Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Effects of *Peganum harmala* L. Seed Extract on *Culex pipiens* (Diptera: Culicidae)

Efeitos do extrato de semente de *Peganum harmala* L. em *Culex pipiens* (Diptera: Culicidae)

F. A. Al-Mekhlafi[a,b*], N. Abutaha[a], S. K. Alhaga[c,d] and M. Al-Wadaan[a]

[a]King Saud University, College of Science, Department of Zoology, Bioproducts Research Chair, Riyadh, Saudi Arabia
[b]Thamar University, College of Agriculture and Veterinary Medicine, Department of Agricultural Production, Thamar , Yemen
[c]King Khalid University, College of Science and Arts, Biology Department, Muhayl Asser, Saudi Arabia
[d]Ibb University, College of Science, Biology Department, Ibb, Yemen

Abstract

Mosquito-borne diseases result in the loss of life and economy, primarily in subtropical and tropical countries, and the emerging resistance to insecticides is increasing this threat. Botanical insecticides are promising substitutes for synthetic insecticides. This study evaluated the larvicidal and growth index of *Culex pipiens* of four solvent extracts of *Terminalia chebula*, *Aloe perryi,* and *Peganum harmala* against *Cx. pipiens*. None of the 12 extracts exhibited larvicidal potential against third instars except the ethyl acetate extract of *P. harmala*. After 24 h of exposure, the LC_{50} value was 314.88 ppm, and the LC_{90} value was 464.19 ppm. At 320 ppm, the hatchability was 25.83%, and it resulted in 100% mortality. In addition, the eggs treated with the EtOAc extract of *P. Harmala* exhibited a long larval period compared with the control. The larval period continued for 12 days, and the pupal period took three days in the treatment groups. The growth index data also exhibited a decrease (0.00–7.53) in the treated groups compare with 8.5 in the control. The transformation of eggs into adults decreased with increasing concentrations. This paper is the first report on the development and growth index of *Cx. pipiens* potential using *P. harmala* seeds.

Keywords: *Peganum harmala*, *Culex pipiens*, solvent extraction, larvicidal activity, growth index.

Resumo

As doenças transmitidas por mosquitos resultam na perda de vidas e economia, principalmente em países subtropicais e tropicais, e a resistência emergente aos inseticidas está aumentando essa ameaça. Os inseticidas botânicos são substitutos promissores dos inseticidas sintéticos. Este estudo avaliou o índice larvicida e de crescimento de Culex pipiens de quatro extratos solventes de Terminalia chebula, Aloe perryi e Peganum harmala contra Cx. pipiens. Nenhum dos 12 extratos exibiu potencial larvicida contra o terceiro ínstar, exceto o extrato de acetato de etila de P. harmala. Após 24 horas de exposição, o valor LC50 era 314,88 ppm e o valor LC90 era 464,19 ppm. A 320 ppm, a eclodibilidade foi de 25,83% e resultou em 100% de mortalidade. Além disso, os ovos tratados com o extrato de EtOAc de P. harmala exibiram um longo período larval em comparação com o controle. O período larval continuou por 12 dias, e o período pupal durou três dias nos grupos de tratamento. Os dados do índice de crescimento também exibiram uma diminuição (0,00-7,53) nos grupos tratados em comparação com 8,5 no controle. A transformação de ovos em adultos diminuiu com o aumento das concentrações. Este artigo é o primeiro relatório sobre o índice de desenvolvimento e crescimento de Cx. potencial de pipiens usando sementes de P. harmala.

Palavras-chave: Peganum harmala, Culex pipiens, extração por solvente, atividade larvicida, Índice de crescimento.

1. Introduction

Mosquitoes are vectors of human parasites that cause many diseases, including yellow fever, filariasis, malaria, dengue, and encephalitis, which are accountable for millions of deaths annually (Gubler, 2009). *Culex* is an important mosquito genus including well known vectors of parasites and pathogens. *Cx. pipiens* is a house mosquito, which causes many diseases, such as West Nile virus, filariasis, and other encephalitides (Thenmozhi et al., 2014). Furthermore, in many people, *Cx. pipiens* bites can cause acute systemic allergic reactions or induce local dermatitis (Peng et al., 2004). For many years, synthetic insecticides have been employed to limit the spread of the vectors and manage the spread of vector-borne diseases (Chareonviriyaphap et al., 2013). However, the widespread

*e-mail: falmekhlafi@ksu.edu.sa
Received: July 23, 2020 – Accepted: December 3, 2020

use of synthetic insecticides has caused the resistance of mosquitoes worldwide. Organophosphate resistance has been detected in *Cx. pipiens* in Brazil, Sri Lanka, Liberia, and Egypt (Curtis and Pasteur, 1981). In addition, *C. pipiens* has been found to be resistant to fenthion and temephos in Turkey (Akiner et al., 2009), todiflubenzuron in Europe, and resistance has also been developed in Saudi Arabia. The fast development of resistance to synthetic insecticides has provoked the need for new insecticides (Zaim and Guillet, 2002, Thomas et al., 2004). Plants are considered a promising natural substitute for controlling mosquitoes. They are affordable, readily available, biodegradable, ecofriendly, safe (Nathan and Kalaivani, 2005), and have different mechanisms of action, which overcome the resistance in mosquito populations (Okumu et al., 2007).

Peganum harmala L. (family Zygophyllaceae) is a plant that grows in Iran, Africa, India, the United States, Mexico, Turkey, China, and the Middle East (Mahmoudian et al., 2002), and it is commonly called "Harmel" in the Middle East. The seed, fruit, bark, and roots of *P. harmala* have been used to treat asthma, coughs, hypertension, diabetes, and rheumatism (Mina et al., 2015; Moloudizargari et al., 2013; Zhao et al., 2011). Several reports have shown angiogenic inhibitory potential (Moloudizargari et al., 2013), antiparasitic (Tanweer et al., 2014), antifungal, antibacterial (Nenaah, 2010), insecticidal (Abbasipour et al., 2010), anti-inflammatory (Davoodi et al., 2015), anti-cancer (Hashemi Sheikh Shabani et al., 2015, Moloudizargari et al., 2013), and antiviral (Moradi et al., 2017) activities.

Considering the interest in developing natural-based insecticides as an alternative to synthetic insecticidal agents, this research was conducted to assess the larvicidal activity of three herbal extracts against *Cx. pipiens*.

2. Material and Methods

2.1. Plant materials and extract preparation

The medicinal plants *Terminalia chebula*, *Aloe perryi,* and *Peganum harmala* were purchased from the Reef Al-Yaman herbal shop in Riyadh province, Saudi Arabia. Voucher specimens were placed in the herbarium at the Bio-product Research Chair, College of Science, King Saud University. A sample of 50 g of powder was extracted from each plant using hexane (Hex), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and methanol (MeOH) in a soxhlet apparatus until colorless. The obtained extracts were filtered and dried using a vacuum evaporator (Heidolph, Germany) and weighted to determine the yield. The evaporated extracts were preserved in a dark flask at −80 °C.

2.2. Mosquito rearing

All assays were conducted in accordance with the national guidelines of Saudi Arabia for the use of laboratory animals. Mosquitoes were obtained from a laboratory colony of *Culex pipiens* at the Zoology Department Insectary that was established in 2008. The mosquitoes were kept in a standard insectary environment of 28 °C ± 1 °C, a 14:10 h light:dark cycle and 75% humidity. Adult mosquitoes were fed *ad libitum* on 10% glucose solution. Female *Cx. pipiens* mosquitoes were blood fed on an albino mouse in the 50 × 50 × 50 cm rearing cage. The newly developed larvae were fed on ground TetraMin fish meal (Tetra GmbH, Germany) and kept in an insect cage after the pupae formation.

2.3. Mortality bioassay test

Different concentrations of plant extracts from 10 to 500 ppm were used to perform the test using sterile six-well plates. Three replicas of 20 Cx. *pipiens* larvae each were treated separately with different concentrations of each extract. The percent mortality was calculated at 24, 48, and 72 h post-treatment. Immobile larvae were counted as dead. Methanol (0.001), and 0.1 ppm permethrin were used as negative control and a positive control respectively.

2.4. Developmental experiments

A bioassay was conducted using egg rafts that were cut into pieces containing 40 eggs of 1–12 h. The eggs were kept in dishes filled with 50 ml tap water and different concentrations of *P. harmala* extract from 40 to 320 ppm. The emerging larvae were kept under standard environments of 28 °C ± 1 °C, a light:dark (14:10 h) cycle, and 75% humidity. The percentage hatchability, larval and pupal mortality, duration of larval and pupal development, and adult emergence were recorded. The growth index was calculated as previously described (WHO, 1996; Su and Mulla, 1999), and each concentration was replicated three times.

2.5. Statistical analysis

Results were expressed as the means ± standard deviation. The analysis was performed using one-way ANOVA, followed by Tukey's test (Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Yields and larvicidal activity

Twelve extracts from three plant species were investigated for larvicidal potential against *Cx. pipiens* third instars. The yields of Hex, $CHCl_3$, EtOAc, and MeOH extracts were calculated (Table 1). None of the 11 extracts exhibited larvicidal potential against the third instars of *Cx. pipiens* even at the highest concentration of 500 ppm, except the *P. harmala* seeds extracts. The EtOAc extract of *P. harmala* seeds showed a larvicidal potential with a higher toxicity at 72 h than that at 48 and 24 h. The LC_{50} value was 314.88 ppm, and the LC_{90} value was 464.19 ppm after 24 h against *Cx. pipiens*. Larvicidal activity was dose- and time-dependent for the extract, with higher toxicity recorded at 48 and 72 h. No mortality was noticed in the control groups (Table 2). The EtOAc extract of *P. harmala* seeds was further tested for development- and growth-related bioassays.

Table 1. Plants evaluated for their larvicidal activity against the *Culex pipiens* third instar.

Species	Family	Voucher number	Mass of Plant Part (g)	Mass of Extract (g) Yield (%)				Methanol (MeOH)
				Hexane	Chloroform (CHCH3)	Ethyl acetate (EtOAC)		
Aloe perryi	*Asphodelaceae*	(KSU-AP30)	leaves (110.50)	0.18(0.16%)	0.23 (0.21%)	0.98 (0.89%)		6.5 (5.88%)
Terminalia chebula	*Combretaceae*	(KSU-TC31)	fruits (65.29)	0.27 (0.41%)	0.09 (0.14%)	0.73 (1.12%)		19.9 (30.48%)
Peganum Harmala	Nitrariaceae	(KSU-PH32)	Seeds (96.5g)	2.22 (2.30%)	1.09 (1.13%)	1.34 (1.39%)		7.54 (7.81%)

Table 2. Larvicidal potential of *Peganum harmala* ethyl acetate extract against *Culex pipiens.*

Time	% Mortality						LC50 (μg/mL)	LC90 (μg/mL)
	Concentration (μg/ml)							
	co	100	200	300	400	500		
24	0.00±00e	3.33± 3.33ed	13.33±3.33d	36.67±3.33c	76.67±3.33b	100±00a	314.88	464.19
48	0.00±00d	6.67±3.33dc	33.33±6.67c	66.67±8.82b	96.67±3.33a	100±00a	279.93	432.60
72	0.00±00d	6.67±3.33dc	33.33±6.67c	66.67±8.82b	96.67±3.33a	100±00a	223.45	348.41

Data for rows with different letters are significantly different P <0.05.

3.2. Effect of Peganum harmala extract on hatching and larval development

The impact of sub-lethal concentrations of the EtOAc extract on larvae were examined. Details of the effects of the EtOAc extract of *P. harmala* from hatchability to adult emergence are presented in Table 3. treatment of the egg with the EtOAc extract of the *P. harmala* seeds affected the life cycle of *Cx. Pipiens*: the hatchability (86.49%), and the mosquito did not completed its life cycle at 240 ppm since the treatment resulted in 100% mortality. In contrast, 98.33% of the eggs hatched in the control. But, *Cx. pipiens* was capable of completing its life cycle at different rates based on the concentrations used. In addition, the eggs treated with the EtOAc extract of *P. harmala* exhibited a longer larval period compared with the control. Similarly, the duration of pupal development was prolonged in the treated groups. The larval period continued for 12 and 10 d, and the pupal period took 3 and 2 d in the treated and control groups, respectively. The total developmental period was 12–15 d in the treated individuals, and it lasted for 10 d in the control group. The growth index data also showed a decrease (0.00–7.53) in the treated groups compare with 8.5 in the control. The transformation of eggs into adults decreased with increasing concentrations. The transformation ratios were 76.67%, 43.33%, and 18.33% at 30, 60, and 120 ppm, respectively, while in the control it was 85%.

4. Discussion

There are several reports of the activity of herbal extracts on *Cx pipiens*, which primarily investigate the larvicidal action of the herbal extracts. However, there are few studies on the effect of herbal extracts on developmental stages. Thus, we found no reports in the literature involving *P. harmala* seed extract and their effects from hatchabilityto adult emergence.

Our findings show that the EtOAc extract of *P. harmala* could be a promising substitute to synthetic insecticides. Phyto-compounds extracted from different plant parts possess different bioactive ingredients with different activities. Plant crude extracts could have higher larvicidal activity than individual compounds due to synergisms that could be effective in controlling populations of resistant mosquitoes (Ghosh et al., 2012). Our results show that the crude EtOAc extract of *P. harmala* was effective against the larvae of important vector mosquitoes, *Cx. pipiens*. These results are similar to previously reported findings (El-Bokl, 2016) that showed that the LC_{50} values of acetone extracts of *Ruta chalepensis* against *Cx pipiens* were 1.08 ppm followed by the aerial part of *P. harmala* (18.75 ppm), *Artemisia herbalba* (73.99 ppm), and *Lavandula multifida* (78.55ppm). Previous studies have shown that the methanol leaf extract of *Delonix elata* acted against the larvae of *A. aegypti* and *A. stephensi* with LC_{50} values of 163.69 and 202.77 ppm and LC_{90} values of 93.59 and 111.83 ppm, respectively. However, the seed extracts had low LC_{50} values against the two mosquitoes of 225.07 and 273.03 ppm and LC_{90} values of 115.28 and 139.04 ppm, respectively.

Earlier researchers reported that the leaf extracts of *Vitex negundo* (LC_{50}: 212.57), *Vitex trifolia* (LC_{50}:212.5), *Vitex peduncularis* (LC_{50}:76.2), and *Vitex* altissima (LC_{50}:128.0) against *Culex quinquefasciatus* fourth instar (Kannathasan et al., 2007). Different solvent extracts of *Euphorbia tirucalli, Nerium indicum, Thuja orientelis, Murraya koenigii, Coriandrum sativum, Ferula asafoetida, and Trigonella foenum graceum, Citrullus colocynthis; Cannabis indica, Cannabis sativus, Momordica charantia; Trichosanthes anguina, Citrullus colocynthis, Cannabis indica, Cannabis sativus, Momordica charantia; Trichosanthes anguina, Momordica charantia, Trichosanthes anguina, Luffa acutangula, Benincasa cerifera, Citrullus vulgaris* showed larvicdal activity against *Culex quinquefasciatus, Anopheles stephensi, Culex quinquefasciatus, Aedes aegypti* (Yadav et al.,

Table 3. Effect of *Peganum harmala* ethyl acetate extract on the development and growth index of *Culex pipiens*.

Parts used	Concentration (ppm)	Hatchabi-lity (%)	Transformation of eggs into larvae (%)	Total larval period in days	Larval Mortality %	Transformation of larvae into pupae (%)	Total pupal Period in days	pupal Mortality %	Adult Emergence (%) (a)	transformation of eggs into adults (%)	Total developmental period in days (b)	Growth index (a/b)
seeds	40	100 ± 00a	96.56 ± 0.85a	10	0.92 ± 0.89d	84.80 ± 1.86b	2	13.71 ± 1.61b	93.78 ± 1.66a	74.17 ± 0.83b	12	6.18 ± 0.07b
	80	95.33 ± 0.83a	87.97 ± 2.20b	10	8.94 ± 3.05c	82.43 ± 2.65b	2	16.13 ± 2.38b	75.02 ± 1.88b	52.50 ± 1.44c	12	4.37 ± 0.12c
	160	68.33 ± 3.63b	42.24 ± 0.74c	11	56.2 ± 0.82b	67.28 ± 2.45c	3	31.55 ± 2.15b	60.66 ± 1.57b	16.17 ± 0.83d	14	1.19 ± 0.06d
	320	25.83 ± 2.20b	00d	00	100a	00d	0	100a	00c	00f	0	0f
	con	100 ± 0a	96.67 ± 0.83a	10	3.33 ± 0.82dc	98.27 ± 0.87a	2	1.73 ± 0.87d	98.25 ± 0.88a	92.5 ± 00a	10	9.25± 0a
	df	4	4		4	4		4	4	4		4
	F	273.38	1338.05		821.35	436.99		562.34	823.55	2148.30		3118.13

Data for columns with different letters are significantly different P <0.05.

2002; Sharma et al., 2005; Harve and Kamath, 2004; Rahuman and Venkatesan, 2008; Prabakar and Jebanesan, 2004). Sixteen harmine derivatives were inveistagated and their insecticidal activities were reported. It was found that 1-phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and methyl 1-phenyl-β-carboline-3-carboxylate were toxic with LC50 values of 20.8 μg/ml and 23.9 μg/ml against 4th instar of *Cx. pipiens* after 24 h treatment (Zeng et al., 2010). *Peganum harmala* extract was also toxic to other insects. Abbassi et al. (2003) and Jbilou et al. (2006) found that *P. harmala* was toxic to *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) and *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), respectively. It was also found to be toxic to several aphids and *Tribolium castaneum* (Salari et al., 2012).

In terms of the effects of the sub-lethal concentration of Harmala seed extract, we observed that there was an immediate response of the EtoAC extracts tested, and when assessing larval development, this extract interfered with the number and timing of the development period of the insect. The benzene extract of *Ervatamia coronaria* leaf was effective against eggs/egg rafts of *Culex quinquefasciatus* (200 ppm), *Anopheles stephensi* (300 ppm), and *Aedes aegypti* (250 ppm) (Govindarajan et al., 2011). The benzene extracts of *Citrullus vulgaris* exerted zero hatchability at 250 ppm and complete ovicidal activity at 300 ppm. The fraction II, III and IV exerted hatchability rate of 6.9%, 4.9% and 5.3% against *Anopheles stephensi* and *Aedes aegypti*, respectively (Mullai et al., 2008). The chloroform fruit extract of *Solenostemma argel* at 10 ppm decreased the eggs hatchability of *Cx. Pipiens* by 20% and adult emergence by 84%. Metamorphosis of larvae treated with chloroform extract was extended to 15 days, as compared to control (10 days). It took 12 days for chloroform extract (1 ppm) treated embryos to develop into adult mosquito as compared to control (10 days) (Al-Mekhlafi et al., 2018).

Although not fatal, Adanan et al. (2005) and Kamaraj et al. (2008) reported that these sub-lethal concentrations could result in morphological and physiological disorders, interfering with total development, which is reflected in the emergence of adults.

5. Conclusions

We believe that this is the first report of the larvicidal potential of *P. harmala* seed extract. Our findings revealed that the *P. harmala* extracts are promising green insecticidal agents, and thus, an alternative to synthetic larvicidal agents for mosquito population management. However, further research is required to isolate the bioactive molecules and determine their impacts on non-target organisms.

Acknowledgements

The authors are grateful to the Deanship of Scientific Research, King Saud University for funding through Vice Deanship of Scientific Research Chairs.

References

ABBASIPOUR, H., MAHMOUDVAND, M., RASTEGAR, F. and BASIJ, M., 2010. Insecticidal activity of Peganum harmala seed extract against the diamondback moth, Plutella xylostella. *Bulletin of Insectology*, vol. 63, no., 2, pp. 259-263.

ABBASSI, K., ATAY-KADIRI, Z. and GHAOUT, S., 2003. Biological effects of alkaloids extracted from three plants of Moroccan arid areas on the desert locust. *Physiological Entomology*, vol. 28, no. 3, pp. 232-236. http://dx.doi.org/10.1046/j.1365-3032.2003.00329.x.

ADANAN, C.R., ZAIRE, J. and NG, K.H., 2005. Efficacy sublethal effects of mosquito mats on Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). In: *Proceedings of the Fifth International Conference on Urban Pests*, 2005, USA. USA: ICUP, p. 265-269.

AKINER, M.M., SIMSEK, F.M. and CAGLAR, S.S., 2009. Insecticide resistance of Culex pipiens (Diptera: Culicidae) in Turkey. *Journal of Pesticide Science*, vol. 34, no. 4, pp. 259-264. http://dx.doi.org/10.1584/jpestics.G09-28.

AL-MEKHLAFI, F. A., ABUTAHA, N., FAROOQ, M., and AL-WADAAN, M., 2018. Insecticidal effect of Solenostemma argel extracts against Culex pipiens. *Journal of the American Mosquito Control Association*, vol. 34, no. 3, pp. 217-223.

CHAREONVIRIYAPHAP, T., BANGS, M.J., SUWONKERD, W., KONGMEE, M., CORBEL, V. and NGOEN-KLAN, R., 2013. Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. *Parasites & Vectors*, vol. 6, no. 1, pp. 1-28. http://dx.doi.org/10.1186/1756-3305-6-280. PMid:24294938.

CURTIS, C.F. and PASTEUR, N., 1981. Organophosphate resistance in vector populations of the complex of Culex pipiens L. (Diptera: culicidae). *Bulletin of Entomological Research*, vol. 71, no. 1, pp. 153-161. http://dx.doi.org/10.1017/S0007485300051129.

DAVOODI, H., GHAEMI, E., MAZANDARANI, M., SHAKERI, F., JAVID, S.N. and AND KLISHADI, M., 2015. Anti-mycobacterial and anti-inflammatory activity of Peganum harmala. *Journal of Chemical and Pharmaceutical Research*, vol. 7, no. 4, pp. 1611-1616.

EL-BOKL, M.M., 2016. Toxicity and bioefficacy of selected plant extracts against the mosquito vector Culex pipiens L.(Diptera: culicidae). *Environment*, vol. 6, pp. 7.

GHOSH, A., CHOWDHURY, N. and CHANDRA, G., 2012. Plant extracts as potential mosquito larvicides. *The Indian Journal of Medical Research*, vol. 135, no. 5, pp. 581-598. PMid:22771587.

GOVINDARAJAN, M., MATHIVANAN, T., ELUMALAI, K., KRISHNAPPA, K., and ANANDAN, A. 2011. Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus (Diptera: Culicidae). *Parasitology Research*, vol. 109, no. 2, pp. 353-367.

GUBLER, D.J., 2009. Vector-borne diseases. *Revue Scientifique et Technique (International Office of Epizootics)*, vol. 28, no. 2, pp. 583-588.

HARVE, G., and KAMATH, V., 2004. Larvicidal activity of plant extracts used alone and in combination with known synthetic larvicidal agents against *Aedes aegypti*. *Indian Journal of Experimental Biology*, vol. 42, no. 12, pp. 1216-1219.

HASHEMI SHEIKH SHABANI, S., SEYED HASAN TEHRANI, S., RABIEI, Z., TAHMASEBI ENFERADI, S. and VANNOZZI, G.P., 2015. Peganum harmala L. *s anti-growth effect on a breast cancer cell line. Biotechnology Reports (Amsterdam, Netherlands)*, vol. 8, pp. 138-143. http://dx.doi.org/10.1016/j.btre.2015.08.007. PMid:28352583.

JBILOU, R., ENNABILI, A. and SAYAH, F., 2006. Insecticidal activity of four medicinal plant extracts against Tribolium castaneum (Herbst) (Coleoptera: tenebrionidae). *African Journal of Biotechnology*, vol. 5, pp. 936-940.

KAMARAJ, C., RAHUMAN, A.A. and BAGAVAN, A., 2008. Antifeedant and larvicidal effects of plant extracts against Spodoptera litura F. Aedes aegypti L. and Culex quinquefasciatus Say. *Journal of Parasitology Research*, vol. 103, no. 2, pp. 325-331. http://dx.doi.org/10.1007/s00436-008-0974-8. PMid:18437424.

KANNATHASAN, K., SENTHILKUMAR, A., CHANDRASEKARAN, M., and VENKATESALU, V., 2007 Differential larvicidal efficacy of four species of Vitex against Culex quinquefasciatus larvae. *Parasitology Research*, vol. 101, no. 6, pp. 1721-1723.

MAHMOUDIAN, M., JALIPOUR, H. and SALEHIAN DARDASHTI, P., 2002. Toxicity of Peganum harmala: review and a case report. *Iranian Journal of Pharmacology and Therapeutics*, vol. 1, no. 1, pp. 1-0.

MINA, C. N., FARZAEI, M. H. and GHOLAMREZA, A., 2015. Review medicinal properties of Peganum harmala L. *Journal of Traditional Chinese Medicine = Chung i Tsa Chih Ying Wen Pan*, vol. 35, no. 1, pp. 104-109.

MOLOUDIZARGARI, M., MIKAILI, P., AGHAJANSHAKERI, S., ASGHARI, M.H. and SHAYEGH, J., 2013. Pharmacological and therapeutic effects of Peganum harmala and its main alkaloids. *Pharmacognosy Reviews*, vol. 7, no. 14, pp. 199-212. http://dx.doi.org/10.4103/0973-7847.120524. PMid:24347928.

MORADI, M.T., KARIMI, A., FOTOUHI, F., KHEIRI, S. and TORABI, A., 2017. In vitro and in vivo effects of Peganum harmala L. seeds extract against influenza A virus. *Avicenna Journal of Phytomedicine*, vol. 7, no. 6, pp. 519-530. PMid:29299435.

MULLAI, K., JEBANESAN, A., and PUSHPANATHAN, T., 2008. Effect of bioactive fractions of *Citrullus vulgaris* Schrad. leaf extract against Anopheles stephensi and *Aedes aegypti*. *Parasitology Research*, vol. 102, no. 5, pp. 951-955.

NATHAN, S.S. and KALAIVANI, K., 2005. Efficacy of nucleopolyhedrovirus and azadirachtin on Spodoptera litura Fabricius (Lepidoptera: noctuidae). *Biological Control*, vol. 34, no. 1, pp. 93-98. http://dx.doi.org/10.1016/j.biocontrol.2005.03.001.

NENAAH, G., 2010. Antibacterial and antifungal activities of (beta)-carboline alkaloids of Peganum harmala (L) seeds and their combination effects. *Fitoterapia*, vol. 81, no. 7, pp. 779-782. http://dx.doi.org/10.1016/j.fitote.2010.04.004. PMid:20398742.

OKUMU, F.O., KNOLS, B.G. and FILLINGER, U., 2007. Larvicidal effects of a neem (Azadirachta indica) oil formulation on the malaria vector Anopheles gambiae. *Malaria Journal*, vol. 6, no. 1, pp. 63. http://dx.doi.org/10.1186/1475-2875-6-63. PMid:17519000.

PENG, Z., BECKETT, A.N., ENGLER, R.J., HOFFMAN, D.R., OTT, N.L. and SIMONS, F.E., 2004. Immune responses to mosquito saliva in 14 individuals with acute systemic allergic reactions to mosquito bites. *The Journal of Allergy and Clinical Immunology*, vol. 114, no. 5, pp. 1189-1194. http://dx.doi.org/10.1016/j.jaci.2004.08.014. PMid:15536430.

PRABAKAR, K., and JEBANESAN, A., 2004. Larvicidal efficacy of some Cucurbitacious plant leaf extracts against *Culex quinquefasciatus* (Say). *Bioresource Technology*, vol. 95, no. 1, pp. 113-114.

RAHUMAN, A.A. and VENKATESAN, P., 2008. Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. *Parasitology Research*, vol. 103, no. 1, pp. 133-139.

SALARI, E., AHMADI, K., DEHYAGHOBI, R.Z., PURHEMATY, A. and TAKALLOOZADEH, H.M., 2012. Toxic and repellent effect of harmal (Peganum harmala L.) acetonic extract on several aphids and Tribolium castaneum (Herbst). *Chilean Journal of Agricultural Research*, vol. 72, no. 1, pp. 147-151. http://dx.doi.org/10.4067/S0718-58392012000100023.

SHARMA, P., MOHAN, L., and SRIVASTAVA, C.N., 2005. Larvicidal potential of *Nerium indicum* and Thuja *oriertelis extracts* against malaria and Japanese encephalitis vector. *Journal of Environmental Biology*, vol. 26, no. 4, pp. 657-660.

SU, T. and MULLA, M.S., 1999. Oviposition bioassay responses of Culex tarsalis and Culex quinquefasciatus to neem products containing azadirachtin. *Entomologia Experimentalis et Applicata*, vol. 91, pp. 337-345.

TANWEER, A.J., CHAND, N., SADDIQUE, U., BAILEY, C.A. and KHAN, R.U., 2014. Antiparasitic effect of wild rue (Peganum harmala L.) against experimentally induced coccidiosis in broiler chicks. *Parasitology Research*, vol. 113, no. 8, pp. 2951-2960. http://dx.doi.org/10.1007/s00436-014-3957-y. PMid:24879014.

THENMOZHI, V., KRISHNAMOORTHY, T., BASKARN, R., KRISHNAMOORTHI, G., BALAJI, R. and TYAGI, B.K., 2014. A first note on Japanese encephalitis virus isolation from Culex quinquefasciatus Say in Northern West Bengal. *International Journal of Mosquito Research*, vol. 1, pp. 1-4.

THOMAS, T.G., RAO, S. and LAL, S., 2004. Mosquito larvicidal properties of essential oil of an indigenous plant, Ipomoea cairica Linn. *Japanese Journal of Infectious Diseases*, vol. 57, no. 4, pp. 176-177. PMid:15329451.

WORLD HEALTH ORGANIZATION – WHO, 1996. Report of the WHO informal consultation on the evaluation and testing of insecticides". Geneva: WHO/HQ. 69 p.

YADAV, R., SRIVASTAVA, V.K., CHANDRA, R., and SINGH, A., 2002. Larvicidal activity of latex and stem bark of *Euphorbia tirucalli* plant on the mosquito *Culex quinquefasciatus*. *Journal of Communication Disorders*, vol. 34, no. 4, pp. 264-269.

ZAIM, M. and GUILLET, P., 2002. Alternative insecticides: an urgent need. *Trends in Parasitology*, vol. 18, no. 4, pp. 161-163. http://dx.doi.org/10.1016/S1471-4922(01)02220-6. PMid:11998703.

ZENG, Y., ZHANG, Y., WENG, Q., HU, M., and ZHONG, G., 2010. Cytotoxic and insecticidal activities of derivatives of harmine, a natural insecticidal component isolated from Peganum harmala. *Molecules*, vol. 15, no. 11, pp. 7775-7791.

ZHAO, T., WANG, Z.T., BRANFORD-WHITE, C.J., XU, H. and WANG, C.H., 2011. Classification and differentiation of the genus Peganum indigenous to China based on chloroplast trnL-F and psbA-trnH sequences and seed coat morphology. *Plant Biology*, vol. 13, no. 6, pp. 947-994. http://dx.doi.org/10.1111/j.1438-8677.2011.00455.x. PMid:21972911.