



Original article

Agro-waste derived compounds (flax and black seed peels): Toxicological effect against the West Nile virus vector, *Culex pipiens* L. with special reference to GC–MS analysis



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ABSTRACT

The development of different approaches to use agricultural residues as a source of high value-added products, become a must, especially after the problems emerged due to their accumulation. This contribution demonstrates the potential of agricultural residues, *Linum usitatissimum* (flax seed) and *Nigella sativa* (black seed) peels, as raw materials for the production of bioactive products, botanical insecticides, against *Cx. pipiens*, with deep analysis to their chemical constituents by gas chromatography-mass spectrometry, the larvicidal efficacies of the three crude extracts (methylene chloride, petroleum ether and methanol 70%) from the two plant waste peels were evaluated for the first time against the late third instar larvae of *Cx. pipiens*. Results indicated different lethal doses in larvae depending on the efficacy of organic solvent used. For both compounds methanol 70% extracts produced the highest dry yield. The most efficient solvent is petroleum ether in case of both flax and Black seed peels. Petroleum ether extract exhibited the highest toxicity against *Cx. pipiens* with an LC50 of 69.6383 ppm. The same results for black seed indicated that petroleum ether was the most efficient against *Cx. pipiens* with an LC50 of 40.7748 ppm. The study revealed for the first time the type of phytochemical constituents presents in peels of flax and black seeds using GC–MS analysis which revealed twenty-eight constituents among extracts of flax and black seed peels ranging from to 58.8711% to 99.99% of the total extracts. GC–MS profiling showed that a five constituents, 9-2-Methyl-Z, Z-3, 13 octadecadienol (terpenoid), 9,17-Octadecadienal, (Z)-, Nonanoic acid, 9-oxo-, methyl ester, 9,12-Octadecadienoic acid Z,Z and Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- have insecticidal activity beside many other biological activities as recorded from a variety of botanical extracts. While the constituents like Hexadecanoic acid, methyl ester and *cis*-9-Hexadecenal, both of them are larvicidal, *cis*-Vaccenic acid and 9-Oxononanoic acid showing only an insecticidal activity beside Undecanoic acid the mosquito repellent. The other six constituents Linoelaidic acid, Oleic Acid, Z-2-Octadecen-1-ol, 1-Methoxy-3-hydroxymethylheptane, Cis-11,14-Eicosadienoic acid-methyl ester and Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- are constituents of other plant extracts which showed as a whole an insecticidal activity.

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1. Introduction

There is an emerging trend on the utilization of biomass from burning of rice husk and sugarcane bagasse to sustainable recycling of agricultural residues. Biomass is still largely underutilized and left to decay or openly burned in the fields, particularly in developing countries that do not have strong regulatory mechanisms to control such practices. As common practice, direct burning of agricultural residue leads to air pollution and thereby posing risk to human and ecological health. Biomass is a renewable

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resource that causes problems when not recycled. Biomass takes the form of residual stalks, leaves, straw, roots, husk, nut or seed shells (peels), animal husbandry waste and waste wood. Widely available, renewable, and virtually free, waste biomass is an important resource. The challenge, therefore, is to convert biomass as a resource for energy and other productive uses to promote ecological solid waste management and environmentally practices [Dtie UNEP, 2009; Baiano 2014].

Researches have focused on the identification and isolation of compounds from waste and by-products generated from cereals processing that of high relevance as they arise in significant amounts in Egypt. During harvesting and processing of these crops, wastes and by-products are generated. These wastes can potentially be upgraded in other production processes and may be used as resources for bioactive compounds [Baiano 2014; Gómez-Caravaca et al., 2014]. Many of these compounds extracted from natural products could be useful lead compounds in the production of Insecticides [Pawelczyk 2005]. By proper methods, these compounds can be isolated and screened for various insecticidal activities. The results will be promising in the toxicological field [Mirabella et al., 2014].

Recently, the insecticidal activities of many plant extracts were tested against various pests [Ahmed 1983; Soliman and Sallam 2009]. The attention has been focused on cheap cereals and associated peels like flaxseed (*Linum usitatissimum*) a rich source with a variety of constituents like of polyunsaturated linolenic acid, α -linolenic acid, phenolic compounds and lignin, it has been reported in many studies for its antimicrobial, antioxidant, and anti-inflammatory effects [Millam et al., 2005].

Amongst the promising medicinal plant waste peels, *Nigella sativa* (black seed) a dicotyledonous of ranunculacea is an amazing herb with a rich historical and religious background. The seeds of *N. sativa* are the source of the active ingredient of this plant [Marwat et al., 2009]. It contains over 100 valuable constituents including 21% protein, 38% carbohydrates and 35% plant fats and oils [Deepak Suri et al., 2011]. The seeds of *N. sativa* L. are the most extensively studied, both phytochemically and pharmacologically, the aqueous and oil extracts of the seeds have been shown to possess antioxidant, analgesic, anticancer, anti-inflammatory and antimicrobial activities [Gali-Muhtasib et al., 2006]. Besides, the essential oil was shown to have antihelminthic [Agarwal et al., 1979], antinematodal [Akhtar and Riffat 1991], antischistosomal [Mahmoud et al., 2002], antimicrobial [Hanafy and Hatem 1991; Aboul-Ela, 2002] and antiviral [Salem and Hossain 2000] effects. Extracts from the plant also exhibited insecticidal and insect repelling activity against *Tribolium castaneum*, *Tuta absoluta*, *Amblyomma americanum* and *Aedes aegypti* [Adil et al., 2015; Moretti et al., 2004].

Insecticidal efficacy of numerous plant extracts and their components has been studied against mosquitoes [Wijayanti et al., 2019; Essa et al., 2019]. Skin reactions and allergy caused by mosquito bites are among health problems associated with the presence of mosquitoes. The West Nile virus (WNV) (family Flaviviridae, genus *Flavivirus*) can infect humans, horses and birds, it is transmitted mainly by the genus *Culex* [Hubálek, 2008]. The West Nile virus is now distributed worldwide. In Egypt, West Nile virus (WNV) caused intermittent outbreaks typically associated with mild febrile illnesses. WNV was first isolated from *Culex* spp. mosquitoes in Egypt in 1952 [Chancey et al., 2015]. Genus *Culex* is the main vectors spreading viruses that cause Japanese encephalitis, St. Louis encephalitis and West Nile fever. Although the eco-friendly control strategies is the best choice for mosquito control, the synthetic insecticides, mainly organophosphates and pyrethroids, are the main tool for the management of mosquitos, including *Cx. pipiens*, which is the most common mosquito insect in rural and urban zones in Egypt [Zahran et al., 2017]. For outdoor

control of *Cx. pipiens*, organophosphate insecticides are commonly applied as larvicides, while for indoor control of adults, pyrethroid insecticides are presently used. However, there are several problems come across the mosquito control programs by using conventional insecticides, such as the development of mosquito resistance, adverse effects on non-target organisms and environmental risks [El-Sabrou et al., 2020].

In the literature, there are no studies on the insecticidal effect of peels of flax and black seed, this work is aimed to investigate the insecticidal bioactivity of crude extracts of three different solvents against the larvae of *Cx. pipiens*. As Gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological for secondary metabolite profiling in both plant and nonplant species [Aftab et al., 2020]. Thus, the present study was aimed to investigate the possible chemical components by preparing (methylene chloride, petroleum ether and methanol 70%) extracts of flax and black seed peels and identification of the compounds by subjecting it to GCMS analysis.

2. Material and methods

2.1. Maintenance of mosquito colony, *Cx. pipiens* L. (Diptera: Culicidae) in the laboratory

The egg rafts of *Cx. pipiens* were obtained from the Research and Training Center on Vectors of Diseases (RTC), Faculty of Science, Ain Shams University. The mosquitoes were reared for minimum eight generations in insectary rooms, under controlled laboratory conditions at temperature 27 ± 2 °C, and relative humidity RH $70 \pm 10\%$, for photoperiods 14:10 (light: dark) hours. Egg rafts were placed in white enamel dishes 35–40 cm in diameter and 10 cm in depth filled with 1500 ml of distilled water. Newly hatched larvae were fed on fish food (Tetra-/Min, Germany) as a diet sprinkled twice daily over the water surface of the breeding pans [Gerberg et al., 1969]. Distilled water in each dish was stirred daily and changed every two days to avoid scum formation on the water surface or on the walls and bottoms of pans. Small air pump was used to aerate the breeding water gently every day for about 5 min. Pupae were collected routinely and separated in plastic containers filled with distilled water then introduced into screened wooden cages until emergence. Adults were reared in (24 × 24 × 24 cm) wooden cages and provided daily with cotton pads soaked in 10% sucrose solution for a period of four days. After this period the females were allowed to take a blood meal from a pigeon host. Sucrose was removed 24 h before blood meal to obtain best blood feeding. Oviposition containers filled with distilled water were placed in adult cages 48 h after blood feeding. Egg rafts were collected routinely and placed in white enamel dishes. When mosquito larvae developed to the 2nd instar, they were poured into clean pans (25 × 30 × 15 cm) containing 3 L of tap water left for 24 h and observed daily [Gerberg et al., 1969; Kasap and Demirhan, 1992]. Late third larval instars were used for toxicological studies.

2.2. Preparation of *Linum usitatissimum* and *Nigella sativa* extracts

The plants *Linum usitatissimum* and *Nigella sativa* seed peels washed to remove any contamination, dried under shade conditions in the laboratory for two days and grinded using electric blender to a coarse powder [Odey et al., 2012]. One hundred grams of the powder materials of each plant were successively extracted in a soxhlet apparatus [Freedman et al., 1979]. Solvents of different polarity were used petroleum ether (60–80 °C), Methylene chloride and methanol 70% {1 kg powder material: 3L solvent}. The whole solution was blended, then filtered and used. Solvent was evapo-

rated by using a rotary evaporator (Labo-Rota C311) at water bath adjusted 40 °C for 2_3 hours in methanol 70% and 40_60 minutes to other solvents. Then, the obtained crude extract was collected, weighted and a sample was sent an aliquot quantity for GC–MS analysis. The rest was kept in deep freezer (–4 °C) in screw capped vials, till used for experiments.

2.3. Toxicological studies

A series of toxicological bioassays were carried out in order to determine the insecticidal activity of the tested extracts, the toxicity was evaluated against the late 3rd instar larvae of *Cx. pipiens*. Batches of 25 third instar larvae were transferred to 30 small disposable test cups, and treated with *N. sativa* and *L. usitatissimum* peel extracts at different concentrations (50, 100, 200, 300 & 400 ppm). Experiments were repeated 3 times. Mortality recorded after 24 and 48 hrs. Statistical analysis: Data were analyzed by statistics package (LDP-line) for goodness of fit (Chi square test) and to detect LC50 and LC95 values with corresponding 95% confidence limits (C.L.), slope, correlation coefficient and standard error [Abbott, 1925].

2.4. Gas chromatography mass spectrometry (GC–MS)

GC–MS analysis was done for the petroleum ether and chloroform extracts of each flax and black seed peels using a Shimadzu GC–MS–QP 2015 plus (Kyoto, Japan). This was performed by injecting 0.5 µl of the examined extract into Hewlett Packard chromatograph model 5970, equipped with flame ionization detector (FID) and 50 m HP capillary column (0.2 mm I.D.). The oven temperature was programmed at 3 °C/minute from 60 °C to 200 °C, then isothermally at 200 °C for 25 min. Detector and injector temperatures were 250 °C and 200 °C, respectively. The carrier gas was helium and gas flow rate was 1 ml/minute. Diluted samples (1% v/v) were injected with split ratio 15:1 and the injected volume was 1 µl. The MS operative parameters were as follows: interface temperature: 280 °C, ion source temperature: 200 °C, EI mode: 70 eV, scan range: 35–500 amu. M. In order to identify obtained peak, this was accomplished through comparing the retention time of each peak with those of the authentic. The quantization of the components was determined through comparing the area of the resulted peaks with data from the WILEY, NIST and Tutor libraries [Beckley et al., 2014].

3. Results

3.1. The mean yield of methylation extraction from the tested agricultural wastes

The extract mean yield obtained by solvent extraction from agro-waste extracts; *Nigella stiva* peels and, *Linum usitatissimum*

Table 1

The mean yield of extraction from the tested agricultural wastes by using different solvents. SE = Standard error. Each value represented mean of 4 replicates ± SE.

Agricultural wastes name	Solvent used	Mean yield (gm) from 100 gm of the plant ± SE
<i>Linum usitatissimum</i> peels	Pet-ether	7.3 ± 0.03 c
	Methylene chloride	5.1 ± 0.03 e
	Methanol 70%	8.9 ± 0.05 b
<i>Nigella sativa</i> peels	Petroleum ether	6.9 ± 0.04 d
	Methylene chloride	4.2 ± 0.03 f
	Methanol 70%	10.1 ± 0.04 a

peels prepared using solvents of different polarities. Data presented in Table 1 showed that the mean yields of the extracts were varied from one waste to another and from one solvent to another. Generally, methanolic extracts yielded the highest yields (weights) followed by petroleum ether extracts. The 70% methanolic extract of *N. sativa* peels gave the highest yield (10.1gm) followed by 70% methanolic extract of *Linum usitatissimum* peels, petroleum ether extract of *Linum usitatissimum* peels, petroleum ether extract of *N. sativa* peels, methylene chloride extract of *Linum usitatissimum* peels, and finally methylene chloride extract of *N. sativa* peels with yields (8.9, 7.3, 6.9, 5.1 and 4.2 gm), respectively.

According to the obtained data, the tested plant extracts could be arranged in a descending order as follows

N. sativa peel 70% methanolic extract > *Linum usitatissimum* peels 70% methanolic extract > *Linum usitatissimum* peels petroleum ether extract > *N. sativa* peels petroleum ether extract > *Linum usitatissimum* peels methylene chloride extract > *N. sativa* peels methylene chloride extract.

3.2. Gas chromatography-mass spectrophotometry (GC–MS) analysis of the tested extracts

The presence of biologically active components from different solvents extracts of *Nigella sativa* and *Linum usitatissimum* peels were evaluated by conducting GC–MS analysis. The principal active compounds, molecular weight (g mol⁻¹, M.W.), molecular formula (M.F.), retention time (R.T.) and peak area (%) are presented in Table 2. The results of GC–MS analysis of the extracts led to the determination of several biological compounds. In the crude extract of *Linum usitatissimum* peels, all solvents showed the presence of major compounds like, (Z,Z)- 9,12-Octadecadienoic acid (Z,Z)- which gave the highest percentage with the solvent methylene chloride 70.606%, 2-Methyl-Z, Z-3, 13 octadecadienol (terpenoid)(giving the highest percentage with methanol 25.868%), and finally, Oleic Acid (minor compounds with relative peak areas ranging from 0.2 to 3.390%). Both methanol and petroleum ether gave 9, 17-Octadecadienal, (Z)- with the percentage of 46.801% and 43.48% respectively. Methanol solvent alone gave *cis*-9-Hexadecenal (1.519%).Petroleum ether alone gave many compounds including: Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (12.628%), Heptasiloxane, 1,1, 3,5,5,7,7,9,9,11,11,13,13 tetradecamethyl- (4.604%), Ethyl 5-(furan-2-yl)-1,2-oxazole-3-carboxylate (6.717%), Cyclopentaneundecanoic acid (2.299%), 1,3-Oxathiane, 2-ethyl-6-methyl-(12.774%), Dodecanoic acid, 1-methylethyl ester (0.601%), 2-Cyclopenten-1-one, 3-ethyl-Bis(carboxymethyl) trithiocarbonate 2TMS derivative (1.290%), Trimethylsilyl-di(time thylsiloxy)-silane (2.517%) and Undecanoic acid (0.322%).

In the crude extract of *N. sativa* peels, all solvents showed the presence of (Z, Z) – 9, 12-Octadecadienoic acid (Z, Z) - with the highest percentage (18.66%) extract from methanol. The two solvents methanol and Methylene chloride gave the compounds Hexadecanoic acid methyl ester and Oleic acid. Alone methanol solvent gave the largest number of *N. sativa* compounds as follow *cis*-Vaccenic acid, Linoelaidic acid, 9,12 Octadecadienoic acid methyl ester, 2-Methyl-Z, Z-3, 13 octadecadienol and 9,17-Octadecadienal, (Z)-. While Methylene chloride alone gave 7,12a-Dimethyl-1,2,3,4,4a,11,12,12a-octahydrochrysene and 1,5,9-Cyclododecatriene, (E,E,Z). Petroleum Ether gave the least number of the compounds of which 1-Eicosene is the highest percentage of 50.331% followed by 9,12-Octadecadienoic acid (Z, Z-(linoleic acid), Nonanoic acid, 9-oxo-methyl ester and Cis-11,14 –Eicosadienoic acid -methyl ester. Nonanoic acid, 9-oxo-, methyl ester was extracted in low quantities using each of the solvents Methylene chloride and petroleum ether.

Table 2
Major phytochemical compounds identified in different solvents extract of *Linum usitatissimum* and *Nigella sativa*.

Phytochemical compound	Molecular formula	Molecular weight g/mol	<i>Linum usitatissimum</i>						<i>Nigella sativa</i>					
			Methylene Chloride		Methanol		Petroleum Ether		Methylene Chloride		Methanol		Petroleum Ether	
			% Area	RT (min)	% Area	RT (min)	% Area	RT (min)	% Area	RT (min)	% Area	RT (min)	% Area	RT (min)
1 Hexadecanoic acid, methyl ester	C ₁₈ H ₃₆ O ₂	284.5	16.9884	14.2821										
2 cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238.41	-		1.519	11.5183	-	-	6.00248	13.504	5.8789	13.4754		
3 cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂	282.468									0.6115	10.9806		
4 9,12-Octadecadienoic acid (Z,Z)-(linoleic acid)	C ₁₈ H ₃₂ O	280.4	70.606	15.6611	23.12	11.901	20.5385	11.5927	0.028	20.0385	18.662	20.2044	6.199	18.4421
5 Linoelaidic acid [55]											24.3424	15.1748		
6 9,12-Octadecadienoic acid, methyl ester, (E,E)-9-	C ₁₉ H ₃₄ O ₂	294.5									25.3036	14.385		
7 2-Methyl-Z, Z-3, 13 octadecadienol (terpenoid)	C ₁₉ H ₃₆ O	280.5	3.0578	12.697	25.868	14.327	7.6221	14.276			9.7202	19.6093	-	
8 9,17-Octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264.4	-		46.8011	15.197	43.485	18.333			11.7474	15.4781		
9 Oleic Acid	C ₁₈ H ₃₄ O ₂	282.47	3.3959	13.3951	2.0115	12.050	0.205	9.9906	1.8428	16.3306	1.7151	18.0243	-	
10 Z-2-Octadecen-1-ol (10)	C ₁₈ H ₃₆ O	268.5	3.4978	16.1188										
11 2-Decenal, (Z)-	C ₁₀ H ₁₈ O	154.25	0.4145	9.7846										
12 1-Methoxy-3-hydroxymethylheptane	C ₉ H ₂₀ O ₂	160.25	0.0416	10.1565										
13 1-Eicosene(Alkene)	C ₂₀ H ₄₀	280.53											50.3312	15.2779
14 Cis-11,14 -Eicosadienoic acid -methyl ester	C ₂₁ H ₃₈ O ₂	322.53											0.1599	20.1873
15 7,12a-Dimethyl-1,2,3,4,4a,11,12,12a-octahydrochrysene	C ₂₀ H ₂₄	264.4	-		-				40.5439	15.2835	-		-	
16 1,5,9-Cyclododecatriene, (E,E,Z)-	C ₁₂ H ₁₈	162.27	-		-				43.2941	14.4996	-		-	
17 Nonanoic acid, 9-oxo-, methyl ester	C ₁₀ H ₁₈ O ₃	186.25							0.4997	10.9634			0.310	11.0608
18 9- Oxononanoic acid	C ₉ H ₁₆ O ₃	172.22											1.871	12.3768
19 Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	C ₁₆ H ₅₀ O ₇ Si ₈	579.2480					12.6284	18.4076						
20 Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-(17)	C ₁₄ H ₄₄ O ₆ Si ₇	505.09406					4.6048	17.1201						
21 Ethyl 5-(furan-2-yl)-1,2-oxazole-3-carboxylate	C ₁₀ H ₉ NO ₄	207.18					6.7172	17.5665						
22 Cyclopentaneundec-anoic acid	C ₁₆ H ₃₀ O ₂	254.41					2.2992	19.9182						
23 1,3-Oxathiane, 2-ethyl-6-methyl-	C ₁₆ H ₃₀ O ₂	254.41					12.7744	20.3645						
24 Dodecanoic acid, 1-methylethyl ester	C ₁₅ H ₃₀ O ₂	242.4					0.6015	19.1343						
25 2-Cyclopenten-1-one, 3-ethyl-	C ₇ H ₁₀ O	110.15					1.2903	9.5157						
26 Bis(carboxymethyl) trithiocarbonate, 2TMS derivative	C ₅ H ₆ O ₄ S ₃	226.3					1.7432	16.5079						
27 Trimethylsilyl-di(trimethylsiloxy)-silane	C ₈ H ₂₇ O ₂ Si ₄	279.65					2.5173	17.0172						
28 Undecanoic acid	C ₁₁ H ₂₂ O ₂	186.29					0.3226	19.4261						
Number of compounds			7		5		14		6		8		5	
Total			98.505		99.319%		99.999		92.2333		97.9811		58.8711	

Table 3
LC₅₀ and LC₉₅ values of the tested extracts of flax and black seed peels.

Plant waste	Extraction	24 hrs			48 hrs		
		LC ₅₀	LC ₉₅	Slope	LC ₅₀	LC ₉₅	Slope
Flax seed	Methylene chloride extract	132.8409	1493.066	1.5655 ± 0.1549	74.3375	1128.345	1.3925 ± 0.1469
	Petroleum ether extract	86.4974	383.2656	2.5443 ± 0.2680	69.6383	328.4647	2.4418 ± 0.2566
	Methanolic extract	355.2265	935.3215	3.9122 ± 0.4324	303.4803	903.0414	3.4771 ± 0.3938
Black seed	Methylene chloride extract	110.1717	1073.692	1.6635 ± 0.1601	60.9653	959.8591	1.374 ± 0.1464
	Petroleum ether extract	74.8587	1952.634	1.6424 ± 0.1597	40.7748	681.5116	1.3449 ± 0.1457
	Methanolic extract	316.404	1035.789	3.1938 ± 0.3695	251.88	893.6158	2.991 ± 0.3430

3.3. Evaluation of the larvicidal activity of agricultural waste

The insecticidal activity of flax and black seed peels was evaluated against the late 3rd instar *Cx. pipiens* larvae in the laboratory. The results are presented in Table 3.

Three solvents (methylene chloride, petroleum ether and methanol 70%) were used for each of the two agro-wastes individually. LC₅₀ and LC₉₅ values of the tested extracts of flax and black seed peels and slope are provided in Table 3.

The results indicated that, petroleum ether are the most efficient in both flax and black seeds extracts with LC₅₀ = 86.4974 and 74.8587 respectively at 24 h exposure. This is the same after 48 h exposure with LC₅₀ equal to 69.6383 and 40.7748 for flax and Black seed respectively. This followed by methylene chloride extract than methanolic extract.

According to the results of the bioassay the most efficient extract of the six used extract is the petroleum ether extract of black seed peels with lowest LC₅₀ and LC₉₀ at both exposure times.

4. Discussion

Due to the problems concerning the use of synthetic chemicals for vector control, there is an urgent need to introduce natural products, mainly agricultural wastes to utilize these waste materials especially after the immense and ever-growing amount of agricultural and food wastes which has come to be a major concern throughout the whole world. Therefore, strategies for their processing and value-added reuse are needed to enable a sustainable utilization of feedstock and reduce the environmental burden. By-products cereals arise in significant amounts in Egypt and are consequently of high relevance.

A compound extraction from plant parts depends upon the type of plant material and solvent used. High-polarity solvents produced a higher yield than that of medium and low-polarity solvents. In our results, methanol (a polar solvent) afforded high extract yields during solvent extraction from both *Linum usitatissimum* and *Nigella sativa* peels (Table 1), which was due to its high polarity. Previously, it was reported that ethanol (a polar solvent) yielded more than other solvents from leaves of *Melastoma malabathricum* [Awang et al., 2016]. Furthermore, a relatively high extract yield from *C. colocynthis* and *C. sativa* using ethanol as an extraction solvent was reported [Ahmed et al., 2019].

Results of this study have shown that phytochemical compounds extracted from flax and black seed peels may be innovative and integrated visualization of bio insecticidal. Thus this study has set as an alternative control measures against *Cx. pipiens* compared to synthetic insecticides. Nature of compounds identified from the Table 2 can be categorized as fatty acids (9- Oxononanoic acid), long chain unsaturated aldehyde (9,17-Octadecadienal, (Z)-), fatty acid esters (hexadecanoic acid,

methyl ester and 9,12 Octadecadienoic acid, methyl ester, (E, E)-9-), polyunsaturated fatty acids (9,12-Octadecadienoic acid (Z,Z)-(linoleic acid)) and Palmitic acid ester (hexadecanoic acid, methyl ester)[Kim et al., 2020].

A literature survey regarding the biological activities of the phytochemical constituents of our studied extracts in GC-MS profiling Table 2 showed that there are many studies reporting many biological activities of the same obtained constituent in our study from a variety of botanical extracts. Many biological activities including the insecticidal activity are recorded for the following constituents: 9-2-Methyl-Z, Z-3, 13 octadecadienol (terpenoid) which act as a Pesticide, herbicide, insecticide and as a pheromone [Adeyemi et al., 2017], the second constituent: 9,17-Octadecadienal, (Z)- an insecticidal Aldehyde compound and act also as an Antieczemic, Nematicide, Antihistaminic and Antimicrobial [Oni et al., 2020], the third constituent, Nonanoic acid, 9-oxo-, methyl ester a Potent antifungal, Antioxidant, Potent Antimicrobial [Sen et al., 2017] beside its larvicidal activity [Sen et al., 2017], fourthly 9,12-Octadecadienoic acid (Z,Z)- known as linoleic acid a Polyunsaturated Fatty acid which has many activities like Insecticidal activity [Christiana et al., 2019], Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic and Anticoronary, [Adeyemi et al., 2017] and finally Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- which has an antimicrobial activity [Ahmed et al., 2020] beside its insecticidal activity [Abdullah, 2019].

While the other constituents Table 2 which showed unique activity according to the literature like Hexadecanoic acid, methyl ester (Fatty acid ester) [Ravindran et al., 2020], *cis*-9-Hexadecenal (Long chain unsaturated aldehyde) [50], both of them are larvicidal, *cis*-Vaccenic acid (*trans*-fatty acid) [Ravindran et al., 2020] and 9- Oxononanoic acid (medium-chain fatty acids)[51–52] showing an insecticidal activity, Undecanoic acid a mosquito repellent [Cantrell et al., 2020] while 2-Decenal, (Z)- recorded nematocidal activity [Caboni et al., 2012].

Six constituents in Table 2 are constituents of other plant extracts, these plant extracts showing as a whole an insecticidal activity. The six constituents are Linoelaidic acid [Ghanem et al., 2013], Oleic Acid [Sini et al., 2005; Farag et al., 2011], Z-2-Octadecen-1-ol [Halawa et al., 2007], 1-Methoxy-3-hydroxymethylheptane [Majdoub et al., 2014] Cis-11,14 -Eicosadienoic acid -methyl ester [Akpuaka et al., 2013a, 2013b] and Hep-tasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- [Ahmed et al., 2020].

No biological activity reported for the following constituents Table 2 as follow 9,12 Octadecadienoic acid, methyl ester, (E,E)-, 7,12a-Dimethyl-1,2,3,4,4a,11,12,12a-octahydrochrysene, 1,5,9-Cyclododecatriene, (E,E,Z)- (Triterpenoids), Ethyl 5-(furan-2-yl)-1,2-oxazole-3-carboxylate, Cyclopentaneundec-anoic acid 1,3-Oxathiane, 2-ethyl-6-methyl-, Dodecanoic acid, 1-methylethyl ester, 2-Cyclopenten-1-one, 3-ethyl-, Bis(carboxymethyl)

trithiocarbonate, 2TMS derivative, Trimethylsilyl-di (trimethylsiloxy)-silane. However, Fatty acid synthetic esters and structural analogs are proved to be a promising source of new mosquito repelling compounds and should be investigated further [Cantrell et al., 2020].

For flaxseed extracts *Linum usitatissimum*, 14 compounds were found in petroleum ether extract, 7 compounds in methylene chloride extract, and 5 compounds in methanolic extract. It can be understood that there was larger amount of compounds found in petroleum ether, compared to that found in methylene chloride and methanolic extract, since the GC–MS technique only performed well for analyzing volatile compounds, which largely contained in the petroleum ether extract compared to that in the methylene chloride and methanolic extract. This simply explained why the insecticidal activity of the petroleum ether extracts against *Cx. pipiens* were the highest giving LC₅₀ 69.6383 ppm and LC₉₅ 328.4647 ppm, Table 3.

For black seed extracts (*N. sativa* Linn), 5 compounds were found in petroleum ether extract, 6 compounds in methylene chloride extract, and 8 compounds in methanolic extract. Although GC–MS Profiling of petroleum ether extract of black seed (*N. sativa* Linn) gave the least number of constituents (5 compounds), it showed the highest LC₅₀ of 40.7748 ppm and LC₉₀ of 681.5116 ppm among all extracts which may be explained to the nature of the extract and its constituents, especially four of the five constituents showed insecticidal and larvicidal activity which are 9,12-Octadecadienoic acid (Z,Z)- [Christiana et al., 2019], Cis-11,14 -Eicosadienoic acid -methyl ester [Akpuaka et al., 2013a, 2013b], Nonanoic acid, 9-oxo-, methyl ester [Sen et al., 2017] and 9-Oxononanoic acid [Gunstone et al., 2007; Amin et al., 2018] while the fifth major constituents 1-Eicosene ((Alkene)(50.331%) (Table 2) which is also a major constituent of *A. altissima* flower oil [Rezae et al., 2006], however, there is a similar compound called 9-Eicosene in *N. sativa* [Choo et al., 2001] and another similar compound called 3-Eicosene in the previous studies which exhibit Insecticidal activity [Anthony et al., 2013], Antimicrobial, Antihyperglycemic [Barathikannan et al., 2016], Cytotoxic Activity [Barathikannan et al., 2016], [Caboni et al., 2012]. The components present in black seed could now be obtained by total synthesis and the study of their insecticidal properties should make very interesting research studies for the future. Finally, although these extracts may be used as bio-larvicides, future evaluation would have to be conducted to validate its long term effects on human health and other organisms in environment.

Additionally, each peel extract may be more effective than a single based active constituent due to its active ingredients synergisms which may be influential in managing resistant population of mosquitoes. We have found total of 28 chemical compounds from which nine chemical compounds have been reported to possess direct effects as insecticidal and larvicidal as mentioned above. All these chemical components have direct effects on the larvicidal activity and there are also many other compounds which may act synergistically in the process of larvicidal activity. So, it would be difficult to think that the insecticidal activity of these extracts is limited only to some of its major constituents or to constituents of already proved insecticidal activity since it could also be due to certain minority constituents or to a synergistic effect of several components [Ravi et al., 2018a, 2018b]. The presence of the reported insecticidal components along with other components present in all of the six plant extracts supports the larvicidal activity of the novel plant extract against *Cx. pipiens*. From the available literature search, the presently studied six plant peel extract as an agro waste is the first study of that kind showing promising potential lethal activity against *Cx. pipiens* larvae. Local community can easily utilize these extracts in the locality for the control of mosquito larvae of *Cx. pipiens*.

5. Conclusion

The present study was able to characterize the phytochemical fingerprint of flax and black seed peels for the first time using GCMS, thus enriching and providing additional information on plant peel bioactive compounds. The study showed the bioactive potentials of these agro-waste collected in Egypt against *Cx. pipiens*, which possessed significant insecticidal properties and could be introduced as botanical insecticides after field evaluations as alternatives to the synthetic chemicals for vector control.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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