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Louicidal Efficacy of Essential Oils against The Dog Louse, *Trichodectes canis* (Mallophaga: Trichodectidae)

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ABSTRACT

Biting lice are widespread ectoparasites of dogs and other animals. Their management is complicated because of growing levels of resistance to commonly applied pediculicides. Thus, the development of novel approaches to their control is of primary clinical interest. Therefore, we examined the chemical composition of garlic, clove, pumpkin, onion and marjoram essential oils through gas chromatography-mass spectrometry (GC-MS) and their individual toxicity against the dog lice *Trichodectes canis* using a contact filter paper bioassay. GC-MS analysis revealed the presence of 45 compounds in garlic oil, 15 compounds in clove oil, 24 compounds in pumpkin oil, 16 compounds in onion oil and 22 compounds in marjoram oil. 2,3,3-trimethyl Hexane (5.33%), (+-) -(1S, 3R)-4(S)(a)-Methyladamantan (44.35%), 2,6,10,14,18,22-Tetracosahexane, Ethyl-2,6,10,15, 19,23-Hexane (31.67%), (E, E)-2,4-Decadienal (18.64%) and 4-methyl-1-(1-methylethyl)-3-Cyclohexen-1-ol (CAS) (21.01%) were the most abundant compounds in garlic, clove, pumpkin, onion and marjoram oils, respectively. All treated groups except marjoram oil showed high levels of toxicity. Clove, garlic and pumpkin oils demonstrated the best loucidal activity reaching 100% mortality within 15 and 20 minutes. The LC_{50} values were 10.757, 9.156, 11.325, 27.296 and 15.059 % for garlic, clove, pumpkin, marjoram and onion oils. Based on the LC_{50} values, the relative efficacies of the tested oils after 35 minutes compared with that of the marjoram oil as a reference material indicated that, clove, garlic, pumpkin and onion oils were 3.0, 2.5, 2.4 and 1.8, respectively more effective than marjoram oil. The LT_{50} values, post-treatment with 25% were 40.659, 48.335, 39.261, 45.744, and 47.974% respectively. The relative speed of killing lice is almost similar in all tested oils. It could be concluded that clove, garlic and pumpkin oils may offer eco-friendly alternatives of veterinary pediculicides for the control of the dog lice *T. canis*.

INTRODUCTION

Canine pediculosis is the infestation of dog lice which could spread over the entire body in case of neglected treatments, *Trichodectes canis* is recorded worldwide and survive by eating skin debris and sebaceous secretions (Mehlhorn *et al.*, 2011).

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Clinical symptoms of pediculosis are characterized by intense itching, heavy scratching, restlessness, rubbing, rough, dry, or matted coat, hair loss, and biting of the infested areas. Severe infestations are more common in young puppies, malnourished/debilitated dogs, or geriatric dogs kept in unsanitary conditions (Torres-Chable *et al.*, 2015 and Benelli *et al*., 2018). *T. canis* grips the dog's fur with its large wide mouthparts and chew the dead skin cells of the infested dogs. it could serve as a vector for the dog tapeworm *Dipylidium caninum* (Benelli *et al*., 2018), severely affecting children and immune-compromised patients after accidental ingestion of a louse parasitized with *D. caninum* cysticercoid (Narasimham *et al.*, 2013 and Torres-Chable *et al.*, 2017)**.**

Dog lice transmission from dog to dog occurs through direct contact (Eckert *et al.*, 2008)**.** **C**onventional insecticides effectively kill lice infesting dogs and work for a long time (Hansen and Londershausen, 2008 and Mehlhorn & Mehlhorn, 2008). However, dog owners do not prefer chemical compounds on their dogs, because of the development of resistance (Naqqash *et al.*, 2016), health hazards of treated dogs especially pregnant dogs and puppies, and the closure contact of humans and dogs. Hence, there is an urgent need for non-toxic and natural alternatives to conventional insecticides to eliminate dog lice. Botanical alternatives, such as essential oils, are currently receiving particular attention (Khater, 2012; Khater, 2013a; Ellse *et al.*, 2016; Ahmed *et al.*, 2021 and Iqbal *et al*., 2021) since the time of the Egyptian pyramids (Khater, 2017)**.**

Several studies proved the insecticidal effects of essential oils of clove, garlic, pumpkin, marjoram, and onion (Khater, 2003; Khater *et al*., 2009; Khater *et al*., 2014; Candy *et al*., 2018; Muturi *et al*., 2018; Abdel-Meguid *et al*., 2019; Prasath *et al*., 2020 and Yang *et al*., 2020), However, none of them investigated their activity against dog lice. So, this study aimed to evaluate the efficacy of the five oils against the dog lice *T. canis*.

MATERIALS AND METHODS

Dog Lice Collection:

Lice were collected from fifteen weaned owned dogs in Qalyubiyya Governorate, Egypt (30° 28′N, 31°11′E). The collection of lice was performed according to the protocol previously described by Ramadan and Abdel-Mageid (2010) as follows: Lice were collected from all of the body areas including the head, ear canal, pinna, elbow and thoracic abdominal areas of each dog by using a fine-toothed comb. After combing, the lice were carefully removed from the teeth of the comb, deposited on a white sheet of paper and placed gently into plastic boxes. The collected lice were identified (Durden, 2019).

Tested Oils:

Five essential oils were evaluated; clove, *Syzygium aromaticum* (Myrtales: Myrtaceae), garlic, *Allium sativum* (Asparagales: Amaryllidaceae), pumpkin, *Cucurbita pepo* (Cucurbitales: Cucurbitaceae), *onion, Allium cepa* (Asparagales: Alliaceae) and marjoram, *Origanum majorana* (Lamiales: Lamiaceae). Oils were obtained from EL CAPTAIN Company for extracting natural oils, plants, and cosmetics "Cap Pharm" El Obor, Cairo, Egypt, were authorized by the Egyptian Ministry of Health for different human uses.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

GC-MS analysis of the tested oils was performed using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium was used as a carrier gas with the

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flow rate of 1.0 ml/min. The injector and MS transfer line temperature was set at 280 ^{0}C. The oven temperature was programmed at an initial temperature 50 ^{0}C (hold 2 minutes) to 150 ^{0}C at an increasing rate of 7 ^{0}C/min, then to 270 ^{0}C at an increasing rate of 5 ^{0}C/min (hold 2 min), then to 310 ^{0}C as a final temperature at an increasing rate 3.5 ^{0}C/min (hold 10 min). The quantification of all the identified compounds was investigated using a peak area percentage. Tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC-MS system.

Contact/Fumigant Bioassays:

Five concentrations of each oil were prepared as follows; 50, 25, 12.5, 6.25, and 3.15%. Oils were diluted in an emulsifier solvent (Tween 80, 5% v/v). After careful examination by using a dissecting microscope, the active lice were collected and classified into 26 groups (10 lice each). Lice were placed on 4.5cm^2 diameter filter paper in disks that had been treated with 400 ul of oil, whereas the control group was treated with the solvent. Petri dishes with treated papers and lice were covered to prevent lice escape (Khater and Geden 2019). Ten lice were used per replicate and three replicates were used for each concentration. Petri dishes were kept at 26±0.5°C and 70±2% humidity. Lice were examined under a dissecting microscope at different time intervals: 5, 10, 15, 20-, 25-, 30-, and 35-min post-treatment (PT). Death was defined as the failure to respond when the legs were stroked with forceps (Khater *et al.,* 2014) and the mortality data were recorded.

Statistical Analysis:

Analyses of data were done using the one-way analysis of variance (ANOVA), Duncan's multiple range tests, as well as the Probit analysis, to calculate the lethal concentration (LC) and lethal time (LT) values through the computer program PASW Statistics 2009 (SPSS version 22). The relative efficacies (RE) were calculated (Khater and Geden, 2018) according to the following formula:

$$\text{RE for LC} = \frac{\text{LC50 (LC90 or LC99)for reference oil}}{\text{LC50 (LC90 or LC99)for essential oil}}$$

$$\text{RE for LT} = \frac{\text{LT50 (LT90 or LT99)for reference oil}}{\text{LT50 (LT90 or LT99)for essential oil}}$$

RESULTS

Chemical Composition of the Essential Oils:

In the present study, the phytocompounds present in garlic, clove, pumpkin, onion and marjoram oils were analyzed by GC-MS and results referring to the identification of components of the tested essential oils are shown in tables (1-5) and figure (1-5). GC-MS analysis revealed the presence of 45 compounds in garlic essential oil (Table 1). The three most abundant compounds obtained were 2,3,3-trimethyl Hexane (5.33%), Tetradecane (4.63%), and 4(Prop-2-enoyloxy) pentadecane (4.52%). According to table 2, the analysis of the clove essential oil allowed the characterization of two components, with identification above 96%, among which (+-)-(1S, 3R)-4(S)(a)-Methyladamantan was identified as the major component at the concentration of 44.35% (peak 1), then N-Ethyl-1,7-Dehydro-2,8-Azacineole (31.65). Twenty-four constituents were detected in the pumpkin essential oil by GC-MS (Table 3), and the major constituent was 2,6,10,14,18,22-Tetracosahexane, Ethyl-2,6,10,15,19,23-Hexane (31.67%). The results of onion oil composition characterized by gas chromatography (Table 4), in this study, indicated (E, E)-2,4-Decadienal (18.64%) as the major component followed by 2,4-Decadienal (11.56%). A total of 22 compounds from the marjoram essential oil were obtained and identified (Table 5). The major component is 4-methyl-1-(1-methylethyl)-3-Cyclohexen-1-ol (CAS) (21.01%) followed by Sabinene (8.39%) and ç-Terpinene (7.9%).

Table 1: Chemical characterization of garlic essential oil through GC-MS analysis

Peak No.	R_t (min.)	MW	MF	Area %	Probabilities of the detected compounds
1	10.67	146	C6H10S2	2.02	Diallyl disulphide
2	11.68	170	C8H14N2O2	0.76	*N,N* dimEthyl- *N'*-(3methyl-2-oxo Tetrahydro-3-furanyl)Iminoformamide
3	12.16	226	C16H34	0.96	7-*n*Propyl tridecane
4	12.80	172	C11H24O	2.31	1-(pentyloxy) - Hexane,
5	12.91	214	C11H18O4	1.11	Rac-5(1-Ethoxyethoy)3-pentyn-2-y-l-acetate
6	13.04	170	C12H26	3.40	4-methy-l –Undecane
7	13.22	170	C12H26	2.61	3-methy-l –Undecane
8	13.77	144	C6H8S2	1.93	2-(Mercaptomethyl)-5-methylthiophene
9	14.07	128	C9H20	5.33	2,3,3-trimethyl Hexane
10	14.43	184	C13H28	0.92	4-methyl Dodecane
11	14.51	144	C6H8S2	1.49	3-Vinyl-1,2-dithiacyclohex-5-ene
12	14.97	184	C13H28	0.84	2,9-dimethyl Undecane
13	15.55	282	C18H34O2	4.52	4(Prop-2-enoyloxy)pentadecane
14	15.68	184	C13H28	1.97	4-methyl Dodecane
15	15.82	264	C13H28O3S	3.92	Sulfurous acid, hexy-l-heptyl ester
16	16.00	170	C12H26	3.18	2,2-dimethyl Decane
17	16.80	198	C14H30	4.63	Tetradecane
18	17.09	198	C5H11I	2.29	2-Iodo-2-methyl Butane
19	17.20	198	C14H30	1.32	2,4-Dimethyldodecane
20	17.47	155	C8H13NO2	1.05	Octahydropyrano[3,2b]Pyridine-6-one
21	17.64	168	C11H20O	1.03	2,2-Dimethylnon-5-en-3-one
22	18.12	254	C18H38	3.67	7-methyl Heptadecane,
23	18.20	226	C16H34	2.24	7-propyl Tridecane
24	18.32	256	C17H36O	1.91	6,10,13-Trimethyltetradecanol
25	18.46	282	C20H42	3.46	10-Methylnonadecane
26	18.63	186	C12H26O	2.72	2-butyl -1-Octanol
27	19.39	198	C14H30	3.28	Tetradecane
28	19.66	212	C15H32	1.55	4-methyl Tetradecane
29	19.76	226	C16H34	0.88	6,9-dimethyl Tetradecane,
31	20.04	192	C7H12O4S	0.77	Dimethylsulfonium-2-methoxy1 (methoxy - carbonyl)-2-oxoethylide
32	20.20	212	C15H32	0.87	3-methyl Tetradecane,
33	20.61	366	C26H54	3.09	3-ethyl-5-(2-ethylbutyl) Octadecane
34	20.71	282	C20H42	1.41	10-Methylnonadecane
35	20.83	198	C14H30	1.21	3-methyl Tridecane
36	20.97	198	C12H22O2	2.01	*ç*Dodecalactone
37	21.14	212	C15H32	1.88	3-methyl Tetradecane
38	21.86	212	C15H32	2.17	Pentadecane
39	22.98	334	C18H38O3S	2.26	butyl ester -6-Tetradecanesulfonic acid
40	23.10	168	C12H24	0.97	*Z*-4-Dodecene
41	23.35	226	C16H34	0.76	2-methyl Pentadecane
42	23.53	226	C16H34	0.79	3-methyl Pentadecane
43	25.27	350	C25H50	0.85	9-octyl -8-Heptadecene
44	36.67	322	C24H15F	0.78	1[(4Fluorophenyl)phenylmethylene]*1H*cyclo propa*[b]*naphthalene
45	37.21	334	C23H23Cl	0.94	1-Chloro-4,6-diethyl-6-*m*Ethyl-5- methylen-2,3-diphenyl-1,3-cyclohexadiene

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Table 2: Chemical characterization of clove essential oil through GC-MS analysis

Peak No.	R_t (min.)	MW	MF	Area %	Probabilities of the detected compounds
1	18.71	179	$C_{12}H_{21}N$	31.65	N-Ethyl-1,7-Dehydro-2,8-Azacineole
2	18.77	452	$C_{26}H_{24}N_6O_2$	12.66	2,2'-1,4-Phenylenedi[5-(4dimethyl amino -phenyl)-1,3,4-oxadiazole]
3	18.86	164	$C_{11}H_{16}O$	44.35	(+-)-(1S,3R)-4(S)(a)-Methyladamantan
4	20.06	204	$C_{15}H_{24}$	3.53	trans-Caryophyllene
5	22.76	206	$C_{12}H_{14}O_3$	1.63	Phenol,2-methoxy-4-(2-propenyl)-, acetate
6	33.28	328	$C_{18}H_{16}O_6$	0.27	Sclerodin
7	33.09	310	$C_{22}H_{46}$	0.36	Docosane (CAS)
8	32.39	310	$C_{22}H_{46}$	0.27	Docosane (CAS)
9	31.60	324	$C_{23}H_{48}$	0.16	9-hexyl Heptadecane
10	31.51	242	$C_{16}H_{34}O$	0.13	2-Hexyl-1-decanol
11	31.38	482	$C_{30}H_{58}O_4$	0.11	Decanedioic acid, dodecyl ester
12	31.18	186	$C_{12}H_{26}O$	0.33	2-butyl- 1-Octanol (CAS)
13	30.63	199	$C_9H_{13}NO_4$	0.17	[(1S,5S)-5-methyl-2-nitro-2-cyclohexenyl]-acetate
14	23.88	220	$C_{15}H_{24}O$	0.16	Caryophyllene oxide
15	20.82	204	$C_{15}H_{24}$	0.25	à-Humulene (CAS)

Table 3: Chemical characterization of pumpkin essential oil through GC-MS analysis

Peak No.	R_t (min.)	MW	MF	Area %	Probabilities of the detected compounds
1	5.14	167	$C_9H_{13}NO_2$	2.41	(1RS,2SR)-9-Oxa-11-azabicyclo [6.3.0] undec-5-en-10-one
2	5.24	100	$C_6H_{12}O$	2.78	2-Methylpent-4-en-1-ol
3	18.45	691	$C_{51}H_{33}NO_2$	1.46	2,6-Bis(2,3,5-triphenyl-4-oxocyclopentadi-enyl) pyridine
4	31.09	310	$C_{22}H_{46}$	1.00	Docosane (CAS)
5	31.67	0	N/A	1.41	HAHNFETT
6	31.97	154	$C_{10}H_{18}O$	1.09	2,5,5-Trimethyl-hepta-1,6-dien-3-ol
7	32.24	362	$C_{20}H_{42}O_3S$	1.19	hexyltetradecyl ester Sulfurous acid
8	32.37	310	$C_{22}H_{46}$	1.72	Docosane (CAS)
9	32.93	310	$C_{22}H_{46}$	1.18	Docosane (CAS)
10	32.99	228	$C_{15}H_{32}O$	1.26	1-Pentadecanol (CAS)
11	33.65	408	$C_{29}H_{60}$	1.24	Nonacosane (CAS)
12	33.75	322	$C_{23}H_{46}$	1.22	(Z)- 9-Tricosene (CAS)
13	34.04	222	$C_{16}H_{30}$	1.98	1-Hexadecyne (CAS)
14	34.15	266	$C_{18}H_{34}O$	2.18	3-Octadecenal (spectrumdisagrees) (CAS)
15	34.24	676	$C_{42}H_{56}N_6O_2$	1.58	2,7,12,18-Tetramethyl-3,8-diethyl-13,17-bis(3-morpholinopropyl)porphyrin
16	34.70	708	$C_{44}H_{36}O_9$	3.17	3,5-Diphenyl-3,5-(9,10- phenanthylene) tricyclo[5.2.1.0]decane-4-one-8-exo-9-endo-dicarboxylic acid diacetoxy methyl ester
17	36.38	490	$C_{35}H_{70}$	2.79	17-Pentatriacontene(CAS)
18	36.47	366	$C_{26}H_{54}$	1.58	11-(1-ethylpropyl)-Heneicosane
19	37.57	182	$C_{13}H_{26}$	1.82	1-Tridecene (CAS)
20	37.80	254	$C_{17}H_{34}O$	1.47	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol
21	38.07	662	$C_{30}H_{70}O_4Si_6$	2.08	1,3,4,4,6,8,8,9,9-Decaisopropyl-2,5,7,10-tetraoxabicyclo[4.4.0]decasilane
22	39.67	678	$C_{36}H_{40}BrI$	1.92	1'''-Iodo-3-bromo[1-[4-(2-phenyl-1,4-dihexylphenyl)phenyl]]benzene
23	41.09	450	$C_{30}H_{58}O_2$	2.05	(Z)-9-hexadecenyl ester Myristic acid (CAS)
24	45.38	410	$C_{30}H_{50}$	31.67	2,6,10,14,18,22-Tetracosahexane,Ethyl-2,6,10,15,19,23-Hexam

Table 4: Chemical characterization of onion essential oil through GC-MS analysis

Peak No.	R_t (min.)	MW	MF	Area %	Probabilities of the detected compounds
1	5.16	664	$C_{38}H_{56}N_4O_6$	4.23	3-[(E)-t-Butoxtcarbonylpropenyl]-2,712,18-tetramethyl-21H,23Hporphine-13,17dipropyl dimethyl ester
2	5.22	630	$C_{21}H_8Cl_4F_6N_6O_2$	2.63	2,2-Bis[4-[(4,6-dichlor1,3,5-triazin-2- yl) oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane
3	5.31	84	CH_2Cl_2	3.78	dichloro Methane (CAS)
4	6.49	216	$C_{15}H_{20}O$	1.25	1á,8à-Dimethyl-1,4,4aà,4bá,5,8,8aá,9aà-octahydro-9H-fluoren-9-one
5	16.59	148	$C_{10}H_{12}O$	3.63	1-methoxy-4-(2-propenyl)Benzene
6	16.69	152	$C_{10}H_{16}O$	11.56	2,4-Decadienal
7	17.29	152	$C_{10}H_{16}O$	18.64	(E,E)-2,4-Decadienal
8	29.15	186	$C_{10}H_{18}O_3$	1.06	trans-Dihydro-4hydroxymethyl-5-pentyl-2(3H)-furanone
9	33.13	272	$C_{20}H_{32}$	2.17	(5á,8à,9á,10à,12à)-Atis-16-ene(CAS)
10	34.03	294	$C_{19}H_{34}O_2$	3.22	(Z,Z)-methyl ester-9,12-Octadecadienoic acid (CAS)
11	34.13	296	$C_{19}H_{36}O_2$	4.23	(Z)-methyl ester-9-Octadecenoic acid (CAS)
12	35.62	348	$C_{25}H_{48}$	7.63	1-hexadecyloctahydro-1H-Indene (CAS)
13	35.69	280	$C_{18}H_{32}O_2$	2.71	(Z,Z)- 9,12-Octadecadienoic acid (CAS)
14	35.76	310	$C_{17}H_{30}OSSi$	2.68	5-Methyl-2-(phenylthio)-2-(trimethylsilyl-methyl)hexan-1-ol
15	37.62	3040	N/A	2.21	DSHAKRHHGYKRKFHEKHHSHRGY/6
16	38.08	282	$C_{18}H_{34}O_2$	1.43	Octadec-9-Enoicacid

Table 5: Chemical characterization of marjoram essential oil through GC-MS analysis

Peak No.	R_t (min.)	MW	MF	Area %	Probabilities of the detected compounds
1	6.37	136	C10H16	1.15	á-Phellandrene
2	6.47	692	C44H28N4OZn	1.07	(2-hydroxy-5,10,15,20-tetraphenylporphinato)zinc(II)
3	7.59	136	C10H16	8.39	Sabinene
4	8.14	660	C42H65ClN2Si	1.76	Bis[(2,4,6-Tri-tert-butylphenyl)amino]phenylchlorosilane
5	8.82	136	C10H16	4.20	1,2,4,6-tetramethyl-1,3-Cyclohexadiene (CAS)
6	9.10	134	C10H14	5.58	3,7,7-Trimethyl-1,3,5-cycloheptatriene
7	9.18	136	C10H16	2.60	1,7,7-trimethyl-Tricyclo[2.2.1.0(2,6)]heptane
8	10.11	136	C10H16	7.90	ç-Terpinene
9	11.31	154	C10H18O	3.21	cis-sabinene hydrate
10	13.71	154	C10H18O	21.01	4-methyl-1-(1-methylethyl)-3-Cyclohexen-1-ol (CAS)
11	14.00	136	C8H8O2	1.47	4-methoxy- Benzaldehyde (CAS)
12	15.71	182	C11H18O2	1.75	linalyl formate
13	19.94	204	C15H24	4.57	trans-Caryophyllene
14	26.78	232	C15H20O2	1.13	(1R,3R,5S,6S,8S)-10-Isopropylidene-5,8-dimethyltricyclo[4.4.0.0(2,8)]decan-2,7-dione
15	27.19	217	C12H11NO3	3.21	6-(4'-methoxyphenyl)-4H,6H-furo[3,4-c]isoxazole
16	27.28	358	C26H46	2.46	1-decylhexadecahydro-Pyrene
17	28.15	246	C13H10O5	1.27	8-Methyl-5-Mrthylbenzo(1,2-B:5,4-B')difuran-2-carboxylic acid
18	28.35	362	C26H50	1.75	octahydro-1-(2-octyldecy l)- Pentalene (CAS)
19	28.50	296	C21H44	2.03	Heneicosane (CAS)
20	28.60	238	C14H22O3	2.26	Acetic acid,2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester
21	29.27	254	C18H38	1.65	8-methyl-Heptadecane (CAS)
22	30.67	254	C18H38	1.21	Octadecane (CAS)

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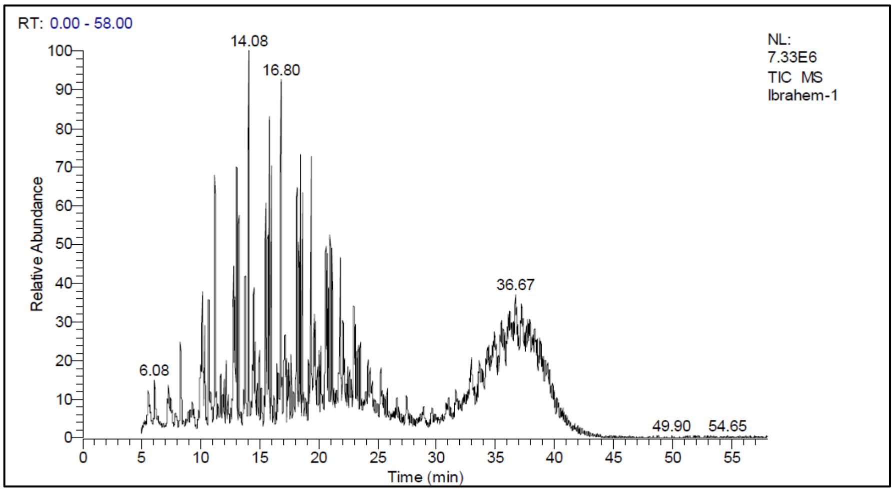

Fig. 1: GC-MS chromatogram of garlic oil.

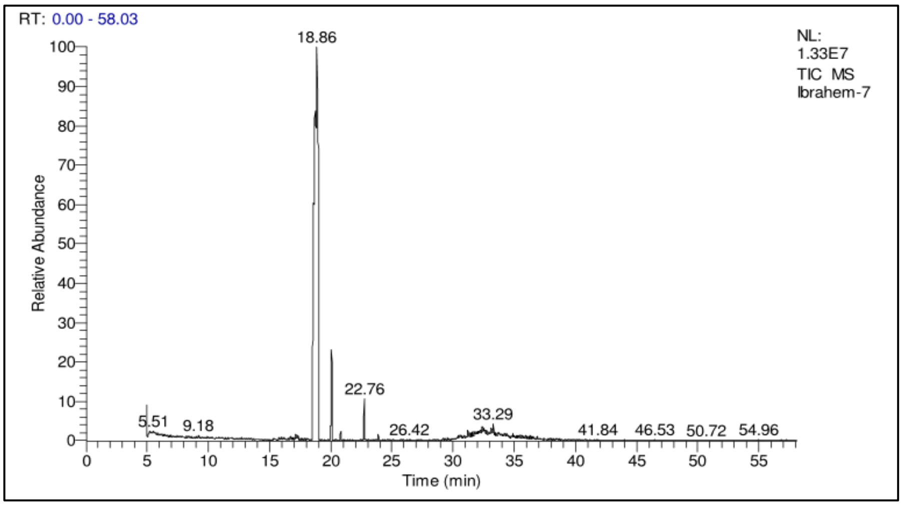

Fig. 2: GC-MS chromatogram of clove oil.

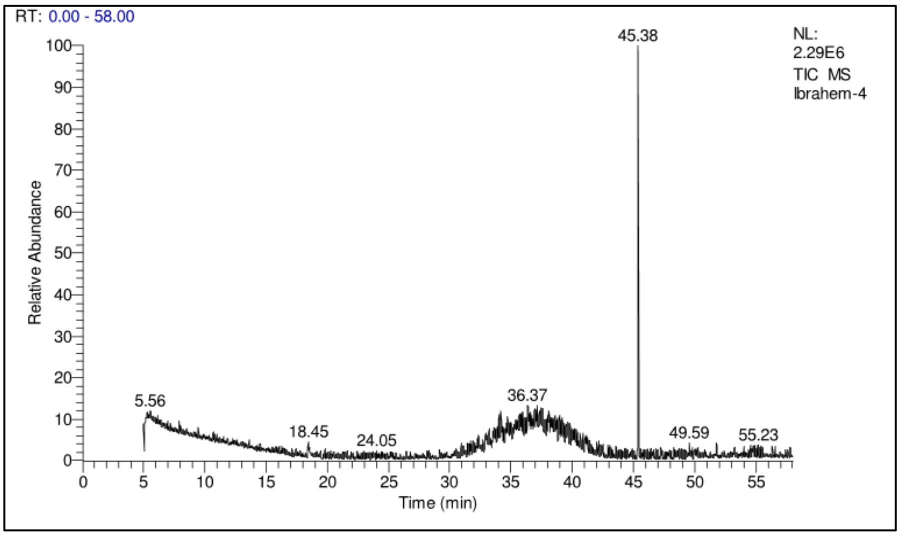

Fig. 3: GC-MS chromatogram of pumpkin oil.

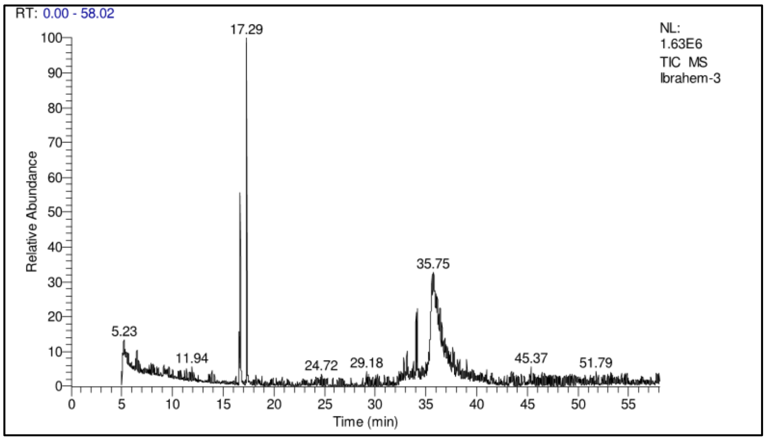

Fig. 4: GC-MS chromatogram of onion oil.

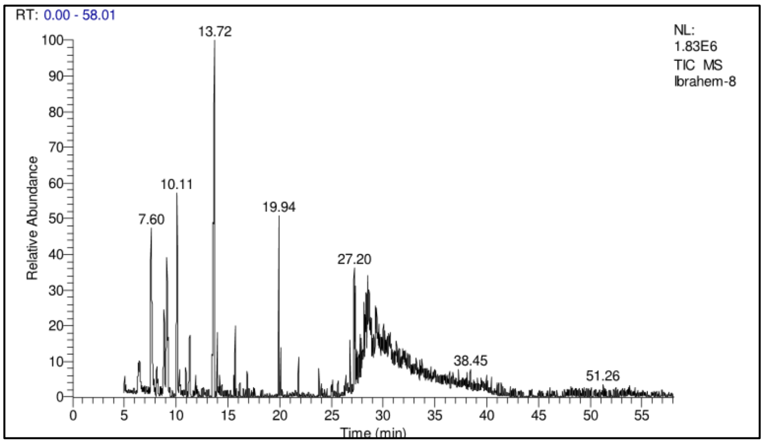

Fig. 5: GC-MS chromatogram of marjoram oil.

Insecticidal Activity of The Tested Oils against *T. canis*:

The mortality percentages (M%) of the applied oils (50% conc.) of garlic, clove, pumpkin, onion and marjoram were 50.0, 33.33, 50.0, 16.67 and 0.00 % 5 min PT, 66.67, 50.0, 76.67, 16.67 and 20.0 % 10 min PT, 100, 83.33, 100, 33.33 and 60% 15 min PT, 100, 100, 100, 50 and 80 % 20 min PT, 100, 100, 100, 50 and 80 % 25 min PT, 100, 100, 100, 66.67 and 80 % 30 min PT, and 100, 100, 100, 100 and 80% 35 min PT, respectively (Figs. 6-10).

The sensitivity of *T. canis* to the used oils was demonstrated by the LC_{50} values. After 35 minutes, the LC_{50} values were 10.757, 9.156, 11.325, 15.059 and 27.296 % for garlic, clove, pumpkin, onion and marjoram oil, respectively; in the meanwhile, their LC_{99} values were 25.840, 19.167, 25.325, 41.934 and 70.947, respectively (Table 6). Based on the LC_{50} values, the relative efficacies of the tested oils after 35 minutes compared with that of the marjoram oil as a reference material indicated that, clove, garlic, pumpkin and onion oils were 3.0, 2.5, 2.4 and 1.8, respectively more effective than marjoram oil.

The LT_{50} values post-treatment with 25% were 40.659, 48.335, 39.261, 45.744, and 47.974% for garlic, clove, pumpkin, marjoram and onion oil, respectively; in the meanwhile, their LT_{99} values were 96.771, 95.966, 101.577, 89.178, and 93.164, respectively. The relative speed of killing lice is almost similar in all tested oils (Table 7).

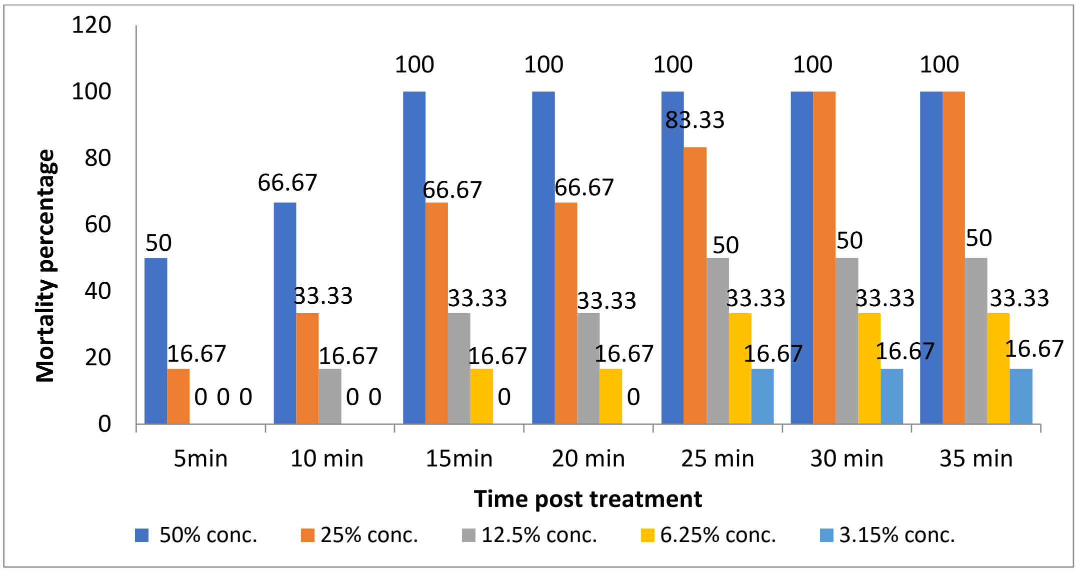

Fig. 6: Mortality % of *T. canis* treated with garlic oil at different times post-treatment.

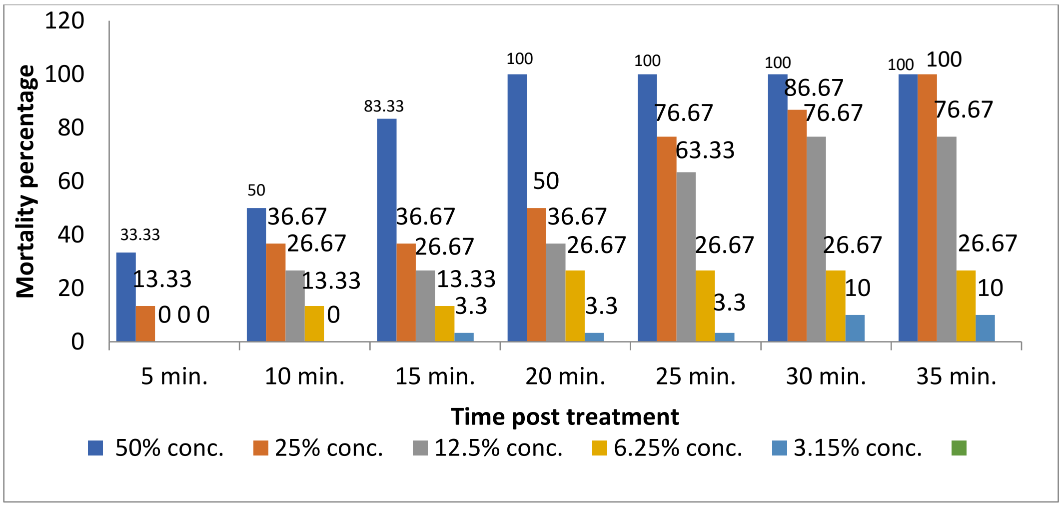

Fig. 7: Mortality % of *T. canis* treated with clove oil at different times post-treatment.

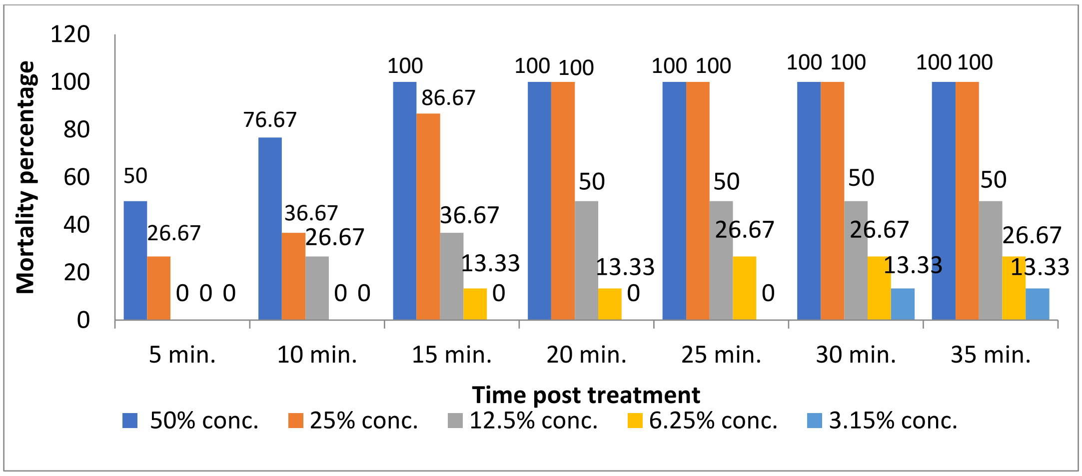

Fig. 8: Mortality % of *T. canis* treated with pumpkin oil at different times post treatment

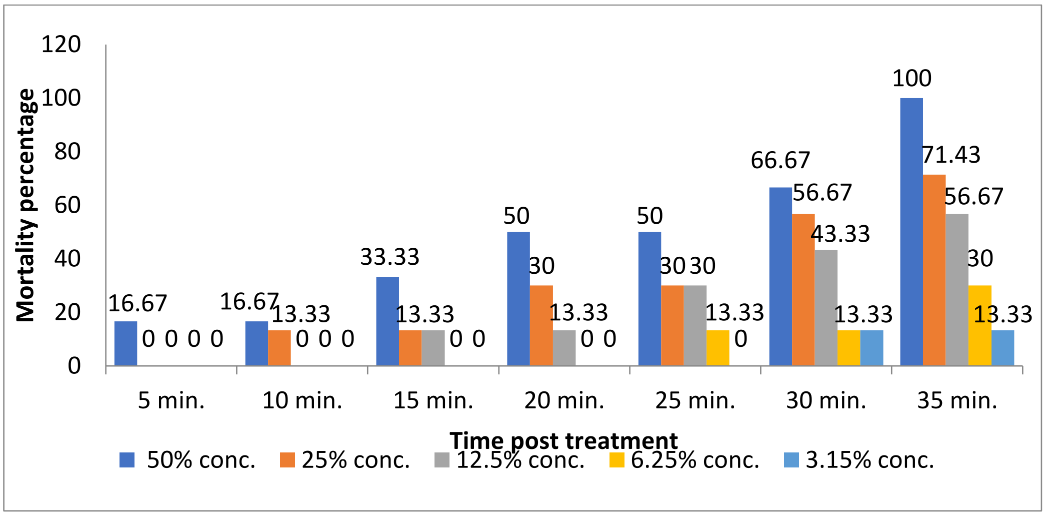

Fig. 9: Mortality % of *T. canis* treated with onion oil at different times post-treatment.

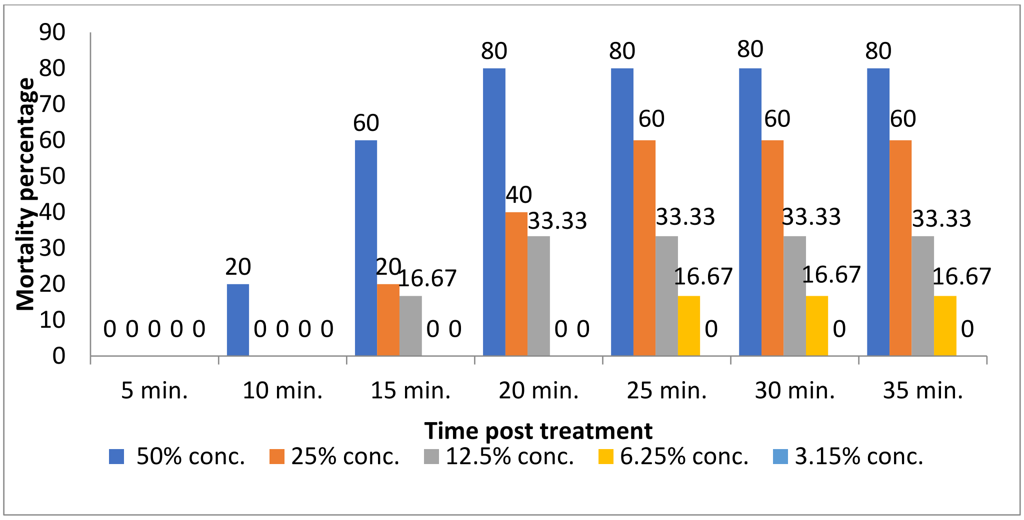

Fig. 10: Mortality % of *T. canis* treated with marjoram oil at different times post-treatment.

Table 6: Lethal concentration values of the applied oils against dog lice,35 min post-treatment.

Oils	LC_{50}	LC_{90}	LC_{95}	LC_{99}	Chi	Relative Efficacy			
	LCL	LCL	LCL	LCL	R^2				
	UCL	UCL	UCL	UCL	Sig	LC_{50}	LC_{90}	LC_{95}	LC_{99}
Clove	**9.156**	**14.671**	**16.234**	**19.167**	**0.735**	3.0	3.5	3.6	3.7
	7.876	12.628	13.880	16.192	1.0				
	10.797	18.214	20.413	24.572	0.947^a				
Garlic	**10.757**	**19.066**	**21.422**	**25.840**	**5.289**	2.5	2.7	2.7	2.7
	9.023	16.072	17.947	21.423	0.937				
	13.063	24.291	27.597	33.841	0.259^a				
Pumpkin	**11.325**	**19.037**	**21.223**	**25.325**	**3.030**	2.4	2.7	2.7	2.8
	9.617	16.143	17.886	21.117	1.0				
	13.643	24.074	27.138	32.926	0.553^a				
Onion	**15.059**	**29.864**	**34.061**	**41.934**	**8.628**	1.8	1.7	1.7	1.7
	12.361	24.923	28.261	34.454	1.0				
	18.562	38.468	44.334	55.406	0.071^a				
Marjoram	**27.296**	**51.343**	**58.160**	**70.947**	**13.398**	1.0	1.0	1.0	1.0
	16.272	35.501	40.000	48.155	1.0				
	53.006	121.92	142.41	181.13	0.009^a				

LCL; lower confidence limit, UCL; upper confidence limit, Chi; Chi-square value

Table 7: Lethal time values of the applied oils against dog lice post treatment with 25%

Oils	LT_{50} / LCL / UCL	LT_{90} / LCL / UCL	LT_{95} / LCL / UCL	LT_{99} / LCL / UCL	Chi / R^2 / Sig	Relative Efficacy			
						LT_{50}	LT_{90}	LT_{95}	LT_{99}
Garlic	**40.659**	**71.570**	**80.333**	**96.771**	**3.944**	1.2	1.0	1.0	1.0
	33.561	55.731	61.886	73.388	6				
	57.058	111.55	127.13	156.40	0.684[a]				
Clove	**48.335**	**71.960**	**80.308**	**95.966**	**2.296**	1.0	1.0	1.0	1.1
	39.343	56.113	61.963	72.896	6				
	70.261	112.30	127.23	155.28	.891[a]				
Pumpkin	**39.261**	**73.590**	**83.322**	**101.57**	**8.030**	1.2	1.0	1.0	1.0
	32.060	56.419	63.166	75.774	6				
	56.442	119.01	136.91	170.53	.236[a]				
Marjoram	**45.744**	**69.671**	**76.454**	**89.178**	**4.810**	1.1	1.1	1.1	1.1
	37.929	54.539	59.170	67.825	6				
	66.445	112.24	125.31	149.84	.568[a]				
Onion	**47.974**	**72.869**	**79.926**	**93.164**	**1.608**	1.0	1.0	1.0	1.1
	39.196	56.207	60.956	69.832	6				
	72.747	122.50	136.68	163.31	.952[a]				

LCL; lower confidence limit, UCL; upper confidence limit, Chi; Chi-square value

DISCUSSION

Chemical characterization of the tested essential oils displayed three major compounds with higher peak area percentage observed in garlic and marjoram oils, two major components in clove and onion oils and one major component in pumpkin. Diallyl disulphide was also previously reported as a component of garlic oil by other authors (Plata-Rueda *et al*., 2017and Muturi *et al.*, 2018) and methylthiophene (Mnayer *et al*., 2014). Similarly, Caryophyllene and Caryophyllene oxide was identified in the phytochemical analysis of clove oil by Liangtiag *et al.* (2015) and Jairoce *et al.* (2016). Pumpkin and onion oils were also analyzed by other authors (Ardabili *et al*., 2011; Rezig *et al*., 2012 and Mnayer *et al.*, 2014). Previous works have been reported the existence of Sabinene and Terpinene in the GC-MS analysis of marjoram (Prabu *et al*., 2020 and Yang *et al.*, 2020).

The doge lice *T. canis* can serve as a vector for the zoonotic dog tapeworm *D. caninum*, severely affecting children (Cabello *et al*., 2011). For this reason, a reliable, effective and eco-friendly control method is urgently required. Essential oils are blends of different plant metabolites: low molecular weight volatile molecules with major terpene constituents (Khater, 2012, 2013) which might have attributed their insecticidal efficacy. Alternatively, the hydrophobic nature of the oils may simultaneously exert a mechanical effect on the lice, such as blocking the spiracles leading to death by suffocation (Burgess, 2009 and Semmler *et al.*, 2010). Botanicals including essential oils are also highly effective in controlling insects (Govindarajan *et al.*, 2016 a, b; Khater *et al.*, 2018; Khater and Geden, 2019; Baz *et al.*, 2021 and Iqbal *et al.*, 2021).

In this study, garlic essential oil was demonstrated to possess strong insecticidal activity against *T. canis*, it causes 100% mortality after 15 min exposure. The same observation had been reported for other insects such as *Blattella germanica* Linnaeus (Tunaz *et al.*, 2009), *Cacopsylla chinensis* (Zhao *et al.*, 2013), *Brevicoryne brassicae* (Baidoo and Mochiah, 2016), *Tenebrio molitor* (Plata-Rueda *et al.*, 2017), *Plutella xylostella* L. (Sangha *et al.*, 2017) and *Culex pipiens* L. (Muturi *et al.*, 2018). 100% lousicidal efficacy was achieved 20 min post-treatment with clove oil. Likewise, high efficacy for the same oil had been reported against *Musca domestica* (Chintalchere *et al.*, 2013), *Cacopsylla chinensis* (Liangtian *et al.*, 2015), the maize weevil *Sitophilus zeamais* and the bean weevil *Acanthoscelides obtectus* (Jairoce *et al.*, 2016) and *Pediculus humanus capitis* (Yones *et al.*, 2016 and Candy *et al.*, 2018). Pumpkin oil-induced pronounced in vitro pediculicidal activity against *T. canis*, all treated lice were killed 15 min PT. Pumkin is toxic against *Cephalopina titillator* (Khater, 2013b), but its louicidal effect was investigated for the first time in this study. Onion oil showed pediculicidal activity against *T. canis*. Onion oil also showed insecticidal activity against some insects such as *Culex pipiens* and *Musca domestica* (Khater 2003) and *Haematopinus tuberculatus* (Khater *et al.*, 2009). This study showed that the essential oil of marjoram was the least effective oil against *T. canis*. Terpinene which was identified in GC-MS analysis may be responsible for the insecticidal activity of marjoram. Marjoram oil was effective in controlling insects other than *T. canis* (Sharma *et al.*, 2009; El-Akhal and Guemmouh, 2014; El-Sherbini *et al.*, 2014; Abd El Meguid *et al.*, 2019 and Prasath *et al.*, 2020). In this study, we found that the major compounds of the tested oils as indicated from GC-MS analysis may be responsible for the insecticidal activity.

Although the essential oils used in this study have been shown to possess insecticidal activity against a wide range of insects, this is the first report of these essential oils' insecticidal activity against the dog lice *T. canis*.

CONCLUSION

It could be concluded that clove, garlic and pumpkin oils exhibited the greatest toxicity against *T. canis*, while marjoram oil showed a limited efficacy. The results also showed the possibility of the use of essential oils, such as clove, garlic and pumpkin oils, as alternatives for the management of dog lice. However, further studies are necessary, with special reference in vivo and toxicological studies as well as their nanoformulations to improve their efficacy and persistence.

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