Chemical Composition and Insecticidal Effects of *Citrus aurantium* of Essential Oil and its Powdery Formulation against *Tuta absoluta*

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ABSTRACT

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The aim of this research was to investigate the chemical composition and to evaluate the insecticidal activities of the bitter orange *Citrus aurantium* essential oil and its major compound pure limonene against adults and larvae of the tomato miner *Tuta absoluta* using contact and fumigation bioassays. Results of chemical analysis of the essential oil using gas chromatography/mass spectrometry (GC-MS) revealed the presence of limonene (87.52%), β-myrcene (1.62%), α-pinene (0.56%) ß-ocimene (0.81%) and ß-pinene (0.61%) as major components. For bioassays, results indicated that both the oil and its major compound were found to be toxic to larvae and adults. In the fumigant assays, median lethal concentrations (LC_{50}) were 10.65 and 37.36 µl/l air respectively for *C. aurantium* essential oil and pure limonene. In contact toxicity assay, the tomato miner adults were more susceptible to the oil than to its major compound even at the lowest concentration: LD_{50} values obtained after 48 h were respectively 0.21 and 0.73 µl. When insects were treated with the essential oil and its aromatized clay powder, significant differences in insect mortality were recorded depending on exposure time. The aromatized clay powder was more toxic (LT_{50} = 101.8 h) than the pure essential oil (LT_{50} = 146.32 h). Hence, bitter essential oil was found to be toxic for *T. absoluta*, and the clay powder could be used to stabilize the essential oil to increase its efficacy and possibly will be used as source of new eco-friendly insecticidal compounds.

Keywords: Aromatized powder, biopesticide, *Citrus aurantium*, essential oil, pure limonene, *Tuta absoluta*

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The tomato miner *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the most devastating pests of tomato (Desneux et al. 2010). Since the time of its initial detection, the pest has caused serious damages to tomato in invaded areas (Germain et al. 2009) and it is currently considered a key agricultural threat to European and North African tomato production. *T. absoluta* can cause 100% yield losses in tomato crops (Estay 2000). In 2008, it was detected for the first time in the region of Akouda in Coastal Tunisia and quickly spread in all Tunisian tomato-cropping areas (EPPO 2009) where it seriously threatened tomato production and trade of tomato fruits. In fact, Chermiti et al. (2009) reported that fruit loss recorded under greenhouses in the region of Bekalta ranged from 11 to 43% of production. In some cases, crops were totally destroyed. The use of synthetic insecticides has been the primary strategy for controlling *T. absoluta* resulting in environmental pollution and resistance in pest population (Desneux et al. 2007). Therefore, research on alternative methods for tomato miner control is needed.

In recent years, essential oils have been developed as potential alternatives for pest control because they are often of low mammalian toxicity and readily biodegradable (Maciel et al. 2010). Nevertheless, their high instability due to their extremely volatile nature handicaps the protection of the plants over a long duration (Ngamo et al. 2007). For that reason, it was necessary to develop new formulations of these active essential oils to improve their use in an integrated pest management approach (Nguemtchouin et al. 2010). Powdered suspensions of processed kaolin were found to be effective against a range of pest insects (Saour 2005; Valizadeh et al. 2013) and to retain the active compounds against insects for a long period (Ndomo et al. 2008).

The present work reported first investigations on insecticidal effects of *C. aurantium* essential oil, pure limonene, and the clay powder aromatized with this oil towards a Tunisian strain of *T. absoluta*.

MATERIALS AND METHODS

Insect rearing.

T. absoluta was provided by the Laboratory of Entomology at the *Centre Régional des Recherches en Horticulture et Agriculture Biologique,* Chott-Mariem, Tunisia. The tomato miner moth was reared on tomato plants placed in a rearing room. The rearing conditions were: 26 ± 2°C, 60 ± 5% RH, and photoperiod cycle of L: D 16 h: 8 h. Third instar larvae (best stage that endures manipulation) and adults were used for bioassays.

Plant material.

Fruits of *C. aurantium* were collected at mature stage from trees located in the Chott-Mariem area (Sousse, Tunisia). They were transferred to the laboratory and rinsed with distilled water. Peels were excised from fruits using a razor blade, which left the white spongy portion (albedo) on the fruit.

Essential oil extraction.

For the extraction of the essential oil, bitter orange peels were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The extracted oil was then stored at 4°C until future use.

Gas chromatography/mass spectrometry analysis conditions.

The quantitative and qualitative analysis of the volatile profile in the essential oil was carried out using a model 5890 Series II plus gas chromatograph (GC) equipped with a 30 m × 0.25 mm HP-5 (cross-linked phenyl–methyl siloxane) column with 0.25 mm film thickness and an Agilent model 5972 inert mass spectrometry (MS) detector (Agilent, Palo Alto, CA). The initial oven temperature was held at 60ºC for 4 min. It was then increased by 3ºC/min to 250ºC; the injection port and ionizing source were kept at 250 and 280ºC, respectively. Helium was used as carrier gas, the flow through the column was 1 ml/min, and the split ratio was 100:1 with 0.1 ml of injected sample. Mass spectra were scanned from m/z 35 to 300, generating 5.27 scan/s. The individual peaks were identified by retention times and the retention index (relative to C6–C17 n-alkanes), compared with those of corresponding reference standards and using the NIST98 library (NIST, Gaithersburg, MD). Percentage compositions of essential oil were calculated according to the area of the chromatographic peaks.

Fumigant toxicity.

To determine the fumigant toxicity of *C. aurantium* essential oil and its major compound pure limonene, Whatman N°1 filter papers (2 cm in diameter) were impregnated with oil at doses calculated to release fumigant concentrations of 5 to 50 µl/l air. Each impregnated filter paper was attached to the screw cap of a 40 ml Plexiglas bottle. Ten larvae on tomato miner were added to each bottle and caps were screwed on tightly. Each treatment and control was replicated five times. The number of dead and alive insects in each bottle was counted after 24 h of exposure.

Contact toxicity.

Essential oil contact toxicity towards newly emerged *T. absoluta* adults was measured according to Liu and Ho (1999). A serial dilution of the essential oil was prepared in acetone (0.1 ml). The doses of oil tested were 0.0125, 0.025, 0.05 and 0.1 µl. A volume of 0.5 ml of each essential oil and pure limonene in acetone solution was applied to the thorax of each adult. Control was treated with 0.5 ml of acetone. Each concentration was repeated five times. Mortality was observed each hour for 48h. The LD_{50} and LD_{90} values were estimated using probit analysis (Finney 1971).

Aromatization of the clay powder with essential oil.

The aim of this test was to resolve the volatilization constraint of the essential oil. For this purpose, the bio-efficacy of essential oil and aromatized clay powder based on the mixture of clay and essential oil extracted from *C. aurantium* were evaluated for their insecticidal activity against *T. absoluta* larvae.

Powdery formulation was prepared as described by Keita et al. (2001). For that, 10 g of kaolin were transferred in a 100 ml flask and 0.75 ml of *C. aurantium* essential oil diluted in 10 ml of acetone was added. Then, the mixture was placed in a water bath thermostated at 30°C for 90 min after 5 min of manual shaking, to total acetone evaporation. Aromatized powder was kept in vials tightly closed using aluminum foil under the cap.

Effect of aromatized kaolin formulation on larval mortality.

A 10 g-sample of powdery formulation previously prepared was introduced into a plastic jar containing a tomato plant infested with *T. absoluta*. All the plant is uniformly coated. Four different control treatments were applied. In the first one, the plants were treated with non-aromatized clay powder. In the second, the plants were treated with pure essential oil. In the third, the plants were treated with aromatized clay powder. For the last control jar, the plants were not treated. Each jar was tightly closed. Five replications were carried out for each set of treatment and dead insects were counted daily. Percentage larvae mortality was calculated using the Abbott formula (Abbott 1925).

Statistical analyses.

Data are presented as means. One-way analysis of variance using Statistical Package for Social Sciences (version 11.0; SPSS, Chicago, III) was performed on the data. A Duncan Multiple Range test was applied to detect significant differences of mortality among concentrations at the 0.05 percent level. Probit analysis (Finney 1971) was used to estimate LC, LD, and LT values.

RESULTS

Chemical analysis.

The oil yield *C. aurantium* was 0.67% on the basis of dry matter weight. Chemical composition of this essential oil is illustrated in Table 1. In fact, GC-MS analysis showed that 25 compounds were identified, which represent 97.69% of total constituents. Limonene (87.52%), β-myrcene (1.62%), α-pinene (0.56%), ß-Ocimene (0.81%), and ß-pinene (0.61%) were the main monoterpene hydrocarbons, whereas sesquiterpene hydrocarbons were represented by ß-caryophyllenne (0.35%) and ß-farnesene (0.12%). Alcohols (0.52%), aldehydes (1.26%) and esters (0.8%) constituted the non terpenic compounds.

Table 1. Chemical composition (%) of essential oil from *Citrus aurantium* peel

N°	Compound	RI	RT	Percentage
1	α-pinene	949	4.097	0.561
2	Camphene	969	4.420	0.007
3	Sabinene	1001	4.998	0.171
4	ß-pinene	1007	5.080	0.453
5	ß-myrcene	1026	5.448	1.628
6	Octanal	1034	5.775	0.381
7	δ -3-carene	1048	5.989	0.021
8	Limonene	1073	6.675	87.523
9	(*E*)- ß -ocimene	1086	7.224	0.325
10	ɣ-terpinene	1109	7.568	0.045
11	Terpinolene	1141	8.660	0.215
12	Linalool	1152	9.141	3.365
13	Nonanal	1157	9.309	0.066
14	(*Z*)- limonene oxide	1173	10.450	0.026
15	(*E*)- limonene oxide	1182	10.643	0.022
16	Camphor	1192	10.870	0.021
17	Citronellal	1201	11.368	0.013
18	Terpinen -4-ol	1220	12.353	0.171
19	α - terpineol	1231	12.982	0.928
20	Neral	1270	15.462	0.754
21	Geraniol	1280	16.157	0.222
22	Geranial	1291	16.896	0.430
23	Neryl acetate	1366	21.658	0.041
24	Geranyl acetate	1386	22.639	0.196
25	Caryophyllene	1420	23.988	0.111
Total		--	97.696	

RT: Retention Time. **RI**: Retention Indices calculated using an apolar column (HP-5).

Fumigant toxicity.

Both *C. aurantium* essential oil and pure limonene were toxic to the third instar larvae of *T. absoluta*. Mortality was directly related to the doses; it increased as the dose of essential oil increased (Table 2). At the highest concentration (50 µl/l air), the oil achieved 100% of mortality while pure limonene caused only 60% of mortality after 24 h of exposure. Results showed that *C. aurantium* essential oil was more toxic to *T. absoluta* larvae than pure limonene. These results were confirmed by lethal concentration values. The corresponding LC_{50} and LC_{90} were respectively 10.65 and 21.16 µl/l air for *C. aurantium* compared to 37.36 and 74.01 µl/l air for pure limonene. Statistical analysis showed significant differences between the oil and its major compound (Table 3).

Table 2. Percentage of mortality of *Tuta absoluta* larvae exposed for 24 h to *Citrus aurantium* essential oil and pure limonene.

Dose (µl/l air)	Mortality (%)	
	***C. aurantium* essential oil**	**Pure limonene**
0	0 a, A	0 a, A
5	30 ± 0.13 b, B	13.33 ± 0.04 b, A
12.5	73.33 ± 0.08 c, B	26.67 ± 0.04 c, A
25	90 ± 0.06 cd, B	43.33 ± 0.04 d, A
50	100 d, B	60 ± 0.06 e, A

Values are means of five replications, each set-up with 10 insects. Within columns, means followed by the same letter (lowercase letters) were not statistically different based on Duncan Multiple Range test at $P < 0.05$. Within rows, means followed by the same letter (uppercase letters) were not statistically different based on Duncan Multiple Range test at $P < 0.05$.

Table 3. LC_{50} and LC_{90} values of fumigant toxicity of *Citrus aurantium* essential oil and pure limonene against *Tuta absoluta* larvae

Fumigant	***C. aurantium* essential oil**	**Pure limonene**
LC_{50} (µl/l air)	10.65 (0.96-24.72)	37.36 (21.83-80.37)
LC_{90} (µl/l air)	21.16 (13.87-80.78)	74.01 (47.43-103.01)
df	3	3
χ^2	30.26	20.97
P	0	0

For LC_{50} and LC_{90} units (µl/l air), lower and upper confidence limits are given in parenthesis.

Contact toxicity.

The results depicted in Fig. 1 point out that *T. absoluta* adults were very susceptible to the contact toxicity of *C. aurantium* essential oil and pure limonene even at the lowest concentration (0.012 µl). Furthermore, the mortality was dose-dependent and increased with increasing concentrations. Indeed, results of the probit analysis revealed that adults were more sensitive to *C. aurantium* essential oil mostly to pure limonene with corresponding LD_{50} and LD_{90} values of 0.21 and 1.92 µl and 0.73 and 2.34 µl, respectively, for the oil and its major compound limonene (Table 4).

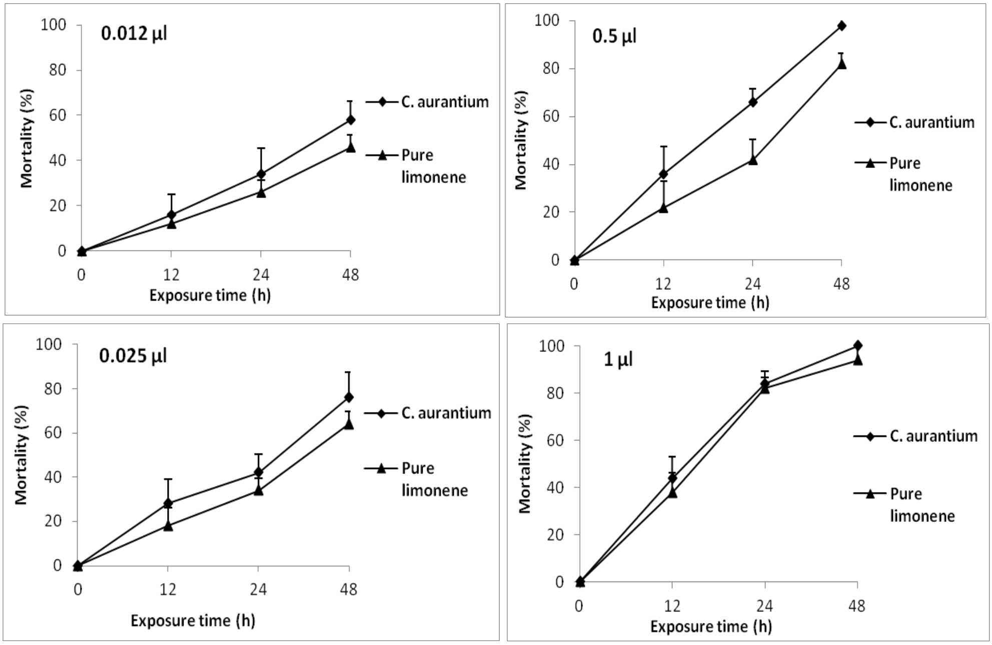

Fig. 1. Percentage of mortality of *Tuta absoluta* adults exposed for various durations to *Citrus aurantium* essential oil and pure limonene applied at different concentrations.

Table 4. LD_{50} and LD_{90} values (µl) of contact bioassay with *Citrus aurantium* essential oil and pure limonene

Fumigant	*C. aurantium* essential oil	Pure limonene
LD_{50} (µl)	0.21 (0.09-1.7)	0.73 (0.12-2.85)
LD_{90} (µl)	1.92 (0.79-4.82)	2.34 (1.32-5.67)
df	3	3
χ^2	8.96	11.61
P	0	0

For LC_{50} and LC_{90} units (µl/l air), lower and upper confidence limits are given in parenthesis.

Effect of aromatized kaolin formulation on larval mortality.

T. absoluta larvae were sensitive to the pure essential oil and its aromatized clay powder. The treatments showed time-response dependence, resulting in significant larvae' mortality when the time increased (Fig. 2). Indeed, mortality values ranging from 27 to 36% and from 57-75% were attained after 24 h and 3 days of exposure, respectively. The highest contact activity was achieved with kaolin powder aromatized at the three exposure durations (24, 48 and 72 h); mortality increased from 36% at 24 h to 75% at 72 h. The mortality observed in the controls and untreated plants was less than 4%. These results were proved by lethal time values (Table 5). The corresponding LT_{50} for bitter orange oil and the powdery formulation were respectively 146.32 and 101.8 h.

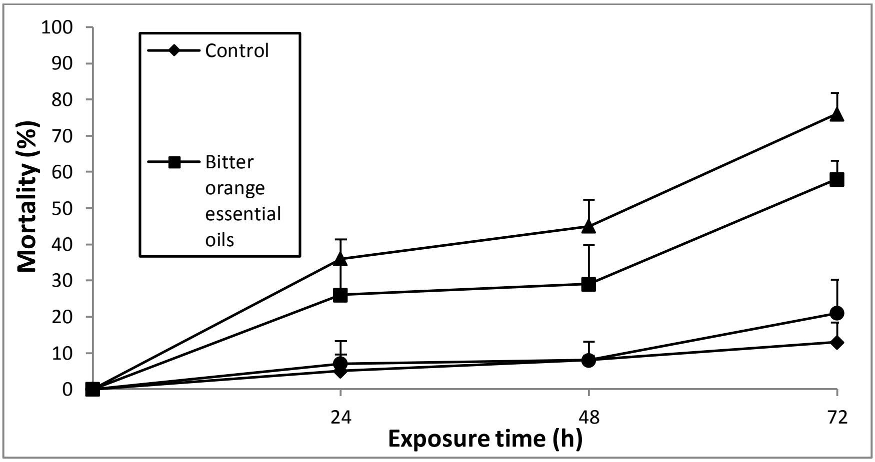

Fig. 2. Evolution of insect mortalities exposed to the essential oil and aromatized clay powder of bitter orange *Citrus aurantium*.

Table 5. LT_{50} and LT_{90} (h) values of *Citrus aurantium* essential oil and its powdery formulation

Treatment	LT_{50}	LT_{90}	χ^2	df	*P*
***C. aurantium* essential oil**	146.32	328.29	25.23	3	0
Powdery formulation	101.8	250.7	17.32	3	0

DISCUSSION

This study revealed that the essential oil of *C. aurantium* was rich in monoterpenoids, and its major compound was limonene (88.52%). Among various compounds of *Citrus* essential oil, limonene is the most important. This result is in agreement with previous investigations on bitter orange essential oil. Indeed, Bousaada and Chemli (2007) reported the predominance of limonene whose level varied from 87% to 92.2%. Additionally, Hosni et al. (2010) showed that bitter orange peel oil comprised mainly monoterpene hydrocarbons with limonene (96.86%), ß-pinene (1.37%), sabinene (0.28%), and α-pinene (0.27%) as major compounds. In the same context, Moraes et al. (2009) proved that Brazilian *C. aurantium* peel oil contained limonene (97.5-98%), myrcene (1.2-1.45%), and octanol (0.34-0.54%). Likewise, Caccioni et al. (1998) reported that *C. aurantium* oil from Italy was richer in limonene (94.3%) than myrcene (1.88%), linalool (0.78%) and α-pinene (0.4%). These qualitative and quantitative differences in compositions of essential oil depended on season, maturational stage, genotype and pedoclimatic factors (Boussaada et al. 2007).

The present study proved also the sensitivity of *T. absoluta* to bitter orange essential oil and pure limonene. In fact, the insecticidal activities of *C. aurantium* essential oil have previously been evaluated against several insect species (Rossi and Palacios 2013). Indeed, Pala and Pathipati (2010) reported that essential oil of *C. aurantium* were highly effective leading to 89 and 76% mortality against respectively *Sitophilus oryzae* and *Rhyzopertha dominica* when applied at 8.5 mg/cm^2. Besides, fumigant toxicity of *C. aurantium* essential oil was investigated on *Callosobruchus* *maculatus* adults (Moravvej and Abbbar 2008). Siskos et al. (2007) suggested that *C. aurantium* contains secondary metabolites that are toxic to the adult olive fruitfly, *Bactrocera oleae*. Additionally, Siskos et al. (2008) studied the effect of the essential oil extracted from *C. aurantium* used at dose of 5 µg/cm^2 for the control of *B. oleae* and obtained 98% mortality after an exposure duration of 72 h. Palacios et al. (2009) reported that essential oil obtained from *C. aurantium* was effective in killing *Musca domestica* and showed that the LC_{50} value was 4.8 mg/dm^3. Moreover, *C. aurantium* presented highly insecticidal toxicity against *Trialeurodes vaporariorum* adults, nymphs and eggs after 24 h of exposure.

The bioactivity of this oil could be mainly allocated to their constituents whose insecticidal activities have previously been proved against several insect species (Rossi and Palacios 2013). Certainly, the insecticidal mode of action of some monoterpene compounds is known (Koul et al. 2008). Indeed, Tripathi et al. (2003) reported the insecticidal activity of d-limonene against *Rhyzoperta domonica*, *Sitophilus oryzae*, and *Tribolium castaneum*. Furthermore, this compound showed promising fumigant toxicity against *S. zeamais* and *T. castaneum* (Rui et al. 2010). Additionally, Ezeonu et al. (2001) indicated the insecticidal properties of limonene against stored-product beetles and mosquitoes. Likewise, α-pinene was reported to be toxic on several insect species (Ceferino et al. 2006; Lucia et al. 2007). Moreover, terpinen-4-ol showed a relatively strong toxicity against the larvae and adults of *Leptinotarsa decemlineata* (Kordali et al. 2007). Also limonene, terpen-4-ol, 1,8- cineole and linalool acted by fumigant toxicity and presented a remarkable insecticide activity against eggs, larvae and adults of *Tribolium confusum* (Stamopoulos et al. 2007).

Our work related to contact toxicity bioassay showed that both bitter orange essential oil and pure limonene displayed an interesting insecticidal potential. As reported elsewhere, *C. aurantium* essential oil revealed a contact toxicity activity. In this context, Palacios et al. (2009) reported the contact toxicity of *C. aurantium* essential oil and pure limonene against *Musca Domestica* adults. Additionally, insecticidal activity of various *Citrus* species was demonstrated by contact toxicity using topical application to various insect pests. Indeed, Chungsamarnyart and Jansawan (1996) evaluated the acaricidial activity of *C. sinensis* oils and registered 49-99% mortality of *Boophilus microplus* after 24 h of exposure. Also, in contact toxicity assay, Kumar et al. (2012) reported that the LC_{50} of *C. sinensis* essential oils against *M. domestica* larvae, varied between 3.93 and 0.71 µl/cm^2 while LT_{50} varied between 5.8 to 2.3 days.

Furthermore, it has been established during the present study the effect of the aromatized clay powder on the mortality of *T. absoluta*. As previous study, kaolin was found to be effective against insects (Barker et al. 2006; Valizadeh et al. 2013) and could prevent damages of insect pests. In fact, our results are in accordance with those of Ndomo et al. (2008) who demonstrated that both aromatized clay powder and pure essential oils showed insecticidal activities against *Acanthoscelides obtectus*. After 2 days of exposure, the aromatized clay powder was more toxic (LD_{50} = 0.069 µl/g grain) than the pure essential oil (LD_{50} = 0.081 µl/g grain). There was, however, a highly significant loss of toxicity after 24 and 36 h following treatment with essential oil and aromatized powder, respectively. Kèita et al. (2001) reported that the application of kaolin powder aromatized with *Thuja occidentalis* essential oils on *C. maculatus* lead to 95% mortality of females and 100% of males with no mortality in the control after 6 h of exposure. When insects were treated with pure oil the mortality rate was 39.1%. Besides, the formulation based on *Xylopia aethiopica* essential oils and powder clay produced mortality rates ranging from 22 to 100% for *Sitophilus zeamais* (Nguemtchouin et al. 2010).

The present work reported the insecticidal activity of the essential oil of *C. aurantium* and pure limonene against *T. absoluta*. Thus, the obtained results suggest the possibility of their use in the sustainable management of greenhouse pests. Although essential oil applied alone provides a good level of control of insect pests, they are very unstable because of their high volatility. The use of clay materials as a support for such oil as shown in the present study could not only increase their stability but also improve their insecticidal activities. Nevertheless, essential oil and clay could be an efficient alternative to synthetic products and therefore a method to reduce pollution.

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RESUME

Zarrad K., Chaieb I., Ben Hamouda A., Bouslama T. et Laarif A. 2017. Composition chimique et effets insecticides de l'huile essentielle de *Citrus aurantium* et de sa formulation poudreuse contre *Tuta absoluta*. Tunisian Journal of Plant Protection 12: 83-94.

Le but de ce travail a été d'étudier la composition chimique et d'évaluer les activités insecticides de l'huile essentielle du bigaradier *Citrus aurantium* et de son composant majoritaire le limonène pur contre les adultes et les larves de la mineuse de la tomate *Tuta absoluta* en utilisant des bioessais de contact et de fumigation. L'analyse chimique de l'huile essentielle, effectuée par chromatographie en phase gazeuse couplée à la spectrométrie de masse, a révélé le limonène (87,52%), le β-myrcène (1,62%), l'α-pinène (0,56%), le ß-Ocimène (0,81%) et le ß-pinène (0,61%) en tant que composants majeurs. Les résultats des bioessais ont montré que l'huile et son composé majoritaire se sont révélés toxiques vis-à-vis des larves et des adultes. Pour les essais de fumigation, les concentrations létales médianes CL_{50} étaient respectivement de 10,65 et 37,36 µl/l air pour l'huile essentielle de *C. aurantium* et le limonène pur. Pour l'essai de toxicité par contact, les adultes de la mineuse de la tomate étaient plus sensibles à l'huile que son composé principal même à la plus faible concentration: les DL_{50} obtenues au bout de 48 h étaient respectivement de 0,21 et 0,73 µl. Lorsque les insectes ont été traités avec l'huile essentielle et de la poudre d'argile aromatisée, des différences significatives de mortalité, en fonction du temps d'exposition, ont été enregistrées. La poudre d'argile aromatisée était plus toxique (TL_{50} = 101,8 h) que l'huile essentielle pure (TL_{50} = 146,32 h). Ainsi, l'huile essentielle du bigaradier a été trouvée toxique pour *T. absoluta* et la poudre d'argile pourrait être utilisée pour stabiliser cette huile essentielle afin d'augmenter leur efficacité et pourrait éventuellement être utilisée comme source de nouveaux composés insecticides respectueux de l'environnement.

Mots clés: Biopesticide, *Citrus aurantium*, huile essentielle, limonène, poudre aromatisée, *Tuta absoluta*

ملخص

الزراد، خولة وإقبال الشايب وأمال بن حمودة وثامر بوسلامة وأسماء لعريف. 2017.التركيبة الكيميائية والفاعلية الإبادية الحشرية للزيت الأساسي لنبتة *Citrus aurantium* وصياغته كمسحوق ضد حشرة *Tuta absoluta*.
Tunisian Journal of Plant Protection 12: 83-94.

تهدف هذه الدراسة إلى تشخيص التركيبة الكيميائية وتقييم الفاعلية الإبادية الحشرية للزيت الأساسي للنارنج *Citrus aurantium* ومكونه الرئيسي الليمونين (limonene) ضد الطور البالغ ويرقات حافرة الطماطم *Tuta absoluta* باستخدام تجارب بيولوجية بالملامسة والتبخير. بينت دراسة التركيبة الكيميائية للزيت الأساسي باستعمال التحاليل الكرومتوغرفية أن limonene (87,52%) وβ-myrcene (1,62%) وß-Ocimene (0,81%) و ß-pinene (0,61%) وα-pinene (0,56%) عناصر أساسية. بينت النتائج نجاعة الزيت الأساسي ومكونه الأهم ضد اليرقات والطور البالغ. في اختبارات السمية بالتبخير بلغت الجرعة القاتلة 10,65 و37,36 مكل/لتر على التوالي بالنسبة للزيت *C. aurantium* والليمونين الصافي. في اختبار السمية بالملامسة، كانت الحشرات البالغة لحافرة الطماطم أكثر حساسية للزيت الأساسي من مكونه الأهم حتى بأدنى جرعة. كانت الجرعة القاتلة المتحصل عليها بعد 48 ساعة على التوالي 0,21 و0,73 مكل. عندما تمت معاملة الحشرات بالزيت الأساسي ومسحوق الطين المنكه، سجلت فوارق معنوية في نسبة الموت وذلك حسب مدة التعرض. وقد كان مسحوق الطين المنكه أكثر سمية حيث بلغ الزمن القاتل LT_{50} = 101,8 ساعة مقارنة بالزيت الأساسي النقي (الزمن القاتل LT_{50} = 146,32 ساعة). وبالتالي فإن الأساسي للنارنج كان ساما لحافرة الطماطم ويمكن استخدام مسحوق الطين لتحقيق استقرار الزيوت الأساسية لزيادة نجاعتها وربما يتم استخدامها مبدئيا كمصدر لمواد مبيدة للحشرات غير مضرة بالبيئة.

كلمات مفتاحية: حافرة الطماطم، زيت أساسي، ليمونين، مبيد حيوي، مسحوق منكه، *Tuta absoluta*، *Citrus aurantium*

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