J. Egypt. Soc. Parasitol. (JESP), 45(2), 2015: 241 -248

REPELLENT EFFECT OF *OCIMUM BASILICUM* AND *GLYCYRRHIZA GLABRA* EXTRACTS AGAINST THE MOSQUITO VECTOR, *CULEX PIPIENS* (DIPTERA: CULICIDAE)

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Abstract

Essential or volatile oils of plants have been variously reported to have many medicinal applications. Methanol, acetone and petroleum ether extracts of *Ocimum basilicum* and *Glycyrrhiza glabra* were screened for their repellency effect against *Culex pipiens* mosquito. The repellent action of the present plants extracts were varied depending on the solvent used and dose of extract. Methanol extract of *O. basilicum* exhibited the lowest repellent activity as it recorded 77.4% at 6.7mg/cm^2. The petroleum ether and acetone extract of *O. basilicum* showed repellency of 98.1 & 84.6% respectively, at dose of 6.7mg/cm^2, while methanolic extract of *G. glabra* recorded 73.8 & 50.3% at dose of 6.7 &1.7mg/cm^2 respectively, the petroleum ether and acetone extract of *G. glabra* showed repellency of 76.3 & 81.6%, respectively at dose of 6.7mg/cm^2, compared with the commercial formulation, N.N. diethyl toulamide (DEET) which exhibited 100% repellent action at dose of 1.8mg/cm^2, respectively. The results may contribute to design an alternative way to control mosquitoes currently based on applications of synthetic insecticides. These extracts could be developed commercially as an effective personal protection measure against mosquito bites and thus to control diseases caused by mosquito–borne pathogens.

Key words: Repellent, Methanol extract, Acetone extract, Petroleum ether extract, *Ocimum basilicum, Glycyrrhiza glabra, Culex pipiens*.

Introduction

Mosquitoes are considered one of the most important serious zoonotic vectors of worldwide distribution even in Egypt (Mikhail *et al*, 2009). Control of mosquitoes represents of the most measures. The extensive use of chemical insecticides and/or repellents resulted in mosquito- resistant chemical measures; this is apart from environmental pollution and, mammalian toxicity (El-Bahnasawy *et al*, 2014). These factors have created the need for environmental safe, degradable and target specific agents as medicinal plants and herbs which are locally available (Abdel Hady *et al*, 2008). Plant extracts have gained importance in insect control, as being environmentally safe, less hazardous to non-target biota, simple inexpensive and applied effectively countries (Soliman and El-Serif, 1995; El-Bokl and Moawed, 1996; Shoukry and Hussein, 1998; Massoud and Labib, 2000; Mohammed and Hafez, 2000; Mohammed *et al*, 2003). The crude plant extracts were screened as natural and biodegradable forms to control pests and vectors of infectious diseases (Omena *et al*, 2007). Plant extracts and herbs proved effective in control of mosquitoes (Ezeonu *et al*, 2001), mosquito repellent, or food deterrents, or growth inhibitors, and toxins (Carlini and Grossi-de-Sá, 2002). Also, some Egyptian plant extracts were successfully used as antioxidant, anticancer and antiparasitic (Abdel Hady *et al*, 2011).

The present work aimed to investigate the repellent efficiency of methanol, acetone and petroleum ether extracts of *Ocimum basilicum* and *Glycyrrhiza glabra* against the adult mosquito vector, *Culex pipiens* (Diptera: Culicidae).

Materials and Methods

The Egyptian strain of *Culex pipiens* were reared and maintained for several generations in the insectary of Medical Entomology using the standard procedures (Kasap and Demirhan, 1992).

Plant extraction: Pure Egyptian cultivated *O. basilicum* and *G. glabra* were used. The extract solvents were 95.0% methanol, acetone and petroleum ether. One hundred grams of powder for each plant was separately extracted with 300ml of aqueous 95.0% methanol, acetone and petroleum ether at room temperature. After 24 h., supernatants were decanted, filtrated through Whatman filter paper No.5 and dried in a rotary evaporator at 40ºC for (2-3) hours to methanol and (40-60) minutes to other solvents. The dry extracts were freeze (-4ºC) till needed (Abdel Halim and Morsy, 2005).

Cages (30×30×30cm) were used to test repellent activity. Different quantities from each extract were dissolved in 2 ml (95% methanol or water with a drop of Tween 80) in 4×4 cm cups to obtain different concentrations. Concentration was directly applied onto 5×6 cm of ventral abdomen of pigeon after removed feathers. After 10 min., pigeons were put for 3 h in cages of starved females. Control tests were carried out with water. Each test was repeated 3 times to get a mean value of repellent activity (El-Sheikh *et al,* 2012). Post treatments number of fed and unfed females was counted and calculated (Abbott, 1925). Repellency% = (A%-B%/ 100-B%)×100. A = treatment unfed females % & B = control unfed females%.

Results

With *Ocimum basilicum*: A- Methanol extracts gave variable degrees (Tab. 1). At the dose of 1.8mg/cm^2, potent repellency was 100% by DEET through the 4hr post treatment. The relative repellency increased with the dose increase, repellency of 77.4% was obtained by 6.7 mg/cm^2 extract and decreased to 68.8% with 33.3 mg/cm^2 after 4hr post treatment, the lowest (55.6%) was obtained with a dose 2.5mg/cm^2 and decreased to 41.7% at a dose 1.7mg/cm^2. B-Acetone extract repellent activity against starved *Cx. pipiens* females varied (Tab. 2). At doses of 6.7 & 3.3 mg/cm^2, the extract induced repellency degrees of 84.6 & 77.5% respectively, while the lower doses induced lower repellency 70.1 & 57.7%, respectively at dose of 2.5 & 1.7mg/cm^2 compared to 100% repellency for DEET at a dose of 1.8mg/cm^2. C-Petroleum ether extract showed a more repellent activity against *Cx. pipiens* females than methanol and acetone extracts. The repellent action was 98.1 & 88.6% at a dose of 6.7,3.3 mg/cm^2, and gave 87.5 & 72.4% at a dose of 2.5,1.7 mg/cm^2, respectively compared to 100% repellency for DEET at a dose1.8mg/cm^2 (Tab. 3).

With *Glycyrrhiza glabra*: A- Methanol extract repellent activity against starved *Cx. pipiens* females varied according to doses used (Tab. 4). At doses of 6.7, 3.3, 2.5 & 1.7 mg/cm^2, extract induced repellency of 73.8, 67.6, 56.7 & 50.3% within 4h post treatment, respectively, compared to 100.0% repellency for DEET at a dose1.8mg/cm^2. B-Acetone extract repellent activity also varied (Tab. 5). At a dose 6.7, 3.3 mg/cm^2 induced repellency to 76.3, & 70.3% respectively, while; at a dose of 2.5, 1.7 mg/cm^2 extract induced lower degree of 60.1& 48.3% respectively compared to 100.0% repellency for DEET at a dose1.8mg/cm^2. C- Petroleum ether extract (Tab. 6) proved to possess highest efficacy. At a dose of 6.7 mg/cm^2 produced the highest protection 81.6 % during the entire testing period of 4h post treatment. Also, gave repellency of 78.1, 66.1 & 55.6% at 3.3, 2.5 &1.7mg/cm^2, respectively, compared to 100.0% repellency for DEET at a dose 1.8 mg/cm^2.

Table 1: Repellency effect of methanol extracts of *Ocimum basilicum* against *Culex pipiens* females.

Plant	Dose (mg/cm^2)	Tested females	Fed females	%	Unfed females	%	Repellency%
Ocimum basilicum	6.7	52	11	21.2	41	78.8	77.4
	33.3	58	17	29.3	41	70.7	68.8
	2.5	60	25	41.7	35	58.3	55.6
	1.7	47	26	55.3	21	44.7	41.7
DEET	1.8	45	0.0	0.0	45	100.0	100.0
Control	----	49	46	93.3	3	6.1	----

Table 2: Repellency effect of acetone extract of *Ocimum basilicum* on females of *Culex pipiens*.

Plant	Dose (mg/cm^2)	Tested females	Fed females	%	Unfed females	%	Repellency%
Ocimum basilicum	6.7	61	9	14.8	52	85.2	84.6
	3.3	48	9	18.8	39	81.2	77.5
	2.5	42	11	26.2	31	73.8	70.1
	1.7	49	20	40.8	29	59.2	57.7
DEET	1.8	45	0.0	0.0	45	100.0	100.0
Control	----	55	53	96.4	22	3.6	---

Table 3: Repellency effect of petroleum ether extract of *Ocimum basilicum* on females of *Culex pipiens*.

Plant	Dose (mg/cm^2)	Tested females	Fed females	%	Unfed females	%	Repellency%
Ocimum basilicum	6.7	53	1	1.9	52	94.3	98.1
	3.3	46	5	10.9	41	89.1	88.6
	2.5	59	9	15.3	50	84.7	87.5
	1.7	53	14	26.4	39	73.6	72.4
DEET	1.8	45	0.0	0.0	45	100.0	100.0
Control	----	47	45	95.7	2	4.3	----

Table 4: Repellency effect of methanol extract of *Glycyrrhiza glabra* on females of *Culex pipiens*.

Plant	Dose (mg/cm^2)	Tested females	Fed females	%	Unfed females	%	Repellency%
Glycyrrhiza glabra	6.7	40	10	25.0	30	75.0	73.8
	3.3	42	13	30.9	29	69.1	67.6
	2.5	46	19	41.3	27	58.7	56.7
	1.7	40	19	47.5	21	52.5	50.3
DEET	1.8	45	0.0	0.0	45	100.0	100.0
Control	----	45	43	95.5	2	4.5	----

Table 5: Repellency effect of acetone extract of *Glycyrrhiza glabra* on females of *Culex pipiens.*

Plant	Dose (mg/cm^2)	Tested females	Fed females	%	Unfed females	%	Repellency%
Glycyrrhiza glabra	6.7	51	11	21.6	40	78.4	76.3
	3.3	48	13	27.1	35	72.9	70.3
	2.5	50	18	36.0	32	64.0	60.1
	1.7	53	25	47.2	28	52.8	48.3
DEET	1.8	45	0.0	0.0	45	100.0	100.0
Control	----	46	42	91.3	4	8.7	-----

Table 6: Repellency effect of petroleum ether extract of *Glycyrrhiza glabra* on females of *Culex pipiens*.

Plant	Dose (mg/cm^2)	Tested females	Fed females	%	Unfed females	%	Repellency%
Glycyrrhiza glabra	6.7	46	8	17.4	38	82.6	81.6
	3.3	53	11	20.7	42	79.3	78.1
	2.5	50	16	32.0	34	68.0	66.1
	1.7	55	23	41.9	32	58.1	55.6
DEET	1.8	45	0.0	0.0	45	100.0	100.0
Control	----	55	52	94.5	3	5.5	-----

Discussion

Generally, *Ocimum basilicum* (Arabic name; *Al-Rehaan*) is known for more than 5,000 years and has a number of different essential oils that come together in different proportions for various breeds, the strong clove scent of sweet basil is derived from eugenol, the same chemical as actual cloves (Nascimento *et al,* 2014). As to the *Glycyrrhiza glabra* (Arabic name *Al Aka' soos*) the isoflavene glabrene and the isoflavane glabridin found in the roots of liquorice, are phytoestrogens (Somjen *et al*, 2004).

A variation in the repellent activity of the plant extracts tested was observed and this may indicate the complexity of the chemi-

cal composition of their constituents (Bisseleua *et al*, 2008).

In the present study, all the concentrations of plant extracts exhibited repellency effect against the starved adult female of *Cx. pipiens*. The repellent activity depended on the solvent used and the dose of the extract tested The most effective was petroleum ether extract of *O. basilicum* which induced 98.1%, while petroleum ether extract of *G. glabra* recorded 81.6% at the dose 6.7 mg/cm^2. These results indicated that the petroleum ether extraction was more effective in exhibiting the repellent action against *Cx. pipiens* as compared with either the methanol, or acetone extracts on one hand and the commercial N. N. diethyl toulamide (DEET) which exhibited 100% repellency action at 1.8 mg/cm^2 on the other hand.

Shaalan *et al*. (2005) stated that the increasing insecticide resistance requires strategies to prolong the use of highly effective vector control compounds. The use of combinations of insecticides with other insecticides and phytochemicals is one such strategy that is suitable for mosquito control. In general several authors worldwide tested different plant extracts against various insect pests. Mansour *et al.* (1998) tested extracts from *Nigella sativa* seeds against *Cx. pipiens*. Al Dakhil and, Morsy (1999) in Saudi Arabia investigated the larviciddal action of three ethanol extracts of peel oils of lemon, grapefruit and navel orange against the early 4th instar larvae of *Cx. pipiens* and emerging pupae. The LC50 were 18.5, 20.3 and 26.5 and the slope functions were 2.9, 2.9 and 3.9 respectively. The action of the lemon extract extended to the pupae which resulted from larvae exposed to sublethal dose. Some of the pupae were unable to escape from the larval exuviae. Govere *et al.* (2000) in South Africa used extracts of fever tea (*Lippia javanica*), rose geranium (*Pelargonium reniforme*) and lemon grass (*Cymbopogon excavatus*) against *Anopheles arabiensis*. Kim *et al.* (2002) in South Korea used ethanol extract of fruits from *Foeniculum vulgarea* against hungry *Aedes aegypti* females; Jeyabalan *et al.* (2003) in India reported that the methanol extracts of *Pelargonium citrosa* leaf exhibited repellency activity (36, 51, 78, 100%) against the adult mosquito of *A. stephensi* at the concentrations (0.5, 1.0, 2.0, and 4.0%); Yang *et al*. (2004) in China found the repellent activity of methanol extracts of *Cinnamomum cassia* bark, *Nardostachys chinensis* rhizome, *Paeonia suffruticosa* root bark and *Cinnamomum camphora* at a dose of 0.1 mg/cm^2 was (91%), (81%), (80%) and (94%) comparable to Deet (82%) against starved *Ae. aegypti*. Amer and Mehlhorn (2006) in Libya tested 41 plant extracts and 11 oil mixtures were evaluated against the yellow fever mosquito, *Aedes aegypti* (Linnaeus), the malaria vector, *An. stephensi* (Liston), and the filariasis and encephalitis vector, *Cx. quinquefasciatus* (Say) (Diptera: Culicidae) using the skin of human volunteers to find out the protection time and repellency. The five most effective oils were those of Litsea (*Litsea cubeba*), Cajeput (*Melaleuca leucadendron*), Niaouli (*Melaleuca quinquenervia*), Violet (*Viola odorata*), and Catnip (*Nepeta cataria*), which induced a protection time of 8 h at the maximum and a 100% repellency against all three species. This effect needs, however, a peculiar formulation to fix them on the human skin. Webb and Russell (2007) in Australia tested the repellency of *Nepeta cataria* (catmint or catnip) was tested against *Aedes aegypti, Ae. vigilax, Cx. annulirostris,* and *Cx. quinquefasciatus*, and compared with a blend of natural plant extracts and N,N-diethyl-3-methylbenzamide (DEET) on the human skin. They found that the catmint and natural plant extract blend did not provide the same level of protection from biting mosquitoes as DEET. There were significant differences in the level of protection provided by catmint to the four spe-

cies of mosquito, with mean protection times ranging from 0 min for *Ae. aegypti* up to 240 +/- 60 min for Cx. quinquefasciatus. Choochote *et al.* (2007) in Thailand used repellent activity of selected essential oils from ten plant species against *Aedes aegypti*. El-Sheikh *et al.* (2012) in Egypt used methanolic extract (leaves, seeds) of *Tribulus terrestris* L. against the malarial vector, *A. arabiensis* and they found that seeds extract was more effective in exhibiting the repellent action (100%) against the mosquito tested as compared with the leaves extract (79.5%) at the dose 1.0 and 2.0mg/cm^2 compared with (100%) of commercial formulation, N. N. diethyl toulamide (DEET). Park *et al*. (2012) in Republic of Korea used *Thymus magnus* against the Asian tiger mosquito, *Ae. albopictus,* they found that the volatile compounds as determined by gas chromatography mass spectrometry were gamma-terpinene (33.0%), thymol (29.9%), beta-bisabolene (8.9%), p-cymene (8.3%), alpha-terpinene (5.0%), myrcene (4.7%), beta-caryophyllene (4.0%), alpha-thujene (2.7%), camphene (1.3%), carvacrol (1.2%) and alpha-pinene (1.1%). The thymol exhibited complete (100%) repellent activity against female *Ae. albopictus*, an effect that was confirmed through evaluating the electrophysiological response on the antenna of *Ae. albopictus*. Hassan *et al*. (2014) in Egypt reported that the petroleum ether extract of *Lagenaria siceraria* leaves showed the same repellency 100% of commercial formulation, N. N. diethyl toulamide (DEET) at the higher dose (3.33 mg/cm2), while petroleum ether extract from stems exhibiting the repellent action (89.6%) at the same dose, respectively. However, the ethanolic extracts of *Lagenaria siceraria* leaves and stems exhibited the lowest repellent activity as it recorded (81.3% and 69.1%) at (6.67 mg/cm2), respectively. Ali et al. (2014) in India studied the repellent activity of *Rhizophoraceae mucronata* stilt root and bark extracts (A3) found maximum percentage of protection (97.5%) with 9.1 h protection time at 4 mg concentration of the stilt root extract. Moreover, ethanolic fraction of the stilt root (E4) extract showed maximum percentage of protection (100%) with 10 h protection time at 4 mg concentration. GC-MS analysis revealed that *R. mucronata* possesses variety of biopesticidal compounds. Gkinis *et al*. (2014) in Greece found that the results of the insect bioassays showed that the *Nepeta parnassica* essential oil and the dichloromethane-methanol extract of *N. parnassica* were very active against *Aedes cretinus* for up to 3 h and against *Cx. pipiens* for up to 2 h post application. The isolated 4aα,7α,7aβ-nepetalactone showed very high mosquito repellency for periods of at least 2 h against both species.

As to the safety of *O. basilicum* for human usage, Ahonkhai *et al.* (2009) in Benin teaching Hospital Nigeria reported that the volatile oils of *O. basilicum* and *O. gratissimum* independently inhibited growth of *Klebisiella pneumonia* at a concentration of 0.51% in the agar; *Streptococcus viridians* and *Staphylococcus albus* at 1.10% and *Pseudomonas aeruginosa* at 10.0%. *Proteus vulgaris* was inhibited at 0.53% by the volatile oil of O. gratissimum and 0.67% by *O. basilicum.* They concluded that as components of mouth washes, the volatile oils completely inhibited the growth of organisms at a concentration of 0.5%. Regarding *Glycyrrhiza glabra* Basar *et al*. (2015) in Malaysia stated *G. glabra* L. (Fabaceae), commonly known as 'liquorice', is a well-known medicinal plant. Roots of this plant have long been used as a sweetening and flavouring agent in food and pharmaceutical products, and also as a traditional remedy for cough, upper and lower respiratory ailments, kidney stones, hepatitis C, skin disorder, cardiovascular diseases, diabetes, gastrointestinal ulcers and stomach ache. Previous pharmacological and clinical studies have revealed its

antitussive, anti-inflammatory, antiviral, antimicrobial, antioxidant, immunomodulatory, hepatoprotective and cardioprotective properties. However, they found that the cytotoxicity of the methanol extracts of nine samples of the roots of *G. glabra*, collected from various geographical origins, was assessed against immortal human keratinocyte (HaCaT), lung adenocarcinoma (A549) and liver carcinoma (HepG2) cell lines using the *in-vitro* 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide cell toxicity/viability assay. Considerable variations in levels of cytotoxicity were observed among various samples of *G. glabra*.

On the other hand, the prevalence and distribution of huge species mosquitoes allover Egypt was reported (El-Bashier *et al*, 2006; Shoukry and Morsy, 2011; Morsy, 2012; Abdel-Hamid, 2012).

Generally speaking, in Egypt *Cx. pipiens* is the main vector of filariasis which has natural and artificial breeding sites in the endemic and non-endemic villages (Harb *et al,* 1993). Apart from filariasis, Culicini, mainly *Cx. pipiens* transmit Rift Valley fever (El Gebaly, 1978), Sindbis virus (Wilson, 1991) and *Cx. pipiens* complex was incriminated as HCV vector (Hassan *et al*, 2002, 2003). Soliman *et al,* (2010) demonstrated that West Nile hemorrhagic fever virus was actively circulating during their study period in different Egyptian areas and caused febrile illness in a considerable proportion of the individuals.

Recommendations

Since the ancient times, plant products were used in various aspects. On the other hand, with the rapid progress of communications, many arthropod-borne infectious diseases are now widely distributed worldwide. The outcome results recommended the use of *Ocimum basilicum* and *Glycyrrhiza glabra* as an effective personal protection measure against mosquito bites and to avoid the zoonotic diseases they transmit.

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