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Larvicidal Activity Of Aedes Albopictus Collected From The Rubber Plantation Natural Ovitrap, Kanniyakumari District India

P. Blessy[1], Dr. E. Grace Marin[2], Dr. R. Mary Suja[3]
[1]Research Scholar, Department of Zoology and Research Centre, Scott Christian College (Autonomous), NagercoilAffiliated with Manonmaniam Sundaranar University Tirunelveli
[2.] Research Guide, Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil, Affiliated with Manonmaniam Sundaranar University Tirunelveli
[3.] Director Charithramalika Kerala Historical and Educational Research Museum, Amaravila Thiruvananthapuram, Kerala

Abstract
Mosquito remained as an important vector of different infectious diseases of viral or bacterial pathogens. Reliable identification and epidemiological investigation may require trustworthy tools with complementary information for their surveillance. Since only a handful of mosquito species play an important role in disease transmission precise mosquito identification and monitoring is essential for risk assessment and implementation of preventative strategies. The control of mosquito at larval stage is necessary and efficient in integrated pest management of mosquitoes. In particular, we focussed on the concept of larvicidal and ovicidal activity of Neustanthus phaseolodies and Hevea brasiliensis parts collected from the ovitrap with mosquito larvae to monitor the growth of mosquito population as these are generally more bio-degradable, less hazardous and rich storehouse of chemicals of diverse biological activity.
Key Words: *Mosquito, Neustanthus phaseolodies, Hevea brasiliensis, larvicidal, ovicidal, ovitrap*

INTRODUCTION

Mosquitoes are the most important vector of human disease in the tropic and are notoriously responsible for causing human health aliments (Qureshi et al., 2017). An alternative approach for mosquito control is the use of natural products such as microorganisms and plants. The microbial pesticides have undergone extensive testing prior to registration. They are essentially non-toxic to humans, so there are no concerns for human health effects. Since early times before the discovery of synthetic insecticides, many herbal products have been evaluated and they are used as natural insecticides. The botanicals such as Chrysanthemum, Pyrethrum, Derris, Quassia, Nicotine, Hellebore, Azadirachtin, Turpentine etc. have been reported to be used as plant-based insecticides in the pre-DDT era (Shaalan et al., 2005).

The natural products of plant origin with insecticidal properties have been used to control different types of insect pests and vectors. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water and thus, it is easy to deal with them in this habitat. During the last decades the effects of plant extracts were evaluated against different mosquito larvae species. The plant products can be used for killing larvae or adult or as repellents for the protection against mosquito (Kovendan et al., 2011).

A steady increase in disease ecology research has taken place in the last two decades (Collins et al., 2022). The world is gradually turning to eradicate mosquitoes using herbal formulations which are known to be effective against a wide range of diseases and ailments. More importantly, they are not known to cause any notable deprecating effects and are readily available at affordable prices. However, adding a note of caution stating that plant remedies is effective and without side effects but they have to be selected properly and taken under proper medical supervision. A decrease in the original land cover and replacement of native vegetation and an increase in urbanization may result in biotic homogenization with a dominant subset of species replacing the original diverse community (Perrin et al., 2022).

Mosquitoes are the most prevalent vector species, which can able to transmit diseases like malaria, dengue fever, yellow fever, filariasis, chickungunya etc. in worldwide (Matasyoh et al., 2011). It also causes some skin and systemic allergic reactions such as angioedema on human beings (Peng et al., 1997). In worldwide, the diseases like malaria, dengue and filariasis are the most significant cause of morbidity and mortality every year

(WHO, 1996). The climate change and human activity may influence mosquito abundance and biodiversity (Schrama et al., 2020 and Chaves et al., 2021). The advent of synthetic insecticides, plants and their derivatives were being used to kill the pests of agriculture, veterinary and public health importance. The insecticidal activity of plant-derived compounds has been evaluated and few of these was exploited commercially (Jacobson & Crosby, 1971). The insecticides of plant origin have been extensively used as agricultural pests to a very limited extent against insect's vectors of public health importance, which deserve careful and thorough screening (Madrigal et al., 1979).

During the immature stage, mosquitoes are relatively immobile (Rutledge et al., 2003). The larval control of mosquitoes either by the source of reduction, use of larvicides or both combinations are the preferred method for reducing adult mosquitoes in many areas of the World (Mulla et al., 2001). The chemical larvicides may also create environmental problems if they are lethal to non-target species (Shililu, 2001). Plants being a natural source of various compounds are known to contain larvicidal agents, which may act in combination or independently.

The average abdomen length of Aedes albopictus is 2.63 mm, with the wings being 2.7 mm and the proboscis being 1.88 mm. Some other morphological features are described by various investigators. In the case of a male, modified mouthparts and plumose antennae are present. The leg is black and each tarsal segment contains the white basal scales. The abdominal tergites are covered by dark scales, colour of scutum to be black that also contains a characteristic distinguished white stripe down the centre, beginning at the dorsal surface of the head that continues along the thorax. The length of this mosquito is about 2.0-10 m 10 mm with a striking black and white pattern, bold black shinny scales and silvery white scales on the tarsi and palpus.

Larvicide is the application of chemicals to kill mosquito larvae or pupae in the water. It is generally more effective and target-specific than applying chemicals to kill adult mosquitoes (adulticide). In controlling mosquito larvae, dichlorodiphenyltrichloroethane (DDT), organophosphatetemehos, methoprene, pyrethroids, phytochemicals and soil bacterium (Bacillus thuringiensis israelensis and Bacillus sphaericus) have been employed (Veerakumar et al., 2013). The conventional insecticides have created a number of ecological problems, such as the development of resistant insect strains, ecological imbalance and harm to mammals. The mosquito larvicidal activity tested against Aedes has been synthesized by using action bacterium (Parasit Dis, 2015).

MATERIALS AND METHODS

The larvicidal bioassay was assessed as per the World Health Organization (WHO) standard protocols (WHO, 1981).

Collection of mosquito egg

The eggs collected from the sampling sites were transferred to 18×22×5 cm wooden tray containing 1 litre of water for hatching.

Mass rearing of mosquitoes

The mosquito larvae were feeded with pedigree dog biscuits and yeast at 3:2 ratios the feeding continued until the larvae developed into the pupal stage. The pupae were collected from the glass beaker containing 300 ml of water with the help of pillar. The plastic glass was kept in 30×30×15 cm mosquito cage for adult emergence. The mosquito larvae were maintained at 27±2°C and 1% of sucrose with 10 µl of vitamin C solution was provided for two days between. The adult female mosquitoes were allowed to feed on the blood of chick (One chick exposed ones in per week) and after blood feeding the trays with water from the culture trays were placed in the cage as an oviposition substrate.

Rearing of sample plants

The collected samples were washed twice in water followed by tap water and double distilled water to remove the sand and other debris. After washing the samples were shade dried for ten days and dried samples are partially powdered by using domestic blender. The powdered samples were stored in air tight glass container for further laboratory uses.

Bioassay

According to the WHO (2005) bioassay test was preferred with the different concentrations to assess the larvicidal activity with slight variation. The bioassay was carried out in the plastic square well box (Size 2×2×2 Cm) (width × length × depth) as per (WHO, 2013) protocol. The different concentration of 0.125ppm, 0.25ppm, 0.50ppm, 1ppm and 2ppm crude were pour into the plastic boxes. Each concentration has maintained at 6 replications each replication contains 5 second and third instar larvae's of Aedes albopictus and pupae of Aedes albopictus were maintained in 4 replications and each replication consists of one pupa. The ovicidal activity of Aedes albopictus were calculated by dividing a single egg raft into four pieces and maintained in four replications. The control mortality was corrected by using Abbott's formula and percentage mortality is calculated as follows. Every 24, 48, 72, 96 and 120 days the mortality was recorded for larva and pupa and ovicidal mortality for Aedes albopictus was done. During the experimental time both the control and treatment larva were feed on yeast solution. The corrected mortality of mosquito larva was calculated using Abbott corrected formula for natural mortality in untreated control. The corrected mortality data was subjected to probit analysis using SPSS. Then alive larvae were maintained till pupation and emerged adult were maintained in plastic container with feed on sugar syrup for further data assessment.

Statistical analysis

The corrected mortality of mosquito larva was calculated using Abbott corrected formula for natural mortality in untreated control. The corrected mortality data was subjected to probit analysis using SPSS. Fiducidal limits (LC30, LC50, and LC90), regression coefficient, Chi – Square value and regression equation were recorded. Then the alive larvae were maintained till pupation and emerged adult were maintained in plastic container with feed on sugar syrup for further data are recorded. All the significance was expressed from 0.001 to 0.005 levels.

RESULTS

The dried leaf extracts of Neustanthus phaseoloides collected from the ovitrap observed on the 120th day showed 100% mortality on 96th day. Hevea brasiliensis dried leaves collected from the ovitrap showed 100% mortality rate on 120th day. Aedes albopictus third instar larvae subjected to the crude extract of Neustanthus phaseoloides and Hevea brasiliensis showed 100% mortality. The probit analysis revealed that the mortality rate of Aedes albopictus second instar tested with Neustanthus phaseoloides leaves showed LC_{50} of 0.324% and Hevea brasiliensis of LC_{50} 0.485% . On the other hand, observation of third instar Aedes albopictus larvae treated with Neustanthus phaseoloides leaves showed LC_{50} of 1.53% and Hevea brasiliensis LC_{50} of 1.984%.

The dose dependent mortality of pupa was also observed in Aedes albopictus. The obtained result of Neustanthus phaseoloides showed 100% mortality on 120th day. Hevea brasiliensis dried leaf extract of 100% mortality on 120th day. The pupa mortality of probit analysis confirmed LC_{50} value in Neustanthus phaseoloides of 1.818 % and Hevea brasiliensis of 2.235%. The ovicidal activity of Neustanthus phaseoloides dried leaf showed higher activity on 96th day and Hevea brasiliensis dried leaves showed higher activity on 24th day. The Probit analysis revealed that the ovicidal activity of Neustanthus phaseoloides dried leaf extract was highly toxic to Aedes albopictus eggs of LC_{50}=1.994% and Hevea brasiliensis extract of LC_{50} 1.205%.

Table: 1 Impact of Neustanthus phaseolodies dried leaves collected from the ovitrap against second instar larva of Aedes albopictus

Concentration	Days	Mean ± SE
0.125	24	5.00±2.24
	48	14.00±2.67
	72	28.00±2.91
	96	70.00±3.65
	120	91.00±3.14
0.25	24	8.00±2.00
	48	19.00±2.77

	72	41.00±3.48
	96	82.00±2.91
	120	96.00±2.21
0.5	24	22.00±4.90
	48	35.00±5.22
	72	67.00±3.35
	96	96.00±2.67
	120	100.00±0.00
1	24	52.00±10.09
	48	73.00±2.13
	72	96.00±1.63
	96	100.00±0.00
	120	100.00±0.00

Table: 2 Impact of Hevea brasiliensis dried leaves collected from the ovitrap against second instar larva of Aedes albopictus

Concentration	Days	Mean ± SE
0.125	24	3.00±1.53
	48	12.00±2.00
	72	20.00±2.58
	96	60.00±5.77
	120	79.00±3.14
0.25	24	5.00±1.67
	48	14.00±2.21
	72	34.00±4.27
	96	66.00±4.52
	120	84.00±2.21
0.5	24	17.00±3.00
	48	27.00±3.96
	72	52.00±4.90
	96	84.00±3.06
	120	94.00±2.21
1	24	42.00±5.12
	48	68.00±3.27
	72	81.00±4.58
	96	90.00±2.58
	120	100.00±0.00

Table: 3 Lethal concentration (LCs) (%) value of Neustanthus phaseolodies and Hevea brasiliensis dried leaves collected from the ovitrap on probit analysis data of Aedes albopictus second instar larva

	LC_{30}	LC_{50}	LC_{90}	CHI-SQ	Df	Significance
Neustanthus phaseolodies	-0.485	0.324	2.301	2.728	2.000	0.256
Hevea brasiliensis	-0.961	0.485	4.108	1.716	2.000	0.424

Table. 4 Impact of Neustanthus phaseolodies dried leaves collected from the ovitrap tested against Aedes albopictus during Third Instar larvae

Concentration	Days	Mean ± SE
0.125	24	1.00±1.00
	48	6.00±2.67
	72	18.00±3.89
	96	51.00±4.07
	120	67.00±5.17
0.25	24	4.00±1.63
	48	13.00±2.13
	72	24.00±2.67
	96	65.00±7.19
	120	78.00±1.33
0.5	24	10.00±1.49
	48	26.00±4.00
	72	43.00±6.67
	96	76.00±6.00
	120	88.00±3.27
1	24	35.00±8.98
	48	64.00±2.21
	72	80.00±4.94
	96	98.00±1.33
	120	97.00±1.53

Table: 5 Impact of Hevea brasiliensis dried leaves collected from the ovitrap against third instar larva of Aedes albopictus

Concentration	Days	Mean ± SE
0.125	24	0.00±0.00
	48	5.00±2.24
	72	14.00±1.63
	96	37.00±1.53
	120	56.00±6.18
0.25	24	2.00±1.33
	48	11.00±1.00
	72	23.00±2.13
	96	48.00±6.46
	120	72.00±2.49
0.5	24	8.00±2.00
	48	23.00±3.35
	72	37.00±4.96
	96	64.00±2.21
	120	79.00±3.79
1	24	27.00±5.59
	48	58.00±3.27
	72	77.00±3.67

	96	80.00±3.65
	120	91.00±2.33

Table: 6 Lethal concentration (LCs) (%) value of Neustanthus phaseolodies and Hevea brasiliensis dried leaves collected from the ovitrap on probit analysis data of Aedes albopictus third instar larva

	LC_{30}	LC_{50}	LC_{90}	CHI-SQ	Df	Significance
Neustanthus phaseolodies	0.152	1.153	3.600	8.412	2.000	0.015
Hevea brasiliensis	0.640	1.984	5.269	0.604	2.000	0.739

Table: 7 Impact of Neustanthus phaseolodies dried leaves collected from the ovitrap tested against pupa of Aedes albopictus

Concentration	Days	Mean ± SE
0.125	24	5.00±2.24
	48	14.00±2.67
	72	28.00±2.91
	96	34.00±2.67
	120	91.00±3.14
0.25	24	8.00±2.00
	48	19.00±2.77
	72	41.00±3.48
	96	49.00±3.14
	120	96.00±2.21
0.5	24	22.00±4.90
	48	35.00±5.22
	72	67.00±3.35
	96	75.00±3.42
	120	100.00±0.00
1	24	52.00±10.09
	48	73.00±2.13
	72	96.00±1.63
	96	97.00±1.53
	120	100.00±0.00

Table: 8 Impact of Hevea brasiliensis dried leaves collected from the ovitrap tested against pupa of Aedes albopictus

Concentration	Days	Mean ± SE
0.125	24	3.00±1.53
	48	12.00±2.00
	72	20.00±2.58
	96	23.00±2.13
	120	79.00±3.14
0.25	24	5.00±1.67

	48	14.00±2.21
	72	34.00±4.27
	96	42.00±1.33
	120	84.00±2.21
0.5	24	17.00±3.00
	48	27.00±3.96
	72	52.00±4.90
	96	68.00±1.33
	120	94.00±2.21
1	24	42.00±5.12
	48	68.00±3.27
	72	81.00±4.58
	96	88.00±2.00
	120	100.00±0.00

Table: 9 Lethal concentration (LCs) (%) value of Neustanthus phaseolodies and Hevea brasiliensis dried leaves collected from the ovitrap on probit analysis data of Aedes albopictus pupa

	LC_{30}	LC_{50}	LC_{90}	CHI-SQ	Df	Significance
Neustanthus phaseolodies	1.052	1.818	3.639	6.007	2.000	0.050
Hevea brasiliensis	1.414	2.235	4.243	0.410	2.000	0.815

Table: 10 The ovicidal mortality of Aedes albopictus in Neustanthus phaseolodies dried leaves collected from the ovitrap

Concentration	Days	Mean ± SE
0.125	24	27.00±2.13
	48	6.00±2.67
	72	18.00±3.89
	96	51.00±4.07
	120	67.00±5.17
0.25	24	54.00±1.63
	48	13.00±2.13
	72	24.00±2.67
	96	65.00±7.19
	120	78.00±1.33
0.5	24	72.00±2.00
	48	26.00±4.00
	72	43.00±6.67
	96	76.00±6.00
	120	88.00±3.27
1	24	83.00±1.53
	48	64.00±2.21
	72	80.00±4.94
	96	98.00±1.33

	120	97.00±1.53

Table: 11 The ovicidal mortality of Aedes albopictus in Hevea brasiliensis dried leaves collected from the ovitrap

Concentration	Days	Mean ± SE
0.125	24	48.00±2.00
	48	5.00±2.24
	72	14.00±1.63
	96	37.00±1.53
	120	56.00±6.18
0.25	24	64.00±2.21
	48	11.00±1.00
	72	23.00±2.13
	96	48.00±6.46
	120	72.00±2.49
0.5	24	76.00±3.06
	48	23.00±3.35
	72	37.00±4.96
	96	64.00±2.21
	120	79.00±3.79
1	24	93.00±1.53
	48	58.00±3.27
	72	77.00±3.67
	96	80.00±3.65
	120	91.00±2.33

Table: 12 Lethal concentration (LCs) (%) value of Neustanthus phaseolodies and Hevea brasiliensis on probit analysis data of Aedes albopictus eggs

Treatment	LC_{30}	LC_{50}	LC_{90}	CHI-SQ	Df	Significance
Neustanthus phaseolodies	0.992	1.994	4.444	1.673	2.000	0.433
Hevea brasiliensis	0.085	1.205	3.940	1.873	2.000	0.392

DISCUSSION

Mosquito larvicidal activity is the use of insecticides to kill mosquito larvae and pupae before they become adult mosquitoes. The leaves of T. procumbens are more effective repellent at 6 percent concentration against An. stephens. Rajkumar et al., (2007) reported the methanol extracts and purified compounds of A. indica leaves were found to have terpenoid, saponin and other pesticide compounds showed the potential source of the leaf extract against Anopheles stephensi (Lingathurai et al., 2011). The dried leaf extracts of Neustanthus phaseoloides and Hevea brasiliensis showed a dose dependent mortality rate of second instar stage larvae, observed on the 120th day showed 100% mortality at a concentration of (0.5 & 1) and the observation of 96th day showed 100% mortality at a concentration of (1) and Hevea brasiliensis dried leaves collected from the ovitrap showed 100% mortality rate on 120th day at a

concentration of 1. On the other hand, 100% mortality was noticed in Aedes albopictus third instar larvae subjected to the crude extract of Neustanthus phaseoloides and Hevea brasiliensis.

The probit analysis revealed that the mortality rate of Aedes albopictus second instar tested with Neustanthus phaseoloides leaves showed LC_{50} of 0.324% and Hevea brasiliensis of LC_{50} 0.485%. On the other hand, observation of third instar Aedes albopictus larvae treated with Neustanthus phaseoloides leaves showed LC_{50} of 1.53% and Hevea brasiliensis of LC_{50} 1.984%. The larvae dose dependent activity, mortality of pupa was also observed in Aedes albopictus. The obtained result of Neustanthus phaseoloides showed 100% mortality on 120^{th} day at a concentration of (0.5 & 1) and Hevea brasiliensis dried leaf extract caused 100% mortality on 120^{th} day of observation at a concentration of (1). The pupa mortality of probit analysis confirmed LC_{50} value in Neustanthus phaseoloides of 1.818 % and Hevea brasiliensis of 2.235 % showed good larval mortality rate. The Probit analysis revealed that the ovicidal activity of Neustanthus phaseoloides dried leaf extract was highly toxic to Aedes albopictus eggs of LC_{50}=1.994% and Hevea brasiliensis extract of LC_{50} 1.205%.

Gokulkrishnan et al., (2012) reported the larvicidal and ovicidal efficacy of different solvent leaf extract of Aritolochia indica against Anopheles stephensi. The larvicidal activity of Croton spariflorus leaf extract showed maximum larvicidal activity in ethyl acetate. The larvicidal activity of Neustanthus phaseoloides and Hevea brasiliensis leaves may be due to the presence of active compounds. Mosquito remained as an important vector of different infectious diseases of viral or bacterial pathogens. Reliable identification and epidemiological investigation require trustworthy tools with complementary information for their surveillance. There are numerous mosquito borne viruses transmitted by Aedes aegypti and Aedes albopictus such as; Dengue virus, chikungunya and they have been reported in Bangladesh.

Summary and Conclusion

Since only a handful of mosquito species play an important role in disease transmission precise mosquito identification and monitoring is essential for risk assessment and implementation of preventative strategies. In conclusion, our findings showed that the plant parts of Neustanthus phaseoloides and Hevea brasiliensis leaves collected from the natural ovitrap can be developed as ecofriendly larvicides. The present findings unlock the possibility for further investigations to assess the efficacy of larvicidal properties from natural products.

REFERENCES

1. Chaves, LF & Friberg, MB 2021, 'Aedes albopictus and Aedes flavopictus (Diptera: culicida) pre-imaginal abundance patterns are associated with different environmental factors along an altitudinal gradient, 'Current Research in Insect Science, 1: 100001.

2. Collins Emma, L, Jody, E, PhelanMagdalena, Hubner , Anton Spadar , Monica Campos , Daniel Ward , Holly Acford-Palmer , Ana Rita Gomes , Keily Silva, Lara Ferrero Gomez , Taane, G, Clark& Susana Campino 2022, 'A next generation targeted amplicon sequencing method to screen for insecticide resistance mutations in Aedes aegypti populations reveals mutation in mosquitoes from Cabo Verde, 13: 16 (12).

3. Jacobson, M & Crosby, DG 1971, 'Insectcidal activities of ar-Turmerone identification in Curcuma longa rhizome against Nilaparvatalugens (Hmopteradelphasidae and Plutellaxylostella (Lepidoptera yponomeutidae.Journal of Asia Pacific Entomology 4:2 181-185.

4. Kovendan K and Murugan K 2011,'Effect of medicinal plants on mosquito vectors from the agro-climatic regions of Tamil Nadu, India. Adv. Environ. Biol. 5: 335-344.

5. Madrigal, RV, Knapp, FE, Soiigafus R and CR, Smith Jr., 1979. Fractitionation of extracts of Lithospermam arvense and their activity against mosquito larae. Mosq. News 39: 536-540.

6.. Matasyoh, JC, Dittrich, B, Schueffler, A&Laatsch, H 2011, 'Larvicidal activity of metabolites from the endophytic Podospora sp. against the malaria vector Anopheles gambiae, Parasitol Res, 108: 561-566.

7. Mulla, S, Thavara, U, Tawatsin, A, Kong-ngamsuk, W, Chompoosri, J and Su, T 2001, 'Mosquito larval control with Bacillus sphaericus reduction in adult population in low income communities in Nonthaburi Province, Thailand, J, of Vec. Ecol, 26 (2): 221-231.

8. Parasit Dis 2015, 39(4) 677-684 'Journal of Parasitic Diseases. Mosquito larvicidal activity of silver nanoparticles synthesised using actinobacterium Streptomyces sp. M25 against Anohelessubpictus, Culex quinquefasciatus and Aedes aegypti.

9. Peng, Z. & Estelle, R 1997, 'Cross reactivity of skin and serum specific IgE responses and allergen analysis for three mosquito, 'Journal of Allergy and Clinical Immunology' 100 (2): 192-198.

10. Perrin Antoine, Olivier Glaizot, Philippe & Christe, 2022, 'Worldwide impacts of landscape anthropization on mosquito abundance and diversity: A meta-analysis. Global Change Biology. 2(3).

11. Qureshi, E.M, Tabinda, A.B. and Vehra, S 2017, 'The distribution of Aedes aegypti (Diptera, Culicidae) in eight selected parts of Lahore using oviposition traps during rainy season, Journal of the Pakistan Medical Association, 67 (10): 1493-1497.
12. Rutledge, CR, Clarke, F, Curtis, A and Sackett, S 2003, 'Larval mosquito control Technical Bulletin of the Florida Mosquito Control Association 4.
13. Schrama, M, Hunting, ER, Beechler, BR, Guaridom, M, Govender, D & Nijland, W, 'Human practices promote presence and abundance of disease-transmitting mosquito species, 'Science Reports, 2020.
14. Shaalan E, Canyon, D, YounesM, Wahab H and Mansour 2005,'A review of botanical phytochemicals with mosquitocidal potential. Environ Int 2005 (8): 149-66.
15. Shililu, J 2001,'Eritrea field studies on efficacy of bacterial larvicides for use in malaria control. Activity Report, 112: 1-26.
16. Veerakumar K, Govindarajan M and M, Rajeswary, 'Green synthesis of silver nanoparticles using Sidaacuta (Malvaceae) leafextractagainst Culex quinquefasciatus, Anophelesstephensi, and Aedesaegypti (Diptera: Culicidae)," Parasitology Research, vol.112,no.12,pp.4073–4085,2013.
17. WHO, 1996. Disease Outbreak News India.