Fumigant and Repellent Activities of Different Essential Oils Alone and Combined Against the Maize Weevil (Sitophilus Zeamais Motschulsky)

Abstract

The insecticidal and repellent activity of five essential oils (EOs) was studied separately and in binary combinations against Sitophilus zeamais. Minthostachys verticillata EO showed the highest fumigant activity with a LC_{50} value of 28.2 µl/l air. A moderate toxicity was observed with Eucalyptus globulus EO (LC_{50} = 335.7 µl/l air), whereas the EOs from Aloysia citriodora, Coriandrum sativum and Mentha sp. did not show insecticidal effect at 600 µl/l air. All combinations that include M. verticillata EO showed strong fumigant activity with LC_{50} values lesser than 78 µl/l air. The co-toxicity coefficient (CCT) of M. verticillata and E. globulus EO combination indicating an aditive effect (CTC = 119.1). Repellent activity was evaluated using two-choice olfactometer assay. All EOs and their combinations had repellent effect on adults of S. zeamais ($P < 0.05$).

Keywords: Biopesticides; Fumigant toxicity; Repellent effect; Stored maize pest

Research Article

Volume 1 Issue 7 - 2017

Julieta Soledad Arena[1,2,3], María Laura Peschiutta[1,2]*, Helena Calvimonte[1] and Julio Alberto Zygadlo[1,2]

[1]*Departamento de Química, Universidad Nacional de Córdoba, Argentina*

[2]*CONICET-Universidad Nacional de Córdoba, Instituto Multidisciplinario de Biología Vegetal (IMBIV), Argentina*

[3]*Universidad Nacional de Córdoba, Argentina*

***Corresponding author:** María Laura Peschiutta, Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET-Universidad Nacional de Córdoba, Avenida Vélez Sarsfield 1611, X5016GCA, Córdoba, Argentina, Tel: +54 351 152023705; Email: mlaurapeschiutta@gmail.com

Received: November 23, 2017 | **Published:** December 22, 2017

Abbreviations: EO: Essential Oil; ANOVA: Analysis of Variance; CTC: Co-Toxicity Coefficients; DDVP: 2,2-Dichlorovinyl Dimethyl Phosphate; RI: Retention Index; GC-MS: Gas Chromatography-Mass Spectrometry; EI-MS: Electron Impact Mass Spectra; TI: Toxicity Index

Introduction

The maize weevil Sitophilus zeamais Motschulsky (Coleptera: Curculionidae) is a worldwide primary pest of stored maize. Both larvae and adults feed on corn grains reducing their weight, nutritional value, commercial value and germination rate [1]. The damage produced on grains also favors the occurrence of secondary pests and fungi [1,2]. Fumigation is the most widespread method to control stored-product pests, however the overuse of conventional fumigants including phosphine and methyl bromide has brought some problems such as the development of resistance by insects, environmental pollution and negative effects on non-target organisms and human health [3,4]. Consequently, the interest in generating different strategies of control has been increased.

Essential oils (EOs) are complex mixtures of volatile secondary metabolites produced by aromatic plants [5]. They constitute an important source of bioactive chemicals [6] and provide interesting alternatives to conventional insecticides due to their limited persistence on the environment low mammalian toxicity and low probability of generate resistance [7,8]. Numerous studies have demonstrated that EOs have a great potential as insecticides and repellents [8,9]. Furthermore, many EOs or their constituents were studied for their fumigant and repellent effect on maize pests, including S. zeamais [10-12].

The activity of an EO generally depends on its major constituents, but sometimes the sum of the activities of individual constituents does not explain the overall activity of the oil, evidencing synergistic or antagonistic effects [13]. These effects also occur among constituents of different EOs [14]. Therefore, combinations of EOs could significantly enhance their biological activity [7,15]. For example, Benelli et al. [16] observed that the binary mixture of EOs from Satureja montana L. and Aloysia citriodora Palau has higher larvicidal toxicity than the individual oils against Culex quinquefasciatus Say.

The aim of the current study was to evaluate the fumigant and repellent activities of EOs from some locally available plants: Aloysia citriodora Palau (Verbenaceae), Coriandrum sativum L. (Apiaceae), Eucalyptus globulus Labill (Myrtaceae), Mentha sp. (Lamiaceae) and Minthostachys verticillata (Griseb.) Epling (Lamiaceae), separately and in binary combinations, against S. zeamais.

Materials and Methods

Essential oils

Leaves of A. citriodora, E. globules, Mentha sp. and M. verticillata and C. sativum seeds were collected in commercial crops in Córdoba, Argentina. The samples were air dried and subjected to hydro-distillation for 2 hours in a Clevenger's apparatus in order to extract their vaporized EOs, which were stored in dark glass tubes under refrigeration (4°C) until evaluation.

Identification of the EOs constituents was determined using electron impact mass spectra (EI-MS) obtained from gas chromatography-mass spectrometry (GC-MS), and by co-injection of standards (Sigma Aldrich Co. Buenos Aires, Argentina), with the mass spectra libraries Adams, NIST and a homemade library being utilized. Compound concentrations were expressed as a percentage of the peaks area, and the retention index (RI) of each compound was obtained for a homologous series of n-alkanes C_9-C_{20} (Sigma Aldrich Co. Buenos Aires, Argentina). Identifications were made by matching both their mass spectra and (RI) values with those reported in the literature and those of pure compounds, whenever possible. GC-MS was performed on a GC-MS Perkin Elmer 600, equipped with a mass selective detector in the electron impact mode (70 eV). The chromatography conditions being as follows: DB-5 capillary column (30 m x 0.25 mm, film thickness 0.25 mm), the oven temperature was programmed linearly at 60°C for 5 minutes, ramped up to 170°C at 4°C/minute, and then to 240°C at 20°C/minute; injector temperature 250°C; detector temperature 250°C; carrier gas, H_2 at 45 cm/second, split into 50 ml/minute and samples of 1 µL (1/100 in n-heptane, v/v) injected manually in the split-less mode.

Insects

Sitophilus zeamais adults were obtained from Metán, Salta, Argentina. Insects were maintained in sealed containers (10 l) with whole maize grains under controlled conditions (26°C and 60 % relative humidity), in darkness. The colony was kept in our laboratory for two years without exposure to insecticides before testing. The unsexed adult weevils used in all the experiments were approximately 2 weeks old.

Fumigant toxicity assay

Susceptibility of S. zeamais adults to volatile compounds from A. citriodora, C. sativum, E. globulus, Mentha sp. and M. verticillata EOs and all their possibly binary combinations were evaluated using fumigant toxicity assay described by Peschiutta et al. [17] with some modifications. Different doses (10-600 µl/l air) of the EOs or their combinations were applied to Whatman filter paper disks of 2 cm diameter placed on the underside of the screw cap of a fumigation chamber (30 ml-glass vial). The EOs was mixed in 1:1 ratio (v/v) in all binary combinations. A piece of voile was also placed under the screw cap to avoid direct contact of the weevils with the EOs. In each vial 5 g of whole maize grains were deposited in order to mimic the natural conditions in a silo. Ten adults of S. zeamais were placed in each fumigation chamber. Control treatments were performed without EO (negative control) and with 2,2-dichlorovinyl dimethyl phosphate (DDVP) at 0.06 µl/l air (positive control). The assays were carried out in complete darkness at 28°C and 60 ± 5% relative humidity. Five replicates per dose were performed and insect mortality was recorded at 24 hours. Co-toxicity coefficients (CTC) were calculated according to Sun et al. [18] to evaluate the effect of the EO combinations. Considering that C indicates the combination of two EOs, and A and B indicates the combined EOs, the CTC were obtained using the following formulas:

Toxicity index of A (TI of A) (using A as standard) = 100

Toxicity index of B (TI of B) = LC_{50} of A / LC_{50} of B × 100

Actual TI of C = LC_{50} of A / LC_{50} of C × 100

Theoretical TI of C = TI of A × proportion of A in C +TI of B x proportion of B in C

CTC = Actual TI of C / Theoretical TI of C × 100

The EO which presented the lesser LC_{50} value was considered as the standard (A). CTC < 80, 80 < CTC < 120 and CTC > 120 indicate antagonism, additive effect and synergism respectively [19].

Two-choice olfactometer assay

The repellent activity of the EOs and their combinations were evaluated against S. zeamais. Behavioral response of S. zeamais adults to these compounds was measured using two-way olfactometer [20]. Two 250 ml-Erlenmeyer were connected by a glass tube 30 x 1 cm diameter in which was opened a small window 1 x 1 cm equidistant from the two Erlenmeyer flasks. Corn kernels (6 g) and a filter paper of 2 cm diameter with the test compound (treatment) or with the solvent alone (control) were placed in each Erlenmeyer flask. The EOs and combinations were tested at 4 µl/l air. Twenty insects deprived of food for at least 12 hours were placed in the center of the tube through the window made for that purpose, which subsequently was closed. The experiments were performed under dark conditions at 28°C and 60 ± 5% relative humidity. The number of insects in each container was recorded after 90 minutes. The experiment was repeated five times per dose. For each test the response index (RI) was calculated with the following equation: RI = [(T-C) / Tot] × 100, where T is the number responding to treatment, C is the number responding to control, and Tot is the total number of insects released [21]. Positive RI indicates attraction to the treatment and negative RI indicates repellency.

Statistical analysis

The concentration-mortality data recorded after 24 hours of exposure to the EOs was subjected to a statistical analysis using the log-logistic model available in the "drc" package [22] and compiled by the statistical software R® [23]. Lethal concentrations causing 50 and 95% of mortality (LC_{50} and LC_{95}) were determined, as well as their confidence limits at 95%.

The significance of the mean RI in each treatment of the two-choice olfactometer bioassay was evaluated by the Student's t test for paired comparisons [21]. Mean values of RI were first analyzed by analysis of variance (ANOVA) followed by a Dunnett's test ($P < 0.05$).

Results and Discussion

The composition of EOs from A. citriodora, C. sativum, E. globulus, Mentha sp. and M. verticillata are shown in Table 1. According to the analysis the main components were geranial

(43.43 %) and nerol (28.89 %) in A. citriodora EO; linalool (93.81 %) in C. sativum EO; 1,8-cineole (32.18 %) and p-cymene (17.04 %) in E. globulus EO; carvone (76.14 %) in Mentha sp. EO; and pulegone (57.09 %) and menthone (36.36 %) in M. verticillata EO.

Table 1: Relative percentage concentrations of the components of the essential oils.

RI (Literature)	RI (Calculated)	Compound Names	Minthostachys Verticillata	Coriandrum Sativum	Aloysia Citriodora	Eucalyptus Globulus	Mentha sp.	Methods of Identification
924	928	α-thujene				2.33		GC-MS, RI
932	935	α-pinene	0.27	0.8	0.17	0.83		GC-MS, RI, Co
969	972	sabinene	0.15				0.32	GC-MS, RI, Co
974	973	1-octen-3-ol			2.71			GC-MS, RI
974	978	β-pinene	0.37			1.25	0.65	GC-MS, RI, Co
988	984	β-myrcene	tr			1.06	0.3	GC-MS, RI
1002	1005	α-phellandrene				9.68		GC-MS, RI
1020	1023	p-cymene	tr	0.52	0.37	17.04	tr	GC-MS, RI, Co
1024	1027	limonene	0.86	0.97	4.56	tr	3.46	GC-MS, RI
1026	1032	1,8-cineole	0.31			32.18	5.19	GC-MS, RI, Co
1054	1056	g-terpinene		1.44		1.07		GC-MS, RI
1086	1084	terpinolene				0.34		GC-MS, RI
1095	1093	linalool		93.81		1.51		GC-MS, RI
1100	1103	undecane			0.23			GC-MS, RI
1137	1150	cis-verbenol			1.97			GC-MS, RI
1141	1151	camphor		2.41				GC-MS, RI
1148	1164	menthone	36.36				0.22	GC-MS, RI
1158	1165	isomenthone	1.7			0.34		GC-MS, RI
1159	1167	menthofuran					0.75	GC-MS, RI
1165	1177	borneol					0.49	GC-MS, RI
1167	1181	isopulegone	0.79					GC-MS, RI
1174	1184	4-terpineol	0.12			5.89		GC-MS, RI
1183	1193	cryptone				9.13		GC-MS, RI
1186	1201	α-terpineol				3.65		GC-MS, RI
1191	1201	cis-dihydrocarvone					2.2	GC-MS, RI
1193	1208	dihydro carveol neo-iso					0.46	GC-MS, RI
1226	1235	carveol cis					0.33	GC-MS, RI
1233	1240	pulegone	57.09			tr	0.41	GC-MS, RI
1235	1243	neral			28.89			GC-MS, RI
1238	1249	cuminaldehyde				2.16		GC-MS, RI
1239	1261	carvone					76.14	GC-MS, RI
1249	1266	piperitone	0.56					GC-MS, RI

1264	1274	geranial			43.43			GC-MS, RI
1274	1285	phellandral				1.97		GC-MS, RI
1283	1349	α-terpinen-7 al	0.52					GC-MS, RI
1374	1378	α-copaene			0.29			GC-MS, RI
1387	1388	β-bourbonene			0.36		0.88	GC-MS, RI
1410	1420	α-cedrene					0.86	GC-MS, RI
1418	1426	β-cariophyllene			1.84		2.78	GC-MS, RI
1434	1427	g-elemene				1.42		GC-MS, RI
1451	1460	allo-aromadendrene			0.35			GC-MS, RI
1452	1464	α-humulene					0.25	GC-MS, RI
1454	1469	cis β-farmasene					tr	GC-MS, RI
1475	1477	g-gurjunene					tr	GC-MS, RI
1478	1480	g-muurolene					4.11	GC-MS, RI
1479	1481	α-curcumene			2.3			GC-MS, RI
1484	1487	germacrene d			0.46			GC-MS, RI
1522	1522	δ-cadinene			0.4			GC-MS, RI
1577	1593	spathulenol	0.66		4.35			GC-MS, RI
1582	1595	cariophyllene oxide	0.14		5.8			GC-MS, RI
1677	1660	nerolidol acetate			1.51			GC-MS, RI
		Total	99.9	99.95	99.99	91.85	99.8	

tr: traces (<0.1%).

The fumigant activity of the EOs and their binary combinations was evaluated against adults of S. zeamais. Minthostachys verticillata EO showed the highest fumigant toxicity with a LC_{50} value of 28.2 µl/l air (Table 2). A moderate toxicity was observed with E. globulus EO (LC_{50} = 335.7 µl/l air), whereas the EOs from A. citriodora, C. sativum and Mentha sp. did not show fumigant activity at 600 µl/l air. Similarly, Herrera et al. [11] found that the EO from M. verticillata was the most bioactive among the tested EOs against S. zeamais, however they registered a higher LC_{50} value that could be attributed to the natural variation in the composition of the EOs. In another previous study M. verticillata EO also was the most toxic against Musca domestica L. equaling the LC_{50} of the reference insecticide DDVP [24]. The strong fumigant toxicity of M. verticillata EO can be due to its elevated content of pulegone and menthone [11].

Combinations of M. verticillata EO with the EOs from Mentha sp., E. globulus, C. sativum and A. citriodora showed high fumigant activity with LC_{50} values of 41.8, 43.7, 57.1 and 77.6 µl/l air respectively (Table 2). On the other hand, all binary combinations of EOs from A. citriodora, C. sativum and Mentha sp. were not toxic against S. zeamais at 600 µl/l air. Due to it was not possible to obtain the LC_{50} values of all the EOs and combinations, we could only calculate the CTC of M. verticillata and E. globulus EO combination (CTC = 119.1), which indicates an additive effect [19].

Table 2: Fumigant toxicity of the essential oils and their combinations against Sitophilus zeamais.

Essential Oils	LC_{50} (µl/l air)	95% CL (µl/l air)	LC_{95} (µl/l air)	95% CL (µl/l air)	$(X^2)^a$
Minthostachys verticillata	28.2	18.4 - 43.1	106.4	40.4 - 280.5	13.45
Eucalyptus globulus	335.7	250.3 - 450.3	896.6	417.2 - 1927.0	8.77
Mentha sp.	>600	-	-	-	-
Aloysia citriodora	>600	-	-	-	-
Coriandrum sativum	>600	-	-	-	-
M. verticillata + Mentha sp.	41.8	31.1 - 56.3	72.7	38.7 - 136.8	2.5

M. verticillata + E. globulus	43.7	32.4 - 58.9	88	50.6 - 153.2	39.05
M. verticillata + C. sativum	57.1	43.7 - 74.6	133.6	57.9 - 308.3	6.71
M. verticillata + A. citriodora	77.6	52.4 - 114.9	262	71.2 - 964.2	7.96
E. globulus + Mentha sp.	>600	-	-	-	-
E. globulus + A. citriodora	>600	-	-	-	-
E. globulus + C. sativum	>300	-	-	-	-
Mentha + A. citriodora	>600	-	-	-	-
Mentha + C. sativum	>600	-	-	-	-
A. citriodora + C. sativum	>600	-	-	-	-

aChi-square values, significant at P < 0.05 level

CL: confidence limits

All the tested EOs and combinations had repellent effect on adults of S. zeamais (P < 0.05) (Table 3). Although there were no statistically significant differences among treatments, the combination of Mentha sp. and A. citriodora EOs showed the highest response index value (-85.75 ± 5.43). This value was even higher than those observed for Mentha sp. and A. citriodora EOs separately (-56.40 ± 10.13 and -44.67 ± 17.72 respectively), which could be due to the synergistic action of their main compounds. Similarly, Liu et al. [25] found that repellent activity of the mixture of EOs from Artemisia princeps Pamp and Cinnamomum camphora (L.) Presl. against adults of Sitophilus oryzae L. and Bruchus rugimanus Bohem was significantly higher than that elicited by individual oils. The mechanisms involved in how the interactions among the components of each EO result in the improvement of the repellent activity need further investigation [26].

Summing up, M. verticillata EO alone or in combination with EOs from A. citriodora, C. sativum, E. globulus or Mentha sp. has strong fumigant activity, while all the tested EOs and combinations have repellent effect on adults of S. zeamais, offering interesting alternatives to traditional pesticides to control S. zeamais.

Table 3: Response of Sitophilus zeamais to five essential oils and their binary combinations at 4 µl/l air in a two-choice olfactometer bioassay.

Essential Oils	Response Index (RI)
Coriandrum sativum	-72.04 ± 13.03 ** a
Eucalyptus globulus	-71.90 ± 5.75 *** a
Mentha sp.	-56.40 ± 10.13 ** a
Aloysia citriodora	-44.67 ± 17.72 ** a
Minthostachys verticillata	-38.40 ± 11.64 * a
A. citriodora + C. sativum	-78.33 ± 1.67 *** a
E. globulus + A. citriodora	-67.34 ± 6.30 *** a
E. globulus + C. sativum	-48.03 ± 5.41 ** a
E. globulus + Mentha sp.	-74.08 ± 3.22 *** a
M. verticillata + A. citriodora	-61.18 ± 2.33 *** a
M. verticillata + C. sativum	-55.85 ± 8.95 ** a
M. verticillata + E. globulus	-43.99 ± 10.18 ** a
M. verticillata + Mentha sp.	-55.80 ± 4.14 *** a
Mentha sp. + A. citriodora	-85.75 ± 5.43 *** a
Mentha sp. + C. sativum	-60.48 ± 12.64 *** a
Control	1.49 ± 1.74 b

*(P < 0.05) and **(P < 0.01); ***(P < 0.001); N = 5 (significant response to experimental stimulus; paired-sample t-test). Mean responses to different treatments followed by different letters are significantly different (ANOVA, P < 0.05, means comparison by Dunnett's test).

Acknowledgement

The authors thank FONCYT, CONICET and SECyT-UNC for financial support. Julieta Soledad Arena and María Laura Peschiutta have fellowships from CONICET. Julio Alberto Zygadlo is researcher of CONICET.

Conflict of Interest

There is no conflict of interest.

References

1. Trematerra P, Ianiro R, Athanassiou CG, Kavallieratos NG (2013) Behavioral responses of Sitophilus zeamais Motschulsky adults to conditioned grain kernels. J Stored Prod Res 53(2013): 77-81.
2. Torres C, Silva G, Tapia M, Rodríguez JC, Figueroa I, et al. (2014) Insecticidal activity of Laurelia sempervirens (Ruiz & Pav.) Tul. essential oil against Sitophilus zeamais Motschulsky. Chilean journal of agricultural research 74(4): 421-426.
3. Benhalima H, Chaudhry MQ, Mills KA, Price NR (2004) Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J Stored Prod Res 40(3): 241-249.
4. Pimentel D (2009) Environmental and Economic Costs of the Application of Pesticides Primarily in the United States. Integrated Pest Management: Innovation-Development Process, Netherlands, pp. 89-111.
5. Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils-A review. Food Chem Toxicol 46(2): 446-475.

6. Kim SI, Park C, Ohh MH, Cho HC, Ahn YJ (2003) Contact and fumigant activities of aromatic plant extracts and essential oils against Lasioderma serricorne (Coleoptera: Anobiidae). J Stored Prod Res 39(1): 11-19.
7. Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19(8-10): 603-608.
8. Koul O, Walia S, Dhaliwal GS (2008) Essential Oils as Green Pesticides: Potential and Constraints. Biopestic Int 4(1): 63-84.
9. Mossa ATH (2016) Green Pesticides: Essential oils as biopesticides in insect-pest management. J Environ Sci Technol 9(5): 354-378.
10. Herrera JM, Zunino MP, Massuh Y, Pizzollito RP, Dambolena JS, et al. (2014) Fumigant toxicity of five essential oils rich in ketones against Sitophilus zeamais (Motschulsky). Agriscientia 31(1): 35-41.
11. Pimienta-Ramírez L, García-Rodríguez YM, Ríos-Ramírez EM, Lindig-Cisneros R, Espinosa-García FJ (2016) Chemical composition and evaluation of the essential oil from Eupatorium glabratum as biopesticide against Sitophilus zeamais and several stored maize fungi. Journal of Essential Oil Research 28(2): 113-120.
12. Soujanya PL, Sekhar JC, Kumar P, Sunil N, Prasad CV, et al. (2016) Potentiality of botanical agents for the management of post harvest insects of maize: a review. J Food Sci Technol 53(5): 2169-2184.
13. Tak JH, Isman MB (2015) Enhanced cuticular penetration as the mechanism for synergy of insecticidal constituents of rosemary essential oil in Trichoplusia ni. Sci Rep 5: 12690.
14. Das NG, Dhiman S, Talukdar PK, Rabha B, Goswami D, et al. (2015) Synergistic mosquito-repellent activity of Curcuma longa, Pogostemon heyneanus and Zanthoxylum limonella essential oils. J Infect Public Health 8(4): 323-328.
15. Pavela R (2008) Insecticidal properties of several essential oils on the house fly (*Musca domestica L.*). Phytother Res 22(2): 274-278.
16. Benelli G, Pavela R, Canale A, Cianfaglione K, Ciaschetti G, et al. (2017) Acute larvicidal toxicity of five essential oils (Pinus nigra, Hyssopus officinalis, Satureja montana, Aloysia citrodora and Pelargonium graveolens) against the filariasis vector Culex quinquefasciatus: Synergistic and antagonistic effects. Parasitol Int 66(2): 166-171.
17. Peschiutta ML, Arena JS, Ramirez Sanchez A, Gomez Torres E, Pizzolitto RP, et al. (2016) Effectiveness of Mexican oregano essential oil from the Dominican Republic (Lippia graveolens) against maize pests (Sitophilus zeamais and Fusarium verticillioides). Agriscientia 33(2): 89-97.
18. Sun YP, Johnson ER (1960) Analysis of Joint Action of Insecticides Against House Flies. J Econ Entomol 53(5): 887-892.
19. Wen H, Zhang Q, Cheng D, Zhang Z, Xu H, et al. (2013) Cassia oil as a substitute solvent for xylene for rotenone EC and its synergistic activities. Pestic Biochem Physiol 105(3): 189-196.
20. Herrera JM, Pizzolitto RP, Zunino MP, Dambolena JS, Zygadlo JA (2015) Effect of fungal volatile organic compounds on a fungus and an insect that damage stored maize. J Stored Prod Res 62: 74-80.
21. Phillips T, Jiang XL, Burkholder WE, Phillips JK, Tran H (1993) Behavioral responses to food volatiles by two species of stored-product Coleoptera, Sitophilus oryzae (Curculionidae) and Tribolium castaneum (Tenebrionidae). J Chem Ecol 19(4): 723-734.
22. Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-Response Analysis Using R. PLoS One 10(12): e0146021.
23. Team RC (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing: Vienna, Austria.
24. Palacios SM, Bertoni A, Rossi Y, Santander R, Urzúa A (2009) Insecticidal activity of essential oils from native medicinal plants of Central Argentina against the house fly, Musca domestica (L.). Parasitol Res 106(1): 207-212.
25. Liu CH, Mishra AK, Tan RX, Tang C, Yang H, et al. (2006) Repellent and insecticidal activities of essential oils from Artemisia princeps and Cinnamomum camphora and their effect on seed germination of wheat and broad bean. Bioresour Technol 97(15): 1969-1973.
26. Nerio LS, Olivero-Verbel J, Stashenko E (2010) Repellent activity of essential oils: A review. Bioresour Technol 101(1): 372-378.