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Chemical Composition and Antimicrobial Activity of Essential Oils, *thymus vulgaris* L. and *Mentha L. pulegium* Against the Major Post Harvest Diseases of Citrus in Morocco

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Abstract: ***Post-harvest diseases ,caused by Penicillium digitatum and Penicillium italicum, lead to considerable losses of citrus fruits. Several research studies have focused on biological properties and antimicrobial activity of essential oils (EO) extracted from the Medicinal and Aromatic Plants (MAP). The main objective of this paper is to study the antifungal effects of two essential oils of the family Lamiaceae (Thymus vulgaris and Mentha pulegium) on the major post harvest disease of citrus. The chemical compositions of EO were analyzed using a gas chromatography / mass spectrometry (GC-MS) showed that EO of T.vulgaris was characterized by the presence of Thymol (41.39 %) Gamma terpinene(22.25%) and p-cymene (15.59%) as the main chemical components. The EO of M.pulegium is formed mainly by Pulegone(78.98 %). The in vitro antifungal activity against both of P.digitatum and P.italicum was evaluated using the method of aromatogram. The results revealed that EO of M.pulegium is most active against both fungi.***

Keywords: Citrus, post harvest, *Penicillium digitatum*, *Penicillium italicum*, antifungal activity, *Thymus vulgaris*, *Mentha pulegium*, *in vitro*.

1. Introduction

Citrus plays a crucial socio-economic role in Morocco. Economically speaking, citrus exports are considered important sources of hard currency, retrieving between 2.5 and 3 billion dirhams a year. On the social level, citrus industry is one of the main source of income for about 10,000 family farmers , In addition, it provides ,either directly (orchards) or indirectly (packing station, ports and other) ,a total of 21 million days of work. Moreover, Citrus fruits provide supply and the continued operation of a large packaging and processing industry (Anonyme, 2011).

In citrus, as in any other agricultural production sector with trade, the final volume of the production is the most important parameter that determines the interest of the culture of a species or of a given variety, thus; improvement and mastery of production techniques, protection and recovery prove necessary for better profitability. Among the objectives to seek to revitalize the national citrus industry is the reduction of post-harvest losses which is less expensive and easier to achieve in a relatively short time since it is easier to increase the volume of exports better than preserving fruit quality after harvest to increase productivity.

Post-harvest losses can be caused by orchard to the packing station, local markets and during transit to the destination country. The sources of damage are diverse, but they are mainly caused by fungi such *as Penicillium digitatum*, *Penicillium italicum*, *Geotrichum citri-aurantii* and *Phytophthora spp.*

Globally, the first two pathogens may be responsible for about 90% of all losses caused by pathogenic fungi post harvest citrus (Holmes et al., 1994).

Chemical control is by far the most practiced means of struggle against all rot, and essentially those caused by *Penicillium spp*. Unfortunately, intensive and repeated use of fungicides in the protection of post-harvest citrus fruits favoured the development of resistant strains worldwide (Harding, 1972; Smoot and Brown, 1974). Similarly, the current trend of consumers to seek more natural products prompted the research, development and application of new natural products with antimicrobial activities in order to use them as alternative to synthetic products in the field of food industries.

Medicinal and aromatic plants (MAP) have been traditionally used for flavouring and extending the shelf life of foods (Wang et al., 2010). Most of their properties are due to essential oils (EO) produced by their secondary metabolism (Rashid et al., 2010). These oils are of growing interest for industries and scientific research because of their antioxidant activity, antibacterial and antifungal (Dung et al., 2008). Furthermore, most EO are classified as GRAS substances, which make them useful as natural preservatives

in food processing plants (Gachkar et al., 2007; Rasooli et al., 2008).

EO extracted from (MAP) to the family of Lamiaceae are known for their antimicrobial activities (Hussain et al., 2010). Thus, we chose two plants of this botanical family which have been used for HE Testerin vitro and in vivo antifungal effect on *Penicillium italicum* and *Penicillium digitatum.* These include *Thymus vulgaris* and *Mentha pulegium.*

It is in this context that our study looks at first extraction by steam distillation of water from ET from WFP: T. vulgaris and *M. pulegium* and identification of various components used in their chemical compositions to the study of the antifungal activity in vitro against *P. italicum* and *P. digitatum*.

2. Materials and Methods

2.1 Plant Material

The samples grown *T. vulgaris* were harvested on a farm in Ifran , while those of *M. pulegium* were harvested in the area of Azrou . The biomass thus obtained was subjected to extraction fresh for obtaining EO.

Essential oil extraction is performed by the drive technology to water vapour using a Clevenger type apparatus, according to the following protocol:

The plant is contacted with sterile distilled water in a flask. The whole is boiled, after the appearance of the first drop of distillate at the exit of the steam condensation tube; EO is then driven by the steam. It is then condensed through a condenser and recovered using a syringe. The time of this extraction is about four hours.

Both EO recovered were dried with anhydrous sodium sulphate and subsequently stored at a temperature of 4 ° C in amber glass vials sealed to preserve against the air and light.

2.2 Microorganisms Studied

The antifungal activity in vitro of EO studied was tested on two pure strains of *Penicillium spp.(Penicillium digitatum, penicillium italicum)* were chosen for their high frequency contaminate citrus and their pathogenicity. They are cultivated on the nutrient medium PDA (Potato Dextrose Agar) for7 days at 25 ° C in the dark.

2.3 Chemical characterization of EO:

The chemical characterization of EO *M.pulegium* and *T. vulgaris* was performed on a gas chromatograph Varian 3400 type, equipped with a polar capillary column BP - 5 (30m x 0.25 mm, film thickness 0.25 .mu.m), an FID detector set at a temperature of 250 ° C and fed with a mixture of H2 / Air and an injector set at 250 ° C. The carrier gas used was nitrogen with a flow rate of 2ml / min. The column temperature is 40 ° C (isothermal for 5 min) at 200 ° C (isotherm for 20 ° C) at 3 ° C / min.

The chemical identity of the various components was carried out by gas chromatography coupled to mass spectrometry (GC / MS); allowing a qualitative and quantitative determination of compounds thereof. The apparatus used is the following Gas chromatograph (Trace GC Ultra) coupled to a mass spectrometer (Polaris Q ion trap MS), ionization was effected by electron impact (70 eV). The data base used: NIST MS Search.

2.4 Microbiological Procedures

This effect was tested using aromatogram method that was described by Maychiew and Devahastin (2008) and Hussain and al.(2010):

20 ml PDA super cooled are cast in Petri dishes. After solidification of the culture medium , 200 µl of the fungal suspension under test (10^7 spores.ml^{-1}) were plated on the surface and left until at total desiccation under aseptic conditions and using sterile forceps, paper discs watman grade 1 of 6 mm in diameter (1disc / box) are sterilized and then deposit on the dried agar inoculated beforehand with the fungal suspensions then these discs are loaded increasing volumes of essential oil (10, 20,30,50,60,80µl) using a micropipette for each volumes, 3 repetitions were performed to minimize the risk of error.

The Petri dishes are kept at 4 ° C for 2 hours so that the essential oil may diffuse into the culture medium. For control, Petri plate had no essential oil. The Petri plates were incubated at 25 ° c for 5 to 14 days until the growth in the control Petri dishes reaches the edges.

3. Results and Discussion

3.1 Chemical Composition

Regarding the chromatographic analysis of HE GC / MS , we found that 10 compounds represent 92.91 % of EO *M. pulegium* with a single major component , while 24 compounds reprsent 99.05% of EO *T.vulgaris* with three major compounds

Table1: chemical composition of the EO of *T.vulgaris:*

Temps de Rétention (min)	Composé	Famille chimique	Valeur Indicative %
5.48	Methyl 2-methylbutanoate	Autre (oxygené)	0.18
11.50	Alpha thujène	Monoterpène hydrocarbonné	1.76
11.74	Alpha pinène	Monoterpène hydrocarbonné	0.85
12.32	Camphène	Monoterpène hydrocarbonné	0.40
13.51	Sabinène	Monoterpène hydrocarbonné	0.33
13.81	2-hexen-1-ol 2-ethyl	Autre (oxygené)	0.41

14.27	Béta pinène	Monoterpène hydrocarbonné	1.63
14.70	Alpha phellandrène	Monoterpène hydrocarbonné	0.28
15.24	Alpha terpinène	Monoterpène hydrocarbonné	3.25
15.61	p-cymène	Monoterpène hydrocarbonné	15.59
17.07	Gamma terpinène	Monoterpène hydrocarbonné	22.25
17.30	p-menth-2-en-1-ol	Monoterpène oxygéné	0.65
18.10	Terpinolène	Monoterpène hydrocarbonné	0.16
18.63	Linalool	Monoterpéne oxygéné	1.79
20.16	Camphre	Monoterpéne oxygéné	0.24
21.04	Bornéol	Monoterpéne oxygéné	0.65
21.48	4-terpinéol	Monoterpéne oxygéné	1.15
23.54	Thymol methyl ether	Autre (oxygené)	1.18
23.86	2-isopropyl-4-methylanisole	Autre (oxygené)	0.88
25.80	Thymol	Monoterpène oxygéné	41.39
26.00	Carvacrol	Monoterpène oxygéné	2.06
27.60	Isothymol	Monoterpène oxygéné	0.27
29.81	Caryophyllène	Sesquiterpène hydrocarboné	1.30
31.71	Germacrène D	Sesquiterpène hydrocarboné	0.40

From these results, the major constituents of thyme EO are Thymol (41.39 %), the Gamma terpinene (22.25 %) and p-cymene (15.59 %).

The comparison of our results with the literature shows considerable qualitative and quantitative differences in the chemical composition. Indeed, our results differ from those obtained by Özcan and chalchat (2004) who studied the thyme of EO composition of a sample of the same species in which thymol (46.2%) , alpha terpinene (14.1%) , p- cymene (9.9%) , the alpha-pinene (3.0%) and carvacrol (2.06 %) were found to be the majority.

This can be explained by the existence of different chemical families or chemotypes Det. vulgaris : thymol , carvacrol , linalool , thuyanol , alpha terpineol , geraniol , and paracymene

Table 2: chemical composition of the EO of *M.pulegium:*

Temps de Rétention (min)	*Composé*	*Famille chimique*	*Valeur Indicative %*
8.30	α-Pinène	Monoterpène hydrocarboné	0.17
9.74	Sabinene	Monoterpène hydrocarboné	0.11
11.65	Limonene	Monoterpène hydrocarboné	1.63
16.50	p-Menthone	Monoterpène hydrocarboné	5.24
17.35	Neomenthyl acetate	Autre (oxygéné)	0.53
19.45	Pulegone	Monoterpène oxygéné	78.98
21.47	3-p-Menthene	Monoterpène hydrocarboné	1.75
21.47	Carene	Monoterpène hydrocarboné	1.75
26.08	α-Caryophyllene	Sesquiterpène hydrocarboné	2.33
26.91	Germacrene-D	Sesquiterpène hydrocarboné	0.42

These results show that pulegone (78.98 %) is the major constituent of EO *M. pulegium*, which justifies the name mint pulegone.

The chemical composition obtained from the EO *M.pulegium*, approximates the results obtained by Lorenzo et al. (2002), who reported a chemical composition dominated by pulegone (73.4 %). Similarly, the majority of work already done in Morocco by Ouraini et al. (2007) and Chebli et al. (2003) showed that pulegone is the major component of *M. pulegium* , with respective concentrations of 44.27 % and 61.11 %

3.2 Antifungal Activity of Essential Oils:

The table 3 showed the result of antifungal activity of essential oils *M.pelegium* and *T.vulgaris .when we test the two oils*, we notice that the greater the volume of EO increases, the greater the diameters of the zones of inhibition increases, This is due to the presence of more active compounds with the increased volume of ET.

One study examined the antifungal effects of thyme EO (Rasooli et al., 2006), especially on the consequences of this oil on the ultra structure of the fungus Aspergillus's Niger . It has everything to first identify by electron microscopy, when A. Niger was exposed to ET, it caused irreversible damage to the cell membrane as well as on the organelles of the fungus (Barrel et al., 2007). So they inhibit spore germination, elongation mycelium, sporulation and toxin production in molds. This antifungal activity may be due to its major constituents: thymol, Gamma terpinene and p-cymene.

Belghazi et al. (2002) agrue that the oil of *M.plegium* whose majority compound is polygon, has a strong antifungal potency against *Penicillium* and *Mucor* .

Indeed, both oils are rich in monoterpenes , which are well known for their antimicrobial activities . According Derwich et al. (2010), the antimicrobial activity of monoterpenes is

explained by the presence of phenolic hydroxyl groups able to form hydrogen bonds with the active sites of the enzymes of the targeted cell.

Table 3: Halos inhibition (diameter in mm) caused by the two essential oils:

		Control	*10µl*	*20µl*	*30µL*	*50µl*	*60µl*	*80µL*
P.digitatum	*M.pulegium*	0	17 (+)	19 (+)	30,1 (+++)	40 (+++)	72 (+++)	100 (+++)
	T.vulgaris	0	16,8 (+)	21 (+)	29,7 (+)	39 (+++)	44 (+++)	88 (+++)
P.italicum	*M.pulegium*	0	16,3 (+)	21(+)	31 (+++)	44 (+++)	69 (+++)	100 (+++)
	T.vulgaris	0	19,8 (+)	24(+)	32(+++)	37(+++)	45(+++)	73,1(+++)

(+): sensitive
(+++): Exemely sensitive

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