J Appl Biotechnol Rep. 2020 June;7(2):111-115

doi 10.30491/JABR.2020.107583

Journal of
Applied Biotechnology
Reports

Original Article

Insecticidal Activity of Essential Oils of *Pistacia atlantica* Desf. and *Pistacia lentiscus* L. Against *Tribolium confusum* Dul.

Leila Tabti[1*], Mohammed El Amine Dib[2], Boufeldja Tabti[2], Jean Costa[3], Alain Muselli[3]

[1]Laboratoire d'Ecologie et Gestion des Ecosystèmes Naturels, Faculté des Sciences de la nature et de la vie, et des Sciences de la terre et l'univers, Algérie
[2]Laboratoire des Substances Naturelles et Bioactives (LASNABIO), Université de Tlemcen, BP 119, 13000, Algérie
[3]UMR CNRS 6134, Campus Grimaldi, Université de Corse, Laboratoire CPN, BP 52, 20250 Corte, France

Corresponding Author: Laboratoire d'Ecologie et Gestion des Ecosystèmes Naturels, Faculté des Sciences de la nature et de la vie, et des sciences de la terre et l'univers, Algérie. Tel : +213-0554378192, Email: tabti2008.leila@gmail.com

Received August 5, 2019; **Accepted** December 15, 2019; **Online Published** May 20, 2020

Abstract

Introduction: Since ancient times, the therapeutic virtues of plants have been a part of the traditional pharmacopoeia of several Mediterranean countries, with various uses depending on the country. Among the plants with a great therapeutic potential, *Pistacia lentiscus* L. and *P. atlantica* Desf. (Anacardiaceae), are found in the Mediterranean circum-country. The present study was conducted in order to identify and compare the chemical compositions of the essential oils of *P. atlantica* and *P. lentiscus* as well as to determine their efficiency as a fumigant toxicity for the control of pest insect *Tribolium confusum*.

Materials and Methods: In this study, the aerial parts of the plants were hydrodistilled in a Clevenger-type apparatus. The isolated essential oil was analyzed using gas chromatography (GC) and mass spectrometry (GC/MS). The fumigation toxicity of essential oils was evaluated against the adults of *T. confusum*.

Results: The essential oils of both plants showed qualitative differences in their chemical compositions. The major compounds identified from *P. lentiscus* were (E)-β-caryophyllene (16.3%) and γ-cadinene (15.6%), while from *P. atlantica* was terpinen-4-ol (35.6%). Results of the fumigant tests of the essential oils revealed that the essential oil of *P. lentiscus* was the most toxic. The estimated concentration to kill 50 % of the treated insects (LC_{50}) was 7.5 µL/L air.

Conclusions: The results showed that *P. lentiscus* essential oil presented an interesting fumigant property and that could be proposed as new potential sources of natural bioinsecticides.

Keywords: Bioinsecticides, Essential Oils, *Pistacia*, Beetles, Mills

Citation: Tabti L, El Amine Dib M, Tabti B, Costa J, Muselli A. Insecticidal activity of essential oils of *Pistacia atlantica* Desf. and *Pistacia lentiscus* L. against *Tribolium confusum* Dul. J Appl Biotechnol Rep. 2020;7(2):111-115. doi:10.30491/JABR.2020.107583.

Introduction

In Algeria, different pests cause serious damages on agricultural food and stored products. *Tribolium confusum* is a beetle that infests stored products. It is widely spread throughout the world and ravages mills, silos and grain stores, but it also infests pantries and kitchen cabinets.[1] A large number of aromatic and medicinal plants possess very interesting biological properties, which find applications in various fields namely medicine, pharmacy, cosmetic and agriculture. The therapeutic properties of plants have been known empirically since antiquity and it is only around the beginning of the 20th century that scientists have become interested in active principles and their actions.[2] Faced with the harmful effects of pesticides and insecticides, essential oils are a natural alternative and respectful of the environment to repel insects, avoid punctures, or to eliminate parasites. The most studied families were Lamiaceae, followed by Asteraceae, Myrtaceae, Apiaceae, and Rutaceae.[3] The genus *Pistacia* belongs to the family of Anacardiaceae. In Algeria, the genus *Pistacia* is represented by four species, namely *Pistacia terebinthus, Pistacia vera*, *Pistacia atlantica* and *Pistacia lentiscu*.[4] Several studies have reported the insecticidal activity of *Pistacia* genus essential oils. The *P. lentiscus* essential oil from Tunisia has been reported to be effective against pest insects similar to the two moths *Ephestia ceratoniae* and *Ephestia kuehniella*.[5,6] The study of the chemical composition of gum, fruit and leaves essential oils of *P. atlantica* subsp. *kurdica* on the fumigant toxicity against *Tribolium beetles* showed the gum essential oil was more toxic than fruits and leaves.[7] The present study was conducted in order to identify and compare the chemical compositions of the essential oils of *P. atlantica* and *P. lentiscus* as well as their fumigant toxicity for the control of pest insect *Tribolium confusum*.

Materials and Methods

Plant Material and Extraction of the Oils

Aerial parts of *P. lentiscus* and *P. atlantica* were collected in May 2017 from the Tiaret region, Algeria. The freshly harvested leaves, were dried in the shade in a dry and ventilated place. The samples were botanically identified by Pr. Noury Benabadji, Laboratory of Ecology and Ecosystem Management Tlemcen (Algeria). Voucher specimens were deposited with the Herbarium of the University of Tlemcen. *P. lentiscus* (P.le.05/2017) and *P. atlantica* (P.al. 05.2017). The oils were isolated by hydrodistillation (400–450 g) for 4 hours using a Clevenger-type apparatus according to the European Pharmacopoeia and yielded 0.19% for *P. atlantica* and 0.12% for *P. lentiscus* w/w of oil. The oils were dried over anhydrous sodium sulfate and were then stored in sealed glass vials at 4°C prior to analysis.

Analysis Conditions

The gas chromatography (GC) analyses were carried out using a Perkin Elmer Autosystem XL GC apparatus equipped with a dual flame ionization detection system and fused Rtx-1 silica capillary columns (60 m × 0.22 mm i.d., 0.25 µm film thickness; polydimethylsiloxane). The oven temperature was programmed to increase from 60-230°C at 2°C/min and was then held isothermally at 230°C for 35 minutes. Injector and detector temperatures were maintained at 280°C. The injection volume was 0.1 µL, as previously reported by Benyoucef et al.[8]

Gas Chromatography-Mass Spectrometry Analysis

Samples were analyzed using a Perkin Elmer Turbo mass detector (quadrupole) coupled to a Perkin Elmer Autosystem XL equipped with Rtx-1 fused silica capillary columns and Rtx-Wax (ion source temperature, 150°C; ionization energy, 70 eV). Ionization energy MS was acquired over a mass range of 35–350 Da (scan time, 1 s). Other GC conditions were the same as described for GC, except the split which was 1/80, as previously reported by Benyoucef et al.[8]

The identification of each compound of the mixtures was carried out by comparison: (*i*) retention indices calculated respectively on polar and apolar columns with those of standard compounds (laboratory library "Arôme") or those reported in the literature; (*ii*) mass spectra (electronic impact) with those of standard compounds (laboratory library) or those present in computerized banks.[9,10] All components were identified by comparing their mass spectra (EI-MS) and retention indices (RIs) with those of mass-spectral library without complementary analysis.

Quantification of constituents was performed using a Flame Ionization Detector by internal standardization of peak areas. This was done using the calculated response factors relative to the tridecane (0.7 g.100 g^{-1}) which was used as an internal standard according to the method described by Benyoucef et al[8] and adapted within the CPN laboratory.

Fumigation Toxicity of Essential oils Against *Tribolium confusum*

To determine the fumigant toxicity of essential oils, appropriate concentrations, were applied separately on the filter papers (Whatman No. 1, 2 cm diameter) to achieve the concentrations ranged from 5 to 20 µL/L air without using any solvent, and the filter papers were attached to under the surface of the lids of plastic jars with 50-mL volumes. The control sets received no oil. The lids were screwed tightly on the jars containing 15 insects, each all of the same age. These were kept at a temperature of 25-26°C and 65% relative humidity.[11] The mortality was checked after 24 hours from the commencement of exposure. The mortality of insects was expressed in % and calculated by using the Abbott correction formula.

Corrected Mortality = (Observed mortality in treatment -Observed mortality in control/100-Control mortality) × 100

Percentage Mortality = (Number of dead larvae / Number of introduced larvae) × 100

Tests were carried out in triplicate.

Statistical Analysis

Statistical analysis of variance (ANOVA) was performed using the SAS software and means were separated using the least significant difference (LSD) test at $P \leq 0.05$. The LC_{50} and LC_{90} values were calculated using probit analysis. Analysis of each test was performed in triplicate.

Results

Chemical Composition of Essential Oils

The essential oil compositions (%) of *P. atlantica* and *P. lentiscus* are listed in Table 1. In *P. lentiscus* essential oil, 34 compounds were identified, accounting for 95.3% of the total oil. The investigated Algerian *P. lentiscus* essential oil consists chiefly of sesquiterpene hydrocarbons (57.2%), accompanied by oxygenated sesquiterpenes (13.7%), monoterpene hydrocarbons (12.5%), and oxygenated monoterpenes (11.5%). The major compounds were (E)-β-caryophyllene (16.3%), γ-cadinene (15.6%), α-terpineol (9.2%), γ-muurolene (6.9%), α-humulene (6.3 %), α-muurolene (5.9%), caryophyllene oxide (5.8%) and τ-muurolol (5.3%) (Table 1).

The essential oil of *P. atlantica* contained 25 compounds, and chiefly consisted of oxygenated monoterpenes (37.1%) and monoterpene hydrocarbons (31.6%). The main components of this oil were terpinen-4-ol (35.6%), followed by bicyclogermacrene (7.8%), α-pinene (6.8%), germacrene-D (6.2%) and Sabinene (5.8%) (Table 1).

Insecticidal Activity

The results of the fumigation toxicity of the essential oils of *P. lentiscus* and *P. atlantica* against *T. confusum* insect are summarized in Figure 1. The efficacy of essential oils varied with their concentrations. The values of insects mortality expressed as percentage (%) caused by the both essential oils were significantly different compared to the control ($P \leq 0.05$). The essential oil of *P. lentiscus* showed a better toxicity than *P. atlantica* essential oil. At 20 µL/L air, the essential oil of *P. lentiscus* caused a mortality of 100%, while, the essential oil of *P. atlantica* of *P. atlantica* with the same concentration caused a mortality of 90% after 24 hours of exposure

Table 1. Chemical Composition of Essential Oils of the Aerial Parts of the *P. lentiscus* and *P. atlantica*

N°[a]	Compounds	lRIa[b]	Ria[c]	Rip[d]	*P. lentiscus*	*P. atlantica*	Identification[e]
01	α-Thujene	921	923	1023	-	0.5	RI, MS
02	α-Pinene	931	931	1022	2.3	6.8	RI, MS
03	Camphene	943	943	1066	0.8	0.9	RI, MS
04	Sabinene	964	965	1120	0.1	5.8	RI, MS
05	β-Pinene	970	971	1110	0.3	3.4	RI, MS
06	Myrcene	977	979	1159	0.1	1.3	RI, MS
07	α-Phellandrene	997	980	1164	0.3	0.3	RI, MS
08	α-Terpinene	1008	1007	1178	-	2.2	RI, MS
09	β-Phellandrene	1020	1027	1208	-	1.9	RI, MS
10	P Cymene	1012	1020	1254	2.2	1.6	RI, MS
11	Limonene	1023	1020	1199	2.6	-	RI, MS
12	(E)-β-Ocimene	1034	1030	1245	0.4	0.4	RI, MS
13	γ-Terpinene	1048	1056	1241	1.5	4.9	RI, MS
14	(E)-Sabinene hydrate	1053	1053	1454	-		RI, MS
15	Terpinolene	1078	1086	1282	1.4	1.6	RI, MS
16	cis-Sabinene hydrate	1083	1080	1541	0.5	-	RI, MS
17	Linalol	1082	1090	1576	0.2	-	RI, MS
18	Terpinen-4-ol	1161	1161	1602	0.2	35.6	RI, MS
19	α-Terpineol	1179	1176	1600	9.2		RI, MS
20	Bornyl acetate	1269	1278	1579	1.9	1.5	RI, MS
21	Undecan-2-one	1270	1278	1604	0.4	-	RI, MS
22	α-Copaene	1379	1385	1515	2.1	-	RI, MS
23	β-Elemene	1389	1392	1553	0.9	-	RI, MS
24	(E)-β-Caryophyllene	1421	1428	1590	16.3	1.5	RI, MS
25	α-Humulene	1456	1454	1668	6.3	-	RI, MS
26	Allooromadendrene	1462	1456	1640	1.5	0.6	RI, MS
27	α-Muurolene	1494	1507	1685	5.9		RI, MS
28	γ-Muurolene	1515	1512	1750	6.9	-	RI, MS
29	Germacrene-D	1480	1488	1705	-	6.2	RI, MS
30	Bicyclogermacrene	1494	1504	1722	0.4	7.8	RI, MS
31	γ-Cadinene	1507	1513	1750	15.6	0.2	RI, MS
32	δ-Cadinene	1516	1524	1754	1.3	1.2	RI, MS
33	Spathulenol	1557	1570	2120	0.8	3.1	RI, MS
34	Caryophyllen oxide	1576	1583	1982	5.8	-	RI, MS
35	Globulol	1589	1590	2068	0.4	1.8	RI, MS
36	Viridiflorol	1591	1589	2085	0.2	-	RI, MS
37	τ-Cadinol	1632	1635	2134	0.9	1.2	RI, MS
38	τ-Muurolol	1634	1638	2145	5.3	-	RI, MS
39	α-Cadinol	1645	1650	2227	0.3	1.6	RI, MS
	% Identification				**95.3**	**93.9**	
	Monoterpene hydrocarbons				12.5	31.6	
	Oxygenated monoterpenes				11.5	37.1	
	Sesquiterpene hydrocarbons				57.2	17.5	
	Oxygenated sesquiterpenes				13.7	7.7	
	Non-terpenic compounds				0.4	-	

[a] Order of elution is given on apolar column (Rtx-1). [b] Retention indices of literature on the apolar column (lRIa). [c] Retention indices on the apolar Rtx-1 column (RIa). [d] Retention indices on the polar Rtx-Wax column (RIp). [e] RI: Retention Indices; MS: Mass Spectra in electronic impact mode.

(Figure 1). After probit analysis, the LC_{50} and LC_{90} values of the *P. lentiscus* essential oil against *T. confusum* were 7.5 and 15 µL/L air, respectively, while those for *P. atlantica* essential oil were 15 and 20 µL/L air, respectively. Based on LC_{50} values, the *P. lentiscus* essential oil was 2-fold more toxic compared to *P. atlantica* essential oils (Table 2).

Corrected Mortality Rates

The corrected mortality after 24 hours is listed in Table 3. The fumigation toxicity of *P. lentiscus* essential oil was higher than that of the *P. atlantica* essential oil, with significant differences. At the dose of 10 µL/L air, the corrected mortality of *P. lentiscus* (60%) was almost 2-fold more toxic of *P. atlantica* (30.33%).

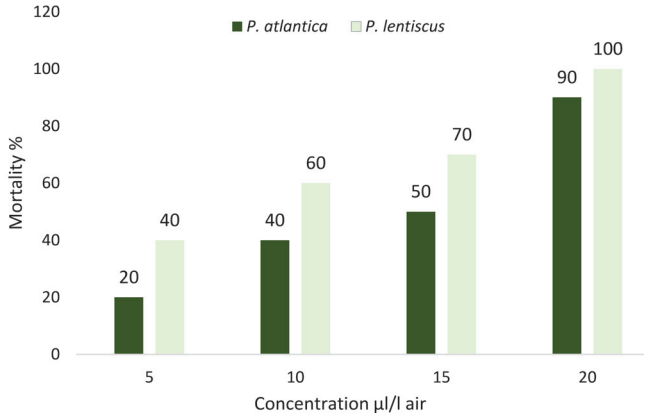

Figure 1. Fumigant Toxicity of Different Concentrations of the Essential Oils of *P. atlantica* and *P. lentiscus* Against *T. confusum* for 24 Hours at 25±1°C ($P \leq 0.05$).

Table 2. LC_{50} and LC_{90} of Essential Oils of *P. atlantica* and *P. lentiscus* against *T. confusum* for 24 Hours at 25±1°C

Insect (N=15)	24-LC_{50}	Slope ± SE	df	Chi-square	24-LC_{90}
P. atlantica	15 ± 1.1	1.21 ± 0.26	2	1.9*	20 ± 1.5
P. lentiscus	7.5 ± 0.8	1.36 ± 0.24	2	0.89*	18.5 ± 1.2

The fumigant toxicity is expressed as the concentration (µL/L air) to kill 50% (LC_{50}) and 90% (LC_{90}) of insects treated.
N: Number of tested insects.
*Since Chi square goodness of fit test is not significant ($P > 0.15$), no heterogeneity factor is used in the calculation of fiducial limits.

Table 3. Determination of Corrected Mortality of Essential Oils of P. atlantica and *P. lentiscus* Against *T. confusum*

Concentrations (µL/L air)	*P. atlantica*	*P. lentiscus*
10	30.33 ± 0.86	60 ± 1.56
15	44.44 ± 1.12	70 ± 1.33
20	88.88 ± 2.18	90 ± 2.43

However, at higher concentrations, the corrected mortality *of P. atlantica* and *P. lentiscus* essential oils were 88.88% and 90%, respectively.

Discussion

Due to the tremendous toxicity of synthetic insecticides to humans and/or animals, plants have gained tremendous power and importance as bioinsecticides in recent years. Many essential oils from various plants have been studied for their repellent properties. Generally, the bioactivity of essential oils is influenced by their chemical composition and are therefore considered as an important aspect to consider before recommending in a pest control program.[12,13] In the present investigation, a total of 34 compounds comprising 95.3% of the oil were identified from the essential oil of *P. lentiscus*, (E)-β-caryophyllene, γ-cadinene and α-terpineol being the major components, comprising 41.1% of the essential oil. This study agrees with the findings of Lo Presti et al[14] and Mecherara-Idjeri et al,[15] who reported β-caryophyllene and γ-cadinene to be the major components of *P. lentiscus* essential oil. A comparison between the composition of *P. atlantica* aerial part essential oil from Algeria with those reported in the literature[16,17] reveals the existence of significant similarities for the main component percentages. Based on the results of the present study, *P. lentiscus* essential oil was found to be effective against *T. confusum* insect pests, as 100% mortality was achieved at a concentration of 20 µL/L air. Results demonstrated that *P. lentiscus* essential oil was characterized by the presence of (E)-β-caryophyllene, γ-cadinene, α-terpineol as major compounds. The action of this essential oil could be attributed to its major compounds. Indeed, (E)-β-caryophyllene is an active and volatile molecule that would act by contact on the seed coat of insects.[18] Moreover, in the previous studies, α-terpineol have been found to possess strong fumigant toxicity against *T. confusum*,[19] while δ-Cadinene have been found to possess strong fumigant toxicity against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*.[20] In the present study, *P. atlantica* essential oil also demonstrated fumigant toxicity against *T. confusum*. By comparing both essential oils based on LC_{50}, it was clear that the *P. atlantica* oil was twice less toxic and 50%of the treated pests could be killed with 15 µL/L air by fumigation. However, the results, the insecticide activity of the essential oil may be in agreement with the concentration of Terpinene-4-ol.[21] Many of the minor compounds of *P. lentiscus* and *P. atlantica*, such as limonene, cymene, α-pinene, and β-pinene, are also well known for their toxicity to insects.[7,22,23]

Conclusions

In the current study, the chemical composition of the essential oils of *P. lentiscus* and *P. atlantica* from Algeria was investigated. The chemical composition of *P. lentiscus* essential oil has been characterized by a significant amount of sesquiterpene hydrocarbons, while, *P. atlantica* essential oil has been characterized by a mixture of monoterpene hydrocarbons and oxygenated monoterpenes compounds. The results of the insecticidal activity showed that the essential oils presented a good insecticide activity. This activity may be partly due to major components or to the effect caused by the interaction of all constituents present in the oils. The present data confirm the usefulness of these essential oils as an insecticide activity. However, further large-scale investigations are required to evaluate its efficacy in the food system.

Authors' Contributions

TL prepared the samples, results interpretation and analyzed the chemical composition; MAD wrote the manuscript; TB performed the antioxidant activities test; JC and AM supervised the project.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

References

1. Mohammad OS, Ali WK, Al-Hulitan AT. The effect of infestation by the confused flour beetle (*Tribolium confusum* Duv.) on specifications of wheat flour. J Agric Sci Tech-Iran. 2012;2:696-701.
2. Petrovska BB. Historical review of medicinal plants' usage. Pharmacogn Rev. 2012;6(11):1-5. doi:10.4103/0973-7847.95849.
3. Campolo O, Giunti G, Russo A, Palmeri V, Zappalà L. Essential oils in stored product insect pest control. J Food Qual.

2018;2018:6906105. doi:10.1155/2018/6906105.
4. Quézel P, Santa S. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Paris: Éditions du Centre National de la Recherche Scientifique; 1963.
5. Bachrouch O, Mediouni-Ben Jemâa J, Wissem AW, Talou T, Marzouk B, Abderraba M. Composition and insecticidal activity of essential oil from *Pistacia lentiscus* L. against Ectomyelois ceratoniae Zeller and Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). J Stored Prod Res. 2010;46(4):242-247. doi:10.1016/j.jspr.2010.07.001.
6. Bachrouch O, Mediouni-Ben Jemâa J, Talou T, Marzouk B, Abderraba M. Fumigant toxicity of *Pistacia lentiscus* essential oil against *Tribolium castaneum* and *Lasioderma serricorne*. Bull Insectology. 2010;63(1):129-135.
7. Sadeghi A, Pourya M, Smagghe G. Insecticidal activity and composition of essential oils from *Pistacia atlantica* subsp. *kurdica* against the model and stored product pest beetle *Tribolium castaneum*. Phytoparasitica. 2016;44(5):601-607. doi:10.1007/s12600-016-0551-0.
8. Benyoucef F, Dib MEA, Arrar Z, Costa J, Muselli A. Synergistic antioxidant activity and chemical composition of essential oils from *Thymus fontanesii, Artemisia herba-alba* and *Rosmarinus officinalis*. J Appl Biotechnol Rep. 2018;5(4):151-156. doi:10.29252/jabr.05.04.03.
9. Jennings W, Shibamoto T. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography. New York: Academic Press; 1980.
10. König WA, Hochmuth DH, Joulain D. Terpenoids and Related Constituents of Essential Oils. Hamburg: Institute of Organic Chemistry; 2001.
11. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925;18(2):265-267.
12. Stojanović-Radić Z, Čomić L, Radulović N, Blagojević P, Mihajilov-Krstev T, Rajković J. Commercial Carlinae radix herbal drug: botanical identity, chemical composition and antimicrobial properties. Pharm Biol. 2012;50(8):933-940. doi:10.3109/13880209.2011.649214.
13. Khanikor B, Parida P, Yadav RNS, Bora D. Comparative mode of action of some terpene compounds against octopamine receptor and acetyl cholinesterase of mosquito and human system by the help of homology modeling and docking studies. J Appl Pharm Sci. 2013;3(2):6-12. doi:10.7324/japs.2013.30202.
14. Lo Presti M, Sciarrone D, Crupi ML, et al. Evaluation of the volatile and chiral composition in *Pistacia lentiscus* L. essential oil. Flavour Fragr J. 2008;23(4):249-257. doi:10.1002/ffj.1878.
15. Mecherara-Idjeri S, Hassani A, Castola V, Casanova J. Composition and chemical variability of the essential oil from *Pistacia lentiscus* L. growing wild in Algeria part I: leaf oil. J Essent Oil Res. 2008;20(1):32-38. doi:10.1080/10412905.2008.9699415.
16. Tzakou O, Bazos I, Yannitsaros A. Volatile metabolites of *Pistacia atlantica* Desf. from Greece. Flavour Fragr J. 2007;22(5):358-362. doi:10.1002/ffj.1805.
17. Barrero AF, Herrador MM, Arteaga JF, et al. Chemical composition of the essential oils of *Pistacia atlantica* Desf. J Essent Oil Res. 2005;17(1):52-54. doi:10.1080/10412905.2005.9698828.
18. Komla S, Koffi K, Wiyao P, Ninkabou I, Binde AA. Pesticidal properties of *Cymbopogon schoenanthus* against the Diamonback Moth *Plutella xylostella* L. (Lepidoptera; Hyponomeutidae). Discov Innov. 2006;18(3):220-225. doi:10.4314/dai.v18i3.15748.
19. Sener O, Arslan M, Demirel N, Uremis I. Insecticidal effects of some essential oils against the confused flour beetle (*Tribolium confusum* du Val) (Col.: Tenebrinoidea) in stored wheat. Asian J Chem. 2009;21(5):3995-4000.
20. Govindarajan M, Rajeswary M, Benelli G. delta-Cadinene, calarene and delta-4-carene from *Kadsura heteroclita* essential oil as novel larvicides against malaria, dengue and filariasis mosquitoes. Comb Chem High Throughput Screen. 2016;19(7):565-571. doi:10.2174/1386207319666160506123520.
21. Abbassy MA, Abdelgaleil SAM, Rabie RYA. Insecticidal and synergistic effects of *Majorana hortensis* essential oil and some of its major constituents. Entomol Exp Appl. 2009;131(3):225-232. doi:10.1111/j.1570-7458.2009.00854.x.
22. Lee BH, Choi WS, Lee SE, Park BS. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). Crop Prot. 2001;20(4):317-320. doi:10.1016/S0261-2194(00)00158-7.
23. Lee BH, Lee SE, Annis PC, Pratt SJ, Park BS, Tumaalii F. Fumigant toxicity of essential oils and monoterpenes against the red flour beetle, *Tribolium castaneum* Herbst. J Asia Pac Entomol. 2002;5(2):237-240. doi:10.1016/S1226-8615(08)60158-2