Effect of Phenological Stages on Yield, Chemical Composition and Biological Properties of Essential Oil Obtained from *Thymus maroccanus* Ball

(Original Research Article)

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Abstract

The effects of phenological stages on the composition, antioxidant, insecticidal and allelopathic properties of essential oils (EOs) obtained from the aerial parts of *Thymus maroccanus* Ball**.** Were studied. The GC-MS analysis identified that analyzed EOs were dominated by carvacrol (63.70 - 68.19 %) followed by p-cymene (6.09 - 9.67 %) and γ-terpinene (3.67 - 8.49 %). When carvacrol and p-cymene increased progressively from the lowest values (63.70% and 6.09%) at the pre-flowering stage to reach the highest ones (68.19% and 9.67%) at post-flowering stage, γ-terpinene decreased gradually from the highest proportion (8.49%) to reach its lowest one (3.67%). EO from post-flowering aerial parts showed higher scavenging ability on DPPH radicals (% inhibition: 25.19 - 95.75 %; IC50 = 0.26 mg/mL), contrast, essential oil from pre-flowering stage exhibited the highest Fe3+ reducing power ability (Absorbance: 0.329 - 0.812; IC50 = 0.14 mg/mL). Insecticidal properties showed no substantial difference between *T. maroccanus* EO extracted at different growth stages. The LD50 and LD90 values were ranged from 0.15 to 0.17 µL/cm^2, and from 0.37 to 0.47 µL/cm^2, respectively in contact assays, as well as from 318.93 to 362.84 µL/L air, and from 725.08 to 739.12 µL/L air, respectively in fumigant

assays. The results indicated that allelopathic activity of EOsnot greatly affected by growth developmental stages.

Keywords: *Thymus maroccanus;* Morocco; Essential oils; Antioxidant; Allelopathic; Insecticidal.

1. Introduction

Thymus species are of great economic and industrial importance, worldwide investigated for their use in folk medicine, culinary, cosmetics and flavoring (Senatore, 1996). The genus *Thymus* comprising around 350 species of perennial, aromatic herbs and shrubs is predominantly found in the Mediterranean region, Asia, Southern Europe and North Africa (Maksimovic et al., 2008). In Morocco, this genus is represented by 21 species, 13 of which are endemic (Fennane et al., 2007). Of these endemic thyme species, *Thymus maroccanus* Ball locally known as *Za'tar* and/or *Za'itra*is a perennial shrub that is widespread in the arid and semi-arid parts of the Moroccan mountains, at altitudes ranging from 100 to 2750 m (Tahiri, 1996).

Leaves and flowering parts of this species have been used as powders, decoctions, or infusions to treat digestive disorders such as diarrhea, fever, coughs, wounds, and numerous infections (Bellakhdar, 2006). In addition, this species is widely used as culinary flavoring agents and its flavor and aroma are familiarized and widely accepted by consumers. *T. maroccanus* is one of the most important Moroccan thyme commercialized as a source of essential oils. Many phytochemical studies so far investigated the chemical composition of *T. maroccanus* essential oils (Jaafari et al., 2007; Alaoui Jamali et al., 2012; Fadli et al., 2012).

As known, the biosynthesis of the volatile compounds is influenced by various environmental factors namely the soil mineral fertilization (Piccaglia and Marotti, 1993; Alaouijamali et al., 2014), the light intensity (Li et al., 1996), the climate conditions (Russo et al., 2013), the culture site (Ben Farhat et al., 2009), the developmental stages (Jordan et al., 2013) and the genetic baggage (Skoula et al., 1999; Li et al., 2015). Plants produce a high diversity of volatile terpenes playing different ecological functions in relation with their interactions with the environment. Terpenoids may serve to attract and guide pollinators but can also act as indirect plant defences against herbivores, or may function as direct repellents or toxicants for herbivores and pathogens, and some have the potential to eliminate reactive oxygen species (Dudareva et al., 2004). Volatiles terpenes may also provide a competitive advantage to several angiosperm species as allelopathic agents (Croteau et al., 2000).The main goal of this work is to assess the essential oil chemical composition and the antioxidant, insecticidal and allelopathic activities of *T. maroccanus* obtained at different plant phenological growth stages (pre-flowering, full-flowering, post-flowering). This investigation will permit the determination of optimal harvesting time

with the desired aromatic and/or biological qualities, which may help increasing economic feasibility of the essential oil production and the obtention of new scientific data on the changes of *T. maroccanus* volatile secondary metabolites in the course of its phenological cycle.

2. Materials and Methods

2.1 Plant Material and Extraction of Essential Oils

T. maroccanus was collected at three different developmental stages, pre-flowering, full-flowering and post-flowering (Table1). The identification of the species was done by Prof. Abdelaziz Abbad from Cadi Ayyad University. The collected aerial parts were air-dried at room temperature (≈ 25 °C) in the shade and subjected to hydro-distillation, using a Clevenger-type apparatus for 3 h until total recovery of oil. The preparation of the EOs was performed three times (3 x 200 g) and oils were dried over anhydrous sodium sulfate, weighed and stored at 4 °C until use.

2.2 Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of Essential Oils

Gas Chromatography (GC/FID) analysis was performed using Hewlett Packard Gas Chromatographer (HP 6890) with electronic pressure control, equipped with HP-5MS capillary column (30 m x0.25 mm, film thickness 0.25µm), FID detector set at 250°C and fed with H2/Air mixture, and a *split-splitless* injector set at 250°C. The injection mode was split (1:50) and the injected volume was 1µl. Nitrogen was used as carrier gas with a flow rate of 1.7 ml/min. The column temperature was programmed from 50 to 200°C at heating rate of 4°C/min. The apparatus was controlled by "ChemStation" software computer system.

Gas chromatography/mass spectrometry (GC/MS) analysis was performed using Hewlett-Packard Gas Chromatographer (HP 6890) coupled with a mass spectrometer (HP 5973). Fragmentation was performed by electron impact at (70eV). The column used was HP-5MS (30 m x 0.25 mm, film thickness 0.25µm). The injection mode was split (1:50). The column temperature was programmed from 50 to 200°C at heating rate of 4°C/min. The components of the essential oils were identified based on their retention indices and their mass spectra by matching with reference spectra database (NIST 98 library).

2.3 Antioxidant Activity

2.3.1 DPPH Free Radical-Scavenging Activity

The antioxidant activity of *T. maroccanus* oils was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Sahin et al., 2004). Samples (EOs and control substance) were series diluted with methanol into 0.08 mg/ml to 1.22 mg/ml. Fifty microliters of various concentrations of the samples were added to 2 mL of a 60 µM methanolic solution of DPPH.

Absorbance measurements were read at 517 nm, after 20 min of incubation in the dark at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. Butylated hydroxytoluene (BHT) was used as positive controls. The percentage inhibition of the DPPH radical was calculated according to the formula:

$$\% \text{ Inhibition} = (Ab\text{-}Aa/Ab) \times 100$$

Where Ab is the absorption of the blank sample and Aa is the absorption of the tested oils. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. The test was carried out in triplicate and IC_{50} values were reported as means ± SD.

2.3.2 Reducing Power Determination

Reductive ability was investigated by the Fe^{+3} to Fe^{+2} transformations in the presence of the oils, using the method of Oyaizu (1986). Samples (EOs and control substance) were series diluted with methanol into 0.07 mg/ml to 1.07 mg/ml for EOs and from 10 µg/ml to 200 µg/ml for control substances. The different sample concentrations were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe\ (CN)_6]$(2.5 mL, 1%).

The mixture was then incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. Finally, the upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. The oil concentration providing 0.5 of absorbance (IC_{50}) was calculated by plotting absorbance at 700 nm against the corresponding oil concentration. BHT was used as reference compounds. The test was carried out in triplicate and IC_{50} values were reported as means ± SD.

2.4 Insecticidal Activity

2.4.1 Insect Cultures

Colonies of the red flour beetle, *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae), were maintained in the laboratory without exposure to any insecticide. They were reared in glass containers (16 cm diameter × 22 cm height) covered by a fine mesh cloth for ventilation. Each container contained a mixture of wheat flour, wheat germ, and yeast extract (13:6:1 w/w/w). The cultures were maintained in a growth chamber at 26 ± 1 °C, with a relative humidity (RH) of 70-85% and 16:8 h light: dark photoperiod.

Only young adults (7-14 days old) were used for the tests. All experimental procedures were conducted under environmental conditions identical to those of the cultures. In all bioassays, insects were considered dead when no leg or antennal movements were observed. The bioassays were designed to assess median lethal doses (LD_{50} and LD_{90} values) (doses that killed 50% and 90% of the exposed insects, respectively).

2.4.2 Contact Toxicity Bioassay

The contact insecticidal activity of EOs obtained from *T. maroccanus* against *T. castaneum* adults was determined by assessing the toxicity using filter paper discs (Whatman No. 1, 9 cm diameter). Oils were dissolved in acetone at concentrations of 0.08, 0.16, 0.24 and 0.31 µL/cm^2. Several preliminary tests were conducted to select the doses to be used for each EO.

One mL of each solution was dispensed on the surface of the filter paper that was then placed in a glass Petri dish. Control filter papers were treated with acetone only. After 10 min, once the solvent had been evaporated, 10 unsexed adults were deposited into each dish. Each EO and control treatment was replicated three times, repeating each assay twice. Mortality was recorded after 24 hours.

2.4.3 Fumigant Toxicity Bioassay

To assess fumigant toxicity of *T. maroccanus* EOs, 2 cm diameter filter papers (Whatman N°. 1) were impregnated with the different oil doses 10, 20, 30 and 40 µL. The impregnated filter papers were then attached to the screw caps of 60 mL Plexiglas bottles to give calculated fumigant concentrations of respectively 166.6, 333.3, 500 and 666.6 µL L^{-1} air. Caps were screwed tightly on the vials, each of which contained 10 unsexed adults. Each EO and control treatment was replicated three times, repeating each assay twice. Mortality was recorded after 24 h.

2.5 Allelopathic Activity

The inhibitory potential of the *T. maroccanus* oils on the seed germination of *Medicago sativa* L. was investigated. The essential oil was emulsified with Tween 80, at the ratio 1:1 (v/v) and dissolved in distilled water to obtain a concentration of 500, 250, 125 and 62.5 mg/L, while a solution of Tween 80 in water was used as a control. Subsequently, aliquot of 5 mL of each concentration was added to glass Petri dish (9 cm) with two layers of filter papers (Whatman N°. 1). Three replicates were prepared for each concentration of the oils, each comprising 20 seeds. The seeds were sterilized for 20 min in 1% NaClO before use. Petri dishes were sealed with a Parafilm® tape and kept at 27 ◦C in a dark growth chamber (Dudai et al., 1999). Germinated seeds (2 mm radicle length) were counted and removed daily. The percentage of germination was calculated after seven days of treatment.

2.6 Data Analysis

All results were expressed as the mean ± standard deviation of three independent experiments. The statistical analysis of essential oils yields was performed using IBM SPSS Statistics version 19. The statistically significant differences were determined by one-way ANOVA, followed by B de Tukey test at the 5% level of significance. Probit analysis (Finney, 1971) was conducted on the corrected mortality data (Abbott,1925) to estimate lethal doses (LD_{50} and LD_{90}) with their 95% confidence intervals by IBM SPSS Statistics version 19.

3. Results and Discussion

3.1 Effect of Phenological Growth Stages on T. maroccanus Essential Oil Yield

The variation of the essential oils yields of *T. maroccanus* at different phenological growth stages as calculated on the basis of dry matter weight are shown in Fig.1. The oil content increased significantly ($p < 0.05$) from pre-flowering stage (2.10 %) to full-flowering stage (2.73 %) and then decreased significantly ($p < 0.05$) in the course of post-flowering stage (1.47 %). These results are in agreement with what has been reported for the same species (Alaoui Jamali et al., 2013) and other *Thymus* species (Nejad Ebrahimi et al., 2008; Nouri and Esmaeilian, 2012). The high essential oil yield observed at full-flowering stage may be explained by the fact that plants may produce substantial amounts of essential oils in order to attract more pollinators (Pala-Paul et al., 2001), however, the low rate of biosynthesis of volatile compounds during the pre-flowering phase may be due to partial inactivation of enzymes necessary to the biosynthesis of certain compounds (Hamrouni Sellami et al., 2009). These finding clearly demonstrated that the harvesting time should be carefully selected to ensure

maximum yield of essential oil. So, for *T. maroccanus*, the full-flowering stage could be favoured.

3.2 Effect of Phenological Growth Stages on T. maroccanus Essential Oil Composition

The chemical composition of *T. maroccanus* essential oils at different phenological stages is listed in Table 2. Thirteen components were identified in the analyzed oil samples, amounting to 97.50 %, 96.87 % and 95.71 % of the total oil, detected at the pre-flowering, full-flowering and post-flowering stages, respectively. Regardless of growth stage, *T. maroccanus* essential oils were dominated by oxygenated monoterpene group (64.76 - 69.35 %) followed by monoterpene hydrocarbons (18.78 - 25.78 %). Analyzed essential oils were dominated by carvacrol (63.70 - 68.19 %) followed by p-cymene (6.09 - 9.67 %) and γ-terpinene (3.67 - 8.49 %). The high carvacrol content observed in *T. maroccanus* oils is in agreement with what has been previously reported for this species collected from other Moroccan regions (Saad et al., 2010; Alaoui Jamali et al., 2012; Fadli et al., 2012). Also, oil extracted from *T. maroccanus* collected in Asni region at Marrakech showed in addition of the major compound, carvacrol, p-cymene and γ-terpinene, considerable amounts of thymol and borneol which were not found in our samples (Jaafari et al., 2007).

Regarding the evolution of the major compounds of *T. maroccanus* oils through the entire vegetative cycle (Table 2), it can be stated that carvacrol and its corresponding precursors (γ-terpinene and p-cymene) quantities have shown a contrasting evolution. In fact, when carvacrol and p-cymene increased progressively from the lowest values (63.70 % and 6.09 %) at the pre-flowering stage to reach the highest ones (68.19 % and 9.67 %) at post-flowering phase, γ-terpinene decreased gradually from the highest proportion (8.49 %) to reach its lowest one (3.67 %). The inverse correlations observed between the production of carvacrol, p-cymene and γ-terpinene have previously been reported (Hudaib et al., 2002; Jordan et al., 2006) and has been considered to be directly and/or indirectly linked to the biosynthetic pathway of these monoterpenes (Saez, 1995).

3.3 Effect of Phenological Growth Stages on the Antioxidant Activity of T. maroccanus Essential Oil

The antioxidant activity of *T. maroccanus* essential oils extracted at pre-flowering, full-flowering and post-flowering stages was assessed by two complementary in vitro antioxidant assays: the DPPH assay and the reducing power capacity. The inhibition percentage, the absorbance at 700 nm and the concentrations that led to 50% inhibition (IC_{50}) are given in Tables 3 and 4. Lower IC_{50} values indicated higher antioxidant activity. The results showed that all essential oils tested expressed interesting

antioxidant potency. This activity increased steadily with increasing essential oils concentration.

For DPPH assay (Table 3), essential oil from post-flowering aerial parts showed higher scavenging ability on DPPH radicals (% inhibition: 25.19 - 95.75 %; IC_{50} = 0.26 mg/mL) when compared to those reported for essential oils of pre-flowering and full-flowering. In contrast, essential oil from pre-flowering stage exhibited the highest Fe^{3+} reducing power ability (Absorbance: 0.329 - 0.812; IC_{50} = 0.14 mg/mL) (Table 4). Nevertheless, the tested oils were less potent than the synthetic antioxidant BHT used as positive control (% inhibition: 96.04 - 97.96 %; IC_{50} = 0.004 mg/mL and Absorbance: 1.003 - 1.087; IC_{50} = 0.008 mg/mL, for DPPH and reducing power tests, respectively). The antioxidant action observed for *T. maroccanus* oil distilled at full-flowering stage was comparable to what has been reported for the same species collected from Ait- Ourir region (Alaoui Jamali et al., 2012; El Bouzidi et al., 2013).

Based on the above results, it seems that harvest time have a significant effect on the antioxidant activity of *T. maroccanus* essential oils. The chemical composition of our plant (Table 2) oils showed that the antioxidant activity is apparently related to the content of carvacrol (63.70 - 68.19 %), which is well known for its high antioxidant action (Ruberto and Baratta, 2000; Kulisic et al., 2004; Safaei-Ghomi et al., 2009). Besides, the fact that oil distilled at pre-flowering stage, which had lower level of carvacrol, exhibited the strongest Fe^{3+} reducing power ability, suggest that this oxygenated monoterpene is not the only compound responsible for the observed activity. In fact, the higher reducing power potency of the oil at this stage can be attributed to the presence of γ-terpinene in higher content than for oils extracted at other stages. This compound is characterized by strong antioxidant activity as was previously reported by many authors (Ruberto and Baratta, 2000; Tepe et al., 2005).

3.4 Effect of Phenological Growth Stages on the Insecticidal Activity of T. maroccanus Essential Oil

The toxicities of essential oils isolated from the aerial parts of *T. maroccanus* at pre-flowering, full-flowering and post-flowering stages, were tested on adults of the important stored product insect pest *T. castaneum*, using contact and fumigant bioassays. The percentage of mortality (%) and lethal doses (LD_{50} and LD_{90} values) obtained in both assays are given in Tables 5, 6 and 7, respectively. All essentials oils tested were toxic against adults of *T. castaneum*. The mortality increased with increasing of the essential oil doses applied. The mortality percentage values were ranged from16.67 to 96.67 % and from 10.00 to 96.67 %, for contact and fumigant toxicity assays, respectively (Table 5). On the basis of the LD_{50} and LD_{90} values (Tables 6 and 7), no substantial difference was registered between *T. maroccanus* essential oils extracted at different growth stages, since no overlap between the 95%

confidence limits was observed. The LD_{50} and LD_{90} values were ranged from 0.15 to 0.17 µL/cm^2 and from 0.37 to 0.47 µL/cm^2, respectively, in contact assays as well as from 318.93 to 362.84 µL/L air and from 725.08 to 739.12 µL/L air, respectively, in fumigant assays. The values obtained for contact toxicity assay were comparable with those reported for the same species collected at flowering stage from Ait-ourir region against adults of *T. castaneum* (Alaoui Jamali et al., 2016). As far as our literature survey could ascertain, this is the first study to provide data regarding the effect of phenological growth stages on the insecticidal potency of *T. maroccanus* essential oil. The above results showed that the tested essential oils presented interesting toxicity effects against adults of *T. castaneum*. The insecticidal potency of *T. maroccanus* essential oils studied is apparently related to the high content of carvacrol and the presence of p-cymene and γ-terpinene. In fact, it was previously shown that the phenolic monoterpene carvacrol, present a significant toxicity against a panel of agricultural and stored-product insects (Shaaya et al., 1990; Regnault-Roger & Hamraoui, 1995; Gonzalez et al., 2002). Also, previous reports have showed that p-cymene and γ-terpinene were toxic against several stored-product insects (Kordali et al., 2008; Kim et al., 2010). In general, the insecticidal activity of essential oils could not be attributed only to their major compounds; other minor compounds may give rise to the insecticidal effect. Since the essential oils are very complex mixtures, the synergistic and antagonistic effect of one compound in minor and/or major percentages in the mixture should also be taken into account (Isman et al., 2001; Kordali et al., 2008).

3.5 Effect of Phenological Growth Stages on the Allelopathic Activity of T. maroccanus Essential

The allelopathic potential of the *T. maroccanus* essential oil obtained at different phenological stages on the germination of *M. sativa* seven days after treatment is illustrated in Figures2 and 3. The results showed that *T. maroccanus* essential oils exhibit interesting allelopathic effect against *M. sativa* seeds. This effect was concentration-dependent and increased with increasing amount of the essential oils. Fig. 2 shows the cumulative germination of the treatments at 1-day intervals compared with the control. The essential oils tested registered less germination percentage than control at all concentrations (0.5 mg/mL to 0.06 mg/mL). At the highest concentration (0.5 mg/mL), the germination of *M. sativa* was completely inhibited. At the seventh day of germination, the highest germination percentage was recorded in control. Treatments with *T. maroccanus* essential oils obtained at different phenological growth stages induced germination percentages varied between 3.33 % and 86.67 %. Data presented in Fig. 3 showed clearly that the allelopathic activity of *T. maroccanus* essential oils was not greatly affected by growth developmental stages. Although, essential oil extracted at pre-flowering stage showed to a lesser degree the lowest

germination percentage (0 - 85 %) compared to the remaining essential oils (0 - 86.67 %).

These findings demonstrated that *T. maroccanus* essential oils were efficient to inhibit seed germination of *M. sativa* seeds. To our knowledge, this is the first report concerning the allelopathic activity of *T. maroccanus* essential oil. The allelopathic activity of the essential oils of several *Thymus* species has been reported previously (Angelini et al., 2003; Arminanteet al., 2006), and this activity has been attributed mainly to their content of monoterpenes especially oxygenated one (Abrahim et al., 2000; Kordali et al., 2007). The high content of carvacrol, an oxygenated monoterpene, in *T. maroccanus* oils therefore likely explains their important allelopathic activity. In fact, it has been previously shown that this compound possesses an inhibition effect on germination of several species (Vokou et al., 2003; Azirak and karaman, 2008; Kordali et al., 2008). In general, the induction of oxidative stress, the inhibition of water uptake, the restriction on reserve mobilization by the alteration in the activities of gibberellic acid and α-amylase are main reported reasons of essential oils and their allelochemical constituents in the prevention of seed germination (Golisz et al., 2008; Das et al., 2012; Poonpaiboonpipat et al., 2013).

4. Conclusion

This work presents the variation of the yield, chemical composition, antioxidant, insecticidal and allelopathic effects of *T. maroccanus* essential oil in the course of its growth cycle. The results revealed that the highest yield in the essential oil was recorded for the aerial part collected at full-flowering stage. Thus, the full-flowering stage could be considered as the favorable stage for important essential oil production.

Also, slight quantitative variation was noted in essential oils chemical composition, especially for the major compounds carvacrol, p-cymene and γ-terpinene. Among the tested oils, the highest antioxidant activity was observed for oil obtained at pre-flowering and full-flowering stages.

This result suggested that *T. maroccanus* oil can be used as natural preservative ingredients in the food industry and as a natural additive in cosmetic and pharmaceutical industries. In addition, *T. maroccanus* oils at all growth stages, showed interesting insecticidal and allelopathic effects. Therefore, this endemic plant is potentially an inexpensive source of natural insecticidal and herbicide substances for insect and weed crops control. This may be also present a promising alternative to the harmful synthetics of the chemical products.

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Tables and Figures

Table1. Collection site and geographical coordinates of the studied *T. maroccanus* Ball.

Samples	Collection site	Latitude/Longitude	Altitude (m)	Harvesting time
Pre-Flowering				February (2015)
Full-Flowering	Ourika	31°15'N/07°42'W	1345	Mai (2015)
Post-flowering				July (2015)

Table 2. Chemical composition of essential oils obtained from aerial parts of *T. maroccanus* at pre-flowering, full-flowering and post-flowering stages.

Compounds[a]	RI[b]	Pre-flowering	Full-flowering	Post-flowering
β-Thujene	938	1.26	1.34	0.74
α-Pinene	950	5.53	3.28	2.00
α-Myrcene	986	1.86	1.62	0.97
α-Terpinene	1019	1.40	1.27	1.14
p-Cymene	1023	**6.09**	**6.39**	**9.67**
Limonene	1031	1.15	0.89	0.59
γ-Terpinene	1062	**8.49**	**7.74**	**3.67**
Linalool	1072	1.06	0.64	1.16
Carvacrol	1299	**63.70**	**66.82**	**68.19**
Caryophyllene	1419	1.78	2.05	2.35
Aromadendrene	1440	1.77	1.34	1.64
α-Bisabolene	1533	3.13	3.19	3.06
(-)-Spathulenol	1576	0.28	0.30	0.53
Monoterpenehydrocarbons		25.78	22.53	18.78
Oxygenatedmonoterpenes		64.76	67.46	69.35
Sesquiterpenehydrocarbons		6.68	6.58	7.05
Oxygenatedsesquiterpenes		0.28	0.30	0.53
Total (%)		97.50	96.87	95.71

[a] Compounds listed in order of elution; [b] Retention indices

Table 3. DPPH scavenging activity (%) and IC_{50} values of *Thymus maroccanus* essential oils and BHT.

Concentrations (mg/mL)	Essential oils			Standard antioxidant
	Pre-flowering	Full-flowering	Post-flowering	BHT
0.08	22.31 ± 0.76[a]	20.81 ± 1.85	25.19 ± 0.33	96.04 ± 0.26
0.15	30.61 ± 1.91	29.61 ± 2.53	33.32 ± 0.38	96.83 ± 0.19
0.30	43.95 ± 0.66	41.66 ± 1.70	48.29 ± 2.23	97.37 ± 0.25
0.61	58.13 ± 2.27	60.30 ± 1.88	69.18 ± 0.91	97.62 ± 0.13
1.22	70.77 ± 1.44	75.52 ± 0.83	95.75 ± 0.13	97.96 ± 0.19
IC_{50} (mg/mL)	0.40 ± 0.03	0.38 ± 0.01	0.26 ± 0.01	0.004 ± 0.080

[a)] Percentage inhibition (%); Values are given as mean ±SD (n = 3).

Table 4. Absorbance and IC_{50} values of *Thymus maroccanus* essential oils and BHT.

Concentrations (mg/mL)	Essential oils			Standard antioxidant
	Pre-flowering	Full-flowering	Post-flowering	BHT
0.07	0.329 ± 0.014[a]	0.295 ± 0.003	0.244 ± 0.003	1.003 ± 0.002
0.13	0.479 ± 0.008	0.453 ± 0.003	0.406 ± 0.005	1.014 ± 0.005
0.27	0.693 ± 0.012	0.631 ± 0.011	0.701 ± 0.010	1.047 ± 0.002
0.53	0.746 ± 0.014	0.696 ± 0.011	0.932 ± 0.006	1.063 ± 0.002
1.07	0.812 ± 0.012	0.748 ± 0.012	0.964 ± 0.005	1.087 ± 0.003
IC_{50} (mg/mL)	0.14 ± 0.00	0.18 ± 0.00	0.16 ± 0.00	0.008 ± 0.100

[a)] Absorbance at 700 nm; Values are given as mean ± SD (n = 3).

Table 5. The percentage of mortality (%) of *T. maroccanus* essential oils against the adults of *T. castaneum* in contact and fumigant toxicity bioassays.

Bioassays		**Mean mortality (%)**		
Contact toxicity	**Concentrations ($\mu L/cm^2$)**	**Pre-flowering**	**Full-flowering**	**Post-flowering**
	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	0.08	16.67 ± 3.17	23.33 ± 2.77	20.00 ± 0.00
	0.16	46.67 ± 5.77	50.00 ± 2.44	36.67 ± 3.28
	0.24	56.67 ± 2.88	70.00 ± 1.32	63.33 ± 4.76
	0.31	96.67 ± 3.47	83.33 ± 4.28	83.33 ± 1.42
Fumigant toxicity	**Concentrations (µL/Lair)**			
	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	166.67	20.00 ± 0.00	13.33 ± 1.45	10.00 ± 4.00
	333.33	43.33 ± 2.56	36.67 ± 2.48	30.00 ± 0.00
	500.00	76.67 ± 4.80	70.00 ± 0.00	73.33 ± 3.12
	666.67	90.00 ± 1.00	93.33 ± 1.43	96.67 ± 2.62

Table 6. Contact toxicity against *Tribolium castaneum* (LD_{50} and LD_{90} values) of the essential oils isolated from the aerial parts of *T. maroccanus* collected at pre-flowering, full-flowering and post-flowering stages.

Essential oils	LD_{50}[a] (95% CL)[b]	LD_{90} (95% CL)	Slope ± SE	Chi square (χ^2)	df
Pre-flowering	0.16 (0.11-0.22)	0.37 (0.26-1.19)	3.57±1.08	2.37	2
Full-flowering	0.15 (0.08-0.22)	0.43 (0.27-3.89)	2.77±0.98	0.06	2
Post-flowering	0.17 (0.11-0.26)	0.47 (0.29-3.81)	2.93±1.01	0.63	2

LD: lethal dose. [c] Concentration: $\mu L/cm^2$. [b] Confidence limits.

Table 7. Fumigation toxicity against *Tribolium castaneum* (LD_{50} and LD_{90} values) of the essential oils isolated from the aerial parts of *T. maroccanus* collected at pre-flowering, full-flowering and post-flowering stages.

Essential oils	LD_{50}[a] (95% CL)[b]	LD_{90} (95% CL)	Slope ± SE	Chi square (χ^2)	df
Pre-flowering	318.93 (205.86-433.00)	739.12 (517.65-2247.85)	3.51±1.05	0.55	2
Full-flowering	353.12 (252.64-465.91)	725.08 (530.24-1731.69)	4.10±1.15	0.96	2
Post-flowering	362.84 (272.55-461.85)	683.03 (520.81-1354.80)	4.88±1.28	1.71	2

LD: lethal dose. [c] Concentration: µL/Lair. [b] Confidence limits.

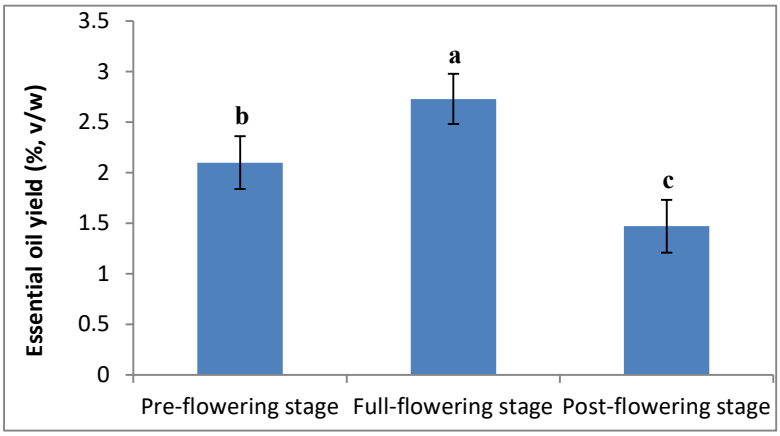
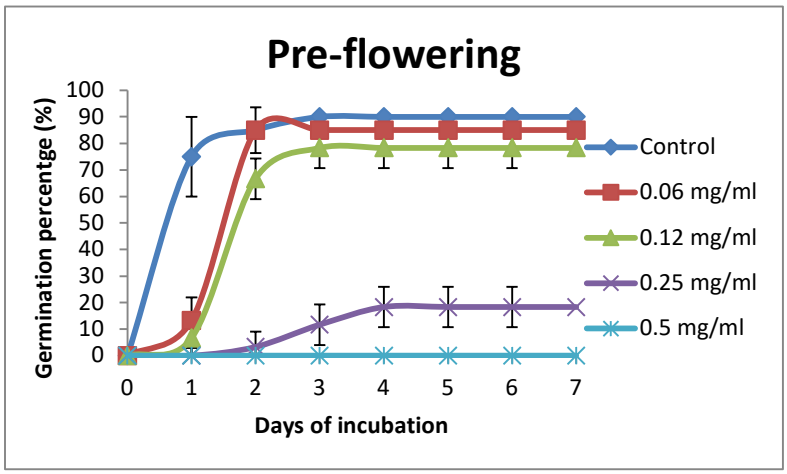


Figure.1. *T.*maroccanusessential oil yields at different growth stages. Values (means of three replicates) with different letters are significantly different at $p < 0.05$ (B de Tukey test).



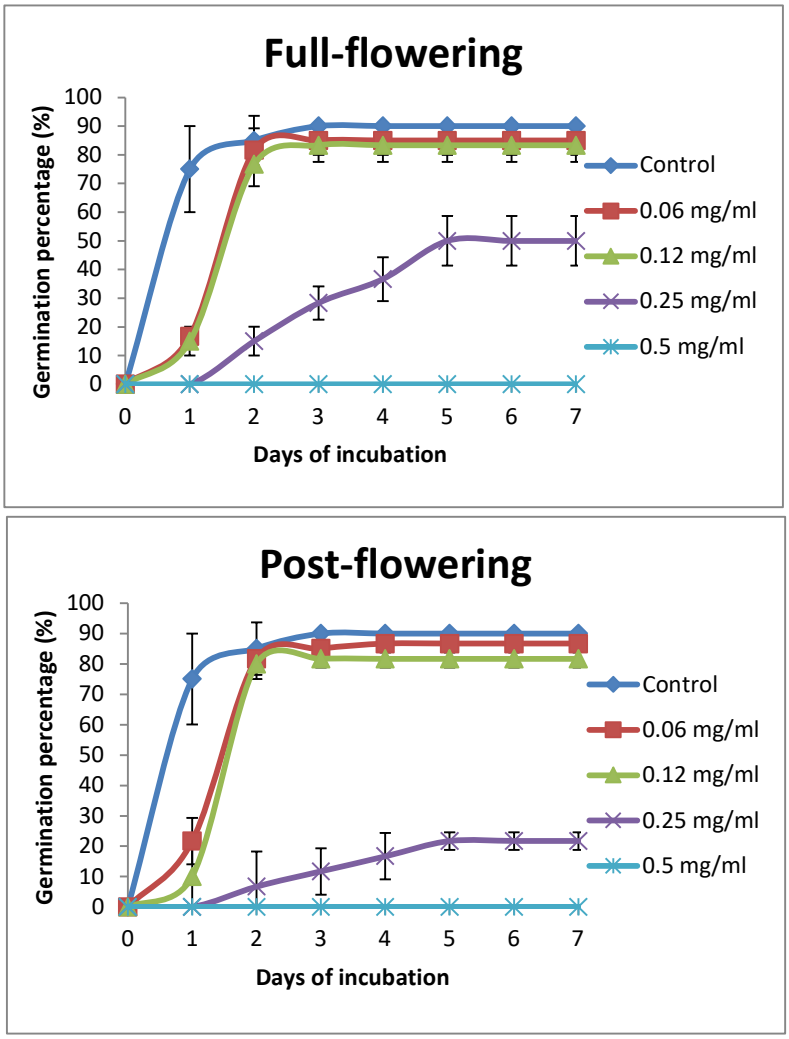


Figure 2. Cumulative germination of *M. sativa* seeds treated with *T. Moroccans* essential oils during the three different phenological stages. Data represent means of three replicates represented by standard error.

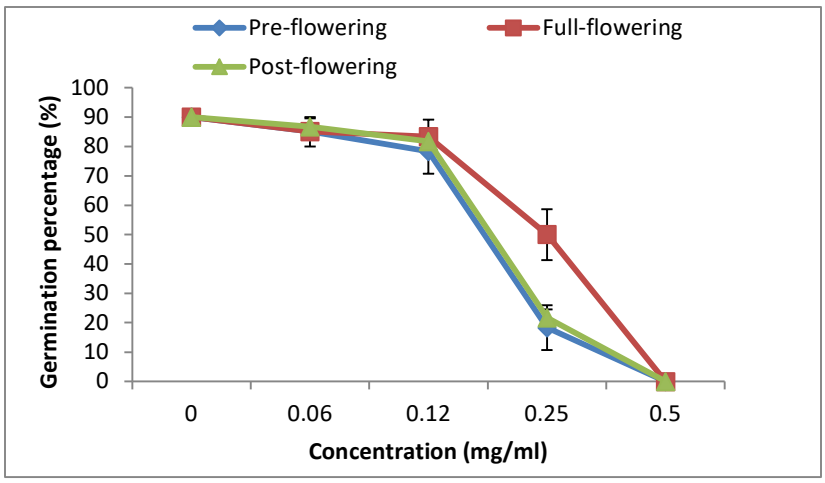


Figure 3. Final germination of *M. sativa* seeds treated with *T. maroccanus* essential oils after 7 days of the experience. Data represent means of three replicates represented by standard error.