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ASSESSMENT OF LARVICIDAL EFFICACY OF TROPICAL PLANTS PARTHENIUM HYSTEROPHORUS, HYPTIS SUAVEOLENS AND MENTHA ARVENSIS AQUEOUS LEAF EXTRACTS AGAINST MOSQUITO AEDES AEGYPTI

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ABSTRACT

Aedes aegypti spreads many dangerous and fatal illnesses like yellow fever, chikungunya and dengue fever. Several tropical plants contain eco-friendly, bioactive molecules act as a valuable supply of alternative mosquito control treatment including specific target insects. Therefore, the larvicidal effectiveness of three tropical plants - pignut (*Hyptis suaveolens*), carrot grass (*Parthenium hysterophorus*), and corn mint (*Mentha arvensis*) against mosquito larvae particularly second and third instars has been evaluated in the current study using aqueous leaf extracts. The fresh plant leave of the above plants were collected locally and identified by Plant taxonomist. Stock solution was prepared 20% of each extract in deionised water. Fully fed larvae were collected from the stagnant water at diverse residential areas of Gorakhpur city. The larvae were exposed to a wide range of test concentrations as mortality in 24, 48, 72 and 96 hours were used to determine LC_{50} and LC_{90} values. The sub-lethal doses 5.88 gm/l of *P. hysterophorus* showed significant larvicidal effect against the mosquito's larva. However, *M. arvensis* and *H. suaveolens* showed comparatively higher sub-lethal concentrations 27.97 gm/l and 29.53 gm/l; respectively against larvae. Our study suggests that *P. Hysterophorus* leave extracts to have promising phytochemicals for larvicidal potential of *Ae. aegypti* mosquitoes.

KEYWORDS: Botanicals, aqueous leaf extract, larval mortality rate, lethal Concentration, *Aedes aegypti*

INTRODUCTION

Mosquitoes spreads infectious diseases to human being and other vertebrate hosts.[1] Mosquito-borne diseases (Such as malaria, filariasis, yellow fever, dengue fever and viral encephalitis) covers a large percentage of health problems in developing countries.[2] In which, dengue is the frequently occurring disease transmitted by mosquito *Aedes aegypti* (*Ae. aegypti*).[3] More than 3.9 billion individuals in over 129 countries are at risk of getting dengue, with approximately 96 million cases of symptomatic dengue followed by 40,000 fatalities every year.[4] About two‑thirds of ecosphere's inhabitants live in areas diseased with dengue vector *Ae. aegypti*. It is an ultimate concern because of its wide distribution in heavily polluted areas such as Asia, America, and some Pacific islands, cities of tropical countries.[5] Recently, In August 23, 2023 reported that over 3.7 million cases and more than 2000 dengue-related deaths have been occurred from 70 countries/territories globally including 31464 cases and 36 deaths from India where 406 cases were reported from Uttar Pradesh.[6] One more *Ae. Agypti* related disease Chikungunya had 56404 suspected and 1794 confirmed cases reported from India.[6]

The climatic condition of India and day to day increasing temperature favours the rise of uncontrolled breeding places of mosquitoes. Mosquito is frequently established in poor drainage system, particularly in rainy session, fish pond, irrigation ditches, and rice fields due to better breeding places for them. After the discovery of DDT, mosquito control has been entirely reliant on synthetic organic compounds.[7] Factually the effect of such control strategies is temporary in nature with number of drawbacks.[8] One of them, prolonged and overuse of synthetic organic pesticides has resulted in environmental risks and development of physiological resistance in vector species.[9] One of the major issues linked with use of chemical insecticides is that they are non-selective to target organism and become hazardous to other organisms.[10] From this point of view, researchers diverted their attention towards the plant kingdom to find alternative agents that possess bioactive chemicals that may act as potential insecticides, antifeedants, oviposition deterrents, repellents as well as growth inhibitors. So, larviciding is the main mosquito control method.[11] Many synthetic chemicals are commonly used with various modes of action; however, plant derived chemicals have a variety of the impact on insect pests and are preferred over synthetic insecticides.[12] Researchers encouraged for the use of natural insecticides and research is underway to identify newer sources of botanicals. [13]

Plants are rich source of alternative agents for control of mosquitoes, because they possess bioactive phytochemicals. Approximately 2000 various kinds of terrestrial vegetation have been reported for their insecticidal characteristics.[14] Several secondary metabolites are also present in plants that serve as a defence mechanism against insect attacks.[15] Three plants selected by us as *Parthenium hysterophorus (P.hysterophorus), Hyptis suaveolens (H. suaveolens)* and *Mentha arvensis (M. arvensis)* were proven as larvicidal. In which, *P. hysterophorus* is a common and easily available annual weed herb belonging to family Asteraceae.[16] Keeping in view the harmful effects and unmanageability of above, the beneficial aspects of the different parts of it were explored in terms of the larvicidal potential against an Indian strain of *Ae. Aegypti.* The assessment of larvicidal potential of this weed may help in the formulation of effective strategies for reduction of mosquito population. Another, *H. suaveolens* (L.) belonging to the family Lamiaceae is a potent medicinal herb of high economic and medicinal value also used widely for its mosquito-repellent properties, possesses larvicidal activity against *Ae. aegypti* and *Culex (Cx.) quinquefasciatus.*[17] The third selected plant *M. arvensis (L).* is one of the important members of the Lamiaceae family, cultivated for menthol worldwide and its essential oil has been often used for various insecticidal assay.[18] Laboratory assessments of these plant extracts offer environmentally benign strategies for *Ae. aegypti* control, reducing ecological damage and pesticide resistance.[19] Such controlled conditions permit precise comparisons between their aqueous extracts, revealing potential bioactive compounds with larvicidal effects. While these studies provide valuable insights, there is still a need for further research to explore the potential of other aqueous leaf extracts and to compare their efficacy against *Ae. aegypti*.

Therefore, the current study was planned to work out the effective larvicidal action of these selected locally available and environmentally safe plant leaf extracts from the simplest formulations against the larvae of *Ae. aegypti.*

MATERIALS AND METHODS

Collection and Identification of Plant's leaf

The fresh plant materials of the Pignut/Vilaiti Tulsi (*Hyptis suaveolens),* carrot grass *(Parthenium hysterophorus)* and Corn mint *(Mentha arvensis)* (**Fig. 1)** were collected locally from Botanical Garden and campus of Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, and identified by plant taxonomist, where a voucher leaf sample was deposited.

Figure 1: Collection of Plants and Mosquito Larvae: (A) *Parthenium hysterophorus,* (B) *Mentha arvensis,* (C) *Hyptis suaveolens* (D) Collected Mosquito Larvae.

Preparation of plant leaf extracts

Preparation of plant leaf extracts were carried out in a simplest way, thinking that it could be easy for the local communities to adopt this method.[20] The collected leaves were washed firstly with tap water and finally they were thoroughly rinsed with sterile distilled water. The plant materials were dried in the shade for 7-14 days under room temperature (27-37°C). The air-dried leaves were grind using electric grinder to fine powder and then packed in air-tight plastic containers.

Preparation of stock solution

Stock solutions (2 lakh ppm/20%) were prepared by dissolving 20 gm of dried leaf powder in 100 ml volume of distilled water, mixed well and kept for 24 hours with periodic shaking. After that, mixture was filtered using a fine muslin cloth. Then, the leaf extract was filtered through a Buchner funnel with Whatman No-1 filter paper, and filtrate was collected. We have prepared previously studied concentrations from 5-25 gm/l for *P. hysterophorus* and 25-125 gm/l for *H. suaveolens* and *M. arvensis.* Serial dilution was done by using the following formula: C1V1 = C2V2. Where, C1=Initial Concentration, V1= Initial Volume, C2- Final Concentration and V2= Final volume.

Collection of experimental animals

Fully fed larvae were collected from the stagnant water at multiple locations of Gorakhpur. The larvae of *Ae. Aegypti* especially 3rd and 4th instar larva were identified and separated by key characteristics comb scales with large median and stout submedian spines; setal support plate of setae 9-12-T with prominent spine and further cross identified by Senior Entomologist of our department with the specification of ventral brush (4-X) with 5 pairs of setae, comb scale with stout, subapical spines and abdomen.[21,22] The colony of larvae was

separated and maintained in the laboratory of department of zoology. They were kept in plastic and enamel trays containing tap water and fed with 3:1 ratio of dog biscuits and yeast.

Procedure for bioassay testing

The larvae were primarily exposed to a wide range (1-15%) of test concentrations and control to establish the activity range of the aqueous leaf extracts. After defining the mortality of larvae in these concentrations, a narrower range of 5 aliquots yielding between 10% and 95% mortality in 24, 48, 72 and 96 hours (h) were used to determine LC_{50} and LC_{90} values. Six replicates and same number of controls were set up for each concentration concurrently with deionized water. The bioassay was performed at room temperature of 26±2°C and relative humidity of 80±2% and a photoperiod of 12 h light and 12 h dark for 96 h.

Larvicidal Bioassay

It was carried out according to standard procedure with slight modifications.[23] Ten *Ae. aegypti* larvae were introduced into the beaker which contains 200 ml of deionized water by wide mouth plastic dropper. Dead larvae, those that cannot be induced to move when probed with a needle in the siphon or the cervical region, and moribund larvae, those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed, were both used to calculate percentage mortality. The mortality percentage was calculated by the formula mentioned below.[24] The Abbott's formula was used to correct for percentage mortality where a minimal proportion of the larvae (between 5% and 20%) in the control batches died during the experiment.

$$\text{Corrected Mortality} = \frac{\text{percentage of alive larvae} - \text{percentage of alive larvae in treated solution}}{\text{percentage of alive larvae in deionized water (as control)}} \text{ X } 100$$

Statistical analyses were carried out for LC values, lower and upper confidence limits, slope value (mean ±S.E.), t-ratio and heterogeneity. LC_{50} and LC_{90} values were calculated using probit analysis by POLO plus programme (LeOra Software version 2.0).

RESULTS

The larvicidal activities of aqueous leaf extracts of three plants tested are summarized in Table 1, 2 and 3. We have observed in the treated larvae became slowly inactive within 10

hours and fall towards the bottom of the beaker. They showed curling up, anxiety and vigorous body activities. More accurate data on effective dose of the toxicity of the plant extracts were obtained in term of gram per litre (gm/l) with mean±S.D for slope value of our data.

Larvicidal activity of *parthenium hysterophorus*

P. hysterophorus were significantly efficient for mosquito larvae control. We have found that LC_{50} and LC_{90} values were 31.55 and 116.80 in 24 h, 18.89 and 94.09 in 48 h, 10.19 and 60.80 in 72 h, 5.88 and 22.71 gm/l in 96 h respectively (**Table 1**). The best suitable dose of aqueous leaf extract of *P. hysterophorus* against larvicidal activity *Ae. aegypti* showed 100% mortality at 30 gm/l (3%) concentration in 96 h exposure as trend is shown in **figure 2 (A).**

Table 1: Toxicity values of LC_{50} and LC_{90} of aqueous leaf extract of *Parthenium hysterophorus* plant against *Ae. aegypti* larvae at 24 h to 96 h exposure period.

Exposure Period (Hours)	Effective dose (gm/l)	Limits (gm/l)		Slope Value	't' ratio	Heterogeneity
		LCL	UCL			
24	LC_{50} = 31.55	24.67	53.60	2.255 ±0.512	4.405	0.28
	LC_{90} =116.80	63.90	541.08			
48	LC_{50} =18.89	15.40	25.14	1.838 ±0.350	5.250	0.23
	LC_{90} =94.09	55.14	292.99			
72	LC_{50} =10.19	7.55	12.66	1.653 ±0.318	5.194	0.47
	LC_{90} =60.80	38.41	160.01			
96	LC_{50} =5.88	4.08	7.36	2.185 ±0.346	6.309	0.59
	LC_{90} =22.71	18.06	33.09			

Abbrevious

Batches of ten larvae were exposed to five diverse concentrations in gram per litre/ppm (parts per million) in the beaker. Each experiment was duplicated six times, and mortality was reported every 24 h for up to 96 h. Regression analysis showed that there was significant value $p < 0.05$ with confidence limits (LCL = lower confidence limit, UCL = upper confidence limit), slope value (±S. E) and t-ratio was applied to locate significant changes with control. Here LC_{50} and LC_{90} means lethal concentration of extract that kills 50% and 90% of mosquito's larvae

Larvicidal activity of *mentha arvensis*

M. arvensis also showed the effective for larvicidal activity in all the experimental concentration as 25, 50, 75, 100 and 125 gm/l. The LC_{50} and LC_{90} values; were 145.74,

477.22; 89.28, 387.80; 44.60, 169.20; and 27.97, 91.46 gm/l observed in 24, 48, 72 and 96 h; respectively (**Table 2**). The larvicidal activity of *M. arvensis* extracts showed 100% mortality at 13% (27.97 gm/l) concentration in 96 h exposure against larvae as trend is shown in **figure 2 (B)**.

Table 2: Toxicity values of LC_{50} and LC_{90} of aqueous leaf extract of *Mentha arvensis* against *Aedes aegypti* larvae at 24 h to 96 h exposure period.

Exposure Period (Hours)	Effective dose (gm/l)	Limits (gm/l)		Slope Value	't' ratio	Heterogeneity
		LCL	UCL			
24	LC_{50} =145.74	117.86	227.09	2.488 ±0.569	4.375	0.24
	LC_{90} =477.22	280.52	1855.59			
48	LC_{50} =89.28	74.02	113.34	2.009 ±0.358	5.616	0.21
	LC_{90} =387.80	245.42	969.36			
72	LC_{50} =44.60	34.97	53.16	2.213 ±0.336	6.596	0.75
	LC_{90} =169.20	129.12	268.30			
96	LC_{50} =27.97	20.10	34.42	2.488 ±0.370	6.733	0.84
	LC_{90} =91.46	75.45	122.42			

Larvicidal activity of *hyptis suaveolens*

The LC_{50} and LC_{90} in gm/l values; are 148.42, 525.92; 105.59, 471.76; 53791.34, 239.50; 29.53, 121.46 gm/l observed in 24h, 48, 72 and 96 h; respectively (**Table 3**). The larvicidal activity of *H. suaveolens* plant against *Ae. aegypti* larvae showed 100% mortality at 13% (29.53 gm/L) concentration in 96 h exposure as similar to *M. arvensis* as trend is shown in **figure 2 (C)**.

Table: 3. Toxicity values of LC_{50} and LC_{90} of aqueous leaf extract of *Hyptis suaveolens* plant against *Ae. aegypti* larvae at 24 h to 96 h exposure period.

Exposure Period (Hours)	Effective dose (gm/l)	Limits (gm/l)		Slope Value	't' ratio	Heterogeneity
		LCL	UCL			
24	LC_{50} =148.42	125.44	262.06	2.459 ±0.552	4.456	0.32
	LC_{90} =525.92	300.48	2144.34			
48	LC_{50} =105.59	86.21	143.07	1.971 ±0.380	5.188	0.30
	LC_{90} =471.76	279.84	1448.29			
72	LC_{50} =53.79	41.53	65.38	1.976 ±0.342	5.777	0.66
	LC_{90} =239.50	167.19	474.96			
96	LC_{50} =29.53	19.73	37.51	2.087 ±0.349	5.985	0.77
	LC_{90} =121.46	94.74	185.44			

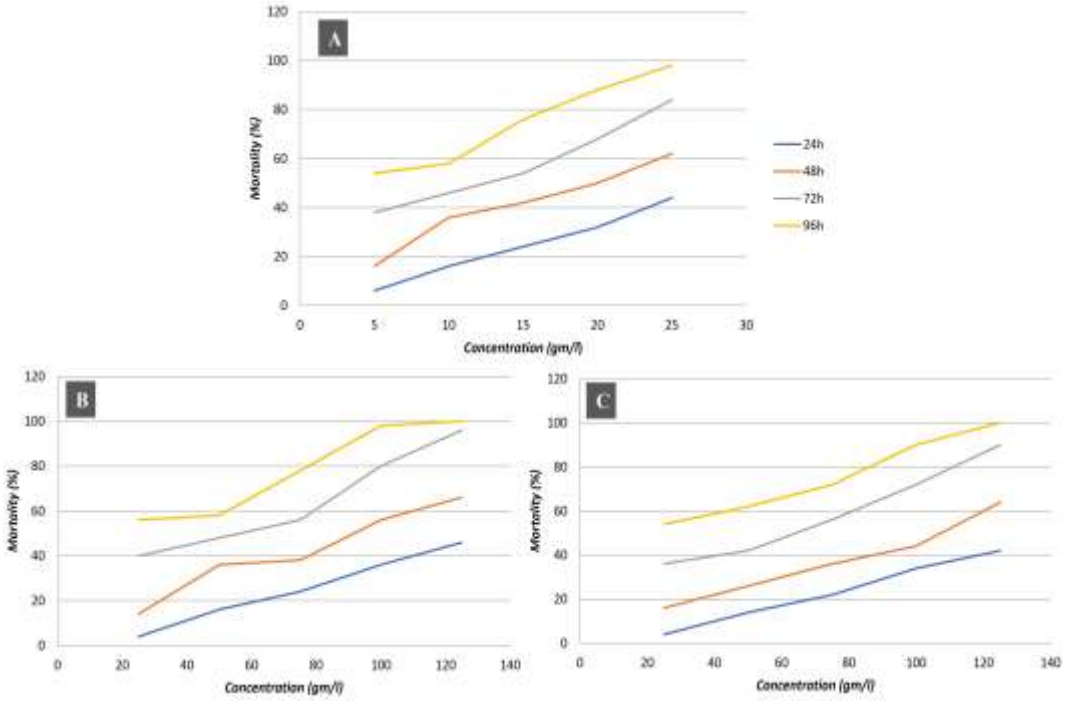

Figure 2: Graphical representation of effectiveness of aqueous leaf extract of (A) *Parthenium hysterophorus*, (B) *Mentha arvensis* and (C) *Hyptis suaveolens* against *Aedes aegypti* larvae; where extract concentration denoted in gram per litre (gm/l) and larvicidal capability denoted in percentage of mortality in 24 hours (h), 48 h, 72 h and 96 h.

DISCUSSION

We found that high rates of larval mortality of *P. hysterophorus* with a LC_{50} of 5.88 gm/l and LC_{90} of 22.71gm/l at 96 hrs exposure indicate the high toxicity of the aqueous leaf extract. Previous studies have also shown that *plant natural* extracts possessed significant larvicidal activity against *Ae. agypti*.[25] Recently, Ahmed A et al. (2023), find that aqueous extracts of four plants especially *P. hysterophorus* showed the lowest LC_{50} values (0.975%) after 24 hrs exposure. The same study also showed after 48 and 72 h the lowest LC_{50} values was found in *Parthenium* (0.420%, 0.120 %), followed by Tobacco (1.103%, 0.876%), *Fagonia indica* (1.412%, 1.104%), and *Melia azedarach* (2.142%, 1.703) respectively. Additionally, Structural modification of *P. hysterophorus* major component as parthenin has potential exploitation in larvicidal management.[26] Natural pesticides may serve as suitable alternative to chemical pesticides in future as they are comparatively safe, low-cost and available everywhere in the world.

We have also found the larvicidal activity of *M. arvensis and H. suaveolens* showed 100% mortality at 13% (27.97 gm/l and 29.53 gm/l) concentration in 96 hrs exposure. A recent study reported that *M. arvensis* contains essential oil acts as a larvicidal and mosquito repellent also showed LC_{50} and LC_{90} were 78.1 and 125.7 ppm, respectively.[27] Besides, the essential oil, aqueous extract of *M. arvensis* has been also testified as sedative-hypnotic, anti-inflammatory, anti-fungal, anti-bacterial and treat postmenopausal osteoporosis.[28] A study reported that at 0.5 ml concentration aqueous extract of *H. suaveolens* had a total mortality of 54.50% against *Anopheles spp.*[29] Dakum YD et al., (2021) also report the LC_{50} of aqueous extract of *H. suaveolens* obtained at the end of 48 hours exposure time of the assay against *Anopheles* species larvae was 323.5 mg/l.[30] Recently, Singh M et al., (2021) find that the *H. suaveolens* (L.) water extracts purified fraction possess remarkable larvicidal activities against two important mosquito species *Ae. aegypti* and *Anopheles stephensi*.[31]

We have targeted at the local and village level use of aqueous extracts of these biopesticides that extract lower quantities of bioactive compounds are more cost effective than the extracts made by organic solvents. Only the leaves of the selected plants were used in this study and they were shown to contain certain components that can had repellent activity, although in different dosages or concentrations for the target species. Similar to our findings, some other studies based on other plants like five species of Philippine plants crude aqueous extract namely- *Anona squarnosa, Eucalyptus globulus, Lansium dornesticum, Azadirachta indica* and *Codiaeurn variegatum* were assayed for *Aedes aegvpti* and *Cx. quinquefasciatus*, were also promising larvicidal agents.[32] According to Khanna and Kannabiran (2007), aqueous extract of *Gymnema sylvestre* causes 31, 45, 45, 71 & 100% mortality against *Cx. quinquefasciatus* at 1, 2, 3, 4 and 5% concentration respectively.[33] Similarly, aqueous extracts of four plants namely and elicited *Cx. quinquefasciatus larva mortality as Azadirachta indica* (70-99%), *Gymnema sylvestre* (44-89%), *Nerium indicum* Mill (41-74%) and *Datura metel* L. (19-54%).[20] Awosolu O et al. (2018), have also noticed larvicidal effects of aqueous extracts of plant *Codiaeum variegatum* (Croton), *Azadirachta indica* (Neem) against *Culex* mosquito larvae with LC_{50} were 5.98g/ml (4.48 –7.51%) and 57.32g/ml (24.72–89.9%) concentrations at 24 h respectively.[34] Murugan K et al. (2007), evaluated larvicidal potential of *Albizzia amara* Boivin and *Ocimum basilicum* Linn against the different instars *Ae. aegypti* with LC_{50} values of *A. amara* and *O. basilicum* for I instar larvae was 5.412 mg/ml and 3.734 mg/ml, II instar 6.480 mg/ml and 4.154 mg/ml, III instar 7.106 mg/ml and 4.664 mg/ml, IV instar 7.515 mg/ml and 5.124 mg/ml; respectively.[35]

Chansang U bioassayed aqueous extracts of nine medicinal plants against larvae of *Cx. quinquefasciatu* and *Ae. Aegypti*. Among these plants, larvicidal activity of aqueous extracts of long pepper (*Piper retrofractum* Vahl) showed the highest level of activity, the LC_{50} were 135 mg/l and 79 mg/l; respectively.[36] One more recently published study on aqueous extracts of leaves of *Plumbago zeylanica* on larvae of *Ae. aegypti* showed LC_{50} values of 275mg/l, 339.7mg/l, and 515.611mg/l after 48 hours exposure period.[37] It is also recommended that other parts of the plants (seeds, stems, fruits, etc.) be investigated as these may carry the active larvicidal ingredients in a more concentrated form. This point of view is the limitation of our study. So, we will test the different part of these plants including some more promising species in various solvents further in the future.

Plant based insecticides are less toxic, delay the development of resistance and easily decomposable.[38] Results of present study are also in line with earlier work done by us.[39] These findings have re-emphasised the need to explore the possibility of using plant based larvicide and reduce the chemical hazards in the environment. Moreover, all these botanicals can be easily collected from the naturally available near locations. Therefore, plant originated insecticides can be used as sustainable larvicide in a mosquito control programme.[40] No antiviral drugs or vaccine of dengue is available up to till date; so, the most commonly chosen solution by killing or stopping *Ae. agypti* mosquitoes from biting human beings to reduce disease virus communication and illness.

In conclusion, *P. hysterophorus* at a LC_{50} 5.88gm/l concentration, hold promising potential as components in eco-friendly integrated Mosquito management strategies. Aqueous leaf extract of *P. hysterophorus* leaves is the most promising one followed by *M. arvensis* (LC_{50}; 27.97 gm/l) and *H. suaveolens* is least efficacious (LC_{50} 29.53 gm/l) after 96 h exposers. This approach would serve as an alternative to the traditional reliance on synthetic pesticides, which often pose environmental and health risks due to their residual toxicity.

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Conflict of interest

None

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