Bio-insecticidal potential of essential oils of two *Citrus* species against two Greenhouse pests *Tuta absoluta* Meyrick and *Spodoptora littoralis* Boisduval

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RÉSUMÉ

L'utilisation de substances naturelles d'origines végétales dont les huiles essentielles constitue une alternative prometteuse. Les potentialités insecticides des huiles essentielles du bigaradier (*C. aurantium*) et du citronnier (*C. limon*) ont été évaluées, par fumigation, à l'égard de deux lépidoptères *Tuta absoluta* et *Spodoptora littoralis*. L'analyse chimique effectuée par chromatographie en phase gazeuse montre que le limonène est le composant principal du bigaradier (88.57%) et du citronnier (70.46%). Tous les traitements ont montré que les huiles du bigaradier sont plus toxiques avec des concentrations létales CL_{50} égales à 14.68 µl/l air et 55.49 µl/l air respectivement pour *T. absoluta* et *S. littoralis* comparées à celles du citronnier qui sont de 24.33 µl/l air et 68.1 µl/l air. La mortalité augmente avec la dose des huiles utilisées. Ces résultats montrent que les huiles essentielles des deux espèces de *Citrus* pourraient être une source potentielle de substances à activité insecticide et respectueuses de l'environnement.

***Mots clés** : Bigaradier, citronnier, huiles essentielles, activité insecticide, Tuta absoluta, Spodoptora littoralis.*

ABSTRACT

The use of plant essential oils in pest control is a suitable alternative for reduction of the side effects of chemical pesticides on the environment. The essential oils extracted from *Citrus aurantium* and *Citrus limon* were tested against third instar larvae of both pest species *Tuta absoluta* and *Spodoptora littoralis* to assess their insecticidal properties. The analysis by gas chromatography showed that limonene was the principal constituent of *C. aurantium* essential oils (88.57%) and *C. limon* (70.46%). Fumigant toxicity tests showed that *C. aurantium* oil was more toxic (LC_{50} was 14.68 µl/l air and 55.49 µl/l air for *T. absoluta* and *S. littoralis* respectively) than *C. limon* (LC_{50} was 24.33 µl/l air and 68.1 µl/l air for *T. absoluta* and *S. littoralis* respectively). The mortality rate of both pest species increased with the increase in essential oils dose. Hence, the essential oils of the two *Citrus* were found to be toxic to both pests, this could be useful for investigation of new natural insecticidal compounds.

***Keywords:** Citrus aurantium, Citrus limon, essential oil, insecticidal potential, Fumigation, Tuta absoluta, Spodoptera littoralis.*

Introduction

The continuous uses of chemical insecticides are the result of serious situations, including the development of resistance by insects, pollution of environment and side effects on human health. Essential oils and their derivatives are recognized as an alternate means of controlling many harmful insects, and being rapidly degradable in the environment and harmless to non target organisms (Pillmoor et *al*., 1993). The essential oils have been extensively used as antiparasitical, bactericidal, fungicidal, antivirus and as well as insecticidal (Pervez et *al*., 2010). The most promising botanicals for use at the present time and in the future are species of families of Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae and Canellaceae (Jacobson, 1989). Some of the *Citrus* species have been reported as a source of botanical insecticides: as a variety of these plants contain secondary metabolites that show insecticidal activity against several coleopteran and dipteran (Su et *al*., 1972; Sheppard 1984; Salvatore et *al*., 2004; Shrivastava et *al*., 2010).

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It also has been investigated that the fruit peels of some citrus species possess insecticidal properties against insect pests (Don-Pedro 1985). In another studies, the essential oils of citrus peels proved to reduce oviposition or larval emergence through parental adult mortality (Elhag 2000). Citrus oils are mixtures of very volatile components as terpenes and oxygenated compounds (Sato et *al*., 1996) and the major active component is Limonene.

Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) is a polyphagous insect pest of cosmopolitan distribution (Pineda and *al*., 2007) and is reported to attack more than 87 different species of cultivated crop plants throughout the world (Salama *et al*., 1970). *S. littoralis* is an economically important polyphagous pest in Africa, Asia, and Europe (Bayoumi et al., 1998, El-Aswad et al., 2003) causing considerable economic loss to many vegetable and field crops since the larvae of *S. littoralis* can defoliate many economically important crops (Pluschkell et al., 1998).

The tomato leafminer *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).

The objectives of this study were to determine the chemical composition of the essential oils of *Citrus aurantium* (sour orange) and *Citrus limon* and to evaluate their fumigant toxicity against third instar larvae of both pest species *Tuta absoluta* and *Spodoptora littoralis*.

MATERIALS AND METHODS

Insect rearing

Both pest species *Tuta absoluta* and *Spodoptora littoralis* were provided by the Laboratory of Entomology at the Regional center for research in Horticulture and Organic agriculture Chott Mariem, Tunisia.

S. littoralis larvae were reared on an artificial diet that of Poitout and Bues (1974) based on agar (1.83%), corn meal (12 .84%), wheat germ (3.21%), brewer's yeast (3.43%), ascorbic acid (0.45%), benzoic acid (0.13%), nipagine (0.11%), aldehyde (0.05%) and water (77.95%). The rearing conditions were: temperature of 25 ± 1°C, photoperiod of 12 h (L) and 60 ± 10% relative humidity.

The tomato leafminer moth was reared on tomato plants placed in a rearing room at the same conditions as *S. littoralis*. Third instar larvae were used for fumigant toxicity tests.

Plant material

Fruits of *Citrus* were collected from trees located in the Chott Mariem area of Sousse, Tunisia. They were immediately 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 oils extraction and analysis

Essential oils were extracted from peels subjected to hydrodistillation for 3hours using a Clevenger-type apparatus. The extracted oils were then stored at 4 °C.

Essential oils were analyzed by gas chromatography (GC) using HP 5090 a gas chromatograph equipped with a flame ionization detector (FID) and DB5 capillary column (30 m x 0.25 mm). Carrier gas flow (N2) was 1 ml/min. Analyses were performed using the following temperature program: oven kept isothermally at 35 °C for 5 min, increased from 50 to 200 °C at the rate of 4 °C/min. Injector and detector temperatures were held, respectively, at 220 and 230 °C.

The composition was reported as a relative percentage of the total peak area. The identification of the essential oils constituents was based on a comparison of their retention times to n alkanes, compared to published data and spectra of authentic compounds.

Fumigant toxicity bioassays

To determine the fumigant toxicity of *C. aurantium* and *C. limon* essential oils, Whatman No1 filter papers (2 cm diameter) were impregnated with each oil at doses calculated to release fumigant concentrations of 5 to 50 µl/l air and 25 to 200 µl/l air for *T. absoluta* and *S. littoralis* respectively. Each impregnated filter paper was attached to the screwcap of a 40 ml Plexiglas bottle. Ten larvae of one or other of the insect species were added to each bottle and caps were screwed on tightly. Each treatment and control were replicated five times. The number of dead and alive insects in each bottle was counted after 24 h of exposure.

Lethal dose bioassays

Trials were designed to assess LC_{50} and LC_{90} values. Ten larvae insects were put into 40 ml Plexiglas bottles with screw lids. Fumigant concentrations were 5 to 50 µl/l air and 25 to 200 µl/l air for *T. absoluta* and *S. littoralis* respectively. Control insects were kept under the same conditions without any essential oil. Each treatment was replicated five times. The mortality was evaluated after 24 h of exposure by direct observation.

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 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_{50} and LC_{90} values.

Results

Essential oils composition

GC and GC-MS analysis of *C. aurantium* and *C. limon* essential oils were respectively reported in Tables 1 and 2. The oil yields based on dry matter weight were respectively 0.32% for *C. aurantium* and 0.41% for *C. limon*.
A total of 96.55% from the constituents of *C. aurantium* peel essential oil were identified (Table 1).

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

N°	Compound	RT	%
1	α-Pinene	11,73	0,31
2	Sabinene	12,76	0,17
3	ß-Pinene	12,86	1,95
4	Limonene	14,49	88,57
5	Cis-limonene oxide	15,04	0,41
6	α-Terpinene	15,32	0,19
7	Decanal	15,41	0,08
8	linalool	15,57	2,95
9	Linalyl acetate	17,71	0,91
10	Terpinene-4-ol	18,51	0,1
11	ß-caryophyllenne	18,85	0,16
12	α-Terpineol	20,82	0,22
13	ß-Farnesene	21,97	0,12
14	Geranial	23,04	0,21
15	Farnesol	24,43	0,20
16	Geranyl acetate	25,79	tr
17	Nerol	28,39	tr
	Total	96,55	

RT: Retention Time; **tr**: traces.

The major compound was limonene (88.57%). The other compounds were present at low contents. Among them, linalool (2.95%), β-pinene (1.95%) and α-Pinene (0.31%).
For *C. limon* peel essential oil, a total of 94.42% of the constituents were identified (Table 2). The oil was rich in limonene (70.46 %). Other compounds were present at low contents such as α-Pinene (1.02%), β-pinene (6.94%), Nonal (3.41%), Terpinene-4-ol (3.79%) and Trans-p-2,8-Menthadien-1-ol (4.97%).

***Table 2:** Chemical composition (%) of essential oil from C. limon peel*

N°	Compound	RT	%
1	α-Pinene	11,77	1,02
2	ß-Pinene	12,87	6,94
3	Limonene	14,50	70,46
4	Nonal	14,81	3,41
5	α-Terpinene	15,28	0,22
6	linalool	15,58	0,62
7	α-Thujone	16,23	0,21
8	Linalyl acetate	17,80	0,71
9	Terpinene-4-ol	18,50	3,79
10	Trans-p-2,8-Menthadien-1-ol	19,10	4,97
11	Neryl acetate	20,54	0,76
12	α-Terpineol	20,87	0,39
13	ß-Farnesene	22,05	0,52
14	Geranial	23,28	0,40
	Total	94.42	

Fumigant toxicity

C. aurantium and *C. limon* essential oils were toxic to third instar larvae of both pest species *Tuta absoluta* and *Spodoptora littoralis.* The results showed that fumigant toxicity varied with insect species and oil concentration. The oils were more toxic to *T. absoluta* compared to *S. littoralis,* both when calculated in terms of lethal concentration (Table 3). Probit analysis also showed that *C. aurantium* was more toxic than *C. limon.* Indeed, for sour orange the corresponding LC_{50} was 14.68 µl/l air and 51.49 µl/l air for *T. absoluta* and *S. littoralis* respectively. LC_{50} values were, respectively 24.33 µl/l air and 52.17 µl/l air for *C. limon* oils.

Table 3: Effects of different doses of *C. aurantium and C. limon* essential oils on the longevity of *S. littoralis* and *T. absoluta* larvae.

	Dose (µL/L air)	Mortality (%)		CL_{50} (µL/L air)		CL_{90} (µL/L air)	
		C. aurantium	***C. limon***	***C. aurantium***	***C. limon***	***C. aurantium***	***C. limon***
S. littoralis	0	0 [a]	0 [a]	55.49	68.1	86.09	101.52
	25	0 [a]	8 [a]				
	50	60 [b]	20 [b]				
	100	92 [c]	90 [c]				
	200	100 [d]	100 [c]				
T. absoluta	0	0 [a]	0 [a]	14.68	24.33	30.18	39.2
	5	23.33 [b]	4 [a]				
	12.5	53.33 [c]	20 [b]				
	25	86.67 [d]	52 [c]				
	50	96.67 [d]	98 [d]				

Value represents mean ± S.D. of five replications, each set-up with 10 insects. Alphabetical letters indicates significant difference between doses in same insects at $P < 0.05$ (Duncan test). Lethal dose calculation with probit analysis method.

DISCUSSION

This study showed that the essential oils of *C. aurantium* and *C. limon* were rich in monoterpenoids, and their major common compound was limonene (88.57- 70.46%). Among various compounds of *Citrus* essential oils, limonene is the most important. This compound is characteristic of the *Citrus* genus and is mainly responsible of its insecticidal proprieties (Rossi and Palacios, 2013). Ezeonu et *al*., (2001) indicated the possess insecticidal properties of limonene against stored-product beetles and mosquitoes. Furthermore, this compound showed promising fumigant toxicity against *S. zeamais* and *T. castaneum* (Rui et *al*., 2010). Additionally, Tripathi et *al*., (2003) reported the insecticidal activity of d-limonene against *Rhyzoperta domonica*, *Sitophilus oryzae* and *Tribolum castaneum*.

Also results reported in this study showed the insecticidal effect of essential oils from *C. aurantium* and *C. limon* on larvae of *T. absoluta* and *S. littoralis* after 24 hours exposure. The mortality rate of both pest species increased with the concentration of the essential oil.

The fumigant activity of *C. aurantium* and *C. limon* essential oils has been evaluated against other insect species. Indeed, Pala and Pathipati (2010) reported that essential oils of *C. aurantium* was highly effective with 89 and 76 % mortality respectively for *S. oryzae* and *R. dominica* at 8.5 mg/cm2. Besides, fumigant toxicity of *C. aurantium* essential oil was investigated on *C. maculatus* adults (Moravvej and Abbbar 2008). Siskos *et al*., (2007) suggested that *C. aurantium* contains secondary metabolites that are toxic to olive fruit fly, *Bactrocera oleae* adults. Additionally, Elias et *al*., (2008), studied the effect of the essential oil extracted from *C. aurantium* in the control of *Bactrocera oleae* and obtained 98% mortality in exposure periods 72 h and 5 µg/cm^2 dose. Sara et *al*., (2009) reported that essential oil obtained from *C. aurantium* was effective in killing *Musca domestica* and showed that the LC_{50} was 4.8 mg/dm3. Moreover, *C. aurantium* presented highly insecticidal toxicity against *Trialeurodes vaporariorum* adults, nymphs and eggs after 24 h of exposure.

On the other hand, *C. limon* essential oil has potent insecticidal activity. In this context, it was indicated that significant effects were detected with using of *C. limon* essential oil against mosquito larvae of *Culex pipiens* (Michaelakis et *al*., 2009). Reda et *al*., (2010) reported that fumigation of the third instar larvae of two museum insect pest: *A. fasciatus* and *L. serricorne* with *C. limon* resulted in malformation and disorientation in of the antennal structures and their associated sensilla in the emerged adults.

Conclusion

The present work reported the insecticidal activity of the essential oils of *C. aurantium* and *C. limon* against *T. absoluta* and *S. littoralis*. Thus, they could be used in sustainable pest management in the greenhouse and will have purely to be advised for the safeguarding of the environment and the health of the user. Especially that application of synthetic insecticides arise development of resistance and pollution of the environment.

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