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Insecticidal Toxicity of Plant Extracts and Green Silver Nanoparticles against *Aedes albopictus*

Hassan Shahzad Khan[1], Muhammad Tariq[1]*, Tariq Mukhtar[2] and Asim Gulzar[1]

[1]*Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi*
[2]*Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi*

ABSTRACT

Aedes albopictus is a known vector of many diseases such as Dengue, Chikungunya, Zika and Yellow fever. Many control measures have been adopted to manage the vector of these diseases. The excessive use of insecticides has led to development of resistance in *Aedes albopictus* and negative impact on the environment and non-target organisms. To overcome these problems, emphasis is being laid for alternatives such as biopesticides which are environmental friendly and economical. Therefore, in the present study, the toxicity of eight plant extracts and their green synthesized nanoparticles was evaluated against *A. albopictus*. The maximum mortality of 88% of 3rd instar larvae of *A. albopictus* was caused by datura extract followed by neem and clove. On the other hand, neem and clove extracts caused the maximum mortality (80%) of 4th instar larvae followed by datura extract (78%). Similarly, the minimum mortalities of 3rd and 4th instar larvae were caused by bitter gourd extract. In case of green silver nanoparticles, the maximum mortalities of 3rd and 4th instar larvae of *A. albopictus* were caused by neem and clove followed by datura, ginger and bakain. All the green silver nanoparticles caused above 80% mortality of both the instars of the mosquito. Bakain and neem extracts showed the minimum LC_{50} values after 72 h of application followed by garlic against the 3rd instar *A. albopictus* larvae. In case of green synthesized silver nanoparticles, the minimum LC_{50} values after 72 h were recorded with neem, bakain and garlic while the values were maximum in the case of clove, bitter gourd and datura. Similar trend of LC_{50} values was observed in case of 4th instar larvae. The LC_{50} values decreased with an increase in the time. It is concluded that the artificially synthesized Ag-nanoparticles can be used as environmental friendly alternative insecticide for the management of *A. albopictus*.

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Authors' Contribution
HSK and MT designed the study, executed experimental work, analyzed the data and prepared the manuscript. TM and AG supervised the experimental work, analyzed the data and edited the manuscript.

Key words
Green synthesized nanoparticles, *Aedes albopictus*, Larvicidal potential, Toxicity, Medicinal plants, LC_{50}

INTRODUCTION

The Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae) is a strong vector of different arboviruses and can transmit 26 viruses belonging to Togaviridae, Reoviridae, Flaviviridae, Bunyaviridae and Nodaviridae families (Moore and Mitchell, 1997; Gratz, 2004; Paupy *et al*., 2009). *A. albopictus* is a serious threat to human health and causes many infectious diseases such as dengue, chikungunya, zika and yellow fever (Zahran *et al*., 2017; Ga'al *et al*., 2018). This mosquito which originated in Asia and spread throughout the world (Benedict *et al*., 2007) can serve as maintenance vector of arboviruses in rural areas breeds in natural habitats of rural and suburban areas, whereas *A. aegypti* is rarely found in such natural habitats (Gratz, 2004).

There is no proper medication and treatment for dengue and zika patients therefore, the vector mosquito is the target to manage these diseases (Moore and Mitchell, 1997; Gratz, 2004; Paupy *et al*., 2009). Many control measures have been used to manage *A. albopictus* but the most promising measure is the use of synthetic chemicals such as pyrethroids and organophosphates (Vontas *et al*., 2012). The excessive use of insecticides has led to development of resistance in *A. albopictus* and has negative impact on the environment and non-target organisms (Devine and Furlong, 2007; Ga'al *et al*., 2018). High resistance of *A. albopictus* against many agrochemicals has also impacted the efficiency of chemicals which are being used for vector control (Khan *et al*., 2011). To overcome these problems, entomologists are looking for alternatives such as biopesticides which are environmental friendly and economical. Nanotechnology has the potential to enhance the efficacy of botanicals. Recent studies have shown that the green synthesized metal nanoparticles showed high efficacy against different insect pests including mosquitoes (Suresh *et al*., 2015; Ga'al *et al*., 2018). These particles are very effective, involve low cost and are environment friendly (Huang *et al*., 2007; Ga'al *et al*., 2018). Various metals are available that enhances the efficacy of green

* Corresponding author: mtariq@uaar.edu.pk
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synthesized nanoparticles but the most important metal is silver (Ag) which has high biocidal effects and is eco-friendly (Suresh *et al*., 2014). The current study was planned to evaluate the toxicity of eight plant extracts and their green synthesized nanoparticles against *A. albopictus*.

MATERIALS AND METHODS

Preparation of plant extracts

The plant extracts were prepared according to the methodology described by Minjas and Sarda (1986).

Synthesis of green silver nanoparticles

For preparation of green silver nanoparticles 10g of the crude extract from each plant was dissolved in 250 ml of distilled water, boiled for five min and then mixed in 80ml of 1mM $AgNO_3$ solution. The mixture was boiled for 5 min until its colour changed to brown. The brown liquid was centrifuged at 5000 rpm for 15 min in Falcon tubes. Excess solution was removed from the falcon tubes and the remainder was transformed in an oven at 50°C for 24 h in a China dish in order to remove moisture from the pellets. The dried pellets were ground to make a pulverized powder. The procedure was done according to the methodology described by Parashar *et al*. (2009).

After the preparation of the green synthesized nanoparticles, their absorbance, signal strength was assessed in the wavelengths range of 300-700 nm using a UV-Vis spectrophotometer at the Alpha Genomics Laboratory, Islamabad.

Rearing of larvae of A. albopictus

Larvae of *A. albopictus* were obtained from the National Institute of Health Sciences, Islamabad. The larvae were shifted to the Insect Molecular Biology Laboratory, Department of Entomology, Arid Agriculture University, Rawalpindi. The rearing was done in plastic trays in rearing jars at 25°C and 85% relative humidity. The front side of rearing cage was covered with a muslin cloth for aeration and feeding. The larvae were fed on commercial based beef liver powder.

Evaluation of plant extracts and green synthesized Ag-nanoparticles against larvae of A. albopictus

Three hundred larvae of 3rd and 4th larval instar of *A. albopictus* were used for each treatment. A total of five concentrations of plant extracts and green synthesized silver nanoparticles excluding one of control with five replications for each plant material were used. For the bioassay of *A. albopictus*, plastic cups were used. The mortality of larvae was recorded after 24, 48, and 72 h of the treatment.

Statistical analysis

The percentage corrected mortality of mosquito larvae was calculated using Abbot's formula (Abbott, 1925) and LC_{50} was calculated using probit analysis. R statistical software was used for data analysis. Probit analysis was conducted using Polo-plus software.

RESULTS

The signal strength of all the nanoparticles synthesized using plant extracts ranged from 0.2-2.5. The maximum peaks for various green synthesized nanoparticles fell into a wavelength range of 420-450 nm.

Larvicidal potential of plant extracts and their nanoparticles

The maximum mortality of 88% of 3rd instar larvae of *A. albopictus* was caused by datura extract followed by neem and clove. On the other hand, neem and clove extracts caused the maximum mortality (80%) of 4th instar larvae followed by datura extract (78%). Similarly, the minimum mortalities of 3rd and 4th instar larvae were caused by bitter gourd extract (Table I).

In the case of green silver nanoparticles, the maximum mortalities of 3rd and 4th instar larvae of *A. albopictus* were caused by neem and clove followed by datura, ginger and bakain. All the green silver nanoparticles caused mortalities of both the instars of the mosquito above 80% (Table I).

Table I. Effect of the highest concentrations of botanical extracts and green-synthesized nanoparticles on the mortality of 3rd and 4th instar larvae of *Aedes albopictus* after 72 h.

Plant	Mortality of *A. albopictus* with botanical extracts against		Mortality of *A. albopictus* with green nanoparticles against	
	3rd instar	4th instar	3rd instar	4th instar
Neem	86%	82%	96%	92%
Ginger	74%	70%	92%	86%
Bitter gourd	66%	60%	82%	74%
Eucalyptus	72%	66%	90%	86%
Datura	88%	78%	94%	90%
Clove	84%	80%	96%	92%
Bakain	74%	70%	92%	86%
Garlic	78%	74%	86%	80%

LC_{50} values of plant extracts and their silver nanoparticles

The LC_{50} values of plant extracts against 3rd instar larvae are shown in Table II. Bakain and neem extracts showed the minimum LC_{50} values after 72 h of application followed by garlic against the 3rd instar *A. albopictus* larvae. The LC_{50} values decreased with an increase in the time. The highest LC_{50} value was observed in case of clove followed by bitter gourd and eucalyptus as shown in Table II. Similar trend of LC_{50} values was observed in case of 4th instar larvae.

The LC_{50} values of the green synthesized silver nanoparticles used against 3rd and 4th instar larvae are shown in Table III. The minimum LC_{50} values after 72 h were recorded in the case of neem, bakain and garlic while the

Table II. LC_{50} values of plant extracts at 3-time intervals against 3rd and 4th instar larvae of *Aedes albopictus.*

Plant extract	LC_{50} with fiducial limit at		
	24 h	**48 h**	**72 h**
3rd instar larvae			
Neem(mg/L)	8.843(6.476-11.605)	6.033(3.037-8.614)	2.419(-1.707-4.947)
Ginger(mg/L)	102.517 (90.396-115.136)	84.168(68.337-96.232)	64.744(46.176-76.718)
Bitter gourd(mg/L)	288.277(262.442-330.923)	267.072(241.293-308.212)	241.903(217.288-276.722)
Eucalyptus(mg/L)	339.987(298.335-402.323)	301.782(261.858-357.359)	229.981(186.150-278.757)
Datura(mg/L)	420.409(374.862-487.494)	309.835(259.614-362.445)	184.453(99.285-237.958)
Clove(mg/L)	530.784(475.707-624.541)	400.139(351.573-460.861)	282.138(216.713-331.076)
Bakain(g/L)	2.615(2.331-3.030)	2.370(2.091-2.728)	2.022(1.711-2.338)
Garlic(g/100ml)	13.610(9.326-27.212)	10.932(6.756-21.635)	5.744(0.084-10.245)
4th instar larvae			
Neem (mg/L)	12.398(9.501-17.146)	10.110(7.280-13.965)	5.721(-0.332-10.131)
Ginger (mg/L)	120.932(107.551-140.624)	97.490(84.064-110.289)	78.174(62.803-89.408)
Bitter gourd (mg/L)	302.620(273.634-353.965)	282.787(252.795-336.563)	257.368(228.871-304.444)
Eucalyptus (mg/L)	371.031(323.842-447.876)	320.021(272.552-394.427)	255.293(211.189-310.375)
Datura (mg/L)	463.070(411.011-548.615)	337.462(293.031-388.854)	246.476(171.969-302.635)
Clove (mg/L)	498.164(454.880-562.589)	445.400(398.355-512.516)	345.821(294.185-396.934)
Bakain (g/L)	2.921(2.639-3.360)	2.743(2.460-3.169)	2.419(2.143-2.783)
Garlic (g/100ml)	15.166(10.655-30.603)	12.239(9.744-16.347)	7.894(3.248-14.386)

Table III. LC_{50} values of green synthesized Ag-nanoparticles at 3-time intervals against 3rd and 4th instar larvae of *Aedes albopictus.*

Plant based Ag-nanoparticles	LC_{50} with fiducial limit at		
	24 h	**48 h**	**72 h**
3rd instar larvae			
Neem (mg/L)	1.197 (0.688-1.649)	0.465(-0.132-0.864)	0.173(-2.939-0.555)
Ginger (mg/L)	16.299 (13.171-18.629)	13.036(9.937-15.153)	8.884(4.732-11.293)
Bitter gourd (mg/L)	50.125 (45.576-56.604)	44.577(40.167-49.877)	36.500(31.192-41.091)
Eucalyptus (mg/L)	54.206 (45.657-65.521)	32.940(23.340-40.90)	19.413(-11.417-33.226)
Datura (mg/L)	70.060 (62.813-78.450)	49.351(39.868-57.272)	35.062(23.933-43.029)
Clove (mg/L)	74.644 (66.182-83.899)	56.474(46.828-64.283)	40.589(29.409-48.410)
Bakain (g/L)	0.433 (0.380-0.492)	0.334(0.276-0.383)	0.255(0.185-0.305)
Garlic (g/100ml)	1.915 (1.051-3.700)	1.303(0.273-2.344)	0.810(-0.621-1.593)
4th instar larvae			
Neem (mg/L)	1.867(1.353-2.499)	0.944(0.467-1.334)	0.246 (-0.492-0.69)
Ginger (mg/L)	19.223(16.796-21.501)	15.070(12.040-17.249)	10.989(6.025-13.833)
Bitter gourd (mg/L)	55.507(50.214-64.255)	50.256(45.311-57.609)	42.524(37.952-47.539)
Eucalyptus (mg/L)	61.236(53.053-72.851)	46.235(38.334-54.946)	33.051(24.645-40.238)
Datura (mg/L)	76.800(68.851-87.120)	61.186(52.753-69.789)	44.541(34.853-52.227)
Clove (mg/L)	85.562(78.406-94.414)	67.650(59.640-75.416)	48.817(38.415-56.575)
Bakain (g/L)	0.494(0.440-0.566)	0.408(0.348-0.469)	0.312(0.248-0.362)
Garlic (g/100ml)	2.542(1.695-5.144)	1.665(0.818-2.951)	1.222(0.232-2.125)

values were the maximum in case of clove, bitter gourd and datura. A similar trend was found in case of 4th instar larvae where neem, bakain and garlic gave the minimum LC_{50} values. On the other hand, the maximum values were observed with clove, datura and bitter gourd. The LC_{50} values decreased with the passage of time. The values were the maximum after 24 h and decreased as the time interval increased.

DISCUSSION

The present findings showed that all the plant extracts caused 50% mortalities of 3rd and 4th instars larvae of *A. albopictus* while Ag-nanoparticles obtained from these plants showed even higher mean mortality values. 4th instar larvae were less susceptible to plant extracts and nanoparticles as compared to the younger larvae.

The mortalities caused by plant extracts and green sliver nanoparticles could be due to certain insecticidal and insect repellant compounds being present in the green synthesized silver nanoparticles of the plants. The clove plant contains the chemical eugenol which is known for its medicinal and antibiotic potential. Medeiros *et al*. (2013) evaluated the larvicidal potential of clove extracts and eugenol against two mosquito species i.e. *Anopheles darlingi* and *A. aegypti*. The results showed that *A. aegypti* was 63 times more susceptible to clove extracts as compared to *A. darlingi* larvae. Similarly, bakain showed good larvicidal potential and required a relatively large concentration to kill 50% of the mosquito population in the present study. The results are supported by the study of Selveraj and Mosses (2011) who evaluated the efficacy of bakain on three different mosquito species i.e. *A. aegypti, Culex quinquefasciatus,* and *A. stephensi*. The dengue mosquito species was found to be the most resistant to bakain extracts. The other two species showed very similar levels of susceptibility.

Ginger was found to cause the maximum mortality of mosquitos. Similar results were obtained by Nasir *et al*. (2017) who tested different plant extracts and confirmed that *Zingiber officinale* had the highest effectiveness in inducing mean mortality in immature *A. albopictus* larvae. The 3rd instar larvae were the least resistant to plant extracts as compared to 4th instar larvae. It was also found that plant oils of eucalyptus gave the highest mean mortality and that of ginger the lowest. The study also showed that *Azadirachta indica* extracts gave the highest LC_{50} values of all the test plants.

In the present study, bitter gourd and eucalyptus extracts proved to be less effective against *A. albopictus* larvae. The present results were in contrast to those reported by Singh *et al*. (2006) in which they treated the 4th instars of three different mosquito species with crude fruit extracts of *Momordica charantia* and obtained good results. However, the difference in results can be explained as follows: *A. albopictus* was not one of the species tested in this study. The closest was *A. aegypti*, which belongs to the same genus, and it was the least susceptible species of the three used in this study. Furthermore, the study utilized hexane extracts of bitter gourd whereas in the current study ethanol extracts were used.

Eucalyptus was also an average performer in the current study, giving similar results to bitter gourd. A study conducted by Nair *et al*. (2014) also reported the larvicidal potential of leaf extracts of eucalyptus as well as of the medicinal plant *Centella asiatica*. According to these authors, eucalyptus was not as effective as its counterpart in inducing mean mortality in the targeted mosquito species i.e. *A. aegypti* and *A. stephensi*. Another conclusion that they drew was that methanol extracts were the least effective out of all the organic solvents used in the study with hexane extracts being the most effective against *A. aegypti* strains.

The extracts and Ag-nanoparticles of garlic did not perform well in this study against *A. albopictus* mosquito larvae. Garlic had by far the highest amount required to reach the LC_{50} value. These results conformed to the findings of Selvam and Durai (2018) who evaluated the larvicidal potential of 11 different plant extracts against *A. albopictus* and an unspecified species of *Anopheles*. In their study, *Allium sativum* bulb extracts in ethyl alcohol gave the worst performance out of all the 11 test plant extracts. Conversely, the extracts of *A. indica* showed the best larvicidal potential. As these were the only two plants out of the 11 used in this study which were the same as the plants used in the current study. In another study, Ali *et al*. (2017) tested five plants against *A. aegypti* larvae. Neem showed the highest larvicidal potential whereas eucalyptus did not prove effective and confirmed the present findings.

In all cases, the Ag-nanoparticles outperformed their plant extract counterparts in causing larval mean mortality of both the larval instars. The effectiveness of Ag-nanoparticles against *A. albopictus* larvae has previously been demonstrated by Ga'al *et al*. (2018). They utilized three different types of silver nanoparticles: first was artificially fabricated while the other two were synthesized using Salicylic acid and 3, 5-dinitrosalicylic acid respectively. It was found that the artificially synthesized Ag-nanoparticles proved to be highly toxic to dengue mosquito larvae as compared to the ones derived from Salicylic acid whereas the ones derived from 3, 5-dinitrosalicylic acid did not cause significant mortality. It is therefore, concluded that the artificially synthesized Ag-nanoparticles can be used as an environmentally friendly alternative insecticide for the management of *A. albopictus*.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. econ. Ent.*, **18**: 165-167. https://doi.org/10.1093/jee/18.2.265a

Ali, M., Khan, H.A.A., Tahir, H.M., Tariq, A., Ashfaq, M., Ali, S.W., Gulzar, A., Aslam, H.U.A., Khalid, U., Yousaf, S. and Mubashar, U., 2017. Larvicidal potenial of diffrent plants extracts against the larvae of mosquito *Aedes aegypti* (L.) (Culicidae: Diptera). *Pak. Entomol.*, **39**: 37-40.

Benedict, M.Q., Levine, R.S., Hawley, W.A. and Lounibos, L.P., 2007. Spread of the tiger: Global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonot. Dis.*, **7**: 76-85. https://doi.org/10.1089/vbz.2006.0562

Devine, G.J. and Furlong, M.J., 2007. Insecticide use: contexts and ecological consequences. *Agr. Hum. Val.*, **24**: 281-306. https://doi.org/10.1007/s10460-007-9067-z

Ga'al, H., Fouad, H., Tian, J., Hu, Y., Abbas, G. and Mo, J., 2018. Synthesis, characterization and efficacy of silver nanoparticles against *Aedes albopictus* larvae and pupae. *Pestic. Biochem. Physiol.*, **144**: 49-56. https://doi.org/10.1016/j.pestbp.2017.11.004

Gratz, N., 2004. Critical review of the vector status of *Aedes albopictus*. *Med. Vet. Ent.*, **18**: 215-227. https://doi.org/10.1111/j.0269-283X.2004.00513.x

Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., Wang, H., Wang, Y., Shao, W. and He, N., 2007. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology*, **18**: 104-105. https://doi.org/10.1088/0957-4484/18/10/105104

Khan, H.A.A., Akram, W., Shehzad, K. and Shaalan, E.A., 2011. First report of field evolved resistance to agrochemicals in dengue mosquito, *Aedes albopictus* (Diptera: Culicidae), from Pakistan. *Parasit. Vectors*, **4**: 146. https://doi.org/10.1186/1756-3305-4-146

Medeiros, E.D.S., Rodrigues, I.B., Litaiff-Abreu, E., da S Pinto, A.C. and Tadei, W.P., 2013. Larvicidal activity of clove (*Eugenia caryophyllata*) extracts and eugenol against *Aedes aegypti* and *Anopheles darlingi*. *Afr. J. Biotechnol.*, **12**: 836-840.

Minjas, J.N. and Sarda, R.K., 1986. Laboratory observations on the toxicity of *Swartzia madagascariensis* (Leguminosae) extract to mosquito larvae. *Trans. R. Soc. Trop. Med. Hyg.*, **80**: 460-461. https://doi.org/10.1016/0035-9203(86)90345-7

Moore, C.G. and Mitchell, C.J., 1997. *Aedes albopictus* in the United States: Ten-year presence and public health implications. *Emerg. Infect. Dis.*, **3**: 329. https://doi.org/10.3201/eid0303.970309

Nair, S.S., Shetty, V. and Shetty, N.J., 2014. Relative toxicity of leaf extracts of *Eucalyptus globulus* and *Centella asiatica* against mosquito vectors *Aedes aegypti* and *Anopheles stephensi*. *J. Insects*, **2014**: 1-7. https://doi.org/10.1155/2014/985463

Nasir, S., Nasir, I., Asrar, M. and Debboun, M., 2017. Larvicidal and pupicidal action of medicinal plant extracts against dengue mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae). *Indian J. Anim. Res.*, **51**: 155-158.

Parashar, U.K., Saxena, P.S. and Srivastava, A., 2009. Bioinspired synthesis of silver nanoparticles. *Dig. J. Nanomater. Bios.*, **4**: 159-166.

Paupy, C., Delatte, H., Bagny, L., Corbel, V. and Fontenille, D., 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Microb. Infect.*, **11**: 1177-1185. https://doi.org/10.1016/j.micinf.2009.05.005

Selvaraj, M. and Mosses, M., 2011. Efficacy of *Melia azedarach* on the larvae of three mosquito species *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Eur. Mosq. Bull.*, **29**: 116-121.

Selvam, J. and Durai, M., 2018. Phytochemical analysis and larvicidal activity of aqueous and ethanol extract of selective medicinal plants against malaria and dengue vector. *Int. J. Sci. Res.*, **9**: 27113-27119.

Singh, R., Dhiman, R. and Mittal, P., 2006. Mosquito larvicidal properties of *Momordica charantia* Linn (Family: Cucurbitaceae). *J. Vector Borne Dis.*, **43**: 88.

Suresh, G., Gunasekar, P.H., Kokila, D., Prabhu, D., Dinesh, D., Ravichandran, N., Balasubramanian, R., Arunagirinathan, K. and Siva, G.V., 2014. Green synthesis of silver nanoparticles using *Delphinium denudatum* root extract exhibits antibacterial and mosquito larvicidal activities. *Spectrochim. Acta A. Mol. Biomol. Spectrosc.*, **127**: 61-66. https://doi.org/10.1016/j.saa.2014.02.030

Suresh, U., Murugan, K., Benelli, G., Nicoletti, M., Barnard, D.R., Panneerselvam, C., Kumar, P.M., Subramaniam, J., Dinesh, D. and Chandramohan, B., 2015. Tackling the growing threat of dengue: *Phyllanthus* niruri-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector *Aedes aegypti* (Diptera: Culicidae). *Parasitol. Res*., **114**: 1551-1562. https://doi.org/10.1007/s00436-015-4339-9

Vontas, J., Kioulas, E., Pavlidi, N., Morou, E., Torre, A. and Ranson, H., 2012. Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. *Pestic. Biochem. Physiol*., **104**: 126-131. https://doi.org/10.1016/j.pestbp.2012.05.008

Zahran, H.E.-D.M., Abou-Taleb, H.K. and Abdelgaleil, S.A., 2017. Adulticidal, larvicidal and biochemical properties of essential oils against *Culex pipiens* L. *J. Asia-Pac. Ent*., **20**: 133-139. https://doi.org/10.1016/j.aspen.2016.12.006