). The project also, offered grants for graduate students to conduct applied research on some of the national wild medicinal, aromatic and herbal species like *Achillea fragrantissima*, *Hypericum triquetrifolium* and *Mintha aquatica*.

2.4.3. Conservation Approaches of *C. capitatus*

The wild populations of *C. capitatus* are in regression in Jordan as well as in most of Mediterranean countries such as Portugal, Italy, Greece, Cyprus and Tunisia (GCEP, 1998; Rodrigues, *et al*., 2006). This is due to various types of threat like urbanization, over exploitation, and pollution.

In Jordan, studies on this important species are scarse. The plant name appears in checklists and in reports of medicinal and aromatic plants. For example, Jordan's Country Study on Biodiversity (GCEP, 1998) listed names and status (common, not common, decreasing, endangered, and rare) of more than 45 plant species labeled as important medicinal plants. *C. capitatus* was included in the list and it described as decreasing species because of over exploitation and habitat destruction. On the other hand, Qasem (2006) cited that populations of *T. capitatus* growing wild in the Mediterranean biogeographic region of Jordan were attacked by *Osyris alba* L., a parasitic weed that form a thick and massive vegetation with long branches, which may lead to death of the host plant. *T. capitatus* populations which affected by the parasitic weed were found in Amman, Na'ur, and Zay.

Conservation of *C. capitatus* as a valuable medicinal and aromatic plant is important. Knowledge of genetic diversity among populations of *C. capitatus* is expected to have a significant impact on conservation and exploitation of its genetic resources. For the *ex situ* conservation strategy, both morphological characterization and molecular markers should contribute to the sampling, development and use of collections (Hawkes, *et al*. 2000), whereas for either on farm or *in situ* conservation strategies, molecular markers should help in the recognition of the most representative populations within the 'gene pool' of a given landrace. In Tunisia, *C. capitatus* classified as a target species for extensive conservation process because it presents

ecological, social and economical value, hence, the conservation process focused on utilization and awareness (Neffati, *et al*., 2006). In Jordan, NCARE gene bank host one population of *C. capitatus* collected in 2004 from Amman (Khalda) from elevation of 1010 masl.

2.5. Diversity Parameters

Thymus is taxonomically a very complex genus with a high frequency of hybridization and introgression among sympatric species and it does not seem to have genetic incompatibility between species (Morales, 1986; Horwath, *et al*., 2008).

The genus Thymus comprises about 150 species (Jalas & Kaleva, 1970), those are distributed throughout the arid, temperate and cold regions of the old world and on the coasts of Greenland (Morales, 1989). In Jordan, two species of the genus Thymus were listed by Al Eisawi (1982), those are: *Thymus bovie* Bentham (Syn: *T. musilii* Velen, *T. serpyllum* auct) and *T. capitatus* (L.) [Syn: *Satureja capitata* L., *Coridothymus capitatus* (L.)]. The known chromosome numbers are 2n=24, 26, 28, 30, 32, 42, 48, 50, 52, 54, 56, 58, 60, 84 and 90, this corresponded to the diploid, tetraploid, and hexaploid levels. Probably, from a basic number X=7, the secondary basic numbers X=14 and X=15 originate. The most frequent numbers are 2n= 28, 30, 56 and 60. Aneuploidy occupies an important place in the evolution of this genus and is responsible for the other numbers (Morales, 1996). Tsigouri *et al*., 2004 cited that thyme honey named *T. capitatus* has n=61. The pollen of the genus Thymus is very homogeneous.

2.5.1. Phenotypic Diversity

Knowledge about species diversity and genetic relationships among species populations could be a valuable aid in species development strategies and for domestication of wild species. Underwood, (1981) mentioned that 71% of studies are using the common statistical test ANOVA for estimation of genetic variation which

based on the contribution of genotype effect among other effects (eg. Environment) influence the phenotype of an individual. Mohammadi and Prasanna, (2003) cited that the analysis of genetic diversity in germplasm accessions and populations could be relied on morphological data, agronomic performance data, biochemical data, and more recently molecular data.

Regarding the biochemical data, the intraspecific diversity of Lamiacae and evident in the heterogeneous composition of the essential oils produced from the various species, is well known (Morales, 1996). Intraspecific diversity is highly influenced by environmental conditions but it based on genetic polymorphism. For instance, within the genus *Thymus* many species present intraspecific chemotypes (Senatore, 1996). Essential oils of *Thymbra capitata* collected from Italy were analyzed to check for chemical variability. The study showed that among the 75 components of the oils the most recurrent ones were thymol and carvacrol, which constituted more than 50% of the oils. Cluster analysis led to the identification of three chemotypes: thymol, carvacrol and thymol/carvacrol; this was presumably a crossbreed between the other two chemotypes. Results of this study agreed with results obtained by Fleisher *et al*., 1984 who studied chemovarieties of *C. capitatus* growing in Israel. They found that wild populations of *C. capitatus* growing in Israel and West Bank of Jordan river were consisted of at least three chemically distinct varieties, differing in the composition of the phenol fraction of essential oil, those were thymol containing, carvacrol containing and contain thymol and carvacrol with 1:2 ratio.

2.5.2. Molecular Diversity

Classical methods of estimating genetic diversity among groups of plants have relied upon morphological or chemical characters, but these characters can be influenced by environmental factors. By looking directly at the genetic material itself, molecular

markers represent a powerful and potentially rapid method for the characterization of diversity *per se* within the *in situ* and *ex situ* conservation of population (Ford-Lloyd 2001). In the case of medicinal and aromatic plants, the deeper insight into the genetic diversity and genetics of the formation of plant secondary metabolites will lead to efficient breeding strategies assisted by molecular markers and allow the indirect selection of traits difficult to observe morphologically (Franz and Novak, 2006).

According to Stansfield (1986), the term marker is used for "locus marker". Each gene has a particular place along the chromosome called locus. Due to mutations, genes can be modified in several forms mutually exclusives called alleles (or allelic forms). All allelic forms of a gene occur at the same locus on homologous chromosomes. When allelic forms of one locus are identical, the genotype is called homozygote (at this locus), whereas different allelic forms constitute a heterozygote. In diploid organisms, the genotype is constituted by the two allelic forms of the homologous chromosomes. Thus, molecular markers are all of loci markers related to DNA (markers can also be biochemical, or morphological).

The most frequent used markers in population genetics are allozymes (biochemical); Restriction Fragment Length Polymorphism (RFLP) (Botstein *et al*., 1980); Single Sequence Repeats (SSR) (Tautz and Renz, 1989); Random Amplified Polymorphic DNA (RAPD) (Williams, *et al*., 1990); Amplified Fragment Length Polymophism (AFLP) (Zabeau and Vos, 1993), Single Nucleotide Polymorphism (SNPs) (Wang, *et al.*, 1998), and Diversity Arrays Technology (DArT) (Jaccoud, *et al*., 2001). AFLP is a recent DNA fingerprinting technique which based on PCR amplification of selected restriction fragments of a total digested genomic DNA. Once labeled, amplified products are separated by electrophoresis and the obtained DNA fragments range from 60-5000 base pair.

Molecular studies dealing with medicinal and aromatic plants are rare in comparison with cultivated plants. This probably due to the presence of large amounts of secondary metabolites and essential oils in medicinal and aromatic plants tissues, which may inhibit DNA amplification in PCR reaction (Khanuja, *et al*., 1999; Mizukami and Okabe, 1999).

Few studies concerned with diversity of Origanum species (a member of the family Labiatae) were carried out. Ricciardi, *et al*. (2002) cited the usefulness of AFLP technique to evaluate DNA polymorphism and estimate the genetic distances among 23 Origanum accessions collected from different areas in Italy. The choice of AFLPs was due to the potential capability of this technique to detect a large level of polymorphism. Another study has been conducted by Ayanoglu, *et al*. (2006) to estimate genetic diversity of Oreganum germplasm collected from different areas in Turkey using AFLP analysis. Forty-four Origanum accessions were analyzed with 10 *Eco*RI-*Mse*I primer combinations. About 294 polymorphic bands were scored for analysis. Seven main groups were identified by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering using Jaccard's pairwise similarity coefficient. Results showed low genetic similarity (0.396 - 0.725%) between accessions indicated that the rate of gene flow between *Origanum* species was high as a result of cross-pollination.

So far, no molecular studies have been reported for estimating the genetic diversity between populations of *C. capitatus* at the molecular level. Most of available studies are concerned with chemodiversity.

2.6. Medicinal and Aromatic Properties of *C. capitatus*

2.6.1. Constituents and their Properties

C. capitatus is a well known edible and medicinal plant. It is widely used in pharmaceutical, cosmetic and perfume industry (Morales, 1996). The essential oil

extracted from the whole plant is used as antiseptic, in aromatherapy like bronchial infections, and as food flavoring (Bown, 1995; Morales, 1996).

Leaves are the edible part of *C. capitatus*. They are used raw (fresh or dry) in salads, cooked foods, and aromatic teas. If the leaves are to be dried, the plant should be harvested in early summer just before flowers opening and the leaves should be dried quickly (Hedrick, 1972; Huxley 1992).

Leaves infusion are used as a pectoral, stomachic, and to treat urinary tract infections (Karim and Quraan, 1986; Al-Khalil, 1995). The essential oil contained in the leaves is a strong antiseptic and disinfectant (Huxley 1992; Bown, 1995).

Al Mustafa and Al-Thunibat (2008) reported the moderate antioxidant and free radical scavenging activity for *C. capitatus* which could explain the traditional use of *C. capitatus* by Jordanians for diabetes treatment.

Studies reported more than 45 constituents extracted from *C. capitatus*, among these are: carvacrol, thymol, α-pinene, β-myrcene, p-cymene, γ-terpinene, caryophyllene, linalool, borneol, camphene, sabinene, and acetyl-thymol, (Arras and Grella, 1992; Hedhili, *et al*., 2005; Miceli, *et al*., 2006).

2.6.2. Essential Oil Parameters

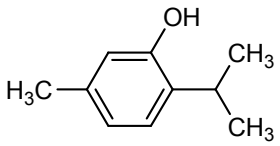
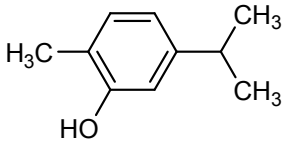
C. capitatus essential oil has been used since ancient times and it is one of the most expensive among the origanum oils (Rodrigues, *et al*., 2006). *C. capitatus* is considered one of the best sources of essential oil in term of its quality and quantity. This is due to the high density of peltate and capitate glandular trichomes present in vegetative organs which their main function is to produce phenolic rich extracts and essential oils (Rodrigues, *et al*., 2006; Sárosi and Bernáth, 2006).

C. capitatus essential oils are industrially extracted by distillation (Morales, 1996). Analysis of the essential oil is very important for the identification of volatile

components. Gas chromatography mass spectrometry (GC/MS) is a practical and reliable method for qualitative and quantitative essential oil analysis (Arras and Grella, 1992; Goren, *et al*., 2003; Hedhili, *et al*., 2005; Miceli, *et al*., 2006). GC/MS analysis of *C. capitatus* essential oil indicated that carvacrol present at 81 to 83%, *p*-cymene at 4.5 to 5%, γ-terpinene at 2.6 to 3.3%, caryophyllene at 1.5 to 1.6%, β-myrcene at 1.6%, and linalool at 1.1 to 1.2%. Carvacrol was found to be the most important fungitoxic compound among the thyme essential oil constituents (Arras and Usai, 2001). Goren *et al*. (2003) analyzed the essential oil components of *C. capitatus* which was obtained by hydrodistillation using a GC/MS method. Carvacrol (35.6%) and thymol (18.6%) were found to be the major components. Using GC and GC/MS method, carvacrol (51-77%) and thymol (9-21%) were identified as the main constituents of the essential oil of *Thymbra capitata* populations which were collected from various sites of Portugal (Rodrigues, *et al*.2006).

Of all identified constituents of the essential oil of *C. capitatus*, thymol and carvacrol were reported as being the principal components and the most bioactive ones (Arras and Grella, 1992; Goren, *et al*., 2003; Hedhili, *et al*., 2005; Miceli, *et al*., 2006). Thymol is a monoterpene phenol derivative of cymene, $C_{10}H_{14}O$, and it is an isomeric with carvacrol, $C_{10}H_{14}O$ (SDBS, 2009), (Table 1).

Table (1): Chemical structures and molecular formulas of thymol and carvacrol.

Compound Name	Molecular Formula	Chemical Structure
Thymol	$C_{10}H_{14}O$	
Carvacrol	$C_{10}H_{14}O$	

Thymol is an antiseptic and disinfectant. It has been employed in inhalations for treatment of bronchial infections of the respiratory organs. By destroying the vitality of organized and living ferments, it prevents the occurrence of putrefaction and arrests it when it has commenced (Felter and Lloyd, 1998). Carvacrol is a powerful antiseptic, an iodide of carvacrol in the form of a light yellow or brownish powder has been used as a substitute for iodoform (De Vincenzi, *et al*., 2004). The investigation study which was conducted by Can Baser (2008) on biological and pharmacological activities of *C. capitatus* essential oils showed that it is rich in carvacrol. The study illustrated that carvacrol is responsible for many biological activities such as antimicrobial, antitumor, antimutagenic, antigenotoxic, analgesic, antispasmodic, antiinflammatory, angiogenic, antiparasitic, antiplatelet, ache inhibitory, antielastase, insecticidal, antihepatotoxic and hepatoprotective activities and for gastrointestinal ailments.

Many studies (Cosentino, *et al*., 1999; Miguel, *et al*., 2003; Can Baser, 2008) reported the use of *C. capitatus* essential oil carvacrol in food systems to prevent bacterial food spoilage and extend the shelf-life of processed foods as it considered a powerful tool in controling species *L. monocytogenes*. Faleiro *et al*. (2005) tested the antilisterial activities of *Thymbra capitata* and *Origanum vulgare* essential oils against 41 strains of *Listeria monocytogenes*. The oil of *Thy. capitata* was constituted mainly by one component, carvacrol (79%), whereas for *O. vulgare* three components constituted 70% of the oil, namely, thymol (33%), γ-terpinene (26%), and *p*-cymene (11%). *Thy. capitata* essential oil had a significantly higher antilisterial activity in comparison to *O. vulgare* oil. The minimum inhibitory concentration values of *T. capitata* essential oil and of carvacrol were ranging between 0.05 and 0.2 μL/mL. In addition the essential oil of *T. capitata* showed significantly higher antioxidant activity than that of *O. vulgare*.

C. capitatus essential oil has important applications in agriculture. A trial was carried out to compare the effectiveness of *T. capitatus* essential oil and thiabendazole (TBZ) on postharvest disease (Green mold) control of mandarin fruit (Arras, *et al*., 1994). While TBZ was used at a concentration of 1000 ppm as water suspension, *T. capitatus* essential oil was fumigated as an ethanolic solution (20 ppm). Fruits treated with *T. capitatus* vapour showed a 30.1% reduction in green mold, while TBZ application gave a 39.1% reduction. *T. capitatus* essential oil caused no injury in flavedo of ripe fruits.

2.7. Cultivation Potential

Consumption of herbal medicines is widespread. Harvesting from the wild where the main source of raw material exists is causing loss of genetic diversity and habitat destruction. Domestic cultivation is a viable alternative and offers the opportunity to overcome the problems that are inherent in herbal extracts: misidentification, genetic and phenotypic variability, extract variability and instability, toxic components and contaminants (Canter, *et al*., 2005). Few medicinal plants are cultivated as crops and plant breeding has only taken place with the commercially most important plants such as *Cinchone* sp., *Chamomilla recutita* and *Mentha piperita* (Schumacher, 1991). Conventional plant-breeding methods can improve both agronomic and medicinal traits, and molecular marker assisted selection of desirable traits (Canter, *et al*., 2005).

In response to popularity increased and greater demand for medicinal plants, a recommendation of bring wild medicinal plants into cultivation system has been adopted by farmers communities in cooperation with pharmaceutical companies (Pank, 1992; WCMC, 1992). In USA, an estimated of 7600 ha was planted by medicinal and aromatic herbs during 2001 with 33.3% of this area under organic cultivation (United State department of Agriculture/Economic Research Service (USDA/ERS), 2002).

Azaizeh, *et al*. (2005) studied the potentiality of several medicinal herbs (*Cichorium pumilum*, *Eryngium creticum*, and *Teucrium polium*) for greenhouse cultivation and assessed the effects of different fertilization regimes on their growth. Seedlings were fertilized with 100%, 50%, and 20% of Hoagland solution or irrigated with tap water. Plant height was measured and the number of green leaves and branches counted and the aboveground parts of plants were harvested. It was found that either 20 or 50% Hoagland solution produced consistent response of the plant growth parameters.

The potentiality of *C. capitatus* for cultivation is still under study and limited scientific studies are available. Hedrick (1972) cited that *C. capitatus* used to be cultivated as a culinary herb in the herb gardens and mentioned that if leaves are to be dried, the plants should be harvested just before the flowers open and the leaves should be dried quickly. Huxley (1992) stated that *C. capitatus* is hardy to about -10°C but at same time prefers light, well drained calcareous soil and a sunny position. *C. capitatus* could be propagated by seeds, cuttings or layering and the best time for seedlings transfer to the field is in late spring or early summer.

Among species that are mainly used as officinal plant, *T. capitatus* show a high aesthetic ornamental value, a long and abundant flowering period, and adaptation to diverse climatic conditions and therefore might have a potential ornamental value (Iapichino, *et al*., 2006). Iapichino, *et al*. (2006) evaluated the phenotypic behavior of *T. capitatus* in order to study it's potentiality for pot cultivation using vegetative cuttings for propagation. The results were positive and *T.capitatus* showed an excellent atitude to pot cultivation.

The yield of the fresh and dry material is critical in the production process of medicinal and herbal plants. The effect of plant density on the yield of Marjoram (*Origanum syriacum*) had been studied (Abu Al Rub, 1996). Results obtained showed

that increasing plant density from 1.9 to 5.3 plants/m² resulted in increasing the total dry yield to 125 g/m². More over, the results obtained by Iapichino, *et al*. (2006) indicated the potentiality of *C. capitatus* for cultivation, which encourage the introduction, cultivation, and biological evaluation of *C. capitatus* species in Jordan.

3. MATERIALS AND METHODS

3.1. Geographical Survey and Sites Designation

Based on bibliography and flora references, populations of *C. capitatus* grow naturally in different areas in Jordan including: Salt, Amman, Madaba, Hisban, and Na'ur, and occupy habitats of non-forest vegetation, batha on rocky ground, calcareous hills and compact soil (Feinbrun, 1978; Al-Eisawi, 1996; Danin, 2004). Areas of habitats mentioned above were scouted in north, middle and south of Jordan during May-July 2005 for sites designation and collection.

The geographical survey started in April 2006. The survey covered sixteen sites where wild populations of *C. capitatus* grow. Coordinates and elevation of each site were recorded using a hand-held Geographical Positioning System (GPS, GARMINOLATHE, KS, USA) and Altimeter (Annex I). A distribution map was developed at the GIS unit- NCARE using Arc GIS; version 9.1 software (Figure 1).

Soil samples were collected from the upper 30cm from each site and analyzed for nitrogen, phosphorus, potash and other elements at NCARE soil analysis laboratories (Annex II).

3.2. Collection of Seeds and Herbarium Specimens

A total of sixteen wild populations of *C. capitatus* from the designated sites were collected from districts of Salt, Suwaylih, Abu Nusayer, Amman, Na'ur, Hisban, and Ajlun (Table 1). The collection of *C. capitatus* herbarium specimens and field data were conducted from wild populations during the blooming stage in the period from May to July 2006. A customized to plant field data registration form for the geographical survey and collection was used (Annex III) to describe site, habitat, herbarium, and seed production in each population (Maxted and Bisby, 1989; Kew, 2002).

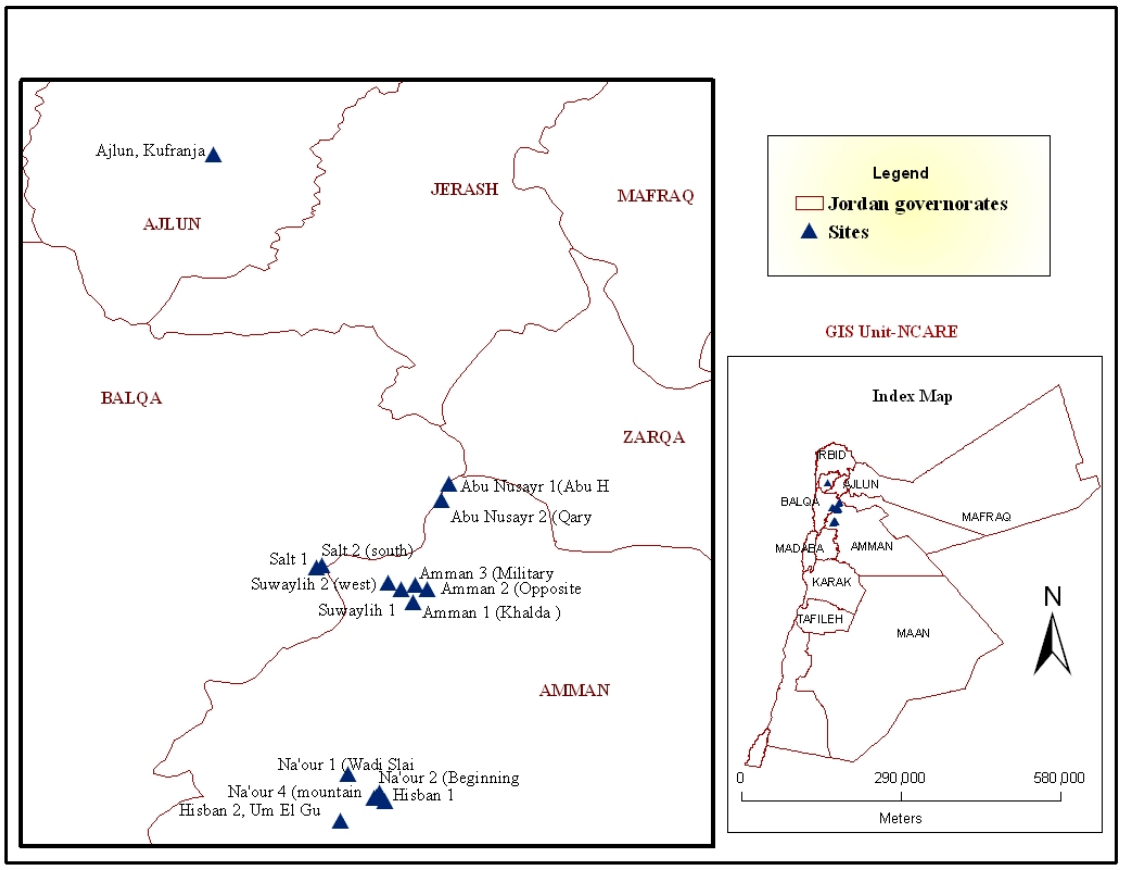


Figure (1): Distribution map of *Coridothymus capitatus* in Jordan

Table (2): Distribution sites of *Coridothymus capitatus* (L.) Reichenb. fil growing wild in Jordan during 2006.

District		Site		Coordinates			Rain fall (mm)*	Habitat
English name	Arabic name	English name	Arabic name	Longitude (E)	Latitude (N)	Altitude (m)		
Salt	السلط	Qasabat AS Salt	قصبة السلط	35° 47′ 30.2"	32° 01′ 41.0"	908	502	Batha hill side
		Al Fuhays	الفحيص	35° 47′ 43.1"	32° 01′ 40.3"	937	502	Batha and open shrub land
Suwaylih	صويلح	Tla' Kaser Khalda	تلاع قصر خلدا	35° 50′ 56.3"	32° 00′ 42.3"	1055	423	Batha adjacent Pinus trees
		Ayn Al Basha	عين الباشا	35° 50′ 24.6"	32° 00′ 46.0"	1040	423	Batha hill side
Abu Nusayr	أبو نصير	Abu Hamid village	قرية أبوحامد	35° 53′ 15.3"	32° 05′ 03.0"	833	423	Batha
		Abu Nusayr village	قرية أبو نصير	35° 52′ 36.6"	32° 04′ 20.6"	858	423	Batha
Amman	عمان	Khalda	خلدا	35° 51′ 27.1"	32° 00′ 10.1"	1047	495	Batha hill side with olive trees
		Amman (West UOJ)	غرب الجامعه الاردنيه	35° 52′ 24.0"	32° 00′ 32.9"	1043	495	Batha hill side
		Khalda military station	قاعده خلدا العسكريه	35° 51′ 38.0"	32° 00′ 43.5"	1062	495	Batha with herbaceous
Na'ūr	ناعور	Wadi Slait	وادي سليط	35° 48′ 42.8"	31° 53′ 10.6"	547	340	Batha , in steep valley
		Bela's village	قرية بلعاس	35° 50′ 07.1"	31° 52′ 23.0"	925	340	Batha with herbaceous
		Edbyan village	قرية ادبيان	35° 49′ 52.0"	31° 52′ 13.6"	880	340	Batha with Pinus trees
		Al Bassah village	قرية البصه	35° 49′ 53.0"	31° 52′ 08.5"	934	340	Batha with herbaceous
Hisban	حسبان	Wadi AS Sir	وادي السير	35° 50′ 16.9"	31° 52′ 36.0"	856	300	Batha in open rocky hills
		Um El Gutain village	قرية أم القطين	35° 48′ 27.0"	31° 51′ 13.0"	877	300	Batha road side
Ajlūn	عجلون	Kufranja	كفرنجه	35° 43′ 18.0"	32° 18′ 28.6"	677	576	Herbaceous with olive trees
		Anjarah Landrace 1	عنجره (سلاله محليه)	35° 45′ 16.0"	32° 18′ 33.4"	911	576	
		Anjarah Landrace 2	عنجره (سلاله محليه)	35° 45′ 16.0"	32° 18′ 33.4"	911	576	

* Rainfall long-term average.

Source: Jordan Meteorological Department (1998-2007).

From each population five herbarium samples were randomly collected for taxonomical verification, and also to be used in various measurements needed to estimate phenotypic diversity. Verification of the samples was done with the help of Prof. Barakat Abu Ermaileh; Faculity of Agriculture, and Prof. Dawood Al Eisawi; Faculty of Science, at the University of Jordan. Among the sixteen wild populations represented in our collection, one population collected from Kufranja was classified as *Thymbra spicata*.

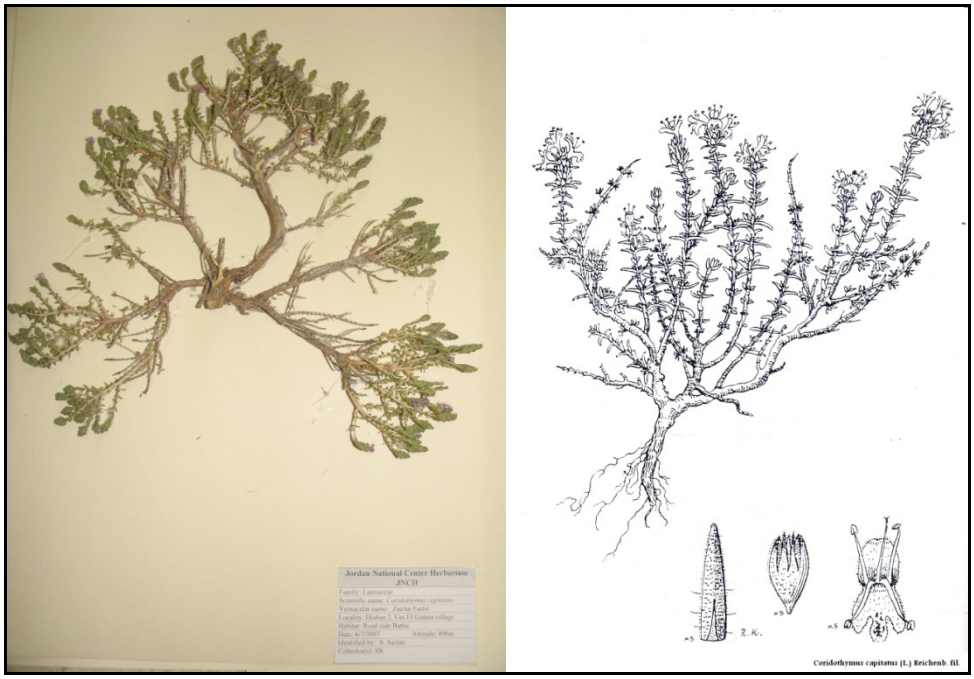
Mature seeds were collected between September and early November based on physiological maturation (Baskin and Baskin, 1998). Seeds were collected as bulk from randomly chosen individuals within each population in each site of distribution. Seeds were collected also from farmers of the local communities and considered landraces. The landraces which are known among local communities as Za'tar Farisi were classified as *Thy. spicata.* At each collecting site, passport data sheet was filled (Annex III).

Collected seeds were deposited at NCARE gene bank for conservation (*ex situ*) and to be used in various experiments. The herbarium specimens for each population were deposited at NCARE national herbarium. Plates (1, 2) show examples of *C. capitatus* and *Thy. spicata* herbarium samples collected from wild Jordan in 2006 compared with reference samples as drawn in Flora Palaestina (Feinbrun, 1978).

3.3. Diversity among Wild Populations

3.3.1. Morphological Diversity

The genetic diversity among wild populations was estimated based on morphological characteristics. In each population, five plants were randomly chosen for data meaurements. Some characters were recorded directly in wild habitat of the distribution sites like growth habit and flowering time (FGDC, 1997).



(a) (b)

Plate (1): Herbarium sample of wild *Coridothymus capitatus* (a), and a reference sample of *C. capitatus* (b) drawn in Flora Palaestina, (Feinbrun, 1978).

(a) (b)

Plate (2): Herbarium sample of wild *Thymbra spicata* (a), and a reference sample of *Thymbra spicata* (b) drawn in Flora Palaestina, (Feinbrun,1978).

The plant height, length, and width were also recorded in the field and measured in centimeters (Gardner and Danneberger, 2003; Taia and El-Etaby, 2006). Other morphological characters were obtained from collected herbarium samples which were deposited at NCARE herbarium, those include: leaf morphology (leaf shape, leaf color, leaf length, leaf width, leaf length: leaf width ratio, leaf arrangement, leaf margin, leaf apices, leaf base, leaf stipules, leaf surface, leaf attachment), stem color, petals color, length of inflorescence and spinescence.

To the best of our knowledge, no descriptors for *C. capitatus* were developed to be used as a reference guide for data measurements. Hence, a description sheet for *C. capitatus* was developed (Annex IV). Various references concerned with flora and plant systematic were used for this purpose (Zohary, 1986; Woodland, 2000).

3.3.1.1. Diversity Parameters and Phenotypic Diversity Index (*H`*)

Means, ranges, standard deviations and coefficient of variations for the sixteen populations were calculated for the measurable characters of plant height, plant length, plant width, leaf length, leaf width, leaf length: width ratio and length of inflorescence according to Steel and Torrie (1980). One-way analysis of variance (ANOVA) and the mean separation (Student's LSD) at probability level 0.05 were run on measured characters using GenStat 10th edition (GenStat, 2007) (Annex V).

Frequency distributions in percentages were calculated for the measuredqualitative characters; growth habit, leaf shape, leaf color, leaf arrangement, leaf margin, leaf apices, leaf base, leaf stipules, leaf surface, leaf attachment, stem color, petals color, and spinescence using SPSS version 13.0 (SPSS, 2004).

In order to estimate phenotypic diversity index (*H`*), continuous data of quantitative traits were converted into categorical data for each variable (i) and this was done by

dividing individuals of each population into five classes based on their values relative to the mean (μ) and the standard deviation (SD) of the population as follows:

1. Class 1 includes individuals with values xi > μ+2*SD
2. Class 2 includes individuals with values μ+1*SD < xi < μ+2*SD
3. Class 3 includes individuals with values μ-1*SD < xi < μ+1*SD
4. Class 4 includes individuals with values μ-1*SD > xi > μ-2*SD
5. Class 5 includes individuals with values xi < μ-2*SD

The Shanon diversity index (H′) was calculated for morphological traits using formula developed by Shanon (1948) and following the procedure described by Hutcheson (1970) and Tolbert *et al*. (1979). The following formula was used for calculating $h_{s.j}$ (Shanon's information statistics) for the j^{th} trait with n categories:

$h_{s.j} = - \sum Pi*\ln Pi$, for n= 1, 2, 3

Where, Pi is the relative frequency in the i^{th} category of the j^{th} trait.

The average diversity (H′) index over K character was estimated as the following:

$H' = -\sum h_{s.j} / k$

Calculations are presented in Annexes (VI, VII).

3.3.1.2. Cluster Analysis

The relationship among the sixteen wild populations was studied using cluster analysis. A proximity matrix (dissimilarity matrix) was developed based on Euclidean distances (genetic distance) then the dendrogram was drawn using UPGMA method of association (Santos *et al*., 2005; Akcin, 2006).

To investigate the separability of the sixteen wild populations of *C. capitatus* based on their morphological characters, the canonical discriminant analysis (multivariate analysis) was performed (Norusis, 1992; Mati'nez, *et al*.; 2003, Monokrousos, *et al*.

2004). Eigen values, variability percentage, and canonical discriminant function coeffients were estimated and a territorial map was developed.

The clustering analysis and the canonical discriminant analysis were performed using SPSS version 13.0 analysis program.

3.3.2. Chemical Diversity of Essential Oil among Wild Populations

3.3.2.1. Plant Materials

The plant materials of (*C. capitatus* and *Thy. spicata*) were collected from sixteen wild populations as outlined in Table (2). A representative sample of the aerial parts was collected during the flowering stage as recommended by Kizil (2005). Besides, from Abu Hamid village site (Table 2) and during the time period from March 2007 to February 2008, a monthly collection was carried out to study the yield and the chemical variability of the essential oil. Samples were spread out on large sheet of paper for dryness under shade at room temperature (22-23 °C) for 24-48 h, then sealed in paper bags and stored in a refrigerator (2-7 °C) protected from light until required for analysis (Danin, 1997; Tonçer and Kizil, 2005, 2005; Miceli, 2006). All plant materials were transported to Jordan University of Science and Technology (JUST) where the biochemical analysis was carried out at the Faculty of Pharmacy laboratories.

3.3.2.2. Essential Oil Extraction

Essential oil was extracted from each air dried plant sample by steam distillation at atmospheric pressure using closed distillation apparatus. Distillation was performed using 10 g of air dried plant material in 2.5 L distilled water for 3 h at 50 °C. The distillate was collected on a flask surrounded by ice to aid for cooling. The essential oil was recovered from the distillate by triple extraction with a mixture of hexane (C_6H_{14}) and dichloromethane (CH_2Cl_2) in a ratio of 75:25 using a separatory funnel. The organic layers were then combined and evaporated using rotary evaporator (35 °C)

leaving the essential oil. The essential oil obtained was dried over anhydrous sodium sulfate (Na_2SO_4), then weighed accurately using analytical balance and stored in amber glass vials at 4 °C until required for GC-MS analysis. The percentage yield of the obtained oil from each site of collection was calculated as weight (mg) of essential oil per 1.00 g of plant dry material (Alali, *et al*., 2004; Lee *et al*., 2005).

3.3.2.3. Determination of Thymol and Carvacrol Content in the Essential Oils

GC-MS method was used for the identification and quantification of the marker compounds: thymol and carvacrol in the essential oil of the plant samples using external reference standards. Thymol and carvacrol were identified by matching their recorded spectra with the database NIST library of mass spectra provided by the instrument software and by comparing their retention indices values with standards measured using the same experimental conditions.

3.3.2.4. Standards Preparation

In separate, two stock solutions of 1000 ppm of thymol and carvacrol (Sigma-Aldrich) were prepared by accurately weighing 100 mg of thymol/carvacrol reference standards into 100 mL volumetric flasks and then diluted to volume using n-hexane (n-hexane 96% for pesticides residue analysis). The stock solutions were then diluted using n-hexane to construct two calibration curves of four points, namely (2, 5, 10, and 15 ppm). Two quality control (QC) points (7 and 17 ppm) for the two calibration curve were also prepared.

3.3.2.5. Chromatographic Conditions and Diversity Estimation

GC (Varian Chrompack CP-3800) equipped with MS detector (Varian Saturn 2000) and DB-5 low bleed GC-MS capillary column (Zebron ZB-5, 30 m length x 0.25 mm ID x 0.25 µm df, Phenomenex, USA) was used for the identification and quantification of thymol and carvacrol. Oven temperature was increased from 40 to 250 °C with a 2

°C/min slope while the temperature of the injector and the detector was fixed at 250 °C. Extra pure Helium was used as a gas carrier, at a flow rate of 1 ml/min, and the total run time was 22 min. MS detector specifications were as follow: emission current was 70 eV and in full scan mode from 40 *m/z* to 400 *m/z*.

Aliquots (1.00 µL) of thymol and carvacrol calibration points and the QC samples were injected into the gas chromatography and two standard calibration curves were obtained for thymol and carvacrol.

From the essential oil of each sample, three sub-samples of 50 ppm were prepared using n-hexane as diluent following the procedures recommended by Lee *et al*. (2005) and Hedhili *et al*. (2005). Aliquots (1.00 µL) were injected into the GC. The concentration of thymol and carvacrol were calculated by interpolation using the constructed external standard calibration curves.

Thymol and carvacrol content in each wild population was expressed as percentage (mg/g) using the following equation:

% Thymol = Total thymol (mg) /Dry weight (1.0 g) × 100

% Carvacrol = Total carvacrol (mg) /Dry weight (1.0 g) × 100

The chemical diversity among wild populations in relation to thymol and carvacrol contents was estimated according to Miceli *et al*., 2006. Experimental design was one factor (population) complete randomized design (CRD) with three replicates. Statistical analysis and ANOVA (Annex V) conducted using Genstat 10th edition (Genstat, 2007).

3.3.2.6. Cluster Analysis

The relationship between the sixteen wild populations based on their essential oil contents was studied using cluster analysis. A proximity matrix (dissimilarity matrix) was developed based on Euclidean distances (genetic distance) then the dendrogram was drawn using UPGMA method of association (Santos *et al*., 2005; Akcin, 2006,

Miceli *et al*., 2006). The cluster analysis was performed using SPSS analysis program, version 13.0.

3.3.2.7. Correlation with Ecogeographical Data

Correlation analysis was performed for thymol and carvacrol contents and the collected ecogeographical data of altitude, rainfall and soil properties using SPSS analysis program, version 13.0.

3.3.3. Essential Oil Seasonal Variation

The essential oil of the twelve samples of *C. capitatus* which were collected on monthly basis from Abu Hamid village site during the time period from March 2007 to February 2008 were studied in terms of yield and composition (thymol and carvacrol content). The same procedures of extraction, GC-MS analysis and quantification were followed as mentioned previously.

Variation in thymol and carvacrol contents was statistically analyzed among months. Experimental design was one factor (month) complete randomized design (CRD) with three replicates. ANOVA analysis (Annex V) and mean separation $LSD_{0.05}$ were carried out using Genstat 10^{th} edition (Genstat, 2007).

3.4. Cultivation of Collected Populations

3.4.1. Plant Material

Cultivation is an important process for studying farming potential, estimating genetic variations and identifying promising populations. A total of 21 populations of Jordan's Za'tar Farisi were introduced for cultivation as follows: 15 wild populations of *C. capitatus*, 1 wild population of *Thy. spicata*, 2 landraces of *Thy. spicata*. In addition, 3 commericial cultivars of *Thymus vulgaris* were introduced to cultivation, those obtained from seed stores of Nabat (Commercial cultivar 1),Taha and Qasho' (Commercial cultivar 2), and Al Hadidi (Commercial cultivar 3) in 2007 (Table 3).

Table (3): Populations of *Coridothymus capitatus* introduced for cultivation.

No.	Site	
	English name	**Arabic name**
1	Qasabat AS Salt	قصبة السلط
2	Al Fuhays	الفحيص
3	Tla' Kaser Khalda	تلاع قصر خلدا
4	Ayn Al Basha	عين الباشا
5	Abu Hamid village	قرية أبوحامد
6	Abu Nusayr village	قرية أبو نصير
7	Khalda	خلدا
8	Amman (West UOJ)	عمان (غرب الجامعه الاردنيه)
9	Khalda military station	قاعده خلدا العسكريه
10	Wadi Slait	وادي سليط
11	Bela's village	قرية بلعاس
12	Edbyan village	قرية ادبيان
13	Al Bassah village	قرية البصه
14	Wadi AS Sir	وادي السير
15	Um El Gutain village	قرية أم القطين
16	Kufranja	كفرنجه
17	Anjarah (Landrace 1)	عنجره (سلاله محليه)
18	Anjarah (Landrace 2)	عنجره (سلاله محليه)
19	Commercial cultivar 1	صنف تجاري
20	Commercial cultivar 2	صنف تجاري
21	Commercial cultivar 3	صنف تجاري

3.4.2. Seed Processing and Seedling Establishment

The seeds collected from populations mentioned in table (3) were extracted from fruit capsules (nutlet) using rubber pads and sieves of 1.4 and 0.6 mm apertures, then seed lot of each population (around 100-150 seed) kept in a paper bag and maintained in refrigerator (4°C) for 2 weeks until sowing. On December 1st , 2006, seeds were planted in the green house of the University of Jordan.

Seeds were sown in nursery beds using polystryene trays containing mixture of peatmoss and perlite (3:1) to a depth of 5 mm then they were covered by a thin layer of peatmoss and irrigated daily, the germination was started after 20 days of sowing.

Seedlings were maintained in moist conditions under temperature of 15-25 °C for about 2 months until the seedlings height reached around 10-15 cm (Estrelles *et al*. 2004; Toncer and Kizil, 2005). Thinning process was conducted to only one seedling per cell before transplanting in permanent field.

3.4.3. Field Cultivation

The developed seedlings were transferred to Mushager Agricultural Research Station where the field conservation (*ex situ*) was established. The station is located in Madaba district, far 28 km at south east of capital Amman with coordinates of 31°42`N latitude, 35°48`E longitude and altitude of 800 masl. The site of the station has Mediterranean semi-arid climate with average annual rainfall of 316 mm. The soil physical and chemical properties of Mushager station are presented in Annex (VIII).

For preparing seedbed, regular plowing was conducted using Disc plow followed by Duck's foot plow. On April 9th ,2007, seedlings of about 120 days old were transplanted to the open field. After a week, a replanting of the missing plants was done.

A randomized complete block design RCBD with one factor and three replicates was used. Each plot consisted of single row 1.5 m long with 5 plants per plot and with a spacing of 1.4 m between rows and 30 cm within a row (Annex IX). Drip irrigation system was used and the irrigation was applied at time of planting and continued with a rate of once per week and as needed. The total amount of water applied to the experiment during first growing season was 190 L/m^2 and the irrigation was terminated on October 2007. In the second growing season (2008), supplementary irrigation was

applied only as needed during the period of June–October. The total amount of water applied during second growing season was 64 L/m^2.

The field was fertilized with an organic fertilizer before planting. Urea and phosphate were applied to 15days old seedlings at a rate of 20 Kg/hectare. A rate of 20 Kg/hectare of the water soluble fertilizer (20N:20P:20K) was applied weekly in the first 2 months through drip irrigation system. During the first (2007) and second (2008) growing seasons, no fungicides or insecticides were applied and the weeds were removed by hand picking whenever needed.

3.4.4. Morphological Characterization of Cultivated Populations

All populations planted in the field were subjected to morphological characterization. Toward blooming, the following characters were recorded following modified procedures mentioned in various publications (Simon *et al*. 1984; FGDC, 1997; Muller *et al*. 1997; Gardner and Danneberger, 2003; Toncer and Kizil, 2005 and; Taia and El-Etaby, 2006):

- Days to flowering: number of days from sowing of the seedling till the first flower opening.

- Plant height (cm): the height of the plant was measured from the ground level to the top of flowers.

- Plant width (cm): plant width was measured from the most far plant edges across the planting row.

- Plant length (cm): plant length was measured from the most far plant edges along with planting row.

- Leaf length (mm): leaf length was measured from leaf base to leaf apices under microscope using an engineering ruler with accuracy of 0.1 mm.

- Leaf width (mm): leaf width was measured from the most far leaf edges across the leaf under microscope using an engineering ruler with accuracy of 0.1 mm.

- Length of inflorescence (mm): the inflorescence (cluster of flowers) length was measured under microscope using an engineering ruler with accuracy of 0.1 mm.

- Number of inflorescence: the number of flower clusters was counted for each plant sampled in the field.

- Fresh weight (g): fresh herbage yield was measured when plant blooming percentage came to about 10% (Toncer and Kizil, 2005), a bulk of each sampled plant including stem, leaves, and flowers was harvested using handheld cutter and the plant material immediately weighed using analytical balance.

- Dry weight (g): the harvested plant material was dried under shade at room temperature (25°C± 5°C) for a week then the dry herbage yield was weighed using the same balance used for fresh weight measurement.

Data were obtained from 3 plant individuals located in the middle of each plot and the collection of data was conducted for all characters during the first and the second growing seasons except for the character days to flowering which was recorded only in the first growing season.

3.4.4.1. Estimation of Variations among Cultivated Populations

Variations among the 21 cultivated populations were estimated based on morphological characteristics. Means, ranges, and standard deviations and coefficient of variations were calculated for each character according to Steel and Torrie formulas (1980).

3.4.4.2. Statistical Analysis

All data were subjected to analysis of variance according to RCBD design and the comparison between means was conducted using least significant differences (LSD $_{0.05}$). Statistical analysis was run using the GenStat 10th edition system.

3.4.3. Chemical Variations of Essential Oil among Cultivated Populations

All cultivated populations mentioned in Table (3) were investigated for their essential oil productivity. During the first growing season (2007) and at the full blooming stage, a representative sample consisted of aerial parts of three random plants from each population was collected. Plant materials were dried following procedure mentioned previously. The same methodology for essential oils extraction, identification and quantification of thymol and carvacrol was applied.

The chemical variation among cultivated populations was estimated based on the output released from data analysis. Means and standard deviations for each population were calculated and the coefficients of variation and mean separation $LSD_{0.05}$ were estimated.

Experimental design was one factor (population) complete randomized design (CRD) with three replicates. ANOVA analysis was carried out using GenStat 10th edition.

3.4.3.1. Thymol and Carvacrol in Relation to Biomass

The content of essential oil, thymol and carvacrol obtained from cultivated the *C. capitatus* populations was studied in relation to the dry herbage yield produced in the field.

3.5. Genetic Diversity among Populations Including AFLP Markers

Variation among *C. capitatus* populations was estimated at the DNA level using AFLP markers. The analysis was conducted in molecular biology laboratories of the

Biodiversity and Integrated Gene Management program at the International Center for Agricultural Research in Dry Areas (ICARDA) headquarters, Tel Hadya, Syria, during the year 2008.

3.5.1. Plant Material

The plant material consisted of the 21 cultivated populations mentioned in Table (3). Young fresh leaves free of apparent pest or diseases were collected randomly from five plants of each population for DNA extraction. Leaves were gently cut using sterilized cutter and sealed in a labeled plastic bag then transferred immediately to ICARDA laboratory in an icebox (0°C). Ice was added frequently to maintain samples temperature at 0°C. At ICARDA, all samples were freeze dried and kept at -80°C until using.

3.5.2. DNA Extraction

The total cellular DNA was extracted from the collected leaves according to Doyle and Doyle method (Doyle and Doyle, 1988) which is based on the CTAB procedure. The protocol used to extract DNA was as follows: from each plant sample, about 200 mg of leaf tissue was first frozen in liquid nitrogen and grounded in mortar and pestle to the fine powder. 700 µL of hot (60°C) 2x CTAB extraction buffer (2% CTAB, 1.4M NaCl, 0.1M Tris-HCl pH 8, 20 mM EDTA, and 0.2% ß-mercaptoethanol) were added, mixed well, and incubated at 60°C in a water bath for 45 min. During incubation, a gentle swirling was made to get homogeneous solution free of clumps which may cause DNA degradation. After incubation, the resulting cell lysate was extracted with an equal volume of chloroform/isoamylacohol (24:1, v/v). The cell lysate was then centrifuged (at 10000_{gn} and 20°C for 10 min). The aqueous phase was transferred into another tube and the DNA then precipitated by addition of 0.7 volume of cold isopropanol (-20°C). The precipitate was collected by centrifugation (at 10000_{gn} and

20°C for 5 min). The obtained DNA pellets were washed with 70% ethanol for 15 min, dried at room temperature, and then dissolved in 1 ml of TE buffer (10mM Tris-HCl, pH 8.0, 1mM EDTA) overnight at 4°C.

3.5.3. Quantification of Genomic DNA Concentration

A preliminary test on the quality and quantity of the extracted DNA was carried out by loading in each well 2 µl of DNA solution and 6 µl of loading dye (0.25% bromophenol blue, 40% (w/v) glycerol in water) and run in gel electrophoresis (using 1% agarose in 1xTE buffer). The concentration of genomic DNA was estimated using 100 bp DNA ladder (GeneRuler™, Fermentas) of concentrations 25 and 50 ng/µL and it was confirmed by using Gene Quant (Pharmacia Biotech) spectrophotometer.

3.5.4. AFLP Analysis

The AFLP analysis was carried out following the method described by Vos, *et al*. (1995) with few modifications suggested by Jubrael *et al*. (2005). Working solutions of 40 ng/ µL were prepared for each sample and these were considered as stocks for conducting AFLP reactions.

3.5.4.1. DNA Digestion

A sample of 250 ng (6.25 µL) of genomic DNA from each population was digested for 4 hrs at 37 °C (in incubator) with 10 U each of two restriction enzymes, *Mse*I (recognition site: 5′ T↓TAA 3′) and *Pst*I (recognition site: 5′ CTGCA↓G 3′), in 20 µL final volume of reaction mix containing 10x one-phor-all (OPA) buffer (Pharmacia Biotech, Uppsala, Sweden) and 1µg/µL BSA. The master mixtures for digestion, ligation, pre-amplification and selective amplification are presented in Annex XII.

3.5.4.2. Ligation of Oligonucleotide Adaptors

After digestion, the specific double- stranded adaptors were ligated to the ends of restricted DNA fragments, generating template DNA for subsequent PCR

amplifications (pre-selective followed by selective). Ligation was carried out by adding 20 µL of a solution containing; 50 pmol of *Mse*I-adaptor and 5- pmol *Pst*I adaptor, 3 U of T_4 DNA ligase (Promega), 10 mM rATP in 10X OPA Buffer. The incubation continue at 37 °C overnight. The sequence of oligonucleotides adapters and primers used in this study are presented in Table (4).

Table (4): Sequences of oligonucleotide adaptors and primers used in the pre-amplification step and the selective AFLP primer combinations.

Name	Reaction	Code	Sequence
Pst I adaptor	Ligation		5`- CTCGTAGACTGCGTACATGCA-3` 3`- CATCTGACGCATGT-5`
*Mse*I adaptor			5`-TACTCAGGACTCAT-3` 3`-GAGTCCTGAGTAGCAG-5`
Pst I primer	Pre-amplification	P 00	5`-GACTGCGTACATGCAG-3`
*Mse*I primer		M 00	5`-GATGAGTCCTGAGTAA-3`
Pst I+ AA	Selective amplification	P- AA	5`-GACTGCGTACATGCAG**AA** -3`
Pst I+ CC		P- CC	5`-GACTGCGTACATGCAG**CC** -3`
Pst I+ GC		P- GC	5`-GACTGCGTACATGCAG**GC** -3`
Pst I+ ACA		P- ACA	5`-GACTGCGTACATGCAG**ACA** -3`
Pst I+ ACG		P- ACG	5`-GACTGCGTACATGCAG**ACG** -3`
Pst I+ AGG		P- AGG	5`-GACTGCGTACATGCAG**AGG** -3`
Pst I+ GGT		P- GGT	5`-GACTGCGTACATGCAG**GGT** -3`
Pst I+ AACC		P- AACC	5`-GACTGCGTACATGCAG**AACC**-3`
Pst I+ AACG		P- AACG	5`-GACTGCGTACATGCAG**AACG**-3`
*Mse*I+ AGT		M-AGT	5`-GATGAGTCCTGAGTAA**AGT** -3`
*Mse*I+ CAA		M-CAA	5`-GATGAGTCCTGAGTAA**CAA** -3`
*Mse*I+ CAC		M-CAC	5`-GATGAGTCCTGAGTAA**CAC** -3`
*Mse*I+ CTA		M-CTA	5`-GATGAGTCCTGAGTAA**CTA** -3`
*Mse*I+ CTT		M-CTT	5`-GATGAGTCCTGAGTAA**CTT** -3`
*Mse*I+ CCCT		M-CCCT	5`-GATGAGTCCTGAGTAA**CCCT**-3`

3.5.4.3. Pre-Amplification

After the ligation, the reaction mixture was diluted to 1:10 using sterilized distilled water and then digested and ligated DNAs were tested on 1% agarose gel. Preselective

PCR amplification was performed in a reaction volume of 20 µL containing; 50 ng of each of two oligonucleotide primers (P00 and M00) corresponding to the *Pst*I and *Mse*I adaptors, 4 µL template-DNA, 2 mM dNTPs, 5 U *Taq* DNA polymerase and 10 X PCR buffer (Roche, Mannheim, Germany). Amplifications were conducted in a ThermoCycler (PE 9600) using PCR program for 30 cycles, each cycle comprising 30 sec. at 94ºC, 30 sec. at 56ºC and 30 sec. at 72ºC.

3.5.4.4. Selective-Amplification

The pre-amplified DNA was diluted to 1:10 times using sterilized distilled water and 4 µL was used as a template for the selective amplification. The selective amplification was carried out using *Pst*I and *Mse*I primer combinations. Ten combinations were developed for polymorphism assessment and the same master mixture reagents of pre-amplification were used. Amplifications were performed in PE 9600 Thermo Cycler programmed for 36 cycles with the following cycling profile: 30 sec. at 94ºC (denaturation step), 30 sec. at 65ºC (annealing step) and a 1 min. at 72ºC (extension step). The touchdown PCR had varying annealing temperatures: in the first cycle it was 65ºC; in the second subsequent cycle for the next 12 cycles it was reduced by 0.7 ºC per cycle (touchdown PCR), and for the remaining 23 cycles, it was 56 ºC. The selective amplified products were stored at temperature of -20 ºC until electrophoresis.

3.5.4.5. Polyacrylamide Gel and Electrophoresis

The selective amplified products (AFLP fragments) were separated on 6% polyacrylamide gels (Maxam and Gilbert, 1980). Each gel was prepared using 6% acrylamide, 0.25% methylene bisacryl and 7 M Urea in 0.5x TBE (50 mM Tris, 50 mM Boric acid, 1mM EDTA pH 8.0). To 75 mL of filtered gel solution 335 µL of 10% APS and 50 µL TEMED were added then the gel solution was poured between two pre-prepared glass plates of 30 x 40 and 38 x 50 cm dimensions. To create wells, a tooth

comb was placed inside gel to about 0.5 cm, the gel dried for 2 h at room temperature and used directly or stored at 4 ºC until use. Prepared gels were protected from the over drying by putting moist tissue and aluminum foil around the two edges of the glasses before storage.

The gel plates were fixed on a Sequi-Gen 0.04 x 38 x 50 cm apparatus (Bio Rad Laboratories Inc., Hercules, CA, USA) in 0.5x TBE running buffer solution which was poured into the apparatus till half up of gel and remaining in the bottom. Before loading samples, the gel was warmed to about 60°C using power supply 1800 V for 30 min. The tooth comb was removed and the formed spaces were washed well with the inside TBE buffer using a syringe, then comb was placed again on a linear polyacrylamide edge in inverted position to prepare for easy loading.

The selective amplified products (3 µL) were mixed with an equal volume (3 µL) of 6x loading buffer (95% formamide, 10 mM EDTA, 0.09% bromophenol blue and 0.09% xylene cyanol as tracking dye), denatured for 3 min at 95 ºC and immediately placed on ice, then samples were loaded on the gel in an ordering system. Electrophoresis was performed at constant current 1800 V for 1.5 hr.

3.5.4.6. Polyacrylamide Gel Silver Staining

After completion of electrophoresis, the DNA fragments were produced by silver staining as described by Promega DNA silver staining system technical manual. Gels were washed 3 times by distilled water and then fixed with gentle agitation in 10% acetic acid (200 mL Acetic Acid, 1800 mL dH_2O) for 25 min followed by three 2 min water rinses. Silver staining was performed in a solution containing 2 g of silver nitrate and 3 mL of 37% formaldehyde in 2 L of water, for 30 min. Gels were then rinsed in water for 10 s and developed in a solution containing 60 g of sodium carbonate (Fisher Chemical); 3 mL formaldehyde; and 400 µL of sodium thiosulfate (Fisher Chemical).

Once bands were visible, the developing process was stopped by adding 10% acetic acid solution. The silver-stained gels were scanned to capture digital images of the gels after air drying.

3.5.4.7. Data Analysis

The banding patterns were analyzed directly on the enlarged scanned image by eye. AFLP bands were scored for absence (0) or presence (1) across 21 Thyme populations presented in Table (3) for each selective primer combination. The level of polymorphism was described for each primer combination as a percentage of variable loci among all analyzed loci. The genetic diversity analysis among 21 populations was performed using the data analysis software, NTSYS- version 2.02 (Numerical Taxonomy and Multivariate Analysis System, Rohlf, 1992). The genetic distance matrix was obtained using 'Nei72' algorithm according to standard genetic distance formula (Nei, 1972);

$$Dp = -\ln [\Box X_i Y_j] / \sqrt{\Box X_i Y_j}$$

Where D = Nei's standard genetic distance, Xi and Yj are frequencies of the ith and jth allele respectively drawn in populations X and Y.

A dendrogram based on genetic distance was generated using unweighted pair group method arithmatic average (UPGMA) clustering.

4. RESULTS AND DISCUSSION

4.1. Collection and Conservation of Wild Populations

Fifteen populations of *Coridothymus capitatus* were collected in a region extended from Abu Hamid village (32°05´03.0" N), district of Abu Nusayr to Um El Gutain village (31° 51´13.0" N), district of Hisban (Figure 1). Populations were distributed in altitudes varying between 547 to 1062 m and precipitation ranged between 300-502 mm (Table 2). In addition, three populations of *Thymbra spicata* were collected, one from wild habitat of Kufranja (32°18´28.6" N) and two landraces from Anjara (32°18´33.4" N) located in Ajlūn district in north Jordan. These are also named Za'tar Farisi, indicating that one common name is used for multiple species in Jordan.

C. capitatus and *Thy. spicata*, representative herbarium specimens were collected from wild populations in each designated site and prepared to be an ideal specimens for conservation at NCARE herbarium (Plates 1, 2). Each voucher plant specimen contained the following information: common name, scientific name, coordinates and altitude, habitat, date of collection, and name of collector. Seeds were collected from wild populations in each distribution site and were conserved at NCARE genebank (*ex situ*). A passport data joint with each seed samples were provided to be included in genebank data base system.

Populations of *C. capitatus* were found in hillsides, where they occupied Batha habitat in a semi–humid Mediterranean climate. According to Feinbrun, (1978), *C. capitatus* is distributed in Jordan in Gilead (Salt), Ammon (Amman) and Moav (Madaba and Na'ūr). Boulos, (1999) and Fragman, *et al*. (2001) reported that the *C. capitatus* grow in Jordan occupy habitats of Batha on rocky ground, calcareous hills and compact soil. Al-Eisawi, (1996) considered *C. capitatus* as one of the leading species in the Mediterranean non-forest vegetation. Information revealed by the

geographical survey conducted in this study, indicate that populations of *C. capitatus* species growing wild in Jordan are fragmentary distributed in a limited region in the middle of Jordan and relatively close to Amman, where urbanization is accelerating. This subjected *C. capitatus* populations to high degradation threat mainly caused by urbanization, over-exploitation, and by air pollution. Rodrigues, (2006), reported that habitat destruction and excessive harvest of *C. capitatus* growing wild in Portuguese are the major factors that lead to disappearing of this species from nature. Troumbis, (1990) reported a direct effect of air pollutants on the reproductive organs of *C.capitatus*. In addition, *C. capitatus* was classified in Jordan Country Study on Biodiversity as a decreasing species threat by over-exploitation (GCEP, 1998). Thus, an efficient conservation strategy based on understanding of the genetic diversity of this species needs to be adopted.

The collected and conserved plant materials of *C. capitatus* are considered a valuable genetic resource. Herbarium specimens are very important references for taxonomical, botanical, and biodiversity studies (Polunin, 1980; Rodrigues, 2006). Seeds are critical source of genetic material conserved (e*x situ*) under controlled conditions and considered a backup of *C. capitatus* populations distributed in wild habitat. The conserved seeds are also available as an identified plant material ready for direct utilization. The conservation process followed in this study could mimic the *ex situ* conservation model developed by Maxted *et al*. (1997) which proposed steps for conservation of a particular species, among these steps, the geographical survey and conservation (*ex situ*) are considered major steps.

4.2. Diversity among Wild Populations

C. capitatus has been reported for its medicinal and ecological value (Morales, 1996; Petanidou, 1996). Attention has been mainly directed toward the composition of

its essential oil and medicinal properties (Hedhili *et al*., 2005; Miceli *et al*., 2006). However, no previous study was conducted on *C. capitatus* phenotypic diversity in Jordan. Estimation of environmental variability and better understanding of diversity among *C. capitatus* populations growing under natural conditions will be very helpful in valorizing and promoting this species as a crop plant, and in developing biodiversity preservation strategies and minimizing genetic erosion.

4.2.1. Phenotypic Variation among Wild Population

The investigated wild populations of *C. capitatus* showed various degrees of variation based on the location and the characters under investigation (Tables 5, 6) which are discussed in the following sections:

Plant height

The coefficient of variation (C.V.) for plant height was high (26.8%), populations showed wide range of plant height (17.3-46.0 cm), indicating high variation among populations. Population of Tla' Kaser Khalda recorded the highest average plant height (46.0 cm) and Al Fuhays population recorded the lowest (17.3 cm). The recorded overall mean was 30.7 cm and it is in agreement with Feinbrun, (1978) and Polunin, (1980) description that *C. capitatus* height ranged between 20-40 cm under natural condition in batha habitat.

Plant length and width

The plant dimensions, both width and length varied among populations as shown in Table (5). Coefficients of variations (C.V.) are 35.4 %, and 39.2 %, with ranges between (25.8-79.8 cm) and (29.0-74.2 cm) respectively, indicating high variation among populations. Among sixteen populations, Um El Gutain population recorded the highest plant width (79.8 cm) exceeding the overall mean by 25.7%, with ranges varying between 30.0 and 130.0 cm. Also this population recorded high plant length

(68.4 cm) exceeding the overall mean by 14.8%, and with ranges varied between 44.0 and 100.0 cm, indicating large dimensions of plant individuals (79.8 cm by 68.4 cm) . Davis, (1985) reported that *C. capitatus* inhabit wild habitat are occupying canopy of about 0.25 m by 0.25 m in dimensions. It is worth to mention that dry rocky slopes are characterizing Um El Gutain site, where population individuals are fragmented due to extensive urbanization and over collection. Hence, the dimentions of individual plant (width, length) could increase due to limited competition among individuals, and in the same time the expanded of plant individuals over rocks searching for water. The long and far-reaching roots and the woody branches growing in high density forming a tangled dome help plant to make a good stand with ideal size (Polunin, 1980). Studying variations of the vegetation morphological characters especially length and width is critical to develop preservation strategy (*in situ*) particularly in restoration technology. According to Taia and El-Etaby, (2006), the most vegetative characters affected by different regions are plant length and width, and color of flowers.

Leaf length and width

Leaves are the edible part of *C. capitatus* and considered the main source of essential oil because they are covered with pits, containing glandular hairs emanating a characteristic smell (Morales, 1996; Polunin, 1980; Hedhili *et al*., 2005). However, as other vegetative morphological characters, they exhibit variations among locations. Our results showed various degrees of variations among population, the recorded C.V.'s for leaf length and width were 24.6 % and 12.6 %, respectively. The highest leaf length was recorded by Kufranja population (11.4 mm), followed by Edbyan population (5.0 mm) and were significantly different. For leaf width, significant difference was recorded between Kufranja population (mean=2.4 mm) and all rest populations (average mean= 1.0 mm), while no significant difference was found among the fifteen

populations. The results obtained for traits of leaf length and leaf width indicated that the wild population growing in Kufranja and named Za'tar Farisi is a different species with different genetic base from other *C. capitatus* populations growing wild in Jordan, and this needs to be confirmed by extra morphological, biochemical and molecular analysis.

Leaf length: width ratio

Information about leaf length: width ratio is important to describe leaf shape and to understand vegetation cover and photosynthesis potential of any species (Taia and El-Etaby, 2006). The coefficient of variation (C.V.) related to this character was (26.3%), indicating high variation among population, this variation could be exploited in developing this species as a crop plant for oil and herb production. Kufranja population recoded the highest leaf length: width ratio (5.02), followed by Edbyan population (5.0) with no significant difference between them. The ratio varied among *C. capitatus* populations between 3.0 and 4.6, indicating narrow leaf shape for all populations and this agreed with *C. capitatus* leaf description cited by Polunin, (1980).

Length of inflorescence

High coefficient of variation (C.V.= 29.30 %) among populations was recorded with average mean of 9.16 mm and range between 6.63 and 12.13 mm. Kufranja population recorded the highest average Length of inflorescence (28.0 mm), followed by Edbyan population (11.8 mm) with highly significant difference. The rest of the populations of *C. capitatus* showed average inflorescence length varying between 4.20 to 10 mm indicating high variations among them.

Flowers of *C.capitatus* are arranged in a dense egg like head and the species name capitata comes from the Latin for 'headed', referring to the flowers grouped at the end of the stems (Feinbrun, 1978; Polunin, 1980). Flowers are hermaphrodite and pollinated

by bees, flies, and Lepidoptera (Davis, 1985). Information about inflorescence length is limited although they are very helpful in biodiversity preservation strategies and ecosystem restoration of dry hill slopes and transitional district called Batha (Margaris, 1976). This character is also important in studies of pollination, palynology, and insect taxonomy (Petanidou, 1996; Tsigouri *et al*. 2004). Petanidou, 1996 cited that *C.capitatus* inhabit wild habitat of east Mediterranean supported an exceptionally high number of flower visitors (123 species) and 50% of the monotropus visitors like large size, and long tongued bees, which may explain the high variation obtained in our study for this character.

Qualitative traits

Variations among sixteen wild populations of *C. capitatus* for qualitative traits varied according to investigated trait (Table 7). Among thirteen studied traits, only five: leaf shape, leaf color, leaf apices, stem color, and petal color, exhibited polymorphism (traits recorded distinct classes). The highest polymorphism referred to petal color (4 distinct classes) followed by stem color (3 distinct classes). It is worth to mention that polymorphism obtained for leaf shape and leaf apices was referred Kufranja population as no variation for these traits found among rest populations. This indicate that Kufranja population is genetically different from other *C. capitatus* populations, also this indicate that trait related to colors is less stable than other descriptive characters and affected by different locations. Taia and El-Etaby, (2006) pointed that floral morphological characters are more stable except traits of flower and fruit color, this may due to number and interaction of genes responsible for expression of flower and fruit color.

The rest eight traits studied showed no variation among population neither between individuals in each population. All population exhibited dwarf shrub growth habit

where all plants showed low-growing not exceeding 1.0 m tall at maturity (FGDC, 1997). Leaves in all populations showed ciliate margin, opposite arrangement, sessile attachment, acute base, absent stipules, and glandular surface. No spinescences were observed in all plants. Most of the obtained results agreed with taxonomical description of *C. capitatus* species reported in various plant systematic references (Feinbrun, 1978; Polunin, 1980; Greuter *et al*., 1986) indicating that these characters are genetically stable and can use for identification of *C. capitatus* taxon.

As it evident from the above discussion, wide interpopulation variability among 16 wild populations of *C. capitatus* in Jordan recorded for most of the characters observed. Significant variations were recorded for quantitative characters, the highest C.V. recorded for plant length character (39.20 %) and the lowest recorded for leaf width character (12.6%) indicating that variation degrees are affected by location where populations are distributed as well as characters under investigation. On the other hand, low allelic frequencies were obtained for a limited number (leaf shape, leaf color, leaf apices, stem color, and petal color) of descriptive traits while other descriptive traits presented monomorphism, indicating strong genetic base associated with qualitative traits. Hence, molecular investigation is needed for better understanding of genetic variation.

4.2.2. Estimates of Diversity Indices (*H`*)

The variation degrees related to characters under investigation could be estimated using Shannon's diversity index (*H`*). Among twenty characters investigated, variation or polymorphism was common in varying degrees for twelve characters. Thus, indicating a wide variability among *C. capitatus* populations growing wild in Jordan. Estimates of (*H`*) for individual trait are presented in Table (8).

Table (5): Phenotypic variation for plant height (cm), plant width (cm), and plant length (cm) of *Coridothymus capitatus* populations growing wild in Jordan during 2006.

Population	Plant height (cm)		Plant width (cm)		Plant length (cm)	
	Mean ± SD	Range	Mean ± SD	Range	Mean± SD	Range
Qasabat AS Salt	28.00 ± 7.12	22.00 - 38.00	38.20 ± 14.39	20.00 - 58.00	32.40 ± 12.58	18.00 - 50.00
Al Fuhays	17.25 ± 7.59	11.00 - 28.00	39.20 ± 10.18	24.00 - 52.00	38.80 ± 13.48	26.00 - 58.00
Tla' Kaser Khalda	46.00 ± 7.87	37.00 - 56.00	53.20 ± 9.42	40.00 - 63.00	51.60 ± 18.57	35.00 - 83.00
Ayn Al Basha	19.75 ± 9.74	10.00 - 30.00	61.40 ± 27.37	32.00 - 97.00	55.60 ± 29.22	21.00 - 93.00
Abu Hamid	21.75 ± 2.06	19.00 - 24.00	25.80 ± 3.90	19.00 - 28.00	31.60 ± 8.62	27.00 - 47.00
Abu Nusayr	20.75 ± 1.50	19.00 - 22.00	26.80 ± 1.79	24.00 - 28.00	31.60 ± 8.62	27.00 - 47.00
Khalda	37.75 ± 9.84	29.00 - 49.00	70.00 ± 30.32	40.00 - 120.00	66.80 ± 27.06	41.00 - 110.00
Amman (West UOJ)	30.50 ± 10.50	24.00 - 46.00	63.00 ± 15.80	37.00 - 80.00	62.00 ± 20.00	28.00 - 80.00
Khalda (military St.)	41.50 ± 10.97	30.00 - 54.00	58.60 ± 23.92	37.00 - 97.00	64.00 ± 18.01	42.00 - 90.00
Wadi Slait	30.25 ± 5.12	23.00 - 35.00	67.40 ± 8.02	55.00 - 76.00	70.20 ± 23.00	36.00 - 100.00
Bela's	36.00 ± 5.94	28.00 - 41.00	69.40 ± 18.23	55.00 - 100.00	74.20 ± 38.64	44.00 - 140.00
Edbyan	40.00 ± 6.88	30.00 - 45.00	59.00 ± 16.34	40.00 - 82.00	51.60 ± 21.38	23.00 - 82.00
Al Bassah	30.50 ± 5.92	24.00 - 36.00	66.20 ± 13.63	52.00 - 86.00	62.80 ± 24.53	41.00 - 100.00
Wadi AS Sir	37.25 ± 6.70	32.00 - 47.00	59.60 ± 20.13	27.00 - 80.00	66.40 ± 16.46	38.00 - 79.00
Um El Gutain	38.00 ± 14.49	27.00 - 59.00	79.80 ± 39.05	30.00 - 130.00	68.40 ± 23.55	44.00 - 100.00
Kufranja	29.25 ± 2.63	27.00 - 33.00	27.40 ± 2.88	23.00 - 30.00	29.00 ± 4.30	23.00 - 35.00
Mean	31.53 ± 7.18	24.50 - 40.19	54.06 ± 15.96	34.69 – 75.44	53.56 ± 19.25	32.13 – 80.88
% C.V.*	26.8		35.40		39.20	
$LSD_{0.05}$**	10.4		24.24		26.54	

*C.V. = Coefficient of variation, **LSD = Least significant difference.

Table (6): Phenotypic variation for leaf length (mm), leaf width (mm), leaf length:width ratio, and inflorescence length (mm) of *Coridothymus capitatus* populations growing wild in Jordan during 2006.

Population	Leaf length (mm)		Leaf width (mm)		leaf length : width ratio		Inflorescence length (mm)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Qasabat AS Salt	3.40 ± 0.55	3.00 - 4.00	1.00 ± 0.00	0.00	3.40 ± 0.55	3.00 - 4.00	7.00 ± 3.67	1.00 - 10.00
Al Fuhays	3.40 ± 0.55	3.00 - 4.00	1.00 ± 0.00	0.00	3.40 ± 0.55	3.00 - 4.00	8.80 ± 1.30	7.00 - 10.00
Tla' Kaser Khalda	4.00 ± 1.00	3.00 - 5.00	1.00 ± 0.00	0.00	4.00 ± 1.00	3.00 - 5.00	7.80 ± 1.30	7.00 - 10.00
Ayn Al Basha	3.60 ± 1.34	2.00 - 5.00	1.00 ± 0.00	0.00	3.60 ± 1.34	2.00 - 5.00	4.20 ± 0.84	3.00 - 5.00
Abu Hamid	4.00 ± 0.71	3.00 - 5.00	1.00 ± 0.00	0.00	4.00 ± 0.71	3.00 - 5.00	7.20 ± 1.30	6.00 - 9.00
Abu Nusayr	4.00 ± 0.71	3.00 - 5.00	1.00 ± 0.00	0.00	4.00 ± 0.71	3.00 - 5.00	7.20 ± 1.64	6.00 - 10.00
Khalda	4.20 ± 1.64	3.00 - 7.00	1.00 ± 0.00	0.00	4.20 ± 1.64	3.00 - 7.00	6.40 ± 2.07	5.00 - 10.00
Amman (West UOJ)	4.60 ± 1.14	3.00 - 6.00	1.00 ± 0.00	0.00	4.60 ± 1.14	3.00 - 6.00	8.00 ± 2.92	5.00 - 12.00
Khalda military st.	4.00 ± 1.22	3.00 - 6.00	1.00 ± 0.00	0.00	4.00 ± 1.22	3.00 - 6.00	7.80 ± 3.11	5.00 - 13.00
Wadi Slait	3.60 ± 1.34	3.00 - 6.00	1.00 ± 0.00	0.00	3.60 ± 1.34	3.00 - 6.00	9.40 ± 2.30	6.00 - 12.00
Bela's	3.80 ± 0.84	3.00 - 5.00	1.00 ± 0.00	0.00	3.80 ± 0.84	3.00 - 5.00	7.40 ± 1.95	6.00 - 10.00
Edbyan	5.00 ± 0.71	4.00 - 6.00	1.00 ± 0.00	0.00	5.00 ± 0.71	4.00 - 6.00	11.80 ± 2.05	10.00 - 15.00
Al Bassah	4.00 ± 0.71	3.00 - 5.00	1.00 ± 0.00	0.00	4.00 ± 0.71	3.00 - 5.00	6.80 ± 1.92	5.00 - 10.00
Wadi AS Sir	3.00 ± 0.71	2.00 - 4.00	1.00 ± 0.00	0.00	3.00 ± 0.71	2.00 - 4.00	8.80 ± 1.10	7.00 - 10.00
Um El Gutain	3.20 ± 0.45	3.00 - 4.00	1.00 ± 0.00	0.00	3.20 ± 0.45	3.00 - 4.00	10.00 ± 2.55	7.00 - 13.00
Kufranja	11.40 ± 2.19	10.00 - 15.00	2.40 ± 0.55	2-3	5.02 ± 1.80	3.30 - 7.50	28.00 ± 6.71	20.00 - 35.00
Mean	4.33 ± 0.99	3.38 – 5.75	1.09 ± 0.03	1.06 -1.13	3.93 ± 0.96	2.96 – 5.28	9.16 ± .30	6.63 -12.13
% C.V.*	24.60		12.60		26.30		29.30	
LSD $_{0.05}$**	1.35		0.17		1.30		3.39	

*C.V. = Coefficient of variation, **LSD = Least significant difference.

Table (7): Phenotypic diversity for thirteen descriptive characters of *Coridothymus capitatus* populations growing wild in Jordan in 2006.

Trait	Leaf shape		Leaf color		Leaf apices		Stem color			Petal color				Growth habit	Leaf margin	Leaf surface	Leaf attachment	Leaf arrangement	Leaf base	Leaf stipules	Spinescence
Population *	Oblong	Lanceolate	Dark green	Light green	Acute	Acuminate	Gray	Whitish	Light red	Dark violet	Light violet	Pink	White								
1		100	40	60	100		100			20	60	20		Dwarf shrub	Ciliate	Glandular	Sessile	Opposite	Acute	Absent	Absent
2		100		100	100		100				80		20								
3		100	20	80	100		100			20	80										
4		100	20	80	100		100			40	60										
5		100		100	100		100			100											
6		100		100	100		100			80	20										
7		100	20	80	100		80	20		40	60										
8		100	20	80	100		80	20		20	80										
9		100	20	80	100		80	20		100											
10		100	20	80	100		100			20	80										
11		100	20	80	100		100			40	60										
12		100	40	60	100		100				80	20									
13		100	60	40	100		100			40	60										
14		100	20	80	100		100				60		40								
15		100	20	80	100		100			40	60										
16	100			100		100			100		100										
Frequency %	6.3	93.8	20.0	80.0	93.8	6.3	90.0	3.8	6.3	20.0	58.8	15.0	6.3	100	100	100	100	100	100	100	100

* Numbers 1-16 are populations of sites: Qasabat AS Salt, Al Fuhays, Tla' Kaser Khalda, Ayn Al Basha, Abu Hamid, Abu Nusayr, Khalda, Amman (West UOJ), Khalda military station, Wadi Slait, Bela's, Edbyan, Al Bassah, Wadi AS Sir, Um El Gutain, and Kufranja, respectively.

Table (8): Phenotypic diversity index (*H`*) of 20 characters for sixteen wild opulations of *Coridothymus capitatus* from Jordan.

Character	Diversity index (*H`*)
Leaf length (mm)	0.42
Leaf width (mm)	0.23
leaf length : width ratio	0.38
Plant height (cm)	1.06
Plant width (cm)	0.99
Plant length (cm)	0.98
Inflorescence length (mm)	0.43
Average quantitative	0.64
Leaf shape	0.23
Leaf color	0.50
Leaf apices	0.23
Stem color	0.39
Petal color	1.09
Average qualitative	0.49
Growth habit	0.00
Leaf margin	0.00
Leaf arrangement	0.00
Leaf base	0.00
Leaf stipules	0.00
Leaf surface	0.00
Leaf attachment	0.00
Spinescence	0.00
Average diversity index*	0.58

* Based on 12 polymorphic traits

These estimates ranged from 0.0 (monomorphic) for traits of growth habit, spinescence, and leaf characters (arrangement, attachment, margin, base, surface, stipules) to 1.09 (polymorphic) for petal color. The higher level of polymorphism was obtained by the traits petal color (*H`*=1.09), plant height (*H`*=1.06), plant width (*H`*= 0.99), and plant length (*H`*= 0.98). The traits leaf (width, shape, and apices) showed the lower level of polymorphism (*H`*=0.23), which may reflect unequal frequencies of different classes. An intermediate level of diversity was obtained by leaf color (*H`*=0.50).

Other traits (leaf length, leaf length: width ratio, Inflorescence length, stem color) showed relatively low polymorphism ($0.23 < H` < 0.50$) in comparison to the average diversity index ($H`$=0.58).

The higher level of polymorphism mainly obtained for quantitative traits (plant height, plant width, and plant length) confirm high level of diversity. Our results agreed with those of Al Nashash *et al*. (2007) that quantitative traits like plant height showed the higher level of polymorphism in comparison to qualitative traits. In this study, the average diversity index ($H`$) recorded for quantitative traits based on seven morphometric traits was 0.64 while the average diversity index ($H`$) based on five qualitative traits was 0.49. On the other hand, the average estimate of diversity in this study ($H`$=0.58) was higher than that reported by Jaradat (1989) for barely landraces ($H`$= 0.55), which was also based on seven quantitative and thirteen qualitative traits.

Excluding petal color, the low diversity calculated for qualitative traits could be due to the fact that these traits are controlled by few genes and consequently the qualitative variation is less limited by natural selection than quantitative variation which is highly affected by environment and selection, and affects plant demography (Al Nashash *et al*., 2007). However, it argued (Bjornstad *et al*., 1997) that phenotypic diversity does not reflect a random and chromosomally balanced sample of genetic variation. Thus, the high phenotypic diversity obtained may not reflect a higher average diversity at biochemical or molecular level (Lefebvre *et al*., 1991). Hence, molecular analysis is necessary to confirm diversity level among populations.

4.2.3. Cluster analysis

Cluster analysis performed with quantitative data according to Weltzinen (1989) and Akcin (2007). The phenotypic relatedness among 16 wild populations of *C. capitatus* assessed using Euclidean distance (Table 9). The mean dissimilarities indices ranged

from 0.40 to 6.69 (≈ 4 – 67 %), indicating high phenotypic distance among populations. When Kufranja population (individuals identified as *Thy. spicata*) was excluded from comparison, the mean dissimilarities indices ranged from 0.40 to 4.02 (≈ 4 – 40 %), indicating that phenotypic distance among populations maintain high and confirming the high C.V. percentages presented in Tables (5, 6). All populations showed an average dissimilarities of 3.2 (≈ 30%), meaning that populations share an average 70 % of the phenotypic traits.

Figure (2) presents the dendrogram resulting from UPGMA using Euclidean distances. The dendrogram resulted in 9 main groups located below the average dissimilarities of 3.2, indicating high phenotypic polymorphism among these populations although they share morphological traits. The constructed tree also revealed 5 separate groups represents populations of: Wadi AS Sir, Edbyan, Abu Nusayr, Um El Gutain, and Kufranja. On the otherhand, SPSS program clearly suggested that two main clusters were appearant; one comprising Kufranja population and other consolidate all other populations in one main cluster. This indicating high genetic distance between Kufranja population and others, this mostly referred to different taxonomy of this wild population. These results are in agreement with those obtained by Akcin (2006) who found that numerical morphological characters were useful to distinguish *Thymus* L. species, and that UPGMA dendrogram used quantitative characters to support morphological evidences.

However, the results showed that populations collected from same district did not tend to group together in the same cluster and this support the need of molecular analysis for comprehensive discussion of the obtained results.

Table (9): The proximity matrix of Euclidean Distance based on morphological traits of *Coridothymus capitatus* populations growing wild in Jordan.

Population*	Euclidean Distance															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0.00															
2	0.58	0.0														
3	3.23	2.86	0.00													
4	2.59	2.33	0.89	0.00												
5	3.46	3.27	2.19	2.08	0.00											
6	2.59	2.47	1.44	0.80	2.08	0.00										
7	2.33	2.19	2.43	1.79	3.07	2.26	0.00									
8	1.13	0.57	2.56	2.19	3.18	2.47	2.19	0.00								
9	3.73	3.46	2.04	2.15	0.57	2.30	3.23	3.27	0.00							
10	1.79	1.27	2.15	2.04	2.97	2.33	2.59	0.80	2.97	0.00						
11	2.08	2.15	2.33	1.44	2.59	1.20	1.65	2.37	2.94	2.62	0.00					
12	2.86	2.56	0.57	0.40	2.04	0.89	2.15	2.37	2.04	2.08	1.79	0.00				
13	1.65	1.20	2.19	2.00	2.88	2.15	2.68	0.89	2.94	0.40	2.47	2.04	0.00			
14	4.02	3.82	2.88	2.86	2.04	2.97	3.56	3.69	2.04	3.51	3.35	2.83	3.49	0.00		
15	5.63	5.80	6.44	6.09	5.56	5.66	6.69	6.01	5.87	6.01	5.70	6.13	5.76	6.13	0.00	
16	3.49	3.25	2.08	2.04	2.86	2.19	2.94	3.10	2.86	2.88	2.68	2.00	2.86	2.00	6.45	0.00

* Numbers 1-16 are populations of sites: 1Qasabat AS Salt; 2 Al Fuhays, 3 Khalda, 4 Tla' Kaser Khalda, 5 Ayn Al Basha, 6 Amman (West UOJ), 7 Wadi AS Sir, 8 Wadi Slait, 9 Abu Hamid, 10 Bela's, 11 Edbyan, 12 Al Bassah, 13 Khalda military station, 14 Abu Nusayr, 15 Kufranja, and 16 Um El Gutain, respectively.

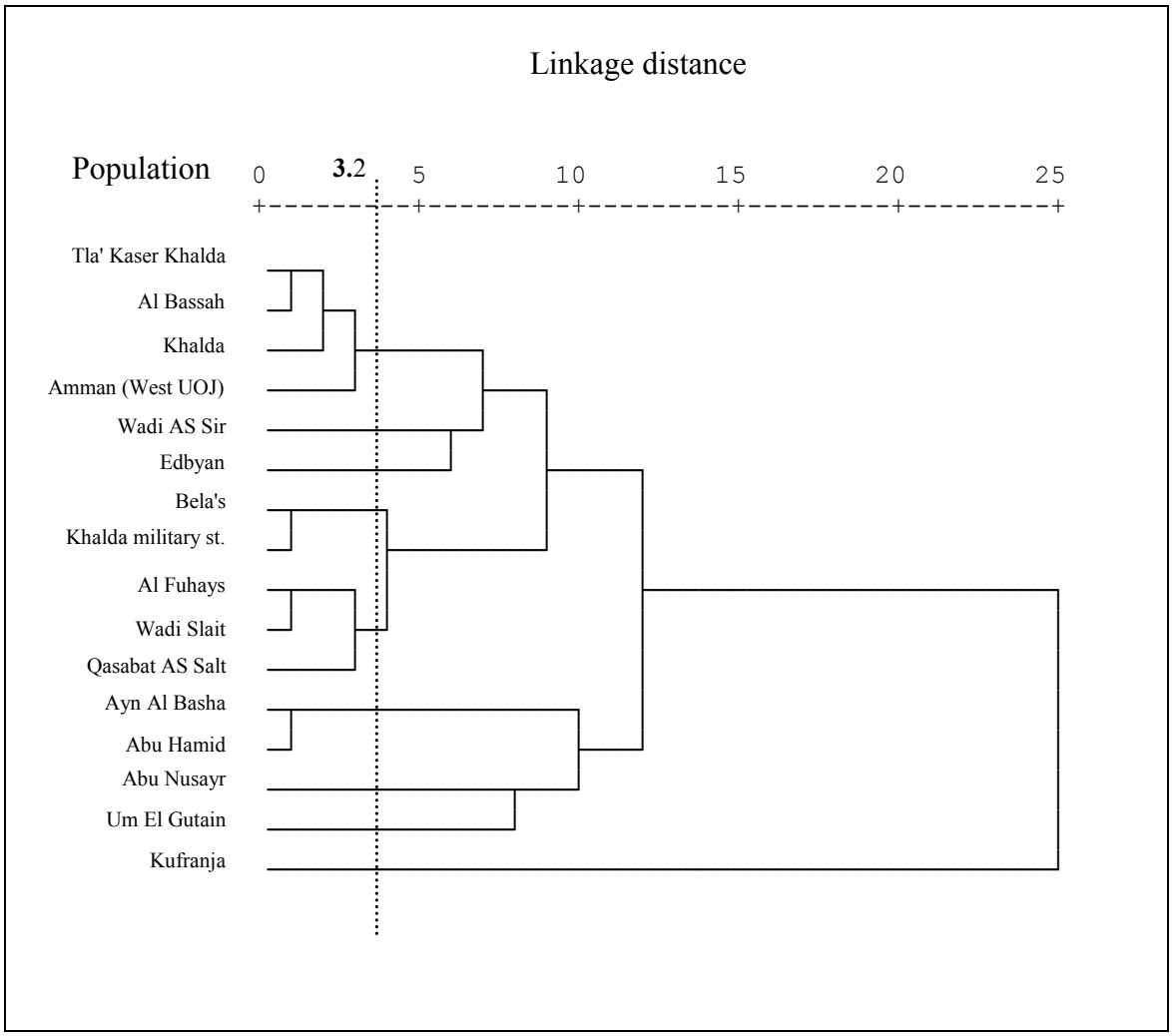


Figure (2): Dendrogram of sixteen populations of *Coridothymus capitatus* growing wild in Jordan based on morphological characters and using Euclidean distances.

4.2.4. Canonical analysis

The canonical discriminant analysis (multivariate analysis) was conducted to investigate the separate ability of the sixteen wild populations of *C. capitatus* based on their morphological traits (Mati'nez *et al*., 2003; Monokrousos, *et al*. 2004). Results of the analysis showed high separation between populations of *C. capitatus* and *Thy. spicata*, and moderate separation among *C. capitatus* populations growing wild in their natural habitat (Figure 3). Overlaps between sites of collection noticed, indicating that these populations share many phenotypic traits.

Canonical discriminant analysis produced three main functions (Table 10). The highest variation percentage caused by function 1 (49.4) with Eigen value equal to 0.40.

Variation caused by functions 2 and 3 were 35.1 and 15.6, respectively and with Eigen values 0.28 and 0.13, respectively. The first two canonical functions explained 84.5 % of the variability referred to the investigated morphological data.

The standardized canonical discriminant functions coefficients (Table 11) for all morphological traits under investigation revealed that the first function, which explained 49.4% of total variability between sites was strongly influenced by stem color (0.99) while other morphological characters showed lower separate ability between populations. The second canonical discriminant function explained 35.1% of total variability between sites was influenced by leaf color followed by petal color and with a standardized canonical discriminant function coefficient equal 0.94 and 0.86, respectively. Third function, which explained the lowest percentage (15.6%) of total variability between population sites, found to be influenced by petal color where the standardized canonical discriminant function coefficient was 0.65.

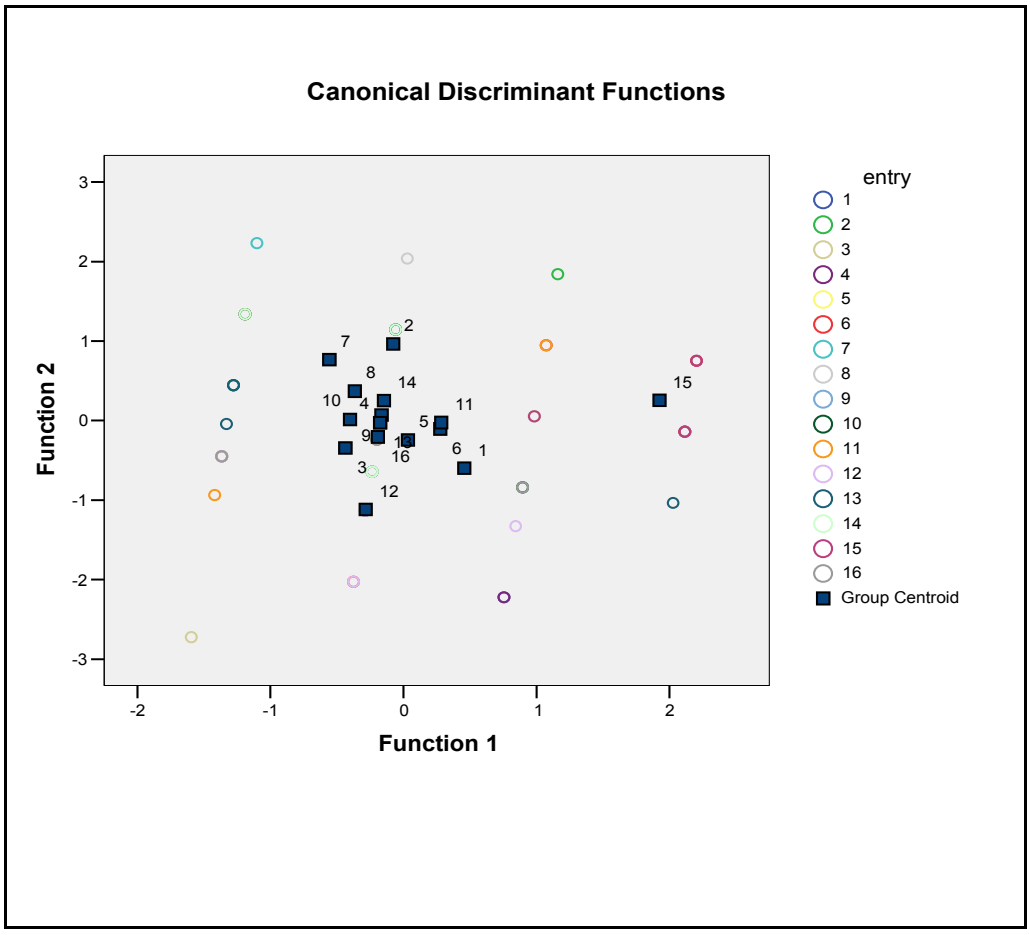


Figure (3): The territorial map showing canonical discriminant functions between the sixteen wild populations and the two main functions. Numbers 1-16 are populations of sites: Qasabat AS Salt, Al Fuhays, Khalda, Tla' Kaser Khalda, Ayn Al Basha, Amman (West UOJ), Wadi AS Sir, Wadi Slait, Abu Hamid, Bela's, Edbyan, Al Bassah, Khalda military station, Abu Nusayr, Kufranja, and Um El Gutain, respectively.

Table (10): Eigen values and variability percentage of canonical discriminant functions.

Function	Eigen value	% of Variance	Cumulative %
1	0.40	49.4	49.4
2	0.28	35.1	84.5
3	0.13	15.6	100.0

Table (11): Standardized canonical discriminant function coefficients for sixteen populations of *C. capitatus*.

Trait	Function		
	1	2	3
Leaf color	0.09	0.94	0.55
Stem color	0.99	0.17	0.06
Petal color	0.06	0.86	0.65

The territorial map (Figure 3) produced was considered as a graphical representation of the distribution of sampled populations in the space of two discriminant functions. The two canonical functions show a separation between plant samples of sixteen populations from each other according to their main population in collection site. Population belonging to district Ajlun, site Kufranja formed one group separated from others while other population presented a moderate distribution in the space of the two discriminant functions.

The first canonical function could successfully and strongly separate Kufranja population from other populations and put it at extreme, which corresponds well to its geographical distribution in Ajloun district. Also first function could separate the other four (1, 6, 5, 11) populations.

The second canonical discriminant function mainly separated samples belonging to populations of 2, 4, 7, 8, 9, 10, and 14. Second function also showed a contribution in separating Ajlun population.

Results obtained by canonical analysis shows that among traits studied, only three traits contributed to the variation in functions; those are leaf color, stem color and petal color. Trait stem color recorded highest contribution to variation in function 1 (0.98)

while it has a low contribution to variation in functions 2 and 3. Petal color trait showed high contribution to variation (0.86, 0.65) but the highest contribution was in function 2 (0.86). Treuren, *et al*. (1993) reported that petal color trait controlled by few genes, but at the same time, colors affected by environmental conditions. Nevertheless, this trait still utilize as an important character to distinguish between plant species and species populations. *C. capitatus* populations showed high variation in petal colors which could refer to the eco-geographical diversity. Segregation could be another cause of variation, where the separation of allelic pairs and their distribution to different cells occurred during meiosis (Fehr, 1987).

4.3. Chemical Diversity of Essential Oil among Wild Population

4.3.1. Qualitative and quantitative analysis

Two main peaks were identified in the volatile components in plant material essential oil using GC-MS technology, indicating the presence of two major compounds. The GC-MS analysis allowed the identification of thymol and carvacrol in the analyzed oil by matching, with high degree of certainty using reverse fit mode, their recorded spectra with the data bank mass spectra provided by the instrument software, and by comparing their retention indices values with standards measured (Annex X). Thymol and carvacrol were reported in literature as being the predominant and the most bioactive volatile components in *C. capitatus* essential oil (Arras and Grella, 1992; Goren *et al*., 2003; Hedhili *et al*., 2005; Miceli *et al*., 2006). Thus, thymol and carvacrol were approved as chemical markers for *C. capitatus*. Two calibration curves of thymol and carvacrol reference standards were prepared in order to calculate thymol and carvacrol contents in each population. Thymol eluted at 8.51 min, while carvacrol eluted at 8.34 min. Two four-points linear calibration curves for thymol and carvacrol, with R^2 values of 0.993 and 0.994 in the range of 2-15 ppm (2, 5, 10, and 15 ppm) were

obtained for thymol and carvacrol, respectively. Two quality control (QC) samples at 7 and 17 ppm were accurate within 3.57, 9.71% and 7.29, and 13.1% (ppm, *RSD* %) from actual concentration based on thymol and carvacrol's calibration curves, respectively (Table 12). Thus, these two linear equations were applied confidently to calculate thymol and carvacrol concentration (mg/gm) in each sample.

Table 12: Accuracy validation using quality control (QC) points

Actual concentration (ppm)	Measured concentration (ppm)	% *RSD**
Thymol		
7	7.25	3.57
12	10.89	9.25
Carvacrol		
7	6.49	7.29
12	10.48	12.92
* Relative Standard Deviation		

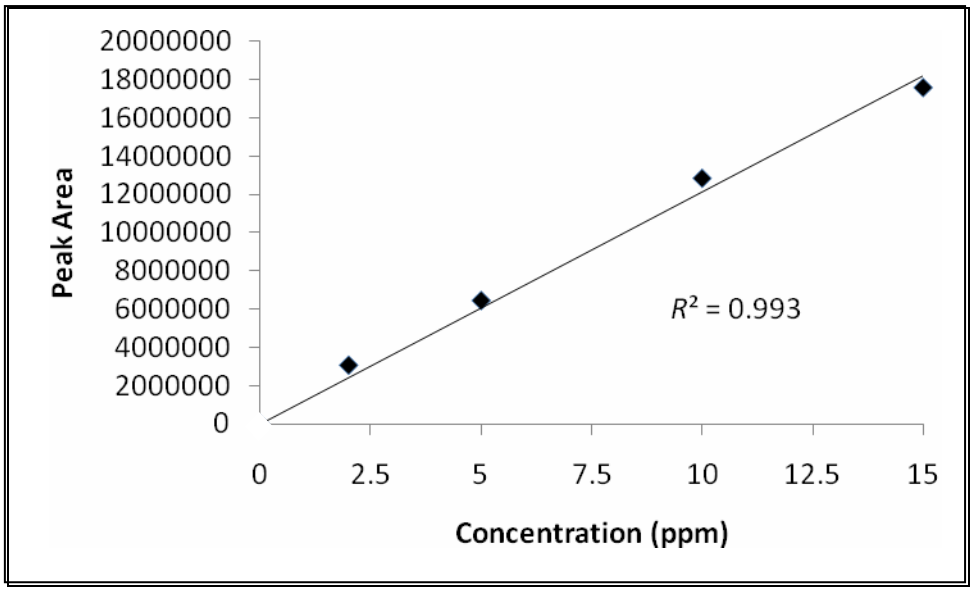


Figure (4): Standard calibration curve of thymol

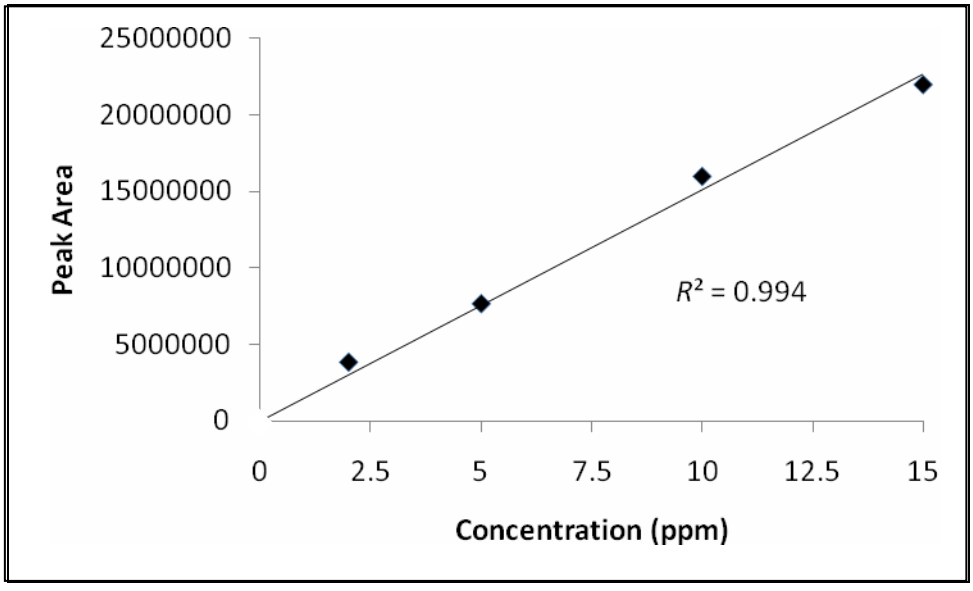


Figure (5): Standard calibration curve of carvacrol

4.3.2. Essential oil variability among wild population

The intraspecific biodiversity of genus *Thymus* evident of heterogeneous composition of the essential oils is well known (Miceli *et al*., 2006). This is important to develop conservation strategies and to initiate breeding programs aiming to develop specific essential oil cultivars. Table (13) shows the amounts of total extracted oil yields, expressed as a percentage of the biomass dry weight of the essential oils coming from the sampled sites hosted *C. capitatus* populations in the study area. The highest yield obtained from populations of Edbyan (2.96 %) while the lowest yield (0.38 %) produced by Kufranja population. Almost all the remaining samples had yielded percentages ranging between 1.04 - 2.85 %, indicating variation in the amounts of aromatic oil obtained from sixteen wild populations. These results are relatively concurring with those obtained by Miceli *et al*. (2006) who reported oil yield percentage ranging between 1.8 - 4.2 % of *Thymbra capitata* growing wild in south Italy. However, the low oil yield percentage obtained in our study, may refer to differences in micromorphology of the trichomes, the structures responsible for the production of the essential oil secreted, which occurred in vegetative organs. Rodrigues *et al*. (2006) cited that most essential oil is believed to be synthesized within the peltate glandular trichomes which their density in fully expanded mature leaves is about 2.5 peltate gland/mm^2 in the adaxial surface and 6 glands/ mm^2 in the abaxial. In this study, the concern was to investigate variation among populations in relation to essential oils, therefore, a bulk of areal vegetative parts were subjected to extraction and not only leaves.

Table (14) shows the essential oil contents of sixteen *C. capitatus* populations grow wild in Jordan including thymol, carvacrol, and their additive percentages. Various degrees of variations obtained by the analysis of the essential oil components.

Table (13): Localization (sites and coordinates), rainfall and soil properties of *Coridothymus capitatus* populations growing wild in Jordan during 2006 and percentage yields (%) of the essential oil obtained.

Sites	Geographical coordinates (GPS system)			Rainfall (mm)*	Soil properties						Yield (%) **
	Altitude (m)	Longitude E	Latitude N		N (%)	P(ppm)	K(ppm)	O.M (%)	$CaCO_3$ (%)	Soil Texture	
Qasabat AS Salt	908	35° 47′ 30.2"	32° 01′ 41.0"	502	0.34	11.73	324.58	4.80	37.20	Loam	1.67
Al Fuhays	937	35° 47′ 43.1"	32° 01′ 40.3"	502	0.15	6.95	135.37	3.29	59.50	Loam	1.32
Tla' Kaser Khalda	1055	35° 50′ 56.3"	32° 00′ 42.3"	423	0.12	6.33	173.22	2.12	46.50	Clay loam	2.85
Ayn Al Basha	1040	35° 50′ 24.6"	32° 00′ 46.0"	423	0.20	8.93	21.85	2.26	37.20	Loam	1.81
Abu Hamid village	833	35° 53′ 15.3"	32° 05′ 03.0"	423	0.12	8.70	122.76	2.21	55.70	Silt loam	2.45
Abu Nusayr village	858	35° 52′ 36.6"	32° 04′ 20.6"	423	0.17	7.33	135.37	1.83	55.70	Silt clay loam	1.67
Khalda	1047	35° 51′ 27.1"	32° 00′ 10.1"	495	0.17	42.67	160.60	2.54	59.50	Loam	1.93
Amman (West UOJ)	1043	35° 52′ 24.0"	32° 00′ 32.9"	495	0.15	7.55	122.76	2.21	70.60	Loam	1.47
Khalda military station	1062	35° 51′ 38.0"	32° 00′ 43.5"	495	0.22	8.15	135.37	1.06	39.00	Silt loam	2.11
Wadi Slait	547	35° 48′ 42.8"	31° 53′ 10.6"	340	0.04	4.65	59.69	0.81	13.00	Sandy loam	2.53
Bela's village	925	35° 50′ 07.1"	31° 52′ 23.0"	340	0.22	12.35	178.11	3.46	61.30	Silt loam	1.04
Edbyan village	880	35° 49′ 52.0"	31° 52′ 13.6"	340	0.20	6.20	97.53	3.15	63.20	Loam	2.96
Al Bassah village	934	35° 49′ 53.0"	31° 52′ 08.5"	340	0.10	6.50	211.06	4.16	59.50	Clay loam	2.56
Wadi AS Sir	856	35° 50′ 16.9"	31° 52′ 36.0"	300	0.20	9.30	198.44	4.23	44.60	Loam	2.28
Um El Gutain village	877	35° 48′ 27.0"	31° 51′ 13.0"	300	0.17	13.07	72.31	2.15	55.70	Loam	1.94
Kufranja	677	35° 43′ 18.0"	32° 18′ 28.6"	576	0.12	6.75	9.24	2.33	61.30	Loam	0.39

* Average long-term. Source: Jordan Department of meteorology (1998-2007).

** Data are yield (mg/g) percentages of total essential oil obtained from 10.0 g dry weight

Table (14): Eessential oils, thymol and carvacrol content of *Coridothymus capitatus* growing wild in Jordan.

Sites	Essential oils		Thymol		Carvacrol		Thymol + carvacrol (mg/g)	Thymol + carvacrol (%)
	Yield (mg/g)	(%)	Yield (mg/g) ± SD	(%)	Yield (mg/g) ± SD	(%)		
Qasabat AS Salt	16.75	1.67	4.39 ± 0.56	0.44	2.08 ± 0.28	0.21	6.47	0.65
Al Fuhays	13.24	1.32	3.38 ± 0.28	0.34	1.50 ± 0.09	0.15	4.88	0.49
Tla' Kaser Khalda	28.52	2.85	5.13 ± 0.48	0.51	5.71 ± 0.39	0.57	10.84	1.08
Ayn Al Basha	18.07	1.81	4.85 ± 0.51	0.48	2.44 ±0.29	0.24	7.29	0.73
Abu Hamid village	24.54	2.45	3.14 ± 0.65	0.31	8.71 ± 0.95	0.87	11.85	1.19
Abu Nusayr village	16.65	1.67	1.34 ± 0.68	0.13	5.30 ± 0.56	0.53	6.64	0.66
Khalda	19.28	1.93	4.69 ± 0.26	0.47	3.01 ± 0.22	0.30	7.70	0.77
Amman (West UOJ)	14.67	1.47	3.20 ± 0.15	0.32	2.07 ±0.10	0.21	5.27	0.53
Khalda military station	21.12	2.11	4.34 ± 0.07	0.43	3.92 ± 0.15	0.39	8.26	0.83
Wadi Slait	25.27	2.53	5.71 ± 0.69	0.57	3.48 ± 0.46	0.35	9.19	0.92
Bela's village	10.39	1.04	2.73 ± 0.17	0.27	0.98 ± 0.12	0.10	3.71	0.37
Edbyan village	29.61	2.96	4.91 ± 0.29	0.49	6.34 ± 0.36	0.63	11.25	1.13
Al Bassah village	25.65	2.56	4.46 ± 0.53	0.45	4.55 ± 0.45	0.45	9.01	0.90
Wadi AS Sir	22.81	2.28	3.39 ± 0.81	0.34	7.23 ± 1.48	0.72	10.62	1.06
Um El Gutain village	19.41	1.94	4.51 ± 0.44	0.45	2.23 ± 0.30	0.22	6.74	0.67
Kufranja	3.89	0.39	0.30 ± 0.04	0.03	1.00 ± 0.12	0.10	1.30	0.13
Over all means	19.37	1.94	3.78 ± 0.41	0.37	3.78 ± 0.4	0.38	7.56	0.76
LSD at 0.05			0.79		0.9			
CV%			12.50		14.30			

* Data are yields of essential oil composition obtained from 10.0 g dry weight

Thymol percentage ranging between 0.03 - 0.57 % with C.V. of 12.5 % while carvacrol percentage ranging between 0.1 - 0.87 % with C.V. of 14.3 %, indicating high level of variation among wild populations. The average percentage of thymol and carvacrol together for all populations was 0.76 %, indicating high contribution (39 %) of thymol and carvacrol essential oils in the average oil yield (1.94 %) of *C. capitatus* which assemble more than quarter of total oil extracted. These results are in agreement with those obtained by Muller *et al*., 1997; Miceli *et al*., 2006; and Rodrigues *et al*., 2006, who reported that thymol and carvacrol are the dominant volatile components in *C. capitatus* essential oil. The study conducted by Miceli *et al*. (2006), shows that among the 75 components of the oils of *Thymbra capitata* (*C. capitatus*) the most recurrent ones were thymol and carvacrol which constituted more than 50% of the oils. Population of Edbyan showed the highest oil yield percentage (2.96 %) and also high percentage of thymol and carvacrol additives (1.13%). It is worth to mention that individuals of Edbyan population exhibit the highest leaf length: width ratio (5.0 mm) and inflorescence length (11.8 mm), these organs are the main plant parts which contributed in oil production (Rodrigues *et al*., 2006). In addition, the calcarious and bedrocky soils characterized Edbyan site may support plant root potential to reach water sources which in turn reflect in producing high oil yield. Monokrousos *et al*. (2004) cited the role of soil properties in the development of *C. capitatus* life form as dimorphic shrub.

The highest percentage of thymol recorded by Wadi Slait population (0.57 %) followed by Tla' Kaser Khalda (0.51%) with no significant difference between them, these two populations exceed the overall mean report by 20 % and 14 %, respectively. Abu Hamid population recorded the highest percentage of carvacrol (0.87 %) with significant difference with all other populations and exceeding the overall mean by

49%. Variation of thymol and carvacrol contents were found among 23 populations of *Thy. capitata* growing wild in Italy, and this variation referred to different climatic conditions (Miceli *et al*., 2006).

The lowest percentage of oil yield, thymol, carvacrol and their additives recorded by Kufranja population, and this lead to assume that this population is taxonomically belonging to different species (individuals identified as *Thy. spicata*).

For instance, within genus *Thymus* many species present intraspecific chemotypes (Senator, 1996). Researchs carried out (Arras and Grella, 1992; Muller *et al*., 1997; Miceli *et al*., 2002) showed that populations of thyme contain carvacrol and small quantities of thymol. In this study, the data shows variability in the amounts of both thymol and carvacrol, which agreed with new findings by Miceli *et al*. (2006) about high percentages of thymol and carvacrol in species *C. capitatus*. This indicate the presence of various chemotypes of this species which confirmed by cluster analysis.

4.3.3. Clustering

Cluster analysis was performed on data related to essential oils content of *C. capitatus*. The chemotype relatedness among 16 wild population of *C. capitatus* assessed using Euclidean distance (Table 15). The dissimilarities indices ranging between 1.26 and 28.47, indicating high chemotype distance among populations and confirming the high C.V. percentages presented in Table (14). The highest dissimilarity (28.47) occurred between Edbyan and Kufranja populations, indicating a divergent genetic base which need to be confirmed by molecular analysis. The lowest dissimilarity (1.26) occurred between Khalda and Um El Qutain populations, indicating analogous genetic base between these two geographically far populations.

Table (15): The proximity matrix of Euclidean Distance based on essential oils content of *Coridothymus capitatus* populations growing wild in Jordan

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0.00															
2	4.03	0.00														
3	13.09	17.02	0.00													
4	1.66	5.67	11.51	0.00												
5	11.63	15.11	5.46	10.24	0.00											
6	4.44	5.77	13.16	4.79	10.21	0.00										
7	2.98	6.96	10.14	1.41	8.93	4.95	0.00									
8	2.68	1.58	15.47	4.30	13.59	4.45	5.50	0.00								
9	5.07	8.96	8.08	3.56	7.00	5.79	2.16	7.43	0.00							
10	9.15	13.14	4.31	7.57	6.45	10.16	6.27	11.66	4.49	0.00						
11	7.21	3.19	20.19	8.86	18.08	8.27	10.15	4.71	12.13	16.33	0.00					
12	14.38	18.28	1.34	12.81	5.90	14.25	11.42	16.74	9.34	5.65	21.44	0.00				
13	9.58	13.47	3.66	8.06	5.32	9.84	6.69	11.93	4.64	1.70	16.63	4.91	0.00			
14	9.03	12.55	6.16	7.66	2.60	7.86	6.36	11.03	4.50	5.25	15.54	7.05	4.36	0.00		
15	2.68	6.58	10.58	1.50	9.81	5.21	1.26	5.14	2.85	6.58	9.76	11.89	7.03	7.27	0.00	
16	14.49	10.49	27.26	16.15	24.60	14.52	17.35	11.90	19.24	23.55	7.35	28.47	23.72	22.21	17.02	0.00

* Numbers 1-16 are populations of sites: Qasabat AS Salt, Al Fuhays, Tla' Kaser Khalda, Ayn Al Basha, Abu Hamid, Abu Nusayr, Khalda, Amman (West UOJ), Khalda military station, Wadi Slait, Bela's, Edbyan, Al Bassah, Wadi AS Sir, Um El Gutain, and Kufranja, respectively.

Miceli *et al*., 2006 and Rodrigues *et al*., 2006 reported that although essential oil composition of genus *Thymus* is highly influenced by environmental conditions, but it is mainly based on genetic make up and on polymorphism among species.

The high relatedness between populations could exploit in developing conservation strategies because these populations could considered as one accession with intraspecific variation.

Populations of *C. capitatus* shows average dissimilarities of 10.68, meaning that populations could divide into chemotypes clustering based on this average. Figure (6) present the dendrogram resulting from UPGMA using Euclidean distances. The dendrogram resulted in 3 main groups located below the average dissimilarities (10.68), indicating polymorphism among populations. The first group solitaire Kufranja population where the lowest percentage of essential oils (thymol and carvacrol) recorded and this support the assumption of different species of this population known by local people as Za'tar Farisi. The second group consisted of carvacrol chemotype which consolidate populations of Abu Hamid, Wadi AS Sir, Tla' Kaser Khalda, Edbyan, Wadi Slait, and Al Bassah, those containing carvacrol percentages more than thymol. However, the second group separated into two sub-groups based on the oil yield extracted beside the high content of carvacrol, which mainly exhibited by the population of Wadi Slait. Third group consisted of thymol chemotype which consolidate populations (Khalda, Um El Gutain, Ayn Al Basha, Qasabat AS Salt, Khalda military station, Abu Nusayr, Al Fuhays, Amman west UOJ, Bela's) containing thymol percentages more than carvacrol. Population of Abu Nusayr has higher percentage of carvacrol but it clustered within thymol chemotype, this could refer to its high oil content.

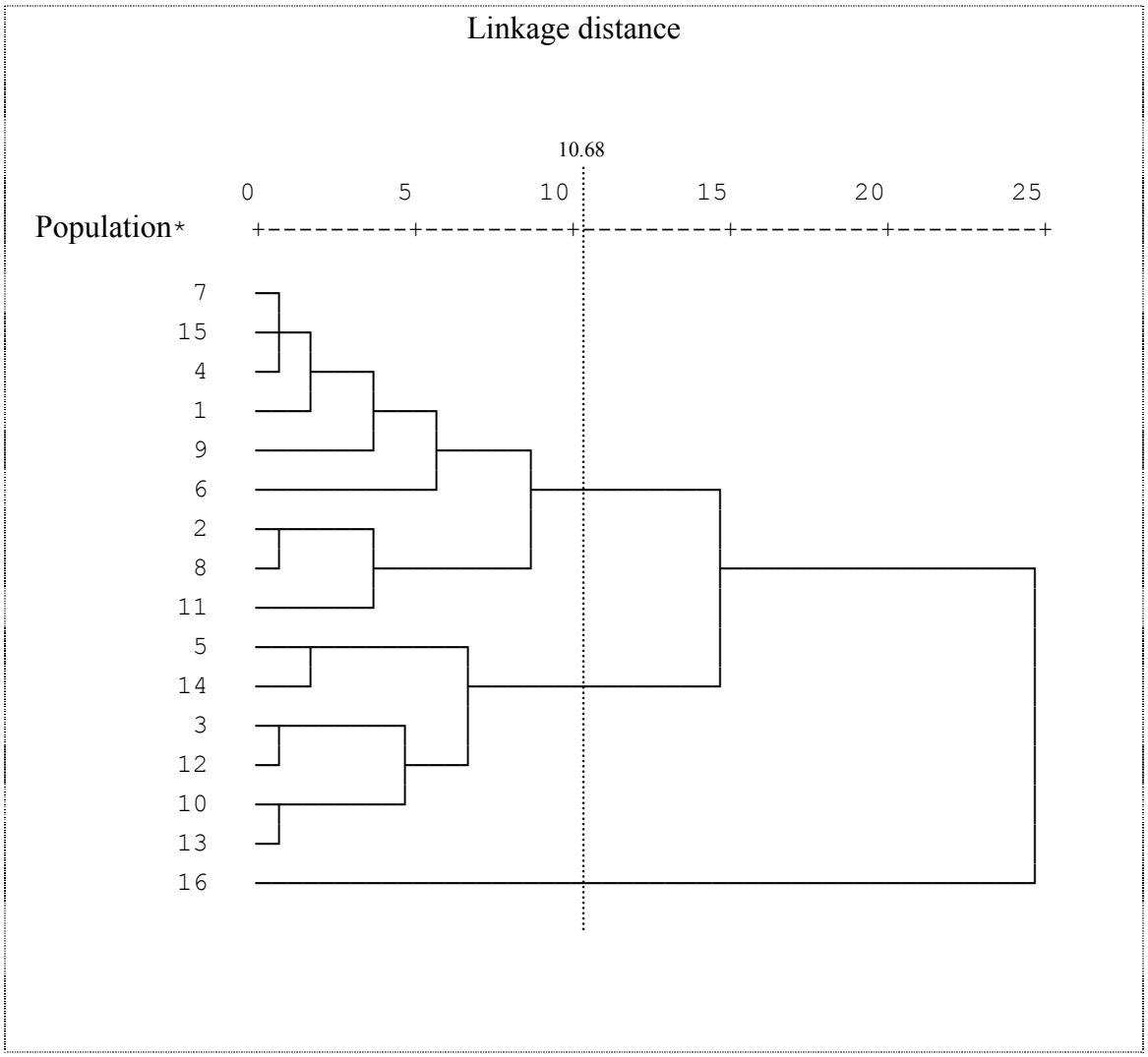


Figure (6): Dendrogram of sixteen populations of *Coridothymus capitatus* growing wild in Jordan based on essential oils content and using Euclidean distances.
* Numbers 1-16 are populations of Qasabat AS Salt, Al Fuhays, Tla' Kaser Khalda, Ayn Al Basha, Abu Hamid, Abu Nusayr, Khalda, Amman (West UOJ), Khalda military station, Wadi Slait, Bela's, Edbyan, Al Bassah, Wadi AS Sir, Um El Gutain, and Kufranja, respectively.

The third group separated into two sub groups mostly based on total oil content. The clustering figure agreed to a certain degree with Miceli *et al*., 2006 whose clustering analysis led to the identification of thymol and carvacrol chemotypes.

The cluster analysis in this study identified two chemotypes depending on the content of carvacrol and thymol in *C. capitatus* populations. The thymol chemotype, which is the most widespread, especially in the north and west of the sampled area, and carvacrol chemotype which is less represented and mostly spread in south of the sampled area where drier conditions are prevailed. This lead to assume a possible connection with particular climatic conditions, which needs to be studied thoroughly. On the other hand, the constructed dendrogram did not consolidate populations collected from same district together in the same cluster supporting the need of molecular analysis.

4.3.4. Correlations with ecogeographical data

Table (16) shows Person's coefficient of correlation between pairs of ecogeographic and essential oils of *C. capitatus* populations growing wild in Jordan. Significant correlation (0.56) recorded between oil yield and rainfall, the relation seems to be negative indicating that potential of *C. capitatus* for oil production expressed more under dry conditions. *C. capitatus* considered a good indicator of the dry Mediterranean area (Fragman, *et al*., 200; Danin, 2004; Miceli, *et al*., 2006). Weather parameters such as atmospheric temperature and rainfall have been reported to influence oil content and and the composition in several aromatic plants (Sangwan, *et al*., 2001).

Results show high significant correlation between oil yield and thymol, carvacrol, and thymol plus carvacrol (0.62, 0.74, and 0.96, respectively) indicating that thymol and carvacrol constitute the major composition of *C. capitatus* essential oils.

Table (16): Person's coefficient of correlation between pairs of eco-geographic and essential oils of *Coridothymus capitatus* populations growing wild in Jordan.

Entry	Elevation	Rain fall	N	P	K	O.M.	$CaCO_3$	Total oil	Thymol	Carvacrol	Thymol + carvacrol
Elevation	1										
Rain fall	0.20	1									
N	0.42	0.15	1								
P	0.32	0.18	0.18	1							
K	0.32	-0.04	0.54*	0.17	1						
O. M.	0.15	-0.14	0.52*	0.07	0.69**	1					
$CaCO_3$	0.36	0.17	0.04	0.18	0.01	0.29	1				
Total oil	0.09	-0.56*	-0.20	-0.08	0.18	-0.07	0.32	1			
Thymol	0.25	-0.36	0.05	0.15	0.17	-0.01	0.48*	0.62**	1		
Carvacrol	0.04	-0.39	-0.18	-0.16	0.14	-0.02	0.07	0.74**	-0.03	1	
Thymol + carvacrol	0.08	-0.50*	-0.15	-0.06	0.19	-0.04	0.30	0.96**	0.45*	0.88**	1

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

Results of Miceli *et al*. (2006) showed that thymol and carvacrol constitute more than 50 % of *C. capitatus* oils. On the other hand, additive of thymol and carvacrol found to be more correlated with carvacrol (0.88) than thymol (0.45), indicating the dominancy of carvacrol which reported as the valuable essential oil characterized *C. capitatus* taxon (De Vincenzi *et al*., 2004; Faleiro *et al*. 2005; Can Baser, 2008). A negative correlation expressed between thymol and carvacrol (- 0.03), which is in agreement with Miceli, *et al*. (2006). Carvacrol is a powerful antiseptic used as a substitute for iodoform and thymol, it is a monoterpene phenol derivative and isomeric of cymene, $C_{10}H_{14}O$ (De Vincenzi *et al*., 2004, SDBS, 2009).

4.4. Essential Oil Seasonal Variation

C. capitatus, the perennial Labiatae aromatic shrub, growing wild in Jordan considered a seasonally dimorphic plant, reducing its transpiring surface at the beginning of fall by changing their big spring and summer leaves with small winter leaves (Muller *et al*., 1997).

Quality and quantity of *C. capitatus* essential oils in relation to thymol and carvacrol reported in the literature as being varied according to corresponding of environmental and growth factors (Arras and Grella, 1992; Muller *et al*., 1997; Tonçer and Kizil, 2005). Table (17), show the monthly variation of the amounts and the compositions of thymol and carvacrol essential oils of *C. capitatus* growing wild in Abu Hamid village during March 2007- February 2008. The site of Abu Hamid was selected because it is fenced, so the marked plants undergo analysis conserved for conducting a frequent monthly harvest. In addition, among fifteen wild populations of *C.capitatus* investigated for oil production, Abu Hamid population presented the highest percentage of oil (2.45 %) and carvacrol (0.87 %), meaning that this site could be ideal to follow seasonal variation in relation to essential oil.

Despite variation in quantities, a common trend identified toward best period for obtaining the highest amount of essential oil (Figure 7). The highest percentages of oil yields, thymol, carvacrol, and thymol plus carvacrol phenolic ingredients recorded in July (2.45, 0.31, 0.87, and 1.19, respectively), and lowest percentages recorded in February followed by March. The full bloom in *C. capitatus* population of Abu Hamid occurred during July where the mean relative humidity was relatively low (42.10 %), indicating a close relationship may exists between maturity stages and production of essential oil and phenolic compounds, this relationship was previously reported by Muller *et al*. (1997).

Table (17): Eessential oil, thymol and carvacrol content of *Coridothymus capitatus* growing wild in Abu Hamid village during March 2007- February 2008.

Month	Total rainfall (mm)	Mean relative humidity (%)	Mean air temperature (˚C)		Oil yield (%)	Thymol			Carvacrol			Thymol plus Carvacrol
			Minimum	Maximum		mg/g	SD	(%)	mg/g	SD	(%)	(%)
March	130.60	74.40	5.70	13.50	0.84	0.25	0.02	0.03	0.91	0.05	0.09	0.12
April	9.70	65.60	9.70	18.70	0.94	0.58	0.08	0.06	1.62	0.40	0.16	0.22
May	9.90	41.10	15.60	26.00	1.12	0.74	0.11	0.07	2.17	0.35	0.22	0.29
June	0.00	43.40	17.20	27.90	2.02	0.70	0.12	0.07	4.15	0.69	0.41	0.49
July	0.00	42.10	18.80	29.00	2.45	3.14	0.65	0.31	8.71	0.95	0.87	1.19
August	0.00	48.60	18.60	29.00	1.95	2.72	0.67	0.27	3.82	0.74	0.38	0.65
September	0.00	58.20	15.90	26.70	1.86	0.25	0.06	0.03	2.75	0.29	0.28	0.30
October	2.80	49.90	14.80	24.90	1.32	0.27	0.07	0.03	2.03	0.50	0.20	0.23
November	72.40	58.00	10.10	17.70	0.95	0.33	0.08	0.03	1.39	0.29	0.14	0.17
December	28.50	62.70	5.30	11.60	0.89	0.60	0.12	0.06	1.56	0.04	0.16	0.22
January	133.60	75.20	0.10	6.20	0.83	0.50	0.04	0.05	1.70	0.28	0.17	0.22
February	88.60	82.30	2.30	10.20	0.80	0.50	0.12	0.05	1.65	0.42	0.17	0.22
C.V. %						28.3			18.2			
$LSD_{0.05}$						0.42			0.83			

Monthly meteorological data obtained from Jordan Department of meteorology (2007-2008).
Data are yield percentages of total essential oil obtained from 10.0 g dry weight.

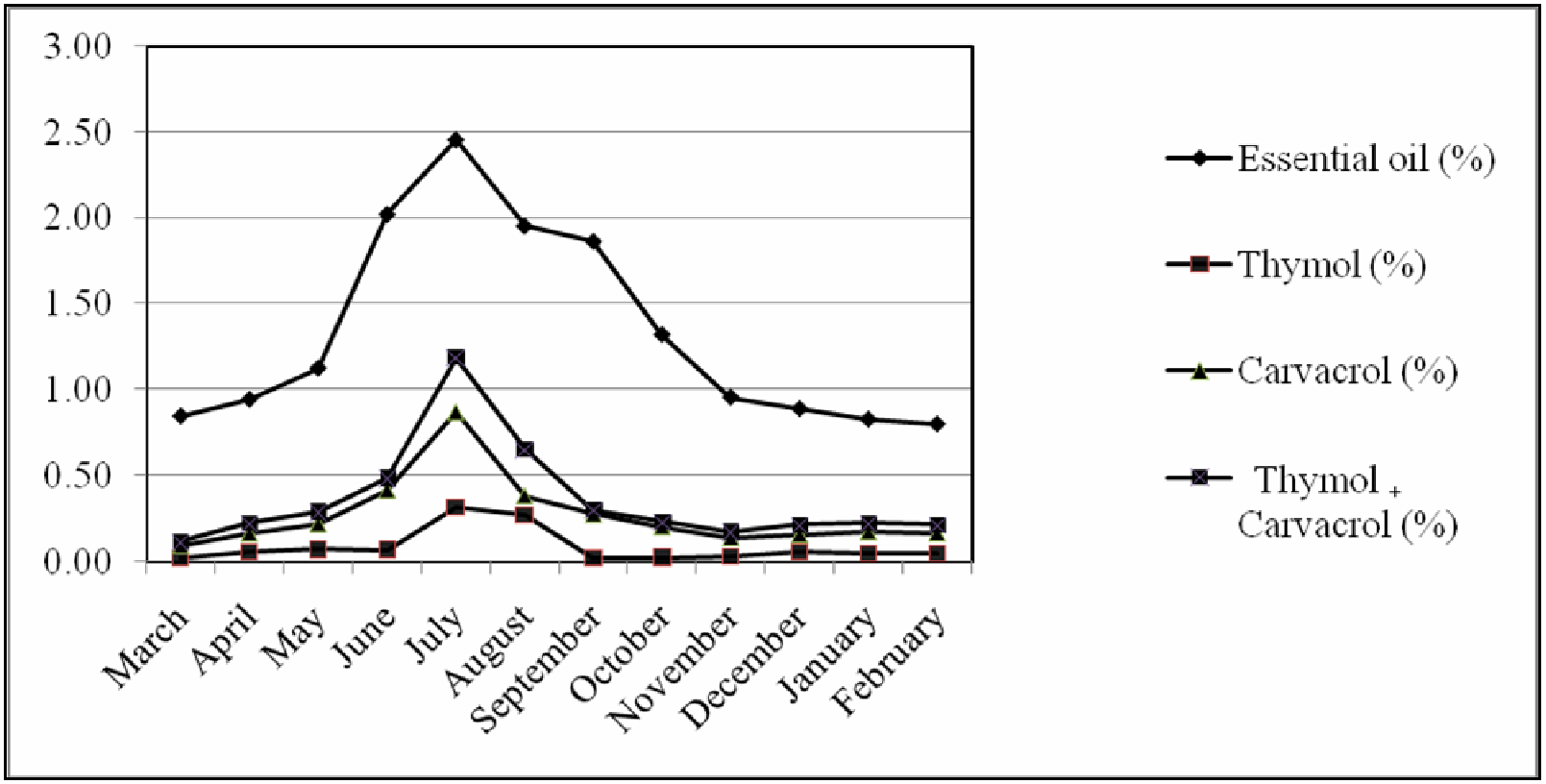


Figure (7): Monthly variation of *Coridothymus capitatus* essential oils, thymol, carvacrol, and thymol plus carvacrol of Abu Hamid village during March 2007 to February 2008.
(Percentages represent oil obtained from 10.0 g dry weight)

The production of aromatic essential oil attract insect pollinators to visit *C. capitatus* populations which have the ability to support an exceptionally high number of flower visitors (123 species) and 50% of monotropous visitors during the hot and rainless summer (Petanidou, 1996). On the other hand, *C. capitatus* species produce phenolic metabolites like carvacrol and thymol (called also phytochemicals), these phenolic secondary metabolites are defensive antimicrobials produced against invading pathogenes and environmental stress like dry, and therefore the methods for exploiting them have to take this into account (Arras, *et al*, 1994; Miguel *et al*., 2003; Can Baser, 2008).

Apparently, major changes occurred during February and March during the process of maturation of the flowering tops which justify the low oil percentages obtained. Furthermore, the air-dried plant material (leaves, top flowers) in June and July had high essential oil content (2.02 %, 2.45 %, respectively), suggesting that this is an optimal time for harvesting. Hence, we can conservatively conclude that the optimum harvesting time for oil production could be during June and July, while the optimum harvesting time for the essential oils of thymol and carvacrol production could be during July.

4.5. Cultivation Potential

Growing *C. capitatus* species under field conditions is important for studying cultivation potential, estimating genetic variations and identifying promising populations (Iapichino *et al*., 2006). The 21 populations (Table 3) of Jordans Za'tar Farisi introduced for cultivation at Mushager research station exhibited a good standing in the field, despite variations of investigated traits. These populations considered as accessions (wild, landraces) conserved at field gene bank (*ex situ*) in Mushager station,

representing different ecogeographical locations, and offering a workable source of characterized plant material ready for different purposes.

Populations of *C. capitatus* distribute in a limited area of Jordan in dry slopes of Mediterranean ecosystem. Almost all *C. capitatus* populations occupying wild habitat in Jordan are subjected to a variety of threatening factors (urbanization, over harvesting, air pollution, grazing, etc.) causing loss of genetic diversity and habitat destruction. Domestic cultivation is a viable alternative and offers the opportunity to overcome the problems that are inherent in herbal extracts: misidentification, genetic and phenotypic variability, extract variability and instability (Canter, *et al*., 2005). In response to increased popularity and greater demand for medicinal plants, bringing wild *C. capitatus* into cultivation system is important (Azaizeh, *et al*., 2005). Few scientific studies are available on the potentiality of *C. capitatus* for cultivation, Hedrick (1972) cited that *C. capitatus* used to be cultivated as a culinary herb in herb gardens.

4.5.1. Phenotypic variation among cultivated populations

Tables (18, 19, and 20) show various degrees of variation obtained according to investigated measurable morphological traits during two subsequent growing seasons (2007, 2008), as follows:

Days to flowering

The number of days from transplanting (9th April 2007) toward blooming recorded only during the year 2007, due to the life form of *C. capitatus* as a perennial shrub. Days to flowering for the 21 populations ranging between 50 to 135 day with C.V. of 0.9 % and over all mean of 108 days. This variation, joint with long period before flowering bring opportunity to harvest thyme populations named Za'tar Farisi as fresh and dry herb in periods extended from June to August, before the full development of flowers. Hedrick (1972) cited that *C. capitatus* is cultivated as a culinary herb, which if

Table (18): Phenotypic variation for days to flowering, number of inflorescence, and length of inflorescence (mm) of *Coridothymus capitatus* populations cultivated at Mushager research station in years 2007 and 2008.

Trait	Days to flowering			No. of inflorescence						Length of inflorescene (mm)					
Year	2007			2007			2008			2007			2008		
Population*	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
1	133.00	2.65	130-135	588.56	386.98	260-1113	593.00	387.31	276-1125	8.78	1.39	7-11	6.33	1.00	5-8
2	135.00	0.00	0.00	232.00	129.34	66-355	236.00	120.01	75-365	12.00	2.74	9-18	5.67	1.12	4-7
3	116.00	0.00	0.00	385.44	194.89	240-652	389.22	194.22	240-655	10.33	2.06	7-14	8.44	2.13	5-12
4	116.00	0.00	0.00	457.33	149.52	255-594	462.67	148.65	255-600	12.56	1.81	10-15	9.89	2.03	7-13
5	106.33	0.58	106-107	668.00	176.07	429-793	672.44	174.24	430-800	13.67	1.00	12-15	12.11	2.80	8-17
6	100.33	2.31	99-103	407.56	157.88	236-649	411.33	154.42	240-605	12.22	3.35	8-20	13.78	5.26	8-22
7	128.00	0.00	0.00	372.22	195.68	110-552	376.44	195.77	113-552	12.33	1.87	10-15	6.22	2.05	4-10
8	127.33	1.15	126-128	589.22	261.53	261-870	592.67	263.50	259-890	10.22	1.86	7-13	9.22	2.73	5-13
9	111.00	0.00	0.00	528.89	92.15	399-619	533.56	91.52	413-620	11.56	2.70	7-15	9.89	3.26	5-15
10	128.00	0.00	0.00	257.11	116.64	98-340	263.44	114.97	105-348	9.78	3.46	6-15	9.33	2.87	6-15
11	108.00	0.00	0.00	428.56	132.41	328-612	433.89	133.25	332-620	12.67	1.87	10-16	11.67	1.50	10-14
12	128.00	0.00	0.00	405.44	395.26	95-940	410.44	395.49	95-945	12.11	1.90	9-15	8.89	3.26	5-14
13	105.00	0.00	0.00	621.67	119.77	482-770	626.00	119.96	485-770	15.00	4.09	10-21	11.78	2.73	8-16
14	107.67	1.15	107-109	357.56	105.15	233-485	361.78	108.20	235-524	9.56	1.81	7-12	9.11	1.62	7-12
15	123.00	1.73	121-124	144.00	75.33	56-235	149.44	74.55	62-238	10.22	2.33	7-15	19.22	14.37	6-42
16	50.00	0.00	0.00	155.11	18.63	130-186	158.33	20.00	125-186	18.56	3.68	14-25	38.11	8.12	29-51
17	87.00	0.00	0.00	122.78	10.06	105-133	129.44	19.76	100-168	22.00	6.12	14-30	46.00	12.16	25-60
18	110.00	0.00	0.00	136.67	21.34	99-162	141.78	20.78	115-170	31.44	11.51	20-55	36.67	8.93	25-50
19	85.00	0.00	0.00	561.11	30.48	513-592	566.89	29.47	525-600	7.11	1.45	5-9	17.00	8.31	10-35
20	85.00	0.00	0.00	555.67	113.06	402-645	559.11	111.81	405-650	14.78	2.68	11-18	20.44	2.79	16-25
21	85.00	0.00	0.00	138.44	7.40	129-150	139.89	8.54	130-155	15.33	4.03	9-21	17.56	2.40	14-22
Overall	108.32		85-135	386.35		56-1113	390.85		62-1125	13.44		5.0-55.0	15.59		5.0-60.0
$LSD_{0.05}$	1.575			315.90			315.20			4.43			7.69		
% CV	0.90			49.50			48.90			20.0			29.90		

* Numbers 1-21 are populations of sites: Qasabat AS Salt, Al Fuhays, Tla' Kaser Khalda, Ayn Al Basha, Abu Hamid, Abu Nusayr, Khalda, Amman (West UOJ), Khalda military station, Wadi Slait, Bela's, Edbyan, Al Bassah, Wadi AS Sir, Um El Gutain, Kufranja, Anjara landrace 1, Anjara landrace 2, commercial cultivar 1, commercial cultivar 2, and commercial cultivar 3, respectively.

Table (19): Phenotypic variation for plant hieght (cm), plant width (cm), and plant length (cm) of *Coridothymus capitatus* populations cultivated at Mushager research station in years 2007 and 2008.

Trait	Plant height (cm)						Plant width (cm)						Plant length (cm)					
Year	2007			2008			2007			2008			2007			2008		
Population*	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
1	23.33	3.57	19-29	18.89	4.04	15-27	31.33	4.92	21-35	43.44	8.14	30-57	26.67	4.24	19-32	31.89	9.14	22-48
2	20.44	3.21	15-26	15.78	2.64	13-22	29.11	8.18	17-39	38.11	9.02	30-58	30.89	7.01	20-43	31.33	7.63	20-40
3	23.33	3.46	18-30	18.67	3.67	14-24	26.78	4.97	19-32	40.33	6.71	33-52	26.78	4.55	18-32	30.00	5.81	23-39
4	25.56	1.88	24-29	19.56	4.80	15-29	33.44	6.56	29-50	38.78	11.20	25-63	27.33	5.12	21-38	30.00	6.22	22-43
5	24.00	3.54	20-29	17.44	5.17	12-28	28.00	5.61	20-40	34.33	5.32	30-44	25.56	4.59	20-34	27.56	7.37	20-44
6	22.78	5.89	18-34	14.56	5.22	10-26	25.00	3.54	20-31	33.89	8.21	22-50	22.22	3.80	15-27	27.44	4.39	21-36
7	26.33	3.97	20-32	20.22	6.34	12-31	30.33	10.30	14-43	39.11	14.62	23-64	30.00	9.67	15-42	35.67	13.27	20-60
8	22.89	3.52	16-28	18.11	5.46	10-28	29.33	4.97	21-38	36.56	9.90	25-51	25.33	2.50	21-28	29.56	5.55	23-40
9	26.56	4.25	21-33	19.11	7.46	10-33	30.44	3.81	26-37	39.89	13.83	20-67	27.56	2.46	25-32	31.89	13.62	20-62
10	22.56	3.40	15-28	19.78	7.73	10-30	33.33	7.78	18-45	34.33	13.14	17-57	29.89	6.19	20-38	32.89	10.87	16-50
11	27.11	4.94	21-33	24.56	5.92	15-32	34.56	11.10	20-55	38.67	10.69	25-60	32.11	7.52	19-41	34.44	11.42	20-50
12	23.22	4.15	18-31	18.00	7.75	12-32	31.33	5.61	19-38	40.11	12.82	30-62	31.56	6.71	23-42	36.56	12.01	25-56
13	25.78	2.33	22-29	19.78	5.95	15-34	30.56	3.47	26-38	43.44	12.56	22-66	26.44	3.88	19-31	33.33	11.00	22-58
14	26.22	5.36	18-34	17.00	3.77	12-23	27.56	4.03	22-35	40.67	10.48	25-56	23.89	6.07	19-39	33.00	5.92	22-39
15	22.33	1.73	20-25	21.00	5.61	15-30	28.89	4.59	20-35	34.11	9.14	25-51	27.78	4.84	21-33	27.22	7.43	20-41
16	29.78	4.09	21-35	29.00	2.69	23-32	28.11	4.31	21-34	42.67	6.26	31-49	26.56	1.94	23-30	38.67	6.02	30-48
17	26.22	4.02	19-31	25.78	4.32	19-32	19.78	8.12	8-35	43.44	5.75	34-50	21.67	7.48	11-30	41.44	8.73	27-56
18	28.22	3.38	22-32	27.33	3.00	23-33	22.89	5.71	15-30	42.11	4.20	36-50	23.56	3.57	19-28	34.22	3.93	31-43
19	21.22	2.49	15-23	29.11	3.26	25-36	27.78	6.82	18-39	42.78	3.93	36-47	23.33	5.70	15-30	38.44	5.03	34-50
20	23.33	4.58	19-30	26.89	8.37	7-35	26.78	6.83	10-33	44.56	4.13	40-50	24.89	3.95	19-30	34.78	8.86	15-46
21	35.00	9.35	20-50	26.11	9.28	15-40	30.11	6.99	20-40	24.44	6.35	15-35	24.78	2.77	20-28	21.11	3.98	15-25
Overall	25.06		15-50	21.27		10-40	28.83		8-55	38.85		15-67	26.61		11-43	32.45		15-62
$LSD_{0.05}$	4.98			5.98			7.47			9.09			7.10			8.82		
% CV	12.00			17.00			15.70			14.20			16.20			16.50		

*Numbers 1-21 are populations of sites: Qasabat AS Salt, Al Fuhays,Tla' Kaser Khalda, Ayn Al Basha, Abu Hamid, Abu Nusayr, Khalda, Amman (West UOJ), Khalda military station, Wadi Slait, Bela's, Edbyan, Al Bassah, Wadi AS Sir, Um El Gutain, Kufranja, Anjara landrace 1, Anjara landrace 2, commercial cultivar 1, commercial cultivar 2, and commercial cultivar 3, respectively.

Table (20): Phenotypic variation for leaf length (mm), and leaf width (mm) of *Coridothymus capitatus* populations cultivated at Mushager research station in years 2007 and 2008.

Trait	Leaf length (mm)						Leaf width (mm)					
Year	2007			2008			2007			2008		
Population*	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
1	4.44	1.42	3-7	3.22	1.48	1-5	1.11	0.22	1-1.5	2.06	1.07	1-4
2	4.44	0.88	3-6	4.00	1.12	2-6	0.98	0.07	0.8-1	1.28	0.44	1-2
3	4.67	0.71	4-6	4.44	1.13	2-6	0.97	0.10	0.7-1	1.33	0.50	1-2
4	4.33	0.87	3-6	4.67	0.71	4-6	0.97	0.10	0.7-1	1.33	0.50	1.2
5	4.33	0.71	3-5	5.11	1.17	4-7	1.11	0.33	1-2	1.44	0.53	1-2
6	4.44	1.13	3-6	4.56	0.88	3-6	1.17	0.35	1-2	1.22	0.44	1-2
7	4.00	0.71	3-5	4.22	0.67	4-6	1.17	0.35	1-2	1.06	0.17	1-1.5
8	4.67	1.00	3-6	4.44	1.51	2-7	1.22	0.36	1-2	1.28	0.44	1-2
9	4.22	1.09	3-6	4.22	0.97	3-6	1.11	0.33	1-2	1.22	0.36	1-2
10	3.89	1.17	3-6	4.33	0.71	3-5	1.22	0.44	1-2	1.22	0.44	1-2
11	5.22	0.97	4-7	4.00	0.87	3-5	1.17	0.35	1-2	1.33	0.50	1-2
12	4.22	0.67	3-5	4.22	1.20	2-6	1.22	0.44	1-2	1.44	0.42	1-2
13	4.67	1.00	3-6	4.44	1.01	3-6	1.33	0.50	1-2	1.31	0.46	1-2
14	3.89	0.78	3-5	4.33	0.71	3-5	0.99	0.25	0.5-1.5	1.39	0.49	1-2
15	5.78	1.48	4-8	4.22	0.67	3-5	1.44	0.73	1-3	1.17	0.35	1-2
16	10.89	1.45	8-13	11.78	3.03	6-15	2.56	0.53	2-3	2.44	0.73	2-4
17	11.00	1.00	10-12	11.89	2.20	10-17	1.89	0.60	1-3	2.22	0.44	2-3
18	10.44	1.67	8-14	12.33	1.58	10-15	1.83	0.35	1-2	2.33	0.50	2-3
19	6.11	1.36	4-8	4.67	1.12	3-6	2.33	0.50	2-3	1.78	0.44	1-2
20	5.78	0.67	5-7	4.22	0.97	3-6	1.78	0.67	1-3	1.78	0.44	1-2
21	10.89	1.17	9-13	10.22	1.64	8-12	1.83	0.43	1-2.5	1.83	0.71	1-3
Overall	5.83		3-14	5.69		1-17	1.40		0.5-3	1.55		1-4
$LSD_{0.05}$	1.34			1.42			0.45			0.57		
% C.V.	14.00			15.10			19.70			22.50		

*Numbers 1-21 are populations of sites: Qasabat AS Salt, Al Fuhays, Tla' Kaser Khalda, Ayn Al Basha, Abu Hamid, Abu Nusayr, Khalda, Amman (West UOJ), Khalda military station, Wadi Slait, Bela's, Edbyan, Al Bassah, Wadi AS Sir, Um El Gutain, Kufranja, Anjara landrace 1, Anjara landrace 2, commercial cultivar 1, commercial cultivar 2, and commercial cultivar 3, respectively.

its leaves are to be dried, the plants should be harvest just before the flowers open.

The first 15 populations of *C.capitatus*, show variation in days to flowering which ranging between 100–135 days. Populations of Qasabat AS Salt and Al Fuhays, exhibit the highest number of days to flowering (133 and 135 day, respectively) followed by populations of Khalda, Wadi Slait, and Edbyan (128 day), while Khalda military station population exhibit the lowest number of days to flowering.

The trait number of days to flowering is known to be affected by environmental conditions, but it could be also inherited (Feher and Walter, 1987). This could explain results obtained in this study because cultivated populations exhibited various numbers of days to flowering in the field where environmental conditions were relatively controlled. For example, individuals of population Wadi Slait collected from elevation of 547 m recorded average number of days to flowering (128) more than Abu Nusayr population (100 day) which was collected from different environment from elevation of 858 m, indicating significant differences among them that could refer to genetic difference. This also indicated the possibility to improve particular population for particular purpose like exploiting the long period of vegetative growth for production of fresh herbs and volatile oil. On the other hand, populations of *C.capitatus* tend toward long time until blooming indicating their ecological value for insect pollinators and solitary bees, Petanidou, (1996) and Tsigouri *et al*. (2004) mentioned that *C. capitatus* is considered an important source for production of unifloral honeys due to late flowering.

Number of inflorescence

High coefficient of variation (C.V.) related to number of inflorescence per plant was recorded during years 2007 and 2008 (50.05 %, 49.0 %, respectively), indicating wide variation among populations. Population of Abu Hamid recorded the highest average

number of inflorescence in two subsequent years (2007 and 2008) which was 668.0 and 672.4, respectively, exceeding the overall mean by 73 % and 72 %, respectively. Knowing that site of Abu Hamid is protected by fence, we suggest that additive accumulation of genetic base related to this character caused the expression of this high number. Hence, we can expect that high oil percentage will be obtained from individuals belonging to this population. This trait is also important for seed production (Tonçer and Kizil, 2005).

Length of inflorescence

High coefficient of variation (C.V.) obtained under field conditions for length of inflorescence during 2007 and 2008 (20.0 %, 29 %, respectively). The highest length during 2007 recorded by Anjara landrace 2 (31.4 mm) followed by Anjara landrace 1 (22.0 mm) and the lowest recorded by the commercial cultivar 1 (7.11). In 2008, the highest length of inflorescence recorded by Anjara landrace 1 (46.00 mm), indicating an improvement occurred for this trait. The recorded overall mean was 13.44 mm in 2007 and 15.59 mm in 2008, indicating an improvement from that obtained in wild (9.16 mm). This trait is important for oil production and in taxonomical studies (Petanidou, 1996; Tsigouri et al. 2004).

Plant height

Table (19) shows high coefficient of variations (C.V.) for plant height in 2007 and 2008 (12 %, 17 %, respectively), indicating variations among populations. The commercial cultivar 3 recorded the largest height during 2007 (35.0 cm) while The commercial cultivar 1 recorded the largest height in 2008 (29.1 cm). In comparison with wild populations (Annex XIII), the trend is coming toward decreasing of plant height, particularly in the absence of particular cultural practices like pruning. For example, *C. capitatus* population of Tla' Kaser Khalda showed the largest height (46.0

cm) among wild populations studied, then height decreased under cultivation in 2007 and 2008 (33.3 cm, 18.7 cm, respectively), indicating that this species tend to distribute horizontally forming dwarf shrub as described by Feinbrun, (1978) and Polunin, (1980).

Plant width and length

No significant differences among populations obtained for plant width trait in the year 2007, this could be justified when consider year 2007 as the first year of field establishment. In 2008, variation among populations for plant width was significant (C.V = 14.2 %), indicating variations among populations and presenting the good stand of this species in the seconed growing season assuming the potentiality of this species to propagate by seeds beside cuttings. Iapichino *et al.* (2006) reported that *C.capitatus* exhibit good aptitude to pot cultivation when propagated by using vegetative cuttings. In 2008, populations of Qasabat AS Salt, Ayn Al Basha, Al Bassah, Edbyan, Wadi AS Sir, and Kufranja show plant width more than 40.0 cm, which exceed the overall mean (38.85 cm).

High coefficient of variation (C.V.) recorded for plant length in 2007 and 2008 (16.20 %, 16.50 %, respectively), indicating variations among populations. Plant length improved in 2008 for all populations. Among *C.capitatus* populations, Edbyan show the highest plant length in 2008 (36.56 cm), while among *Thy. spicata* populations (Kufranja, Anjara landrace 1, Anjara landrace 2), no significant difereces were record.

Data shows that almost all population tend to have plant width more than length, this attitude did not recognized in wild population, indicating that this difference may be due to cultivation process. Monokrousos, *et al*. (2004), reported that the soil biochemical processes are factors affect life form of *C. capitatus*. Huxley (1992) stated that *C. capitatus* is hardy to about -10°C but at same time prefers light, well drained

calcareous soil and a sunny position. Hence, we suggest that plants cultivated in the same plot compete for the micronutrients and other environmental growth factors, and consequently increase their width where more space available for catching nutrient sources. Hence, we suggest increase the distance between plants within same row for better expression of plant width and length traits.

Leaf length and width

The coefficient of variation (C.V.) related to leaf length was high during years 2007 and 2008 (14.0 %, 15.1 %, respectively), indicating high variation among populations (Table 20). Relatively, *C.capitatus* populations show consequent increase for leaf length in 2008 (Figure 8). However, *C.capitatus* populations varied in their improvements, for example, Um El Gutain population present the highest average of leaf length in year 2007 (5.78 mm), while in 2008 Abu Hamid population present the highest value (5.11 mm). On the other hand, populations of Kufranja, Anjara landrace1, and Anjara landrace 2, those identified as *Thy. spicata,* show leaf length more than 10 mm in 2007 and more than 11 mm in 2008, this due to the genetic base which differenciate these populations from the rest. This genetic difference is important to confirm at the molecular level.

The trait leaf width expressed high variation among 21 thyme populations grow under cultivation. The C.V. obtain in 2007 and 2008 was 19.70 % and 22.50 %, respectively (Table 19). Toward 2008, all populations growing under field conditions show an increase in their leaf width except the populations of Khalda, Um El Gutain, and the three commercial cultivars (Figure 8), indicating the ability of thyme species to increase their leaf surface area and improve photosynthesis process and sequentially the productions of biproducts (Taia and El-Etaby, 2006). Among *C.capitatus* populations, the highest leaf length recorded in 2008 belong to Qasabat AS Salt (2.06 mm), followed

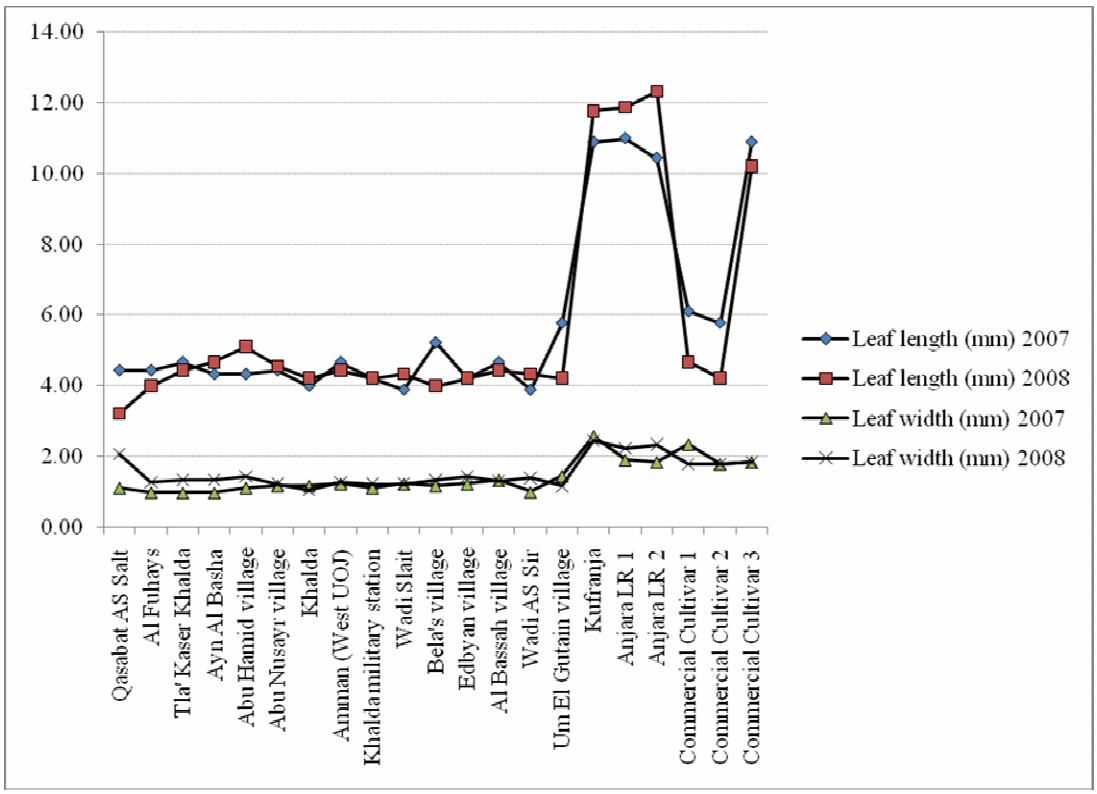


Figure (8): Leaf length (mm), and leaf width (mm) of *C. capitatus* populations cultivated at Mushager research station in years 2007 and 2008.

by Abu Hamid and Edbyan (1.44 mm), indicating that these populations could be a promissing populations for production herbal materials and oil.

Moreover, the cultivated populations of *C.capitatus* present leaf length and width higher than those grow in wild (Annex XIII), indicating the potential of these population to improve for leaf length and width. Leaves (fresh or dried) of thyme species considered the main vegetative organs used as a spice add aroma and flavor to food, and the essential oils extracted from leaves and flowers can be used as pharmaceuticals, and cosmetics (Morales, 1996; Senatore, 1996; Goren, *et al*. 2003; Lee, *et al*. 2005). Thus, variation exists among thyme populations for leaves length and width found is important to initiate breeding program.

4.5.2. Fresh and dry weight

Tables (21, 22) and Figures (9, 10) present variation in productivity expressed by fresh and dry weight of all cultivated populations during years 2007 and 2008.

In 2007, no significant differences among populations obtained for fresh weight, the (C.V. = 20.2 %). However, Bela's population recorded the highest amount of fresh yield (253.22 g/plant), followed by Edbyan population (217.22 g/plant). In 2008, the C.V. raised to 21.20 %, indicating high variation among populations.

Among *C. capitatus* populations, Ayn Al Basha present the highest fresh weight (209.56 g/plant) followed by Bela's and Edbyan populations (178.33 g/plant, 163.89 g/plant, respectively) with no significant difference, indicating the ability of these populations to present high fresh herbage yield. In fact, almost all *C. capitatus* populations produced lower fresh yield in 2008 than in 2007 (Figure 9).

In the contrary, the fresh yield dramatically increased during 2008 for populations of Kufranja, Anjara landraces 1, and Anjara landraces 2 (363.33 g/plant, 265.00 g/plant, and 251.11 g/plant, respectively), suggesting the ideality of these populations for production of fresh herb. All commercial cultivars show higher progress toward fresh yield than dry yield production.

Regarding dry weight, high C.V. obtained in years 2007 and 2008 (23.2 %, 22.90 %, respectively) indicating variations among populations. In 2008, all populations show an increase in dry weight (Figure 10). Out of expectation, all *C. capitatus* populations show increase of dry yield during the second year (2008) of establishment. This may be due to the tendency of species *C. capitatus* to accumulate secondary metabolites (mainly essential oil) in a dry form during plant life.

Table (21): Fresh herb production of *Coridothymus capitatus* populations cultivated at Mushager research station during 2007 and 2008.

Year	2007				2008			
Population	g plant^{-1}	SD	Range	*** Yield (Kg ha^{-1})	g plant^{-1}	SD	Range	Yield (Kg ha^{-1})
Qasabat AS Salt	173.89	42.02	105-250	4140.2	156.67	29.22	110-205	3730.2
Al Fuhays	205.56	57.31	120-290	4894.2	185.11	50.00	95-250	4407.4
Tla' Kaser Khalda	166.11	45.87	110-245	3955.0	152.11	36.09	110-210	3621.7
Ayn Al Basha	205.00	33.99	155-270	4881.0	209.56	53.60	125-295	4989.4
Abu Hamid village	182.78	47.38	115-270	4351.9	142.78	31.09	110-205	3399.5
Abu Nusayr village	165.56	26.21	125-215	3941.8	154.33	19.70	125-186	3674.6
Khalda	172.78	50.79	110-265	4113.8	130.33	39.80	74-210	3103.2
Amman (West UOJ)	180.00	32.66	130-235	4285.7	137.22	27.82	110-190	3267.2
Khalda military station	140.56	45.64	75-215	3346.6	133.33	54.14	55-230	3174.6
Wadi Slait	160.56	33.86	100-290	3822.8	126.33	18.46	96-160	3007.9
Bela's village	253.22	74.38	135-350	6029.1	178.33	48.33	110-260	4246.0
Edbyan village	217.22	81.66	115-340	5172.0	163.89	45.74	125-255	3902.1
Al Bassah village	202.78	49.50	140-295	4828.0	175.00	79.18	85-310	4166.7
Wadi AS Sir	191.11	56.84	125-320	4550.3	175.56	39.72	110-240	4179.9
Um El Gutain village	161.33	27.35	125-195	3841.3	131.44	22.13	108-180	3129.6
Kufranja	193.56	45.03	132-290	4608.5	363.33	133.37	195-590	8650.8
Anjarah (Landrace 1)	148.89	27.06	125-220	3545.0	265.00	52.26	190-335	6309.5
Anjarah (Landrace 2)	150.44	36.79	90-220	3582.0	251.11	69.23	145-345	5978.8
Commercial cultivar 1	187.22	50.48	125-270	4457.7	329.44	151.58	105-545	7843.9
Commercial cultivar 2	185.00	42.62	130-245	4404.8	325.56	109.13	160-555	7751.3
Commercial cultivar 3	167.78	42.75	120-230	3994.7	396.11	113.02	180-550	9431.2
Mean	181.49			4321.3	203.93			485.5.5
*LSD $_{0.05}$	59.26				71.33			
**C.V. %	20.20				21.20			

* LSD = Least significant difference.

** C.V. = Coefficient of variation.

***Estimated yields (2.4 plant m^{-2}).

Table (22): Dry herb production of *Coridothymus capitatus* populations cultivated at Mushager research station during 2007 and 2008.

Year	2007				2008			
Population	g plant^{-1}	SD	Range	*** Yield (Kg ha^{-1})	g plant^{-1}	SD	Range	Yield (Kg ha^{-1})
Qasabat AS Salt	74.44	19.95	35-105	1772.5	130.56	21.86	55-120	3108.5
Al Fuhays	81.11	25.13	50-115	1931.2	172.22	28.59	65-145	4100.5
Tla' Kaser Khalda	56.67	18.00	35-90	1349.2	128.11	19.30	35-90	3050.3
Ayn Al Basha	86.11	16.76	65-115	2050.3	158.33	19.00	70-125	3769.8
Abu Hamid village	83.89	26.64	25-115	1997.4	116.67	24.49	30-105	2777.8
Abu Nusayr village	66.11	14.22	40-90	1574.1	119.44	10.00	60-85	2843.9
Khalda	63.89	33.21	20-120	1521.2	100.78	13.58	35-79	2399.5
Amman (West UOJ)	78.89	15.98	55-105	1878.3	116.11	21.62	40-115	2764.6
Khalda military station	44.44	22.09	15-80	1058.2	88.22	14.92	30-74	2100.5
Wadi Slait	67.44	16.21	52-100	1605.8	90.78	19.41	30-90	2161.4
Bela's village	104.44	33.33	30-135	2486.8	153.00	26.02	40-130	3642.9
Edbyan village	85.56	33.78	30-140	2037.0	140.56	27.95	35-135	3346.6
Al Bassah village	90.56	20.58	60-120	2156.1	156.11	41.74	45-160	3716.9
Wadi AS Sir	93.89	19.69	70-125	223.5.4	166.11	20.78	70-140	3955.0
Um El Gutain village	53.33	11.92	38-80	1269.8	81.67	10.93	30-70	1944.4
Average production				1794.9				3045.5
Kufranja	84.11	21.12	57-130	2002.6	248.89	48.99	90-230	5925.9
Anjarah (Landrace 1)	57.78	21.58	30-105	1375.7	183.89	17.70	85-135	4378.3
Anjarah (Landrace 2)	64.44	21.90	35-100	1534.4	170.56	36.81	65-185	4060.8
Commercial cultivar 1	73.89	30.24	20-120	1759.3	208.33	53.94	55-220	4960.3
Commercial cultivar 2	77.78	21.85	20-100	1851.9	194.44	46.53	40-205	4629.6
Commercial cultivar 3	28.33	5.13	20-35	674.6	261.67	83.92	25-265	6230.2
Mean	72.24			1720.1	151.74			3612.7
*LSD $_{0.05}$	27.65				57.36			
**C.V. %	23.20				22.90			

* LSD = Least significant difference, ** C.V. = Coefficient of variation, *** Estimated yields (2.4 plant m^{-2})

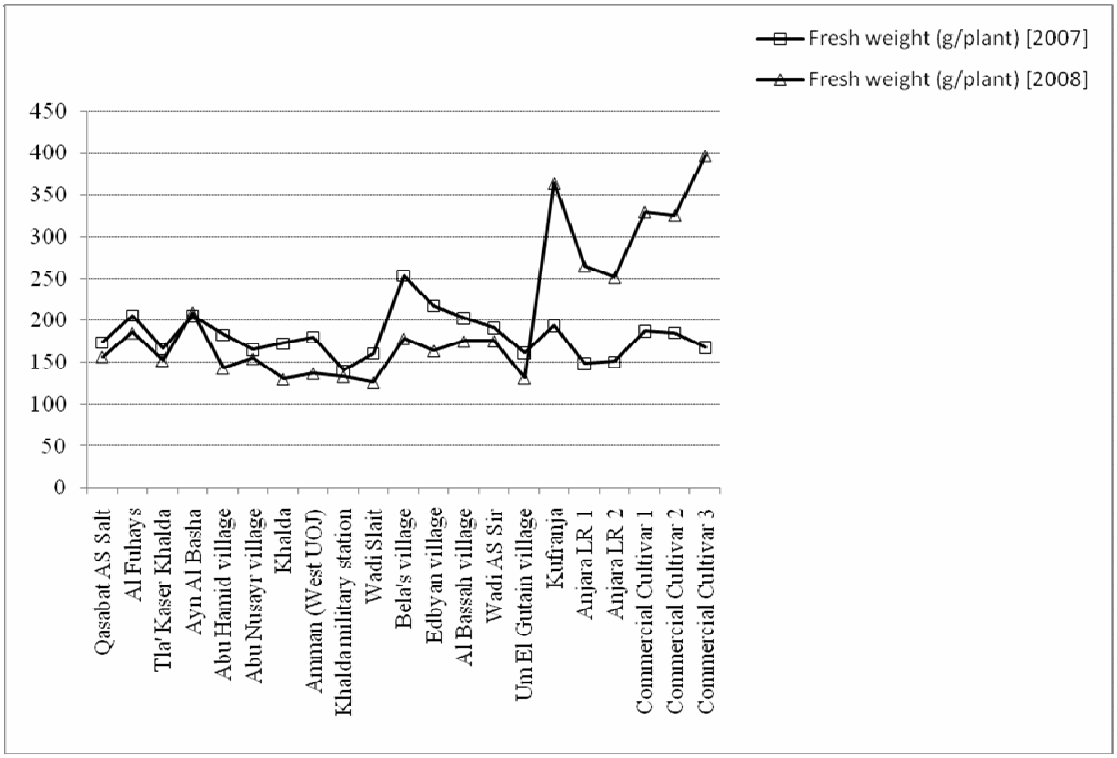


Figure (9): Fresh weight (g/ plant) of *Coridothymus capitatus* populations cultinvated at Mushager research station in years 2007 and 2008.

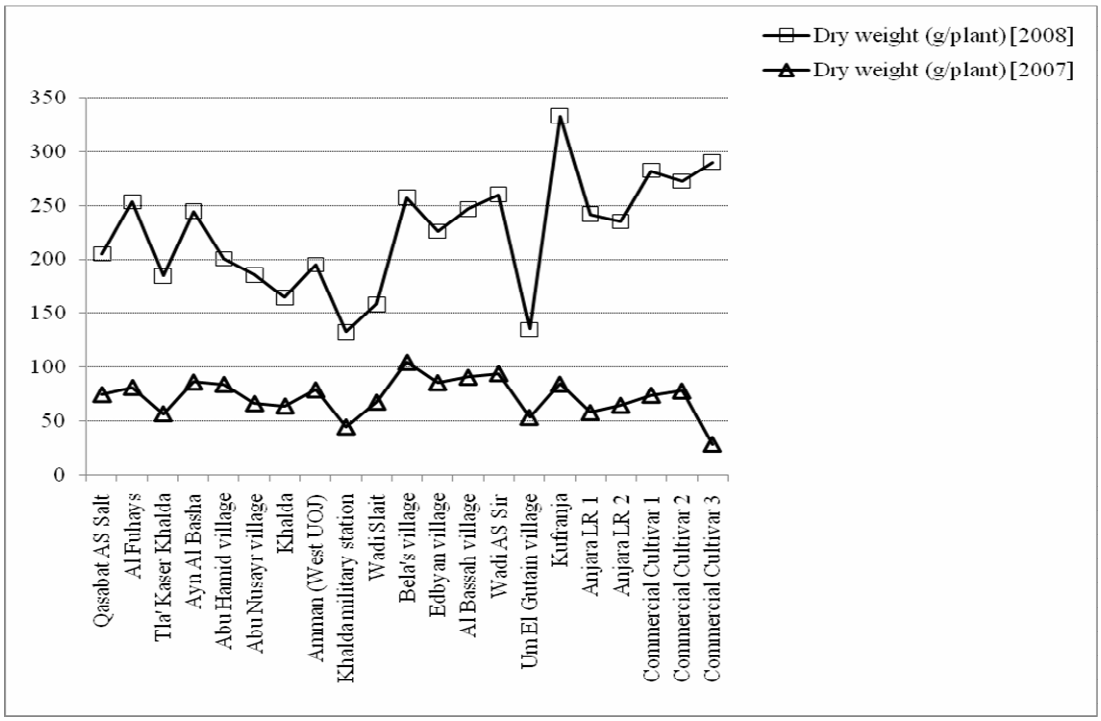


Figure (10): Dry weight (g/ plant) of *Coridothymus capitatus* populations cultivated at Mushager research station in years 2007 and 2008.

Rodrigues, *et al*. 2006, cited that the material secreted (mainly carvacrol) by the glandular cells in species *C. capitatus* passes through the apical walls and accumulates within a large space formed by detachment of the cuticle together with the pectin layer of the secretory cell walls.

Among *C. capitatus* populations: Khalda, Khalda military station, Wadi Slait, and Um EL Gutain, shows the lowest dry yield with no significant differences, while Al Fuhays population show the highest dry yield (172.22 g/plant) with no significant difference between this population and the rest.

In order to valorize and promote *C.capitatus* as a crop plant, it is critical to investigate variation concerning potentiality of *C.capitatus* populations to produce fresh and dry herbage yield, which is utilize in various purposes (Rodrigues, *et al*. 2006). Considering the life form of *C.capitatus* as a dimorphic shrub (Monokrousos, *et al*. 2004), the fresh and dry weight estimated as g/plant. However, the fresh and dry herbage yield (Kg/ha) could be improved by increasing number of plants per unit area for more than 2.4 plant/ m^2 which applied in this study, and this needs to be studied thoroughly. The total dry yield of *Origanum syriacum* increased to 125 gm/m^2 by increasing plant density from 1.9 to 5.3 plants/ m^2 (Abu Al Rub, 1996).

Data obtained in this study, shows variations among *C.capitatus* for production of both fresh and dry yield. However, almost all *C.capitatus* populations show potential and progress toward producing dry herbage yield as the average production during 2008 was 3045.5 kg/ha (127.9 g/ plant). Knowing that the cost of 250 g of dried leaves is about 2.5 J.D. in local markets of Jordan, make it logic to give *C.capitatus* more attention toward development as a crop. In addition, the demand for dry material of *C.capitatus* increase dramatically for essential oil production and this oil being one of

the most expensive oil utilize in pharmaceutical, food industry, cosmetic and perfumery (Rodrigues, *et al*., 2006).

On the other hand, populations of Kufranja, Anjara landrace 1, and Anjara landrace 2, show high potential to produce fresh herbal material. During 2008, the individuals of these populations identified as *Thy. spicata* and named Za'tar Farisi in Ajloun district exhibit an average fresh herbage yield of 6979.7 kg/ha (293.15 g/plant) which encourage cultivation of this species for fresh herb production and also promote investigation of its diversity.

4.5.3. Variation of essential oils among cultivated populations

In recent years, thyme has started to be cultivated as a new crop for production of oregano essential oils particularly those rich in carvacrol; the oil responsible for the biological activities of oregano such as antimicrobial, antitumor, antimutagenic, etc. (Toncer and Kizil, 2005; Can Baser, 2008). In this study, almost all populations of *C. capitatus* grow in wild habitat in Jordan exhibit high content of the oregano essential oil. However, results varied under cultivation, Table (23) shows the amount of essential oil, thymol, carvacrol and thymol plus carvacrol of *C.capitatus* cultivated at Mushagar agricultural research station. The highest essential oil percentage (4.99%) obtained from Wadi AS Sir population followed by Edbyan population (4.23%), while the lowest percentage (1.04 %) produced by Bela's population. Comparing with oil percentage obtained from wild *C. capitatus,* some populations show progress while other show retreat (Figures 11, 12, 13, and 14). For example, Wadi AS Sir and Edbyan populations produced higher percentages of oil under field conditions exceeding the wild production by 188.86 %, and 127.0 % respectively. In the contrary, Abu Hamid population exhibit oil percentage in the field less than in wild habitat. This may be because individuals grow in Abu Hamid site were protected, thus they grow

Table (23): Eessential oil, thymol and carvacrol content of *C. capitatus* growing under cultivation at Mushager agricultural research station.

Population	Total essential oil		Thymol		Carvacrol		Thymol+Carvacrol	
	(mg/g)	(%)	(mg/g) ± SD	(%)	(mg/g) ± SD	(%)	(mg/g)	(%)
Qasabat AS Salt	12.82	1.28	3.53 ± 0.83	0.35	2.96 ± 0.60	0.30	6.49	0.65
Al Fuhays	31.56	3.16	0.32 ± 0.07	0.03	0.08 ± 0.02	0.01	0.40	0.04
Tla' Kaser Khalda	25.70	2.57	4.17 ± 1.27	0.42	1.77 ± 0.54	0.18	5.94	0.59
Ayn Al Basha	27.43	2.74	0.40 ± 0.05	0.04	4.58 ± 0.51	0.46	4.99	0.50
Abu Hamid village	21.90	2.19	2.49 ± 0.41	0.25	1.92 ± 0.32	0.19	4.42	0.44
Abu Nusayr village	35.28	3.53	0.58 ± 0.02	0.06	6.96 ± 0.20	0.70	7.53	0.75
Khalda	22.63	2.26	4.26 ± 0.20	0.43	1.49 ± 0.07	0.15	5.75	0.57
Amman (West UOJ)	33.74	3.37	7.34 ± 1.41	0.73	5.30 ± 0.42	0.53	12.64	1.26
Khalda military station	40.23	4.02	9.03 ± 2.04	0.90	1.72 ± 0.39	0.17	10.74	1.07
Wadi Slait	24.17	2.42	4.46 ± 1.64	0.45	1.58 ± 0.58	0.16	6.04	0.60
Bela's village	38.46	3.85	5.29 ± 2.16	0.53	4.22 ± 2.07	0.42	9.50	0.95
Edbyan village	42.30	4.23	0.46 ± 0.06	0.05	9.04 ± 1.11	0.90	9.51	0.95
Al Bassah village	28.89	2.89	0.38 ± 0.03	0.04	0.46 ± 0.03	0.05	0.84	0.08
Wadi AS Sir	49.93	4.99	0.50 ± 0.05	0.05	9.0 ± 0.96	0.90	9.53	0.95
Um El Gutain village	22.57	2.26	0.31 ± 0.09	0.03	5.58 ± 1.57	0.56	5.88	0.59
Kufranja	24.42	2.44	0.51 ± 0.03	0.05	4.66 ± 0.29	0.47	5.17	0.52
Anjarah (Landrace 1)	25.46	2.55	0.26 ± 0.04	0.03	4.02 ± 0.55	0.40	4.28	0.43
Anjarah (Landrace 2)	24.52	2.45	0.12 ± 0.01	0.01	4.25 ± 0.13	0.42	4.37	0.44
Commercial cultivar 1	12.71	1.27	0.14 ± 0.03	0.01	2.60 ± 1.41	0.26	2.74	0.27
Commercial cultivar 2	17.05	1.71	0.25 ± 0.03	0.03	2.76 ± 0.31	0.28	3.01	0.30
Commercial cultivar 3	14.04	1.40	0.20 ± 0.04	0.02	2.28 ± 0.42	0.23	2.48	0.58
Over all	27.42	2.74	2.14	0.21	3.69	0.37	5.82	0.85
$^{*}LSD_{0.05}$			1.475		1.275			
** % C.V.			41.70		21.00			

Data are yields of essential oil composition obtained from arial parts of 10.0 g dry weight. * Least significant difference. ** Coefficient of variation.

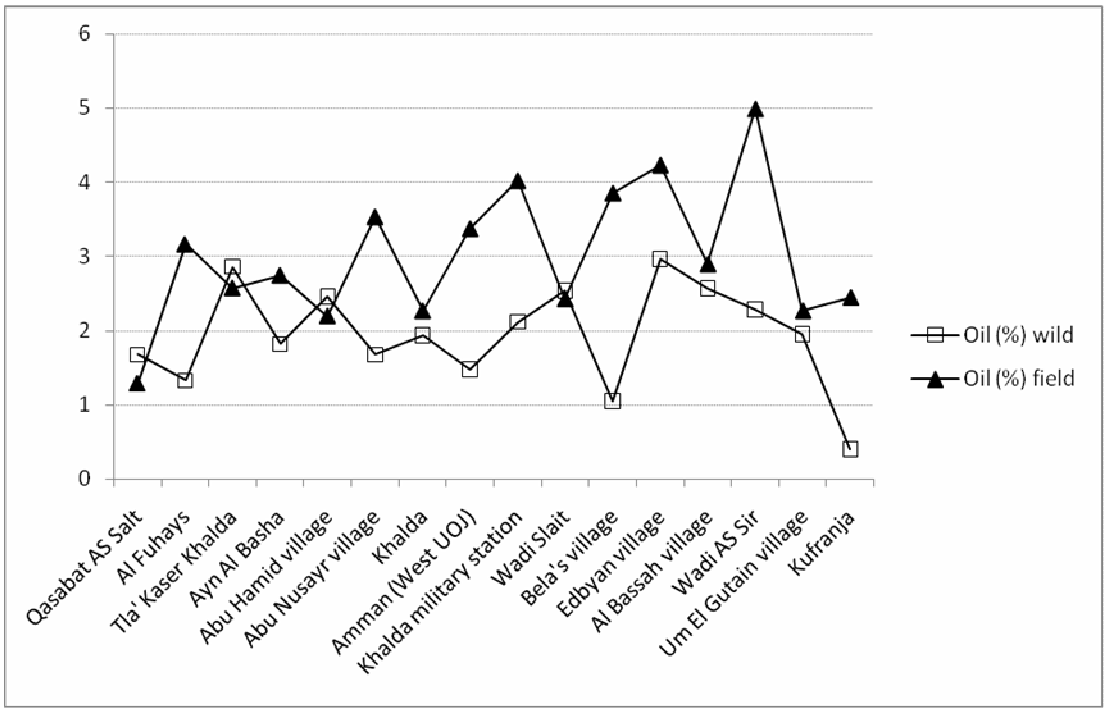


Figure (11): Percentages of oil yield of *Coridothymus capitatus* populations growing in wild habitat in Jordan and cultivated under field conditions during 2007.

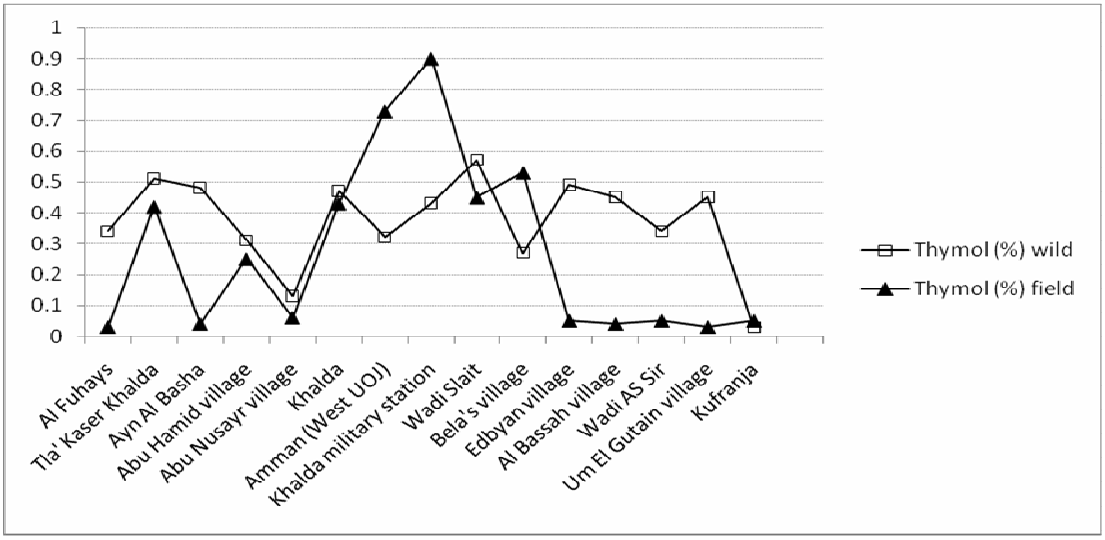


Figure (12): Percentages of thymol of *Coridothymus capitatus* populations growing in wild habitat in Jordan and cultivated under field conditions during 2007.

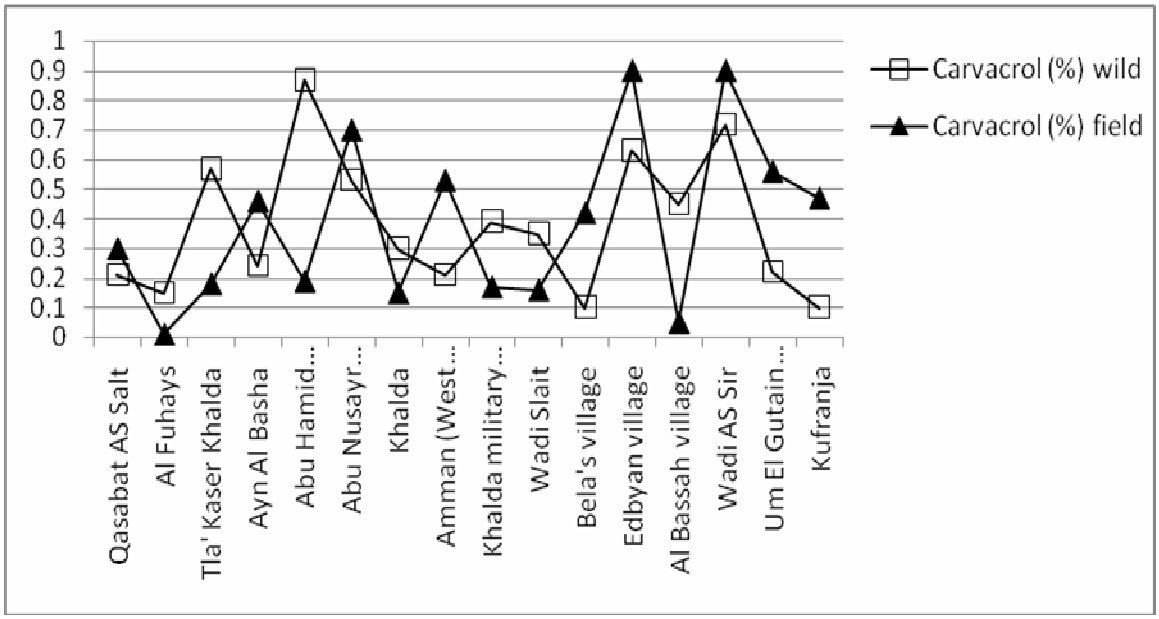


Figure (13): Percentages of carvacrol of *Coridothymus capitatus* populations growing in wild habitat in Jordan and cultivated under field conditions during 2007.

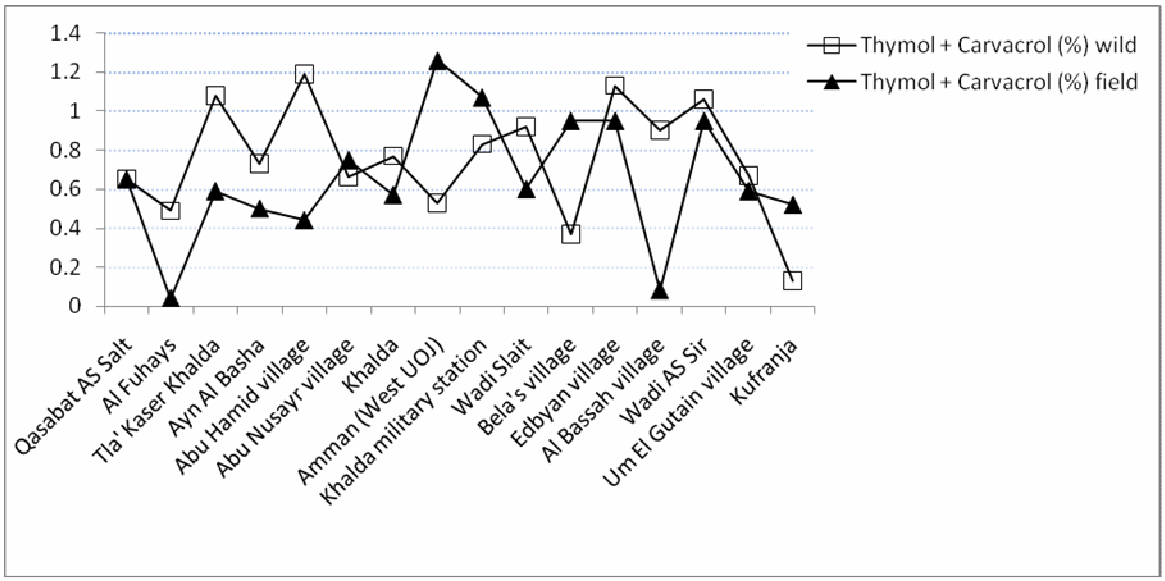


Figure (14): Percentages of thymol plus carvacrol of *Coridothymus capitatus* populations growing in wild habitat in Jordan and cultivated under field conditions during 2007.

in optimum conditions and not subjected to pollution neither urbanization threat unlike other population in this study, consequently Abu Hamid population was able to express its potential to produce superior oil yield. In the field, where environmental conditions are relatively controlled, all populations have same opportunity to exhibit their real potential toward oil production, thus Wadi AS Sir and Edbyan populations present the highest oil yield.

Populations of Kufranj, Anjara landrace 1, and Anjara landrace 2, represent species *Thy. spicata,* produced oil percentage ranging between 2.44 and 2.55 %, indicating the potential of these populations to be developed for high oil yield. Toncer and Kizil (2005) results show essential oil percentage ranged from 1.58 to 2.33 % when evaluated *Thymbra spicata* L. var. *spicata*, under field conditions.

Various degrees of variation among populations grow under field conditions obtained by the analysis of the essential oil components. Thymol percentage ranging between 0.01 - 0.9 % with C.V**.** of 41.70 %. The carvacrol percentage ranging between 0.1 - 0.87 % with C.V. of 21.0 %, indicating high level of variation among populations in relation to their contents of thymol and carvacrol.

The highest percentage of thymol recorded by Khalda military station (0.90 %), followed by Amman (0.72 %), suggesting that some populations could be exploited to produce mainly the essential oil thymol, this was confirmed in cluster analysis conducted for wild populations as these two populations were belonged to thymol type group. The lowest percentage recorded by Anjara landrace 2 and commercial cultivar supporting our previous suggestion to promote cultivation of these species for fresh herb and for total oil yield production.

The highest percentage of carvacrol recorded by Edbyan and Wadi AS Sir populations (0.90 %). In cluster analysis (Figure 6), these two populations consolidated

in one cluster characterized by high carvacrol content, meaning that production of carvacrol essential oil is an inherited trait with a specific genetic base. Thus, conservatively we recommend exploiting Edbyan and Wadi AS Sir populations for production of the valuable essential oil carvacrol. However, molecular analysis is important to confirm our findings that these two populations are genetically diverse from other *C. capitatus* populations.

One of the most important characteristics of oil accumulation in cultivated thyme is the amount of thymol and carvacrol contained in the oil (Toncer and Kizil, 2005; Can Baser, 2008). Among 21 populations, grow under field conditions in this study, populations of Amman (1.26 %), Khalda military station (1.07 %), Wadi AS Sir, Edbyan, and Bela's (all 0.95 %), considerd the top five populations produced the maximum additive percentage, of thymol and carvacrol. These results indicate the potentiality of the species *C.capitatus* to produce thymol and carvacrol under field conditions.

The essential oil yield paralleled to the herbage yield is shown in table (24). The yields of essential oil, thymol, carvacrol, and thymol carvacrol additive varied among cultivated population and correlated significantly (0.73) with dry herbage yield (Annex XIX). The highest essential oil yield was obtained from Wadi AS Sir population followed by Belas and Edbyan (111.6, 95.6, and 86.0 Kg/ha, respectively) while the commercial cultivar 3 shows the lowest essential oil yield (9.5 Kg/ha). In general, populations studied exhibit higher carvacrol yield than thymol, for example, population of Wadi AS Sir recorded an additive of thymol and carvacrol yield equal 21.3 Kg/ha, and carvacrol yield of 20.1 Kg/ha. In addition, high significant correlation (0.76) was found between thymol carvacrol additive yield and carvacrol yield. To a certain limit, these results are in agreement with those obtained by Fleisher, *et al*. (1984), who found a phenol fraction of the essential oil contain thymol and

Table (24): Essential oil production (Kg/ha) of *Coridothymus capitatus* populations cultivated at Mushager research station in 2007.

Population	Dry herbage yield (Kg/ha)	Essential oil yield	Thymol yield	Carvacrol yield	Thymol + carvacrol yield
Qasabat AS Salt	1772.5	22.7	6.3	5.2	11.5
Al Fuhays	1931.2	60.9	0.6	0.2	0.8
Tla' Kaser Khalda	1349.2	34.7	5.6	2.4	8.0
Ayn Al Basha	2050.3	56.2	0.8	9.4	10.2
Abu Hamid village	1997.4	43.7	5.0	3.8	8.8
Abu Nusayr village	1574.1	55.5	0.9	11.0	11.9
Khalda	1521.2	34.4	6.5	2.3	8.7
Amman (West UOJ)	1878.3	63.4	13.8	10.0	23.7
Khalda military station	1058.2	42.6	9.6	1.8	11.4
Wadi Slait	1605.8	38.8	7.2	2.5	9.7
Bela's village	2486.8	95.6	13.2	10.5	23.6
Edbyan village	2037.0	86.2	0.9	18.4	19.4
Al Bassah village	2156.1	62.3	0.8	1.0	1.8
Wadi AS Sir	2235.4	111.6	1.1	20.1	21.3
Um El Gutain village	1269.8	28.7	0.4	7.1	7.5
Kufranja	2002.6	48.9	1.0	9.3	10.4
Anjarah (Landrace 1)	1375.7	35.0	0.4	5.5	5.9
Anjarah (Landrace 2)	1534.4	37.6	0.2	6.5	6.7
Commercial cultivar 1	1759.3	22.4	0.2	4.6	4.8
Commercial cultivar 2	1851.9	31.6	0.5	5.1	5.6
Commercial cultivar 3	674.6	09.5	0.1	1.5	1.7

Data are yields of essential oil composition obtained from arial parts of 10.0 g dry weight.

carvacrol in the ratio of approximately 1:2 in wild populations of *C. capitatus*.

The results of this study led to the conclusion that essential oils produced from cultivated *C. capitatus* are rich in carvacrol essential oil but also contain thymol in a good percentage, indicating that improving particular population to produce carvacrol and thymol is possible. Also promoting *C. capitatus* as a crop plant for carvacrol production is economically efficient, particularly carvacrol reported as being one of the most expensive among the origanum oils (Rodrigues, *et al*. 2006). Results obtained are paving the road for a potential commercial and large-scale essential oil production from this species.

4.6. Molecular Diversity among *C. capitatus* Populations

4.6.1. Quality and Quantity of DNA

The genomic DNA isolated from one plant per population for all 21 thyme populations revealed proper quality and quantity for proceeding AFLP analysis (Annex XI). The concentrations ranged between 25-50 ng/µL. All samples adjusted to a concentration 40 ng/µL for conducting AFLP reactions.

In this study, the CTAB isolation method worked better when compared to a short isolation method using NucleoSpin® Plant II isolation kit (Machery-Nagel, Germany) which was successfully used for microsatellite markers; this might due to the limited modification facilities in the plant isolation kit particularly when dealing with new species and high number of samples. It was shown that, when different plant organs are used as source of DNA, AFLP markers display different banding profile (Donino *et al*., 1997). Thus, DNA isolation was performed on the leaf materials.

The large amounts of secondary metabolites and essential oils in medicinal and aromatic plants tissues cause difficulties in obtaining large amount of DNA and sometimes can inhibit the DNA amplification in PCR reaction (Khanuja, *et al*., 1999;

Mizukami and Okabe, 1999). Thus, preliminary PCR reaction tests using the DNA concentration 40 ng/µL were performed before proceeding AFLP analysis.

4.6.2. Genetic relationship among thyme populations

AFLP banding pattern produced by ten selective primer combinations on a series of 21 thyme populations in Jordan are shown in Figures 15 to 19. AFLP markers generated from the ten selective primer combinations and their distribution across thyme populations are presented in Table (25).

Table (25): Number of fragments amplified and polymorphism percentage convened by 10 primer combinations employed to detect genetic diversity among 21 thyme populations.

Primer combinations	Total fragments (no.)	Polymorphic fragments (no.)	Monomorphic fragments (no.)	Polymorphism (%)
Pst I+ GC/ *Mse*I+ CTT	29	27	2	93.1
Pst I+ CC/ *Mse*I+ CAA	28	23	5	82.1
Pst I+ ACA/ *Mse*I+ CAC	12	11	1	91.7
Pst I+ AGG/ *Mse*I+ CAC	11	11	0	100.0
Pst I+ GGT/ *Mse*I+ CAC	20	17	3	85.0
Pst I+ AACG/ *Mse*I+ CCCT	28	23	5	82.1
Pst I+ AACC/ *Mse*I+ AGT	32	29	3	90.6
Pst I+ AACC/ *Mse*I+ CAC	28	25	3	89.3
Pst I+ ACG/ *Mse*I+ CTA	19	17	2	89.5
Pst I+ CC/ *Mse*I+ CTT	28	26	2	92.9
Overall	235	209 (89%)	26 (11%)	89.6

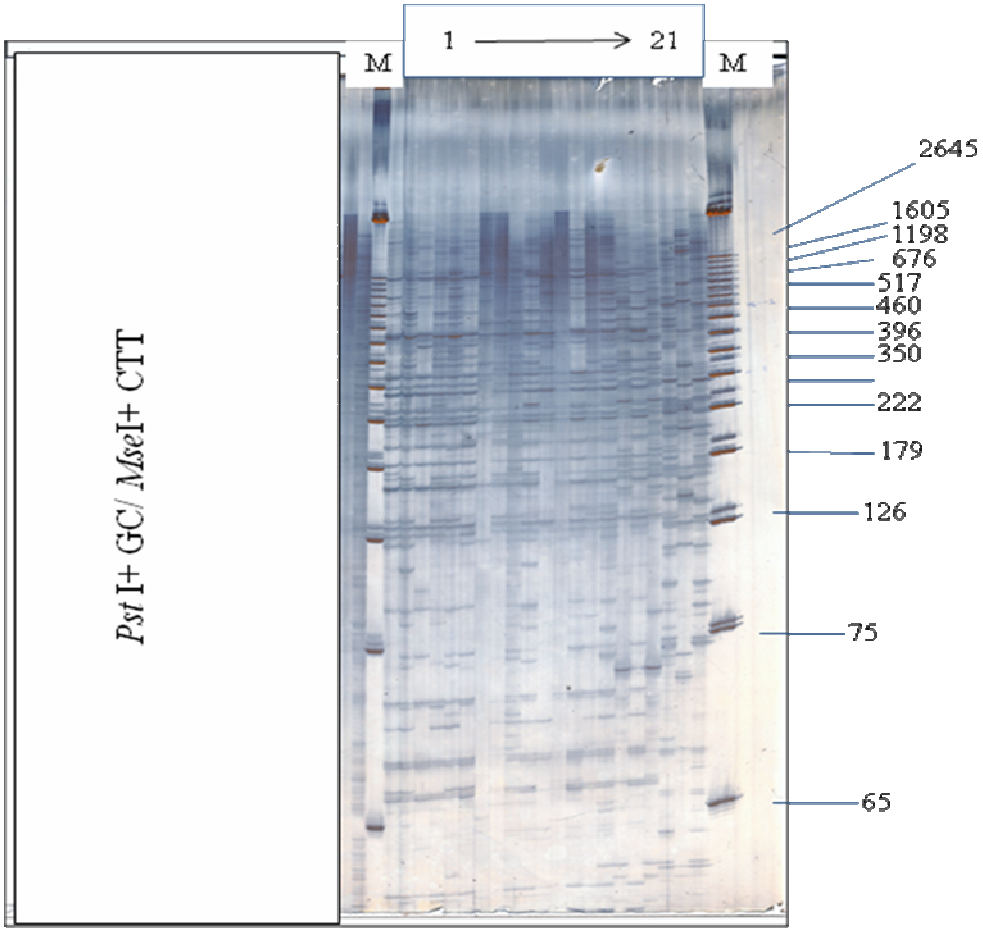


Figure (15): AFLP banding pattern of 21 thyme populations of Jordan as revealed by primer combination *Pst* I+ GC/ *Mse*I+ CTT. Lane M indicated the fragment size of molecular weight markers in base pair (bp). The lanes 1 to 21 are the banding pattern of thyme populations (presented in Table 3) of: Qasabat AS Salt; Al Fuhays; Khalda; Tla' Kaser Khalda; Ayn Al Basha; Amman 'West UOJ'; Wadi AS Sir; Wadi Slait; Abu Hamid village; Bela's village; Edbyan village; Al Bassah village; Khalda military station; Abu Nusayr village; Um El Gutain village; Kufranja; Anjarah (Landrace 1); Anjarah (Landrsce 2); Commercial cultivar 1; Commercial cultivar 2; and Commercial cultivar 3, respectively.

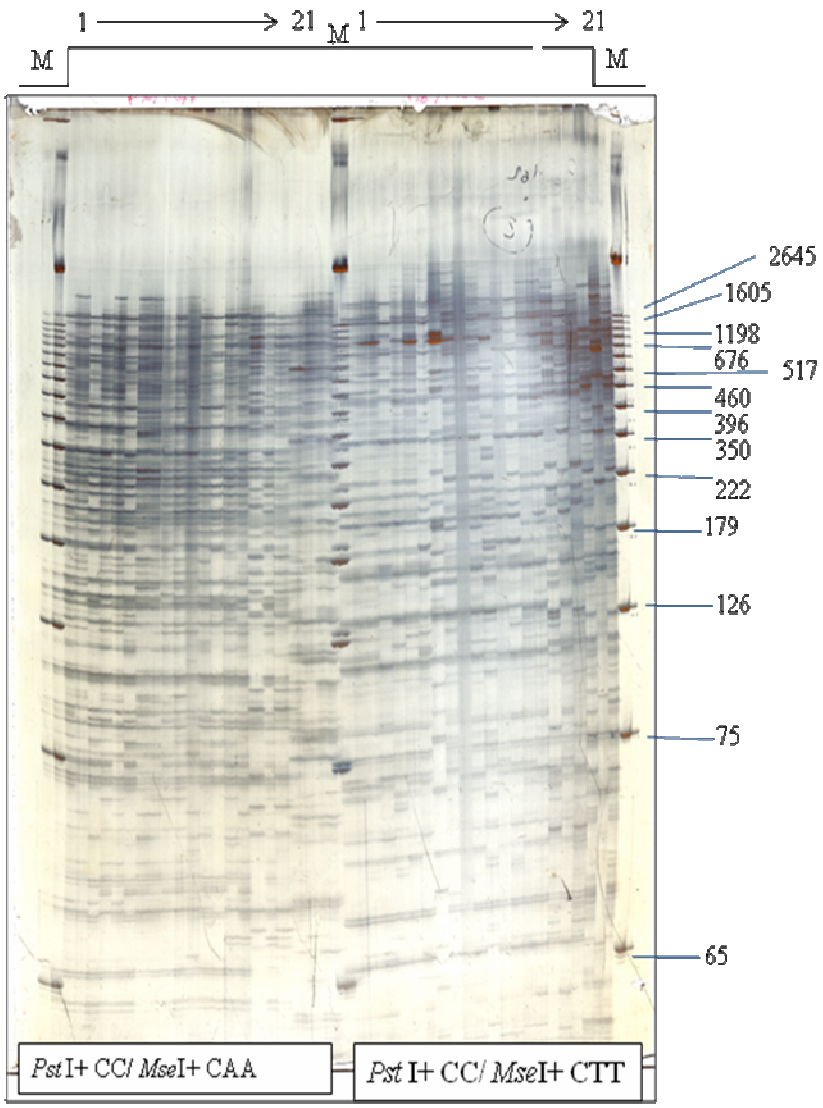


Figure (16): AFLP banding pattern of 21 thyme populations of Jordan as revealed by primer combinations *Pst* I+ CC/ *Mse*I+ CAA and *Pst* I+ CC/ *Mse*I+ CTT. Lane M indicated the fragment size of molecular weight markers in base pair (bp). The lanes 1 to 21 are the banding pattern of thyme populations (presented in Table 3) of: Qasabat AS Salt, Al Fuhays, Khalda, Tla' Kaser Khalda; Ayn Al Basha, Amman 'West UOJ', Wadi AS Sir, Wadi Slait, Abu Hamid village, Bela's village, Edbyan village, Al Bassah village, Khalda military station, Abu Nusayr village, Um El Gutain village, Kufranja, Anjarah (Landrace 1), Anjarah (Landrsce 2), Commercial cultivar 1, Commercial cultivar 2, and Commercial cultivar 3, respectively.

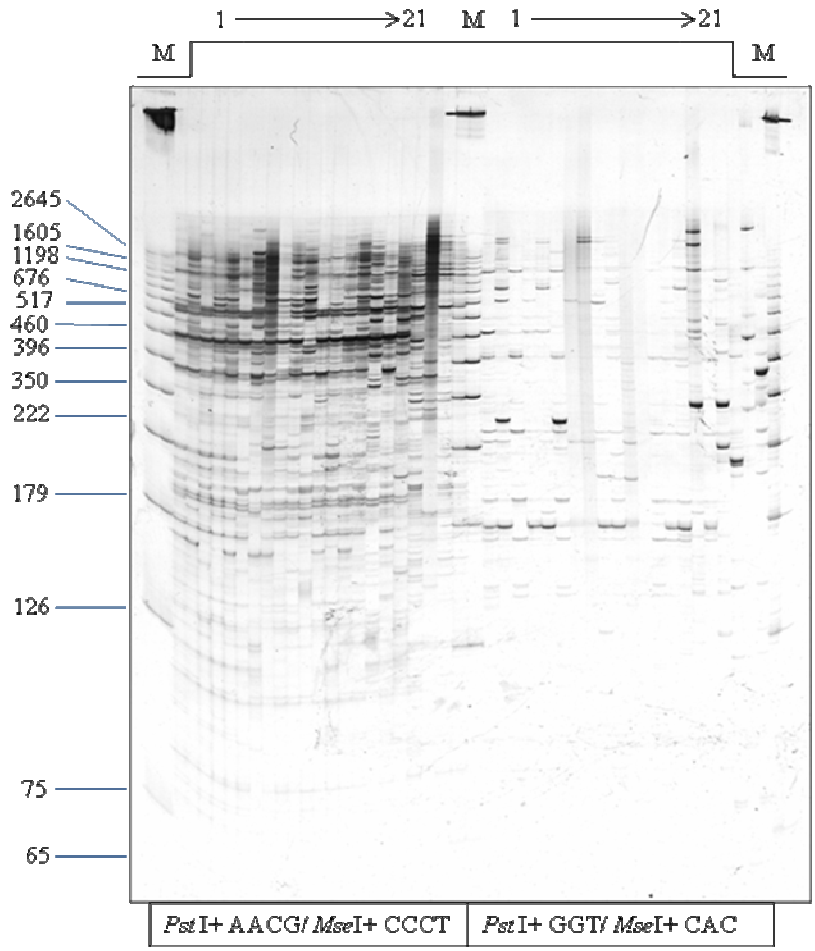


Figure (17): AFLP banding pattern of 21 thyme populations of Jordan as revealed by primer combinations *Pst* I+ AACG/ *Mse*I+ CCCT and *Pst* I+ GGT/ *Mse*I+ CAC. Lane M indicated the fragment size of molecular weight markers in base pair (bp). The lanes 1 to 21 are the banding pattern of thyme populations (presented in Table 3) of: Qasabat AS Salt, Al Fuhays, Khalda, Tla' Kaser Khalda, Ayn Al Basha, Amman 'West UOJ', Wadi AS Sir, Wadi Slait, Abu Hamid village, Bela's village, Edbyan village, Al Bassah village, Khalda military station, Abu Nusayr village, Um El Gutain village, Kufranja, Anjarah (Landrace 1), Anjarah (Landrsce 2), Commercial cultivar 1, Commercial cultivar 2, and Commercial cultivar 3, respectively.

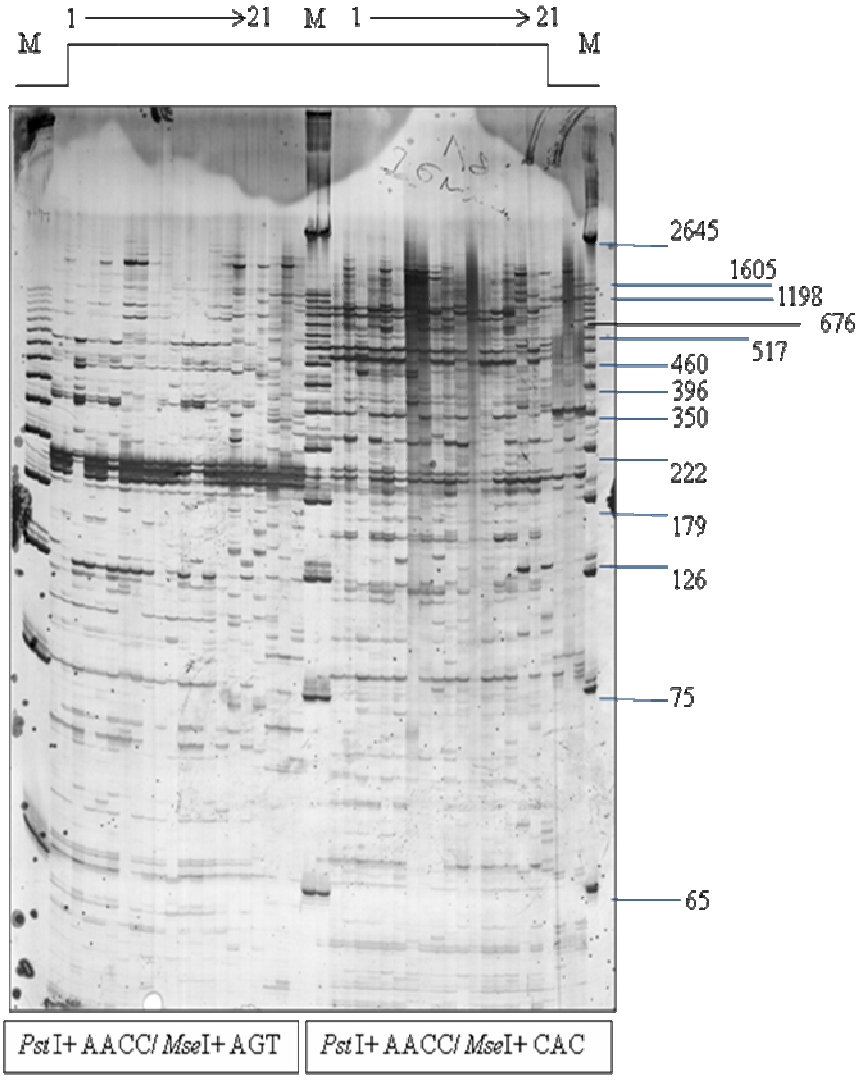


Figure (18): AFLP banding pattern of 21 thyme populations of Jordan as revealed by primer combinations *Pst* I+ AACC/ *Mse*I+ AGT and *Pst* I+ AACC/ *Mse*I+ CAC. Lane M indicated the fragment size of molecular weight markers in base pair (bp). The lanes 1 to 21 are the banding pattern of thyme populations (presented in Table 3) of: Qasabat AS Salt, Al Fuhays, Khalda, Tla' Kaser Khalda, Ayn Al Basha; Amman 'West UOJ', Wadi AS Sir, Wadi Slait, Abu Hamid village, Bela's village, Edbyan village, Al Bassah village, Khalda military station, Abu Nusayr village, Um El Gutain village, Kufranja, Anjarah (Landrace 1), Anjarah (Landrsce 2), Commercial cultivar 1, Commercial cultivar 2, and Commercial cultivar 3, respectively.

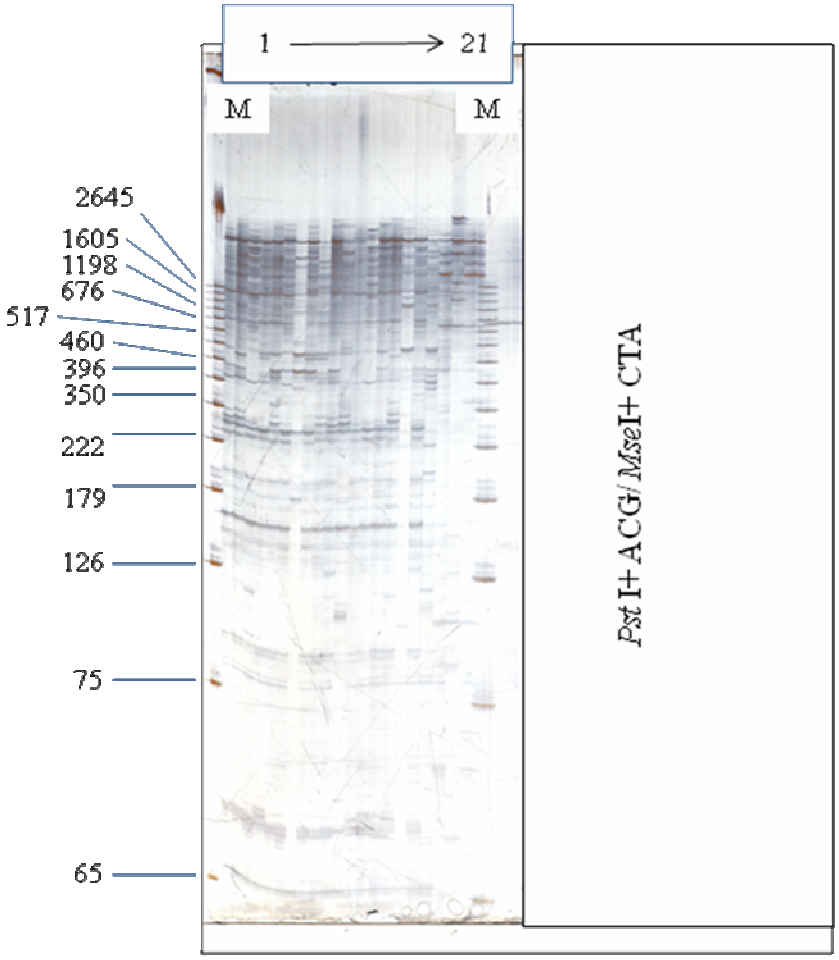


Figure (19): AFLP banding pattern of 21 thyme populations of Jordan as revealed by primer combination *Pst* I+ ACG/ *Mse*I+ CTA. Lane M indicated the fragment size of molecular weight markers in base pair (bp). The lanes 1 to 21 are the banding pattern of thyme populations (presented in Table 3) of: Qasabat AS Salt, Al Fuhays, Khalda, Tla' Kaser Khalda, Ayn Al Basha, Amman 'West UOJ', Wadi AS Sir, Wadi Slait, Abu Hamid village, Bela's village, Edbyan village, Al Bassah village, Khalda military station, Abu Nusayr village, Um El Gutain village, Kufranja, Anjarah (Landrace 1), Anjarah (Landrsce 2), Commercial cultivar 1, Commercial cultivar 2, and Commercial cultivar 3, respectively.

A total of 235 bands were generated from the ten selective primer combinations (Table 23). Vos, *et al.* (1995) and Vos and Kuiper, (1997), cited that AFLP technique is able to produce DNA fragments range from 60 to 500 base pairs. The number of markers detected varied from 11-32 with an average of 23.5 fragments per primer combination. Among the 235 scored bands across all populations, 209 bands (89%) were polymorphic. The remaining 26 bands (11%) were conserved through all populations. The percentage of polymorphism varied from 82.1- 100 % with an average of 89.6 % polymorphism per primer combination.

The ability of individual AFLP primer combinations to amplify polymorphic bands differed (Table 25). The primer combination *Pst* I+ AACC/ *Mse*I+ AGT amplified 29 polymorphic bands while primer combinations *Pst* I+ ACA/ *Mse*I+ CAC and *Pst* I+ AGG/ *Mse*I+ CAC amplified only 11 polymorphic bands. On the other hand, the highest polymorphism percentage was obtained by using primer combination *Pst* I+ AGG/ *Mse*I+ CAC (100%), followed by *Pst* I+ GC/ *Mse*I+ CTT (93.1%).

So far, the AFLP analysis dealing with medicinal and aromatic plants is limited in comparison with cultivated crops. Hence, the selective primer combinations were chosen randomly for the AFLP marker generation. However, some selective primers combinations were tested among the ones of Shasany, *et al*. (2005) and Ayanoglu, *et al*. (2006) shown to be useful specifically for Mentha and Origanum, these primer combinations failed to produce fragments for the species *C. capitatus* or the fragments were not polymorphic. The essential oils mainly carvacrol contained in species *C. capitatus* may affect the process of DNA amplification during PCR reaction (Mizukami and Okabe, 1999). Thus, new primer combinations are needed to be investigated for their ability to amplify easily scorable polymorphic bands for thyme species. In this study, the ten primer combinations with 2, 3, and 4 selective nucleotides were able to

produce a scorable AFLP fragments with high polymorphism percentages (82.1- 100%) which in turn used for investigating the genetic diversity among the 21 thyme populations presented in Table (3).

Table (26), shows the estimates of genetic distances between the populations which were calculated to help in studying genetic relationships and genetic divergence (Dp) between pairs of populations (Nei, 1972; Udupa, *et al*., 1998; Incirli, *et al*., 2001). The standard genetic distances among 21 thyme populations varied from 0.06 to 0.68, indicating diverse relationships. The population of Qasabat AS Salt is highly divergent from all of commercial cultivars and very closely related to Ayn Al Basha population.

Genetic distances among the fifteen *C. capitatus* populations which were collected from wild habitat in Jordan and cultivated in open field varied from 0.06 to 0.34, and it is relatively high compared with the limited geographical distribution of *C.capitatus* species growing in Jordan, and indicated that the rate of gene flow between *C.capitatus* populations was high, as a result of cross-pollination (Ayanoglu, *et al*, 2006; Rodrigues, *et al*., 2006). The population of Edbyan village is divergent from other 14 *C. capitatus* populations particularly those of Khalda, Amman (West UOJ), Abu Hamid village, and Al Fuhays (Dp; 0.34, 0.33, 0.33, 3.32, respectively). The population of Edbyan village is located in south of Jordan with long-term average rainfall 340 mm while the other four *C. capitatus* populations are located in the north of the country. Sternberg and Shoshany (2001) found that species and sub species diversity changed significantly in a short distance separating the north and south facing slopes. Thus, variation in eco-geographical conditions may explain this genetic divergence which worth to be conserved.

The Nei 72 genetic distances among populations of Kufranja, Anjarah (Landrace 1), and Anjarah (Landrace 2), which named locally as Za'tar Farisi and identified as

Thymbra spicata (plate 2) varied from 0.23 to 0.52. The highest divergence was between Anjara landrace 1 and the Anjara landrace 2, meaning that thyme populations produced by farmers of Anjara are heterogeneous. The genetic distance recorded between Kufranja population and Anjara landrace 2 was 0.23 which is considered low because population of *Thy. spicata* was collected from wild habitat of Kufranja while population of Anjara landrace 2 was collected from farmers who produced their own seeds. This means that farmers of Anjara started to produce their own seeds of *Thy. spicata* not long time ago and still depend on the wild habitat for collecting seeds and herbage materials which add threat on the distribution of this species.

Populations represents commercial cultivars named Za'tar Farisi and available in seed store markets show genetic distance varied from 0.09 to 0.23 indicating that thyme species which selling well in Jordan markets are not pure varieties and need to be included in breeding program. This observation is in agreement with the morphological variation observed among these commercial cultivars.

The dendrogram constructed by UPGMA cluster analysis revealed 3 main clusters at 48% dissimilarity levels (Figure 20). The first cluster consists of the three commercial cultivars of thyme (*Thymus vulgaris*). The second cluster consists of Kufranja; Anjarah landrace 1; and Anjarah landrace 2 populations at 41% dissimilarity levels, these thyme populations (*Thymbra spicata*) were collected from Ajlun district where the highest average annual rainfall among the surveyed sites was recorded (576 mm). The third cluster was constructed at 37% dissimilarity levels and consisted of the remaining fifteen *C. capitatus* populations collected from wild habitat in Jordan. This cluster was subdivided into five sub-clusters. In the first one Edbyan population was separated from the other fourteen *C. capitatus* populations at 30% dissimilarity levels.

Table (26): Diagonal matrix, based on Nei's (1972) genetic distance among 21 thyme populations of Jordan as estimated by AFLP analysis.

Population	Qasabat AS Salt	Al Fuhays	Khalda	Tla' Kaser Khalda	Ayn Al Basha	Amman (West UOJ)	Wadi AS Sir	Wadi Slait	Abu Hamid	Bela's	Edbyan	Al Bassah	Khalda (military station)	Abu Nusayr	Um El Gutain	Kufranja	Anjarah (Landrsce 1)	Anjarah (Landrsce 2)	Commercial cultivar 1	Commercial cultivar 2	Commercial cultivar 3
Qasabat AS Salt																					
Al Fuhays	0.06																				
Khalda	0.16	0.21																			
Tla' Kaser Khalda	0.08	0.08	0.17																		
Ayn Al Basha	0.06	0.06	0.17	0.07																	
Amman (West UOJ)	0.12	0.14	0.11	0.13	0.13																
Wadi AS Sir	0.14	0.16	0.22	0.13	0.12	0.19															
Wadi Slait	0.16	0.17	0.27	0.13	0.14	0.24	0.21														
Abu Hamid	0.14	0.14	0.23	0.14	0.13	0.20	0.22	0.17													
Bela's	0.13	0.11	0.18	0.08	0.09	0.16	0.16	0.17	0.14												
Edbyan	0.29	0.32	0.34	0.27	0.26	0.33	0.28	0.24	0.33	0.27											
Al Bassah	0.20	0.22	0.21	0.19	0.20	0.23	0.29	0.18	0.24	0.23	0.33										
Khalda (military station)	0.15	0.19	0.11	0.13	0.14	0.15	0.18	0.22	0.18	0.15	0.31	0.17									
Abu Nusayr	0.12	0.15	0.17	0.11	0.11	0.14	0.15	0.18	0.14	0.13	0.28	0.20	0.11								
Um El Gutain	0.12	0.12	0.19	0.09	0.09	0.16	0.11	0.14	0.16	0.10	0.26	0.22	0.17	0.13							
Kufranja	0.37	0.37	0.41	0.30	0.33	0.39	0.32	0.35	0.42	0.37	0.42	0.39	0.38	0.34	0.36						
Anjarah (Landrsce 1)	0.37	0.33	0.50	0.34	0.33	0.48	0.39	0.28	0.37	0.37	0.42	0.47	0.50	0.45	0.37	0.49					
Anjarah (Landrsce 2)	0.37	0.37	0.42	0.33	0.35	0.42	0.29	0.38	0.42	0.34	0.48	0.42	0.36	0.36	0.36	0.23	0.52				
Commercial cultivar1	0.42	0.45	0.47	0.39	0.38	0.46	0.34	0.44	0.47	0.36	0.50	0.49	0.44	0.41	0.36	0.49	0.65	0.41			
Commercial cultivar2	0.55	0.56	0.64	0.49	0.50	0.58	0.49	0.47	0.58	0.48	0.58	0.52	0.55	0.53	0.48	0.54	0.63	0.43	0.23		
Commercial cultivar3	0.44	0.45	0.51	0.40	0.39	0.50	0.38	0.43	0.47	0.35	0.49	0.52	0.46	0.44	0.39	0.49	0.68	0.42	0.09	0.19	

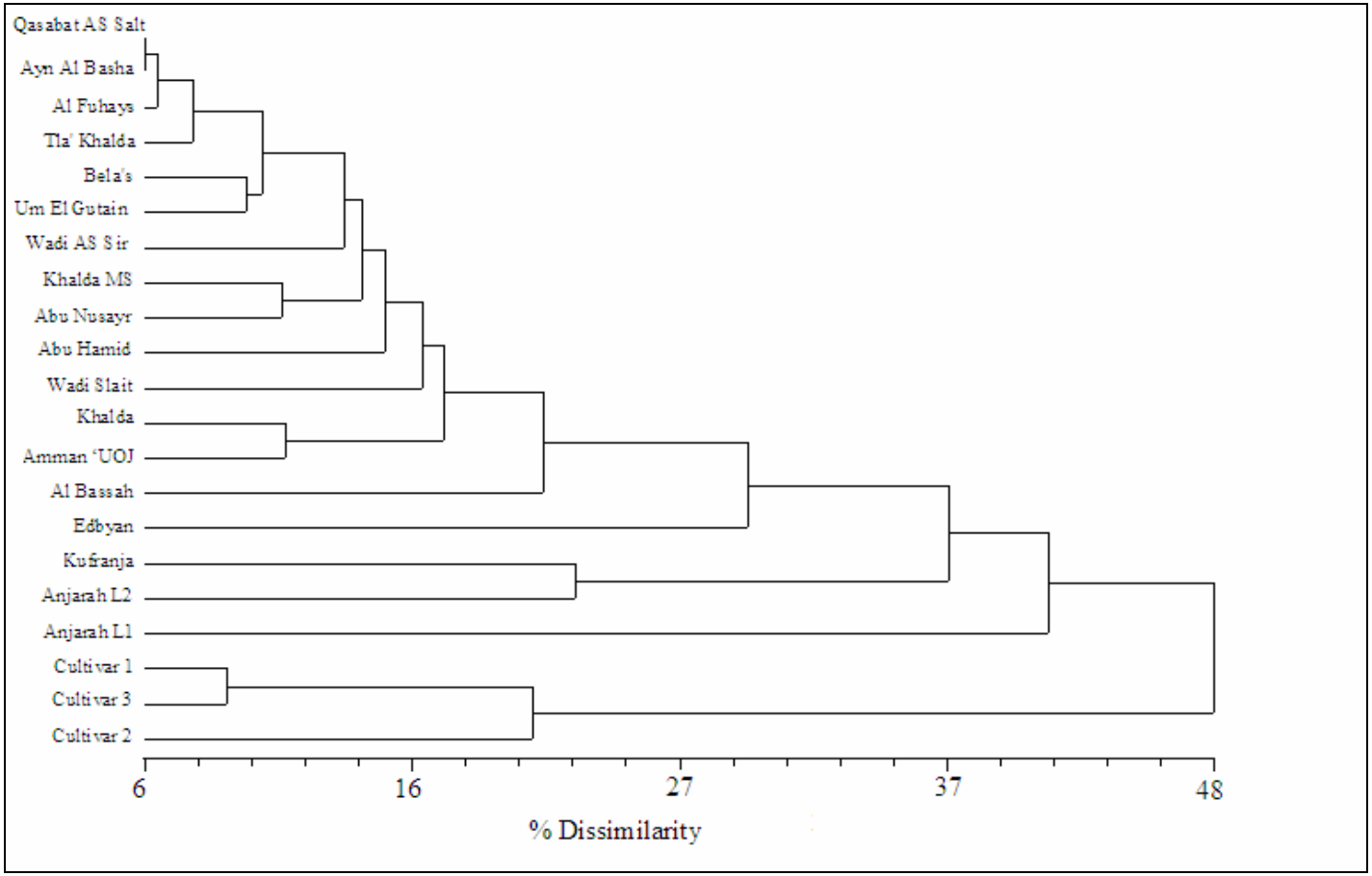


Figure (20): UPGMA-based dendrogram showing genetic relationship among 21 thyme populations. The dendrogram was based on the genetic dissimilarity calculated according to Nei 's standard genetic distance (Nei, 1972).

The second one separated Al Bassah population from the other thirteen populations at 22% dissimilarity levels, and grouped Khalda and Amman 'West UOJ' populations in concert with genetic distance equal 11%. The third one separated Wadi Slait population, and grouped Abu Hamid, Khalda military station and Abu Nusayr populations at 14.5% dissimilarity levels. The fourth sub-cluster separated populations of Wadi AS Sir, Um El Gutain, and Bela's from the rest of *C.capitatus* populations at 12.5% dissimilarity levels. The fifth sub-cluster consists populations of Tla' Kaser Khlda, Al Fuhays, Ayn Al Basha, and Qasabat AS Salt at 7% dissimilarity levels. These four populations were collected from two districts (Salt and Suwaylih) among the seven surveyed districts for distribution and collection of *C. capitatus*.

Understanding the amount and distribution of genetic variation present in genetic pool is critical for any successful conservation strategy or breeding program (Ford-Lloyd, 2001; Ojeda, *et al*., 2001; Jubrael, *et al*., 2005). The morphological characters and mainly chemical traits were used to describe such genetic variation in *C. capitatus* (Arras and Grella, 1992; Goren, *et al*., 2003) but these traits are influenced by environment. Various DNA marker analysis techniques have been used to determine genetic diversity in crop plants (Powell, *et al*., 1996). AFLP technique was reported to be useful for studying genetic relationship in some medicinal species (Ricciardi, *et al*., 2002; Shasany, *et al*., 2005; Ayanoglu, *et al*., 2006). In this study AFLP technique was used as a dominant molecular markers characterized by their ability to produce high amount of polymorphism and their efficient in separating closely related genotypes, beside, no prior DNA sequence information is needed and small amount of DNA is required for conducting the analysis (Vos, *et al*., 1995; Mueller and Wolfenbarger,1999).

The cluster analysis separated the 21thyme populations which all named in Jordan as Za'tar Farisi into three main clusters: the first cluster contained three commercial cultivars of *Thymus vulgaris*, the second contained three populations of *Thymbra spicata*; the Kufranja wild population and the two Anjara landraces, and the third cluster contained the remaining fifteen populations of *C. capitatus* collected from different sites in Jordan. The *C. capitatus* populations were distributed within the third cluster and constructed five sub-clusters. Hence, a total of eight groups were identified by the UPGMA- based dendrogram, which were helpful in better understanding of genetic diversity among thyme populations. AFLP technique reliably distinguished all 21 thyme populations included in this study indicating that some of the populations showed no genetic differences like Qasabat AS Salt and Ayn Al Basha, Bela's and Um El Gutain, Khalda military station and Abu Nusayr, Khalda and Amman 'West UOJ', Kufranja and Anjarah (Landrace 2), and Commercial cultivar 1 and Commercial cultivar 3. Consequently, 15 thyme populations rather than 21populations could be considered for conservation and breeding program initiation. Excluding the commercial cultivars, 11 populations of *C. capitatus* (among 15) and 2 populations (among 3) of *Thy. spicata* should be considered in the conservation strategy of thyme species in Jordan, as their degradation is exceeding their regeneration process (Ojeda, *et al*., 2001). To initiate a selective breeding program for *Origanum onites* L. in Turkey, AFLP analysis was conducted and seven main groups were identified by UPGMA clustering for forty-four *Origanum onites* L. accessions conserved in a gene bank (Ayanoglu, *et al*., 2006).

Results obtained from the UPGMA cluster indicated that the eco-geographical variation influenced the distribution of *C. capitatus* populations in Jordan. In the third cluster, the first sub cluster separated Edbyan population located in south Jordan from

all other populations including those collected from the south of the country. Edbyan site characterized by habitat of batha with Pinus trees with average annual rainfall 340 mm, elevation range between 860 -890 m, and slopes mainly subjected to the west. Sternberg and Shoshany (2001) found a significant relationship between slope aspect, vegetative composition and density of *C. capitatus*. On the other hand, the subcluster 5 consolidated *C. capitatus* populations collected from north Jordan where open shrub land and batha habitat is dominated with average annual rainfall range between 423-502, elevation range between 820-1060 m and slopes subjected to north west of Jordan. The relationships between plant distribution and eco-geographical variation recognized in this study disagrees with findings of Ayanoglu *et al*. (2006) who stated that no close genetic similarity among *Origanum* accessions related to their growing region was observed.

In conclusion, AFLP markers exhibited high efficiency in producing a detectable polymorphism among 21 thyme populations distributed in Jordan and named Za'tar Farisi. The results of this study indicate that a broad range of genetic variation exists among populations of *C. capitatus* collected from wild habitats in Jordan, also variability exists among *Thy. spicata* populations. This means that a conservation strategy (*in situ* and *ex situ*) needs to be applied to maintain these variation and to decrease degradation. Also future breeding programs could be initiated based on the data obtained, by selecting materials with maximally different AFLP fingerprints.

5. CONCLUSIONS

This study showed that

- In Jordan, the Arabic common name Za'tar Farisi (زعتر فارسي) is used for the species *Coridothymus capitatus*, *Thymbra spicata*, and *Thymus vulgaris*.
- *Coridothymus capitatus* populations are distributed in a limited regions in Jordan, however, high phenotypic diversity was obtained among them through applying various genetic variation parameters.
- The DNA analysis confirmed the genetic variation among *Coridothymus capitatus* populations. AFLP markers were efficient in producing a detectable polymorphism among thyme populations as they separated *Thymbra spicata* landraces and *Thymus vulgaris* commercial cultivars from *C. capitatus* populations, and also clustering *C. capitatus* populations according to their geographical distribution.
- Thymol and carvacrol oils are considered biochemical markers found in various percentages in all *Coridothymus capitatus* populations. Clustering based on essential oils showed two main chemotypes: thymol chemotype and carvacrol chemotype. Hence, improvement of specific chemotype population is possible.
- The highest amount of essential oils were obtained during the month of July at full bloom.
- *Coridothymus capitatus* populations have high cultivation potential toward production of fresh and dry herb as well as essential oil, while *Thymbra spicata* populations have potential toward fresh herb production.
- *Coridothymus capitatus* populations of Wadi AS Sir and Edbyan could be elected as a promising populations (accessions) to initiate a breeding program for essential oil production.

6. RECOMMENDATIONS

Regarding research level, it is recommended to:

- Exploit the broad genetic base of *Coridothymus capitatus* populations obtained in this study to promote this species as a crop plant and develop diversity preservation strategy.
- Conduct association analysis to study genetic markers to specify gene (s) responsible for thymol and carvacrol production, this will facilitate selection of promising populations.
- Initiate breeding program based on selecting materials with maximally different AFLP fingerprint.
- Establish for thymol and carvacrol production at commercial level.
- Conduct cultural research to determine best plant spacing, fertilizer type and rate, irrigation, and other cultural practices to insure maximum yield.
- Identify other essential oil constituents and secondary metabolites of *Coridothymus capitatus*, and investigate their antioxidant and antimicrobial properties.
- Conduct comprehensive socio-economic studies, and ethno-botanical surveys on species of *Coridothymus and Thymbra spicata* in order to encourage local inhabitants to adopt recommended conservation techniques.

Regarding government and institutional level, it is recommended to:

- Adopt C*oridothymus capitatus* species as one of the most important medicinal, aromatic, and ecological plant, which could be developed at national and international level.
- Enhance collaboration between research institutes, universities, and pharmaceutical companies to evaluate the feasibility of commercial production of thymol and carvacrol.

- Introduce *Coridothymus. capitatus* as an alternative crop to improve farmer income.
- Set collaboration between ministries of Environment, Agriculture, and Manucipalities to protect and conserve (*in situ*) this species from degradation as urbanization and air pollution are considered the main threat.

7. REFERENCES

Abu Al Rub, I. (1996). The effect of plant density and cutting height on the yield of Marjoram (*Origanum syriacum* L.) under open field conditions. M. Sc. thesis, University of Jordan.

Abu-Harfeil, M., Maraqa, A. and Von Kleist, S. (2000). Augmentation of natural killer cell activity in vitro against tumor cells by wild plants from Jordan. **J. Ethnopharmacol**., 71, 55-63.

Abu-Irmaileh, B. (1988). **Poisonous plants of Jordan,** (1st ed.). Jordan University press.

Abu-Zarqa, M., Sabri, S., Firdous, S. and Shamma, M. (1987). Fumadensine a phthalideisoquinoline from *Fumaria densiflora*. **Phytochemistry**, 26, 1233- 1234.

Afifi, F. and Abu-Ermaileh, B. (2000), Herbal medicine in Jordan with special emphasis on less commonly used medicinal herbs. **J. Ethnopharmacol,** 72, 101-110.

Akcin, A. (2006). Numerical taxonomic studies on some species of the genus *Thymus* L. (Labiatae) in Turkey. **Asian J. of Plant Science**, 5, 782-788.

Alali, F., Tahboub, Y., Ibrahim, E., Tawaha, A. Qandil, A., Tawaha, K., Burgess, J., Arlene Sy., Nakanishi, Y., Kroll, D. and Oberlies N.(2008). Pyrrolizidine alkaloids from *Echium glomeratum* (Boraginaceae). **Phytochemistry,** 69, 2341–2346.

Alali, F., Tawaha, T., El-Elimat, T., Syouf, M., El-Fayad, M., Abulaila, Kh, Nielsen, S., Wheaton, W., Falkinham, J. and Oberlies, N. (2007). Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants. **Natural Products Research,** 21, 1121-1131.

Alali, F., El-Elimat, T., Li, C., Qandil, A., Alkofahi, A., Tawaha, K., Burgess, J., Nakanishi, Y., Kroll, D., Navarro, H., Falkinham , J., Wani, M. and Oberlies, N. (2005). New colchicinoids from a native Jordanian meadow saffron, *Colchicum brachyphyllum*: Isolation of the first naturally-occurring dextrorotatory colchicinoid. **J. Nat. Prod**. 68, 173-178.

Alali, F., Hudaib, M., Aburjai, T., Khairallah, K. and Al-Hadidi, N., 2004. GC/MS analysis and antimicrobial activity of the essential oil from the stem of the Jordanian toothbrush tree (*Salvadora persica* L.). **Pharmaceutical Biology**, 42, 577-580.

Al-Eisawi, D. (1982). List of Jordan vascular plants. **Mitt. Bot. Müchen**, 18, 79-182.

Al-Eisawi, D., 1996. **Vegetation of Jordan**. UNESCO, Cairo office.

Al-Eisawi, D. and Takruri, H. (1989), A checklist of wild edible plants in Jordan. **Arab Gulf J. Research: Agric. Biol. Sci.**, 1, 79-102.

Al-Khalil, S., Aqel, M., Afifi, F. and Al-Eisawi, D. (1990), Effects of aqueous extract

of *Ferula ovina* on smooth muscles of rabbit and guinea pig. **J. of Ethnopharmacology**, 30, 35-42.

Al-Khalil, S. (1995), A survey of plants used in Jordanian traditional medicine. **International J. of Pharmacognosy**, 33, 317-323.

Al-Kofahi, A. and Atta, A. (1999). Pharmacological screening of the anti-ulcerogenic effects of some Jordanian medicinal plants in rats. **J. Ethnopharmacol**, 67, 341-345.

Al-Mustafa, A. and Al-Thunibat O. (2008). Antioxidant activity of some Jordanian medicinal plants used traditionally for treatment of diabetes. **Pakistan J. of Biological Science**, 11, 351-358.

Al-Nashsh, A., Migdadi, H., Shatanawi, M., Saoub, H. and Masoud, S. (2007). Assessment of phenotypic diversity among Jordanian barely landraces (*Hordum vulgare* L.). **Biotechnology**, 6, 232-238.

Ayanoglus, F., Erglu, A. and Arslan, M. (2006). Assessment of genetic diversity in Turkish Oregano (*Origanum onites* L.) germplasm by AFLP analysis. **J. of Horticulural Science and Biotechnology,** 81,45-50.

Arras, G. and Grella, G. (1992). Wild thyme, *Thymus capitatus*, essential oilseasonal Changes and antimycotic activity. **J. of Horticultural Science**, 67, 197-202.

Arras, G., Piga, A. and D'Hallewin, G. (1994). Effectiveness of *Thymus capitatus* aerosol application at subatmospheric pressure to control Green mold on 'Avana' mandarin fruit. **Acta Horticulturae**, 368, 382-386.

Arras, G. and Usai, M. (2001). Fungitoxic activity of 12 essential oils against four postharvest citrus pathogens: chemical analysis of *Thymus capitatus* oil and its effect in Subatmospheric pressure conditions. **J. Food Prot.**, 64, 1025-1029.

Ayanoglus, F., Erglu, A. and Arslan, M. (2006). Assessment of genetic diversity in Turkish Oregano (*Origanum onites* L.) germplasm by AFLP analysis. **J. of Horticulural Science and Biotechnology**, 8, 45-50.

Azaizeh, H. Fulder, S., Khalil K., and Said O. (2003). Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region. **Fitoterapia**, 74, 98-108.

Azaizeh, H., Ljubncic, P., Portnaya, I., Said, O., Cogan, U. and Bomzon, A. (2005). Fertilization-induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine**. Evid. Based Complement. Alternat. Med.,** 2, 549-560.

Barberan, F., Hernández , L. and Tomas F. (1986). A chemotaxonomic study of flavonoids in *Thymbra capitata*. **Phytochemistry,** 25, 561-562.

Baskin, C. and Baskin, J. (1998). **Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination.** Academic Press. San Diego.

Bhadula, K., Anoop Sing, H., Lata, P. and Purohit, N. (1996). Genetic resources of *Podophyllum hexandrum Royle*, an endangered medicinal species from Garhwal Himalaya, India. **Plant Genetic Resources Newsletter**, 106, 30-38.

Bjornstad, A., Demissie, A., Kilian, A. and Kleinfos, A. (1997). The distinctness and diversity of Ethiopian barleys. **Theor. Applied. Genet**, 94, 514-521.

Botstein, D., White, L., Skolnick, M. and Davis, W. (1980). Construction of a genetic linkage map in man using Restriction Fragment Length Polymorphism. **Am. J. Hum. Genet**., 32, 314-331.

Boulos, L. (1999). **Flora of Egypt**. Volume one. Al hadara publishing. Egypt.

Bown. D. (1995). **Encyclopaedia of herbs and their uses**. Dorling Kindersley, London.

Can Baser, K. (2008). Biological and Pharmacological Activities of Carvacrol and Carvacrol Bearing Essential Oils. **Current Pharmaceutical Design**, 14, 3106-3119.

Canter, H., Thomas, H. and Ernst, E. (2005). Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology.**Trends Biotechnol.,** 23, 180-185.

Cowan, M. (1999). Plant products as antimicrobial agents. **Cli. Microbio. Rev**., 12, 594-582.

Cunningham , B. (1993). African medicinal plants: setting priorities at the interface between conservation and primary healthcare. **People and Plants** working paper I. UNESCO , Paris p.92.

Danin, A. (2001), Near east ecosystem and plant diversity. **Encyclopedia of biodiversity**, 4: 1-12.

Danin, A. (2004). **Distribution Atlas of Plants in the Flora Palaestina Area**. Israel academy of science and humanities, Jerusalem.

Danin, A., Ravid, U., Umano, K. and Shibamoto, T. (1997). Essential oil composition of *Origanum ramonense* Danin leaves from Israel. **J. Essent. Oil Res**., 9, 411-417.

Davis, P. (1985). **Flora of Turkey and the east Aegean islands**. Volume 9. Edinburgh University Press. Scotland.

De Vincenzi, M., Stammati, A., De Vincenzi, A., Silano, M. (2004). Constituents of aromatic plants: carvacrol. **Fitoterapia**, 75, 801– 804.

Della, A. (1995). **Cyprus country report** to the international conference and program on plant genetic resources. (Leipzig, 1996). FAO. 1996. Food and Agriculture Organization of the United Nations, Rome, Italy.

Diamond, J. (1989). **Overview of recent extinctions**. In: Conservation of for the twenty-first century (eds. Western, D. and Pearl, M.). Oxford University Press, London.

Donino, P., Elias M. L., Bougourd S. M. and Koebner R. M. D. (1997). AFLP fingerprinting reveals pattern differences between template DNA extracted from different plant organs. **Genome**, 40, 521-526.

DOS. (2004), **Jordan Environmental statistics book 2003**. National Department of Statistics**.**

Doyle, J. and Doyle, J. (1988). Isolation of plant DNA from fresh tissue. **Focus**, 12, 13-15.

Dubey, N. K., Kumar, R. and Tripathi, P. (2004). Global promotion of herbal medicine: India's opportunity. **Current Science**, 86, 37-41.

El-Oqlah, A. and Lahham, J. (1985). A checklist of vascular plants of Ajlun mountain (Jordan). **Candollea**, 40, 377-387.

Eleuch, L., Jilal, A., Grando, S., Ceccarelli, S., Schmising, M. K. Tsujimoto, H., Hajer, A., Daaloul, A. and Baum, M. (2008). Genetic diversity and association analysis for salinity tolerance, heading date and plant height of barley germplasm using Simple Sequence Repeat Markers. **J. of Integrative Plant Biology**, 50, 1005-1015.

Elujoba, A., Odeleye, O. and Ogunyemi, C. (2005). Traditional Medical Development for medical and dental primary healthcare delivery system in Africa. **Afr. J. Traditional, Complementary and Alternate Med**. 2, 46-61.

Estrelles, E. Albert, F., Navarro, A., Prieto, J. and Ibars, A. (2004). **Germination behaviour of *Labiatae* SW distributed in the Iberian Peninsula**. Proceeding of the 4th European conference on the conservation of wild plants, Valencia, Spain. Planta Europa IV Proceedings. Retrived January, 1, 2009, from, www.nerium.net.

Faleiro, L., Miguel, G., Gomes, S., Costa, L., Venâncio, F., Teixeira, A., Figueiredo, A., Barroso, J. and Pedro, G. (2005). Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L. **J. Agric. Food Chem.**, 19, 8162-8168.

Farnsworth, R. (1988). **Screening plants for new medicines**. In: Wilson, O. (Ed.), Biodiversity. National Academy Press, Washigton.

Farnsworth, R. and Soyjarto, D. (1991). **Global importance of medicinal plants**. In: Akerele, O., Heywood, V. and Synge, H. (Eds.), the conservation of medicinal plants. Proceedings of an international consultation 21-27. Cambridge University Press. London.

Fehr, R. and Walter, R. (1987). **Principles of cultivar development**. 2nd edition. Macmillan Publising Company, New York.

Feinbrun, N. (1978). **Flora Palaestina**. Vol. III. The Israel Academy of Sciences and Humanities. Jerusalem.

Felter, H. and John Uri Lloyd, J. (1998). **Thymol (U.S.P.)**. Retrieved June, 13, 2008 from www. henriettesherbal.com.

FGDC, (1997). **National Vegetation Classification Standard**. Federal Geographic Data Committee, Vegetation Subcommittee FGDC-STD. Retrieved in July, 3, 2006, from www.fia.fs.fed.us.

Fleisher A., Fleisher, Z. and Abu-Rukun, S. (1984). Chemovarieties of *Coridothymus capitatus* L. Rchb. Growing in Israel. **J. of the Science of Food and Agriculture**, 35(5), 495-499.

Ford-Lloyd, B. (2001). **Genotyping in plant genetic resources**. Pp. 59-81 in Plant genotyping, the DNA fingerprinting of plants (R.J. Henry, ed.). CABI Publishing, CAB International, Wallingford,UK/New York, USA.

Foster, S. (1993). Medicinal plant conservation and genetic resources: examples from the temperate northern hemisphere. **Acta Hort.,** 330, 67-74.

Foster, C. (2006). **Complete health solutions**. Retrieved, February, 2, 2009, from www.startthehealing.com.

Fragman, O., Yamamori, R. and Christodoulou, P. (2001). **Flowers of Eastern Mediterranean**. A.R.G. Gantner Verlag K.G. Germany.

Frankel, H., Brown, D. and Burdon, J. (1995). **The conservation of plant biodiversity**. Cambridge University Press.

Franz, Ch. and Novak, J. (2006). Molecular support of genetic improvement. Proceeding of international symposium, **The *Labiatae*: advances in production, biotechnology and utilization**, Sanremo, Italy.

Gardner, S. and Danneberger, K. (2003). Relative fitness of glyphosate resistant creeping bentgrass cultivars in kentucky bluegrass. **Plant Research News**, Retrieved in July, 3, 2006, from http://www.isb.vt.edu/news/2003/news03.dec.html.

GCEP. (1998), **Jordan country study on biological diversity**. The General Cooperation for Environment Protection, Amman, Jordan.

Genstat 10 Committee (2007). Genstat 10 Release 1.0.71, Lawes agricultural trust. VSN International Ltd. United Kingdom. Web: www.vsni.co.uk.

Goren, A., Bilsel, G., Bilsel, M., Demira, H. and Kocabas, E. (2003). Analysis of essential oil of *Coridothymus capitatus* (L.) and its antibacterial and antifungal activity. **J. of bioscience**, 58, 687-690.

Guarino, R. (1996). Mapping the ecogeographic distribution of biodiversity. In: Guarino, R., Rao V. R., and reid, R. (Eds), **collecting plant genetic diversity**, (pp, 287-314). CAP International, UK.

Haddad, N. and Turk, M. (2002), **Medicinal and herbal plants cultivation**. Ministry of Agriculture, National Center for Agricultural Research and Technology Transfer (NCARTT). Conservation of medicinal and herbal project preparation grant, Global Environment Facility (GEF), Ammn. Jordan.

Hamdan, I. and Afifi,F. (2004). Studies on the in vitro and in vivo hypoglycemic activities of some medicinal plants used in treatment of diabetes in Jordanian traditional medicine. **J. Ethnopharmacol**., 93, 117-121.

Hawkes, J., Maxted, N. and Ford-Lloyd, B. (2000). **The *Ex Situ* conservation of plant genetic resources**. Kluwer Academic Publishers, London.

Hedhili, L., Romdhane, M., Planche H. and Abderrabba M. (2005). Towards gas chromatography-mass spectrometry coupling protocols for both identifying and quantification essential oils of *Thymus capitatus* Hoff et Link. **J. of Chromatography,** 1064, 129-134.

Hedrick. U. (1972). **Edible Plants of the World**. Dover Publications, US. Retrived May, 20, 2008, from www.ibiblio.org/pfaf/cgi-bin/arr.

HMPP, (2003). **Tunisia, supervision mission**: 8-14, June 2003. Herbal and Medicinal Plant Project. Retrived October, 11, 2008, from www.icarda.org/PAM/Publications.

Hutcheson, K. (1970). A test of comparingdiversities based on the Shanon formula. **J. Theo. Biolo**. 29, 151-154.

Huxley. A. (1992). **The new herb dictionary of gardening**. MacMillan Press.

Iapichino, G., Arnone, C., Bertolino, M. and Amico Roxas, U. (2006). Potentiality of three *Thymus* species to pot cultivation. international symposium the *Labiatae*: **Advances in Production, Biotechnology and Utilization**. 22-25 February 2006, Sanremo, Italy.

Incirli, A. and Akkaya, M. (2001). Assessment of genetic relationships in durum wheat cultivars using AFLP markers. **Genetic Resources and Crop Evolution,** 48, 233–238, 2001.

IPGRI (1993). **Diversity for development**. International Plant Genetic Resources Institute, Rome.

Iqbal, M. (1993). **International trade in non-wood forest products**: Working paper. Food and Agriculture Organization of the United Nations (FAO). FO: Misc/93/11. Rome.

Izuakor, M. (2005). **Bio-resources conservation: The Role of Agro-forestry**. Heritage Printers, Nigeria.

Jaccoud, D., Peng, K., Feinstein, D. and Kilian, A. (2001).Diversity Arrays: a solid state technology for sequence information independent genotyping. **Nucleic acid research**, 29, 24-25.

Jalas, J. and Kaleva, K. (1970). Supraspezifische gliederung und verbreitungstypen in dergattung *Thymus* L. *(Labiatae)*. **Feddes Repert**, 81, 93-106.

Jaradat, A. (1989). Diversity within and between populations of two symmetrically distributed *Hordeum* species in Jordan. **Theor. Applied Genet.**, 78, 653-656.

Jordan Meteorological department. (2008). Retrieved April, 20, 2008, from http://www.jmd.gov.jo.

Jubrael, J., Udupa, S. and Baum, M. (2005). Assessment of AFLP-based genetic relationships among Date Palm (*Phoenix dactylifera* L.) varieties of Iraq. **J. Amer. Soc. Hort. Sci**, 130, 3, 442-447.

Karim, M. and Quraan, A. (1986). **Medicinal plants of Jordan**. Center for Jordanian studies. Jordan natural history museum. Yarmouk University. Jordan.

Kew, Royal Botanical Garden. (2002). National plant collection at Wakehurst Place. **Information sheet.** Retrived June, 5, 2005, from www.kew.org.

Khairallah, K. (2005). **A botanical survey of wild medicinal and aromatic plants in the eastern upper slopes of the Jordan Rift valley**. Ministry of Planning and International Cooperation, Research fellowship documents, Amman, Jordan.

Khanuja, S., Shasany, K., Darokar, P. and Kumar, S. (1999). Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. **Plant Molecular Biology Reporter,** 17, 1-7.

King, R. (1992). **Conservation and tropical medicinal research**. Shaman Pharmaceutical Incorporated.

Lee, J., Umano, K., Shibamoto,T. and Lee, K.G. (2005). Identification of volatile components in Basil (*Osmium basilicum* L.) and thyme (*Thymus vulgaris* L.) and their antioxidant properties. **Food chemistry**, 91, 131-137.

Lefebrvre, V., Palloix, A. and Reieves, M. (1993). Nuclear RFLP between pepper cultivars (Capsicum annum L.). **Euphytica**, 71, 189-199.

Lewington, A. (1993). **A review of importation of medicinal plants and plant extracts into Europe**. WWF International Plant Programme/IUCN.

Lev, E. and Amar, Z. (2002). Ethnopharmacological survey of traditional drugs sold in the kingdom of Jordan. **J. Ethnopharmacol**, 82, 131-145.

Luiz, S. (2005). An integrated approach to identification and conservation of medicinal plants in the tropical forest - a Brazilian experience. **Plant Genetic Resources**,3,199-205.

Mahasneh, M. and El-Oqlah, A. (1999). Antimicrobial activity of extracts of herbal plants used in the traditional medicine of Jordan. **J. Ethnopharmacol**., 64, 271-276.

Marti'nez, F., Cuevas, G., Calvo, R. and Walter, I. (2003). Ecosystem restoration: Biowaste effects on soil and native plants in a semiarid ecosystem. **J. Environ. Qual**., 32,472–479.

Margaris, S. (1976). Structure and dynamics in a phryganic (East Mediterranean) ecosystem. **Journal of biogeography**, 3,249-259.

Marti'nez, F., Díaz, T., Fernández-González, F., Izco,J., Loidi, J., Lousã, M. and Penas, A. (2002). **Worldwide bioclimatic classification system**, Vascular plant communities of Spain and Portugal. Addenda to the Syntaxonomical checklist of 2001. Itinera Geobotanica 15(1-2), 5-922. Retrieved January, 20, 2009, from www.globalbioclimatics.org.

Maxam, M. and Gilbert, W. (1980). **Sequencing end-labeled DNA with base-specific chemical cleavages.** Methods Enzymol. 65, 499-560.

Maxted, N. and Bisby, A. (1989). **Accurate identification of wild forage species**. ECP/GR forage working group meeting, Montpelleier, France. Appendix 5, 62-75. IBPGR, Rome.

Maxted, N., Ford-Llyod, V. and Hawkes, G. (1997). **Complementary conservation strategies**. In: plant genetic conservation: the in situ approach (eds. Maxted, N., Ford-Llyod, V. and Hawkes, G.). Chapman and Hall, London.

Miceli, A., Tommasi, L., Negro C., and De Leo, P. (2002). Composition variability in essential oils of *Thymus capitatus*. In : Proceeding of LXV congress of SIFV, Riva del garda (TN), 20-23 September.

Miceli, A., Negro C. and Tommasi, L. (2006). Essential oil variability in *Thymbra capitata* (L.) Cav. Growing wild in southern Apulia (Italy). **Biochemical Systematics and Ecology**, 34, 528-535.

Miguel, G., Figueiredo, A., Costa, L., Martins, D., Duarte, J., Barroso, J. and Pedro, G. (2003). Effect of the volatile constituents isolated from *Thymus albicans, Th. mastichina, Th. carnosus* and *Thymbra capitata* in sunflower oil. **Nahrung**, 47, 397-402.

Miski, M., Ulubelan, A. and Mabry, J. (1983). 6-Hydroxyflavones from *Thymbra spicata*. **Phytochemistry**, 22, 2093–2094.

Mizukami, H. and Y. Okabe. (1999). A simple and rapid protocol for preparation of crude drug DNA suitable for PCR. **Biol. Ohar. Bull**. 22, 765-766.

Mohammadi, A. and Prasanna, M. (2003). Analysis of genetic diversity in Crop plants salient statistical tools and considerations. **Crop Science,** 43, 1235-1248**.**

Monokrousos, N., Papatheodorou, M., Diamantopoulos, D. and Stamou, P. (2005) Temporal and spatial variability of soil chemical and biological variables in a Mediterranean shrubland. **Forest Ecology and Management**, 202, 83–91.

MoPIC, (2005). **Annual progress report**, Conservation of medicinal and herbal plants project in Jordan. Ministry of Planning and International Cooperation, Amman.

Morales, R. (1996). Studies on the genus *Thymus* L. Real Jardín Botánico, CSIC, Plaza de Murillo 2, 28014 Madrid, Spain. **Lamiales news letter**, 4, 6-8.

Morales, R. (1989). El género Thymus L. en la región mediterránea occidenta (Lamiaceae). **Biocosme Mésogéen,** 6, 205-211.

Morales, R. (1986). Taxonomía de los géneros Thymus (excluida la sección Serpyllum) Thymbra en la Península Ibérica. **Ruizia**, 3, 1-324.

MPCP. (2006). **Medicinal Plants Conservation Project in Egypt**. Retrieved January, 15th , 2009, from www.mpcpegypt.com.

Muller, J., Berger, B., Yegen, O. and Cakir, C. (1997). Seasonal variation in chemical compositions of essential oils of selected aromatic plants growing wild in Turkey. **J. Agric. Food Chem**., 45, 4821-4825.

Mueller, U.G., and Wolfenbarger, L. L. (1999). AFLP genotype and fingerprinting. **TREE**, 14, 389-394.

NCSA. (2006). **Environmental profile of Jordan 2006**. Ministry of Environment. National Capacity Self Assessment for Global Environmental Management (NCSA) Project. Amman. Jordan.

Neffati, M., Belgacem, A. and El Mourid, M. (2006). **The medicinal and aromatic plants sector in the drylands**: A promising alternative for sustainable development and combating desertification in Tunisia. In: The future of drylands (eds. Lee, C. and Schaaf, T.). Springer Netherlands.

Neffati, M. and Belgacem, A. (2006). **A multidisciplinary study of herbal, medicinal and aromatic plants in Southern Tunisia**: a new approach. Regional consultation on linking producers to markets: lessons learned and successful practices, Egypt.

Nei, M. (1972) Genetic distance between populations. **American Naturalist**, 106:283-292.

Newman, J. and Cragg, M. (2007). Natural products as sources of new drugs over the last 25 years. **J. of Natural Products**, 70, 461-477.

Ojeda, M., Coirini, R., Cosianasi, J., Zapata, R.,Zygadlo, J. (2001). Evaluation of variability in natural populations of peperina (*Menthostachys mollis* (Kunth.) Griseb.), an aromatic species from Argentina. **Plant Genetic Resources Newsletter**, 126, 27-30.

Okigbo, N., Eme, E. and Ogbogu, S. (2008). Biodiversity and conservation of medicinal and aromatic plants in Africa. **Biotechnology and Molecular Biology Reviews**, 3, 127-134.

Pank, F. (1992). The influence of chemical weed control on quality characters of medicinal and aromatic plants. **Acta Horticulturae**, 306, 145-154.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP, and SSR (microsatellite) markers for germplasm analysis. **Mol. Breeding**, 2, 225-238.

Oran, S. and Al-Eisawi, D. (1998). Check-list of medicinal plants in Jordan. **Dirasat,** 25, 84-112.

Prajapati, D., Purohit, S., Sharma, K. and Kumar T. (2003). **A Hand Book of Medicinal Plants**, Agrobios (India).

Petanidou, T. (1996). Labiatae: a key family for wild bees and the pollination ecology in Mediterranean phryganic communities. **Lamiaceae news letter, 4, 4-6.**

Polunin. O. (1980). **Flowers of Greece and the Balkans**. Oxford University Press.

Ricciardi, L., Mastro, G., Giovanni, C., Lotti, C., and Armenise, L. (2002). **Morphological, biochemical and molecular characterisation of *origanum* accessions endemic of southern Italy**. Proceedings of the XLVI Italian Society of Agricultural Genetics - SIGA Annual Congress, Giardini Naxos, Italy.

Rodrigues, S., Monteiro, P., Maldoa-Martins, M., Monteiro, A., Povoa, O. and Teixeira, G. (2006). Biodiversity studies on Portuguese *Thymbra capitata***. Acta Horticulturae**, 723, 127-132.

Rohlf, J. (1992). NYTSYS-pc numerical taxonomy and multivariate analysis system, version 2.02. Exeter Publications, New York.

RSCN, (2002). **An Inventory study on medicinal and herbal plants in Jordan**. Royal Society for Conservation of Nature. Amman. Jordan.

Said, O., Khalil, K., Fulder, S. and Azizeh, H. (2002). Ethnoparmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. **Ethnopharmacology**, 83, 251-256.

Sangwan, N., Farooqi, A., Shabih, F. and Sangwan, R. (2001). Regulation of essential oil production in plants. **Plant Growth Regulation**, 34, 21-22.

Santos, P., Barroso, J.,Figueiredo, A., Pedro, L., Salgueiro, L., Fontinha, S., Deans, S. and Scheffer, J. (2005).Chemical polymorphism of populations of *Thymus caespititius* grown on the islands Corvo, Flores, São Miguel and Terceira (Azores) and on Madeira, assessed by analysis of their essential oils. **Plant Science**, 169, 1112-1117.

Sárosi SZ. and Bernáth, J. (2006). **Comparative evaluation of the antioxidant *Prunella vulgaris* L. and *Thymus vulgaris* L**. International symposium on the *Labiatae*: Advances in production, biotechnology and utilization, 22-25 February 2006, Sanremo, Italy.

Satil F., Dirmenci T., and Tumen G. (2002). **Natural Situation of commercial *Satureja* Species' in Turkey**. 16th Natl. Congr. Biol. September 1-7, Malatya, Turkey.

Schumacher, H. M. (1991). Biotechnology in the production and conservation of medicinal plants. In: akerele, O., Heywood, V. and Synge, H. (eds), **The conservation of medicinal plants**. Cambridge University Press, Cambridge.

SDBS, 2009. National Institute of Advanced Industrial Science and Technology. Retrived February, 11th from www. http://riodb01.ibase.aist.go.jp/sdbs.

Senatore, F. (1996). Influence of harvesting time on yield and composition of the essential oil of a Thyme (*Thymus pulegioides* L.) growing wild in Campania (Southern Italy). **J. Agric. Food Chem**. 44, 1327-1332.

Shannon, C.E. (1948). A mathematical theory of communication. **Bell System Technical Journal**, 27, 623–656.

Shasany, K., Darokar, P., Dhawan, S., Gupta, K., Gupta, S., Shukl, K., Patra, K., Khanuja, S. (2005). Use of RAPD and AFLP markers to identify inter- and intraspecific hybrids of Mentha. **Journal of Heredity**, 96, 542–549

Shoaib, A. and Arabi, E. (2006) .Genetic diversity among Syrian cultivated and landraces wheat revealed by AFLP markers. **Genetic Resources and Crop Evolution**, 53: 901–906.

Simon, E., Chadwick, F. and Craker, E. (1984). Herbs: An Indexed Bibliography. 1971-1980. The scientific literature on selected herbs, and aromatic and medicinal Plants of the Temperate zone. Archon Books, Hamden, CT. Retrieved June, 15, 2007, from www.hort.purdue.edu.

Skoula, M., Renée, J. Grayer, and Kite C. (2004). Surface flavonoids in *Coridothymus capitatus* and *Thymbra calostachya* (Lamiaceae). **Biochemical Systematics and Ecology**, 32, 1197-1200.

SPSS, (2004). SPSS 13.0 for windows. Marija J. Norusis /SPSS Incorporate. U.S.A.

Stahl-Biskup, E. and Sáez, F. (2002). **Thyme: the genus *Thymus***. CRC Press, London.

Stansfield, D. (1986). **Theory and problems of genetics**. McGraw-Hill book company, New York.

Sternberg, M. and Shoshany, M. (2001). Influence of slope aspect on Mediterranean woody formations: comparison of semi arid and an arid site in Israel. **Ecological Research**, 16, 335-345.

Taia, W. and El-Etaby, O. (2006). Taxonomical study in the desert plant *Calligonum comosum* L Her from two different locations in Saudi Arabia. **Asian J. of Plant Science**, 5, 570-579.

Tautz, D. and Renz, M. (1989). Simple sequences and ubiquitous components of eukaryotic genomes. **Nucleic Acid Res.** 18, 6531-6535.

Tawaha, K., **Alali**, F., Gharaibeh, M.,Mohammad, M. and El-Elimat, T. (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species. **Food Chemistry**, 104, 1372-1378.

Tenkate, K. and Laird, S.A. (1999). **The commercial use of biodiversity: access to the genetic resources and benefit sharing**. Earthscan, London.

Tolbert, D. M., Qualset, C. O., Jain, S. K., and Carddock, J. L. (1979). A diversity analysis of the worlds collection of barley. **Crop Sci**., 19, 789-794.

Tomas-Barberan, A., Grayer-Barkmeijer, J., Gil, I. and Harborne, B. (1988). Distribution of 6-hydroxy-, 6-methoxy- and 8-hydroxyflavone glycosides in the Labiatae, the Scrophulariaceae and related families. **Phytochemistry**, 27, 2631-2645.

Tonçer, Ö. and Kizil, S. (2005). Determination of yield and yield components in wild thyme (*Thymbra spicata* L. var. *spicata*) as influenced by development stages. **HORT. SCI.** (PRAGUE), 3, 100–103.

Troumbis, Y. (1990). Regulation of reproduction in *Thymus capitatus* under air pollution stress. **Acta Oecologica**, 3, 385-390.

Tsigouri, A., Passaloglou, M. and Sabatakou, O. (2004). Palynological characterisitics of Different unifloral honeys from Greece. **Grana,** 43, 122-128.

Udupa, M., Weigand, F., Saxena, C. and Kahl, G. (1998). Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the ascochyta blight pathogen of chickpea. **Theor. Appl. Genet.**, 299-307.

Underwood, A. (1981). Techniques of analysis of variance in experimental marine biology and ecology. Ann. Rev. **Oceanogr. Mar. Biol**.19, 513-605

UNESCO (1994). **Traditional knowledge in Tropical Environment. Nature and Resource**, 39(1) UNESCO, Paris.

United State Department of Agriculture/Economic Research Service (USDA/ERS). (2002). Organic production overview. Retrived April, 20, 2006. From http:// **www.ers.usda.gov/Data/organic**.

Vos, P., Hogers R., Bleeker M., Reijans M., Van De Lee T., Hornes M., Fritjers A., Pot J., Peleman J., Kuiper M. and Zabeau M. 1995. AFLP: a new technique for DNA fingerprint. **Nucleic Acid Res.,** 23, 4407–4414.

Vos, P. and Kuiper, M. (1997). AFLP analysis. **DNA markers: protocols, applications and overviews**, in Caetano, G. and Gresshoff (eds.). Wiley-VCH, NewYork.

Walter K. S. and Gillett H. J. (1998). **IUCN Red list of threatened plants**,1997. IUCN, Gland, Switzerland.

Wang, D., Fan, J., Siao, C., Berno, A., Young, P., Sapolsky, R., Ghandour, G., Perkins, N.,Winchester, E. and Spencer, J. (1998). Large scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome. **Science**, 1077-1082.

WCMC (1992). **Global biodiversity: Status of the earth living resources**. World Conservation Monitoring Center. Chapman and Hall, London, p 594.

Weltzinen, E. (1989). Differentiation among barley landraces populations from Near East. **Euphytica**, 43, 29-39.

WHO (1993). **The Promotion and Development of Traditional Medicine**: technical report. World Health Organization, Geneva.

Williams K., Kubelik, A., Livak, J., Rafalski, J. and Tingey, S. (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. **Nucleic Acid Res.** 18, 6431-6436.

Wilson, O. (1992). **The diversity of life**. Allan Lane, Penguin Press. London.

Woodland, D. (2000). **Contemporary plant systematic** (3rd ed.). Andrews University Press, U.S.A.

WRI, UNEP, UNDP, (1992). **Global biodiversity strategy**: guidelines for action to save, study and use Earth's biotic wealth sustainably and equitably. WRI,Washington.

Zabeau, M. and Vos, P. (1993). Selective restriction fragment amplification: a general method for DNA fingerprinting. **European Patent Application**, EP 534858A1.

Zohary, M. and N. Feinbrun. (1966). **Flora Palaestina**. The Israel Academy of Sciences and Humanities. Jerusalem.

Zohary, M. (1986). **Flora palaestina**. The Israel Academy of Sciences and Humanities Jerusalem.

8. APPENDICES

Annex (I): Distribution sites of *Coridothymus capitatus* (L.) Reichenb. fil growing wild in Jordan during 2005- 2006 and used in developing the distribution map by GIS.

No.	Site no.	District	Site Name	Longitude E	Latitude N	Altitude m	Pop. Size Palnt/4m²
1	1	Salt	Qasabat ASSalt	35 47 29.8	32 01 42.0	936	3
2	1	Salt	Qasabat ASSalt	35 47 29.9	32 01 42.9	928	3
3	1	Salt	Qasabat ASSalt	35 47 30.5	32 01 42.5	928	3
4	1	Salt	Qasabat ASSalt	35 47 29.7	32 01 42.8	928	3
5	1	Salt	Qasabat ASSalt	35 47 31.2	32 01 34.8	820	3
6	2	Salt	Al Fuhays	35 47 44.3	32 01 40.1	941	4
7	2	Salt	Al Fuhays	35 47 44.6	32 01 38.8	938	4
8	2	Salt	Al Fuhays	35 47 41.3	32 01 40.5	935	4
9	2	Salt	Al Fuhays	35 47 41.2	32 01 41.7	943	4
10	2	Salt	Al Fuhays	35 47 44.3	32 01 40.6	928	4
11	7	Amman	Khalda	35 51 26.4	32 00 7.1	1035	5
12	7	Amman	Khalda	35 51 25.9	32 00 9.1	1038	5
13	7	Amman	Khalda	35 51 26.9	32 00 11.2	1048	5
14	7	Amman	Khalda	35 51 27.1	32 00 13.8	1058	5
15	7	Amman	Khalda	35 51 29.4	32 00 10.2	1058	5
16	3	Suwaylih	Tla' Kaser Khalda	35 50 57.7	32 00 40.2	1054	2
17	3	Suwaylih	Tla' Kaser Khalda	35 50 56.7	32 00 41.4	1055	2
18	3	Suwaylih	Tla' Kaser Khalda	35 50 54.5	32 00 43.6	1051	2
19	3	Suwaylih	Tla' Kaser Khalda	35 50 53.3	32 00 44.9	1055	2
20	3	Suwaylih	Tla' Kaser Khalda	35 50 59.4	32 00 41.2	1060	2
21	4	Suwaylih	Ayn Al Basha boarders	35 50 29.1	32 00 56.4	1026	2
22	4	Suwaylih	Ayn Al Basha boarders	35 50 26.7	32 00 59.1	1032	2
23	4	Suwaylih	Ayn Al Basha boarders	35 50 14.4	32 00 48.4	1048	2
24	4	Suwaylih	Ayn Al Basha boarders	35 50 25.8	32 00 58.6	1049	2
25	4	Suwaylih	Ayn Al Basha boarders	35 50 27.0	32 00 57.2	1045	2
26	8	Amman	Opposite UOJ	35 52 49.6	32 00 37.9	1065	2
27	8	Amman	Opposite UOJ	35 52 58.3	32 00 43.6	1053	2
28	8	Amman	Opposite UOJ	35 52 01.9	32 00 39.4	1031	2
29	8	Amman	Opposite UOJ	35 52 06.1	32 00 01.4	1031	2
30	8	Amman	Opposite UOJ	35 52 04.3	32 00 42.2	1037	2
31	14	Hisban	Wadi As Sir boarders	35 50 04.5	31 52 59.2	826	4
32	14	Hisban	Wadi As Sir boarders	35 50 20.1	31 52 59.5	855	4
33	14	Hisban	Wadi As Sir boarders	35 50 20.3	31 52 59.7	860	4
34	14	Hisban	Wadi As Sir boarders	35 50 20.7	31 52 00.1	865	4
35	14	Hisban	Wadi As Sir boarders	35 50 19.0	31 52 01.3	875	4
36	10	Na'ūr	Wadi Slait	35 48 34.3	31 53 12.3	504	4
37	10	Na'ūr	Wadi Slait	35 48 42.0	31 53 12.1	527	4
38	10	Na'ūr	Wadi Slait	35 48 43.7	31 53 12.1	548	4
39	10	Na'ūr	Wadi Slait	35 48 45.3	31 53 08.9	562	4
40	10	Na'ūr	Wadi Slait	35 48 48.5	31 53 07.6	596	4
41	5	Abu Nusayr	Abu Hamid Village	35 53 57.6	32 05 00.1	844	2
42	5	Abu Nusayr	Abu Hamid Village	35 53 04.5	32 05 04.3	833	2

43	5	Abu Nusayr	Abu Hamid Village	35 53 04.5	32 05 04.3	833	2
44	5	Abu Nusayr	Abu Hamid Village	35 53 04.5	32 05 04.3	833	2
45	5	Abu Nusayr 1	Abu Hamid Village	35 53 05.5	32 05 0.3	823	2
46	11	Na'ūr	Bel'as village	35 50 08.8	31 52 23.5	910	4
47	11	Na'ūr	Bel'as village	35 50 08.2	31 52 23.1	920	4
48	11	Na'ūr	Bel'as village	35 50 07.6	31 52 23.2	925	4
49	11	Na'ūr	Bel'as village	35 50 06.2	31 52 22.7	930	4
50	11	Na'ūr	Bel'as village	35 50 04.8	31 52 22.6	940	4
51	12	Na'ūr	Edbyan	35 49 40.6	31 52 17.8	872	4
52	12	Na'ūr	Edbyan	35 49 47.6	31 52 15.11	860	4
53	12	Na'ūr	Edbyan	35 49 54.3	31 52 12.5	887	4
54	12	Na'ūr	Edbyan	35 49 58.6	31 52 11.9	890	4
55	12	Na'ūr	Edbyan	35 49 58.8	31 52 11.1	892	4
56	13	Na'ūr	Al Bassah village	35 49 52.3	31 52 08.7	941	4
57	13	Na'ūr	Al Bassah village	35 49 51.8	31 52 08.5	942	4
58	13	Na'ūr	Al Bassah village	35 49 51.9	31 52 09.0	936	4
59	13	Na'ūr	Al Bassah village	35 49 57.6	31 52 05.9	930	4
60	13	Na'ūr	Al Bassah village	35 49 51.4	31 52 10.3	920	4
61	9	Amman	Khalda military station	35 51 36.3	32 00 28.0	1062	3
62	9	Amman	Khalda military station	35 51 35.6	32 00 50.9	1046	3
63	9	Amman	Khalda military station	35 51 47.5	32 00 39.7	1051	3
64	9	Amman	Khalda military station	35 51 36.4	32 00 47.0	1070	3
65	9	Amman	Khalda military station	35 51 34.4	32 00 52.0	1080	3
66	6	Abu Nusayr	Abu Nusayr village	35 52 36.6	32 04 20.7	854	2
67	6	Abu Nusayr	Abu Nusayr village	35 52 36.8	32 04 20.6	855	2
68	6	Abu Nusayr	Abu Nusayr village	35 52 36.8	32 04 20.6	863	2
69	6	Abu Nusayr	Abu Nusayr village	35 52 36.5	32 04 20.7	853	2
70	6	Abu Nusayr	Abu Nusayr village	35 52 36.7	32 04 20.4	867	2
71	16	Ajlūn	Kufranja	35 43 17.2	32 18 28.7	686	1
72	16	Ajlūn	Kufranja	35 43 18.5	32 18 28.5	669	1
73	16	Ajlūn	Kufranja	35 43 18.2	32 18 28.6	673	1
74	16	Ajlūn	Kufranja	35 43 18.0	32 18 28.4	670	1
75	16	Ajlūn	Kufranja	35 43 17.9	32 18 29.0	689	1
76	15	Hisban	Um El Gutain village	35 48 27.0	31 51 13.9	883	3
77	15	Hisban	Um El Gutain village	35 48 26.3	31 51 13.8	873	3
78	15	Hisban	Um El Gutain village	35 48 25.9	31 51 12.9	868	3
79	15	Hisban	Um El Gutain village	35 48 26.6	31 51 11.8	870	3
80	15	Hisban	Um El Gutain village	35 48 29.0	31 51 12.5	890	3
81	17	Ajlūn	Anjarah (Landrace)	35 45 28.6	32 18 34.8	906	
82	18	Ajlūn	Anjarah (Landrace)	35 46 03.1	32 18 32.0	915	

Annex II: Soil characteristics of distribution sites of *Coridothymus capitatus* (L.) Reichenb. fil growing wild in Jordan during 2006.

Site name	pH	E.C. (ds/m)	N (%)	P(ppm)	K(ppm)	O.M. (%)	$CaCo_3$ (%)	Soil Texture
Qasabat AS Salt	7.7	1.1	0.3	11.7	324.6	4.8	37.2	Loam
Al Fuhays	7.7	0.8	0.2	7.00	135.4	3.3	59.5	Loam
Tla' Kaser Khalda	7.7	0.4	0.1	6.3	173.2	2.1	46.5	Clay loam
Ayn Al Basha	7.7	0.5	0.2	8.9	121.9	2.3	37.2	Loam
Abu Hamid village	7.8	0.5	0.1	8.7	122.8	2.1	55.7	Silt loam
Abu Nusayr village	7.7	0.8	0.2	7.3	135.4	1.8	55.7	Silt clay loam
Khalda	7.7	0.5	0.2	7.7	160.6	2.5	59.5	Loam
Amman (West UOJ)	7.7	1.1	0.2	7.6	122.8	2.2	70.6	Loam
Khalda military station	7.9	0.6	0.2	8.2	135.4	1.1	39.0	Silt loam
Wadi Slait	7.9	0.5	0.1	4.7	159.7	0.8	13.0	Sandy loam
Bela's village	7.6	0.7	0.2	12.4	178.1	3.5	61.3	Silt loam
Edbyan village	7.8	1.0	0.2	6.2	197.5	3.2	63.2	Loam
Al Bassah village	7.7	0.7	0.1	6.5	148.0	4.2	59.5	Clay loam
Wadi AS Sir	7.7	1.3	0.2	9.3	198.4	4.2	44.6	Loam
Um El Gutain village	7.7	1.8	0.3	4.3	159.7	2.2	55.7	Loam
Kufranja	7.7	0.5	0.1	6.8	109.2	2.3	61.3	Loam

Annex (III): Form of field data registration for geographical survey and collection of *Coridothymus capitatus* (L.) Reichenb. fil. growing wild in Jordan

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Date …/…. / 200 **Site no.** ( ) **Serial no**. ( )

I. SITE DATA

District name

Site name and description

Latitude N 3 °/ '/ **Altitude (m)**

Longitude E 3 ° / '/ **Rainfall (mm)**

II. HABITAT DATA

Habitat and Assoc. Species **Habitat:** *Batha, shrub land, wood land, Semi-steppe shrubland, Mountane vegetation*
Associated species:

Modifying factors *Mown, Burnt, Grazed, Flooded, Trampled, Urbanization,Others*

Land Form *Hill side. Plateaue. vallev….* **Drainage** *Free , Moderate*

Land Use **Slope°** **Aspect**

Geology *Limestone, sandstone, basalt , laterite, granite ,Chalky, hygromorph, granite*

Soil texure *Sand clay loam sandy-loam clay-loam* **Soil color**

II. COLLECTION DATA **Date of seed collection** ……/…../ 200

Population size (Plant/ m^2) **No. of plants sampled** **Seeds collected from** *Plants, Ground*

IV. HERBARIUM DATA

Growth habit *Prostrate.Creeping. Erect Herb. Dwarf Shrub. Shrub* **Spinescence** *Present, absent*

Plant Height (cm) **Plant width (cm)** **Plant length (cm)**

Leaf arrangement *Opposite. Alternate*

Flower colour *White, Pink, Violet, purple* **No. of voucher duplicates**

V. ETHNOBOTANICAL DATA

Local name

Community *Women society, farmers, Bedouin, herbalists*

Use *Food, Food Additive, Medicine, Bee Plant, Fuel, Social, Vertebrate Poison, Non-Vertebrate Poison, Ornamental, Environmental Use, Gene Source*

Annex (IV): Some descriptive characters of *Coridothymus capitatus* (L.) Reichenb. fil.

Growth Habit

Erect Herb, Dwarf Shrub, Shrub

Spinescence

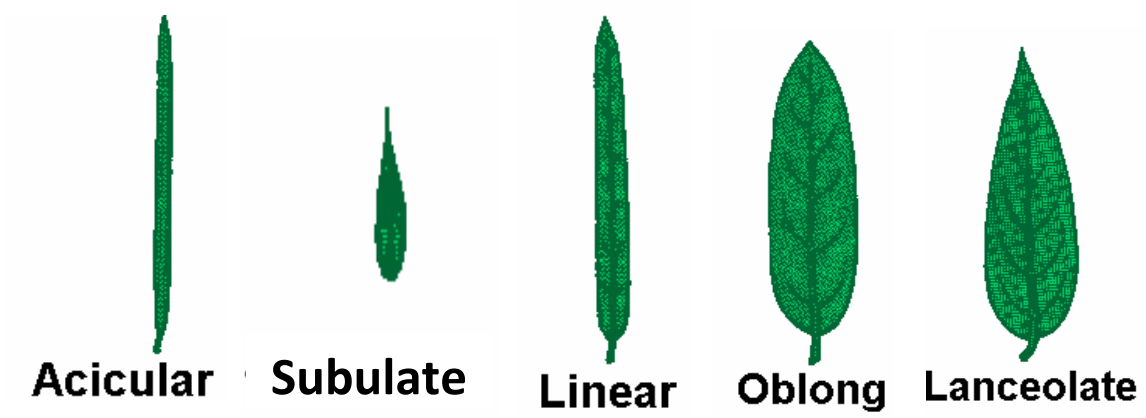
Present, Absent

Leaf Color

(1) Dark green, (3) Light green, (5) Gray, (7) Whitish

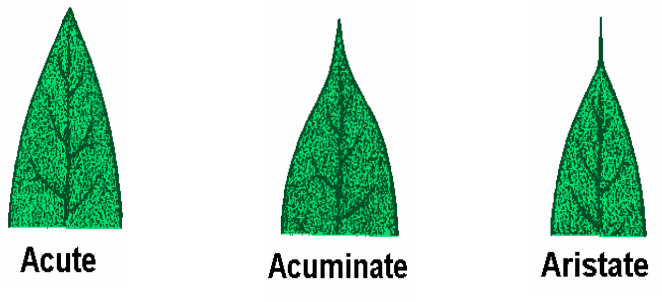
Leaf Shape

(1) Acicular, (3) Subulate, (5) Lineaer, (7) Oblong, (9) Lanceolate



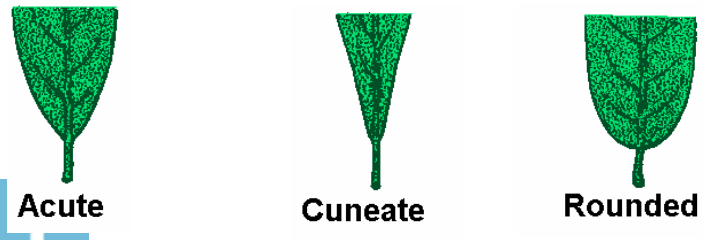
Leaf Apices

(1) Acute, (3) Acuminate, (5) Aristate



Leaf Bases

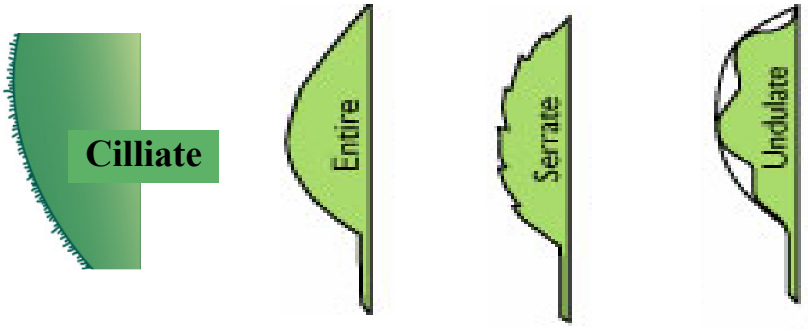
(1) Acute, (3) Cuneate, (5) Rounded



Cont. Annex (IV)

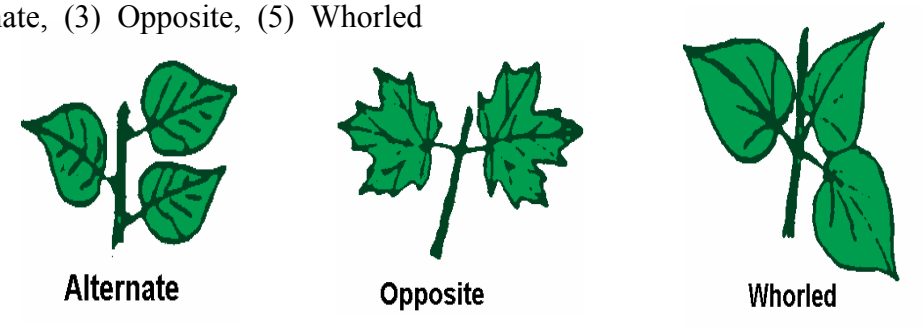
Leaf Margins

(1) Cilliate, (3) Entire, (5) Undulate, (7) Serrate



Leaf Arrangement

(1) Alternate, (3) Opposite, (5) Whorled



Stem Color

(1) Dark green, (3) Light green, (5) Gray, (7) Whitish, (9) Light red

Petal Color

(1) Dark violet, (3) Light violet, (5) Pink, (7) White

Leaf Surface

(1) Glabrescent, (3) Glabrous, (5) Glandular, (7) Semi Glandular, (9) Glaucous

Leaf Attachment

(1) Petiolate, (3) Sessile, (5) Nearly Sessile, (7) Sheathing

Leaf Stipules

Present, Absent

Annex (V)
Analysis of variance (ANOVA) tables

ANOVA of leaf length of wild *C. capitatus* populations in 2006.

Source of variation	D. F.	F value	F pr.
Replicate	4		
Population (P)	15	16.84	< 0.001
Error = R X P	60		
Total	79		

ANOVA of leaf width of wild *C. capitatus* populations in 2006.

Source of variation	D. F.	F value	F pr.
Replicate	4		
Population (P)	15	32.67	< 0.001
Error = R X P	60		
Total	79		

ANOVA of leaf length : width ratio of wild *C. capitatus* populations in 2006.

Source of variation	D. F.	F value	F pr.
Replicate	4		
Population (P)	15	1.59	0.10
Error = R X P	60		
Total	79		

ANOVA of plant height of wild *C. capitatus* populations in 2006.

Source of variation	D. F.	F value	F pr.
Replicate	4		
Population (P)	15	4.50	< 0.001
Error = R X P	60		
Total	79		

ANOVA of plant width of wild *C. capitatus* populations in 2006.

Source of variation	D. F.	F value	F pr.
Replicate	4		
Population (P)	15	4.01	< 0.001
Error = R X P	60		
Total	79		

ANOVA of plant length of wild *C. capitatus* populations in 2006.

Source of variation	D. F.	F value	F pr.
Replicate	4		
Population (P)	15	2.86	0.00
Error = R X P	60		
Total	79		

ANOVA of length of inflorescence of wild *C. capitatus* populations in 2006.

Source of variation	D. F.	F value	F pr.
Replicate	4		
Population (P)	15	19.54	< 0.001
Error = R X P	60		
Total	79		

ANOVA of thymol content of wild *C. capitatus*			
Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	15	27.6	< 0.001
Error = R X P	30		
Total	47		
ANOVA of carvacrol content of wild *C. capitatus*			
Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	15	55.20	< 0.001
Error = R X P	30		
Total	47		

ANOVA of seasonal thymol content of *C. capitatus*			
Source of variation	D. F.	F value	F pr.
Replicate	2		
Month	11	45.76	< 0.001
Error = R X P	22		
Total	35		
ANOVA of seasonal carvacrol content of *C. capitatus*			
Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	11	55.71	< 0.001
Error = R X P	22		
Total	35		

ANOVA of thymol content of cultivated *C. capitatus*			
Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	26.81	< 0.001
Error = R X P	40		
Total	62		
ANOVA of carvacrol content of cultivated *C. capitatus*			
Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	31.31	< 0.001
Error = R X P	40		
Total	62		

ANOVA of plant height of cultivated *C. capitatus* populations in 2007.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	3.55	< 0.001
Error = R X P	40		
Total	62		

ANOVA of plant width of cultivated *C. capitatus* populations in 2007.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	1.77	0.062
Error = R X P	40		
Total	62		

ANOVA of plant length of cultivated *C. capitatus* populations in 2007.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	1.45	< 0.001
Error = R X P	40		
Total	62		

ANOVA of leaf length cultivated *C. capitatus* populations in 2007.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	29.49	< 0.001
Error = R X P	40		
Total	62		

ANOVA of leaf width of cultivated *C. capitatus* populations in 2007.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	8.33	< 0.001
Error = R X P	40		
Total	62		

ANOVA of length of inflorescence cultivated *C. capitatus* populations in 2007.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	11.73	< 0.001
Error = R X P	40		
Total	62		

ANOVA of number of inflorescence of cultivated *C. capitatus* populations in

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	2.66	0.004
Error = R X P	40		
Total	62		

ANOVA of fresh weight of cultivated *C. capitatus* populations in 2007.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	1.29	0.244
Error = R X P	40		
Total	62		

ANOVA of dry weight of cultivated *C. capitatus* populations in 2007.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	3.39	< 0.001
Error = R X P	40		
Total	62		

ANOVA of plant height of cultivated *C. capitatus* populations in 2008.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	4.55	< 0.001
Error = R X P	40		
Total	62		

ANOVA of plant width of cultivated *C. capitatus* populations in 2008.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	2.20	0.017
Error = R X P	40		
Total	62		

ANOVA of plant length of cultivated *C. capitatus* populations in 2008.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	2.22	0.016
Error = R X P	40		
Total	62		

ANOVA of leaf length cultivated *C. capitatus* populations in 2008.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	35.39	< 0.001
Error = R X P	40		
Total	62		

ANOVA of leaf width of cultivated *C. capitatus* populations in 2008.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	4.23	< 0.001
Error = R X P	40		
Total	62		

ANOVA of length of inflorescence of cultivated *C. capitatus* populations in 2008

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	17.42	< 0.001
Error = R X P	40		
Total	62		

ANOVA of number of inflorescence of cultivated *C. capitatus* populations in

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	2.67	0.004
Error = R X P	40		
Total	62		

ANOVA of fresh weight of cultivated *C. capitatus* populations in 2008.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	11.27	< 0.001
Error = R X P	40		
Total	62		

ANOVA of dry weight of cultivated *C. capitatus* populations in 2008.

Source of variation	D. F.	F value	F pr.
Replicate	2		
Population (P)	20	6.03	< 0.001
Error = R X P	40		
Total	62		

Annex (VI): Shanon diversity index (*H`*) calculations for qualitative traits of *Coridothymus capitatus* (L.) Reichenb. fil wild populations.

Trait	Description	Attribute %	Pi	Ln Pi	Pi * Ln Pi	∑Pi * Ln Pi	*H`* = - (∑Pi * Ln Pi)
Growth habit	Dwarf Shrub	100.00	1.00	0.00	0.00		0.00
Leaf shape	Oblong	6.25	0.06	-2.77	-0.17	-0.23	0.23
	Lanceolate	93.75	0.94	-0.06	-0.06		
Leaf color	Dark green	20.00	0.20	-1.61	-0.32	-0.50	0.50
	Light green	80.00	0.80	-0.22	-0.18		
Leaf margin	ciliate	100.00	1.00	0.00	0.00		0.00
Leaf arrangement	Opposite	100.00	1.00	0.00	0.00		0.00
Leaf apices	Acute	93.75	0.94	-0.06	-0.06	-0.23	0.23
	Acuminate	6.25	0.06	-2.77	-0.17		
Leaf base	Acute	100.00	1.00	0.00	0.00		0.00
Leaf stipules	Abscent	100.00	1.00	0.00	0.00		0.00
Leaf surface	Glandular	100.00	1.00	0.00	0.00		0.00
Leaf attachment	Sessile	100.00	1.00	0.00	0.00		0.00
Stem color	Gray	90.00	0.90	-0.11	-0.09	-0.39	0.39
	Whitish	3.75	0.04	-3.28	-0.12		
	Light red	6.25	0.06	-2.77	-0.17		
Petal color	Dark violet	20.00	0.20	-1.61	-0.32	-1.09	1.09
	Light violet	58.80	0.59	-0.53	-0.31		
	Pink	15.00	0.15	-1.90	-0.28		
	White	6.30	0.06	-2.76	-0.17		
Spinescence	Abscent	100.00	1.00	0.00	0.00		0.00
Over all *H`* =							0.49

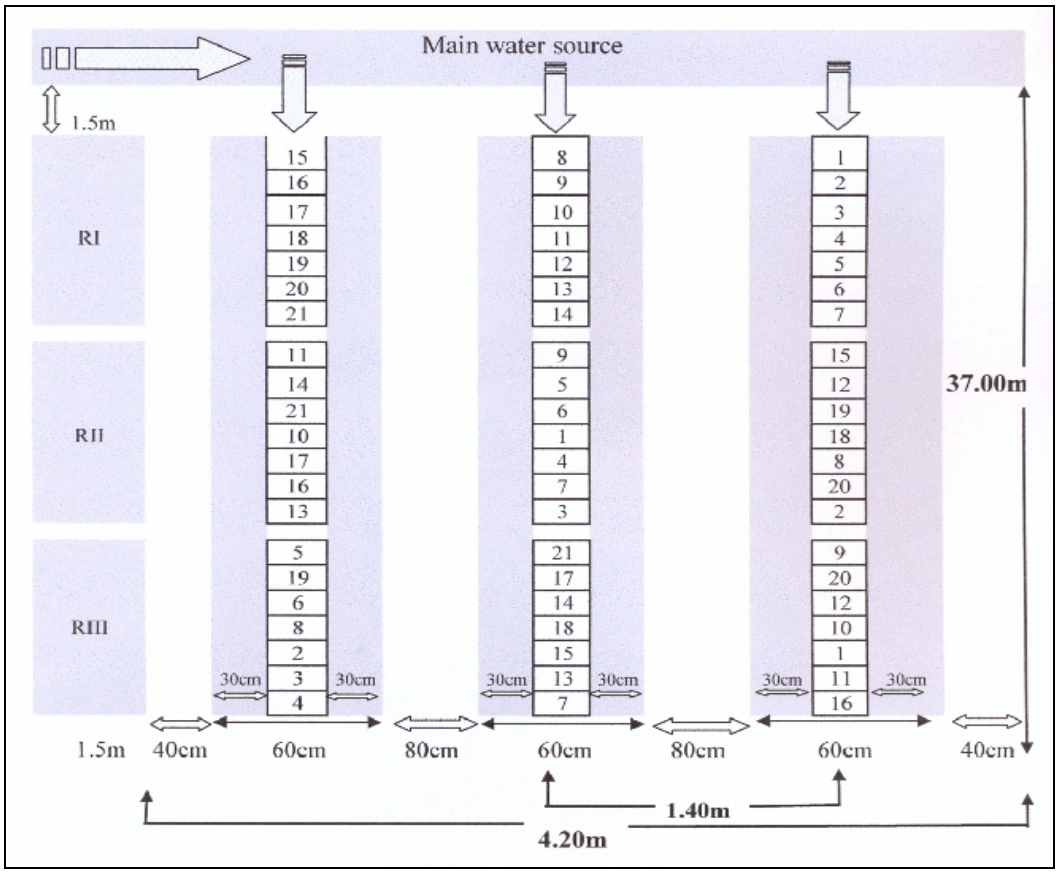
Annex (VII): Shanon diversity index (*H`*) calculations for quantitative traits of *Coridothymus capitatus* (L.) Reichenb. fil wild populations.

Classes	Formula	Leaf length (mm)	Leaf width (mm)	L:W ratio (mm)	Plant height (cm)	Plant width (cm)	Plant length (cm)	Inflorescence length
1	xi > μ+2*SD	5	5	2	3	2	2	4
2	μ+1*SD < xi < μ+2*SD	1		4	8	10	10	2
3	μ-1*SD < xi < μ+1*SD	72	75	73	52	49	50	72
4	μ-1*SD > xi > μ-2*SD	2		1	14	19	18	2
5	xi < μ-2*SD				3			
	N = ∑xi	80	80	80	80	80	80	80
Pi								
1		0.06	0.06	0.03	0.04	0.03	0.03	0.05
2		0.01	0.00	0.05	0.1	0.13	0.13	0.03
3		0.90	0.94	0.91	0.65	0.61	0.63	0.90
4		0.03	0.00	0.01	0.18	0.24	0.23	0.03
5		0.00	0.00	0.00	0.04	0.00	0.00	0.00
	Total Pi	1	1	1	1	1	1	1
ln Pi								
1		-2.77	-2.77	-3.69	-3.28	-3.69	-3.69	-3.00
2		-4.38	0.00	-3.00	-2.30	-2.08	-2.08	-3.69
3		-0.11	-0.06	-0.09	-0.43	-0.49	-0.47	-0.11
4		-3.69	0.00	-4.38	-1.74	-1.44	-1.49	-3.69
5		0	0	0	-3.28	0	0	0
Pi*lnPi								
1		-0.17	-0.17	-0.09	-0.12	-0.09	-0.09	-0.15
2		-0.05	0.00	-0.15	-0.23	-0.26	-0.26	-0.09
3		-0.09	-0.06	-0.08	-0.28	-0.30	-0.29	-0.09
4		-0.09	0.00	-0.05	-0.31	-0.34	-0.34	-0.09
5		0	0	0	-0.12	0	0	0
∑ Pi*lnPi		-0.42	-0.23	-0.38	-1.06	-0.99	-0.98	-0.43
Shanon index (SDI) = -∑ Pi*lnPi		0.42	0.23	0.38	1.06	0.99	0.98	0.43
Overall $H`$ = 0.64								

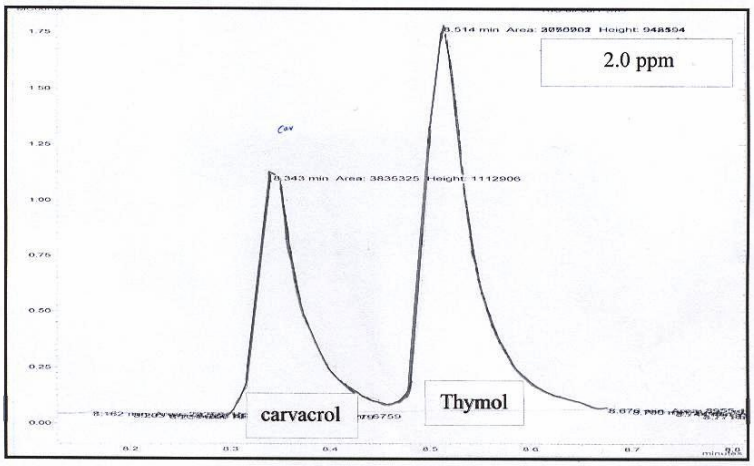
Annex (VIII): Some soil physical and chemical characters of Mushager research station.

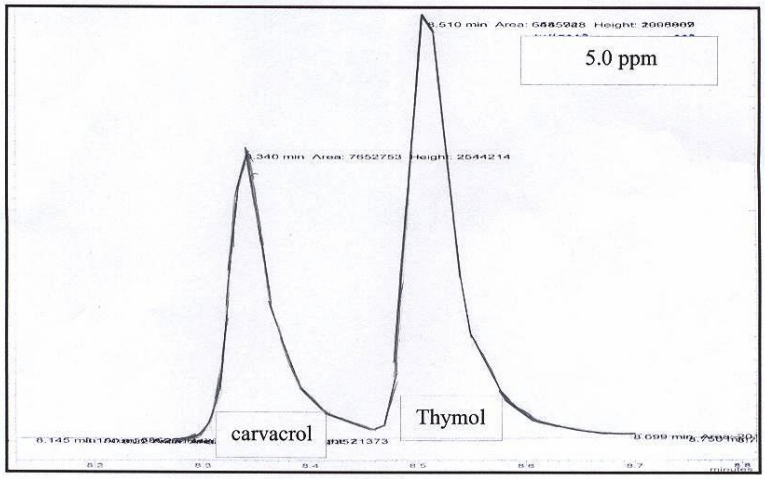
Character	value	Unit
pH	7.9	
EC	1.53	ds/m
$CaCo_3$	18.4 %	%
O.M	1.14 %	%
N	0.056 %	%
P	21.4	ppm
K	431	ppm
Sand	6.7 %	%
Silt	31.6	%
Clay	61.7	%
Texture	Clay	

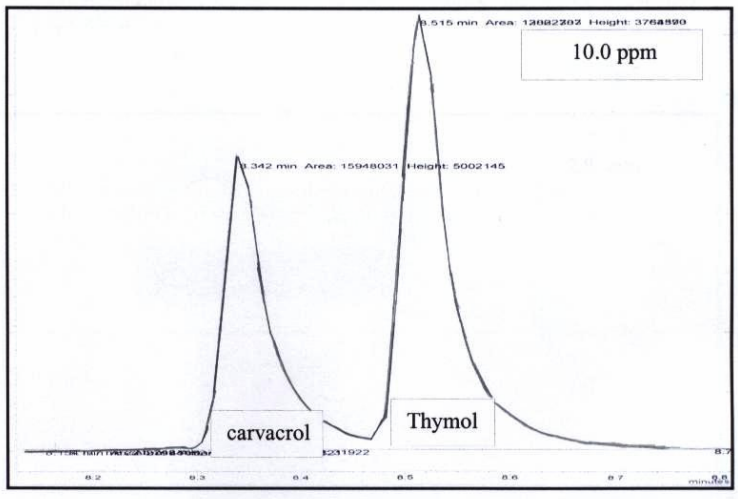
Annex (IX): Randomized complete block design layout for *Coridothymus capitatus* (L.) Reichenb. fil cultivation.



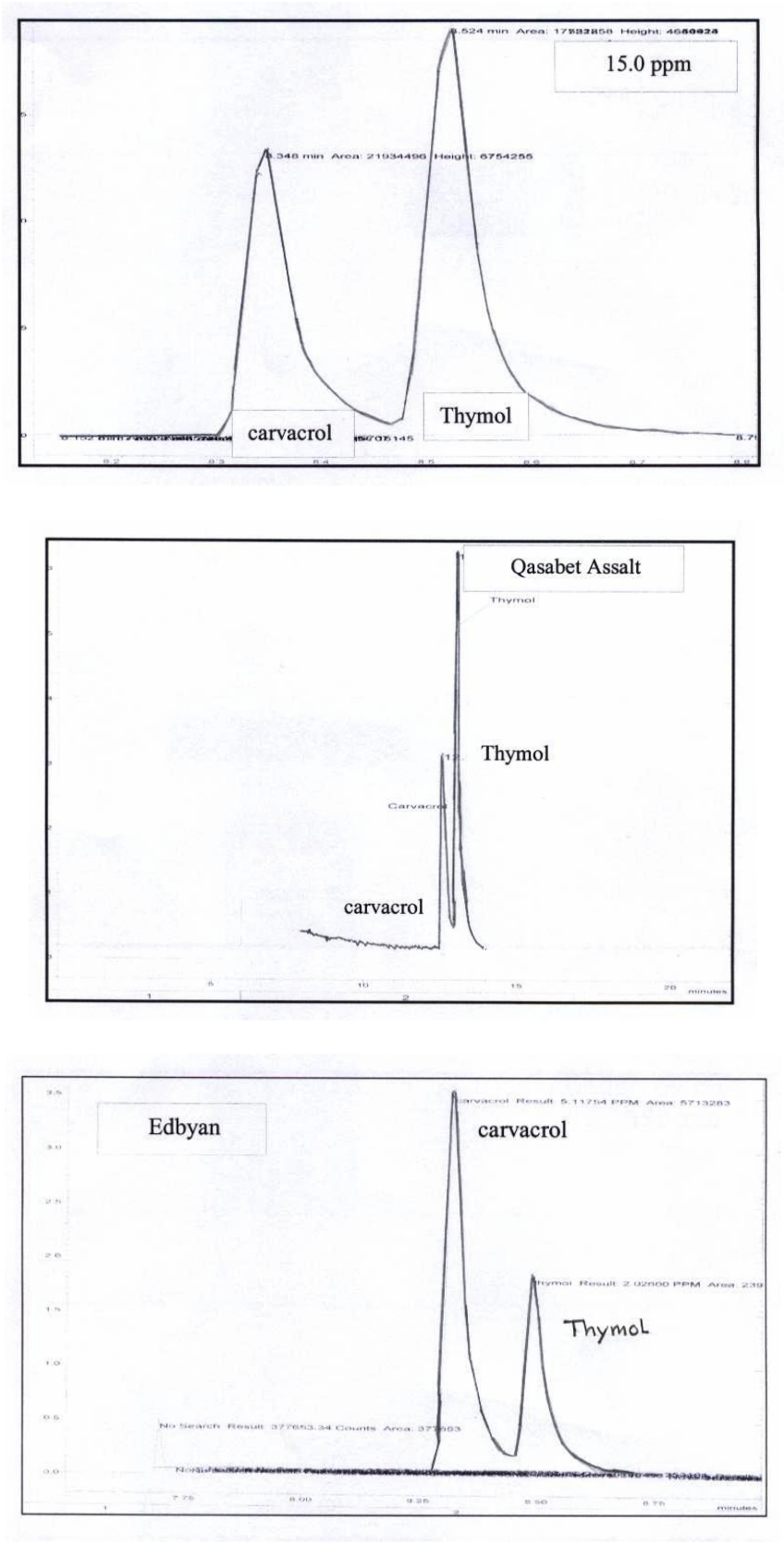
Annex (X): Mass spectra identifiedying thymol and carvacrol, the GC-MS chromatogram of thymol and carvacrol in standards, and in two sample population of *Coridothymus capitatus* (L.) Reichenb. fil.



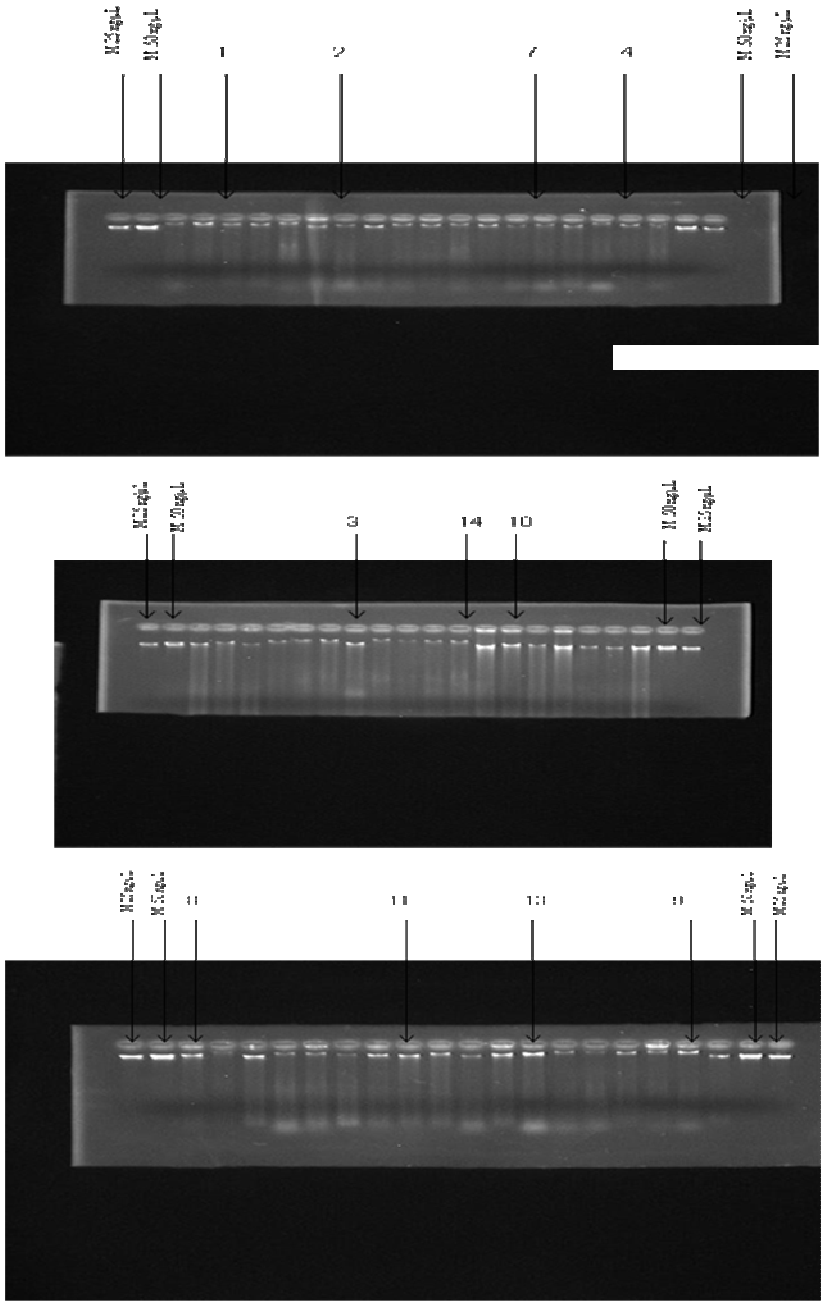




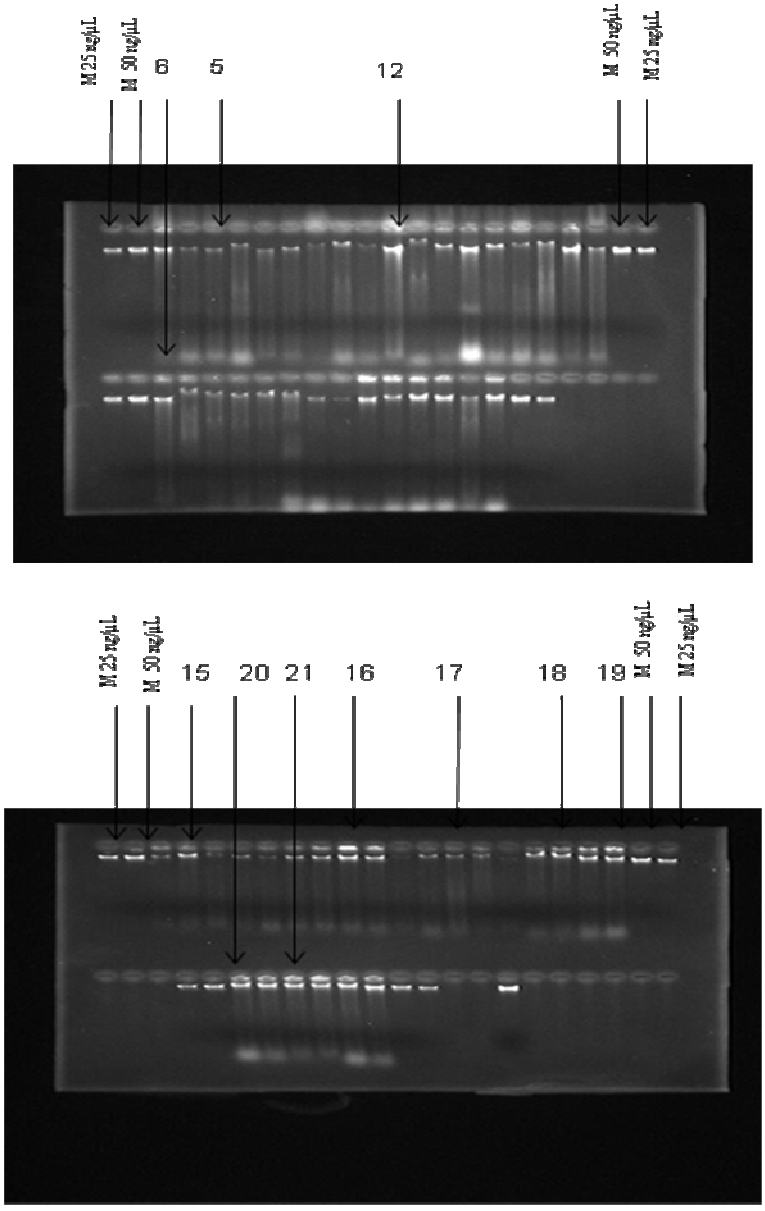
Cont. Annex (X)



Annex (XI): Agarose gel (1.0%) showing total genomic DNA isolated from 21 thyme populations and used in AFLP analysis.



Cont. Annex (XI): Agarose gel (1.0%) showing total genomic DNA isolated from 21 thyme populations and used in AFLP analysis.



Annex (XII): AFLP master mixtures

DNA digestion master mixture

Reagents	**Volumes** (µL)
250 ng of DNA	6.25
10X (OPA) Buffer	2.00
BSA (1µg/µL)	2.00
*Pst*I (10 U/µL)	0.50
*Mse*I (10 U/µL)	0.50
S.D H2O to 20 µL	8.75
Final volume	20.00

DNA ligation master mixture

Reagents	**Volumes** (µL)
Digested DNA	15
Buffer OPA 10X	0.5
ATP (10mM)	0.5
*Pst*I adapter (5 pmol/µL)	0.5
*Mse*I adapter (50 pmol/µL)	0.5
T4 DNA ligase (3U/µL)	0.4
S.D H2O to 20 µL	2.6
Final volume	20

Pre-amplification master mixture

Reagents	**Volumes** (µL)
Diluted ligated DNA	4
10X PCR Buffer	2
dNTPs (2mM/µL each)	2.5
*Pst*I + P00 (50ng/µL)	1
*Mse*I + M00 (50ng/µL)	1
Taq polymerase (5U/µL)	0.2
S.D H2O to 20	9.3
Final volume	20

Selective-amplification master mixture

Reagents	**Volumes** (µL)
Diluted pre-amplified products	4
10X PCR Buffer	2
dNTPs (2mM/µL each)	2
*Pst*I selective primer (50ng/µL)	1
*Mse*I selective primer (50ng/µL)	1
Taq polymerase (5U/µL)	0.2
S.D H2O to 20	9.8
Final volume	20

Annex (XIII): Summary of variations of wild and cultivated (2007, 2008) thyme populations known in Jordan as Za'tar Farisi, for nine morpholocal and four biochemical traits.

Trait	No. of inflorescence		Length of inflorescene (mm)			Plant height (cm)			Plant width (cm)			Plant length (cm)		
Pop*	2007	2008	Wild	2007	2008	Wild	2007	2008	Wild	2007	2008	Wild	2007	2008
1	588.56	593.00	7.00	8.78	6.33	28.00	23.33	18.89	38.20	31.33	43.44	32.40	26.67	31.89
2	232.00	236.00	8.80	12.00	5.67	17.25	20.44	15.78	39.20	29.11	38.11	38.80	30.89	31.33
3	385.44	389.22	7.80	10.33	8.44	46.00	23.33	18.67	53.20	26.78	40.33	51.60	26.78	30.00
4	457.33	462.67	4.20	12.56	9.89	19.75	25.56	19.56	61.40	33.44	38.78	55.60	27.33	30.00
5	668.00	672.44	7.20	13.67	12.11	21.75	24.00	17.44	25.80	28.00	34.33	31.60	25.56	27.56
6	407.56	411.33	7.20	12.22	13.78	20.75	22.78	14.56	26.80	25.00	33.89	31.60	22.22	27.44
7	372.22	376.44	6.40	12.33	6.22	37.75	26.33	20.22	70.00	30.33	39.11	66.80	30.00	35.67
8	589.22	592.67	8.00	10.22	9.22	30.50	22.89	18.11	63.00	29.33	36.56	62.00	25.33	29.56
9	528.89	533.56	7.80	11.56	9.89	41.50	26.56	19.11	58.60	30.44	39.89	64.00	27.56	31.89
10	257.11	263.44	9.40	9.78	9.33	30.25	22.56	19.78	67.40	33.33	34.33	70.20	29.89	32.89
11	428.56	433.89	7.40	12.67	11.67	36.00	27.11	24.56	69.40	34.56	38.67	74.20	32.11	34.44
12	405.44	410.44	11.80	12.11	8.89	40.00	23.22	18.00	59.00	31.33	40.11	51.60	31.56	36.56
13	621.67	626.00	6.80	15.00	11.78	30.50	25.78	19.78	66.20	30.56	43.44	62.80	26.44	33.33
14	357.56	361.78	8.80	9.56	9.11	37.25	26.22	17.00	59.60	27.56	40.67	66.40	23.89	33.00
15	144.00	149.44	10.00	10.22	19.22	38.00	22.33	21.00	79.80	28.89	34.11	68.40	27.78	27.22
16	155.11	158.33	28.00	18.56	38.11	29.25	29.78	29.00	27.40	28.11	42.67	29.00	26.56	38.67
17	122.78	129.44		22.00	46.00		26.22	25.78		19.78	43.44		21.67	41.44
18	136.67	141.78		31.44	36.67		28.22	27.33		22.89	42.11		23.56	34.22
19	561.11	566.89		7.11	17.00		21.22	29.11		27.78	42.78		23.33	38.44
20	555.67	559.11		14.78	20.44		23.33	26.89		26.78	44.56		24.89	34.78
21	138.44	139.89		15.33	17.56		35.00	26.11		30.11	24.44		24.78	21.11
Overall	386.35	390.85	9.16	13.44	15.59	31.53	25.06	21.27	54.06	28.83	38.85	53.56	26.61	32.45
$LSD_{0.05}$	315.90	315.20	29.30	4.43	7.69	26.80	4.98	5.98	35.40	7.47	9.09	39.20	7.10	8.82
% CV	49.50	48.90	3.39	20.00	29.90	10.40	12.00	17.00	24.24	15.70	14.20	26.54	16.20	16.50

*Numbers 1-21 are populations of sites: Qasabat AS Salt; Al Fuhays;Tla' Kaser Khalda; Ayn Al Basha; Abu Hamid; Abu Nusayr; Khalda; Amman (West UOJ); Khalda military station; Wadi Slait; Bela's; Edbyan; Al Bassah; Wadi AS Sir; Um El Gutain; Kufranja;Anjara landrace 1; Anjara landrace 2; commercial cultivar 1; commercial cultivar 2; and commercial cultivar 3, respectively.

Cont. Annex (XIII): Summary of variations of wild and cultivated (2007, 2008) Thyme populations named in Jordan Za'tar Farisi for nine morpholocal and four biochemical traits.

Trait	Leaf length (mm)			Leaf width (mm)			Fresh weight (g)/ plant		Dry weight (g)/ plant	
Pop*	Wild	2007	2008	Wild	2007	2008	2007	2008	2007	2008
1	3.40	4.44	3.22	1.00	1.11	2.06	173.89	156.67	74.44	130.56
2	3.40	4.44	4.00	1.00	0.98	1.28	205.56	185.11	81.11	172.22
3	4.00	4.67	4.44	1.00	0.97	1.33	166.11	152.11	56.67	128.11
4	3.60	4.33	4.67	1.00	0.97	1.33	205.00	209.56	86.11	158.33
5	4.00	4.33	5.11	1.00	1.11	1.44	182.78	142.78	83.89	116.67
6	4.00	4.44	4.56	1.00	1.17	1.22	165.56	154.33	66.11	119.44
7	4.20	4.00	4.22	1.00	1.17	1.06	172.78	130.33	63.89	100.78
8	4.60	4.67	4.44	1.00	1.22	1.28	180.00	137.22	78.89	116.11
9	4.00	4.22	4.22	1.00	1.11	1.22	140.56	133.33	44.44	88.22
10	3.60	3.89	4.33	1.00	1.22	1.22	160.56	126.33	67.44	90.78
11	3.80	5.22	4.00	1.00	1.17	1.33	253.22	178.33	104.44	153.00
12	5.00	4.22	4.22	1.00	1.22	1.44	217.22	163.89	85.56	140.56
13	4.00	4.67	4.44	1.00	1.33	1.31	202.78	175.00	90.56	156.11
14	3.00	3.89	4.33	1.00	0.99	1.39	191.11	175.56	93.89	166.11
15	3.20	5.78	4.22	1.00	1.44	1.17	161.33	131.44	53.33	81.67
16	11.40	10.89	11.78	2.40	2.56	2.44	193.56	363.33	84.11	248.89
17		11.00	11.89		1.89	2.22	148.89	265.00	57.78	183.89
18		10.44	12.33		1.83	2.33	150.44	251.11	64.44	170.56
19		6.11	4.67		2.33	1.78	187.22	329.44	73.89	208.33
20		5.78	4.22		1.78	1.78	185.00	325.56	77.78	194.44
21		10.89	10.22		1.83	1.83	167.78	396.11	28.33	261.67
Overall	4.33	5.83	5.69	1.09	1.40	1.55	181.49	203.93	72.24	151.74
LSD $_{0.05}$	24.60	1.34	1.42	12.60	0.45	0.57	59.26	71.33	27.65	57.36
% CV	1.35	14.00	15.10	0.17	19.70	22.50	20.20	21.20	23.20	22.90

*Numbers 1-21 are populations of sites: Qasabat AS Salt; Al Fuhays;Tla' Kaser Khalda; Ayn Al Basha; Abu Hamid; Abu Nusayr; Khalda; Amman (West UOJ); Khalda military station; Wadi Slait; Bela's; Edbyan; Al Bassah; Wadi AS Sir; Um El Gutain; Kufranja;Anjara landrace 1; Anjara landrace 2; commercial cultivar 1; commercial cultivar 2; and commercial cultivar 3, respectively.

Cont. Annex (XIII): Summary of variations of wild and cultivated (2007, 2008) thyme populations named in Jordan Za'tar Farisi, for nine morpholocal and four biochemical traits.

Trait	Oil yield (%)		Thymol (%)		Carvacrol (%)		(%) Thymol + carvacrol	
pop*	Wild	2007	Wild	2007	Wild	2007	Wild	2007
1	1.67	1.28	0.44	0.35	0.21	0.30	0.65	0.65
2	1.32	3.16	0.34	0.03	0.15	0.01	0.49	0.04
3	2.85	2.57	0.51	0.42	0.57	0.18	1.08	0.59
4	1.81	2.74	0.48	0.04	0.24	0.46	0.73	0.50
5	2.45	2.19	0.31	0.25	0.87	0.19	1.19	0.44
6	1.67	3.53	0.13	0.06	0.53	0.70	0.66	0.75
7	1.93	2.26	0.47	0.43	0.30	0.15	0.77	0.57
8	1.47	3.37	0.32	0.73	0.21	0.53	0.53	1.26
9	2.11	4.02	0.43	0.90	0.39	0.17	0.83	1.07
10	2.53	2.42	0.57	0.45	0.35	0.16	0.92	0.6
11	1.04	3.85	0.27	0.53	0.10	0.42	0.37	0.95
12	2.96	4.23	0.49	0.05	0.63	0.90	1.13	0.95
13	2.56	2.89	0.45	0.04	0.45	0.05	0.90	0.08
14	2.28	4.99	0.34	0.05	0.72	0.90	1.06	0.95
15	1.94	2.26	0.45	0.03	0.22	0.56	0.67	0.59
16	0.39	2.44	0.03	0.05	0.10	0.47	0.13	0.52
17		2.55		0.03		0.40		0.43
18		2.45		0.01		0.42		0.44
19		1.27		0.01		0.26		0.27
20		1.71		0.03		0.28		0.30
21		1.40		0.02		0.23		0.58
Overall	1.94	2.74	0.37	0.21	0.38	0.37	0.76	0.85
$LSD_{0.05}$				1.48		1.27		
% CV			12.50	41.70	14.30	21.00		

*Numbers 1-21 are populations of sites: Qasabat AS Salt; Al Fuhays;Tla' Kaser Khalda; Ayn Al Basha; Abu Hamid; Abu Nusayr; Khalda; Amman (West UOJ); Khalda military station; Wadi Slait; Bela's; Edbyan; Al Bassah; Wadi AS Sir; Um El Gutain; Kufranja;Anjara landrace 1; Anjara landrace 2; commercial cultivar 1; commercial cultivar 2; and commercial cultivar 3, respectively.

Annex (XIX): Person's coefficient of correlation between pairs of dry herbage yield and essential oil yields (kg/ha) of *Coridothymus capitatus* populations cultivated at Mushagar research station in 2007 .

Trait	Dry herbage yield	Essential oil yield	Thymol yield	Carvacrol yield	Thymol + carvacrol yield
Dry herbage yield	1				
Essential oil yield	0.73**	1			
Thymol yield	0.13	0.2	1		
Carvacrol yield	0.48*	0.72**	0.55	1	
Thymol + carvacrol yield	0.47*	0.70**	0.60**	0.76**	1

** correlation is significant at 0.01 level

** correlation is significant at 0.05 level

التنوع الجيني، الحفظ، وامكانية زراعة الزعتر الفارسي *Coridothymus capitatus* (L.) Reichenb. fil في الأردن

اعداد

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المشرف

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المشرف المشارك

الأستاذ الدكتور فراس قاسم علعالي

ملخص

ينمو نبات ال *Coridothymus capitatus* والمعروف محلياً بالزعتر الفارسي في الموائل البرية في الأردن وهو نبات عطري. تمت دراسة التباين الوراثي والحفظ وإمكانية زراعة النبات في الحقل. شملت الدراسة خمسة عشر عشيره برية من ال *Coridothymus capitatus*، وعشيرة برية من ال *Thymbra spicata*، بالإضافة الى عشيرتين مستزرعتين من ال *Thymbra spicata*. أظهرت عشائر ال *Coridothymus capitatus* البرية درجات مختلفة من التباين الظاهري بالإعتماد على الصفات الشكليه المدروسة، وأظهرت الصفات الكمية تباينات معنوية بين العشائر حيث تراوحت نسبة معامل التباين (CV%) من 12.60 % إلى 39.20 %. قدر معدل معيار التباين شانون (H') ب 0.58 وكان الإختلاف بين أزواج العشائر قليلاً.

أظهر التحليل الكيميائي للزيوت الأساسية باستخدام تقانات التفريق اللوني الغازي (Gas chromatography)، والتفريق اللوني الغازي - قياس الطيف الكتلي (Gas chromatography-Mass spectrometry) تباينا معنويا بين عشائر ال *Coridothymus capitatus* البريه. تراوحت نسبة زيت الثايمول من 0.03 إلى 0.57 %

وتراوحت نسبة زيت الكارفاكرول من 0.10 إلى 0.90 %. قدر معدل الإختلاف بين أزواج العشائر البريه بالإعتماد على الزيوت ب 10.68 كما أظهر تحليل المجموعات باستخدام طريقة المتوسطات الحسابية غير المرجحة للأزواج [Unweighted Pair Group Method with Arithmatic Mean (UPGMA)] وجود عشائر متخصصة لإنتاج الثايمول وأخرى للكارفاكرول. أظهرت الدراسة تباينا شهريا بالنسبه لمحتوى النبات من زيوت الثايمول والكارفاكرول.

أظهرت الدراسه إمكانية زراعة عشائر ال *Coridothymus capitatus* البريه المجموعه في هذه الدراسه حيث بلغ إنتاج المادة الجافة 3046 كغم/هكتار، هذا وتبين وجود إختلاف وراثي ظاهري وإختلاف في محتوى الزيوت الأساسيه للعشائر المزروعه في الحقل وتراوحت نسبة زيت الثايمول بين 0.01 إلى 0.90 % والكارفاكرول من 0.1 إلى 0.87 %. تم تقدير التباين الوراثي بين العشائر المزروعه على مستوى الحمض النووي الرايبوزي الخالي الأكسجين DNA باستخدام تقانة مكاثرة القطع المتباينة الناتجة عن التجزيء الإنزيمي (AFLP). تم رصد 235 قطعة وراثية (band) باستخدام عشرة ازواج متحدة من البادئات المنتخبه، ولقد أمكن تمييز خمسة مجاميع من ال *Coridothymus capitatu* باستخدام تحليل المجموعات (UPGMA) مما يشير إلى وجود تباين وراثي جيني بين عشائر النبات.

تشير نتائج الدراسة الى وجود تنوع وراثي بين عشائر نبات ال *Coridothymus capitatus* المجموعه من البيئات البرية في الأردن وكذلك بين عشائر ال *Thymbra spicata*. تم حفظ الماده الوراثيه لعشائر ال *Coridothymus capitatus* وال *Thymbra spicata* في البنك الوراثي وفي البنك الحقلي في المركز الوطني للبحث والإرشاد الزراعي. تمهد الدراسة الطريق لزراعة نبات الزعتر الفارسي *Coridothymus capitatus* على مستويات عالية وتجارية ولإنتاج الزيوت العطرية الاساسية.