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Long-Term Toxic Effect of Inhaling Smokes of Cigarette and Incense on different stages of *Culex pipiens*

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ABSTRACT

The toxic effects of inhaling cigarette and industrial incense smoke on the different stages of the aquatic insect *Culex pipiens* (L.) for two generations had been investigated. Eggs, larvae, pupae, and adult stages were exposed to the smoke daily for 82 days. The results showed that in the 1st generation, cigarette smoke affected the mortality rate of larvae (29.80 %) and adults (57.95 %), while industrial incense smoke recorded a loss rate on the larvae (47.56 %) and to the adults (32.61 %). In the 2nd generation, the highest loss rate was recorded for adult mortality (75.68 %) from cigarette smoke. Larval and adult mortality was affected by industrial incense smoke by 86.67 % and 54.55 %, respectively. Moreover, egg production of the 1st to 2nd generations was affected negatively by cigarettes (88.06 %) and positively by industrial incense (36.99 %). X-ray fluorescence spectrometer analysis showed incense had a major amount of calcium (78.24 %) followed by ferrous (10.04 %) and potassium (6.12 %). The cigarette had calcium and potassium as major components represent (60.86%) and (31.39%), respectively. Using the GC-MS technique, toxic material of crude material of cigarette and incense was found that cigarette had 60 chemicals and industrial incense had 33 chemicals. Glycerol, 3TMS derivative, and dimethyl phthalate recorded the most dominant chemicals in cigarettes by 19.64% and 16.75%, respectively. Dimethyl phthalate was the most dominant chemical (75.68%) in industrial incense. Most cigarette components were recorded as toxic materials while those in the incense had a low toxicity.

INTRODUCTION

Mosquitoes are the world's most severe public health concern spreading a variety of diseases, such as filariasis, avian malaria, Japanese encephalitis, dengue, etc., and causing countless deaths every year **(Das *et al.*, 2007)**. Frequent use of artificial insecticides for mosquito control led to the growth of resistance, adverse effects on non-target species, and environmental and human health problems have also contributed to search for alternative control measures **(Brown, 1986; Hayes and Laws, 1991)**.

Naturally occurring substances of plant origin with insecticidal qualities have recently been evaluated for the control of numbers of insect pests and vectors. For example, oils of leaf and bark from *Cryptomeria japonica* displayed high larvicide efficacy toward *Aedes aegypti* (Diptera: Culicidae) larvae **(Cheng *et al.*, 2003)**.

Azadiractin which is the active ingredient from neem plant and was recognized for its larvicide mosquito capacity **(Alouani *et al.*, 2009)**. The extracts of *Oriandrum sativam*, *Murraya koenigii*, *Trigonella foenum graceum* and *Ferula asafetida* had been found to be successful agents against *Ae. aegypti* and *Culex* mosquito larvae **(Isman 1999, Harve and Kamath 2004;)**. Phytochemicals extracted from plant origin exhibited larvicide, insect growth regulator, oviposition attractant and repellent **(Babu and Murugan 1998; Venkatachalam and Jebanesan 2001a; 2001b)**.

The tobacco plant (*Nicotiana tabacum*) contains a high amount of alkaloid which has an insecticidal effect and for decades, people use its mixture with water as a homemade remedy to control garden insects **(Booker *et al.*, 2010)**. The potential larvicidal effects of *N. tabacum* plant extracts against *Cx. quinquefasciatus* were evaluated **(Ullah *et al.*, 2018)**. Also, repellent activities of its bio-oil on mosquitoes were reported **(Jufri *et al.*, 2016)**. Cigarette smoke is commonly known as an air pollutant and has had severe side effects on public health. Recently, cigarette smoke has been associated with the severity of coronavirus disease 2019 **(Guo, 2020)**. Whenever industrial incense is burned, it emits smoke containing pollutants and gas products as well as other organic molecules. The fire from burned incense was distinguished as CO, CO_2, NO_2, SO_2, and many others that will be emitted to the surrounding environment **(Lin *et al.*, 2008)**.

Popular synthetic bug sprays used for mosquitoes such as DEET, N,N diethyl-m-toluamide that was approved for use as a pesticide, but the potential side effects including, eyes and skin irritation, insomnia and the promotion of angiogenesis which increases tumor growth had also been reported **(Legeay *et al.*, 2016)**. Permethrin, resmethrin, and sumithrin, as well as other industrial pyrethroids that are widely used in mosquito control systems to combat adult mosquitoes showed side effect in altering the nerve function by changing the biochemistry of nerve membrane of sodium channels **(Paul *et al.* 2005; EPA 2009; Trivedi *et al.*, 2018)**.

Thus, The authors speculated that the long-term exposure to cigarette smoke and industrial incense on *Cx. Pipiens* would have a negative effect on the yield at various life stages, which might be related to the toxic substances emitted from both of them. Consequently, the current study aimed to investigate the chemical-related abnormality in the numbers of yielded stages of *Cx. pipiens* upon the long-term exposure to smokes of cigarette and industrial incense for two generations.

MATERIALS AND METHODS

Insect rearing

Laboratory colonies of *Cx. pipiens* were established by collecting egg patches from drain wells situated in Qena Campus of South Valley University, Egypt. Mosquito specimens were reared and identified by following the key of **Harbach (1985)**. In the laboratory, hatched larvae were kept in glass jars (50×100 mm) with a sufficient amount of water. Larvae were provided with finely ground wheat bread crumbs and the jars were covered with fine white mesh allowing ventilation. For all jars, water, and food were replaced daily with clean water and food for the mosquito larvae. Adults were kept in cages under laboratory conditions 25 ± 5 °C, 25 ± 10% humidity a photoperiod of 16L:8D at 10 lx.

Smokes effect

The smoke effect resulted from the burning of industrial incense and cigarettes lasted continuously through all life stages for two generations of *Cx. pipiens* from February 2019 to May 2019. It was accomplished in isolated chambers 4 m × 2 m × 3 m in length, width, and height, respectively. Tests were conducted daily by burning 1.5 g of cigarette materials and 3.5 g of industrial incense for each treatment chamber. The burning process of cigarette and incense stick lasted for 15 min, while insects exposed to the emitted smoke for about 12 h inside each chamber. The same protocol was applied daily starting from the eggs stage to the adult stage for two constitutive generations which extended to 82 days. Data were recorded every day of *Cx. pipiens* various stages.

XRF analysis

Crude materials of cigarette and commercial incense were subjected to X-ray fluorescence spectrometer analysis at the Central Laboratory of South Valley University, Qena, Egypt. Internal instrument calibration was performed prior to the sample study. Cigarette and incense contents were examined for 300 s through a small plastic cup filled with SDI mylar film to evaluate the main and trace elements **(Ene *et al.*, 2010; Mccomb *et al.*, 2014)**.

Sample derivatizations and GC-MS analysis

Sample derivatization and GC-MS analysis were done by taking 30 mg from each sample which was extracted by 6 ml ethyl acetate following by 3 ml diethyl ether to characterize polar and non-polar components. The organic phase from each extraction was pooled and dried in liquid nitrogen. The dried extract was derivatized with bistrimethylsily ltriflouroacetamide; BSTF 100 ul, trimethylchlorosilane; TMCS 20 ul and pyridine 20 ul at 70 °C for 30 min (**Villas-Bôas *et al.*, 2006)**. After cooling the samples were processed for GC-MS analysis. The GC-MS Agilent Technologies device was equipped with gas chromatograph 7890B and mass spectrometer detector 5977A at Central Laboratory Network, National Research Centre, Cairo, Egypt. The GC was packed with HP-5MS column 30 m × 0.25 mm internal diameter and 0.25 μm film thickness. Analyses were accomplished using helium as the carrier gas at 1.0 ml/min flow rate, at a split 50:1, injection volume of 0.5 µl and the following temperature program: 50 °C for one min; rising at 8 °C /min to 300 °C and held for 20 min. The detector and injector were held at 250 °C. Mass spectra were obtained by electron ionization; EI at 70 eV; using a spectral range of m/z 30-700 and solvent delay 8 min. The mass temperature was 230 °C and Quad 150 °C. The identification of different constituents was determined by matching the spectrum fragmentation pattern with those stored in NIST Mass Spectral Library data.

Statistical analysis

Results were subjected to one-way variance analysis; ANOVA, $P < 0.05$ was considered to be significant, and mean values were measured using the Least Significant Difference (LSD) using post hoc test.

RESULTS

Fresh air and stages of *Cx. pipiens*

Data through the two successive generations for numbers of eggs, live larvae, larval moulting and mortality, live pupae, live adults and mortality of *Cx. pipiens* under the condition of fresh air, cigarette, and incense smokes are shown in (Table 1). Results showed that under fresh air a very low loss rate has been observed at all stages. Generally, it was found that in the 1st generation there was no adult mortality while at the 2nd generation some adults had been lost. First generation of *Cx. pipiens*in each stage to the next showed zero loss rates except larval moulting and mortality. In the second generation, the data revealed that live larvae, larval mortality, pupal number, and live adults showed a variable loss rate. It had also been shown that the different rates observed for live larvae, molting of larval, larval mortality, live pupa and live adults were varied between the two generations in the range between 9.52 and 12.02 %.

Cigarette smoke and stages of *Cx. pipiens*

It was evident that cigarette smoke caused loss rates for live larvae, larval mortality, live pupae, live adults, and adult mortality, respectively. In the second generation, cigarette smoke caused a loss in live larvae, larval mortality, pupal numbers, live adults and mortality. Numbers of eggs hatching, live larvae, larval molting, larval mortality, emerged live adults, and adults mortality were found to be in the range between 3.41 and 75.68 %. Exposure to cigarette smoke significantly caused disorder in most stages of *Cx. pipiens*, whereas the difference rate between 2nd and 1st generations of hatched eggs under the cigarette smoke was found to be 88.06 %, and larval mortality 96.39 % and the other stages in the range between 38.62 % and 70.26 % (Table 1).

Table 1. Impact of smokes from cigarette and industrial incense on the yield numbers of various stages of *Cx. Pipiens*for two generations

Treat-ment	Generation	Eggs	Live larvae	Larval moulting	Larval mortality	Pupal No.	Live adults	Adult mortality
Control	1st	7.09 (291)	7.09 (291 ± 9.61)	6.15 (252 ± 2.46)	0.46 (19 ± 0.44)	5.68 (233 ± 2.16)	5.68 (233 ± 2.46)	0.00
	Loss %		0.00 %	13.40 %	7.54 %	0.00 %	0.00 %	0.00 %
	2nd	6.76 (277)	6.39 (262 ± 10.41)	5.51 (226 ± 3.21)	0.51 (21 ± 0.75)	5.00 (205 ± 3)	5.00 (205 ± 3.21)	0.71 (29 ± 1)
	Loss %		5.42 %	13.74 %	8.02 %	10.24 %	0.00 %	14.15 %
	Df 1st/2nd	4.81 %	9.97 %	10.32 %	9.52 %	12.02 %	12.02 %	1.00 %
Smoke from *cigarette*	1st	17.98 (737)	13.59 (557 ± 43.85)	25.29 (1037 ± 9.54)	4.05 (166 ± 3.67)	9.54 (391 ± 3.67)	4.76 (195 ± 2.65)	2.76 (113 ± 2.84)
	Loss %		24.42 %	46.29 %	29.80 %	27.09 %	50.13 %	57.95 %
	2nd	2.15 (88)	2.07 (85 ± 5.41**)	2.01 (86 ± 4.40**)	0.15 (6 ± 0.36**)	2.85 (80 ± 2.15)	1.41 (58 ± 1.09)	1.07 (44 ± 1.09**)
	Loss %		3.41 %	1.16 %	7.06 %	6.98 %	27.50 %	75.68 %
	Df 1st/2nd	88.06 %	52.05 %	91.71 %	96.39 %	38.62 %	70.26 %	61.06 %
Smoke from *industrial incense*	1st	12.34 (506)	17.15 (471 ± 57.34)	24.76 (1015 ± 23.83)	11.68 (247 ± 15.87)	5.46 (224 ± 3.14)	4.49 (184 ± 2.21)	1.46 (60 ± 1.07)
	Loss %		6.92 %	53.59 %	47.56 %	9.31 %	17.86 %	32.61 %
	2nd	19.59 (803)	2.61 (107 ± 3.06**)	1.97 (81 ± 2.19)	1.10 (45 ± 1.13)	1.34 (55 ± 1.04*)	0.80 (33 ± 0.89*)	0.44 (18 ± 0.63)
	Loss %		86.67 %	24.29 %	42.06 %	48.60 %	4.00 %	54.55 %
	Df1st/2nd	36.99 %	77.28 %	92.02 %	81.78 %	75.45 %	82.07 %	70.00 %

*P-value significant (<0.05); **P-value significant (<0.01)

Industrial incense smokes and stages of *Cx. pipiens*

Results show smoke effect of industrial incense on the two generations of *Cx. pipiens*. 1st generation of smoke effect from industrial incense caused loss rate for the larval mortality and adult mortality. The 2nd generation of *Cx. pipiens* had been affected by loss rate for live larvae, larval mortality, pupal numbers and adult mortality. Live larvae, larval moulting and mortality, adult mortality and live adults negatively decreased from 86.67 and 4.00%. However, difference in rates between the two generations on eggs showed increasing by 36.99%. That means, egg production of the 1st to 2nd generations was affected negatively by smoke of cigarette and positively by industrial incense (Table 1).

XRF analysis

Cigarette and industrial incense substances were analyzed for their mineral phases by XRF, which showed variable quantities (Fig. 1, 2). Dominant and non-dominant elements were quantified in cigarette and industrial incense substances. Incense showed a major amount of Calcium followed by Ferrous and Potassium. Silica, Manganese, Zinc, and Titanium were identified to be minor components in the range between 3.32 and 0.68%. Cigarette had Calcium and Potassium as major components representing 60.86% and 31.39% respectively. Ferrous, Silica, Manganese, Bromine, Strontium, and Sulfur found to be minor components in the range between 2.96 and 0.10% (Table 2).

Phytocomponents in cigarette and industrial incense raw materials

Identification and characterization of the phytocomponents found in the ethyl acetate and diethylether extracts to know polar and nonpolar chemicals present in cigarette and incense are shown (Tables 3 and 4; Figs 2 and 3). Sixty peaks were recognized from the GC-MS chromatogram for the cigarette of which the most common compounds found are Glycerol, 3TMS derivative, followed by Dimethyl phthalate, 5.alpha.-Androstan-1.alpha.-methyl-3.alpha.,17.beta.-diol, di-trimethylsilyl, alpha.-Linolenic acid, TMS derivative, Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-, D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 2). The remaining fifty three chemicals were found in less amount of in the range between (0.20 to 2.79%) (Table 3, Fig. 2). Industrial incense had thirty-three peaks and the most dominant chemical is Dimethyl phthalate representing, followed by Methyl trimethylsilyl phthalate. Chemicals found in fewer amounts are Decanoic acid, TBDMS derivative, Phthalic acid, 2TMS derivative, Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester. Also, there were chemicals found in very less amount in the range between 0.08 % to 0.71 % (Table 4, Fig. 4).

Table 2. XRF analysis of raw materials of cigarette and industrial incense

Element %	Raw materials of cigarette %	Raw materials of industrial incense %
Si	1.4909	3.3248
K	31.3946	6.1237
Ca	60.8629	78.2430
Mn	0.6791	0.8506
Fe	2.4062	10.0409
Zn	Not determined	0.7371
Ti	Not determined	0.6799
Br	0.1083	Not determined
Sr	0.0985	Not determined
S	2.9594	Not determined

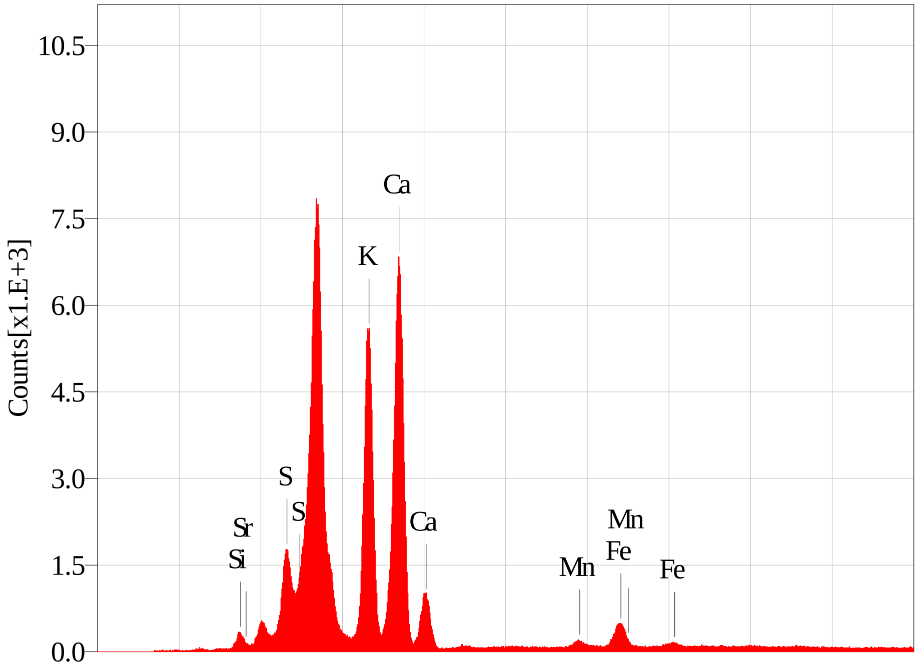

Fig. 1 XRF material output of cigarettes.

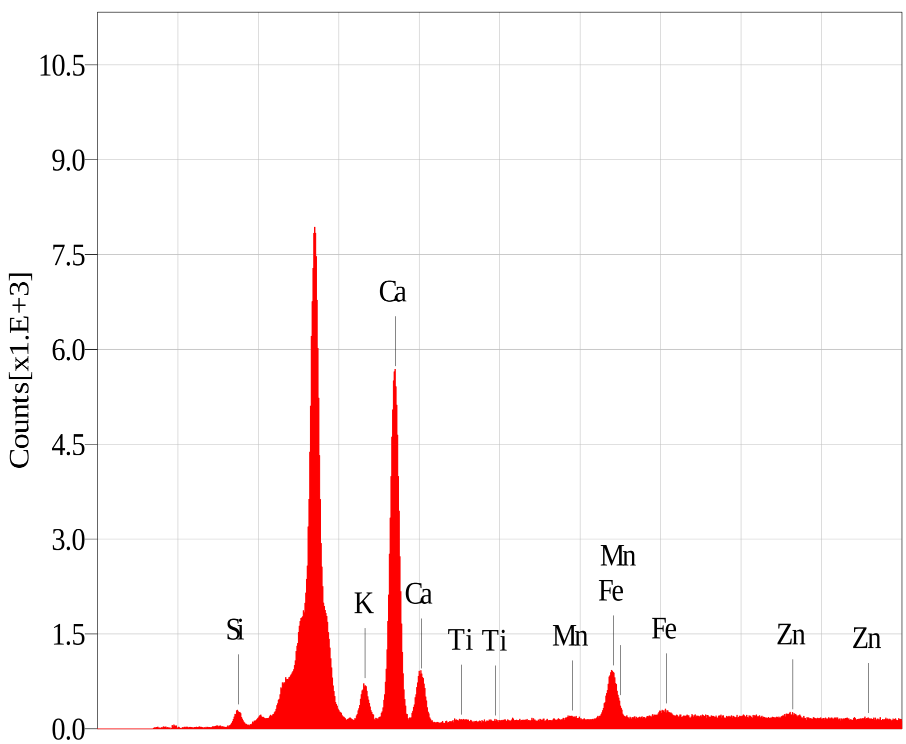

Fig. 2 XRF material output of industrial incense.

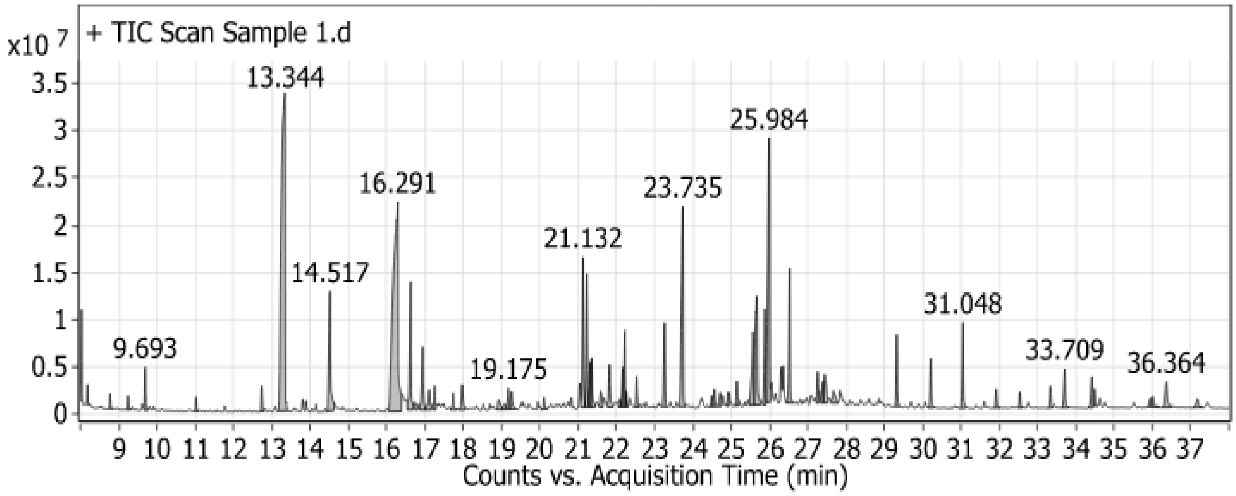

Fig. 3 Typical chromatograph of GC-MS with identified chemical from extract of ethyl acetate and diethyletherof raw material of cigarette.

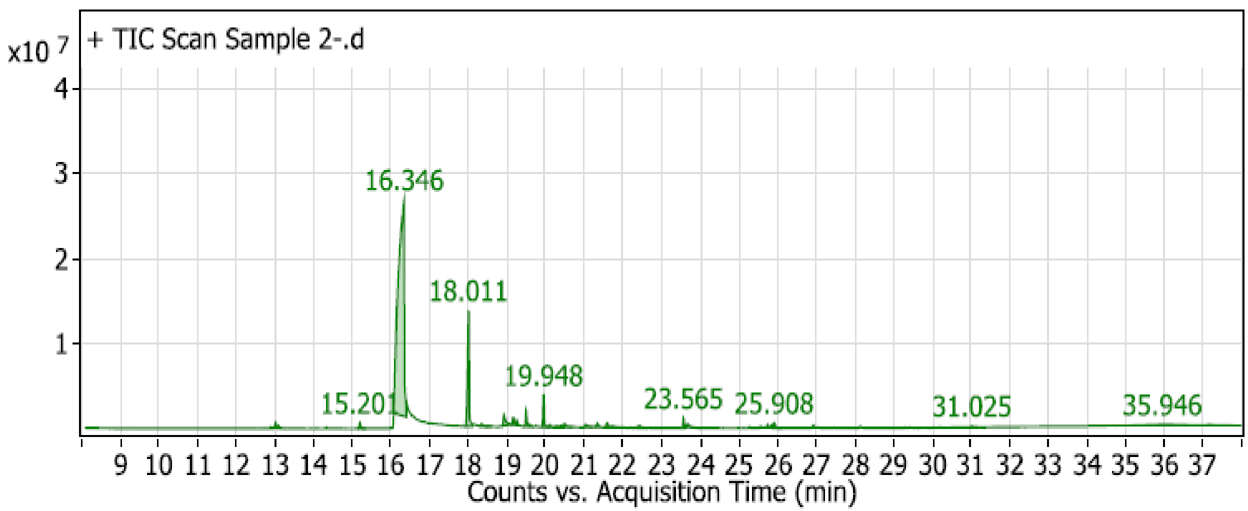

Fig. 4 Typical chromatograph of GC-MS with identified chemical from extract of ethyl acetate and diethylether of raw material of industrial incense.

Table 3. GC-MS analysis of ethyl acetate and diethylether extracts of raw materials of cigarette

Peak	RT	Name	Formula	Area	Area Sum %
1	8.772	Propylene glycol, 2TMS derivative	$C_9H_{24}O_2Si_2$	2774659.7	0.23
2	9.253	Tris(trimethylsilyl)carbamate	$C_{10}H_{27}NO_2Si_3$	2518988.3	0.21
3	9.693	D-(-)-Lactic acid, 2TMS derivative	$C_9H_{22}O_3Si_2$	7642719.1	0.63
4	11.021	Hydracrylic acid, 2TMS derivative	$C_9H_{22}O_3Si_2$	2492883.8	0.21
5	12.737	Benzoic Acid, TMS derivative	$C_{10}H_{14}O_2Si$	5485902	0.45
6	13.344	Glycerol, 3TMS derivative	$C_{12}H_{32}O_3Si_3$	237671251	19.64
7	14.517	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	$C_{10}H_{14}N_2$	40498040	3.35
8	16.291	Dimethyl phthalate	$C_{10}H_{10}O_4$	202674517	16.75

9	16.623	Malic acid, 3TMS derivative	$C_{13}H_{30}O_5Si_3$	34482905	2.85
10	16.76	1-Benzenesulfonohydrazide, N'-tricyclo[4.2.2.0(1,5)dec-8-ylidene	$C_{16}H_{20}N_2O_2S$	2514927.4	0.21
11	16.937	2'-Deoxyribolactone, 2TMS derivative	$C_{11}H_{24}O_4Si_2$	18584116	1.54
12	17.115	L-5-Oxoproline, , 2TMS derivative	$C_{11}H_{23}NO_3Si_2$	7083156.5	0.59
13	17.252	3-Aminothiophenol, 2TMS derivative	$C_{12}H_{23}NSSi_2$	5649964.2	0.47
14	17.733	L-Threonic acid, tris(trimethylsilyl) ether, trimethylsilyl ester	$C_{16}H_{40}O_5Si_4$	4697027.2	0.39
15	17.967	Monomethyl phthalate, TMS derivative	$C_{12}H_{16}O_4Si$	8001740.7	0.66
16	18.923	α-d-Xylopyranoside, methyl-2,3,4-tris-O-[9-borabicyclo[3.3.1]non-9-yl]-	$C_{30}H_{51}B_3O_5$	4567488.8	0.38
17	19.095	10,12-Tricosadiynoic acid, TMS derivative	$C_{26}H_{46}O_2Si$	2382974.6	0.2
18	19.175	4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	$C_{15}H_{22}O_2$	6213514.5	0.51
19	19.255	Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihy	$C_{15}H_{24}O_3$	5618365.7	0.46
20	20.113	3-(2-Hydroxyethyl)phenol, TBDMS derivative	$C_{14}H_{24}O_2Si$	3176540.8	0.26
21	21.132	D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 2)	$C_{21}H_{52}O_6Si_5$	38794419	3.21
22	21.229	D-Psicofuranose, pentakis(trimethylsilyl) ether (isomer 2)	$C_{21}H_{52}O_6Si_5$	35250604	2.91
23	21.32	D-(-)-Fructopyranose, 5TMS derivative (isomer 1)	$C_{21}H_{52}O_6Si_5$	10173939	0.84
24	21.349	Myristic acid, TMS derivative	$C_{17}H_{36}O_2Si$	12179520	1.01
25	21.584	1,5-Anhydrohexitol, 4TMS derivative	$C_{18}H_{44}O_5Si_4$	6040132.5	0.5
26	21.818	Quininic acid (5TMS)	$C_{22}H_{52}O_6Si_5$	11007784	0.91
27	22.093	D-Fructose, 5TMS derivative	$C_{21}H_{52}O_6Si_5$	2449105.6	0.2
28	22.167	D-(-)-Tagatose, 5TMS derivative	$C_{21}H_{52}O_6Si_5$	9901431.5	0.82
29	22.219	D-Glucose, 5TMS derivative	$C_{21}H_{52}O_6Si_5$	17073798	1.41
30	22.259	L-(-)-Sorbose, 5TMS derivative	$C_{21}H_{52}O_6Si_5$	2888303.1	0.24
31	22.528	Acrylic acid, 2,3-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{12}H_{28}O_4Si_3$	7443298.9	0.62
32	23.255	D-Glucopyranose, 5TMS derivative	$C_{21}H_{52}O_6Si_5$	19075624	1.58
33	23.735	Palmitic Acid, TMS derivative	$C_{19}H_{40}O_2Si$	61244523	5.06
34	24.49	Doconexent, TMS derivative	$C_{25}H_{40}O_2Si$	2622457.6	0.22
35	24.553	Myo-Inositol, 6TMS derivative	$C_{24}H_{60}O_6Si_6$	4411643.9	0.36
36	24.708	Doconexent, TBDMS derivative	$C_{28}H_{46}O_2Si$	4092456.1	0.34
37	24.92	Epitestosterone, TMS derivative	$C_{22}H_{36}O_2Si$	3392316.7	0.28
38	25.149	Epimethendiol-diOTMS	$C_{26}H_{48}O_2Si_2$	6376744.2	0.53
39	25.566	9,12-Octadecadienoic acid (Z,Z)-, TMS derivative	$C_{21}H_{40}O_2Si$	25318828	2.09
40	25.664	.alpha.-Linolenic acid, TMS derivative	$C_{21}H_{38}O_2Si$	44963501	3.72
41	25.875	Stearic acid, TMS derivative	$C_{21}H_{44}O_2Si$	22453372	1.86
42	25.984	5.alpha.-Androstan-1.alpha.-methyl-3.alpha.,17.beta.-diol , di-trimethylsilyl	$C_{26}H_{50}O_2Si_2$	85638137	7.08
43	26.528	Epitestosterone, TMS derivative	$C_{22}H_{36}O_2Si$	33788945	2.79
44	27.254	Eicosapentaenoic Acid, TBDMS derivative	$C_{26}H_{44}O_2Si$	7652859.9	0.63
45	27.397	5,8,11-Eicosatriynoic acid, TMS derivative	$C_{23}H_{36}O_2Si$	5988980.6	0.49
46	27.437	11-Hydroxyetiocholanolone (3.alpha.,5.alpha.,11.beta.)-, 2TMS derivative	$C_{25}H_{46}O_3Si_2$	13234467	1.09

47	27.666	5,8,11-Eicosatriynoic acid, tert-butyldimethylsilyl ester	$C_{26}H_{42}O_2Si$	6614646.3	0.55
48	29.326	1-Monopalmitin, 2TMS derivative	$C_{25}H_{54}O_4Si_2$	14119055	1.17
49	30.213	Sucrose, 8TMS derivative	$C_{36}H_{86}O_{11}Si_8$	11206862	0.93
50	31.048	Glycerol monostearate, 2TMS derivative	$C_{27}H_{58}O_4Si_2$	18603304	1.54
51	31.918	Nonacosane	$C_{29}H_{60}$	4444265.7	0.37
52	32.536	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	3725700.5	0.31
53	33.337	Heptacosane	$C_{27}H_{56}$	5611588.9	0.46
54	33.709	Hentriacontane	$C_{31}H_{64}$	11036525	0.91
55	34.418	.alpha.-Tocopherol, TMS derivative	$C_{32}H_{58}O_2Si$	9393527.6	0.78
56	34.487	Triacontane, 1-bromo-	$C_{30}H_{61}Br$	4826384.4	0.4
57	35.94	Campesterol, TMS derivative	$C_{31}H_{56}OSi$	3198913	0.26
58	36.009	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	$C_{33}H_{54}O_3$	4193104	0.35
59	36.364	Stigmasterol, TMS derivative	$C_{32}H_{56}OSi$	11207903	0.93
60	37.176	.beta.-Sitosterol, TMS derivative	$C_{32}H_{58}OSi$	3783643.3	0.31

Table 4. GC-MS analysis of ethyl acetate and diethylether extracts of raw materials of industrial incense

	RT	**Name**	**Formula**	**Area**	**Area Sum %**
1	13.023	Tripropylene glycol monomethyl ether, TMS derivative	$C_{13}H_{30}O_4Si$	1480969.2	0.41
2	15.201	.beta.-Eudesmol, TMS derivative	$C_{18}H_{34}OSi$	1433694.7	0.39
3	16.346	Dimethyl phthalate	$C_{10}H_{10}O_4$	275222001	75.68
4	18.011	Methyl trimethylsilyl phthalate	$C_{12}H_{16}O_4Si$	39369759	10.83
5	18.35	2-Propenal, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{12}H_{18}O$	1166609.7	0.32
6	18.524	1-Benzenesulfonohydrazide, N'-tricyclo[4.2.2.0(1,5)dec-8-ylidene	$C_{16}H_{20}N_2O_2S$	303696.12	0.08
7	18.923	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	$C_{13}H_{22}O_3$	5456772.7	1.5
8	19.172	4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	$C_{15}H_{22}O_2$	2088256.6	0.57
9	19.255	4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	$C_{15}H_{22}O_2$	1247646.6	0.34
10	19.496	Phthalic acid, 2TMS derivative	$C_{14}H_{22}O_4Si_2$	4133074.5	1.14
11	19.594	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	656475.49	0.18
12	19.948	Decanoic acid, TBDMS derivative	$C_{16}H_{34}O_2Si$	7102461.5	1.95
13	20.106	Benzoic acid, 2-methoxy-4-methyl-3-nitro-, methyl ester	$C_{10}H_{11}NO_5$	643245.73	0.18
14	20.28	Falcarinol	$C_{17}H_{24}O$	763529.81	0.21
15	20.468	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	893127.04	0.25
16	20.506	Doconexent	$C_{22}H_{32}O_2$	1130214	0.31
17	21.041	3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-3a,4,7,8,9,1	$C_{15}H_{20}O_4$	1207428.4	0.33
18	21.334	5,8,11-Eicosatrienoic acid, (Z)-, TMS derivative	$C_{23}H_{42}O_2Si$	1058153.2	0.29
19	21.598	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	$C_{18}H_{26}O$	1605190	0.44
20	21.734	3.beta.,17.beta.-dihydroxyestr-4-ene	$C_{18}H_{28}O_2$	582729.56	0.16

21	22.435	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	1111454.5	0.31
22	23.565	Ethylene brassylate	$C_{15}H_{26}O_4$	2586271.1	0.71
23	23.67	2,4-Difluorobenzene, 1-benzyloxy-	$C_{13}H_{10}F_2O$	1027027.8	0.28
24	25.652	7,10-Epoxytricyclo[4.2.1.1(2,5)]decane, 1-trimethylsilyl-	$C_{13}H_{22}OSi$	322667.04	0.09
25	25.75	9,12-Octadecadiynoic acid, trimethylsilyl ester	$C_{21}H_{36}O_2Si$	1000782.7	0.28
26	25.848	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	811544.86	0.22
27	25.908	Bicyclo[5.3.0]decan-2-one, 9-trimethylsilylmethylene	$C_{14}H_{24}OSi$	1939257.5	0.53
28	26.926	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]m	$C_{27}H_{52}O_4Si_2$	1140693.6	0.31
29	31.025	t-Butyl-(2-[3-(2,2-dimethyl-6-methylene-cyclohexyl)-propyl]-[1,3]dithian-2-yl)-dimethyl-silane	$C_{22}H_{42}S_2Si$	384914.45	0.11
30	35.946	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3.alpha.,5.alpha.)-	$C_{29}H_{43}NO_3Si$	2581664.8	0.71
31	37.151	Cyclobarbital	$C_{12}H_{16}N_2O_3$	301337.68	0.08
32	45.094	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]m	$C_{27}H_{52}O_4Si_2$	2578951	0.71
33	50.165	1,4-Bis(trimethylsilyl) benzene	$C_{12}H_{22}Si_2$	337876.47	0.09

DISCUSSION

To the best of our knowledge, no studies have been published about the effect of the long-term toxic effect of inhaling cigarette and industrial incense smokes on mosquitos. Results of the current study indicated that mosquito biology behaviors were more negatively affected by cigarette smoke than industrial smokes. Effects of cigarette smoke on the first and the second generations of *Cx. pipiens*exhibited loss rates towards the hatched numbers of eggs, alive larvae, larval moulting and mortality, alive pupae, alive adults, and adult mortality more than industrial incense. This may be due to the differences between cigarette and incense chemical components as indicated from GC-MS and XRF results. Regardless of the lack of overall differences between the impact of cigarette and industrial incense smokes, there was some interesting complexity in the results. For instance, under cigarette smoke, eggs in the 1st generation were seen to be not affected. However, under incense smoke, the number of eggs increased. These results may be explained by the nature of the insect egg shell which may be less affected with smoke or gases from industrial incense smoke/or it causes mutation that allows adult female to lay an increased numbers of eggs (need more investigations). It has been verified that the egg stage of the insect is difficult to monitor due to its unusual egg shell structure, which consists of several layers to allow the embryo to breathe industrial incense smoke, and previously has been shown to be as a barrier to insecticides (**Campbell *et al.*, 2016)**. In addition, the sensitivity of how the egg shell hardens varies depending on the age of the egg, as the hardness changes over time, and the embryo may develop more enzymes that break down insecticides **(Jacobs *et al.*, 2013)**.

The negative impact of cigarette smoke may be from the genetic mutation on various stages of *Cx. pipiens*. Many other mutation patterns associated with smoking **(Blackford *et al.*, 2009)**.

The susceptibility level of mosquito's stages to cigarette and industrial incense smokes through the two generations was different from each other. It was found that the effect of incense smokes on the live larvae produced a loss rate that was higher compared with the effect of cigarette smoke. The metal component of incense should be taken into consideration. It was proved by XRF analysis the presence of Zn but not in cigarette which was reported to be used in different forms to manage mosquito larvae **(Mostafa *et al.*, 2018).** The differences in the loss rate of the live larvae, larval moulting and mortality, live pupae, pupal mortality, and adult mortality through the exposure to smokes for two constitutive generations may have occurred because of the possible morphological malformation.

Regarding GC-MS analysis of cigarette, there were sixty peaks, some of these peaks referred to toxic chemicals found such as D-(-)-Lactic acid, 2TMS derivative, Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-, Dimethyl phthalate and 3-Aminothiophenol, 2TMS derivative, which was reported as good insect repellent **(Brown and Hebert, 1997; Jantan and Zaki, 1998).**

CONCLUSION

This is the first demonstration of cigarette and industrial incense smokes directly inhaled by *Cx. pipiens* for continuous 82 days, which is important to investigate the alteration in the physiological response associated with smoking to other organisms. Our works suggested that long–term inhaling of the smoke from cigarette continuously could alter all successive stages of *Cx. pipiens* more severely than the industrial incense smoke. This is probably related to the toxicity of the chemicals as revealed by GC-MS and XRF analysis. Our results may be beneficial for further exploration of the toxicological mechanism of identified chemicals from cigarette and incense smokes against *Cx. pipiens* and human.

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