International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 12-11-2022, Accepted: 30-11-2022, Published: 15-12-2022
Volume 7, Issue 12, 2022, Page No. 92-96

# Larvicidal action of *Prunus persica* against third instar larvae of *Aedes aegypti* (Diptera: Culicidae)

**Anil Kumar[1], Hridayesh Arya[2]**
[1] Research Scholar, Department of Zoology, N.R.E.C. College, Khurja, Bulandshahr, Uttar Pradesh, India
[2] Associate Professor, Department of Zoology, N.R.E.C. College, Khurja, Bulandshahr, Uttar Pradesh, India

**Abstract**
*Aedes (Stegomyia) aegypti* is the primary vector of the dengue virus and infects millions of people throughout the world. The dengue vector has become resistant due to the extensive use of synthetic chemical insecticides and has greatly diminished insecticide efficacy to control mosquito vectors. Thus, the search for new alternative control measures is essential, that can prevent insecticide resistance or decrease the utilization of such chemicals. The aim of the present investigation was to evaluate the larvicidal potential of *Prunus persica (Thunb.)* leaf extracts against the third instar of *Aedes aegypti* larvae. The aqueous, ethanolic, and petroleum ether extracts of the leaf were successively tested against the dengue vector. The aqueous extract of the leaf of *Prunus persica* exhibits the highest larvicidal activity at 500 ppm with $LC_{50}$ and $LC_{90}$ values of 179.78 and 524.80 ppm, respectively. Similarly, the petroleum ether extract of the leaf exhibits the highest larvicidal potential at 150 ppm with $LC_{50}$ and $LC_{90}$ values of 48.97 and 93.32 ppm, respectively and the ethanolic extract of the leaf showed the highest larvicidal activity at 300 ppm with $LC_{50}$ and $LC_{90}$ values of 79.43 and 181.97 ppm, respectively within 24 h post-exposure. All the treatments showed significant ($P<0.05$) larvicidal activity. Log probit analysis at 95% confidence level reveals the $LC_{50}$ and $LC_{90}$, and the results of regression analysis were shown the mortality rate (Y- dependent variable) was positively correlated with the concentration (X- Independent variable). In conclusion, the plant *Prunus persica* may be a good candidate to be utilized in control programs against the dengue vector, *Aedes aegypti*.

**Keywords:** aqueous extract, *Prunus persica*, *Aedes aegypti*, larvicidal action, ethanolic extract, petroleum ether extract

## Introduction

*Aedes aegypti* is a primary vector of the viral disease dengue and constitutes a severe public health threat on a global scale (WHO, 2009) [35]. Globally, an estimated million people get infected with dengue (WHO, 2021) [34] and about ten lakh cases and three hundred deaths were reported in India, in the last five years (MOHFW, 2021) [24].

Extensive use of chemical insecticides has led to Insecticide resistance in mosquitoes and has greatly diminished insecticide efficacy to control mosquito vectors (Fonseca-Gonzalez *et al.*, 2011; Grisales *et al.*, 2013; David *et al.*, 2014) [13, 15, 10]. Chemical insecticides are nonbiodegradable and cause environmental pollution (Benelli, 2015) [4], affecting the nontarget organism including human beings (Sharma *et al.*, 2016) [29]. Thus, the search for alternative control measures that can prevent insecticide resistance or decrease the utilization of such chemicals in insect vector control is essential. Plant-based bioinsecticides are good alternatives to chemical insecticides, biodegradable, and less toxic to non-target organisms (Sukumar *et al*, 1991) [32]. Approximately 344 species of plants have been reported to encompass diverse activities against mosquitoes (Ghosh *et al.*, 2012; Ali *et al.*, 2015; Hikal *et al.*, 2017) [14, 2, 18].

According to previous research on different plants, using different solvents, phytochemicals extracted from *Argemone mexicana* (Warikoo and Kumar, 2014) [33] *Asparagus setaceus* (Chakraborty, 2021) [7] *L. camara*, *H. suaveolens*, *N. oleander* and *T. stans* (Hari and Mathew, 2018) [17] *Maerua siamensis* (Nobsathian *et al.*, 2018) [25] have been shown to have larvicidal potential against the larvae of different mosquitoes also including *Aedes aegypti*.

*Prunus persica (L.)* Batsch is a member of the family -Rosaceae and the subfamily -Amygdaloidea and is mainly found in India, China, and Spain. Commonly known as peach, for its edible fruits largely grown in warm, temperate, and subtropical climates. Peach has numerous anti-disease qualities, including antimicrobial, antiallergic, anticancer, antibacterial, antitumor, antioxidant, and anti-inflammatory properties. The peach plant's leaves are used to cure leukoderma and whooping cough, as well as an antihelmintic, insecticidal, vermicidal, sedative, and laxative. (Dhingra *et al.*, 2014; Kant *et al.*, 2018; Haleema *et al.*, 2020; Catarina *et al.*, 2022) [11, 20, 16, 6].

With the aforesaid qualities and prospective mosquito control potential of *Prunus persica* in mind, the leaves were tested for their larvicidal activity against *Aedes aegypti* larvae. The investigations were carried out with the goal of fostering research aimed at the development of novel eco-friendly alternatives for mosquito control based on biologically active plant sources.

## Material and methodology

### Collection and preparation of plant extracts

The plant *Prunus persica* was chosen based on its local availability and known medicinal properties. The leaves were collected from healthy plants from the district Bulandshahr region, U.P, India, in the month of October 2021 and brought to the laboratory for subsequent processing. The leave was washed through distilled water and allowed to dry in a shaded place for three weeks at 30ºC. An electric grinder was used to make a fine powder of dried leaves. *Prunus persica* 500 g powder was extracted in two solvents, ethanol, and petroleum ether by using the glass Soxhlet extraction apparatus, separately. The extraction was carried out for 24 h and the temperature of the Soxhlet extractor was set according to the boiling points of the solvents (Kasiramar, 2018) [21]. After 24 h, the residual solvent evaporated to make the solvent-free extract. The total residue of the leaf was weighed and redissolved in distilled water to obtain the desired concentration. A stock solution containing 2000 ppm was stored at 4ºC for future use. The control consisted of distilled water with 5% ethanol. To prepare the aqueous extract, fresh leaves of the plant were crushed in a Remi-Udyog glass homogenizer to form a thick paste. Leaf paste diluted with distilled water and stirred on a magnetic stirrer for 4 h at 30ºC and allowed to stand for 48 h. Later this suspension was well agitated and filtered through a Whatman filter paper-1. A stock solution of 20% concentration was prepared. The control consisted of distilled water only.

### *Aedes aegypti* larvae culture

*Aedes aegypti* eggs and larvae were collected with the help of oviposition traps from district Bulandshahr and subdistrict region Khurja, Utter Pradesh, India. Larvae reared in the research laboratory of the department of zoology, N.R.E.C. College, Khurja. Identification of *Aedes aegypti* larvae was done morphologically (Christophers, 1960; Rueda, 2004) [9, 27]. Eggs were hatched into larvae in 500 mL of dechlorinated water for 30 to 60 minutes. A plastic tray (20 cm × 15 cm × 5 cm) was used to culture the larvae and powdered brewer's yeast, powdered rice, and soybean powder in a ratio of 3:1:1 was used to feed the larvae. Maintain at 27 ± 5°C, Relative humidity 75 ± 5%. A photoperiod of 14 h light and 10 h dark appears to allow the best uniform development (Kamraj *et al*., 2009) [19].

### Larvicidal bioassay

The larvicidal activity of the selected plant *Prunus persica* was assessed by the protocol of WHO (WHO, 2005) [36]. Five replicates of *Aedes aegypti* third-stage larvae were used for the bioassay testing. Ten mL of Tween-20 (emulsifying agent) was added to a 2000 ppm stock solution and desired concentrations were prepared from this solution. Plant extracts (from stock solution) that produced 100% larval mortality during the initial testing were serially diluted (25, 50, 75, 100, 125, 150 ppm and 25, 50, 100, 150, 200, 250, 300 ppm, and 50, 100, 200, 300, 400, 500 ppm) in distilled water and LC50 determined. Twenty, third-instar larvae were kept in 250 mL of a glass beaker with 150 mL of plant extracts, for the bioassay. After 24 h post-exposure, the mortalities of *Aedes aegypti* larvae were determined and percentage mortality was computed from the five replicates. Two control groups, one with distilled water and the second with 5% ethanol and distilled water, were used for the testing of aqueous and ethanolic extracts, respectively.

### Statistical analysis

Probit analysis (Finney, 1952) [12] was performed on *Aedes aegypti* third instar larvae mortality data collected after 24 h post-exposure to extracts from plant species using the software MS-Excel 2021. The percentage of mortality was corrected by using Abbott's formula (Abbott, 1925) [1].

### Result

The obtained results documented in table 1 revealed that the aqueous, petroleum ether, ethanolic extract of *Prunus persica* showed significant larvicidal action against the third instar of *Aedes aegypti* larvae. The aqueous extract of the leaf of *Prunus persica* exhibits the highest larvicidal activity at 500 ppm with LC50 and LC90 values of 179.78 and 524.80 ppm, respectively (P<0.001, Y=2.6607x-0.9783). Similarly, the petroleum ether extract of the leaf exhibits the highest mortality at 150 ppm with LC50 and LC90 values of 48.97 and 93.32 ppm, respectively (P<0.009, Y=4.5831x-2.7678) and the ethanolic extract of the leaf were showed highest larvicidal activity at 300 ppm with LC50 and LC90 values of 79.43 and 181.97 ppm, respectively (P<0.002, Y=3.5217x-1.6494). No mortality was observed in the control group. The petroleum ether extract of the leaf was shown the highest larvicidal potential than the aqueous and ethanolic extracts (petroleum ether extract>ethanolic extract>aqueous extract). The result of larvicidal bioassay at different concentrations presented in figure 1-3, showed a significant increase in mortality with the increase of concentration in all treatments.

**Table 1:** Larvicidal action of leaf extract of *Prunus persica* against the third instar of *Aedes aegypti* larvae.

| Mosquito species | Solvents | Conc. (PPM) | Mortality (%) | LC50 (PPM) | LC90 (PPM) | 95% confidence interval | | Regression equation | $R^2$ | P-value (P<0.05) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 24 h | 24 h | 24 h | Lower bound | Upper bound | | | |
| *Aedes aegypti* | Aqueous extract | 500 | 100±0.0 | 173.78 | 524.80 | 1.6897 | 3.3631 | Y=2.6607x-0.9783 | 0.94 | 0.001 |
| | | Control | 00±00 | | | | | | | |
| | Petroleum ether | 150 | 100±0.0 | 48.97 | 93.32 | 1.8712 | 7.2950 | Y=4.5831x-2.7678 | 0.85 | 0.009 |
| | | Control | 00±00 | | | | | | | |
| | Ethanolic extract | 300 | 100±0.0 | 79.43 | 181.97 | 1.9451 | 5.0984 | Y=3.5217x-1.6494 | 0.87 | 0.002 |
| | | Control | 00±00 | | | | | | | |

### Discussion

Plants are a rich source of complex phytochemicals, act synergistically and exhibit different biological activities on mosquitoes (Maurya *et al.*, 2012) [23]. This involves the development of bioactive substances that interact at various stages of an insect's life cycle, which includes the egg, larva, pupa, and adult stages. Plant-derived bioinsecticides rarely cause the insect to become resistant. Phytochemicals may serve as a suitable alternative to synthetic chemical insecticides and are eco-friendly, inexpensive, and readily available throughout the world (Sukumar *et al.*, 1991; Ghosh *et al.*, 2012) [32, 14].

This study showed that the *Prunus persica* aqueous, petroleum ether, and ethanolic extract of leaves might have larvicidal properties that can kill *Aedes aegypti* larvae. Aqueous, petroleum ether and ethanolic extracts achieved the highest larval mortality at 500, 150, and 300 ppm, and the least larvicidal activity was observed at 50, 25, and 25 ppm respectively (figure 1-3). In comparison, petroleum

ether extract showed more larvicidal potential at a lower concentration than the ethanolic and aqueous extracts.
Previous studies on *Prunus domestica* have reported that the petroleum ether, chloroform, and methanolic extracts of leaf demonstrated larvicidal activity against *C. pipiens* third instar larvae with LC50 values of 33.30, 70.8, and 132.70 ppm, respectively in 24 h (Shehata, 2019) [30]. In another example, extracts prepared from *Prunus persica* have previously been tested against the house fly *Musca domestica (L.)*, which showed moderate larvicidal activity (Seo and Park, 2011) [28].

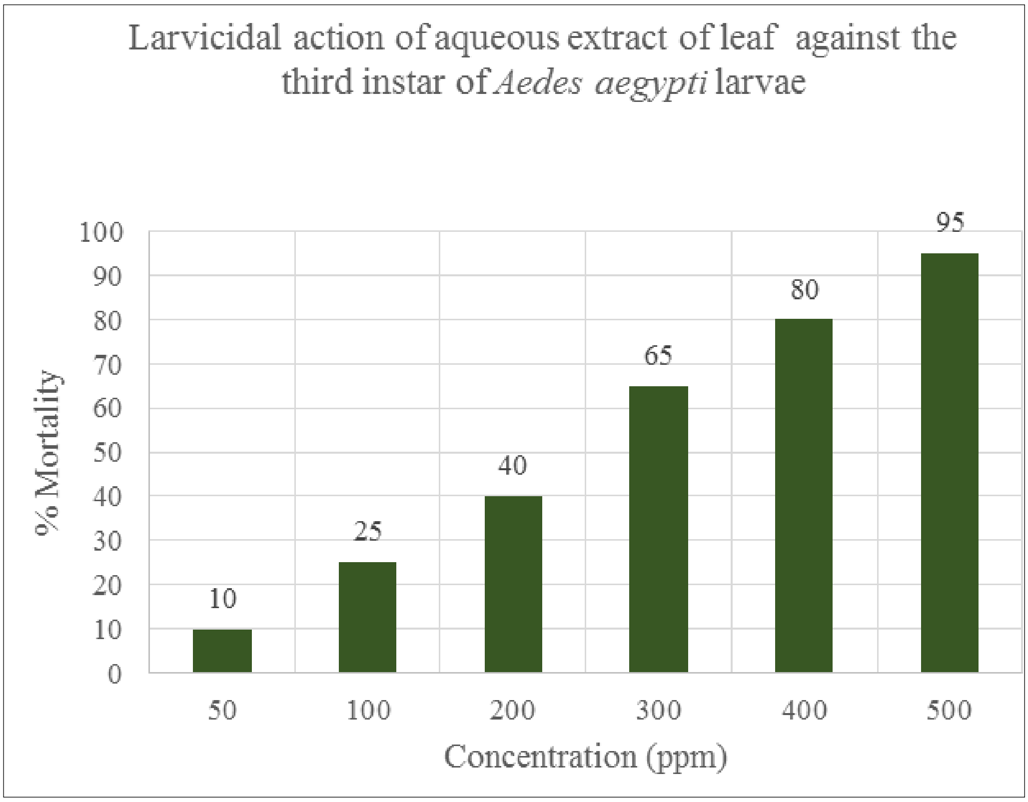


**Fig 1:** Larvicidal action of aqueous extract.

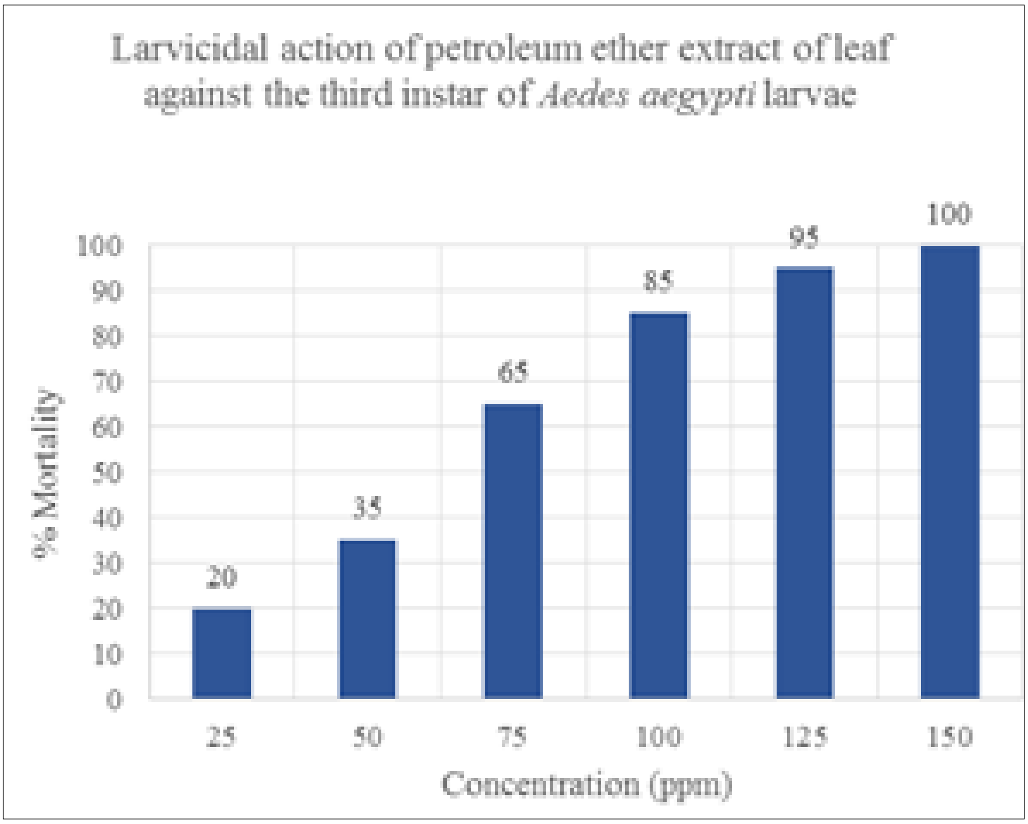


**Fig 2:** Larvicidal action of Petroleum ether extract.

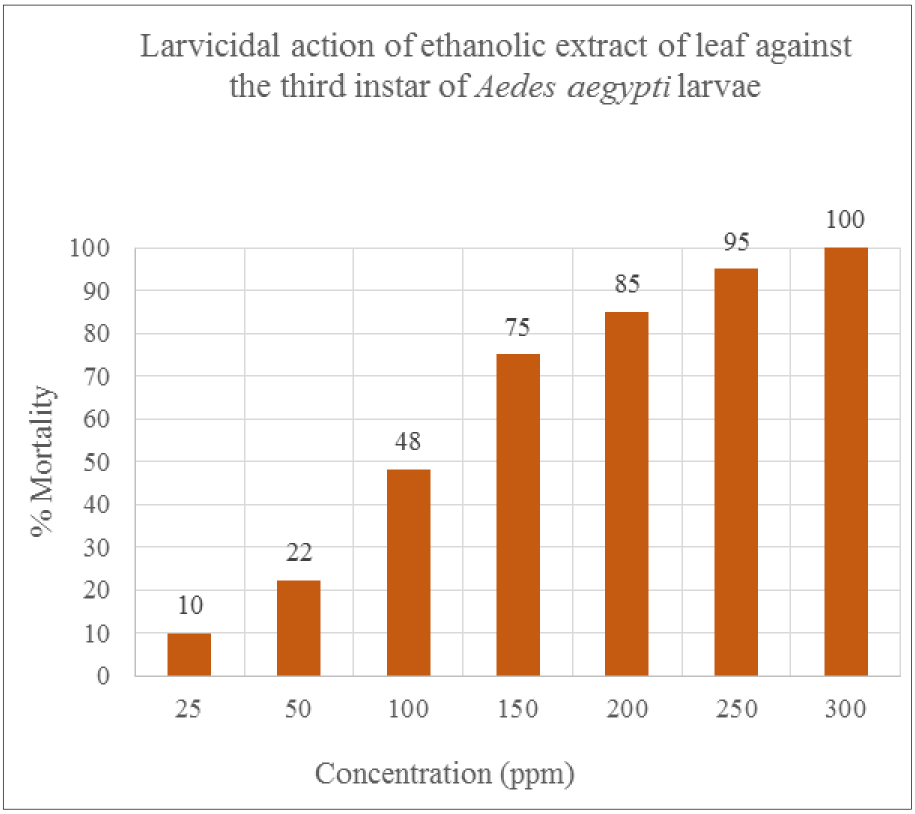


**Fig 3:** Larvicidal action of ethanolic extract.

However, many researchers have found that phytochemicals derived from different plants showed larvicidal potential against different medically significant mosquito species including *Aedes aegypti* (Kumar *et al.*, 2012) [22]. *Vitex ovata* methanolic extract at 5000 ppm and 10000 ppm achieved 76% and 84% larval mortality within 24 h, against *Aedes aegypti* (Aziz, 2021) [3], and the leaf and seed extracts of *Ricinus cummunis* prepared in solvent methanol exhibit larvicidal potential with LC50 and LC90 values of 9.37, 31.1 ppm, and 15.52, 45.24 ppm respectively against *Aedes aegypti* (Sogan, 2018) [31]. Furthermore, Chandrasekaran (2019) [7] reported that V. trifolia oils have good larvicidal potential against *Aedes aegypti* and *C. quinquefasciatus*. And methanolic extract prepared from the flower of *Clitoria ternatea* was shown to have larvicidal activity against *Aedes aegypti* (Ravindran, 2020) [26].

## Conclusion
In conclusion, the plant *Prunus persica* here studied, ethanolic and petroleum ether extracts of leaf showed significant larvicidal potential. Maybe a good candidate to be utilized in vector control programs against the dengue vector, *Aedes aegypti*. Nowadays, environmental protection is seen as critical. Eco-friendly insecticides with adequate mortality on target species to keep insect populations below the threshold level should be encouraged. However, the mechanism involved in causing the mortality of mosquito larvae is still unknown and needs to be studied further and needed to identify, purify, and isolate the key chemicals that kill mosquitos.

## Acknowledgment
The authors acknowledge N.R.E.C. College, Management, Dr Vipin Singh, principal and Head, Zoology Department and we are thankful to all our colleagues and friends for their help and encouragement during this work.

## Conflict of interest
In relation to the publishing of this paper, the authors certify that there are no conflicts of interest.

## References
1. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 1925:18(2):265–267.
2. Ali A, Tabanca N, Demirci B, Blythe EK, Ali Z, Baser KHC, *et al.* Chemical composition and biological activity of four *Salvia* essential oils and individual compounds against two species of mosquitoes. Journal of Agriculture and Food Chemistry,2015:63:447-456.
3. Aziz M, Hashan AEI, Muhammad DNI, Ishak IH, Hamdan RH, Syazwan SA, *et al*. Larvicidal effect of *Vitex ovata* Thunb. (lamiales: Lamiaceae) leaf extract towards *Aedes (stegomyia) aegypti* (Linnaeus, 1762) (Diptera: Culicidae). *Parasitologia*,2021:1(4):210–217.
4. Benelli G. Research in mosquito control: Current challenges for a brighter future. Parasitology Research,2015:114(8):2801–2805.
5. Brathwaite Dick O, San Martín JL, Montoya RH, del Diego J, Zambrano B, Dayan GH. The history of dengue outbreaks in the Americas. Am J Trop Med Hyg.,2012:87:584-93.
6. Catarina Bento Ana C, Gonçalves BS & Luís RS. Peach (*Prunus Persica*): Phytochemicals and Health Benefits, Food Reviews International,2022:38:8:1703-1734.
7. Chakraborty, S., Singh, A., & Chandra, G. (2021). Larvicidal activity of different plant parts of asparagus setaceus against dengue vector *Aedes aegypti*. International Journal of Mosquito Research, 2021:*8*(5):01–06.

8. Chandrasekaran T, Thyagarajan A, Santhakumari PG, Pillai AKB, Krishnan UM. Larvicidal activity of essential oil from *Vitex negundo* and *Vitex trifolia* on dengue vector mosquito *Aedes aegyptii*. *Revista Da Sociedade Brasileira de Medicina Tropical*,2019:*52*. https://doi.org/10.1590/0037-8 (Placeholder1)682-0459-2018
9. Christophers SR. *Aedes aegypti* (L.) The yellow fever mosquito. Its life history, bionomics and structure. *Cambridge University Press*, 1960.
10. David JP, Faucon F, Chandor-Proust A, Poupardin R, Riaz MA, Bonin A, *et al*. Comparative analysis of response to selection with three insecticides in the dengue mosquito *Aedes aegypti* using mRNA sequencing. *BMC Genomics*, 2014:15:174.
11. Dhingra N, Sharma R, Kar A. Towards further understanding on the antioxidative activities of *Prunus persica* fruit: A comparative study with four different fractions, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*,2014:132:582-7.
12. Finney DJ. Probit analysis; statistical treatment of the sigmoid response curve. Macmillan, 1947.
13. Fonseca-González I, Quiñones ML, Lenhart A, Brogdon WG. Insecticide resistance status of *Aedes aegypti* (L.) from Colombia. *Pest Manag Sci*.,2011:67:430-7.
14. Ghosh A, Chowdhury N, & Chandra G. Plant extracts as potential mosquito larvicides. The Indian journal of medical research,2012:*135*(5):581–598.
15. Grisales N, Poupardin R, Gomez S, Fonseca-Gonzalez I, Ranson H, Lenhart A. Temephos resistance in *Aedes aegypti* in Colombia compromises dengue vector control. *PLoS Negl Trop Dis*.,2013:7(9): e2438.
16. Haleema R, Shenoy A, Shabaraya AR. A review on pharmacological activities of *Prunus persica*. *Int J Pharma Sci Rev Res*.,2020:60(2):38-40.
17. Hari I, Mathew N. Larvicidal activity of selected plant extracts and their combination against the mosquito vectors *Culex quinquefasciatus* and *Aedes aegypti*. Environmental Science and Pollution Research, 2018:25(9):9176–9185.
18. Hikal WM, Baeshen RS, & Said-Al Ahl HAH. Botanical insecticides as simple extractives for pest control. Cogent Biology,2017:3:1.
19. 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.
20. Kant R, Shukla R, Shukla A. A Review on Peach (*Prunus persica*): An Asset of Medicinal Phytochemicals. International Journal for Research in Applied Science and Engineering Technology, 2018:6:2186-2200.
21. Kasiramar G. Significant Role of Soxhlet Extraction Process in Phytochemical Research. Mintaje Journal of Pharmaceutical & Medical Sciences,2018:7:43-47.
22. Kumar S, Wahab N, Mishra M, Warikoo R. Evaluation of 15 local plant species as larvicidal agents against an Indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). Frontiers in Physiology, 2012:3.
23. Maurya P, Sharma P, Mohan L, Verma MM, Srivastava CN. Larvicidal efficacy of *Ocimum basilicum* extracts and its synergistic effect with neonicotinoid in the management of *Anopheles stephensi*. Asian Pacific Journal of Tropical Disease,2012:*2*(2):110–116.
24. Ministry of Health & Family Welfare-Government of India (MOHFW). National Center for Vector Borne Diseases Control (NCVBDC), 2021. From https://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715.
25. Nobsathian S, Bullangpoti V, Kumrungsee N, Wongsa N, Ruttanakum D. Larvicidal effect of compounds isolated from *Maerua siamensis* (Capparaceae) against *Aedes aegypti* (Diptera: Culicidae) larvae. *Chem Biol Technol Agric*, 2018:5:1-7.
26. Ravindran DR, Bharathithasan M, Ramaiah P, Rasat MSM, Rajendran D, Srikumar S, *et al.* Chemical composition and larvicidal activity of flower extracts from *Clitoria ternatea* against *Aedes* (Diptera: Culicidae). Journal of Chemistry, 2020, 1–9.
27. Rueda L. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission. *Zootaxa*, 2004:589.
28. Seo SM, Park IK. Larvicidal activity of medicinal plant extracts and lignan identified in *Phryma leptostachya* var. asiatica roots against housefly (*Musca domestica* L.). Parasitology research. 2011:110:1849-53.
29. Sharma AK, Tiwari U, Gaur MS, & Tiwari RK. Assessment of malathion and its effects on leukocytes in human blood samples. Journal of Biomedical Research, 2016:*30*(1):52–59.
30. Shehata A. Biological Activity of *Prunus Domestica* (Rosaceae) and *Rhamnus Cathartica* (Rhamnaceae) leaves extracts against the Mosquito Vector, *Culex Pipiens* L. (Diptera: Culicidae). Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control,2019:11:65-73.
31. Sogan N, Kapoor N, Singh H, Kala S, Nayak A, Nagpal BN. Larvicidal activity of *Ricinus communis* extracts against mosquitoes. Journal of Vector-Borne Diseases, 2018:55(4):282-290.
32. Sukumar K, Perich MJ, & Boobar LR. Botanical derivatives in mosquito control: a review. Journal of the American Mosquito Control Association, 1991:7(2):210–237.
33. Warikoo R, Kumar S. Impact of the *Argemone mexicana* stem extracts on the reproductive fitness and behavior of adult dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). International Journal of Insect Science, 2014:*6*, IJIS.S19006.
34. World Health Organization (WHO). Dengue and Severe Dengue, 2021. Available online: https://www.who.int/news-room/q-adetail/dengue-and-severe-dengue.
35. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization; 2009. [Online] Available from: https://www.who.int/csr/resources/publications/dengue_9789241547871/en
36. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides, 2005. Available online: https://apps.who.int/iris/handle/10665/69101