International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2022; 9(6): 27-32

www.dipterajournal.com
Received: 13-09-2022
Accepted: 15-10-2022

Sudip Paramanik
Epidemiology, Vector Biology and Environmental Monitoring Research Units, Entomology Laboratory, Department of Animal Science, Kazi Nazrul University, Asansol, Paschim Bardhaman, West Bengal, India

Sagarika Mukherjee
1. Epidemiology, Vector Biology and Environmental Monitoring Research Units, Entomology Laboratory, Department of Animal Science, Kazi Nazrul University, Asansol, Paschim Bardhaman, West Bengal, India
2. Department of Zoology, Bidhan Chandra College, Asansol, Paschim Bardhaman, West Bengal, India

Ranjit Kapuri
1. Epidemiology, Vector Biology and Environmental Monitoring Research Units, Entomology Laboratory, Department of Animal Science, Kazi Nazrul University, Asansol, Paschim Bardhaman, West Bengal, India
2. Department of Zoology, Rampurhat College, Rampurhat, Birbhum, West Bengal, India

Suman Dasmodak
Epidemiology, Vector Biology and Environmental Monitoring Research Units, Entomology Laboratory, Department of Animal Science, Kazi Nazrul University, Asansol, Paschim Bardhaman, West Bengal, India

Dr. Manas Paramanik
Epidemiology, Vector Biology and Environmental Monitoring Research Units, Entomology Laboratory, Department of Animal Science, Kazi Nazrul University, Asansol, Paschim Bardhaman, West Bengal, India

Corresponding Author:
Manas Paramanik
Epidemiology, Vector Biology and Environmental Monitoring Research Units, Entomology Laboratory, Department of Animal Science, Kazi Nazrul University, Asansol, Paschim Bardhaman, West Bengal, India

Evaluation of mosquito larvicidal potency of leaf and fruit extracts of *Olax scandens* Roxb. against the vectors of dengue and lymphatic filariasis

Sudip Paramanik, Sagarika Mukherjee, Ranjit Kapuri, Suman Dasmodak and Manas Paramanik

DOI: https://doi.org/10.22271/23487941.2022.v9.i6a.637

Abstract
The emergence of resistance in insect vectors and destabilization of the ecosystem by excessive use of synthetic insecticides suggest an environmentally familiar alternative to suppress vector-borne diseases. Nowadays, phytochemicals play a major role in the biocontrol of vector populations. The present work evaluated the mosquito larvicidal potential of leaf and fruit extracts of *Olax scandens* Roxb. against the 3rd instar larvae of *Aedes albopictus* and *Culex quinquefasciatus*. The result of this assay indicated that the LC_{50} values of crude extracts of leaf and fruit 72 h post-exposure against *Aedes albopictus* larvae are 0.496%, and 0.879% respectively. While, LC_{50} values of leaf and fruit crude extracts against *Culex quinquefasciatus* are 0.354%, and 0.572% respectively in 72 h of exposure. Both extracts were able to create adverse effects in both the mosquito species and gave satisfactory mortality. Regarding the effectiveness of *Olax scandens*, the experiment reveals larvicidal toxicity of fruit extract is higher than the leaf extract and toxicity against the larvae of *Culex quinquefasciatus* is higher than the larvae of *Aedes albopictus*. The plant, *Olax scandens* can be used as a potent, plant-derived, eco-friendly, broad-spectrum mosquitocidal agent and it may become an important tool in mosquito management programs.

Keywords: Larvicidal potential, *Olax scandens*, Leaf, Flower, *Aedes albopictus*, *Culex quinquefasciatus*

1. Introduction
Mosquito is one of the most incontrovertible hematophagous insect vectors of vector-borne diseases. Most part of the world faces the frown of some nocuous mosquito-borne diseases. The tiger mosquito, *Aedes albopictus* Skuse (Diptera: Culicidae) is a transmitter of the Dengue virus [1], and the southern mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae) predominantly spreads lymphatic filariasis [2]. Dengue is endemic in more than 120 nations with 3.9 billion people at high risk of infection globally and it imposes morbidity and mortality [3]. However, lymphatic filariasis is an avoidable disease with 51.4 million people infected globally [4]. Lymphatic filariasis does not impose mortality but its impact on associated morbidity and disability in different groups is a matter of concern [5, 6]. Although some therapeutic strategies are available [1, 3, 7], the outburst of these diseases should be restricted by controlling the vector mosquito population.
Chemical control using larvicides, pupicides, and adulticides (organophosphates, organochlorines, synthetic pyrethroids, etc.) is the most popular method of vector management. These mosquitocides are highly efficacious and act quickly but due to the imprudent application, resistance develops in the vector population [8]. Synthetic insecticides damage our environment, cause the destruction of some non-target organisms, and are hazardous to humans also. So, there is a need for a proper vector control strategy to overcome these detrimental problems. Familiar vector control involves attacking the earlier life stages (larva and pupa) in a specific way, without using unnecessary synthetic chemicals, implementing insecticides only in breeding habitats of mosquitoes like drains, septic tanks, rice paddy fields, fish ponds, cesspools, agricultural trenches, irrigation wells, etc [9].
One of the best accepted eco-friendly approaches of vector control method is the application of plant extracts as they are biodegradable and their nontoxic nature towards the non-target organisms.

Olax scandens Roxb. is a shrub and edible plant included in the family of Olacaceae, found in tropical India. Leaves of this plant are being used by tribal peoples for medicinal and culinary purposes [10]. A number of phytochemicals like glucosides of b-sitosterol, oleanolic acid, and octacosanol are found in the leaves of this plant. Some of which show anticancer, antimicrobial, or both activities [11]. The main purpose of this study is to investigate the larvicidal potential of leaf and fruits crude extract of *Olax scandens* Roxb. against *Aedes albopictus* and *Culex quinquefasciatus* larvae.

2. Materials and Methods

The current investigation was carried out in the Epidemiology, Vector biology and Environmental monitoring research unit, Entomology Laboratory, Department of Animal Science, Kazi Nazrul University, Asansol.

2.1. Collection of plant materials

Fresh plant parts were randomly collected in the morning from Disergarh road, the outskirts part of Barakar, West Bengal, India (23°43′32.1″N, 86°48′52.5″E) in May 2022. The green mature leaves and unripe fruits are screened and selected for larvicidal bioassay.

2.2. Rearing of test mosquito larvae

The colonies of two vector mosquitoes, *Culex quinquefasciatus* and *Aedes albopictus* were established separately in the laboratory. These colonies were well maintained with a diet of dried yeast powder, dog biscuits, and algae (3:1:1) mixture at 25-30 °C temperature and 80-85% humidity, and carefully kept free from any kind of harmful pathogens, and insecticides [12, 13]. Larvae for the experiments were taken from these colonies.

2.3. Preparation of crude Phyto-extracts

All the collected plant parts were rinsed in distilled water for removing the impurities present on the outer surface and then transferred on a paper towel to dry. To release fluid, the fresh leaves and unripe fruits were crushed using a mechanical grinder separately and then filtered with Whatman No. 1 filter paper. After filtration, the clear filtrates were collected and preserved at 4°C as a stock solution. The required concentrations (0.5%, 1%, 1.5%, and 2%) were prepared by combining the stock extract with varying volumes of sterile distilled water [13].

2.4. Evaluation of the crude extract by dose-response bioassay

The larvicidal bioassay followed the standard protocols recommended by WHO [14] with slight modifications. 100 ml of crude extracts of four different concentrations (0.5%, 1%, 1.5%, and 2%) were taken in glass bowls and 20 early 3rd instar larvae of vector mosquitoes (*Culex quinquefasciatus* and *Aedes albopictus*) were transferred to each bowl. Each experiment was carried out in four replicates and one control was set up with only distilled water (devoid of plant extract). During the experiment, no food was supplied to the glass bowls containing the tested larvae. The mortality and survival were recorded on 24 h, 48 h, and 72 h of post-exposure periods. Dead larvae were recognized when they stopped moving after being pricked with a blunt needle in the cervical area or siphon. The glass bowls were maintained at a temperature of 27±2 °C, 80-90% relative humidity, and a photoperiod of 12 h light: 12 h dark phase [13, 15].

2.5. Toxicity on non-target organisms

The non-target organism was selected for test according to its presence in the same habitat as the target mosquito species. The effect of leaf and fruit crude extracts of *Olax scandens* was assessed on Chironomid larvae which share common habitat with larvae of *Culex quinquefasciatus* and *Aedes albopictus*. Non-target organisms were exposed in four concentrations (0.5%, 1%, 1.5%, and 2%) of crude extract and their mortalities were recorded 24 h, 48 h, and 72 h of post-exposure [13, 16].

2.6. Statistical analysis

For each concentration, percentage mortalities with standard deviations were calculated after 24 h, 48 h, and 72 h of exposures. Lethal concentrations (LC_{50} and LC_{90}) were computed using probit analysis at a 95% confidence level. When control mortality was between 5% to 20%, the observed percentage mortality was corrected by Abbott's formula [17].

$$\text{Corrected larval mortality (\%)} = \frac{\text{Treated Mortality (\%)} - \text{Control Mortality(\%)}}{100 - \text{Control Mortality (\%)}} \text{ X } 100$$

3. Results

Results for the potentiality of the crude extract of leaf and fruit of *Olax scandens* against the larvae of two mosquito vectors, *Culex quinquefasciatus* and *Aedes albopictus* are shown in Table 1. Among these two vectors, the highest larvicidal activity was observed against *Culex quinquefasciatus* in both leaf and fruit crude extracts with corresponding LC_{50} and LC_{90} values of 0.354%, 0.572%, and 0.197%, 0.386% respectively after 72 h of exposure. While mortality rates of *Aedes albopictus* in leaf and fruit extracts are much lower than *Culex quinquefasciatus* with corresponding LC_{50} and LC_{90} values of 0.496%, 0.879%, and 0.469%, 0.770% respectively (Table 2).

Leaf extract of 2% concentration showed 100% mortality against *Aedes albopictus* larvae after 72 h of exposure and against *Culex quinquefasciatus* larvae 100% mortality was achieved in 1% and 1.5% concentrations in 72 h and in 2% after 48 h of exposure. Fruit extract showed 100% mortality against *Aedes albopictus* larvae in 1.5% and 2% concentrations after 72 h of exposure and against *Culex quinquefasciatus* larvae 100% mortality was observed in 1% concentration after 72 h and in 1.5%, 2% concentrations after 48 h of exposure (Table 1).

Analysis of variance (ANOVA) of this *in vitro* bioassay displayed that the larvicidal effectiveness of both leaf and fruit extracts excelled with increased concentration and time of exposure against both mosquito species. No compelling difference was observed when two factors (concentration and period of exposure) were taken (Table 3-6).

4. Discussion

The immense application of synthetic insecticides is boosting the immunity of vector mosquito populations and toxifying the habitat of non-target organisms [18]. Thus, plant-derived mosquitocides may be employed as biocontrol agents due to their target selectivity, biodegradable nature, and being less lethal to non-target organisms [19]. Secondary metabolites like alkaloids, flavonoids, phenols, steroids, terpenoids, etc. synthesized by plants have insecticidal properties which control the insect vector population. The efficacy of these phytochemicals depends on several factors like plant species, parts used and solvents used for extraction, and geographical area from which the plant has been collected [20, 21].

Since some decades several plant families are reported to have potency and may play crucial roles in the mosquito control program. Some phytochemicals from the Olacaceae plant family have also been documented as having mosquito larvicidal properties. Mavundza *et al*. reported that the extract of the bark of *Olax dissitiflora* possesses larvicidal activity against *Anopheles arabiensis* with an LC_{50} value of 25.24 µg/ml after 24 h of exposure [22]. *Ximenia americana* leaf extract and leaf-mediated silver nanoparticles showed their efficiency as a promising larvicidal agent against *Aedes aegypti* [23].

In this study, the larvicidal activity of leaf and fruit crude extracts of *Olax scandens* were assessed against *Aedes albopictus* and *Culex quinquefasciatus* larvae which is the first-ever observation under laboratory conditions. Both the leaf and fruit extracts showed the potential of 100% mortality in larvae of both mosquito species in a short time. Study shows the fruit extracts of *Olax scandens* are more efficient larvae killer than the leaf extracts and at the same time larvae of *Culex quinquefasciatus* is more prone to the *Olax scandens* extracts than the larvae of *Aedes albopictus* (Table 1, Fig 1 & 2).

Table 1: Mean larval mortality with standard error (SE) of 3rd instar larvae of *Aedes albopictus* and *Culex quinquefasciatus* in different concentrations of leaf and fruit crude extracts of *Olax scandens.*

Parts of Plant	Mosquito Species	Concentration (%)	Mean Mortality ± SE (%)		
			24 h	48 h	72 h
Leaf	*Aedes albopictus*	Control	0.00	0.00	0.00
		0.5	6.25±1.25	35±2.04	73.75±2.39
		1.0	16.25±1.25	48.75±1.25	83.75±1.25
		1.5	22.50±1.44	61.25±2.39	93.75±1.25
		2.0	26.25±1.25	76.25±2.39	100±0.00
	Culex quinquefasciatus	Control	0.00	0.00	0.00
		0.5	25±2.04	62.5±2.5	83.75±1.25
		1.0	32.5±3.22	72.5±1.44	100±0.00
		1.5	40±2.04	83.75±2.39	100±0.00
		2.0	61.25±2.39	100±0.00	100±0.00
Fruit	*Aedes albopictus*	Control	0.00	0.00	0.00
		0.5	16.25±1.25	52.5±1.44	63.75±4.26
		1.0	40±2.04	67.5±2.5	87.5±3.22
		1.5	53.75±1.25	81.25±1.25	100±0.00
		2.0	76.25±2.39	88.75±3.14	100±0.00
	Culex quinquefasciatus	Control	0.00	0.00	0.00
		0.5	43.75±2.39	67.5±2.5	91.25±2.39
		1.0	68.75±1.25	93.75±1.25	100±0.00
		1.5	81.25±2.39	100±0.00	100±0.00
		2.0	90±2.04	100±0.00	100±0.00

Table 2: Probit and regression analysis of mortalities of 3rd instar larvae of *Aedes albopictus* and *Culex quinquefasciatus* to different concentrations of leaf and fruit crude extracts of *Olax scandens.*

Parts of Plant	Mosquito Species	Time of Exposure	LC_{50} Value (%)	LC_{90} Value (%)	Regression Equation	R^2 Value
Leaf	*Aedes albopictus*	24 h	4.908	33.818	Y = 1.527x + 3.945	0.984
		48 h	0.923	5.009	Y = 1.742X + 5.061	0.938
		72 h	0.496	0.879	Y = 4.69X + 6.542	0.655
	Culex quinquefasciatus	24 h	1.737	13.802	Y = 1.422X + 4.659	0.823
		48 h	0.563	1.024	Y = 4.929X + 6.23	0.581
		72 h	0.354	0.572	Y = 6.142X + 7.769	0.776
Fruit	*Aedes albopictus*	24 h	1.220	3.681	Y = 2.668X + 4.77	0.969
		48 h	0.505	2.439	Y = 1.872X + 5.555	0.972
		72 h	0.469	0.770	Y = 6.715X + 7.044	0.863
	Culex quinquefasciatus	24 h	0.612	2.111	Y = 2.379X + 5.508	0.993
		48 h	0.461	0.728	Y = 6.454X + 7.171	0.899
		72 h	0.197	0.386	Y = 4.404X + 8.103	0.776

Table 3: Two-way ANOVA analysis using concentrations of leaf extract and time period of exposure (hours) as the parameters for *Aedes albopictus* larval mortality.

Source of Variation	DF	Sum of Squares	Mean Squares	F value	P-value
Concentration (C)	11	23045.36	2095.03	383.09	0.00
Time Period (T)	1	65887.76	65887.76	12048.05	0.00
C*T	11	22462.86	2042.08	373.41	0.00
Within	72	393.75	5.47		
Total	95	111789.7			

Table 4: Two-way ANOVA analysis using concentrations of leaf extract and time period of exposure (hours) as the parameters for *Culex quinquefasciatus* larval mortality.

Source of Variation	DF	Sum of Squares	Mean Squares	F value	P-value
Concentration (C)	11	17117.86	1556.17	232.82	0.00
Time Period (T)	1	119356.5	119356.5	17856.97	0.00
C*T	11	16540.36	1503.67	224.96	0.00
Within	72	481.25	6.684028		
Total	95	153496			

Table 5: Two-way ANOVA analysis using concentrations of floral extract and time period of exposure (hours) as the parameters for *Aedes albopictus* larval mortality

Source of Variation	DF	Sum of Squares	Mean Squares	F value	P-value
Concentration (C)	11	14475.21	1315.93	128.47	0.00
Time Period (T)	1	110026	110026	10741.53	0.00
C*T	11	13600.21	1236.38	120.70	0.00
Within	72	737.5	10.24		
Total	95	138839			

Table 6: Two-way ANOVA analysis using concentrations of floral extract and time period of exposure (hours) as the parameters for *Culex quinquefasciatus* larval mortality

Source of Variation	DF	Sum of Squares	Mean Squares	F value	P-value
Concentration (C)	11	7633.33	693.94	166.55	0.00
Time Period (T)	1	176816.7	176816.7	42436	0.00
C*T	11	7123.33	647.58	155.42	0.00
Within	72	300	4.17		
Total	95	191873.3			

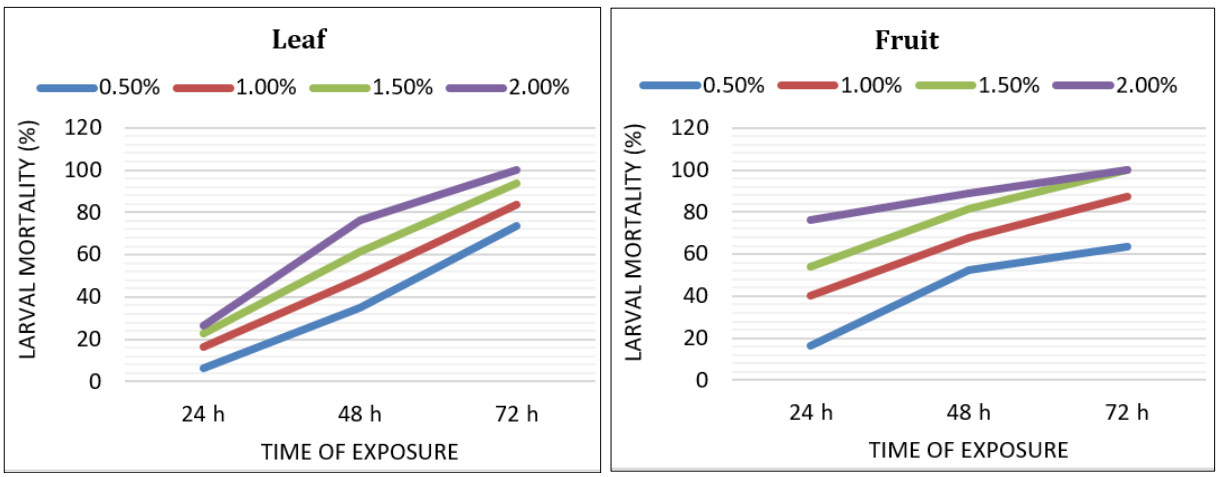

Fig 1: Mortality of 3rd instar larvae of *Aedes albopictus* to different concentrations of leaf and fruits crude extracts of *Olax scandens.*

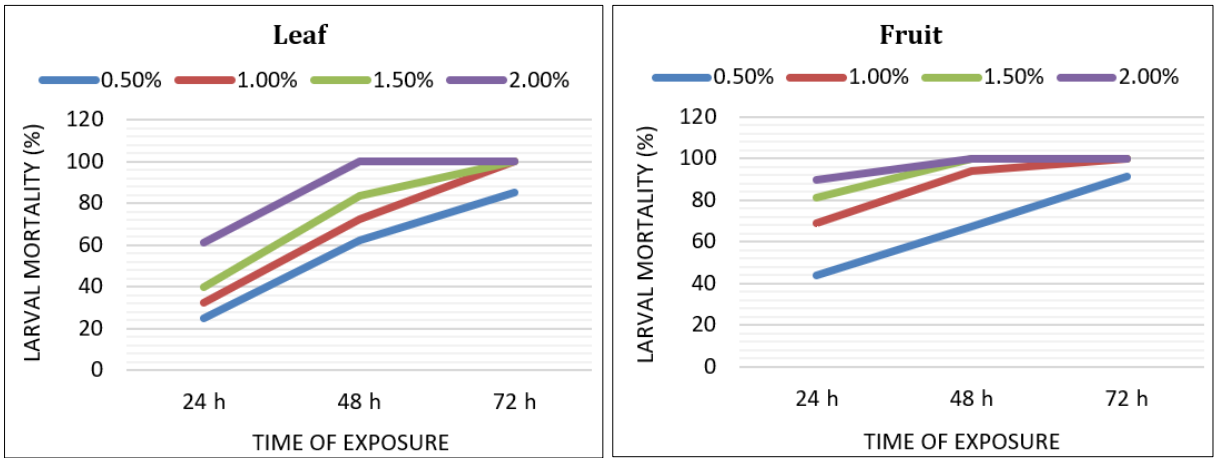

Fig 2: Mortality of 3rd instar larvae of *Culex quinquefasciatus* to different concentrations of leaf and fruits crude extracts of *Olax scandens.*

5. Conclusion

Every year vector-borne diseases take the lives of more than 7 lakhs peoples globally, of which about 40 thousand are due to dengue. The intensity of lymphatic filariasis has decreased due to MDA drive but still has concerned epidemiology in many parts of the world [3, 4, 24, 25]. The number of infected people is many folds bigger and so is the burden of these vector-borne diseases. To attend the goal of killing vectors, environmentally hazardous methods for vector control now become a threat to us. Integrated Vector Management (IVM) provides some decision-making criteria for vector population control and reducing the transmission of vector-borne diseases. The significance of phytoproducts in the sustainable management of the vector mosquito population is inevitable. The plant tested in this study, *Olax scandens* is easily available, cheap, and edible and the result of its bioassay is encouraging for future research. It can be used as an eco-friendly, bio-alternative agent in place of synthetic insecticides and can become a valuable tool in the mosquito control program. Further studies are undergoing on recognizing the active compounds, field trials etc.

6. Conflict of interests

The author declares that they have no conflict of interest.

7. References

1. Gratz NG. Critical review of the vector status of *Aedes albopictus*. Medical and Veterinary Entomology. 2004;18(3):215-227.
2. Paramanik M, Chandra G. Studies on seasonal fluctuation of different indices related to filarial vector, *Culex quinquefasciatus* around foothills of Susunia of West Bengal, India. Asian Pacific Journal of Tropical Medicine. 2010;3(9):727-730. https://doi.org/10.1016/S1995-7645(10)60174-5
3. WHO (World Health Organization). Dengue and severe dengue; c2022. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue (accessed 20.10.2022).
4. WHO. Global programme to eliminate lymphatic filariasis: progress report, 2021. Weekly epidemiological record No. 41. 2022;97:513-524.
5. Paramanik M, Sarkar N, Chandra G. Awareness and impact of Lymphatic Filariasis among the school children in rural endemic areas of West Bengal in India. Journal of Vector Borne Diseases. 2020;57(4):295-300. https://doi.org/10.4103/0972-9062.313970
6. Paramanik M, Sarkar N, Chandra G. Impact of Lymphatic Filariasis on married women from rural areas of Bankura District, West Bengal, India. Journal of Communicable Diseases. 2021;53(1):23-26. https://doi.org/10.24321/0019.5138.202105
7. Chandra G, Paramanik M. Effect of single to triple dose DEC on microfilaremics up to 5 years. Parasitology Research. 2008;103(6):1279-1282. https://doi.org/10.1007/s00436-008-1126-x
8. Senthil-Nathan S. A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes. Frontiers in Physiology. 2020;10:1591.
9. Paramanik M, Bhattacharjee I, Chandra G. Studies on breeding habitats and density of postembryonic immature filarial vector in a filarial endemic area. Asian Pacific Journal of Tropical Biomedicine. 2012;2(3):S1869-S1873. https://doi.org/10.1016/S2221-1691(12)60511-5
10. Raghavendra N, Sneha BD, Harisha CR, Acharya RN. A detailed pharmacognostical evaluation on leaf of *Olax scandens* Roxb. Global Journal of Research on Medicinal Plants & Indigenous Medicine. 2013;2(4):246.
11. Mukherjee S, Vinothkumar B, Prashanthi S, Bangal PR, Sreedhar B, Patra CR. Potential therapeutic and diagnostic applications of one-step in situ biosynthesized gold nanoconjugates (2-in-1 system) in cancer treatment. RSC Advances. 2013;3(7):2318-29.
12. Kamaraj C, Bagavan A, Rahuman AA, Zahir AA, Elango G, Pandiyan G. Larvicidal potential of medicinal plant extracts against *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). Parasitology Research. 2009;104(5):1163-1171.
13. Paramanik M, Paramanik S, Mukherjee S, Kapuri R, Dasmodak S. Larvicidal efficacy of the leaf and flower extracts of *Tropaeolum majus* L.(Tropaeolaceae) against the vector of lymphatic filariasis. International Journal of Mosquito Research. 2022;9(2):26-29. https://doi.org/10.22271/23487941.2022.v9.i2a.597
14. WHO. Guidelines for laboratory and field testing of mosquito larvicides. WHO: Geneva WHO/CDS/WHOPES/GCDP/13. 2005, 1-41.
15. Ishak AR, Dom NC, Hussain H, Sabri NH. Biolarvacidal potential of *Ipomoea cairica* extracts against key dengue vectors. Procedia-Social and Behavioral Sciences. 2014;153:180-188.

16. Govindarajan M, Benelli G. Facile biosynthesis of silver nanoparticles using *Barleria cristata*: mosquitocidal potential and biotoxicity on three non-target aquatic organisms. Parasitology Research. 2016;115(3):925-35.
17. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925;18(2):265-267.
18. Huang HT, Lin CC, Kuo TC, Chen SJ, Huang RN. Phytochemical composition and larvicidal activity of essential oils from herbal plants. Planta. 2019;250(1):59-68.
19. Rawani A, Haldar KM, Ghosh A, Chandra G. Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitology Research. 2009;105(5):1411-7.
20. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. The Indian Journal of Medical Research. 2012;135(5):581.
21. Ukoroije RB, Otayor RA. Review on the bio-insecticidal properties of some plant secondary metabolites: Types, formulations, modes of action, advantages and limitations. Asian Journal of Research in Zoology. 2020;3(4):27-60. http://dx.doi.org/10.9734/ajriz/2020/v3i430099
22. Mavundza EJ, Maharaj R, Chukwujekwu JC, Finnie JF, Van Staden J. Larvicidal activity against *Anopheles arabiensis* of 10 South African plants that are traditionally used as mosquito repellents. South African Journal of Botany. 2013;88:86-89.
23. Madpathi S, Bannoth RN. The efficacy of *Ximenia americana* plant mediated silver nanoparticles against dengue vector mosquito larvae [*Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Diptera: Culicidae)]. International Journal of Mosquito Research. 2021;8(5):48-56.
24. Paramanik M, Chandra G. Lymphatic filariasis in the foothill areas around Susunia of West Bengal in India. Asian Pacific Journal of Tropical Medicine. 2009;2:20-25. https://oaji.net/articles/2016/3555-1469588266.pdf
25. WHO. Vector-borne diseases. 2020. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases (accessed 20.10.2022).