Original Research Article

Biological activity of *Cupressus sempervirens* extracts against *Musca domestica*.

4 Abstract:

1

5 The biological activity against the larval, pupal and adult stages, moreover, the efficacy on 6 reproductive potential, antifeedant and repellency activity of ethanolic, acetone and 7 petroleum ether, leaves and stems extracts of C. sempervirens against the housefly, M. 8 domestica was evaluated. The larval and pupal duration, pupal mortality, adult emergence 9 and growth index were highly affected by different extracts tested. The antifeedant and 10 repellent activity of was varied depending on solvent, plant parts used in extraction and 11 the dose of extract. Tested extracts significantly reduced the fecundity and increased the 12 sterility. Based on LC_{50} , the toxicity may be arranged as follows: leaves > stems. Petroleum 13 ether extract from leaves was more effective in inducing the fecundity, antifeedant, 14 repellent actions and egg-hatchability than those from stems. These results may provide an 15 opportunity to develop alternatives to costly organic pesticides using some available cheap 16 plants, which are usually safe to the environment and to other living organisms.

17 Keywords: C. sempervirens, M. domestica, toxicity, fecundity, antifeedant, pesticides.

18 Introduction

19 The housefly, M. domestica (Diptera: Muscidae) is cosmopolitan, it generally breeds in 20 decaying organic matter and feeds in manure, garbage and food left out by humans (Morey 21 and Khandagle, 2012). The housefly is an important medical insect pests that causes 22 irritation, spoils food and acts as a vector for more than 100 human and animal pathogenic 23 organisms such as entomopathogenic bacteria, enterovirus and protozoa cysts (Hanan, 24 2013). Adult houseflies have been shown to transmit pathogens from their sponging 25 mouthparts, through vomitus on the sticky parts of the feet and through the intestinal tract, 26 thereby contaminating food and propagating disease (De Jesús et al., 2004).

27 Control of housefly largely relies on chemical insecticides. Unfortunately, houseflies 28 have developed resistance to most of synthetic insecticides (Khan et al., 2013). In addition, 29 synthetic insecticides have adverse effect on environment, health and threat of persistence 30 the bio-magnifications through the food chain (Kumar et al., 2012; Ito et al., 2015). Recently, 31 the application of botanical products has drawn much attention as effective alternatives to 32 the synthetic pesticides; these plant products are reported to be more effective, less 33 expensive, biodegradable and safe for mankind and environment than synthetic 34 counterparts (Singh et al., 1996). Therefore, alternatives to conventional insecticides are 35 required to be developed from the active ingredients of plant origin, and these compounds 36 may serve as insecticides, antifeedants, repellents as well as attractants (Murugesan et al., 37 2016; Ito and Ighere, 2017). Cupressus sempervirens is a medicinal plant has antiseptic, aroma 38 therapeutic, astringent, balsamic or anti-inflammatory, antispasmodic, astringent, 39 antiseptic, deodorant and diuretic activities. Several monoterpenes, di-terpenes, 40 polyphenols, flavonoids, flavonoid glycoside and bioflavonoids have been isolated from 41 this plant (Khan et al., 2017).

In the present study we aimed at evaluating the biological, antifeedant and repellent
activities of *C. sempervirens* extracts against the larvae and adults of the housefly, *M. domestica*.

45 Materials and Methods

46 **1.** Laboratory maintenance of *M. domestica*.

The culture of the housefly was maintained for several generations under controlled conditions of 27±2°C and 70-75 RH and 12-12 light/dark. The emerged flies were fed on dry diet (milk powder) and sucrose solution. Eggs were collected from paper strips or from cotton pads of feeding. Larvae were reared on an artificial diet (wheat bran, milk, powder yeast; 200:100:5gm) per 200ml of distilled water according a method described by (Busvine, 1962).

53 **2.** Extraction of plant materials.

54 Cupressus sempervirens was collected from Sadat City, Cairo-Alexandria desert road, 55 El-monofiya Governorate and left to dry away from sun rays at room temperature (27±2°C) 56 for 5 to 10days. Then, C. sempervirens leaves and stems pulverized to powder separately in a 57 hammer mill. One hundred grams of powder from C. sempervirens (leaves and stems) for 58 each solvent separately were extracted using 300ml of 70% ethanol, acetone and petroleum 59 ether solvents at room temperature. After 24 h., the supernatants were decanted, filtrated 60 through Whatman filter paper (No. 5) and dried in a rotary evaporator at 40°C for 61 (2-3hours) for ethanol and (40-60minutes) for the other solvents. The dry extracts were 62 weighed and kept at -4°C till using for experiments.

63 **3.** Experimental bioassay.

64 In order to study the toxicity of the concerned plant extracts, the tested material of the 65 70% ethanol extracts was dissolved in 0.1ml of ethanol, while the tested material of acetone and petroleum ether extracts was dissolved in 2drop of Tweenso as emulsifier to facilitate 66 67 the dissolving oils of tested material in 250ml water. Larval artificial diet was mixed with 68 different concentrations of each concerned extract to detect mortality percent. Then, twenty 69 five of third 3rd instar larvae were put immediately into plastic cups contained media mixed 70 with different concentrations of extracts. Three replicates were usually used for each tested 71 concentration. All plastic cups were incubated under controlled conditions of temperature 72 27±2°C, 70-75% RH and 12-12 light-dark regime. Control larvae received 0.1 ml of ethanol 73 or 2 drop of Tweenso in 250ml water. Mortality was recorded daily and dead larvae and 74 pupae were removed daily.

75 Larval mortality percent was estimated using the following equation (Briggs 1960): 76 larval mortality $\% = A - B / A \times 100$ (where: A = number of tested larvae, B = number of 77 tested pupa). The pupation rate was estimated by using the following equation: pupation % 78 = $A/B \times 100$. Where: A = number of pupae, B = number of tested larvae. The pupal mortality 79 percentage was estimated by using the following equation: pupal mortality % =A-B/A× 80 100.Where: A = number of produced pupae, B = number of observed adults. Pupal duration 81 was calculated as interval between the commencement of pupation and the commencement 82 of adult emergence. The emerged adult males and females were counted and the adult 83 emergence was calculated by using the following equation: adult emergence %= A/B×100. 84 Where: A = number of emerged adults, B = number of tested pupae. All values calculated 85 for each one and then the mean value was taken

86 Adult females that succeeded to emerge from the 3rd instar larvae treated with each 87 concentration were collected and transferred with untreated adult males obtained from the 88 colony to the wooden cages (20×20×20 cm) by using an electric aspirator recommended by 89 (WHO) and fed with dry diet (milk powder) and sucrose solution (cotton pads soaked in 90 10% sucrose solution) for four days. The eggs were counted by using binocular and then 91 the mean values were taken. The Egg-hatchability percentage was calculated by using the 92 following equation: Egg hatchability $\% = A/B \times 100$. Where: A = total no. of hatched eggs, B = 93 total no. of eggs laid. The Sterility percentage was estimated according to the formula of 94 (Toppozada *et al.*1966): Sterility percentage = 100 – [a×b /A×B] ×100.

95 Standard cages (20×20×20cm) were used to test the repellent activity of plant extracts. 96 Cotton pieces soaked in 10% sucrose solution from each concentration added to the 97 wooden cages containing 40 starved individuals (5-7 d-old) for three hours. Control tests 98 were carried out alongside with the treatments using cotton pieces soaked in 10% sucrose 99 solution with 2 drops of 70% ethanol or Tweenso. Each test was repeated three times to get a 100 mean value of repellent. Repellency % was calculated according to Abbott (1925): 101 Repellency % = [%A -%B /100 -%B] ×100. Where: A = percent of unfed females in treatment, 102 B = percent of unfed females in control.

103 **4.** Statistical analysis.

104 Statistical analysis of the data was carried out according to the method of (Lentner *et al.,* 1982). The analysis was revised and graphics were drawn by Excel for Microsoft office 106 2010.The obtained data were assessed by calculation of the mean (M) and standard 107 deviation (SD). The LC₅₀ was calculated using multiple linear regression (Finney, 1971).

108 Results

109 1- Biological activity of *C. sempervirens* against *M. domestica*.

110 1-1- Ethanolic extract.

Data given in (Table 1) shows the effect of ethanol extract of *C. sempervirens* (leaves) against different biological aspects of *M. domestica*. The larval mortality percent and the mean larval duration were concentration-dependent. There was a negative correlation between the P% and the concentration used. The mortality percent of pupae were recorded 12.5, 11.8, 8.4 and 8.7% at the concentrations of 2000, 1700, 1400 and 1100 ppm; respectively.

Table (1): Effect of ethanol extract of *C. sempervirens* (leaves) on some biological aspects of *M. domestica*.

Conc. ppm	Larval mort. %	Larval duration	Pupation %	Pupal mort. <mark>%</mark>	Pupal duration	Larval and Pupal mort. %	Emergence <mark>%</mark> (a)		<mark>Growth Index</mark> (a/b)
2300	100.0	-				100.0	0.0		
2000	84.0	4.2±0.3 ^d	16.0	12.5	4.6±0.2 ^a	96.4	87.5	8.8±0.5 ^a	9.9
1700	68.0	3.7±0.11 ^c	32.0	11.8	4.0±0.7 ^a	79.6	88.2	7.7±0.8 ^a	11.5
1400	52.0	3.5±0.20 ^b	48.0	8.4	4.3±1.1 ^a	60.4	91.6	7.9±1.5 ^a	11.6
1100	36.0	3.2±0.19 ^a	64.0	8.7	3.8±0.3 ^a	44.5	91.3	7.0±1.0 ^a	13.0
800	20.0	3.0±0.12 ^a	80.0	0.0	4.1±0.6 ^a	20.0	100.0	7.1±1.61 ^a	14.1
Control	2.8	2.9±0.19	97.2	0.0	3.9±0.45	2.8	100.0	6.8±0.64	14.7

Conc.: concentration, ppm : part per million, means with different letter are significantly different (P<0.05).

118 The mean pupal duration was non-significantly (P>0.05) affected. The adult 119 emergence % was recorded 87.5 % for adults resulted from larvae treated with 2000 ppm 120 and this percent was increased as the concentrations decreased. The growth index for 121 larvae and pupae was not affected at all concentrations used as compared with the control 122 group.

123 Table (2): Effect of ethanol extract of C. sempervirens (stems) on some biological aspects of M. 124 domestica.

Conc. ppm	Larval mort, <mark>%</mark>	Larval duration	Pupation %	Pupal mort. <mark>%</mark>	Pupal duration	Emergence <mark>%</mark> (a)	Developmental Period (b)	Growth Index (a/b)
2300	100.0					0.0		
2000	84.0	4.2±0.37 ^b	16.0	0.0	3.8±0.22 ^a	100.0	8.0±0.59 ^a	12.5
1700	62.8	3.7±0.12 ^a	37.2	0.0	4.0±0.20 ^a	100.0	7.7±0.32 ^a	13.0
1400	38.8	3.5±0.40 ^a	61.2	0.0	4.0±0.13 ^a	100.0	7.5±0.53 ^a	13.3
1100	29.2	3.2±0.78 ^a	70.8	0.0	3.8±0.28 ^a	100.0	7.0±1.06 ^a	14.3
800	16.0	3.0±0.93 ^a	84.0	0.0	4.1±0.14 ^a	100.0	7.1±1.07 ^a	14.1
Control	8.0	2.9±0.24	92.0	0.0	3.9±0.19	100.0	6.8±0.43	14.7
See footn	ote of table (1).					•	125

tootnote of table (1).

126 The effect of ethanol extract of *C. sempervirens* (stems) against different biological 127 aspects of M. domestica given in (Table 2). Complete larval mortality 100.0% was caused at 128 the highest concentration 2300ppm, meanwhile the lowest value 16.0% was occurred at 129 the lowest concentration used 800ppm compared to 8.0% for the control group. The larval 130 duration was insignificantly (P>0.05) affected by all concentrations used except the highest 131 concentration 2000 ppm, which prolonged it to 4.2±0.37 (days) against the control group. 132 There was a negative correlation between the pupation % and the concentration used. The 133 pupal mortality % was insignificantly (P>0.05) affected by all concentrations used. The 134 growth index for larvae and pupae was not affected at all concentrations used as 135 compared with the control group.

136 1-2- Acetone extract.

137 The biological activity of acetone extract of C. sempervirens (Leaves) against the 3rd instar larvae of *M. domestica* was recorded in (Table 3). Results obtained revealed that, the 138 139 larval mortality % was concentration-dependent; the highest larval mortality % (100.0) 140 was caused by the concentration 1500 ppm. The larval duration was insignificantly 141 (P>0.05) affected by all concentrations used. A reduction in pupation % was recorded at all 142 concentrations used.

Conc. ppm	Larval mort. <mark>%</mark>	Larval duration	Pupation <mark>%</mark>	Pupal mort. <mark>%</mark>	Pupal duration	Emergence <mark>%</mark> (a)	Developmental Period (b)	<mark>Growth Index</mark> (a/b)
1500	100.0					0.0		
1300	89.2	1.9±0.10 ^c	10.8	74.1	3.8±0.25 ^d	25.9	5.7±0.35 ^d	4.5
1100	72.0	2.5±0.24 ^a	28.0	56.0	3.9±0.23 ^d	44.0	6.4±0.47 ^c	6.9
900	48.0	2.6±0.22 ^a	52.0	76.9	3.7±0.19 ^d	23.1	6.3±0.41 °	3.7
700	36.0	2.8±0.17 ^a	64.0	68.8	3.8±0.11 ^d	31.2	6.6±0.28 °	4.7
500	22.8	3.0±0.23 ^a	77.2	62.2	3.8±0.14 ^d	37.8	6.8±0.37 ^b	5.6
Control	6.8	2.8±0.41	93.2	0.0	5.4±0.29	100.0	8.2±0.70	12.2
See foot	note of table (1).		•				145

143 Table (3): Effect of acetone extract of C. sempervirens (leaves) on some biological aspects of M. 144 domestica.

There was a toxic effect of acetone extract on the pupae resulted from treated larvae, 146 147 the highest pupal mortality % (74.1 %) was induced at the concentration 1300 ppm. The 148 pupal duration was significantly (P<0.05) affected by all concentrations used. A sharp 149 reduction in AE % among the adults developed from the treated larvae at all 150 concentrations was exhibited. A very retarded effect on growth of larvae, pupae and adult 151 was observed especially at the highest concentration 1300ppm.

152 Data given in (Table 4) shows the biological activity of acetone extract of C. 153 sempervirens (stems) against the 3rd instar larvae of *M. domestica*. The highest larval mortality 154 % (100.0 %) was occurred at the concentration of 1500 ppm, while the lowest percent 17.2 155 % was occurred at the concentration of 500 ppm. The larval duration was insignificantly 156 (P>0.05) affected by all concentrations used except the highest concentration 1300ppm.

157 Table (4): Effect of acetone extract of C. sempervirens (stems) on some biological aspects of M. 158 domestica.

Conc. ppm	Larval mort. <mark>%</mark>	Larval duration	Pupation <mark>%</mark>	Pupal mort. <mark>%</mark>	Pupal duration	Emergence <mark>%</mark> (a)	Developmental Period (b)	<mark>Growth Index</mark> (a/b)
1500	100.0	- ->	-		-	0.0		
1300	90.8	1.9±0.10 ^b	9.2	10.6	3.8±0.29 ^a	89.4	5.7±0.39 ^a	15.7
1100	69.2	2.5±0.24 ^a	30.8	10.0	3.9±0.21 ^a	96.1	6.4±0.45 ^a	15.0
900	48.0	2.6±0.19 ^a	52.0	9.5	3.7±0.14 ^a	90.5	6.3±0.33 ^a	14.4
700	30.8	2.8±0.33 ^a	69.2	0.0	3.8±0.10 ^a	100.0	6.6±0.43 ^a	15.2
500	17.2	2.7±0.15 ^a	82.8	0.0	3.8±0.15 ^a	100.0	6.5±0.30 ^a	15.4
Control	12.0	2.7±0.27	88.0	7.7	4.6±0.65	92.3	7.3±0.92	12.6
See footn	ote of table (<mark>1).</mark>						159

160 There was a negative correlation between the pupation % and the concentration used. 161 No significant effect on the pupal duration was recorded. The growth index did not 162 affected by acetone extract at all concentrations used as compared with the control group.

163 **1-3-** Petroleum ether extract.

164 Table (5): Effect of Petroleum ether extract of *C. sempervirens* (leaves) on some biological aspects of165 *M. domestica.*

Conc. ppm	<mark>Larval mort.</mark> <mark>%</mark>	Larval duration	Pupation <mark>%</mark>	Pupal mort. <mark>%</mark>	Pupal duration	Emergence <mark>%</mark> (a)	Developmental Period (b)	<mark>Growth Index</mark> (a/b)
1000	100.0					0.0		
800	84.0	4.2±0.22 ^a	16.0	81.1	7.3±0.41 °	18.9	11.5±0.63 °	1.6
600	66.7	3.9±0.18 ^a	33.3	84.3	6.9±0.19 °	15.7	10.8±0.37 °	1.5
400	53.3	3.3±0.36 ^a	46.7	73.2	7.1±0.91 °	26.8	10.4±1.27 b	2.6
200	28.0	2.8±0.21 ^a	72.0	59.7	6.6±0.78 °	40.3	9.4±0.99 ^a	4.3
Control	12.0	2.7±0.27	88.0	7.7	4.6±0.65	92.3	7.3±0.92	12.6
See footn	ote of table (1	l).				1		166

167 Data given in (Table 5) indicated the biological activity of petroleum ether extract of 168 C. sempervirens (Leaves) against the 3^{rd} instar larvae of M. domestica. Complete larval 169 mortality % (100.0%) was caused at the highest concentration used 1000ppm. Meanwhile, 170 the LM % decreased to 28.0 at the lowest concentration 200 ppm. The larval duration was 171 insignificantly (P>0.05) affected by all concentrations used. The pupation % was 0.0 at the 172 highest concentration 1000 ppm and 72.0 at the lowest concentration 200ppm. A toxic effect 173 on the pupae resulted from treated larvae was observed. The pupal duration affected by 174 petroleum ether extract tested. A remarkable reduction in the adult emergence % was also 175 observed. The growth index was greatly affected by tested extract.

On the other hand, the biological activity of stems petroleum ether extract wasrevealed in (Table 6).

Table (6): Effect of Petroleum ether extract of *C. sempervirens* (stems) on some biological aspects of *M. domestica*.

Conc. ppm	Larval mort, <mark>%</mark>	Larval duration	Pupation <mark>%</mark>	Pupal mort. <mark>%</mark>	Pupal duration	Emergence <mark>%</mark> (a)	Developmental Period (b)	Growth Index (a/b)
1000	100.0					0.0		
800	84.0	3.9±0.98 ^a	16.0	100.0		0.0		
600	66.7	3.5±0.12 ^a	33.3	47.6	4.0±0.7 ^a	52.4	7.7±0.8 ^a	6.8
400	53.3	3.4±0.96 ^a	46.7	47.3	4.3±1.1 ^a	52.7	7.9±1.5 ^a	6.7
200	28.0	2.9±0.23 ^a	72.0	27.6	3.8±0.3 ^a	72.4	7.0±1.0 ^a	10.3
Control	6.8	2.2±0.40	93.2	0.0	4.5±0.21	100.0	6.7±0.61	14.9
See footn	ote of table (1).	-			-		180

Data obtained showed that, the larval mortality% was concentration-dependent. The larval duration was insignificantly prolonged at all the concentrations used. The pupation% of the treated larvae was decreased as the concentration increased. The pupal mortality% and adult emergence% were found to be affected by extract used. The pupal duration was insignificantly (P>0.05) affected by all concentration used as compared with the untreated group. The growth index was greatly affected by petroleum ether stems
extract, where it recorded 6.8, 6.7 and 10.3 at the concentrations 800, 700 and 600ppm;
respectively, compared to 14.9 for the control group.

189 From the aforementioned results and based on the LC⁵⁰ values Fig. (1) it is obvious
190 that, the toxicity values of the tested ethanolic, acetone and petroleum ether extracts of *C*.
191 sempervirens (leaves and stems) may be arranged in a descending order as follows:

192 Petroleum ether extract > Acetone extract > Ethanolic extract.



193

Fig. (1): LC₅₀ values for ethanol, acetone and petroleum ether extracts from leaves and stems of *C*.
 sempervirens against 3rd instar larvae of *M. domestica*.

196 2- Reproductive potential.

197 The fecundity of females resulted from the larvae treated with leaves petroleum ether 198 extract of *C. sempervirens* was significantly (P<0.001) decreased from 42.3 ± 1.7 eggs/female at 199 the lowest concentration 200 ppm to 38.0 ± 1.4 at the highest concentration 800 ppm 200 compared to 58.8 ± 2.1 eggs/ female for control (Table 7). There was a slight decrease in the 201 hatchability percent of eggs laid by females resulted from treated larvae. Also, a marked 202 increase in the percentage of sterility for all females emerged from treated larvae was 203 recorded.

Table (7): Effect of petroleum ether extract of *C. sempervirens* (leaves) on fecundity, fertility and sterility index of female *M. domestica*.

Conc.	No. of tested	Fecundity		Hatched	l eggs	Non-hate	ched eggs	Sterility Index
(ppm)	females	Total	Mean±SD	Total	%	Total	%	(SI)
800	2	76	38.0±1.4 ^d	72	94.7	4	5.3	38.2
600	4	159	39.8±1.7 ^d	154	96.8	5	3.2	33.9
400	7	291	41.6±2.1 ^d	283	97.3	8	2.7	30.5
200	8	338	42.3±1.7 ^d	332	98.2	6	1.8	28.7
Control	18	1059	58.8±2.1	1049	99.1	10	0.9	0.0
See footr	ote of table (1).			1			1	206

On the other hand, the fecundity of females resulted from the larvae treated with stems petroleum ether extract of *C. sempervirens* was decreased by increasing the concentration and the statistical analysis revealed that, there was a significant (P<0.001) decrease in the mean number of eggs laid by females resulted from treated larvae at the all concentrations used (Table 8). A marked decrease in the hatchability percent and
 remarkable increase in the percentage of sterility index were also recorded.

Table (8): Effect of petroleum ether extract of *C. sempervirens* (stems) on fecundity, fertility and sterility index of female *M. domestica*.

Conc.	No. of	Fecundity		Hatched eggs		Non-hat	ched eggs	Sterility Index
(ppm)	tested	Total	Mean±SD	Total	%	Total	%	(SI)
900	2	77	38.5±2.1 ^d	73	94.8	4	5.2	39.0
800	3	117	39.3±1.2 ^d	111	94.9	6	5.1	37.7
700	6	264	44.1±3.3 ^d	253	95.8	11	4.2	29.4
600	7	327	46.7±1.7 ^d	318	97.2	9	2.8	24.1
Control	20	1205	60.2±2.9	1198	99.4	7	0.6	0.0
See footnote	of table (1).		•	•	•	4		215

216 3- Antifeedant and repellency activities.

The antifeedant and repellent activity of ethanolic, acetone and petroleum ether leaves extracts of *C. sempervirens* was shown in (Table 9), data obtained revealed that at the LC₅₀ concentrations, the repellency percent recorded 45.6, 61.4 and 78.9 % for the tested extracts; respectively as compared with the untreated group.

Table (9): Effect of LC₅₀ concentration from ethanol, acetone and petroleum ether extracts of *C*.
 sempervirens (leaves) as antifeedant or repellent for *M. domestica*.

Extract	LC ₅₀ value	Fed No.	Fed%	Non-fed No.	Non-fed %	Repellency action (%)
70% Ethanol	1363.0	10.3±0.64	51.7±2.91	9.7±0.63	48.3±2.92	45.6
Acetone	860.4	7.3±1.23	36.7±5.80	12.7±1.24	63.3±5.74	61.4
Petroleum Ether	412.01	3.6±1.52	18.3 ±7.63	16.3 ±1.53	80.0±5.01	78.9
Control	0.0	19.0±1.20	95.0 ±5.01	1.0 ±1.12	5.0±5.03	0.0

222

In contrast, the antifeedant and repellent activity of ethanolic, acetone and petroleum ether stems extracts of *C. sempervirens* against starved *M. domestica* adults were varied according to the solvents used in extraction (Table 10).

226

Table (10): Effect of LC₅₀ concentration from ethanol, acetone and petroleum ether extracts of *C. sempervirens* (stems) as antifeedant or repellent for *M. domestica*.

Extract	LC ₅₀ value	Fed No.	Fed%	Non-fed No.	Non-fed %	Repellency action (%)
70% Ethanol	1462.5	14.7±1.54	73.3±7.61	5.3±1.52	26.7±7.63	21.4
Acetone	893.7	10.7±1.51	53.3±7.63	9.3±1.54	46.7±7.61	42.9
Petroleum Ether	706.1	5.3±1.20	26.7±5.82	14.7±1.21	73.3±5.84	71.4
Control	0.0	18.7±1.52	93.3 ±7.64	1.3 ±1.51	6.7±7.60	0.0

The petroleum ether extract was more effective than acetone and ethanolic extracts

229

231

229 230 dur

during the entire testing period of 2h post treatment. The repellency was 71.4% for the petroleum ether extract, while it recorded 42.9 and 21.4% for acetone and ethanolic extracts;

respectively compared with the control group.

233 Discussion and conclusion

The plant tested in this study is known to be eco-friendly and non-toxic to vertebrates (EL-Sheikh *et al.*, 2011). Moreover, it is clearly proved that crude or partially purified plant extracts are less expensive and highly efficacious for the control of *M. domestica* rather than the purified compounds or extracts (Jang *et al.*, 2002 and Cavalcanti *et al.*, 2004). The results of this study may offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides.

240 An insecticide dose not has to cause high mortality on target organisms in order to be 241 acceptable. Sukumar et al., (1991) suggested the existence of variations in toxicities of 242 phytochemical compounds on target species depending on the plant part from which they 243 were extracted. In addition, Maurya et al., (2009) noted that other variations were due to 244 responses by species and developmental stages of species to the specified extract, solvent of 245 extraction, geographical origin of the plant, photosensitivity of compounds in the extract, 246 effect on growth and reproduction and other factors. The larval mortality percent was 247 increased as concentration increased for all extracts tested. Based on LC₅₀ values, the 248 toxicity tested of ethanolic, acetone and petroleum ether extracts of leaves were more 249 effective than those of stems. Also, the petroleum ether extracts were more effective than 250 acetone and ethanolic extracts for all plant parts used. These results are in agreement with 251 the previously mentioned suggestions of Maurya et al., (2009).

252 Different extracts tested varied the larval and pupal duration depending on plant part, 253 solvent and concentration of the extract. Prolongation of the larval duration was similar to 254 that reported in M. domestica by Gad-Allah, (1991), using Melia azedarach, Ande, (2001) using 255 Peganumharmala, Acalyphaindica and Calotropis gigantic and El-bermawy et al. (2011) for 256 Cupressus macrocarpa (leaves) powders. Prolongation in the pupal duration was also 257 recorded in this study, Similar observation was also recorded on *M. domestica* by Assar, 258 (2003) using Atriplex inflate and Bakr et al. (2003) using Artemisia monosperma. The pupation 259 rate was varied according to plant part and solvent used in extraction, moreover, the 260 pupation percent was decreased as the concentration of plant extract increased. Similar 261 effects of some botanical plant extracts have been reported on *M. domestica* by (Ande, 2001; 262 Assar, 2003; Bakret al. 2003 and El-bermawy et al. 2011).

263 The decrease in the percentage of adult emergence of *M. domestica* due to treatment 264 with the tested plant extracts was similar to those of Muse et al., (2003) where, the mean 265 number of males and females of Chrysomya chloropyga emerging from larvae feeding diet 266 containing 5 % of *Lantana camara* powder, were significantly less than those of the control, 267 Khalaf et al., (2009) who found that, high reduction in Synthesiomyi anudiseta adult 268 emergence was induced by larval treatment with C. macrocarpa volatile oils. The growth 269 index of *M. domestica* was clearly affected by the plant extracts tested. It decreased as the 270 concentration increased. Retardation in growth was induced by different parts of plant 271 tested, such results are in agreement with earlier studies using different plant extracts 272 against other dipteran species by Jeyabalan et al. (2003) using Pelargonium citrosa leaf 273 extracts on Anopheles stephensi, Nathan et al. (2006) using Melia azedarach on An. stephensi and 274 Sharma et al. (2006) sing Artemisia annua extract against Culex autnauetesctetus,

275 The results obtained also indicated that, treatment of *M. domestica* larvae with plant 276 extracts caused a decrease in egg production. Different authors reported some illustrations 277 revealing the possible reasons for the reduction of fecundity and as a result increasing 278 sterility following the treatment with plant extracts. The weakened physical stage of the 279 treated insects (Tripathi et al., 2003). Mild suppressing effect exerted by the plant extract on 280 the insect's mating-decisive factor influencing the subsequent number of eggs laid by the 281 insect (Engelmann, 1970). Partial sterilization of females and/or males, or the inability of the 282 sperms to be transferred to the females during copulation (Ismail, 1980). Reduction in the 283 number of normal sperms produced by male insect (El-Meniawi et al., 1999).Blockage of 284 ovarian activity, as the tested botanical products may interfere with oogenesis, which in 285 turn, results in a complete and irreversible sterility of insect female flies (Khan et al., 2007).

286 Reduction in the egg hatching percent by plant extracts was similar to findings 287 reported by many authors against M. domestica, among these are: Melia azedarach extract 288 Radwan, (2000), leaves and flowers extracts of Daturainnoxia Al-Zubaidi et al., (2002), A. 289 inflate Assar, (2003). Tested extracts displayed various degree of repellency at various 290 concentrations against *M. domestica* and this may reflect the complexity of the chemical 291 composition of their constituents and the petroleum ether extract was more effective in 292 repellent action as compared with the acetone and ethanol extracts, These results are in 293 consistence with Bisseleua et al. (2008) using petroleum ether extracts of Griffonia 294 simplicifolia.

It could be concluded that, toxicity of tested extracts varied according to plant part, solvent used in extraction and concentration of the extract. Based on LC_{50} , the toxicity values were arranged as follows: leaves > stems. Petroleum ether extract from leaves was more effective in inducing the fecundity, antifeedant, repellent actions and egg-hatchability than those from stems. So, the plant extracts used may be considered as new promising controlling agents for the housefly, *M. domestica*.

301 Ethical Approval:

302 As per international standard or university standard ethical approval has been collected and303 preserved by the author(s).

- 304
- 305 References

- 306 Al-Zubaidi, FS, Al Rubaie, HM and Al Okaily, LN (2002): The effects of terpenoid compounds
- 307 extracted from Daturainnoxia Mill on the biological performance of Musca domestica L. (Diptera:
- 308 Muscidae). Proc. of the 2nd. Inter. Conf. on Biological Sciences, Fac. Sci. Tanta Univ.
- 309 Ande, AT (2001): Biological activities of some plant materials against the housefly, *Musca domestica*.
- 310 Ni. S. Exp. Bio. J. 1(4):293-296.
- 311 Assar, AA (2003): Biological effects of the water extract of *Atriplex* inflate (Chenopodiaceae) on the
- 312 housefly, Musca domestica vicina. (Diptera: Muscidae). J. Egypt. Ger. Soc. Zool. 41 (E): 15-28.
- 313 Bakr, ME, Nassef, NE, Assar, AA, El-Sobky, MM and Shams El-Din, SA (2003): Biological and
- 314 morphological effects of water extracts of some plants on the housefly, *Musca domestica* vicina.
- 315 (Diptera: Muscidae). J. Egypt. Ger. Soc. Zool. 41 (E): 29-47.
- 316 Bisseleua, HBD, Gbewonyo, SWK and Obeng-Ofori, D. (2008): Toxicity, growth regulatory and
- 317 repellent activities of medicinal plant extracts on *Musca domestica* L. (Diptera: Muscidea). *Afr. J. Biot.*
- 318 7(24): 4635-42.
- 319 Busvin, JR (1962): A laboratory technique for measuring the susceptibility of houseflies and blow 320 flies to insecticides. *Lab. Prot. London*. (11): 464-468.
- 321 Cavalcanti, ES, Morais, SM, Lima, MA and Santana, EW (2004): Larvicidal activity of essential oils
- 322 from Brazilian plants against Ae. aegyptiL. Mem. Inst. Oswaldo. Cruz. 99(5): 541-544.
- 323 De Jesús, AJ, Olsen, AR, Bryce, JR and Whiting, RC (2004): Quantitative contamination and transfer
- 324 of *Escherichia coli* from foods by houseflies, *Musca domestica* L. (Diptera: Muscidae). *Inter. J. Food* 325 *Microbial*.93: 259-262.
- 326 El-bermawy, SM, Elkattan, NAI, Ahmed, SK and Abdel-Gawad, RM (2011): Screening for bioactive
- materials against housefly, *Musca domestica* L. (Diptera: Muscidae). *Functional Plant Biotech*. 5(1):
 45-51.
- 329 El-Meniawi, FA,Hashem, M, El-Mesieri, SM and Rawash, IA (1999): Physiological effects of neem
- 330 seed extracts on the reproductive activities of the cotton leaf worm, *Spodoptera littoralis* Boisd. *Alex. J.*
- 331 Agric. Res. 44(1): 79-96.
- 332 EL-Sheikh, TMY, Hassan, MI, Moselhy, WA, Amer, MS and Shehata, AZ (2011): Evaluation of the
- 333 biological activity of *Cupressus sempervirens* (Cupressaceae) extracts against the mosquito vector
- 334 Culex pipiens L. (Diptera: Culicidae). Egypt. Acad. J. biolog. Sci., 4 (1): 33 48
- 335 Engelmann, F (1970): The physiology of insect reproduction. Pergamon Press. New york.
- 336 Finney, DJ (1971): Probit analysis Third edition. *Cambridge Univ. Press.* 333 p.
- 337 Gad-Allah, SZ (1991): The effect of some plant extracts on Musca domestica vicina. Unpublished M. Sci.
- 338 Thesis, Fac. Sci., Cairo Univ., Egypt.
- 339 Hanan, BA (2013): Evaluation of insecticidal activities of MenthapipertaandLavandulaangustifolia
- 340 essential oils against housefly, Musca domesticaL. (Diptera: Muscidae). J. Entomol. Nematol. 5: 50-54.
- 341 Ismail, IE (1980): Physiological studies on the effect of juvenile hormone analogues upon the cotton

- 342 leaf worm Spodoptera littoralisBoisd (Lep., Noctuidae). Unpublished Ph.D. Thesis, Cairo, Univ. Egypt.
- 343 Ito EE, Nmor, JC, Ake JEG, and Uteboe K (2015). Larvicidal Activities of *Pistiastratiotes* (water lettuce)
- 344 against 3rd and 4th instar larvae of Aedes aegypti. Advances in Research, 3(6): 589-595.
- 345 Ito EE and Ighere EJ (2017):Bio-insecticidal Potency of Five Plant Extracts against Cowpea Weevil,
- 346 Callosobruchusmaculatus (F.), on Stored Cowpea, Vignaunguiculata (L). Jordan Journal of Biological
- 347 *Sciences*, 10(4): 34-39.
- Jang, YS, Baek, BR, Yang, YC, Kim, MK and Lee, HS (2002): Larvicidal activity of *leguminous* seeds
 against *Ae. aegypti* and *C. pipiens*pallens. *J. Am. Mosq. Control Assoc.* 18(3): 210-213.
- 350 Jeyabalan, D, Arul, N and Thangamathi, P (2003): Studies on effects of Pelargonium citrosa leaf
- 351 extracts on malarial vector, A. stephensi Liston. Biores. Tech. 89(2): 185-189.
- 352 Khalaf, AA, Hussein, KT and Shoukry, KK (2009): Biocidal Activity of two botanical volatile oils
- against the larvae of *Synthesiomyianudiseta* (Wulp) (Diptera: Muscidae). *Egypt Acad. J. Bio. Sci.* 2 (1):
- **354 89-101**.
- 355 Khan, M. Hossain, MA and Islam, MS (2007): Effects of neem leaf dust and a commercial formulation
- 356 of a neem compound on the longevity, fecundity and ovarian development of the melon fly,
- Bactoceracucurbitae(Coquillett) and the oriental fruit fly, Bactroceradorsalis (Hendel) (Diptera:
 Tephritidae). Pak. J. Biol. Sci. 10: 3656-61.
- 359 Khan, HAA, Shad, SA and Akram, W (2013): Resistance to new chemical insecticides in the housefly,
- 360 *Musca domestica* L., from dairies in Punjub, Pakistan. *Parasito*. *Res*.12: 2049-54.
- 361 Khan, MF, Ahamad, T and Rawat, P (2017): Biomedicinal and chemical Profile of *Cupressus* 362 *sempervirens*: A Mini Review. Insights Biomed. 2: 3-16.
- 363 Kumar, PS, Mishra, A and Malik, SS (2012): Housefly (*Musca domestica* L.) control potential of
- 364 *Cymbopogon citratus* Staff (Poales: Poaceae) essential oil and monoterpenes. *Parasitol Res.* 112: 69-76.
- Lentner, C, Lentner, C and Wink, A (1982): Students t-distribution tables. In Geigy scientific Tables
 Vol. 2.International Medical and Pharmaceutical information, Ciba–Geigy Limited, Basal,
 Switzerland.
- 368 Maurya, P, Sharma, P, Mohan, L, Batabyal, L and Srivastava, CN (2009): Evaluation of the toxicity of
- 369 different phytoextracts of Ocimumbasilicum against Anopheles stephensi and Culex quinquefasciatus.
- 370 Journal of Asia-Pacific Entomology.12: 113-115.
- Morey, RA and Khandagle, AJ (2012): Bioeffeicacy of essential oils of medicinal plants against
 housefly, *Musca donestica* L. *Parasito*. *Res.* 111: 1799-05.
- 373 Murugesan, S, Senthilkumar, N, Babu, DS and Rajasugunasekar, D (2016): Chemical constituents
- 374 and toxicity assessment of the leaf oil of Lantana camara Linn from Tamilnadu regions. Asian J. Plant
- 375 Sci. Res. 6(3): 32-42.
- 376 Muse, WA, Lajide, L and Adediri, CO (2003): Effect of some Nigerian plants on survival, oviposition,
- 377 and emergence of adult blowfly, Chrysomya chloropyga (Wied.) (Diptera: Calliphoridae). J. Asia-Pacific
- 378 Entomol. 6 (1): 69-75.

- 379 Nathan, SS, Savitha, G, George, DK, Narmadha, A, Suganya, L and Chung, PG (2006): Efficacy of
- 380 Melia azedarach L. extract on the malarial vector A. stephensi Liston (Diptera: Culicidae). Biores. Tech.
- 381 79: 1316-23.
- 382 Radwan, MEH (2000): Effect of crude methanolic extracts of leaves and fruits of chinaberry tree Melia
- 383 azedarach L. on the adult female housefly Musca domestica vicina. Unpublished M.Sc. Thesis, Fac. Sci.
- 384 Alex. Univ., Egypt.
- Sharma, P, Mohan, L and Srivastava, CN (2006): Growth inhibitory nature of *Artemisia annua* extract
 against *Culex autnauetesctetus*(Say). *J. Asia-Pacific Entomol.* 9(4): 389-395.
- 387 Singh, A, Singh, DK, Mishra, TN and Agarwal, RA (1996): Molluscicides of plant origin. *Biol.*388 *Agri.Horti.* 13: 205-252.
- 389 Sukumar, K, Perich, MJ and Boobar, LR (1991): Botanical derivatives in mosquito control: A review.
- 390 Am. Mosq. Contr. Assoc. 7(2): 210-237.
- 391 Toppozada, A, Abdallah, S and El-Defrawi, ME (1966): Chemo-sterilization of larvae and adults of
- 392 the Egyptian cotton leafworm, *Prodenialittura* by apholate, metepa and hempa. *J Econ. Entomol.59*:
- 393 1125-28.
- 394 Tripathi, AK, Prajapati, V, Khanuja, SPS and Kumar, S (2003): Effect of d-limonene on three
- 395 stored-product beetles. J. Econ. Entomol. 96(3): 990-995.