

2 **The biological activity of *Cupressus sempervirens* extracts**
3 **against *Musca domestica*.**

4 **Abstract:**

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* were 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 the solvent, plant parts used in extraction
11 and the dose of extract. Tested extracts significantly reduced the fecundity and increased
12 the sterility. Based on LC₅₀, the toxicity may be arranged as follows: leaves > stems.
13 Petroleum ether extract from leaves was more effective in inducing the fecundity,
14 antifeedant, repellent actions and egg-hatchability than those from stems. These results
15 may provide an opportunity to develop alternatives to costly organic pesticides using some
16 available cheap plants, which are usually safe to the environment and to other living
17 organisms.

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

19 **Introduction**

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

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

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

46 **Materials and Methods**

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

48 The culture of the housefly was maintained for several generations under controlled
49 conditions of $27\pm 2^{\circ}\text{C}$ and 70-75 RH and 12-12 light/dark. The emerged flies were fed on dry
50 diet (milk powder) and sucrose solution. Eggs were collected from paper strips or from
51 cotton pads of feeding. Larvae were reared on an artificial diet (wheat bran, milk, powder
52 yeast; 200:100:5gm) per 200ml of distilled water according a method described by (Busvine,
53 1962).

54 2. Extraction of plant materials.

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

64 3. Experimental bioassay.

65 In order to study the toxicity of the concerned plant extracts, the tested material of the
66 70% ethanol extracts was dissolved in 0.1ml of ethanol, while the tested material of acetone
67 and petroleum ether extracts was dissolved in 2drop of Tween₈₀ as emulsifier to facilitate
68 the dissolving oils of tested material in 250ml water. Larval artificial diet was mixed with
69 different concentrations of each concerned extract to detect mortality percent. Then, twenty
70 five of third 3rd instar larvae were put immediately into plastic cups contained media mixed
71 with different concentrations of extracts. Three replicates were usually used for each tested
72 concentration. All plastic cups were incubated under controlled conditions of temperature
73 $27\pm 2^{\circ}\text{C}$, 70-75% RH and 12-12 light-dark regime. Control larvae received 0.1 ml of ethanol
74 or 2 drop of Tween₈₀ in 250ml water. Mortality was recorded daily and dead larvae and
75 pupae were removed daily.

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

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

96 Standard cages (20×20×20cm) were used to test the repellent activity of plant extracts.
 97 Cotton pieces soaked in 10% sucrose solution from each concentration added to the
 98 wooden cages containing 40 starved individuals (5-7 d-old) for three hours. Control tests
 99 were carried out alongside with the treatments using cotton pieces soaked in 10% sucrose
 100 solution with 2 drops of 70% ethanol or Tween₈₀. Each test was repeated three times to get a
 101 mean value of repellent. Repellency % was calculated according to Abbott (1925):
 102 Repellency % = [%A -%B /100 -%B] ×100. Where: A = percent of unfed females in treatment,
 103 B = percent of unfed females in control.

104 4. Statistical analysis.

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

109 **Results**

110 **1- Biological activity of *C. sempervirens* against *M. domestica*.**

111 **1-1- Ethanolic extract.**

112 Data given in (Table 1) shows the effect of ethanol extract of *C. sempervirens* (leaves)
 113 against different biological aspects of *M. domestica*. The larval mortality percent and the
 114 mean larval duration were concentration-dependent. There was a negative correlation
 115 between the P% and the concentration used. The mortality percent of pupae were recorded
 116 12.5, 11.8, 8.4 and 8.7% at the concentrations of 2000, 1700, 1400 and 1100 ppm; respectively.

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

Conc. ppm	Larval mort. %	Larval duration	Pupation %	Pupal mort. %	Pupal duration	Larval and Pupal mort. %	Emergence % (a)	Developmental Period (b)	Growth Index (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).

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

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

Conc. ppm	Larval mort. %	Larval duration	Pupation %	Pupal mort. %	Pupal duration	Emergence % (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 footnote of table (1).

126

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

137 **1-2- Acetone extract.**

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

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

Conc. ppm	Larval mort. %	Larval duration	Pupation %	Pupal mort. %	Pupal duration	Emergence % (a)	Developmental Period (b)	Growth Index (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 ^c	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 ^c	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 footnote of table (1).

146

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

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

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

Conc. ppm	Larval mort. %	Larval duration	Pupation %	Pupal mort. %	Pupal duration	Emergence % (a)	Developmental Period (b)	Growth Index (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 footnote of table (1).

160

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

164 **1-3- Petroleum ether extract.**

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

Conc. ppm	Larval mort. %	Larval duration	Pupation %	Pupal mort. %	Pupal duration	Emergence % (a)	Developmental Period (b)	Growth Index (a/b)
1000	100.0	--	--	--	--	0.0	--	--
800	84.0	4.2±0.22 ^a	16.0	81.1	7.3±0.41 ^c	18.9	11.5±0.63 ^c	1.6
600	66.7	3.9±0.18 ^a	33.3	84.3	6.9±0.19 ^c	15.7	10.8±0.37 ^c	1.5
400	53.3	3.3±0.36 ^a	46.7	73.2	7.1±0.91 ^c	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 ^c	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 footnote of table (1).

167

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

177 On the other hand, the biological activity of stems petroleum ether extract was
 178 revealed in (Table 6).

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

Conc. ppm	Larval mort. %	Larval duration	Pupation %	Pupal mort. %	Pupal duration	Emergence % (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

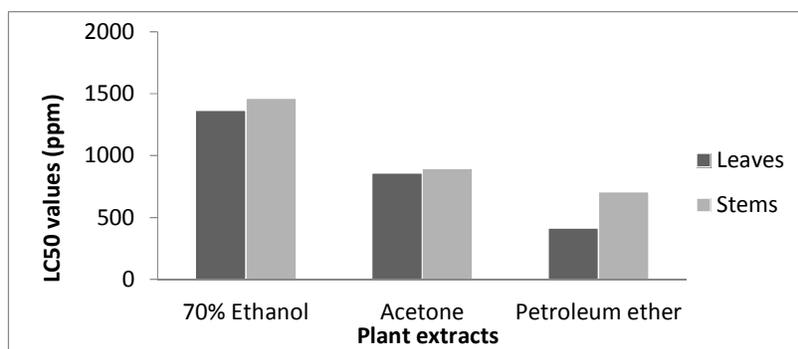
See footnote of table (1).

181

182 Data obtained showed that, the larval mortality% was concentration-dependent. The
 183 larval duration was insignificantly prolonged at all the concentrations used. The
 184 pupation% of the treated larvae was decreased as the concentration increased. The pupal
 185 mortality% and adult emergence% were found to be affected by extract used. The pupal
 186 duration was insignificantly ($P>0.05$) affected by all concentration used as compared with

187 the untreated group. The growth index was greatly affected by petroleum ether stems
 188 extract, where it recorded 6.8, 6.7 and 10.3 at the concentrations 800, 700 and 600ppm;
 189 respectively, compared to 14.9 for the control group.

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



194

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

197 2- Reproductive potential.

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

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

Conc. (ppm)	No. of tested females	Fecundity		Hatched eggs		Non-hatched eggs		Sterility Index (SI)
		Total	Mean±SD	Total	%	Total	%	
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 footnote of table (1).

207

208 On the other hand, the fecundity of females resulted from the larvae treated with
 209 stems petroleum ether extract of *C. sempervirens* was decreased by increasing the
 210 concentration and the statistical analysis revealed that, there was a significant ($P < 0.001$)
 211 decrease in the mean number of eggs laid by females resulted from treated larvae at the all

212 concentrations used (Table 8). A marked decrease in the hatchability percent and
 213 remarkable increase in the percentage of sterility index were also recorded.

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

Conc. (ppm)	No. of tested	Fecundity		Hatched eggs		Non-hatched eggs		Sterility Index (SI)
		Total	Mean±SD	Total	%	Total	%	
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).

216

217 3- Antifeedant and repellency activities.

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

222 Table (9): Effect of LC₅₀ concentration from ethanol, acetone and petroleum ether extracts of *C.*
 223 *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

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

227

228 Table (10): Effect of LC₅₀ concentration from ethanol, acetone and petroleum ether extracts of *C.*
 229 *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

230 The petroleum ether extract was more effective than acetone and ethanolic extracts
 231 during the entire testing period of 2h post treatment. The repellency was 71.4% for the
 232 petroleum ether extract, while it recorded 42.9 and 21.4% for acetone and ethanolic extracts;
 233 respectively compared with the control group.

234 Discussion and conclusion

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

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

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

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

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

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

296 It could be concluded that toxicity of tested extracts varied according to a plant part,
297 the solvent used in extraction and concentration of the extract. Based on LC₅₀, the toxicity
298 values were arranged as follows: leaves > stems. Petroleum ether extract from leaves was
299 more effective in inducing the fecundity, antifeedant, repellent actions and egg-hatchability
300 than those from stems. So, the plant extracts used may be considered as new promising
301 controlling agents for the housefly, *M. domestica*.

302 Ethical Approval:

303 As per international standard or university standard ethical approval has been collected and
304 preserved by the authors.

305

306 References

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