Larvicidal Activity of Artemesia Campestris L Oil on Culex Pipiens (Linnaeus, 1758) and Ceratitis Capitata (Wiedemann) (Diptera: Tephritidae)

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Abstract

Essential oil of *Artemisia campestris* L (Asteraceae) collected in Ain Temouchent region, Algeria were evaluated for its chemical composition and larvicidal toxicity on *Culex pipiens* (Diptera: Culicidae) and *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) larvae. The main compounds of *A. campestris* oil were β-pinene ,α-pinene, limonene, γ-terpinene and α-phellandrene. LC50 and LC90 values were 0.041 and 0.092 µl/L for *C. pipiens* and 0.063 and 0.139 µl/L for *C. capitata*, respectively. The active compounds of *A. campestris* oil are important to be used as natural alternative especially against Tephritidae.

Keywords:*A. campestris*, essential oil, *C. pipiens, C. capitata*, larvicidal.

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1. Introduction

The most important of Asteraceae family is Artemesia, with 500 species found in worldwide (Aljaiyash et al., 2018). *Artemisia campestris* L is famous by its medicinal properties with biological properties (Aloui et al., 2016) including antimicrobial, antihypertensive, anticancer, antifungal, anthelmintic, antivenom, insecticidal and antidiabetic properties (Erel et al., 2012; Houicher et al., 2016).

Essential oils obtained from aromatic plants have are known by their insecticides activities. They have repellent, insecticidal, antifeedants, growth inhibitors, oviposition inhibitors, ovicides, and growth-reducing effects on a variety of insects (Elzen& Hardee, 2003; Tripathi et al., 2003; Pereira et al., 2006; Sithisut et al., 2011; Regnault-Roger et al., 2012;) EO of *A. campestris* well known by its activities as antibacterial, antitumor, anthelmintic, insecticidal, antioxidant, antifungal, antimutagenic properties (Dib et al., 2017).

Insects inflict damage to humans, farm animals and crops (Hikal et al., 2017). The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), commonly named medfly, is one

of the world's most destructive pests attaching both wild and cultivated plants, and it is considered to be the most invasive of all the Tephritidae (Zucchi, 2001). This species is multivoltine and highly polyphagous, being able to attack more than 350 different species of fruits (McQuate and Liquido 2017), thus causing quantitative and qualitative losses to several crops (Ghabbari et al., 2018).

Insect vectors as Culex genus are responsible for the transmission of several human diseases like malaria, lymphatic filariasis West Nile encephalitis, dengue fever, yellow fever and chikungunya fever (Aguiar, 2015). Asteraceae family are well known by its toxic and repellent activity against mosquitoes.

In this study, we analyzed the constituents of essential oil of *A. campestris* with it insecticidal activity on *C. capitata* (Wiedemann) (Diptera: Tephritidae), and *C. pipiens* (Diptera: Culicidae) larvae.

2. Materials and Methods

2.1. Essential Oil extraction:

Artemesia campestris was chosen for essential oil extraction. It was collected from Ain Temouchent region of Algeria. After rinsing the leaves of th plant with water, they were dried in the shade at 26 °C for 7 days prior to distillation in the laboratory. We conducted essential oil extraction from the plant using hydrodistillation with the Clevenger apparatus (Clevenger, 1928). Fifty grams of leaves from each plant were distilled in 500 mL of water (at a ratio of 1:10 plant material to water) for 3 hours. The extracts were then stored at 4°C for analysis.

2.2. Gas Chromatography/mass Spectrometry analysis (GC-MS)

The bioactives compounds present in the essential oil were identified and quantified on a Shimadzu QP2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector. The injection were performed in splitless mode at a temperature ranging from 60°C to 220°C, with a gradient of 1 mL/ min. 0.5 L of the liquid samples were dissolved in an appropriate volume of hexane. The soluble extract was injected into the column using a 1:5 split mode, with helium as the carrier gas. Essential oil composition was determined based on peak areas and calculated as a percentage of the total compounds detected in the sample. Compound identification was performed using NIST 27.

2.3. Bioassays

2.3.1. Insect rearing

- ***Culex pipiens*:**

From Ain Temouchent region, we collected *C. pipiens* eggs and take to the laboratory to the University of Ain Temouchent, Algeria.

Under laboratory conditions (14:10 light and dark photo period cycle, 25-27°C) the larvae were reared in plastic bottle water.

A fresh food containing Biscuit-dried yeast (75:25 by weight) was used as fed larvae (Alouani et al., 2009). After transformation to pupae, we placed it in screened cages (20x20x20cm), where the adult emerged. After emergence, The egg-masses were kept to continue next generation.

-Ceratitis capitata:

Laboratory conditions used for rearing and bioassays were 25°C ± 1°C, 60% ± 10% relative humidity (RH) and 12 h of light and 12 h of darkness. *C. capitata* larvae from 0.5 ml of eggs were raised on an artificial diet containing 800 cm3 carrot pulp slurry, 16 g of yeast powder, anti- fungal 1.04 and 15 cm3 2 N HCl (Finney, 1956).

Third-instar larvae were transferred into a pupation chamber with sand and were maintained there for at least 1 week before they were collected using a sieve. Adult flies were fed an artificial diet comprised of 20% (wt/wt) yeast autolysate and 80% (wt/wt) sucrose.

3.2. Bio essais larvicides

- Culex pipiens:

The tests are carried out in the laboratory in 5cm diameter bottles. However, for 60 ml of formulated water, the essential oil was diluted to three concentrations: 0.05 µl/L; 0.1 µl/L and 0.15µl/L.

In each bottle containing 20 mosquito larvae (Fourd instar) is put 1ml of diluted solution + 99 ml of distilled water to have a volume of 100 ml, with the exception of the control bottle which contains 100 ml of distilled water. Three repetitions are carried out for all tests. Result's reading were at 24 and 48.The percentage mortality was corrected by Abbot's (1925) formula:

$$\text{Corrected mortality (\%)} = \frac{(\text{\% mortality in treatment} * \text{\% mortality in control}) \times 100}{(100 * \text{\% mortality in control})}$$

-Ceratitis capitata:

Ten third instar larvae were transferred a recipients (30 mm diam × 70 mm depth) containing 10 g of diet mixed with the essential oil, except for the control group, which had just received water with the diet. Three concentrations were used in three repetitions : 0.05 µl/L; 0.1 µl/L and 0.15µl/L.

The recipients were covered with nylon mesh, and fixed around the edges with a rubberband. After 48 h of exposure, the number of dead larvae was counted. The percentage mortality was corrected by Abbot's (1925) formula:

$$\text{Corrected mortality (\%)} = \frac{(\text{\% mortality in treatment} * \text{\% mortality in control}) \times 100}{(100 * \text{\% mortality in control})}$$

2.5. Statistical Analysis

The LC50 regression equation was calculated by using Probit analysis. Percentages of mortality obtained from bioassay tests were analyzed using one-way analysis of variance (ANOVA, $p < 0.05$).

Results:

1. Essential oil composition:

The results of the EOs are presented in Table 1.The main compounds found in the essential oils of *A. campestris* were β-pinene (22.32 %),α-pinene (16.13 %), Limonene (13.99 %), γ-terpinene (13.56 %) and α-phellandrene (10.22 %). Also, we found other componds like: Germacrene D (6.52 %), β-ocimenetrans (4.56 %), β-ocimene (5.23 %), Sabinene (1.52 %), α-thujene (1.32 %), Davanone (1.23 %) and Terpinen-4-ol (1.01 %). The total compound identified in *A. campestris* was 92.22%.

Table 1 : Chemical constituents of *A. campestris*, EO (Relative percentages).

Compounds	Relative percentages
α-pinene	16.13
α-phellandrene	10.22
Sabinene	1.52
β-myrcene	0.54
β-pinene	22.32
Germacrene D	6.52
Terpinen-4-ol	1.01
α-humullene	0.33
Limonene	13.99
β-caryophyllene	0.33
Terpinolene	0.25
Aromadendrene	0.1
p-cymen	0.99
β-ocimenetrans	4.56
α-terpinolene	0.32
γ-terpinene	13.56

Linalool	0.09
β-ocimene	5.23
α-thujene	1.32
Camphene	0.13
Davanone	1.23

2. Bioassays

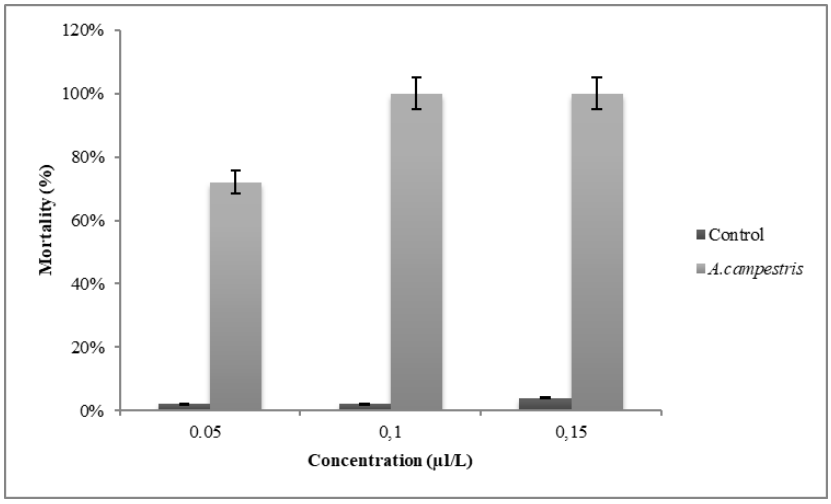

Figure 1: Larvicidal activity of *A. campestris* oil against larvae of *C. pipiens.*

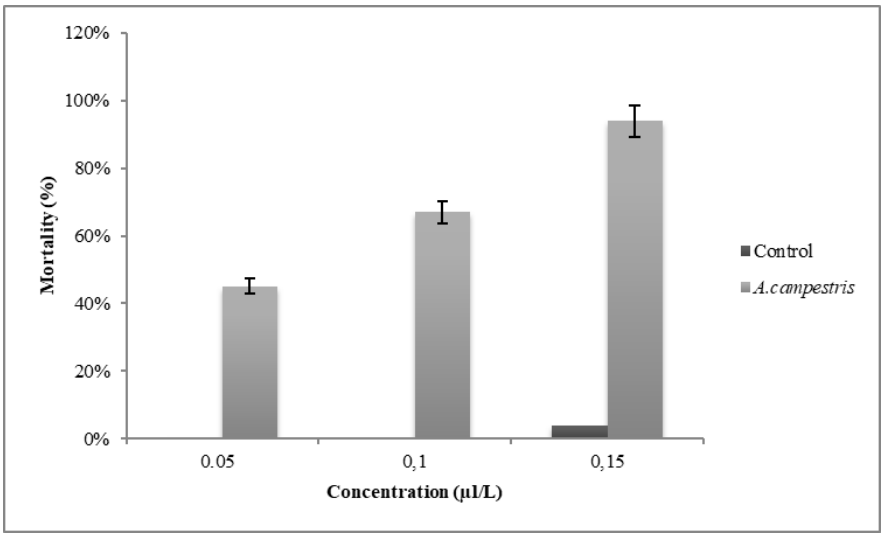

Figure 2: Larvicidal activity of *A. campestris* oil against larvae of *C. capitata.*

Correction mortality of *C. pipiens* and *C. capitata* larvae differed significantly between concentration of *A. campestris* oil (Figure 1and 2), ($p < 0.001$). At a concentration of 0.05 µl/L, between 72 ± 0.12 % of *C. pipiens* larvae and 44 ± 0.56 % of C. capitata larvae. For concentrations 0.1ml/L and 0.15µl/L, the mortality was 100 ± 0.00% for *C. pipiens*. *C. capitata* presented a mortaliy of 71 ± 0.48 % and 94 ± 0.03% for concentrations 0.05µl/L and 0.15 µl/L, respectively. Insecticidal activity of *A. campestris* oil on larvae of *C. pipiens* was more strong than larvae of and *C. capitata*.

Table 2: Larvicidal activity of A. campestris oil applied for 24h and 48h on larvae of C. pipiens and C. capitata.

	Mortality of *C. pipiens* (%)		Mortality of *C. capitata* (%)	
Concentrations (µl/L)	**24h**	**48h**	**24h**	**48h**
0.05	41±0.33	72±0.42	23±0.01	45±0.33
0.1	79±0.06	100±0.00	51±0.45	67±0.73
0.15	92±0.11	100±0.00	61±0.12	94±0.13

The table 2 present the mortality of larvae of *C. pipiens* and *C. capitata* for 24h and 48h. For concentration of 0.05µl/L, we observed a high mortality for larvae of *C. pipiens* compared to larvae of *C. capitata* with 41% and 72% after 24h and 48h respectively, for larvae of *C. pipiens* and 23% and 45% after 24h and 48h respectively, for larvae of *C. capitata*.
Concentration of 0.1µl/L, presented a mortality of 100% for larvae of *C. pipiens* after 48h and was 67% for larvae of *C. capitata*. The last concentrations 0.15µl/L was the most effect on larvae of *C. pipiens* and *C. capitata* with a mortality of 100% and 94%, respectively. *A. campestris* present high larvicidal activity against *C. pipiens* than *C. capitata*.

Table 3: Lethal concentrations (LC50 and LC90) and the median lethal time (TL50) for larvae of *C. pipiens* and *C. capitata*

	LC_{50} (µl/l) P > 0	LC_{90} (µl/l) P > 0	TC_{50} (h)
C. pipiens	0.0410.011	0.0920.013	23h 44mn
C. capitata	0.0630.032	0.1390.014	52h 13mn

Basis on the LC50 and LC90 (Table 3), *A. campestris* oil was more toxic towards *C. pipiens* than *C. capitata*. LC50 was 0.041 and 0.063 for *C. pipiens* and C. capitata, respectively and LC90 was 0.092 and 0.139 for *C. pipiens* and C. capitata, respectively.
TC50 present 23h 44mn to obtain mortality of 50% for larvae of *C. pipiens* and 52h 13mn for larvae of *C. capitata*. Regarding the results of table 2, *A. campestris* oil present a high mortality to larvae of *C. pipiens* and *C. capitata* with low concentration and fast time.

Discussion

The analysis results of GC-MS of *A. campestris* found that the major components are β-pinene (22.32 %),α-pinene (16.13 %), Limonene (13.99 %) and γ-terpinene (13.56 %). According to Touil et al., (2014), the major components found in *A. campestris* oil in Algeria are β-pinene, limonene and γ-terpinene with 20.75%, 10.46%, 10.18%, respectively. Bakchiche et al., (2014) the found as important componds -pinene (25.6%), and Sabinene (17%). In Tunisia, Aichaet al.,(2008), demonstrate as main componds -pinene (41.0%), p-cymene (9.9%), Terpinene (7.9%), and Limonene (6.5%).

Chemical composition and variability are due to many conditions like extraction or distillation technique, the parts of the plant. Also, region, climate, geographic location and harvest season. Essential and aromatic oils produce from the plant are depends on physiological, biochemical, metabolic regulations (Masotti et al., 2003; Figueiredo et al., 2008).

EOs have anti-feedant effects, thus affecting growth, moulting, fertility and development of insects and mites. In general, essential oils are known as neurotoxicants with acute effects interfering with octopaminergic transmitters of arthropods (Bastien, 2008).

Many insects are affected by botanical insecticides depending on physiological characteristics of the insect species.

Also, β-caryophyllene extracted from *F. carica* leaf , is known to stimulate strong antennal response on *C. capitata* (Cossé et al. 1995). Several studies improved the larvicidal activity of the genus Artemisia (Bailen et al., 2013; Julio et al., 2015; Aljaiyash et al., 2018) including tephritidae species.

Rajashekar et al., (2012), present some groups of botanical insecticidal components that are chemosterilants, repellents, attractants, feeding deterrents or antifeedants and toxicants.

Our results are similar to Ramzi et al., (2022) that found the mortality of *C. pipiens* increases with dose and contact time . *A. campestris* have known by its insecticidal activity most important against *C. pipiens* larvae (Masotti et al., 2012; Murugesan et al., 2016; Ammar et al., 2020; Diniz et al., 2020).

The main results of our results is that *A. campestris* oil have a larvicidal activity and can be used to control the most important species of tephritidae familly like *C. capitata* and *C. pipiens*.

References:

[1] Aguiar, R.W.S; Dos Santos, S.F; Da Silva Morgado, F; Ascencio, S.D; De Mendonça Lopes, M; Viana, K.F (2015). Insecticidal and Repellent Activity of *Siparuna guianensis* Aubl. (Negramina) against *Aedes aegypti* and *Culex quinquefasciatus*. PLoS ONE 10(2): e0116765. doi:10.1371/journal. pone.0116765.

[2] Aicha, N; Ines, S; Mohamed, B. S (2008). Chemical composition, mutagenic and antimutagenic activities of essential oils from (Tunisian) *Artemisia campestris* and *Artemisia herba alba*. Journal of Essential Oil Research. (20): 471–477.

[3] Aljaiyash, A., Kasrati, A., Alaoui Jamali, C., Chaouch, A (2018). Effect of cultivation on chemical composition and bioactivities of essential oils from *Artemisia herba-alba* Asso grown in Morocco," Biochemical Systematics and Ecology. (81): 74–79.

[4] 4-Aloui, Z., Messaoud, C., Haoues, M (2016). Asteraceae *Artemisia campestris* and *Artemisia herba-alba* essential oils trigger apoptosis and cell cycle arrest in *Leishmania infantum* promastigotes," Evidence-based Complementary and Alternative Medicine.

[5] Alouani, A;Rehimi, N; Soltani, N (2019). Larvicidal Activity of a Neem Tree Extract (Azadirachtin) Against Mosquito Larvae in the Republic of Algeria. Jordan Journal of Biological Sciences. (2):15 - 22.

[6] Ammar, S; Noui, H; Djamel, S (2020). Essential oils from three Algerian medicinal plants (*Artemisia campestris*, *Pulicaria arabica*, and *Saccocalyx satureioides*) as new botanical insecticides. Environmental Science & Pollution Research. (27): 26594–26604.

[7] Bailen, M; Julio, L.F; Diaz, C. E(2013). Chemical composition and biological efects of essential oils from *Artemisia absinthium* L. cultivated under different environmental conditions. Industrial Crops and Products. (49): 102– 107.

[8] Bakchiche, B; Gherib, A; Maatallah, M; Miguel, M. G (2014). Chemical composition of essential oils of *Artemisia campestris* and *juniperus phoenicea* from Algeria. International Journal of Innovation and AppliedStudies. (9) 4.

[9] Bastien, F (2008). Effet larvicide des huiles essentielles sur *Stomoxys calcitrans* à la Reunion. These docteur veterinaire. Université Paul-Sabatier de Toulouse, France.

[10] Clevenger, J(1928). Apparatus for the Determination of Volatile Oil. J. Am. Pharm. Assoc. 17, 345–349.

[11] Cossé, A.A; Todd, J.L; Millar, J.G; Martinez, L.A; Baker, T.C (1995). Electroantennographic and coupled gas chromatographic-electroantennographic responses of the Mediterranean fruit fly, *Ceratitis capitata*, to male-produced volatiles and mango odor. J Chem Ecol 21(11):1823–1836.

[12] 12-Dib, I; Angenot, L; Mihamou, A; Ziyyat, A; Tits, M (2017). *Artemisia campestris* L.: ethnomedicinal, phytochemical and pharmacological review. Journal of Herbal Medicine. (7): 1–10.

[13] 13- Diniz do Nascimento, L; Moraes, A. A. B. D; Costa, K. S. D (2020). Bioactive natural compounds and antioxidant activity of essential oils from spice plants: new :ndings and potential applications. Biomolecules. (10):988.

[14] 14- Elzen, G. W., Hardee, D. D (2003). United state department of agricultural- research on managing insect resistance to insecticides. Pest Management Science, 59: 770–776.

[15] 15- Erel, S. B., Reznicek, G., Senol, S. G., Yavasoglu, N. ¨U. K., Konyalioglu, S., Zeybek, A. U (2012). Antimicrobial and antioxidant properties of *Artemisia* L. species from western Anatolia. Turkish Journal of Biology, vol. 36.

[16] 16-Figueiredo, A.C; Barroso, J.G; Pedro, L.G; Scheffer, J.J.C (2008). Factors affecting secondary metabolite production in plants: Volatile components and essential oils. Flavour Fragr. J. (23): 213–226.

[17] 17- Finney, G. L (1956). A fortified Carrot Medium for Mass-Culture of the Oriental Fruit Fly and Certain Other Tephritidis. Journal of Economic Entomology. (49): 134.

[18] 18-Ghabbari, M; Guarino, S; G; Caleca, V; Saiano, F; Sinacori, M; Baser, N; Mediouni Ben Jemaa, J; Lo Verde, G (2018). Behavior-modifying and insecticidal effects of plant

extracts on adults of *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae). Journal of Pest Science. https://doi.org/10.1007/s10340-018-0952-6.

[19] 19- Hikal, W. M., Baeshen R.S., Said-Al Ahl. H.A.H| (2017). Botanical insecticide as simple extractives for pest control, Cogent Biology, 3:1. 1404274, DOI: 10.1080/23312025.2017.1404274.

[20] 20-Houicher, A., Hechachna, H., Ozogul, F (2016).In Vitro determination of the antifungal activity of *Artemisia campestris* essential oil from Algeria," International Journal of Food Properties, vol. 19 (8): 1749–1756.

[21] 21- Julio, L. F; Burillo, J; Gimenez, C (2015). Chemical and biocidal characterization of two cultivated *Artemisia absinthium* populations with different domestication levels. Industrial Crops and Products. (76): 787–792.

[22] 22-Masotti, V; Juteau, F; Bessiere, J.M; Viano, J (2003). Seasonal and Phenological Variations of the Essential Oil from the Narrow Endemic Species *Artemisia molinieri* and Its Biologial Activities. J. Agric. Food Chem. (51): 7115–7121.

[23] 23- Masotti, V; De Jong, L; Moreau, X; Rabier, J; Laffont- Schwob, I; Thiery, A (2012). Larvicidal activity of extracts from *Artemisia* species against *Culex pipiens* L. mosquito: comparing endemic versus ubiquist species for effectiveness. ComptesRendusBiologies. (335): 19–25.

[24] 24-McQuate, G.T; Liquido, N.J (2017). Host plants of invasive tephritid fruit fly species of economic importance. International Journal of Plant Biology and Research. 5(4):1072.

[25] 25- Murugesan, S; Gunasekaran, P; Tenzin, G (2016). Laboratory Evaluation of Asteraceae Species *Tagetes erecta* Linnaeus and *Tridax Procumbens* Linnaeus for teir Toxicity against the Larvae of *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae), International Journal of Mosquito Research, Delhi, India.

[26] 26- Pereira, S. G., Sanaveerappanavar, V. T., Murthy, M. S (2006). Geographical variation in the susceptibility of the diamond back moth *Ptlutella xylostella* L. to *Bacillus thuringiensis* products and acylurea compounds. Pest Management, 15: 26–26.

[27] 27- Ramzi, A; Farah, A; EzZoubi, Y; Annemer, S; El Ouali Lalami, A (2022). Aroma profile and fumigant toxicity of two Moroccan Lavandula species essential oils against *Culex pipiens* (Diptera: Culicidae). International Journal of Tropical Insect Science. (42): 2663–2672.

[28] 28- Rajashekar, Y; Bakthavatsalam, N; Shivanandappa, T (2012). Botanicals as grain protectants. Psyche. 1–13.

[29] 29- Regnault-Roger, C., Vincent, C., Arnason, J. T (2012). Essential oils in insect control: Low-risk products in a high-stakes world. Annual Review of Entomology, 57: 405–424. https:// doi.org/10.1146/annurev-ento-120710-100554

[30] 30- Sithisut, D., Fields, P. G., Chandrapathya, A (2011). Contact toxicity, feeding reduction and repellency of essential oils from three plants from the ginger family (Zingiberaceae) and their major components against *Sitophilus zeamais* and *Tribolium castaneum*. The Journal of Stored Products, 104: 1445–1454.

[31] 31-Touil, S; Benrebiha, F.Z (2014). Composition chimique et activité antimicrobienne des huiles essentielles d'*Artemisia herba alba* Asso et *Artemisia campestris* L de la region aride de Djelfa. Agrobiologia. 4: 40-45.

[32] 32-Tripathi, A. K., Prajapati, V., Khanuja, S. P. S., Kumar, S (2003). Effect of d-limonene on three stored-product beetles. Journal of Economic Entomology, 96: 990–995. https://doi.org/10.1093/jee/96.3.990.

[33] 33- Zucchi, R.A (2001). Mosca do Mediterrâneo, *Ceratitis capitata* (Diptera: Tephritidae). In: Vilela EF, Zucchi RA, Cantor F (eds) Historico e impacto das pragas introduzidas no Brasil Holos, Ribeiro Preto, Brazil, pp 15–41.