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Larvicidal and repellent potential of *Sesamum indicum* hull Peels Extracts Against *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

Pest control is facing economic and ecological challenges around the world as a result of human and environmental threats caused by many synthetic pesticide chemicals. Alternative pest management tools that are biodegradable and less harmful to humans, non-target species, and the environment, in general, are highly required. Plant extracts with significant insecticidal activity hold promising effects. On this basis, this present study intended to assess the efficacy of methanol, acetone, chloroform, and petroleum ether extracts of Sesamum indicum hull peels against the 3rd instar larvae of mosquito, Culex pipiens which are considered as a disease vector. The prepared plant extracts were tested for their ability to repel Cx. pipiens adults. All plant extracts had larvicidal activity against Cx. Pipiens larvae. The petroleum ether extract, on the other hand, was found to be the most efficient. The median lethal concentration (LC₅₀) values of methanol extract were 161.66, 158.17, and 144.52 ppm after 24hrs, 48 hrs, and 72 hrs, respectively. For acetone extracts, LC50 values were 131.88, 117.45, and 104.59 ppm after 24hrs, 48 hrs, and 72 hrs, respectively. While chloroform extracts revealed LC50 values 146.89, 139.86 and 133.20 ppm at 24hrs, 48 hrs and 72 hrs, respectively. Finally, petroleum ether extracts recorded 125.95, 88.32, and 67.25 ppm at 24 hrs, 48 hrs, and 72 hrs, respectively. The extracts were found to have repellent activity against Cx. pipiens adults using LC₅₀. In comparison to chloroform, acetone, and methanol extracts, petroleum ether was the most effective plant extract in terms of antifeedant and repellent action. Those results proved that the four extracts of Sesamum indicum hull peels have the potential to be developed as natural insecticides and repellents for the control of mosquitoes even in their simplest form. Hence, those findings may facilitate the development of low-cost alternatives to expensive organic pesticides that are relatively safe for the environment and living organisms.

INTRODUCTION

Mosquitoes are well known for their impact on public health since they cause major health problems and are considered to be the most formidable cause of fatalities in the world, being responsible for the death of approximately 750,000 humans annually (Calzolari, 2016). In Egypt, *Culex pipiens* Linnaeus (Diptera: Culicidae), has been declared as a vector of several diseases (Abd El-Samie & Abd El-Baset, 2012; El-

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Zayyat et al., 2017). It transmits Rift valley fever virus (Dodson et al., 2012), Japanese encephalitis (Chancey et al., 2015), Wuchereria bancrofti attributed to the transmission of human lymphatic filariasis (Joseph et al., 2011), and West Nile virus (Ahmed, 2016; Bassal et al., 2017). Considerably, *Culex pipiens* is the major filarial vector in Egypt (Dyab et al., 2015; El-Naggar et al., 2017) and has been recorded throughout all the governorates (Ammar et al., 2012; Abd El-Shafie et al., 2016).

The use of chemical insecticides to prevent diseases vectored by mosquito resulted in problems related to human and environmental hazards as well the development of insecticide resistance among the vectors (Acevedo *et al.*, 2009; Bonner & Alvanja, 2017). Alternatively, biopesticides of plant origin including plant extracts and essential oils are an appealing counter strategy to combat vectors due to their larvicidal, adulticidal, and repellent properties (Bekele, 2018). Accordingly, plant extracts with significant insecticidal activity have been considered as a new source of pesticides (Kamel *et al.*, 2005a, 2005b; Sosa *et al.*, 2018). But most of the implemented studies were restricted to preliminary screening (Abdul Rahuman *et al.*, 2009; Deepa *et al.*, 2015; Mukandiwa *et al.*, 2016).

Furthermore, agricultural wastes are natural organic materials that are wasted, lost or disregarded (FAO, 2011). Though the Food and Agricultural Organization (FAO) recorded an increased rate of food losses in Egypt that led to an increased rate of food wastes (Rutten & Kavallari, 2013). Yet, Several previous studies showed that some agricultural wastes were efficient in the control of insects (El-Maghraby *et al.*, 2012; Fahim *et al.*, 2013; Eldiasty *et al.*, 2014; Jouhara *et al.*, 2017).

Sesame (*Sesamum indicum* L.), for instance, is an essential seed oil crop in the genus *Sesamum* and family Pedaliaceae. It is cultivated mainly in Africa and in slightly less numbers in India. *Sesamum indicum* is known in English as sesame, in Bantu as Benne seed, in Igbo as Isisa, in Hausa as Ridi, in Yoruba as Ekuku, and in Obudu as Kana. It is a plant well-known for its medicinal properties (**Morebise** *et al.*, 2002). Since the hulls contain large amounts of indigestible fiber and unpalatable oxalic acid, that provide the sesame products their dark color, dehulling is needed in regions where sesame seeds are eaten by humans (**Inyang & Nwadimkpa**, 1992). Dehulling gets rid of oxalates and is the first step in producing a light-colored, low-fiber, non-bitter and high-protein flour. Notably, dehulling improves both nutritive value and taste of the sesame products (**Inyang & Ekanem**, 1996; Abou-Gharbia *et al.*, 1997). The extract from hull is tested in this study for its efficacy as mosquitocides.

Thus, the goal of the present research was to estimate the larvicidal and repellent potential of the four extracts of *Sesamum indicum* hull peels against the larvae of *Culex pipiens* (Diptera: Culicidae) under laboratory conditions.

MATERIALS AND METHODS

Tested mosquitoes' culture:

The Medical Entomology Institute supported the current research with *Culex pipiens* larvae, and self-perpetuating colonies were developed and maintained in the Entomology Department of the Faculty of Science at Ain Shams University. Mosquitoes were cultivated in a stable environment of temperature $(27\pm2^{\circ}C)$, relative humidity (70%), and light-dark loops (16: 8 hrs.) (**Kauffman** *et al.*, **2017**). Late third larval instars were used in toxicological experiments.

Collection and extraction of plant materials:

The extract used for this research was acquired from hull peels of dehulled *Sesamum indicum* obtained from *Sesamum* agricultural food waste. *Sesamum* was collected from food factories at different localities in Egypt, especially from El-Sharkia and Qalyubia governorates in February 2021. It was washed in dechlorinated water, dried under shade conditions in the laboratory for five days, and grinded with the aid of an electric blender to a coarse powder (**Odey et al., 2012**). Different solvents of increasing polarity were used (petroleum ether (60-80°C) then chloroform, acetone and finally methanol 70%) {1kg powder material: 3L solvent}. The solvents were evaporated using a rotary evaporator (Labo-Rota C311) in a water bath adjusted at 40°C for 2-3 hrs in methanol 70% and (40-60 minutes) to the remaining solvents. Then, the obtained crude extract was weighed and kept in a deep freezer (-4°C) in screw capped vials till used for experiments (**Aina et al., 2007**).

Larvicidal activity:

Different concentrations of the four extracts were applied. The mortality data were recorded after 24, 48 and 72 hrs and evaluated by a probit analysis (**Finney, 1971**) to calculate $LC_{50} \& LC_{90}$. The percent of larval mortality was calculated using the equation of **Briggs (1960)**. Larval mortality % = A-B/A×100, where (A) is the number of tested larvae and (B) is the number of tested pupae. The intervals between the beginning of the first instar larvae and the beginning of pupation were calculated. The percent of the pupation was estimated by **El-Sheikh (2002)**. The percent of pupation= A/B×100, where (A) represents the number of pupae and (B) represents the number of tested larvae. Pupal duration was determined by calculating the time between the start of pupation and the start of adult emergence. The adult emergence percentage was calculated by using the equation mentioned by **El-Sheikh (2002)**. The percent of adult emergence= A/B×100, where A is the number of adults which emerged, and B is the number of pupae that were tested. The Growth index was calculated by using the following equation cited by **El-Sheikh (2002)**. Growth index = a/b, where (a) is the percentage of adult emergence and (b) is the mean development (days).

Reproductive potential of resulted females:

Fecundity

According to the protocol of **WHO** (2005), the emerged adult females were gathered and then released in wooden cages (20x20x20cm) with normal adult males. For three days, they were fed cotton pads soaked in a 10% sugar solution, then the adults were kept to starve for one day. The starving females were provided with a pigeon to take a blood meal on day five. Females laid egg rafts above the waterline. Using stereo microscopes, the number of eggs in each egg raft was counted.

Egg – hatchability:

The eggs were organized into two groups (hatched and unhatched) according to **Hassan** *et al.* (1996). Under a dissecting microscope, the presence of an embryo was used to further classify the unhatched eggs into embryonated and unembryonated eggs. Fertilized eggs were considered hatched and unhatched embryonated eggs, while unfertilized eggs were considered unhatched and unembryonated eggs (**Rak & Ishii**, 1989). The hatching eggs were calculated using the following equation referred to **El-Sheikh** (2002). The percent of hatching eggs= $A/B \times 100$, where (A) is the whole number of hatched eggs and (B) is the total number of the laid eggs.

Sterility index (SI):

Percent of sterility was assessed by the equation described in the study of **Toppozada** *et al.* (1966). The percent of sterility = $100 - [a \times b/A \times B] \times 100$, where a represents the number of eggs laid per female in treatment, and b represents the percentage of hatched eggs in treatment. (A) represents the number of eggs laid per untreated female and (B) represents the proportion of hatched eggs in the control group. Antifeedant and repellent activity:

The repellent behavior of the four extracts was tested in standard cages (20x20x20 cm). For three hours, cotton pads immersed in 10% sucrose solution from each concentration were introduced to forty starving adults (5-7 days old). Control experiments were conducted alongside the treatments with cotton pads soaked in a 10% sucrose solution with a few drops of methanol or Tween 80. To get a mean repellent value, each test was replicated thrice. The percent of repellency was measured according to the method of **Abbott (1925)**. Repellency % = [%A-%B/100-%B] ×100, where A is the percentage of unfed treated female, and B is the percentage of unfed control females. **Statistical analysis:**

Multiple linear regressions were used to measure the LC_{50} (Finney, 1971). The calculations were performed according to the method adopted from Lentner *et al.* (1982).

RESULTS

The susceptibility of the 3^{rd} instar larvae of *Cx. pipiens* to extracts of *Sesamum* from methanol, acetone, chloroform and petroleum ether at different time intervals and coefficient limits are shown in Table (1&2). Data revealed that the extracts showed a larvicidal effect on the 3^{rd} instar larvae of *Cx. pipiens*. However, the most efficient extract was revealed to be petroleum ether. The median lethal concentration values for that extract were 125.95, 88.32 and 67.25 ppm at 24 hrs, 48hrs and 72 hrs, respectively.

Table 1. Susceptibility of the 3^{rd} instar larvae of *Cx. pipiens* to extracts of *Sesamum* from methanol and acetone at different time intervals and coefficient limits.

Concentration			Percentage of	mortalities %		
Ppm	Sesamun	n peel methano	l extract	Sesamu	<i>m</i> peel acetone	extract
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
Untreated	0.0	0.0	0.0	0.0	0.0	0.0
35	12.33	16	18.66	21.33	24	26.66
75	28	29.33	30.66	32	34.66	37.3
150	45.33	46.66	48	50.66	53.33	56
250	62.66	64	65.33	66.66	69.33	72
350	72	73.33	74.66	77.33	80	82.66
I.C. (nnm)	61.39	65.51	53.96	48.67	43.13	38.46
LC ₂₅ (ppm) (co. limit)	(45.83-	(50.69-	(39.31-	(34.73-	(30.10-	(26.31-
(co. mmt)	75.94)	79.42)	67.70)	61.80)	55.48)	50.08)
I.C. (nnm)	161.66	158.17	144.52	131.88	117.45	104.59
LC ₅₀ (ppm) (co. limit)	(136.68-	(135.51-	(121.73-	(110.57-	(97.80-	(86.37-
(co. mint)	193.95)	186.41)	172.61)	157.17)	139.68)	124.34)
	1017.78	844.16	939.26	876.35	788.07	699.87
LC ₉₀ (ppm)	(693.65-	(606.55-	(644.64-	(605.29-	(551.36-	(497.32-
(co. limit)	1810.41	1364.37)	1652.57	1526.85)	2654.30)	1162.10
Slope ± SE	1.60±0.17	1.76±0.18	1.58±0.17	1.56±0.17	1.55±0.17	1.55±0.17

Concentration			Percentage of	mortalities %		
Ppm	Sesamum	peel chlorofor	m extract	Sesamum pe	el petroleum e	ther extract
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
Untreated	0.0	0.0	0.0	0.0	0.0	0.0
35	10.66	12	13.33	13.33	22.66	30.66
75	25.33	26.66	28	29.33	40	53.33
150	50.66	52	53.33	53.33	72	74.66
250	73.33	74.66	76	74.66	81.33	82.66
350	74.66	76	77.33	85.3	86.66	88
	68.91	65.08	61.57	61.67	40.01	26.91
LC ₂₅ (ppm)	(55.55-	(52.09-	(48.94-	(49.86-	(30.07-	(17.87-
(co. limit)	81.50)	77.32)	73.48)	72.82)	49.50)	35.80)
I.C. (mmm)	146.89	139.86	133.20	125.95	88.32	67.25
LC ₅₀ (ppm)	(128.12-	(121.75-	(115.71-	(110.19-	(74.86-	(53.80-
(co. limit)	168.82)	160.74)	153.09)	143.40)	102.16)	80.40)
	618.76	598.35	577.04	489.16	397.56	383.22
Lc ₉₀ (ppm)	(478.49-	(463.35-	(447.69-	(391.51-	(316.57-	(296.16-
(co. limit)	882.53)	851.48)	818.65)	659.85)	541.62)	551.57)
Slope ± SE	2.05±0.19	2.03±0.19	2.01±0.18	2.16±0.19	2.0±0.18	1.7±0.18

Table 2. Susceptibility of the 3^{rd} instar larvae of *Cx. pipiens* to extracts of *Sesamum* from chloroform and petroleum ether at different time intervals and coefficient limits.

Table 3. Changes in biological aspects of *Cx. pipiens* after treatment with methanol extract of *Sesamum*.

Conc. ppm	Larval mort. %	Mean Larval Period (days)±SE	Pupation %	Mean Pupal duration (days)±SE	Adult Emergence % (a)	Mean adult longevity (days) (b)±SE	Growth Index (a/b)
Untreated	0.0	4.6 ± 0.2^{a}	100	1.6±0.2 ^a	100	6.2 ± 0.4^{a}	16.1
35	12.33	4.3 ± 0.05^{a}	87.67	1.6±0.2 ^a	100	5.8±0.9 ^a	17.24
75	29.33	4.1 ± 0.05^{b}	70.67	1.5±0.0 ^a	95	5.8 ±0.3 ^a	16.37
150	46.66	4.4 ± 0.2^{a}	53.34	1.7±0.2 ^a	87.5	5.9±0.2 ^a	14.83
250	64	4.4 ± 0.1^{a}	36	1.5 ± 0.0^{a}	77.8	5.8±0.3 ^a	13.41
350	73.33	4.9±0.3 ^a	26.67	$1.4{\pm}0.2^{a}$	33.4	5.6 ± 0.9^{b}	5.96

Note: Means with the same letters are not significantly different. Each value represents mean of 3 replicates \pm SE (standard error).

		No.	of eggs laid	No. of ha			No. c	f unhatched	l eggs		Sterility
Conc. ppm	No. of tested						Embry	onated	Unembry	onated	index (S.I.)
	females	Total	Mean ±SE	Total	%	Total	No	%	No	%	%
Untreated	18	4050	225±10.2 ^a	3985	98.4	65	12	18.5	53	81.5	0.0
50	10	2165	216.5±13.3 ^b	2037	94.1	128	33	25.8	95	74.2	28.2
100	10	1950	195±14.6 ^b	1823	93.5	127	38	29.9	89	70.1	35.7
200	12	2150	179.2±14.3°	2003	93.2	147	48	32.6	99	67.4	41.1
300	8	1260	157.5±9.6°	1144	92.5	116	40	34.5	76	65.5	48.6

Table 4. Impact of methanol extract of *Sesamum* on reproductive potential of *Cx. pipiens* female.

Note: Means with the same letters are not significantly different. Each value represents mean of 3 replicates \pm SE.

Table 5. Changes in biological aspects of *Cx. pipiens* after treatment with acetone extract of *Sesamum*.

Conc. ppm	Larval mort. %	Mean Larval Period (days)±SE	Pupation %	Mean Pupal duration (days)±SE	Adult Emergence % (a)	Adult longevity (days) (b)±SE	Growth Index (a/b)
Untreated	0.0	4.8±0.11 ^a	100	1.6±0.20	100	6.4±0.31 ^b	14.1
35	24.0	4.5±0.20 ^a	76.0	1.8±0.05	100	6.3±0.25 ^b	15.8
75	34.66	4.3±0.10 ^b	65.34	1.9±0.10	88.9	6.2±0.20 ^b	14.3
150	53.33	4.6±0.15 ^a	46.67	2.1±0.12	75.0	6.7±0.27 ^b	11.2
250	69.33	4.3±0.05 ^b	30.67	2.3±0.20	66.7	6.6±0.15 ^a	10.1
350	80	5.5±0.25 ^a	20	2.5±0.10	0.0	8±0.45 ^a	0.0

Note: Means with the same letters are not significantly different. Each value represents mean of 3 replicates \pm SE.

Table 6. Impact of acetone extract of *Sesamum* on reproductive potential of *Cx. pipiens* female.

Conc.	No. of	No.	of laid eggs	No. of l eg	natched gs		No. o	f unhatch	ed eggs		Sterility index
ppm	tested females	Total	Mean ±SE	Total	%	Total	Embry	onated	Uneml	bryonated	(S.I.)
	Temates	Total	Mean ±SE	Total	70	Total	No	%	No	%	%
Untreated	20	4400	220±9.8 ^a	4268	97.0	132	20	15.2	112	84.8	0.0
35	12	2275	189.6±11.1 ^b	2108	92.7	167	49	29.4	118	70.6	32.6
75	10	1685	168.5±9.7 ^c	1447	92.5	109	34	31.2	75	68.8	40.2
150	7	980	140±11.2 ^c	847	90.4	81	28	34.6	53	65.4	51.4
250	4	505	126.2±7.5 ^c	444	88.0	61	24	39.3	37	60.7	57.4
350	1	110	110±0.0 ^c	94	85.6	16	7	43.7	9	56.3	62.4

Note: Means with the same letters are not significantly different.

Each value represents mean of 3 replicates \pm SE.

Conc. Ppm	Larval mort. %	Mean Larval Period (days)±SE	Pupation %	Mean Pupal duration (days)±SE	Adult Emergence % (a)	Mean adult longevity (days) (b)±SE	Growth Index (a/b)
Untreated	0.0	4.5 ± 0.15^{b}	100	1.5±0.20 ^b	100	6.0±0.35 ^b	16.5
35	12	4.3±0.47 ^b	88	1.4±0.37 ^b	100	5.8±0.84 ^b	17.5
75	26.66	4.6±0.20 ^b	73.34	1.7±0.15 ^b	88.9	6.3±0.35 ^b	14.0
150	52	4.9 ± 0.15^{b}	48	2.0±0.10 ^b	70.0	6.9±0.25 ^b	10.0
250	74.66	5.0±0.15 ^b	25.34	2.1±0.11 ^a	71.5	7.1±0.26 ^b	9.9
350	76	5.2±0.15 ^a	24	2.2±0.11 ^a	66.7	7.4±0.26 ^a	8.9

Table 7. Changes in biological aspects of *Cx. pipiens* after treatment with chloroform extract of *Sesamum*.

Note: Means with the same letters are not significantly different. Each value represents mean of 3 replicates \pm SE.

Table 8. Impact of chloroform	extract of Sesamum	on reproductive potential of
Cx. pipiens female.		

Conc.	No. of	No	o. of eggs laid	No. of l eg	natched gs		No.	of unhatched	eggs		Sterility index
ppm	tested females	Total	Mean ±SE	Total	%	Total	Embr	yonated	Unembr	yonated	(S.I.)
	Temates	Total	Wiean ESE	Totai	/0	Total	No	%	No	%	%
Untreated	10	2200	220 ± 8.8^{a}	2112	96.0	88	13	14.8	75	85.2	0.0
35	10	2135	213.5±15.5 ^a	1959	91.8	176	47	26.7	129	73.3	7.2
75	9	1850	205.5 ± 10.1^{a}	1690	91.4	160	46	28.8	114	71.2	11.1
150	12	2275	189.6±13.2 ^b	2070	91.0	205	64	31.2	141	68.8	18.3
250	6	1100	183.3±14.7 ^b	998	90.8	102	35	34.3	67	65.7	21.2
350	4	630	157.5±11.9 ^c	570	90.5	60	22	36.7	38	63.3	32.5

Note: Means with the same letters are not significantly different. Each value represents mean of 3 replicates \pm SE.

Table 9. Changes in biological aspects of *Cx. pipiens* after treatment with petroleum ether extract of *Sesamum*.

Conc. Ppm	Larval mort. %	Mean Larval Period (days)±SE	Pupation %	Mean Pupal duration (days)±SE	Adult Emergence % (a)	Mean adult longevity (days) (b)±SE	Growth Index (a/b)
Untreated	0.0	5.6 ± 0.10^{a}	100	$1.8{\pm}0.15^{a}$	100	7.4 ± 0.35^{a}	10.9
35	22.66	$4.4{\pm}0.15^{\rm b}$	77.34	1.5±0.15 ^b	100	5.9±0.30 ^b	16.9
75	40	4.3±0.20 ^b	60	1.3±0.17 ^b	100	5.6±0.25 ^b	17.8
150	72	4.1 ± 0.11^{b}	28	1.3±0.05°	91.7	5.4±0.28 ^c	16.9
250	81.33	4.7±0.20 ^c	18.67	$1.2\pm0.15^{\circ}$	88.9	5.9±0.30 ^c	15.1
350	86.6	4.0±0.64 ^c	13.4	1.1±0.11 ^c	75.0	5.1±0.75 ^c	14.7

Note: Means with the same letters are not significantly different. Each value represents mean of 3 replicates \pm SE.

Conc.	No. of	No.	of laid eggs	No. hatcl egg	ned		No. of	unhatche	d eggs		Sterility index
ppm	tested females	Total	Mean ±SE	Total	%	Total	Embr	yonated		lbryon ed	(S.I.) %
							No	%	No	%	
Untreated	17	3825	225±14.4 ^a	3733	97.6	92	16	17.4	76	82.6	0.0
35	8	1335	166.9±12.5 ^b	1121	95	57	16	28.1	41	71.9	36.7
75	10	1470	147±12.5°	1243	92.2	97	31	32.0	66	68.0	45.8
150	7	985	140.7±6.7 ^c	838	90	84	29	34.5	55	65.5	49.4
250	4	505	126.2±13.8 ^c	430	87.7	53	21	39.6	32	60.4	55.8
350	1	110	110±0.0 ^c	94	85.6	16	7	43.7	9	56.3	62.4

Table 10. Impact of petroleum ether extract of Sesamum on reproductivepotential of Cx. pipiens female.

Note: Means with the same letters are not significantly different. Each value represents mean of 3 replicates \pm SE.

Table 11. Relative efficiency of <i>Sesamum</i> hull peels extracts against <i>Cx. pipiens</i> adults.

Extract	LC ₅₀ (ppm)	Slope (b)	Correlation Coefficient (r)		
Methanol	158.17	1.762±0.180	0.999		
Acetone	117.45	1.550±0.170	0.9881		
Chloroform	139.86	2.0302±0.185	0.9923		
Petroleum ether	88.32	1.96±0.180	0.99		

Table 12. LC_{50} concentration of *Sesamum* hull peels extracts as antifeedant and repellent for *Cx pipiens*.

Extract	LC50 value (ppm)	Fed flies		Unfed flies		Repellency
		No.	%	No.	%	action (%)
Methanol	158.16	17	29.3.	41	70.7	68.8
Acetone	117.44	11	21.20	41	78.8	77.4
Chloroform	139.86	9	14.8	52	85.2	84.6
Petroleum Ether	88.318	9	15.3	50	84.7	87.5
untreated	0.0	47	95.7	2	4.3	0.0

Data in Tables (3, 5, 7 & 9) show the effect of the four extracts on different biological aspects of *Cx. pipiens* larvae. At the concentration of 350 ppm, extract from *Sesamum* hull peels induced a larval mortality equal to 80%, while petroleum ether extract induced the highest degree of larval mortality (86.6%) at the same concentration compared to 76% and 73.33% mortality for chloroform and methanol extracts, respectively. Concerning the number of the emerged adults from the treated larvae, at low

concentrations (50 ppm, 75 and 150 ppm) of the four extracts, there were no significant effects on adult emergence while at 350 ppm, acetone extract was the most efficient with 0% of adult emergence followed by methanol extract with 33.4% emergence.

Data in the Tables (4, 6, 8 & 10) show the effect of the four extracts on the fecundity, fertility and sterility index of female *Culex pipiens*. The results indicated that, among the extracts of *Sesamum* hull peels, both petroleum ether and acetone extract showed maximum effect on reproductive potential for females of *Cx. pipiens* with the sterility value equal to 62.4 at 350 ppm. Both petroleum ether and acetone extracts had significant effects on the fecundity of females with number of eggs laid at 350 ppm equal to half the number laid by untreated females. The same table revealed that the percent of unhatched embryonated eggs with conc. 350 ppm increased in the case of using both petroleum ether and acetone extracts.

Moreover, antifeedant and repellent efficacy of the four used extracts of *Sesamum* hull peels are given in Table (12). Among the extracts, petroleum ether showed the highest toxicity having an LC₅₀ value of 88.32 ppm. Acetone extract showed LC₅₀ value of 117.45 ppm, chloroform 139.86 ppm and methanol 158.17 ppm (Table 11).

DISCUSSION

Killing larvae as well as preventing bites of *Culex pipiens* are the more effective ways to reduce disease transmission (Shehata, 2019). The susceptibility of the 3rd instar larvae of Cx. pipiens to the toxic action of the tested Sesamum hull peels extracts were not the only feature to determine its efficiency. The delayed effects of the tested extracts, which occurred during the developmental processes, played an important role due to their efficacy. Comparing values of those biological parameters in treated insects with their control suggested that the treated insects showed various toxicity effects. In this study, the data clarified that the larval mortality was positively related to the concentrations of the tested compounds and the solvents used for extraction. The current findings showed that, all plant extracts from Sesamum indicum hull peels presented a larvicidal action on the 3rd instar larvae of Cx. Pipiens. Markedly, the most effective extract with larval mortality of 86.6 % at a concentration of 350 ppm was petroleum ether. In addition, the median lethal concentration values of methanol, acetone, chloroform and petroleum ether extracts were 158.17, 117.45, 139.86 and 88.32 ppm, respectively. The response of larval mortalities caused by the tested *Sesamum* hull peels extracts was similar to the results showed by other authors on Cx. pipiens (Al-Mehmadi & Al-Khalaf, 2010; Al-Mehmadi, 2011; Al-Keridis 2017; Mahyoub et al., 2017) and on other insect species (Candido et al., 2013; Rami et al., 2014).

Remarkably, treatment with plant extracts reduced the percentage of pupation and adult emergence of *Cx. pipiens*, which is close to data obtained from some authors (Eldiasty *et al.*, 2014; Farag & Emam, 2016). Other researchers used different plant

extracts and obtained similar findings (Abduz Zahir *et al.*, 2010; Candido *et al.*, 2013; Farag *et al.*, 2021). It is wothy to consider that, plant extracts block the maturation of imaginal discs, which are the precursors of many adult integumentary structures in endopterygota insects. This may explain why the percentage of adult emergences showed a decrease (Schneidermann, 1972; Suh *et al.*, 2000). This may also be related to the deformation of adult chitin (Abo El-Mahasen *et al.*, 2010) or as a result of two or more of the following factors: adults were unable to free themselves from the pupal exuvia due to unsaturated fatty acids that enhanced the melanization process and hardening of the larvae; as a result, adults were unable to free themselves from the pupal exuvia, insufficient pressure in the ptilinum, and the hardening of opercular suture (Hussien, 1995).

The extract from *Sesamum* hull peels induction of larval mortality was equal to 80%, while Petroleum ether extract induced the highest degree of larval mortality (86.6%) at the same concentrations compared to the 76% and 73.33% mortality of chloroform and methanol extracts, respectively. Moreover, results recorded a reduction in larval duration of *Cx. pipiens* following treatment extract from *Sesamum* hull peels, that finding agrees with that of **Salama and Shehata (2017)** and **Sosa** *et al.* (2018). The detected reduction may be due to the accelerated moulting process, where those compounds are capable of disrupting the endocuticle deposition during building up a new cuticle (Abdalla & Sammour, 1991). Similarly, the effect of the tested compounds against the pupal duration followed the same pattern obtained for the larval duration. Considering the prolongation of pupal duration of *Cx. pipiens*, following treatment with *Sesamum* hull peels, petroleum ether extract is similar to the data obtained in the study of **Khater and Shalaby (2007)** and **Granados-Echegoyen (2014)**.

The number of eggs laid per female mosquito of *Cx. pipiens*, together with the percentage of egg-hatch, were decreased due to treatment with the tested extracts. The mentioned results agree with those obtained by **Jayarama and Pushpalatha (2008)**. The reduction in both fecundity and fertility is attributed to the inability of sperm to pass to females during copulation or partial sterilization of females or males (**Ismail, 1980**). Additionally, the egg maturation in culicinae species is under hormonal control. This may indicate that the used plant extracts interfered with the hormonal system of *Cx. pipiens*. **Riddiford (1970)** demonstrated that, in endopterygota insects, the juvenoids might cause female sterility when applied at a certain stage of oocyte development.

The tested extracts significantly reduced adult fecundity of Cx. *pipiens*. The decreased effect on the potential fecundity of those females, which survived larval treatment with extracts, might either be due to the direct interference with the hormonal system or to the loss of appetite due to insecticidal poisoning. This, in turn, affected the hormones and thus directly affected the fecundity of the insect. The used extracts might directly affect the corpora allata, which in turn, resulted in a decrease in number of eggs formed from the germanium. The loss of appetite of insects during the 48 hours following

treatment might probably inactivate the corpora allata (**Yamashita** *et al.*, **1961**). The extirpation of the corpora allata from the 5th stage larvae of the silkworm, *Bombyx mori*, caused a decrease of some 12% of the number of eggs produced by the female moth. The present results supposed that the tested *Sesamum* hull peels extracts could interfere with the accumulation of protein in the eggs, which might also explain the reduction in number of eggs laid of *Cx. pipiens* females, a result that agrees with that of **Bouaziz** *et al.* (**2017**). The inability of sperm to be transferred to females during copulation, or partially sterilizing females and/or males, is responsible for the decrease in fecundity and fertility. In addition, the egg maturation in lepidopteran insects was under hormonal control.

The results of the present study revealed that all tested extracts from *Sesamum indicum* hull peels recorded a variable repellent activity against *Cx. pipiens* starved females. The concentration and the type of the solvent affected the repellency strenght. Generally, petroleum ether extract was more effective against *Cx. pipiens* starved females than chloroform, acetone and methanol extractions. Those results agree with those of **Maia and Moore (2011)**, **Deletre** *et al.* (2013), **Costa** *et al.* (2017) and **Shoukat** *et al.* (2020).

CONCLUSION

Generally, all plant extracts revealed a larvicidal effect on the larvae of *Cx. Pipiens*, however, the petroleum ether extract was the most effective one. Petroleum ether extract of *Sesamum indicum* hull peels was more effective against *Culex pipiens* unfed females compared to chloroform, acetone and methanol extractions. Thus, *Sesamum indicum* hull peels tested extracts are proved to be new promising larvicidal and repellent agents against the mosquito vector, *Cx. pipiens*.

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticides. Journal of Economic Entomology, 18:266-267.

Abdalla, E. F. and Sammour, E. A. (1991). Comparative study on the efficacy of neem seed extract and diflubenzuron against the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.). Bulletin of the Entomological Society of Egypt / Economic series, 19:53-61.

Abd El-Samie, E. and Abd El-Baset, T. (2012). Efficacy of some insecticides on field populations of *Culex pipiens* (Linnaeus) from Egypt. The journal of Basic and Applied Zoology, 65(1): 62-73.

Abd El-Shafie, I.; Shoeib, E.; Attia, S; Rubio, J.; Edmardash, Y. and Badry, A. (2016). Mosquito identification and molecular xenomonitoring of lymphatic filariasis in selected endemic areas in Giza and Qualioubiya governorates, Egypt. Journal of the Egyptian Society of Parasitology, 46(1):87-94.

Abdul Rahuman, A.; Bagavan, A.; Kamaraj, C.; Vadivelu, M.; Abduz Zahir, A.; Elango, G. and Pandiyan, G. (2009). Evaluation of indigenous plant extracts against larvae of *Culex quiquefasciatus* (Say) (Diptera: Culicidae). Parasitology Research, 104:637-643.

Abduz Zahir, A.; Rahuman A.; Bagavan, A. and Thirunavukkarasu, S. (2010). Evaluation of botanical extracts against *Haemaphysalis bispinosa* Neumann and *Hippobosca maculata* Leach. Parasitology Research, 107(3):585-92.

Abo El-Mahasen, M. M.; Assar, A. A.; Khalil, M. E. and Mahmoud, S. H. (2010). Biological effects of some insect growth regulators on the house fly, *Musca domestica* (Diptera: Muscidae). Egyptian academic journal of biological sciences (A. Entomology), 3(2): 95-105.

Abou-Gharbia, H. A.; Shahidi, F.; Shehata, A. A. and Youssef, M. M. (1997). Effect of processing on oxidative stability of sesame oil extracted from intact and dehulled seed. Journal of the American Oil Chemists' Society, 74:215-221.

Acevedo, G.; Zapater, M. and Toloza, A. (2009). Insecticide resistance of house fly, *Musca domestica* (L.) from Argentina. Parasitology Research, 105:489-493.

Ahmed, M. (2016). Incidence History of West Nile virus in Africa and Middle East, with an emphasis on Egypt: A Review. Journal of Dairy, Veterinary and Animal Research, 3(3):1-4.

Aina, O. O.; Dixon, A. G. and Akinrinde, E. A. (2007). Genetic variability in cassava as it influences storage root yield in Nigeria. Journal of Biological Sciences, 7:765-770.

Al-Keridis, L. A. (2017). Larvicidal Potency of Arta (*Calligonum comosum* L'Her.) Extract on the Biology of *Culex pipiens* Mosquito. Asian Journal of Applied Sciences, 10:39-44.

Al-Mehmadi, R. and Al-Khalaf, A. (2010). Larvicidal and histological effects of *Melia azedarach* extract on *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Journal of King Saud University (Science), 22:77–85.

Al-Mehmadi, R. (2011). Larvicidal, histopathological and ultrastructure studies of *Matricharia chamomella* extracts against the rift valley fever mosquito *Culex quiquefasciatus* (Culicidae: Diptera). Journal of Entomology, 8(1):63-72.

Ammar, S.; Kenawy, M.; Abdel Rahman, H.; Gad, A. and Hamed, A. (2012). Ecology of the mosquito larvae in urban environments of Cairo Governorate, the Journal of the Egyptian Society of Parasitology, 42(1):191-202.

Bassal, R.; Shohat, T.; Kaufman, Z.; Mannasse, B.; Shinar, E.; Amichay, D.; Barak, M.; Ben-Dor, A; Bar-Haim, A.; Cohan, D.; Mendelson, E. and Lustig, Y. (2017). The sero prevalence of West Nile Virus in Israel: A nationwide cross-sectional study. PLOS ONE, 12(6):1-10.

Bekele, D. (2018). Review on insecticidal and repellent activity of plant products for malaria mosquito control. Biomedical Research and Reviews, 2(2):1-7.

Bonner, A. and Alvanja, M. (2017). Pesticides, human health and food security. Food and Energy Security, 6(3):89-93.

Bouaziz, A.; Djeghader, N.; Lynda, A. and Boudjelida, H. (2017). Impact of an insect growth regulator on the development and the reproduction potency of mosquito. Journal of Entomology and Zoology Studies, 5(3):1662-1667.

Briggs, J. (1960). Reduction of adult house fly emergence by the effective *Bacillus sp.* On the development of immature forms. Journal of Insect Pathology, 2:418-432.

Calzolari, M. (2016). Mosquito-borne diseases in Europe: an emerging public health threat. Reports in Parasitology, 5:1-12.

Candido, L. P.; Cavalcanti, M. T. and Beserra, E. B. (2013). Bioactivity of plant extracts on the larval and pupal stages of *Aedes aegypti* (Diptera, Culicidea). Revista da Sociedade Brasileira de Medicina Tropical, 46(4):420-42.

Chancey, C.; Grinev, A.; Volkova, E. and Rios, M. (2015). The global Ecology and epidemiology of West Nile Virus. Bio Medical Research International, 1-20.

Costa, A. A.; Naspi, C. V.; Lucia, A. and Masuh, H. M. (2017). Repellent and larvicidal activity of the essential oil from *Eucalyptus nitens* against *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology, 54:670–676.

Deepa, J.; Gokulakrishnan, J.; Baranitharan, M. and Dhanasekaran, S. (2015). Larvicidal activity of Indian medicinal plants on the dengue fever mosquito, *Aedes aegypti* Linn. International Journal of Pure and Applied Zoology, 3(2):130-136.

Deletre, E.; Martin, T.; Campagne, P.; Bourguet, D.; Cadin, A.; Menut, C.; Bonafos, R. and Chandre, F. (2013). Repellent, Irritant and Toxic Effects of 20 Plant Extracts on Adults of the Malaria Vector *Anopheles gambiae* Mosquito. Plos One, 8(12):e82103.

Dodson, B. L.; Kramer, L. D. and Rasgon, J. L. (2012). Effects of larval rearing temperature on immature development and west Nile virus vector competence of *Culex tarsalis*. Parasites & Vectors, 5:199.

Dyab, A.; Galal, L.; Mahmoud, A. and Mokhtar, Y. (2015). Xenomonitoring of different filarial nematodes using single and multiplex PCR in mosquitoes from Assiut Governorate, Egypt. Korean Journal of Parasitology, 53(1):77-83.

Eldiasty, J.; Hassan, M. and Kamel, O. (2014). Evaluation of some agricultural waste extracts against mosquito larvae, and some types of microorganisms as insecticidal and antibiotic agents. Egyptian Academic Journal of Biological Sciences. 6 (1):1-16.

El-Maghraby, S.; Nawwar, G.; Bakr, R.; Helmy, N. and Kamel, O. (2012). Toxicological studies for some agricultural waste extract on mosquito larvae and experimental animals. Asian Pacific Journal of Tropical Biomedicine, 558 – 563.

El-Naggar, A.; Elbanna, S.; Kaiser, M. and Gabre, R. (2017). Mosquito larval habitat mapping remote sensing and GIS for monitoring the filarial infection regions in Alkorin village, Sharkia Governorate (Egypt). International Journal of Mosquito Research, 4(4):135-139.

El-Sheikh, T. (2002). Effects of application of selected insect growth regulators nd plant extracts on some physiological aspects of the black cutworm, *Agrotis ipsilon*, (Huf). Ph.D. Thesis, Faculty of Science, Ain Shams University. P 186.

El-Zayyat, E.; Solimn, M.; Elleboudy, N. and Ofaa, S. (2017). Bio efficacy of some Egyptian aromatic plants on *Culex pipiens* (Diptera: Culicidae) adults and larvae. Journal of Arthropod Borne Diseases, 11(1):147-155.

Fahim, A.; Kamel, O. and Nawwar, G. (2013). Facile synthesis of in vitro insecticidal and antimicrobial evaluation of bis heterocyclic moiety from PET waste recycling. Egyptian Academic Journal of Biological Sciences, 6(2):132-144.

FAO (2011). Global food losses and food waste: extent, causes and prevention. Study conducted for the save food. International Congress. Düsseldorf, Germany, Interpack.

Farag, R. and Emam, S. (2016). Insecticidal activities of pomegranate peels and leave crude juices. Bulletin of Environment, Pharmacology and Life Sciences, 5:68-75.

Farag, S. M.; Hussein, M. A.; Hafez, S. E.; Khaled, A. S.; Kamel, O. M. and Zyaan, O. H. (2021). Larvicidal, biological, and histopathological alterations induced by pomegranate peel extract, *Punica granatumagainst* against *Culex pipiens* L. (Diptera:Culicidae). Egyptian Journal of Aquatic Biology & Fisheries, 25(2):139–161.

Finney, D. J. (1971). Probit analysis. 3rd Edn., Cambridge University press, London, pp:318.

Freedman, B.; Nowak, L. J.; Ewolek, W. F.; Berry, E. C. and Guthric, W. D. (1979). A bioassay for plant-derived pest control agents using the European corn borer. Journal of Economic Entomology, 72(4):541-545.

Granados-Echegoyen, C.; Pérez-Pacheco, R.; Soto-Hernández, M.; Ruiz-Vega, J.; Lagunez-Rivera, L.; Alonso-Hernandez, N. and Gato-Armas, R. (2014). Inhibition of the growth and development of mosquito larvae of *Culex quinquefasciatus* (Diptera: Culicidae) treated with extract from leaves of *Pseudocalymma alliaceum* (Bignonaceae). Asian Pacific Journal of Tropical Medicine, 7(8):594-601.

Hassan, M.; Zayed, A. and Ahmed, M. (1996). The influence of symbiotic bacteria on digestion and yolk protein synthesis in *Cx. pipiens* L. (Diptera: Culicidae). Journal of Egyptian German Society of Zoology. 21(E):269-284.

Hussien, K. T. (1995). Effect of some plant extracts in the control of a non-biting muscoid fly. Ph. D. Thesis. Cairo University, Cairo, pp. 237.

Inyang, U. E. and Ekanem, J. O. (1996). Effect of dehulling methods and desolventizing temperatures on proximate composition and some functional properties of sesame (*Sesamum indicum* L.) seed flour. Journal of the American Oil Chemists' Society,73(9):1133-1136.

Ismail, I. E. (1980). Physiological studies on the effect of juvenile hormone analogues upon the cotton leafworm *Spodoptera littoralis* Boisd (Lep., Noctuidae). Ph.D. Thesis, Cairo, Univ. Egypt.

Jayarama, M. and Pushpalatha, E. (2008). Effects of plant extracts on fecundity and fertility of mosquitoes. Journal of Applied Entomology, 125(1-2):31-35.

Joseph, H.; Maiava, F.; Naseri, T.; Silva, U; Lamnie, P. and MelRose, W. (2011). Epidemiological assessment of continuing transmission of lymphatic filariasis in Samoa. Annals of Tropical Medicine and Parasitology, 105(8):567-578.

Jouhara, H.; Czajczyriska, D.; Ghazal, H.; Krzyzyriska, R.; Anguilano, L.; Reynolds, A. and Spencer, N. (2017). Municipal waste management systems for domestic use. Energy, 139:485-506.

Kamel, O.; Abd El-Baky, S.; Hassan, M.; Hafez, J. and Hamed, M. (2005 a). Synergistic and joint actions of some plant extracts on their larvicidal activity against the mosquito *Ochlerotatus caspius* (Diptera: Culicidae). Egyptian Academic Society of Environmental Development. (A-Entomology), 6(2):277-289.

Kamel, O.; Hassan, M.; Abd El-Baky, S.; Hafez, J. and Hamed, M. (2005 b). Effect of the salinity and temperature on the larvicidal activity of some insecticidal agents the mosquito *Ochlerotatus caspius* (Diptera: Culicidae). Egyptian Academic Society of Environmental Development. (A-Entomology), 6(2):307-321.

Kauffman, E. K.; Payne, A.; Franke, M. A.; Schmid, M. A.; Harris, E. and Kramer, L. D. (2017). Rearing of *Culex* spp. and *Aedes* spp. Mosquitoes. Bio Protoc, 5; 7(17):e2542.

Khater, H. F. and Shalaby, A. A. S. (2007). Potential of biologically active plant oils for controlling of *Culex pipiens* (Diptera: Culicidae). Acta Scientiae Veterinariae, 35(2):153-160.

Lentner, C. and Wink, A. (1982): Students t- distribution tables. In Geigy scientific Tables Vol. 2. International Medical and Pharmaceutical information, CibaGeigy Limited, Basal, Switzerland.

Mahyoub, J. M.; Al-Saidi, H.; Assagaf, I. P. and Al-Solami, H. M. (2017). Biological effects of *Salicornia fruiticosa* aqueous extract and its synthesized silver nanoparticles against *Culex pipiens* L. (Diptera: Culicidae). Egyptian Journal of Biological Pest Control, 26(4):839-844.

Maia, F. M. and Moore, S. J. (2011). Plant-based insect repellents: a review of their efficacy development and testing. Malaria Journal, 10(Suppl1): S11.

Morebise, O.; Fafunso, M. A.; Makinde, J. M.; Olajide, O. A. and Awe, E. O. (2002). Antiinflammatory property of *Gongronema latifolium*. Phytotherapy Research, 16:S75-S77.

Mukandiwa, L.; Eloff, J. and Naidoo, V. (2016). Repellent and mosquitocidal effects of leaf extracts of *Clausena anisata* against the *Aedes aegypti* mosquito (Diptera: Culicidae). Environmental Science and Pollution Research, 23(11):11257-11266.

Odey, M. O.; Iwara, I. A.; Udiba, U. U.; Johnson, J. T.; Inekwe, U. V.; Asenye, M. E. and Victor, O. (2012). Preparation of plant extracts from indigenous medicinal plants. International Journal of Science and Technology, 1(12):688-692.

Rak, S. and Ishii, T. (1989). Characters eggs and egg masses obtained by induced oviposition in *Cx. pipiens* L. Journal of Science. College of General Education. University of Tokushima. 22:25-31.

Rami, A.; Zuharah, F.; Thiagaletchumi, M. and Subramaniam, S. (2014). Larvicidal Efficacy of Different Plant Parts of *Railway Creeper*, *Ipomoea cairica* Extract Against Dengue Vector Mosquitoes, *Aedes albopictus* (Diptera: Culicidae) and *Aedes aegypti* (Diptera: Culicidae). Journal of Insect Science, 14(180): 2014.

Riddiford, L. M. (1970). Prevention of metamorphosis by exposure of insect to juvenile hormone analogue. Science, N.Y., 167:287-288.

Rutten, M. and Kavallari, A. (2013). Can reductions in agricultural food losses avoid some of the trade-offs involved when safeguarding domestic food security? A case study of the Middle East and North Africa. GTAP Resource. Paper presented at the 16th Annual Conference on Global Economic Analysis, Shanghai, China.

Salama, S. H. and Shehata, E. I. (2017). Bioactivity and repellency of some plant extracts against the tomato leaf miner *Tuta absoluta* (Meyrick 1917) (Lepidoptera: Gelechiidae). Research Journal of Pharmaceutical, Biological and Chemical Sciences, 8:1021-1036.

Schneidermann, H. A. (1972). Insect hormone and insect control (In: Insect Juvenile Hormone, Chemistry and Action, J.J. Mennand M. Beroza (Eds), Academic press, New York, London. 3:27.

Shehata, A. I. (2019). Biological activity of *Prunus domestica* (Rosaceae) *and Rhamnus cathartica* (Rhamnaceae) leaves extracts against the mosquito vector, *Culex pipiens* L. (Diptera: Culicidae). Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control, 11(1):65-73.

Shoukat, R. F.; Shakeel, M.; Rizvi, S. A. H.; Zafar, J.; Zhang, Y.; Freed, S.; Xu, X. and Jin, F. (2020). Larvicidal, Ovicidal, Synergistic, and Repellent Activities of *Sophora alopecuroides* and Its Dominant Constituents Against *Aedes albopictus*. Insects, 11:246.

Sosa, A.; Diaz, M.; Salvatore, A.; Bardon, A.; Borkosky, S. and Vera, N. (2018). Insecticidal effects of *Vernonanthura nebularum* against two economically important pest insects. Saudi Journal of Biological Sciences, 26(5):881-889.

Suh, C. P. C.; Orr, D. B. and Van Duyn, J. W. (2000). Effect of insecticides on *Trichogramma exiguum* (Hymenoptera: Trichogrammatidae) preimaginal development and adult survival. Journal of Econonomic Entomology, 93:577-583.

Toppozada, A.; Abdallah, S. and El-Defrawi, M. (1966). Chemosterization of larvae and adults of the Egyptian cotton leaf worm, *Prodenia littura* by apholate, metepa and hempa. Journal of Economic Entomology, 59:1125-1128.

Yamashita Y.; Tani K. and KoBaYashi, M. (1961). The effects of allatectomy on the number of eggs in the silkworm, *Bombyx mori*. Acta Serology. 39:12–15.

WHO (2005). Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization, WHO/CDS/WHOPES/GCDPP/2005.13.