

# Insecticidal Activity Assessment of *Thymus capitatus* Essential Oils in Combination with Natural Abrasives against *Myzus persicae*

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## ABSTRACT

**Khaled, W., Ben Fekih, I., Chaieb, I., Souissi, R., Harbaoui, I., and Boukhris-Bouhachem, S. 2017. Insecticidal activity assessment of *Thymus capitatus* essential oils in combination with natural abrasives against *Myzus persicae*. Tunisian Journal of Plant protection 12: 49-59.**

This study was performed to evaluate the insecticidal activity of *Thymus capitatus* essential oils and two natural abrasives to control *Myzus persicae*. The in vitro application of thyme essential oils showed a significant toxic effect by fumigation as well as by spraying. The LC<sub>50</sub> for both application methods recorded after 24 h were about 20.01 and 13.26 µl/l air, respectively. In addition, in vivo experiment based on bioinsecticide formulation (LC<sub>50</sub> thyme essential oils + kaolin or diatomaceous earth) were carried out. The mortality rates registered after 24 h were 74.19 and 97.84% for the combination with kaolin and diatomaceous earth, respectively. Meanwhile, emulsions with 1 µl of these oils have been tested on the target aphid. This treatment has lead after 24 h to a mortality rate of 55.55%. The mechanical effect of both abrasive powders has been highlighted through dehydration, shrinkage and deformation of the aphid cuticle. Interestingly, the combination of diatomaceous earth with the *T. capitatus* essential oils was significantly the most effective to control aphid populations.

*Keywords:* Diatomaceous earth, essential oils, kaolin, *Myzus persicae*, *Thymus capitatus*

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Since 1940's, chemical pesticides have been excessively used within the green revolution concept for the development of an intensive agriculture

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(Davies 2003). Previous studies have shown that the intensive use of chemical insecticides has created several negative effects on non-targeted organisms and environment, which may affect human health (Davies 2003; Dich et al. 1997). In addition, resistance phenomena among pest insects have been severely increased to different chemical groups (Charaabi et al. 2008; 2016; Robert 2013). The use of

essential oils as biopesticides has shown to hold a promising future as one of the alternatives to chemical insecticides (Pavela et al. 2010). Indeed, previous reports have highlighted the potential of the essential oils and their derivatives as agents to control pest insects under the Integrated Pest Management (IPM) concept (Bachrouch et al. 2010; Chaieb 2011; Mediouni Ben Jemâa et al. 2012; Regnault-Roger 1997). The importance of the essential oil compounds was also shown by their ability to be more promptly degraded in the environment than synthetic chemicals (Kalita et al. 2013). Fumigant and contact toxic potential of essential oils have been reported against different biological stages of pest insects (adults, larvae and eggs) (Sahaf and Moharramipour 2008). In addition to their anti-feeding and repelling activities (Hernández-Lambraño et al. 2014), essential oils may inhibit reproduction, affect larvae development and adult emergence (Ayvaz et al. 2010). Despite the noteworthy potential of essential oils, their applications as biopesticides remain challenging in practice due to their volatility, poor water solubility, and aptitude for oxidation (Keita et al. 2001). Beside essential oils, mineral powders were used successfully to control insect populations (Nguemtchouin et al. 2010). Pure kaolin (i.e. clay powder) is recognized by some traditional societies for its ability to control insects. Previous study showed the important insecticidal activity of the combined effect of these flavored powders with essential oils against *Callosbruchus maculatus* (Awojide and Fayemiwo 2010).

The green peach aphid, *Myzus persicae* (Hemiptera, Aphididae), is one of the most economically important aphids infesting a wide range of host plants (Charaabi et al. 2016). *M. persicae*

is a cosmopolitan species causing direct damage as phloem feeders and indirect damage as vectors of several viruses (Boukhris-Bouhachem et al. 2007). The aim of this work is to investigate the insecticidal effects of *Thymus capitatus* essential oils against *M. persicae*. Moreover, this study inspects the biocidal activity of two natural abrasives (kaolin and diatomaceous earth) in combination with thyme essential oils.

## MATERIALS AND METHODS

### Plant material and essential oil extraction.

The spontaneous aromatic medicinal plant, *Thymus capitatus* (Lamiaceae), was used as plant material for the extraction of the essential oils. *T. capitatus* was gathered at the vegetative stage from the Kef region (N: 36°11', E: 8°42'), in the north-west of Tunisia. Sampling was performed in June 2014. Freshly harvested leaves were left in the shade in a dry and aerated place for 4 weeks. The dried materials were grounded to a fine powder used for the extraction of essential oils. Then, 100 g dried-leaves were placed with 1000 ml of distilled water in a round bottom flask and submitted for hydrodistillation for 2 to 3 h in Clevenger-type apparatus. The essential oils were driven by water vapor. This gas mixture was condensed by the condenser and separated into two liquid phases; the higher organic phase was the essential oils, containing the majority of the odorous compounds and the lower aqueous phase, containing aromatic water. The essential oils samples were then stored in sterile glass bottles at 4°C. Essential oils yield was calculated using the following formula:  $Y = (wo/wp) \times 100$ , where Y: Essential oils yield, wo: Weight of essential oils, and wp: Dry weight of plant (Akrouit 2004).

### **Chemical composition.**

The chemical composition of the essential oils was analyzed by gas chromatography/mass spectrometry (GC/MS). The chromatographic analysis was carried out with a Hewlett-Packard type gas chromatograph (HP 6890) coupled to a mass spectrometer (HP 5973). The fragmentation was carried out by electronic impact at 70eV, by using an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The temperature of the column was set from 50 to 250°C at a rate of 4°C/min. The injected volume was 0.2 µl with a split ratio of 1:70. The device was connected to a computer system managing a NIST 98 mass spectrum library and piloted by "HP ChemStation" software to monitor the evolution of chromatographic analyses.

### ***M. persicae* rearing.**

Sampling of *M. persicae* was performed from potato crops in the Cap Bon region: site of Soliman (N: 36°42', E: 10°29'). Rearing of *M. persicae* was conducted on tobacco plants in a controlled growth chamber at 23°C, 60% RH and LD 16:8 h photoperiod. Three individuals were collected to be placed on tobacco plants with height growth of 5 cm. The plants were placed in hermetic cages under the same condition as described previously.

### **In vitro bioassays.**

**Fumigant toxicity.** Ten adults of *M. persicae* were placed on a peach leaf in a Petri plate and treated with different concentrations of thyme essential oils: 16.66, 33.33, and 66.66 µl/l air. Filter paper discs (1 cm<sup>2</sup>) were impregnated with the appropriate oil concentration using a micropipette and glued to the lid. The Petri plate was hermetically closed and placed under the same conditions

described above for mass rearing. For each concentration, the assay was repeated 5 times. Control insects were maintained under the same conditions without any essential oil. The mortality rate was determined 24 h post-treatment. Aphid was considered dead when it was completely immobilized under binocular.

**Contact toxicity.** As for the fumigant assay, 10 adults of *M. persicae* were placed on a peach leaf in a Petri plate of 60 ml of volume to be treated with the same concentrations. In this experiment, the aphids are sprayed with a solution of 100 µl of Tween (1%) combined with the essential oils at the appropriate concentration. Under the same conditions, control insects were treated only with Tween (1%). Each treatment was replicated five times and the mortality rate was determined 24 h post-treatment.

### **In vivo essential oil formulations.**

This experiment was performed using 10 adults of *M. persicae* infesting pepper plants. Both fumigant and spraying assays were conducted. For fumigant toxicity, 6 different treatments based on the preparation of flavored powder with thyme essential oils were used:

- (1) 0.1 g of kaolin: the powder was sprinkled on the infested plants. The plants were then placed in hermetically sealed bottles (1 liter volume).
- (2) 0.1 g of diatomaceous earth: this treatment was carried out under the same conditions as the first treatment.
- (3) 0.1 g of kaolin + LC<sub>50</sub> of thyme essential oils: the LC<sub>50</sub> of essential oils after 24 h was revealed by the in vitro fumigation test. The two products were mixed thoroughly by a vortex. The obtained powder was homogenized and sprinkled on the leaves of plants infested

by *M. persicae*. Afterwards, treated plants were placed in hermetically sealed bottles (1 liter volume).

(4) 0.1 g of diatomaceous earth + LC<sub>50</sub> of thyme essential oils: this treatment was carried out by the same methodology of the third treatment.

(5) Imidacloprid used at the homologated dose (35 ml/hl) as a reference insecticide: the purpose of this treatment was to compare the mortality rates observed following the application of a chemical insecticide to those of the abrasive + essential oil combinations.

(6) Under the same conditions, untreated plants were used as control.

Regarding the contact toxicity assay, 3 different treatments were conducted based on the preparation of an emulsion of thyme essential oils:

(1) Solution of Tween 1% + LC<sub>50</sub> of thyme essential oils: the LC<sub>50</sub> was revealed after 24 h by the in vitro contact test. Each plant infested with 10 aphids was sprayed with 1 ml of this product.

(2) Imidacloprid was used at the homologated dose as a reference insecticide.

(3) Controls were treated with 1 ml of a Tween solution (1%).

### Microscopic investigation.

Investigation of the abrasive treatments effects was performed on 80 adults of *M. persicae* infesting tobacco leaves. The different treatments were applied by sprinkling 0.1 g of kaolin or diatomaceous earth powder on *M. persicae* placed in Petri plates. Control includes the same number of aphids with no treatment. Aphid mortality was recorded 30 min, 1 h, 90 min, 2 h, 4 h, 24 h, and 48 h post-treatment. From each treatment, 10 treated aphids were selected and stored directly in alcohol (70°) to be mounted in the Canada balsam according to Remaudière (1992). Observations were

made under an "Olympus" optical microscope at 4× magnification.

### Statistical analyses.

*M. persicae* mortality was corrected using Abbott formula (Abbott 1925). Data were analyzed using one-way ANOVA using Statistical Package for Social Sciences (version 20.0; SPSS). Means were separated using the Duncan Multiple Range test at  $P \leq 0.05$ . LC<sub>50</sub> and LC<sub>90</sub> at 24 h were calculated by probit analysis according to Finney's equation (Finney 1971).

## RESULTS

### Essential oils yield and chemical composition.

Essential oils yield recorded from *T. capitatus* leaves was 2.03%. GC/MS analysis revealed the presence of 21 compounds (Table 1). Carvacrol was the major compound with 84.13%, followed by *p*-cymene (4.36%), caryophyllene (2.6%), gamma-terpinene (1.44%),  $\beta$ -caryophyllene (1.32%), and linalol (1.29%). Therefore, *T. capitatus* essential oils contained monoterpenes as well as sesquiterpene compounds.

### In vitro insecticidal activity.

The fumigant activity was shown to be correlated to the concentrations of thyme essential oils. Mortality rates of 58.14 and 62.79% were obtained after 24 h of exposure using the concentrations 16.66 and 33.33  $\mu$ l/l air, respectively. The analysis of the variance shows no significant difference between these two concentrations. However, a significant effect was recorded with the highest concentration used. Indeed, the maximum mortality (83.72%) was recorded with the concentration of 66.66  $\mu$ l/l air.

Concerning the in vitro spraying test, mortality rates recorded after 24 h of exposure at the concentrations 16.66 and

33.33 µl/l air were estimated at 72.09 and 76.74%, respectively. The highest

mortality rate (97.67%) was with the concentration of 66.66 µl/l air (Table 2).

**Table 1.** Chemical composition (%) of *Thymus capitatus* essential oils

N°	RT (min)	Compound	Percentage (%)
1	5.49	$\alpha$ -pinene	0.18
2	6.52	$\beta$ -pinene	0.18
3	6.8	$\beta$ -myrcene	0.19
4	6.93	3-octanol	0.12
5	7.55	$\alpha$ -terpinene	0.35
6	7.78	<i>p</i> -cymene	4.36
7	7.9	$\beta$ -phellandrene	0.06
8	8.76	gamma terpinene	1.44
9	9.04	(Z)-sabinene hydrate	0.11
10	10.02	Linalol	1.29
11	12.21	Borneol	0.72
12	12.56	4-carvomenthenol	0.86
13	13.02	$\beta$ -fenchyl alcohol	0.12
14	16.25	o-cymen-5-ol	0.14
15	16.48	2-isopropyl-5-methylphenol	0.39
16	16.95	Carvacrol	84.13
17	17.26	5-isopropyl-2-methylphenol	0.26
18	18.87	2-isopropyl-5-methylphenyl acetate	0.93
19	20.4	Caryophyllene	2.6
20	25.21	Spathulenol	0.25
21	25.34	(-)- $\beta$ -caryophyllene epoxide	1.32
<b>Total</b>	-	-	100

**Table 2.** Corrected mortality rates (%) of *Myzus persicae*, LC<sub>50</sub> and LC<sub>90</sub> values recorded 24 h post-treatment with *Thymus capitatus* essential oils

Bioassay	Concentration (µl/l air)	Mortality (%)	LC <sub>50</sub> (µl/l air)	LC <sub>90</sub> (µl/l air)
Fumigation	16.66	58.14±1.81a	20.01	64.81
	33.33	62.79±0.83a		
	66.66	83.72±0.54b		
Spraying	16.66	72.09±0.54a	13.26	39.55
	33.33	76.74±1.41a		
	66.66	97.67±0.44b		

For each essay, values followed by similar letters are not significantly different according to Duncan' Multiple Range test (at  $P \leq 0.05$ ).

### In vivo essential oil formulations (powder and emulsion).

The treatment with the abrasive kaolin or diatomaceous earth against *M. persicae* has led to a mortality rate of 26.88 and 19.35%, respectively. The combination of thyme essential oils with these abrasives induced a mortality rate of 74.19 and 97.84% for kaolin and diatomaceous earth, respectively. A

mortality rate of 95.69% was noted after treatment with chemical insecticide (Fig. 1-A). The treatment with Tween (1%) combined to thyme essential oils led to a mortality rate of 55.55%. However, the effect of this bio-treatment was significantly lower than the chemical insecticide which causes more than 90% mortality (Fig. 1-B).

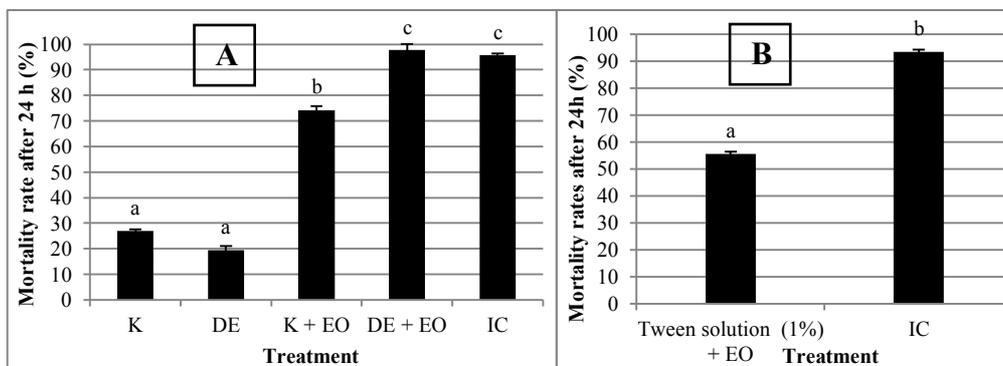


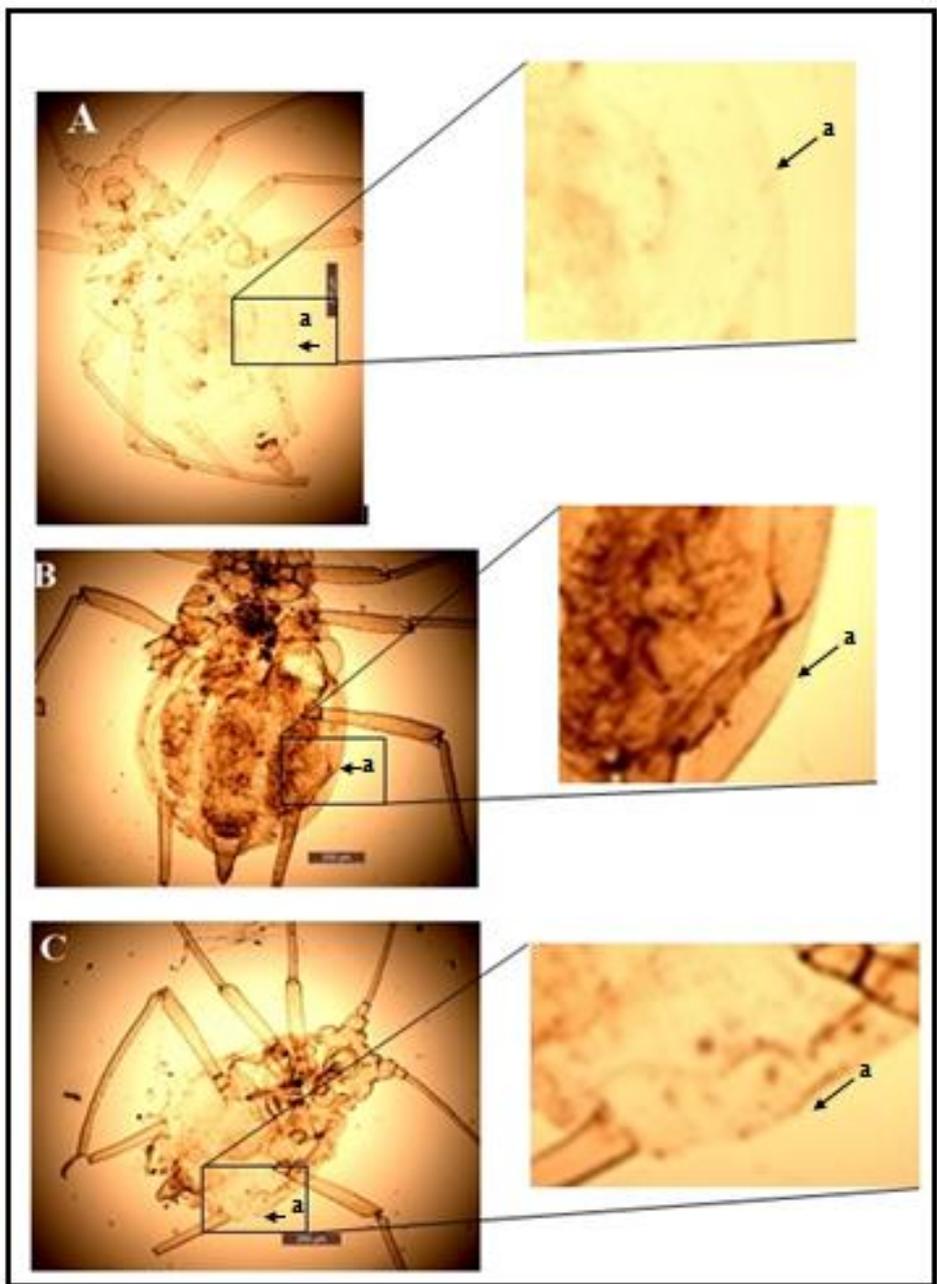
Fig. 1. Effect of *Thymus capitatus* essential oil (EO) on *Myzys persicae* mortality in vivo using fumigation assay (A) and spraying assay (B). K: kaolin; DE: diatomaceous earth; IC: Imidacloprid. Values with the same letter are not significantly different according to Duncan's Multiple Range test (at  $P \leq 0.05$ ).

### Treatments effects of abrasives on *M. persicae*

Both kaolin and diatomaceous earth induced an interesting effect on size and color of treated *M. persicae* individuals. After treatment, aphid cadavers showed reduced size due to cuticle dehydration. These effects reflect the direct action of both treatments on the cuticle of *M. persicae*. In fact, the cuticle of control aphids was smooth and hydrated (Fig. 2-A), while the treated ones were distorted, rough and dry. *M. persicae* treated with diatomaceous earth were drier and more rigid (Fig. 2-B) than those treated with kaolin (Fig. 2-C). Therefore, the dehydrating action of diatomaceous earth was more important than that of kaolin.

### DISCUSSION

This research study was performed to evaluate the biocidal activity of the thyme essential oils. The extraction process of the thyme has provided a yield of 2.03% which is similar to the one reported from a similar study performed in Morocco (Amarti et al. 2008; El Ajjouri et al. 2008). However, this reported yield was slightly less important than the one from *T. capitatus*, collected from Matmata region (South-East of Tunisia), evaluated at 2.75% (Akrouit 2004). Regarding the biochemical composition, the results were similar to previous studies, showing that carvacrol was the main component of *T. capitatus* with 70.92% (El Ajjouri et al. 2008) and 66% (Akrouit 2004).



**Fig. 2.** Abrasive impact on *Myzus persicae* cuticle. Aa: Cuticle of the control aphid presenting smooth and hydrated texture; Ba: Cuticle of *M. persicae* treated with diatomaceous earth presenting a dry and rigid texture; Ca: Cuticle of *M. persicae* treated with kaolin showing a dry texture.

Our study reported also an important insecticidal activity of the thyme essential oils against *M. persicae* through both contact and fumigant experiments. The same activity was registered for both rosemary and thyme essential oils through an important repellent activity of 70% at the dose of 1 µl against *M. persicae* (Masatoshi 1998). Other studies showed the important toxic activities of thyme essential oils against several aphid species including *M. persicae* (Masatoshi 2008). The investigation of the insecticidal activity of the thyme essential oils has been also tested against other insect pests. In fact, a previous study has demonstrated the repulsive, contact and fumigant insecticidal activities of terpenes and phenols from thyme essential oils on insects (Isman 2000). The same author noted that components such as monoterpenes and phenols were responsible of the insecticidal activity against *Spodoptera litura* and *M. persicae*. Moreover, further investigation has also shown a higher biocidal activity of *T. capitatus* essential oils than those of *Tetraclinis articulata* against first instar larvae of *Tuta absoluta* (Ben yahia 2015). Beside the interesting toxic activity, *T. capitatus* essential oils has been also proved to reduce the egg production of *Tineola bisselliella* compared to the control (Bouchikhi-Tani et al. 2013).

Our research study also demonstrated the mechanical effect of kaolin and diatomaceous earth through the dehydration, shrinkage and deformation of aphid cuticle. The diatomaceous earth mechanical action has been also reported in a previous study (Ndiaye 2014). In fact, every insect that comes into contact with diatomaceous earth powder died within 24 to 72 h of dehydration (Fields et al. 2002). The

mechanical action of kaolin was also reported against *M. persicae*.

The bio-insecticide formulation assays based on thyme essential oils, either by making emulsions or by combining them with natural substances such as diatomaceous earth and kaolin were significantly important against *M. persicae*. In the same context, it was reported that essential oils of some plant species have greater toxicity effects when combined with powdered products such as kaolin (Keita et al. 2001). The same study also showed that the direct sprinkler of the kaolin powder flavored in combination with *Ocimum basilicum* essential oils on *Callosobruchus maculatus* had a significant effect on adult mortality (100%) recorded 48 h post-treatment (Keita et al. 2001). Furthermore, the use of 0.4 g of essential oils in the formulation (essential oils + kaolin) has led to 100% mortality in *C. maculatus* within one hour post-treatment (Awojide and Fayemiwo 2010). The mechanical and chemical actions of the flavored powders once combined with the essential oils were previously reported by Ramaswamy et al. (1995). Concerning the mechanical action, the effect was mainly presented by blocking the insect articulation and filling the intergranular spaces at high dosages (Ramaswamy et al. 1995). However, the chemical effect was mainly acting on the insect glandular cells (Ramaswamy et al. 1995).

In conclusion, our study demonstrated the high effectiveness of the thyme essential oils in combination with the abrasive powder, diatomaceous earth, as bio-insecticide against *M. persicae*. Therefore, diatomaceous earth powder seems to improve the efficacy of thyme essential oils and make it more stable. Such findings may enhance knowledge about the use of essential oils and the

**RESUME**

**Khaled W., Ben Fekih I., Chaieb I., Souissi R., Harbaoui I. et Boukhris-Bouhachem S. 2017. Evaluation de l'activité insecticide des huiles essentielles de *Thymus capitatus* en combinaison avec des abrasifs naturels contre *Myzus persicae*. Tunisian Journal of Plant Protection 12: 49-59.**

Cette étude a pour objectif d'évaluer l'activité insecticide des huiles essentielles de *Thymus capitatus* et de deux abrasifs naturels pour le contrôle de *Myzus persicae*. L'application de ces huiles a montré un effet toxique et fumigant très important in vitro. La CL50 des essais de fumigation après 24 h est de l'ordre de 20,01 µl/l d'air et de 13,26 µl/l d'air pour les essais de pulvérisation. En outre, des essais in vivo de formulation de bio-insecticides ont été abordés. En effet, les huiles essentielles de thym ont été associées à une argile naturelle (le kaolin) et à un minéral naturel (la terre de diatomée). Les résultats ont montré qu'après 24 h, la poudre de kaolin ou de la terre de diatomée associés aux huiles essentielles de thym ont donné des taux de mortalité respectivement de 74,19 et 97,84%. Parallèlement, des émulsions de 1 µl de ces huiles ont été testées sur le puceron cible. Après 24 h, le taux de mortalité a été de l'ordre de 55,55%. L'observation microscopique a montré une action mécanique sur les aphides montés, notamment la déshydratation, le rétrécissement et la déformation de leur cuticule. Ainsi, la terre de diatomée additionnée aux huiles essentielles de *T. capitatus* a été l'association la plus efficace pour lutter contre les populations de pucerons.

*Mots clés:* Huiles essentielles, kaolin, *Myzus persicae*, terre de diatomée, *Thymus capitatus*

**ملخص**

خالد، وفاء وابتسام بن فقيه وإقبال الشايب ورايحة السويسي وإيمان حرباوي وسنية بوخرص-بوهاشم. 2017. تقييم فاعلية الزيوت الأساسية لنبته الزعتر (*Thymus capitatus*) ممزوجا بمواد طبيعية كاشطة، كمبيد حشري لمكافحة من الخوخ (*Myzus persicae*). Tunisian Journal of Plant Protection 12: 49-59.

تهدف هذه الدراسة إلى تقييم فاعلية الزيوت الأساسية لنبته الزعتر (*Thymus capitatus*) مع اثنين من المواد الكاشطة الطبيعية وذلك لمقاومة حشرة من الخوخ (*Myzus persicae*). أظهرت النتائج أن استعمال هذه الزيوت عن طريق التبخير أو الرش المباشر له تأثيراً سلبياً مهماً جداً في المخبر حيث كانت درجة التركيز القاتلة لـ 50% من الحشرات التي وقع عليها الاختبار (LC50) بعد 24 ساعة في حدود 20,01 مل/ل من الهواء بالتبخير وفي حدود 13,26 مل/ل بالرش. تم اختبار هذه الزيوت على مجموعة نباتات تحتوي على حشرات المن حيث وقع مزج الزيوت الأساسية للزعتر مع الكاولين (kaolin) أو التربة الدياتومية (diatomaceous earth). أظهرت النتائج بعد 24 ساعة موت 97,84% من الحشرات المعاملة بالزيوت مع التربة الدياتومية و 74,19% بالزيوت مع الكاولين، في حين لم يسبب 1 مل من الزيوت المستخلبة في الماء سوى 55,55% من السمية لحشرات المن. قمنا بفحص مجهرى للتغيرات النسيجية لحشرات المن المعاملة بالمواد الكاشطة فثبتت أن لهذه المواد القدرة على تجفيف الأنسجة مع انكماش وتشوه في الجليدة عند الحشرات. هكذا، تظهر هذه الدراسة أن الجمع بين الزيوت الأساسية للزعتر مع المواد الكاشطة وخاصة التربة الدياتومية، يمكن أن يكون طريقة ناجعة لمكافحة حشرة من الخوخ.

كلمات مفتاحية: تربة دياتومية، زيوت أساسية، كاولين، *Thymus capitatus*، *Myzus persicae*

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