Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.42A, No.1,
January-June 2023: P.170-189

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI: 10.48165/bpas.2023.42A.1.16

Original Research Article *Content Available online at: https://www.bpasjournals.com/zoology*

Bioassay of Larvicidal Efficacy of Selected Plant Extracts Against Mosquito Larvae *Anopheles Culicifacies* and *Aedes Aegypti L.*

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Article Info:
Received on 12.03.2023
Revised on 19.05.2023
Approved on 22.04.2023
Accepted on 31.05.2023
Published on 16.06.2023

ABSTRACT:
Mosquitoes act as a vector and are the most significant nuisance for spreading many diseases, viz. malaria, dengue and filariasis encephalitis. The extensive use of chemical insecticides to control these vector-borne diseases causes adverse environmental impacts, pest resurgence, vertebrate toxicity, and physiological resistance to vectors. The present research work was undertaken to study the efficacy of ethanolic extracts of commonly available plants, viz. *Paederia foetida, Murraya koenigii, Zingiber officinale* and *Allium sativum* against the larvae of the mosquitos, *Aedes aegypti* and *Anopheles culicifacies* at different sub-lethal concentrations (50 mg, 100 mg, 150 mg per 100 ml of water) for 1 hour, 10 hours, 24 hours, 48 hours, 72 hours and one week of exposure. The study was based on the behavioural changes, mortality rate and larval development following standard technique. The phytochemical screening for these plants was carried out following standard methods. The investigation results showed abnormal changes in the behaviour, such as hyperactive wriggling movement in the initial period. In contrast, sluggish and motionless movements were also observed in the later days of exposure associated with an upward mouth posture on the water surface indicative of the moribund condition. The impairment of the larval development and attainment of fly size was also noted in that they respond variedly to the different plant extracts in a dose and time-dependent manner. The mortality rates in these two mosquito larvae with respect to these plant extracts have also recorded an increasing trend, i.e., *A. sativum*> *P. foetida*> *Z. officinale*> *M. koenigii*.

Keywords: Mosquito Larvae, Plant Extracts, Larvicidal Activity, Phytochemicals.

How to cite this article: Dutta U. and Dey S. (2023). Bioassay of Larvicidal Efficacy of Selected Plant Extracts Against Mosquito Larvae *Anopheles Culicifacies* and *Aedes Aegypti L. Bulletin of Pure and Applied Sciences-Zoology*, 42A (1), 170-189.

INTRODUCTION

Mosquitoes are blood-sucking arthropods that act as a vector in transmitting pathogens or parasites of many dreaded diseases like malaria, dengue, filarial, Japanese encephalitis, yellow fever, West Nile Viral Fever etc. (Service, 2008). These mosquito-borne diseases are species-specific, which cause annoyance and harmful impacts not only on humans but also on birds and other mammals (Goddard, 2007). In addition, it imparts to the developing burden of death, diseases, social debility, and poverty in tropic zones (Jang et al., 2002; Balraju et al., 2009).

India contributes alone 77% of total malaria in South East Asia (Kumar et al., 2007). Therefore, controlling these mosquito-borne diseases in South East Asian countries is of great concern for human health in the present day. In most cases, the mortality rate of infant children and adults is directly caused by malaria, with approximately two to three million new cases arising yearly by different mosquito species (Kumar et al., 2007; Kamaraj et al., 2011). In 2011, 2012, 2013 and 2014, malaria incident cases were reported around 1.31 million, 1.01 million, 0.88 million and 0.85 million, respectively. Out of these, 754, 519, 440, and 316 deaths were recorded (NVBDCP, 2011-15). About 91% of malaria and 99% of malarial deaths were reported from North Eastern states, Andhra Pradesh, Chattisgarh, Gujrat, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, and West Bengal (NVBDCP, 2014-15).

There are nine species of malaria vector found in India, out of which the primary vector is *A. culicifacies,* mainly distributed in the rural and deforested areas of North East India as well as all over the country (NVBDCP, 2014-15). *A. culicifacies* is the vector of rural malaria in the country. It generates about 65% of malaria cases annually (Dev V., & Sharma V. P., 2013). In addition, other crucial malarial vector species found in North Eastern region, namely *A. fluviatilis, A. minimus,* and *A. dirus,* are breeders in the forests, rural areas, and urban areas with organic pollutants (Prakash A et al., 2004, NVBDCP, 2014-15).

It was also found that India has merely 6 million clinically diagnosed dengue cases annually. Between 2006 and 2012, it was reported that the number of dengue cases was 300 times more than that have been officially reported (Sephard D.S et al., 2014).

Dengue has become endemic in 16 states of India from 2010-2013. In most areas, dengue cases fluctuate, but in North Eastern states, especially in Assam, the cases are alarmingly rising (Cecilia D., 2014). Among the different dengue vectors, *A. aegypyti* is the major arbovirus responsible for diseases such as dengue, dengue haemorrhagic fever, chikungunya, etc. Reports showed that more than 100 million people are infected by this disease yearly. Despite the control strategy taken by the government or other agencies on a worldwide basis, dengue fever has become a severe public health issue as the number of reported cases is continuously increasing at an alarming rate, especially with more and more severe forms such as dengue haemorrhagic fever, dengue shock syndrome or with manual manifestations (Hendarto and Hadinegoro, 1992; Pancharoen et al., 2002). Although the number of dengue and malaria cases steadily rises every year, the mortality rate is reduced. This reduction is probably due to the cumulative effect of better patient management, better reporting, diagnostic capabilities, and social awareness. But in a real sense, on a worldwide basis, government and private agencies have taken an active part in controlling these mosquito-borne diseases, but success has not yet been achieved fully. Some cases of malaria and dengue go off-record because of their occurrence in remote areas.

North Eastern states are mostly remote because of their topography and geography; sometimes, government-aided control may reach partially or not. Therefore, if larval stages can be properly controlled, much success can be achieved in eradicating mosquito-borne disease to a greater extent.

Most of the control strategy adopted globally uses synthetic chemical pesticides and insecticides such as organophosphates like fenthion, temephos, diflubenzuron, methoprene etc., for mosquito larva control (Yang et al., 2002). Although it is an effective measure, their repeated use disrupts natural biological control and leads to adverse effects on the ecosystem, causing an ecological disturbance and undesirable effects on non-target organisms, human health, and the environment. Moreover, more prolonged exposure to these pesticides or insecticides leads to the development of resistance in the vectors (Pitasawat et al., 2007).

Adverse consequences have been reported whenever common synthetic pesticides such as carbamates, organochlorines and organic phosphates are released into the environment, causing severe toxicity to human health or contaminated agricultural products and contamination and development of resistance to commonly used pesticides (Nicolopoulou-Stamati et al., 2016). Due to the toxicity of synthetic pesticides to humans, the management of breeding sites is now a widely adopted method of combating additional vectors. This systematic way to reduce mosquito larvae and pupae numbers leads to larvicidal treatments, which require the constant use of insecticides (Ayidé et al., 2017). Insecticide resistance is an insurmountable obstacle for government health authorities around the world. This problem makes the concentration of insecticides in the environment increasingly high, polluting the environment and threatening human health. Plants have been used for centuries for pest control and constitute an ecological resource to search for molecules with larvicidal activity (Felipe Oliveros-Díaz et al., 2022). Attempts have been made to reduce or eliminate mosquito populations using various insecticides and chemical compositions. These pesticides are threatened by mosquitoes, which develop resistance to chemical insecticides, leading to retransmission, despite being highly effective against the target species. The long-term stability of many of these pesticides and their propensity to bioaccumulate in non-target organisms has raised many environmental and human health concerns. Because of the abundance of bioactive chemicals, their easy availability, their environmental safety, etc., secondary plant metabolites are a promising alternative against many mosquito species and their various juvenile stages (Kumar et al., 2023). These secondary metabolites are called green pesticides because they are known to be effective and safe for other vertebrates. Different phytochemicals have different potentials, affecting insect behaviour, repelling pests, impairing foraging, affecting pest physiology, inhibiting respiration and growth, reducing fertility, and impairing epidermal formation (Ganesan et al., 2023).

Due to the dramatic increase in chemical resistance, natural plant extracts are meant to be better alternative means of control. These plants are readily available in our vicinity to control the mosquitoes at their larval stages. Plant products are given more importance as; they are bio-degradable, readily available, and have a specific effect on the target organism without affecting the non-target organism sharing the same ecological niche. The plant extract was used based on its use in traditional medicine, having a history of anti-helminthic, anti-bacterial, anti-fungal and strong pungent smell characteristics.

Based on this background, the present project was undertaken to study the efficacy of ethanolic extracts of commonly available plants, viz. *Paederia foetida, Murraya koenigii, Zingiber officinale* and *Allium sativum* against the larvae of the mosquitos, *Aedes aegypti* and *Anopheles culicifacies* at different sub-lethal concentrations (50 mg, 100 mg, 150 mg per 100 ml of water) for 1 hour, 10 hours, 24 hours, 48 hours, 72 hours and one week of exposure. The study was based on the behavioural changes, mortality rate and larval development following standard technique. The phytochemical screening for these plants was carried out following standard methods.

MATERIALS AND METHODOLOGY

1. Collection of plant materials

The leaves of *M. koenigii* and *P. foetida* were collected from the Zoology and Botany Department, Cotton University campus, in April 2022. The taxonomic identification was made at the Botany Departments of Cotton University and Gauhati University.

Z. officinale and *A. sativum* were collected from the weekly market of Beltola, Guwahati and was taxonomically identified at the Botany Department of Cotton University.

The leaves of *M. Koenigii* and *P. foetida* and the roots of *Z. officinale* and *A. sativum* were washed and dried for 7- 10 days in a shaded environment (23-27°±2C). The dried roots (gm) and leaves (gm) were powdered in an electric stainless grinder machine. They were submitted to successive extraction by soxhlet apparatus with 100% ethanol and methanol at room temperature. The extracts were then filtered through a membrane filter and dried at room temperature. The dried extracts were further diluted in methanol and ethanol, respectively. The extracts were further sterilized by filtration (0.22µm) (Shipra Bhargava et al*.,* 2012)

2. Collection of mosquito larvae

A. culicifacies and *A. aegypti* were collected from selected municipality areas around Guwahati, where reserved water bodies are located. The mosquito larvae were collected in April 2022 from the sites given below (Fig. 1) -

- Panbazar Area: Dighalipukhuri area (DGHP Ar; 2611'16.8''N, 9145'03.7''E), Cotton College Campus area (CCC Ar; 2611'12.7''N, 9144'60.0''E),
  Naakkata Pukhuri area (NKP Ar; 2611'09.1''N, 9144'40.6''E)
- Guwahati Club Area: Silpukhuri area (SLP Ar; 2611'04.4''N, 9145'48.8''E)
- Fancy Bazar Area: Near Fancy Ghat area (FC Ar; 2611'03.1''N, 9145'12.8''E)
- Uzan Baazar Area: Jorpukhuri Area (JP Ar; 2611'20.1''N, 9145'15.6''E)
- Bharalu River Area: (BH Ar; 2610'03.3''N, 9145'19.7''E)

Among other sites, the sites selected from the Panbazar area, viz. DGHP Ar, CCC Ar and NKP Ar were located closest to the Department of Zoology, Cotton University, where the research was conducted.

3. Identification of mosquito specimen collected

The 3rd and 4th instar larvae were collected from the drains, ponds, and other water bodies from the abovementioned areas where the mosquitoes breed abundantly. The larvae were collected using insect collecting nets and then brought to the laboratory in sample-collecting plastic bags, along with some water where they were initially found.

For identification, some mosquito larvae were prepared in cold 70% alcohol, and the slide was prepared following the standard method (Widerholm, 1983) as follows-
Washed in 10% KOH solution, dehydrated three times consecutively in 95% alcohol and finally in absolute alcohol. Cleared in xylene, mounted in DPX, and slides are ready for observation.

The larvae were also identified as belonging to different genera by observing their posture of staying in the water with respect to the water surface and with the help of entomological books (Meigen, 1803; Linnaeus, 1878).

4. Culture of mosquitoes

In the laboratory, selected specimens were put down in the clean tray, and preferably 3rd and 4th instars were sorted out from the other larvae, especially from chironomous larvae, by hand, using droppers and forceps under naked eye observation. They were then transferred to clean petri-dishes containing clean, dechlorinated tap water. The larvae were acclimatized in laboratory conditions (25-27°±2C), 12 hours day/night cycle for two days prior to the experimental setup, and then reared to the 4th instar larval stage. The larvae were fed twice daily on a mixture of bread, powdered milk and yeast powder crushed together in the proportion of 1:1:1 by weight. The food was sprinkled onto the water surface in small amounts that were enough to be taken by all the larvae in order not to foul the water.

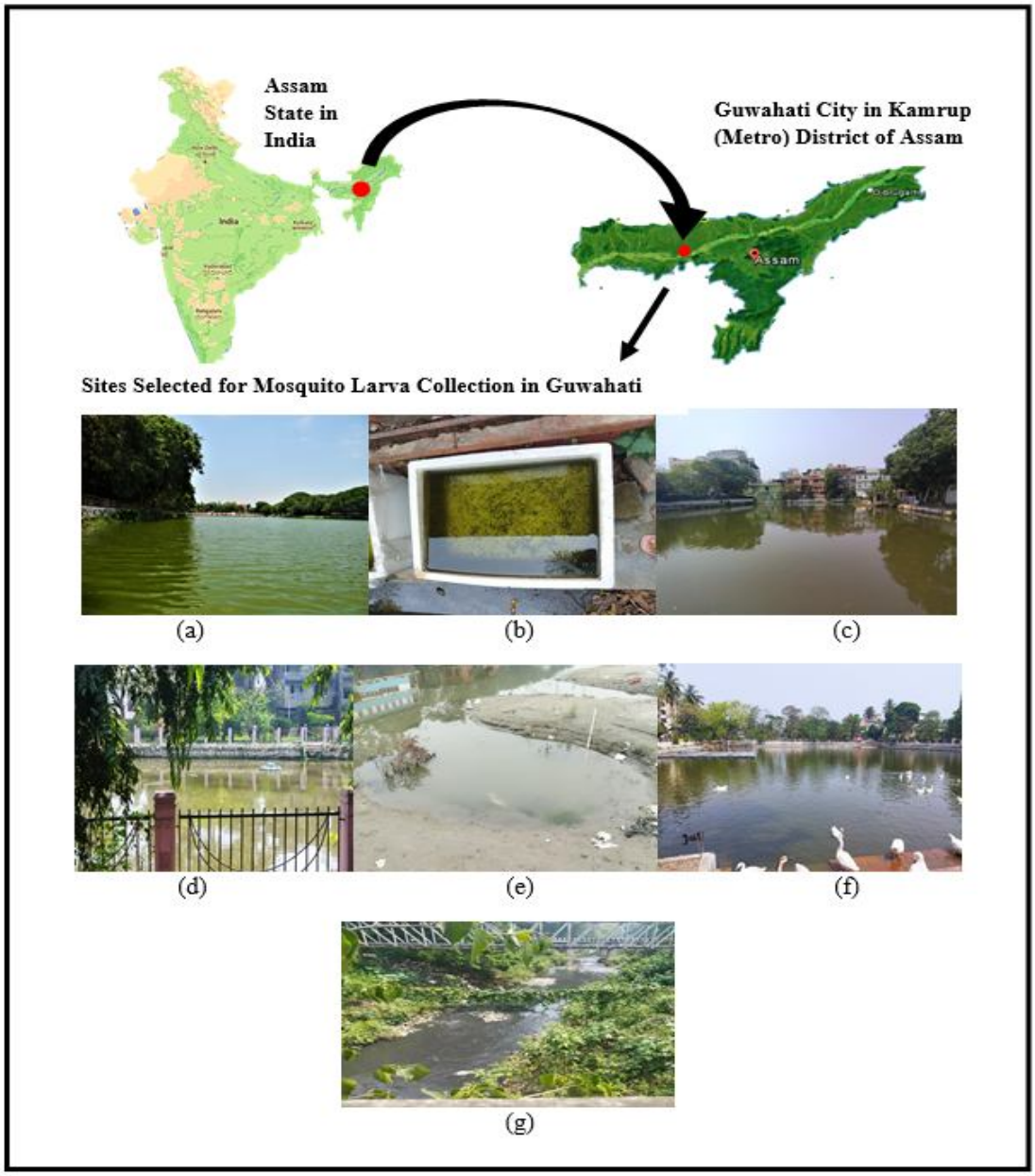


Figure 1: Map of Assam showing the sites selected for mosquito larva collection in Guwahati city – (a) Dighalipukhuri area, (b) Cotton College Campus area, (c) Naakkata Pukhuri area, (d) Silpukhuri area, (e) Near fancy ghat area, (f) Jorpukhuri area, (g) Bharalu River area

5. Experimental design

In the experimental setup, a minimum of 20 identified 4th instars larvae were placed in each of the four 500 ml beakers containing 100 ml of clean tap water, which was already acclimatized as earlier and maintained in uniform laboratory conditions, as mentioned earlier. Four groups were maintained for the experiment, out of which Group 1 is pure control (PuC) for all the plant extracts. Groups 2, 3 and 4 contain all three lethal concentrations of three doses, viz. 50mg, 100 mg and 150 mg.

- Group1-pure control (PUC)
- Group 2-50 mg plant extract / 100 ml
- Group 3-100 mg plant extract / 100 ml
- Group 4-150 mg plant extract / 100 ml

A minimum of 20 larvae per concentration were used for all the experiments for testifying the plant extract to maintain uniform batches of larvae. Application concentrations were 0.5 mg/ml, 1 mg/ml, and 1.5 mg/ml, which were applied only once and observed immediately after a one-hour duration and then onward 10 hours, 24 hours, 48 hours, and 72 hours periods. However, the larvae were also observed for one week to understand the effect of chronic exposure to the plant extracts.

As the number of *A. aegypti* were found in a limited number of areas throughout the experimental period, this species was exposed only to *A. sativum* and *P. foetida* in the way mentioned above.

The mosquito larvae were also exposed to combined extracts of all the plants mixed in the ratio 1:1:1:1 to study the combined efficacy of the plant extracts compared to their individual doses at 1 hour, 10 hours, 24 hours, 48 hours, and 72 hours. The effectiveness of combined extracts of all four plants was studied in the case of *A. culicifacies* larvae. In comparison, the efficacy of the combined extract of two plants, i.e., *A. sativum* and *P. foetida,* was studied in the case of *A. aegypti.*

6. Dose preparation for experimental studies

From the dried and evaporated ground plant samples collected either manually or by soxhlet apparatus, the concentration of different doses was prepared by dissolving them in a concentration of 50 mg, 100 mg, and 150 mg in 100 ml of ethanol, and they were stored in airtight, dark coloured glass bottles and at 4c in the refrigerator. The concentrations of the applied dosage are 0.5 mg/ml, 1 mg/ml, and 1.5 mg/ml.

7. Phytochemical screening test

Qualitative analysis of different phytochemical constituents in the selected plant extracts was carried out on the powdered plant extracts by using standard protocols following the methods described by Harborne (1973), Trease and Evans (1989), Sofowora (2003).

The following test has been performed for phytochemicals like Alkaloids, Flavonoids, Tannins, Saponins, Glycosides and Steroids for *M. koenigii* (Maheshwari N. Uma and N. Cholarani, 2013), *Z. officinale* (Bhargava Shipra et al., 2012), *P. foetida* (Kumar Vikas et al., 2009) and *A. sativum* (Harsha. N et al., 2013).

8. Bioassay and mortality of larvae

After the exposure of the plant extract to the larvae, behavioural changes of the larvae, that is, their movement and other activities, were observed immediately after one hour and to the later period of the exposure paradigm. Mortality of larvae was assessed immediately after 1hr, 10 hr, 24 hr, 48 hr and 72 hrs of exposure. Moribund larvae were counted as dead, followed by the method of Azmi et al., 1998. Experiments were repeated three times for each concentration of plant extract, and an average percentage of mortality was calculated using the following formula:

$$\% \text{ of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of total larvae}} \times 100$$

Mortality correction was done by the following formula given by Abbot (1925);

$$\text{Corrected \% of mortality} = \frac{1 - \text{n in T after treatment}}{\text{n in C after treatment}}$$

Where, n = number of larvae
T= treated group
C= control concentration

9. Statistical analysis

Probit analysis was used for the determination of LC50 (ppm) of different plant extracts against *A. culicifacies* and *A. aegypti* larvae in individual and combined formulations.

RESULT

1. Detection of phytochemicals

Table 1 showed that alkaloids and flavonoids were present in all the extracts of *A. sativum, P. foetida, Z. officinale* and *M. koenigii,* while terpenoids, saponins, tannins and steroids were present in some plant extracts and absent in others. The amount of phytochemicals mentioned above was found to be higher in the plant extracts of *A. sativum.*

Table 1: Presence of different phytochemicals in the selected plant species extracts

Phytochemicals	*A. sativum*	*P. foetida*	*Z. officinale*	*M. koenigii*
Alkaloids	+ (***)	+ (**)	+ (***)	+ (*)
Flavonoids	+ (***)	+ (***)	+ (**)	+ (*)
Saponins	+ (*)	+ (**)	+	+
Steroids	-	+	-	-
Tannins	+ (**)	+	+	+ (**)
Terpenoids	+ (***)	+ (**)	+	+ (*)
Cardiac Glycosides	+ (***)	-	+ (***)	+ (*)

(+)- Present, (-) Absent, (*) - Minimum intensity of reaction, (**) - Moderate intensity of reaction and (***) - Highest intensity of reaction

2. Behavioral growth and developmental study

On application of the plant extract in the beaker containing the larvae, the larvae showed wriggling movement. They wriggled downwards and then stayed on the bottom of the beaker for 2-5 minutes before wriggling up and assuming their normal positions again. In higher concentrations of the prepared plant extract application, the larvae became irritated and showed hyperactive wriggling movements. However, in the exposure paradigm's later days, the larva slowed down in its movement and appeared to be sluggish and motionless. They also showed an upward posture of the mouth on the water surface, indicative of moribund conditions.

Exposure to the plant extract causes the impairment of larval development. In most cases, attainment of the full adult size was impaired, especially in the case of *A. culicifacies* and *A. aegypti* with respect to *A. sativum, P. foetida* and *Z. officinale*. In some occasions, larvae attained the pupa stage, but morphologically the pupae were affected, and on another occasion, the 4th instar larvae followed the delayed development by moulting from larva to the pupa and fly stage, but they were unable to fly and sluggishly sat down on the water surface as the wings were not fully developed on account of exposure to the plant extracts of *Z. officinale* and *M. koenigii*. In contrast, the attainment of adulthood was almost absent in the case of *A. sativum* and *P. foetida* in a decreasing trend.

3. Study of mortality rate in mosquito larvae

Results showed varied mortality rates in the mosquito species viz. *A. Culicifacies* and *A. aegypti* with regard to the selected plant extracts and their doses and length on the time of exposure level. The impact of the different plant extracts on the mortality rates of different types of larvae has been depicted in Tables 2 to 9.

Analysis of the mortality rates in the mosquito larvae with respect to different plant extracts has been observed in the following trend:

A. sativum > P. foetida > Z. officinale > M. koenigii

Table 2: Effect of *A. sativum* on the mortality of *A. culicifacies*

Time period of observation	Plant Extract Used (*A. sativum*)	Total number of *A. culicifacies*	Number of dead larvae	% of mortality	Corrected % of mortality
1 hour	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	0	0	-0.05
10 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	1	5	5
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	1	5	5
24 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	6	30	30.25
	Group 3 (1 mg / ml)	20	8	40	40.35
	Group 4 (1.5 mg / ml)	20	11	55	55.5
48 hours	Group 1 (pure)	20	1	5	5
	Group 2 (0.5 mg / ml)	20	7	35	35.3
	Group 3 (1 mg / ml)	20	9	45	45.4
	Group 4 (1.5 mg / ml)	20	12	60	60.55
72 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	15	75	75.7
	Group 3 (1 mg / ml)	20	19	95	95.9
	Group 4 (1.5 mg / ml)	20	20	100	100.95

Table 3: Effect of *A. sativum* on the mortality of *A. aegypti*

Time period of observation	Plant Extract Used (*A. sativum*)	Total number of *A. aegypti*	Number of dead larvae	% of mortality	Corrected % of mortality
1 hour	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	0	0	-0.05
10 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	1	5	5
	Group 4 (1.5 mg / ml)	20	1	5	5
24 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	3	15	15.1
	Group 3 (1 mg / ml)	20	6	30	30.25
	Group 4 (1.5 mg / ml)	20	8	40	40.35

48 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	6	30	30.25
	Group 3 (1 mg / ml)	20	6	40	30.25
	Group 4 (1.5 mg / ml)	20	10	50	50.45
72 hours	Group 1 (pure)	20	1	5	5
	Group 2 (0.5 mg / ml)	20	15	75	75.7
	Group 3 (1 mg / ml)	20	17	85	85.8
	Group 4 (1.5 mg / ml)	20	18	90	90.85

Table 4: Effect of *P. foetida* on the mortality of *A. culicifacies*

Time period of observation	**Plant Extract Used (*P. foetida*)**	**Total number of *A. culicifacies***	**Number of dead larvae**	**% of mortality**	**Corrected % of mortality**
1 hour	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	0	0	-0.05
10 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	1	5	5
	Group 4 (1.5 mg / ml)	20	1	5	5
24 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	4	20	20.15
	Group 3 (1 mg / ml)	20	7	35	35.3
	Group 4 (1.5 mg / ml)	20	9	45	45.4
48 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	4	20	20.15
	Group 3 (1 mg / ml)	20	9	45	45.4
	Group 4 (1.5 mg / ml)	20	10	50	50.45
72 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	10	50	50.45
	Group 3 (1 mg / ml)	20	16	80	80.75
	Group 4 (1.5 mg / ml)	20	18	90	90.85

Table 5: Effect of *P. foetida* on the mortality of *A. aegypti*

Time period of observation	Plant Extract Used (*P. foetida*)	Total number of *A. aegypti*	Number of dead larvae	% of mortality	Corrected % of mortality
1 hour	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	0	0	-0.05
10 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	1	5	5
	Group 4 (1.5 mg / ml)	20	0	0	-0.05
24 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	2	10	10.05
	Group 3 (1 mg / ml)	20	4	20	20.15
	Group 4 (1.5 mg / ml)	20	8	40	40.35
48 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	2	10	10.05
	Group 3 (1 mg / ml)	20	4	20	20.15
	Group 4 (1.5 mg / ml)	20	7	35	35.3
72 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	7	35	35.3
	Group 3 (1 mg / ml)	20	12	60	60.55
	Group 4 (1.5 mg / ml)	20	14	70	70.65

Table 6: Effect of *Z. officinale* on the mortality of *A. culicifacies*

Time period of observation	Plant Extract Used (*Z. officinale*)	Total number of *A. culicifacies*	Number of dead larvae	% of mortality	Corrected % of mortality
1 hour	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	0	0	-0.05
10 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	0	0	-0.05
24 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	2	10	10.05
	Group 3 (1 mg / ml)	20	4	20	20.15
	Group 4 (1.5 mg / ml)	20	8	40	40.35

48 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	2	10	10.05
	Group 3 (1 mg / ml)	20	6	30	30.25
	Group 4 (1.5 mg / ml)	20	8	40	40.35
72 hours	Group 1 (pure)	20	1	5	5
	Group 2 (0.5 mg / ml)	20	4	20	20.15
	Group 3 (1 mg / ml)	20	9	45	45.4
	Group 4 (1.5 mg / ml)	20	14	70	70.65

Table 7: Effect of *M. koenigii* on the mortality of *A. culicifacies*

Time period of observation	**Plant Extract Used (*M. koenigii*)**	**Total number of *A. culicifacies***	**Number of dead larvae**	**% of mortality**	**Corrected % of mortality**
1 hour	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	0	0	-0.05
10 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	1	5	5
24 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	2	10	10.05
48 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	3	15	15.1
	Group 3 (1 mg / ml)	20	4	20	20.15
	Group 4 (1.5 mg / ml)	20	4	20	20.15
72 hours	Group 1 (pure)	20	1	5	5
	Group 2 (0.5 mg / ml)	20	6	30	30.25
	Group 3 (1 mg / ml)	20	8	40	40.35
	Group 4 (1.5 mg / ml)	20	10	50	50.45

Table 8: Effect of *combination extract* on the mortality of *A. culicifacies*

Time period of observation	Plant Extract Used - *A. sativum, P. foetida, Z. officinale,* and *M. koenigii* (1:1:1:1)	Total number of *A. culicifacies*	Number of dead larvae	% of mortality	Corrected % of mortality
1 hour	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	0	0	-0.05
10 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	2	10	10.05
	Group 3 (1 mg / ml)	20	3	15	15.1
	Group 4 (1.5 mg / ml)	20	6	30	30.25
24 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	8	40	40.35
	Group 3 (1 mg / ml)	20	10	50	50.45
	Group 4 (1.5 mg / ml)	20	14	70	70.65
48 hours	Group 1 (pure)	20	1	5	5
	Group 2 (0.5 mg / ml)	20	10	50	50.45
	Group 3 (1 mg / ml)	20	13	65	65.35
	Group 4 (1.5 mg / ml)	20	15	75	75.7
72 hours	Group 1 (pure)	20	2	10	10.05
	Group 2 (0.5 mg / ml)	20	16	80	80.75
	Group 3 (1 mg / ml)	20	20	100	100.95
	Group 4 (1.5 mg / ml)	20	20	100	100.95

Table 9: Effect of *combination extract* on the mortality of *A. aegypti*

Time period of observation	Plant Extract Used - *A. sativum* and *P. foetida* (1:1)	Total number of *A. aegypti*	Number of dead larvae	% of mortality	Corrected % of mortality
1 hour	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	0	0	-0.05
	Group 3 (1 mg / ml)	20	0	0	-0.05
	Group 4 (1.5 mg / ml)	20	0	0	-0.05
10 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	1	5	5
	Group 3 (1 mg / ml)	20	3	15	15.1
	Group 4 (1.5 mg / ml)	20	4	20	20.15
24 hours	Group 1 (pure)	20	0	0	-0.05
	Group 2 (0.5 mg / ml)	20	6	30	30.25
	Group 3 (1 mg / ml)	20	9	45	45.4
	Group 4 (1.5 mg / ml)	20	11	55	55.5

48 hours	Group 1 (pure)	20	0	0	5
	Group 2 (0.5 mg/ml)	20	9	45	45.4
	Group 3 (1 mg/ml)	20	12	60	60.55
	Group 4 (1.5 mg/ml)	20	16	80	80.75
72 hours	Group 1 (pure)	20	1	5	-0.05
	Group 2 (0.5 mg/ml)	20	16	80	80.75
	Group 3 (1 mg/ml)	20	18	90	95.85
	Group 4 (1.5 mg/ml)	20	20	100	100.95

The highest mortality rates were observed in all two mosquito larvae with exposure to A. *sativum,* and the maximum death of larvae occurred within 48 hours (Figure 2 a, b). In contrast, in the case of *A. aegypti,* mortality response was high only with *A. sativum* as compared to P. *foetida* (Figure 2b, 3b). Overall, the lowest mortality rate was observed with *M. koenigii,* even at 72 hours of exposure, where 100% mortality was not attained in any case (Figure 4b). Most exposure to the other three plant extracts for 72 hours showed the maximum rate of moribund conditions.

The exposure of *A. culicifacies* to combined plant extract of *A. sativum, P. foetida, Z. officinale,* and *M. koenigii* (1:1:1:1) has resulted in the highest mortality of larvae, with the death of 50% larvae within 24 hours and 100% death within 72 hours (Figure 5a). In the case of *A. aegypti*, maximum mortality was obtained when exposed to combined plant extract of *A. sativum* and *P. foetida* (1:1) compared to their individual plant extract doses (Figure 5b).

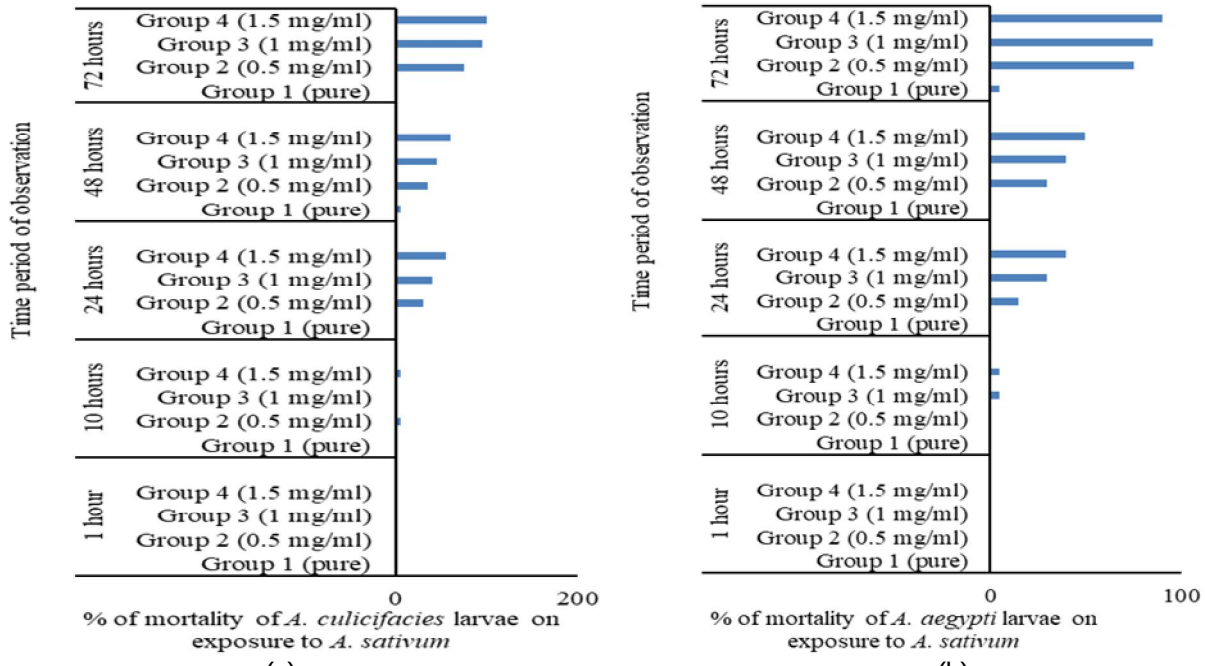
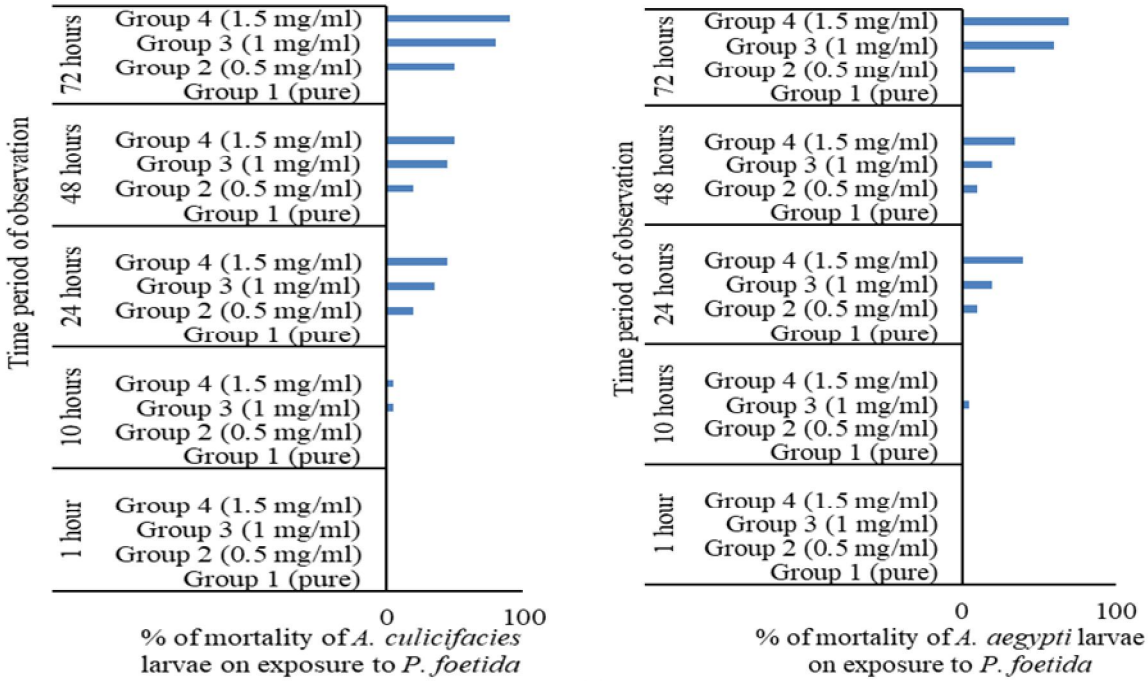
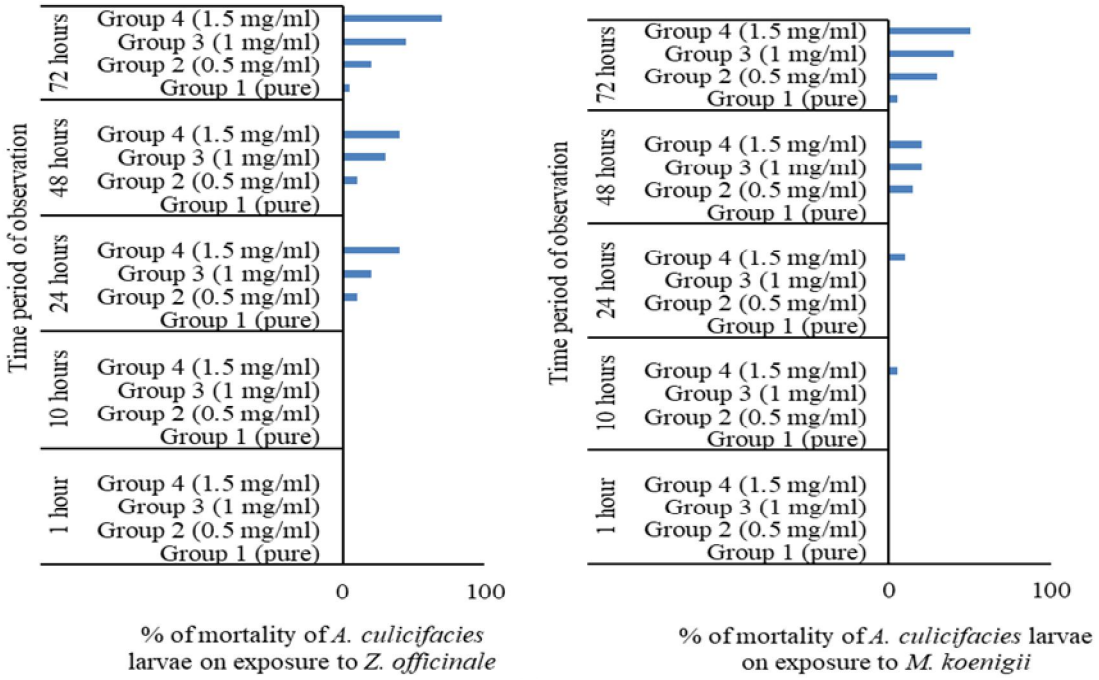


Figure 2 (a), (b) Percentage of mortality of *A. culicifacies* and *A. aegypti* larvae on exposure to various doses of *A. sativum* extracts at time periods of 1, 10, 24, 48, and 72 hours



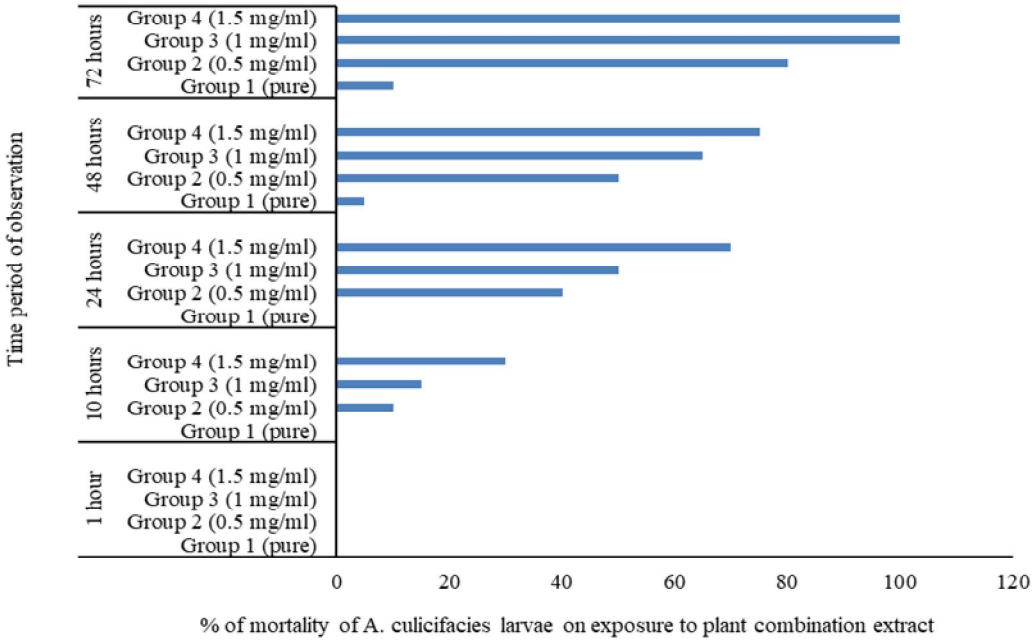
(a) (b)

Figure 3 (a), (b): Percentage of mortality of *A. culicifacies* and *A. aegypti* larvae on exposure to various doses of *P. foetida* extracts at time periods of 1, 10, 24, 48, and 72 hours



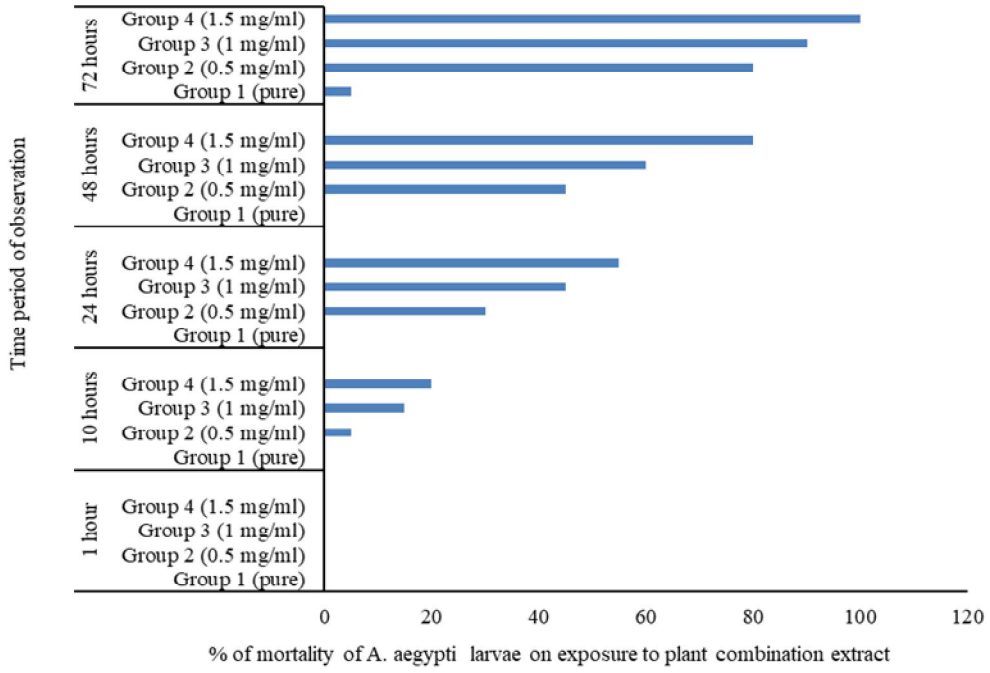
(a) (b)

Figure 4 (a), (b): Percentage of mortality of *A. culicifacies* larvae on exposure to various doses of *Z. officinale* and *M. koenigii* at time periods of 1, 10, 24, 48, and 72 hours



(a)

Figure 5 (a): Percentage of mortality of mosquito larva of *A. culicifacies* on exposure to various doses of combined plant extract viz. *A. sativum, P. foetida, Z. officinale,* and *M. koenigii* (1:1:1:1) at time periods of 1, 10, 24, 48, and 72 hours



(b)

Figure 5 (b): Percentage of mortality of mosquito larva of A. aegypti on exposure to various doses of combined plant extract viz. *A. sativum* and *P. foetida,* (1:1) at time periods of 1, 10, 24, 48, and 72 hours

The results of probit analysis for larvicidal efficacy of different plant extracts against *A. culicifacies* and *A. aegypti* larvae have been shown in Tables 10 to 11. *A. sativum* showed promising larvicidal activity against both the larval species, with LC50 (ppm) of 426.58 in the case of *A. culicifacies* and 151.36 in *A. aegypti* at 72 hours, which was followed by *P. foetida* with LC50 (ppm) of 489.78 and 776.25 for *A. culicifacies* and *A. aegypti* respectively. The combined plant extract formulations have also shown potential for use as a larvicide against both mosquito species.

Table 10: Probit analysis of larvicidal efficacy of different plant extracts against *A. culicifacies and A. aegypti* larvae

Plant Extracts	*A. culicifacies*	*A. aegypti*
	LC50 (ppm) at 72 hours	**LC50 (ppm) at 72 hours**
A. sativum	426.58	151.36
P foetida	489.78	776.25
Z. officinale	1047.1	-
M. koenigii	1548.8	-

Table 11: Probit analysis of larvicidal efficacy of combined plant extract against *A. culicifacies and A. aegypti* larvae

Time period	*A. culicifacies*	*A. aegypti*
	Combined plant extract of *A. sativum, P. foetida, Z. officinale,* and *M. koenigii* (1:1:1:1)	Combined plant extract of *A. sativum,* and *P. foetida* (1:1)
	LC50 (ppm)	**LC50 (ppm)**
24 hours	831.76	1258.9
48 hours	512.86	602.56
72 hours	331.13	407.38

The analysis of the result showed that the mentioned concentrations and exposure of the plant extracts caused the death of mosquito larvae and their impairment of larval growth.

DISCUSSION

In agriculture and public health programs, synthetic chemicals play a significant role. Its constant use has led to the cause of many public health issues and ecological imbalance. Botanical insecticides are relatively safe, biodegradable, and readily available in many parts of the world and may serve as viable alternatives to synthetic pesticides in the future (Sivagnaname and Kalyanasundaram, 2004). These plant extracts are known to be toxic to different mosquito species and could be used to control the vector-borne diseases they transmit (Wilcox et al., 2004).

The abundance of mosquito larvae in the areas studied is due to the high amount of organic matter in the water bodies, which acts as food matter for the growing larvae. The mosquitoes find it suitable for laying their eggs in such areas; therefore, more larvae were found in such selected areas. At the same time, the larvae belonging to *A. aegypti* were found in fewer numbers in limited areas like Jorpukhuri and Naakkata pukhuri as the water was relatively less polluted in these locations and thus served to be a breeding ground for these species of mosquito.

Plant products have traditionally been used against insect vectors and pest species by human communities in many parts of the world (Jacobson, 1958; Pavela, 2007). According to Bowers et al. (1995), screening locally available medicinal plants for mosquito control would create local jobs, reduce reliance on expensive

imported products and stimulate local efforts to improve public health.

In the preliminary screening, the local plant extract showed potential larvicidal activity. The plant extracts exhibited a concentration-dependent activity against mosquito larvae since the percentage mortality was observed to increase with increasing concentration of plant extract. A minor percentage of mortality was observed in the control group. The highest mortality of the larvae was seen with the application of 1 ml of 1.5 mg/ml plant extract during 72 hours of exposure. Larvicidal efficacy was found to be highest in *A. sativum* than in *P. foetida* and, in turn, followed in the order of *Z. officinale* and *M. koenigii*.

When the mosquito larvae of both species were exposed to combined plant extract formulations, it caused mortality of maximum larvae (>50%) within 24 hours of exposure. 100% larval death was obtained in both larval species on exposure to combined plant extract doses at 72 hours. Deterioration in larval development was seen more quickly, within 10-24 hours of exposure. The increased efficacy of combined formulations might be attributed to the presence of terpenoids, one of the phyto-active components, present in all the plants selected for the study. Terpenoids are already established to have insecticidal activity, supported by the study of Bhabesh et al., 2022. Therefore, the combination of plant extracts shows promising activity as an insecticide against *A. culicifacies* and *A. aegypti* larvae.

The percentage mortality was observed to increase with the increase in the concentration of the applied dose of plant extracts. The increased percentage of treated mosquito larvae is supported by a high amount of phytochemicals in the plant extract, which have larvicidal activities. The presence of several bioactive chemicals like alkaloids, saponins, tannins, flavonoids and steroids can be attributed to the susceptibility of the plant extracts as killing agents against mosquito larvae (Gutierrez et al., 2014). The highest mortality percentage seen in *A. sativum* might be due to its excessive pungent smell and other phytochemicals, as shown in the plant extract's phytochemical screening.

The moulting of the larvae was delayed, and the pupa which moulted to fly was unable to fly and was found in the control beakers. The delay in the moulting process indicated the impairment of the development process of larval growth as the plant extracts interacted with the normal growth of the larvae.

The investigation results also showed abnormal changes in the behaviour, such as hyperactive wriggling movement in the initial period. In contrast, sluggish and motionless movements were also observed in the later days of exposure associated with an upward mouth posture on the water surface indicative of the moribund condition. The impairment of the larval development and attainment of fly size was also noted in that they respond variedly to the different plant extracts in a dose and time-dependent manner. The analysis of the results showed that the different concentrations and hours of exposure paradigm for these selected plant extracts caused the death and impairment of larval growth. Therefore, further investigation into the biochemical mechanism of these plant extracts must be carried out, thereby suggesting an active measure for the biological control of mosquito larvae, which, in turn, can control the diseases of malaria or dengue.

CONCLUSION

Analysis of the current project showed that different plant extracts show variation in activity in controlling larval growth as well as interfering in the development of mosquito flies from the larval stage. Plant extracts have a selective impact on the moulting rate of the different mosquito species in different proportions, which act in a time-dependent and dose-dependent manner. Analysis of the mortality rates in the mosquito larvae with respect to different plant extracts has been observed in the following trend:
A. sativum > *P. foetida* > *Z. officinale* > *M. koenigii*

After analysis of the results obtained from exposure to both individual and combined formulations, it has been seen that combined

formulations of plant extracts are more effective than individual doses and cause targeted death of the mosquito larvae (>50%) within 24 hours of exposure.

Plant extract (combined formulation doses) > Plant extract (individual doses)

Therefore, it can be concluded that available or indigenous plant species can be a suitable measure for controlling the larval growth of mosquitoes, which, in turn, can help manage or eradicate malaria and dengue disease to a greater extent.

SUGGESTIONS

Available or indigenous plants can be a suitable or promising measure to control the larval growth of mosquitoes and, in turn, control or eradicate mosquito-borne diseases to a greater extent. Further investigation should be conducted regarding a better understanding of the biochemical and neurochemical activities of the mosquito larvae towards various plant extracts. Plant species should be judiciously used as biological control measures, encouraging the growth and flourishing of these plants to maintain proper ecological balance, which, in turn, protects the species diversity.

The chemical control measure is not to be very cost-effective or readily available to the people of remote areas or tribes, so an awareness campaign or educating the people about the importance of the biological controls of mosquitoes should be conducted.

Above all, "prevention is better than cure". All preventive measures, viz. well, maintained drainage system and hygienic surroundings, must be strictly followed to check mosquito breeding.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgement

The authors are thankful to the Department of Zoology, Cotton University, for providing administrative and laboratory support to carry out the research work and the Department of Botany, Cotton University and Gauhati University, for help in the identification of the plant leaves used in the research work.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

The study was conceptualized by Uma Dutta. Data collection and analysis were done by Uma Dutta and Sonali Dey. The first draft of the manuscript was written by Uma Dutta, and Sonali Dey commented on the previous manuscripts. The final draft was read and finalised by Uma Dutta and Sonali Dey.

REFERENCES

1. Ahouansou, C. A., Fagbohoun, L., Sèdami, M. F., Alban, H. G., Siméon, K., & Fernand, G. A. (2017). Inhibition effects of *Elaeis oleifera* (Arecaceae) and *Launaea taraxacifolia* (Asteraceae) on two genotypes of *Anopheles gambiae* larvae. International. *Journal of. Current. Research. Bioscience and. Plant Biology*, 4, 39-46.
2. Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M., & Farombi, E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. Sci Res Essay, 2(5), 163-166.
3. Azmi, W., Sani, R. K., & Banerjee, U. C. (1998). Biodegradation of triphenylmethane dyes. *Enzyme and microbial technology*, 22(3), 185-191.
4. Balaraju, K., Maheswaran, R., Agastian, P., & Ignacimuthu, S. (2009). Egg hatchability and larvicidal activity of Swertia chirata Buch.-Hams. ex Wall. against *Aedes aegypti L.* and *Culex quinquefasciatus Say. Indian J. Sci. Technol*, 2(12), 46-49.
5. Bhargava, S., Dhabhai, K., Batra, A., Sharma, A., & Malhotra, B. (2012). *Zingiber officinale*: Chemical and phytochemical screening and evaluation of its antimicrobial activities. *Journal of chemical and pharmaceutical research,* 4(1), 360-364.
6. Bowers, W. S., Sener, B., Evans, P. H., Bingol, F., & Erdogan, I. (1995). Activity of Turkish medicinal plants against

mosquitoes *Aedes aegypti* and *Anopheles gambiae*. *International Journal of Tropical Insect Science*, 16(3-4), 339-342.

7. Cecilia, D. (2014). Current status of dengue and chikungunya in India. *WHO South-East Asia Journal of Public Health*, *3*(1), 22.

8. Deka B., Borah A., Dutta U., Babu A. (2022). Terpenoids: The Phyto-Active Components as Sources of Insecticide. In: Terpenes and Terpenoids: Sources, Applications and Biological Significance. Editor: Davies, C. A. (Ed.). (2022, January 20). Nova Science Publisher Inc. New York. ISBN: 978-1-68507-559-0.

9. Dev, V., & Sharma, V. P. (2013). The dominant mosquito vectors of human malaria in India. In: Anopheles mosquitoes-New insights into malaria vectors. IntechOpen.

10. Ganesan, P., Samuel, R., Mutheeswaran, S., Pandikumar, P., Reegan, A. D., Aremu, A. O., & Ignacimuthu, S. (2023). Phytocompounds for mosquito larvicidal activity and their modes of action: A review. *South African Journal of Botany,* 152, 19-49.

11. Goddard, J., & Zhou, L. (2007). Physician's Guide to Arthropods of Medical Importance, 5th Edition. Emerging Infectious Diseases, 13(9), 1442.

12. Gutierrez, P. M., Antepuesto, A. N., Eugenio, B. A. L., & Santos, M. F. L. (2014). Larvicidal activity of selected plant extracts against the dengue vector *Aedes aegypti* mosquito. *Int Res J Biol Sci,* 3(4), 23-32.

13. Harborne, J. B. (1973). A guide to modern techniques of plant analysis. Chapman and hall.

14. Harsha, N., Sridevi, V., Lakshmi, M. V. V. C., Rani, K., & Vani, N. D. S. (2013). Phytochemical analysis of some selected spices. *Int J Innov Res Sci Eng Technol,* 2(11), 6618-6621.

15. Hendarto, S. K., & Hadinegoro, R. (1992). Dengue encephalopathy. *Pediatrics International,* 34(3), 350-357.

16. Jacobson, M. (1958). Insecticides from Plants: A Review of the Literature, 1941-1953. United States: U.S. Department of Agriculture.

17. Jang, Y. S., Baek, B. R., Yang, Y. C., Kim, M. K., & Lee, H. S. (2002). Larvicidal activity of leguminous seeds and grains against *Aedes aegypti* and *Culex pipiens pallens*. *Journal of the American Mosquito Control Association,* 18(3), 210-213.

18. Kamaraj, C., Bagavan, A., Elango, G., Zahir, A. A., Rajakumar, G., Marimuthu, S., ... & Rahuman, A. A. (2011). Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus*. *The Indian journal of medical research,* 134(1), 101.

19. Kumar, A., Valecha, N., Jain, T., & Dash, A. P. (2007). Burden of malaria in India: retrospective and prospective view. Defining and Defeating the Intolerable Burden of Malaria III: Progress and Perspectives: Supplement to Volume 77 (6) of American Journal of Tropical Medicine and Hygiene.

20. Kumar, S., & Srivastava, S. K. (2023). Mosquito Larvicidal Effects of 33 Plants Aqueous Extracts of 14 Different Plants Against Larva of *Culex* Mosquito. *International Journal for Research in Applied Science & Engineering Technology (IJRASET).*

21. Maheswari, N. U., & Cholarani, N. (2013). Pharmacognostic effect of leaves extract of *Murraya koenigii* Linn. *Journal of chemical and pharmaceutical Research*, 5(4), 120-123.

22. National Vector Borne Disease Control Programme (NVBDCP) Annual Report 2014-15, Government of India, Directorate General of Health Services, Ministry of Health & Family Welfare. https://nvbdcp.gov.in/Doc/Annual-report-NVBDCP-2014-15.pdf. Accessed 13 April 2023.

23. Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., & Hens, L. (2016). Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. *Frontiers in public health*, 4, 148.

24. Oliveros-Díaz, A. F., Pájaro-González, Y., Cabrera-Barraza, J., Hill, C., Quiñones-Fletcher, W., Olivero-Verbel, J., & Castillo, F. D. (2022). Larvicidal activity of plant extracts from Colombian North Coast against *Aedes aegypti L.* mosquito larvae. *Arabian Journal of Chemistry*, 15(12), 104365.

25. Pancharoen, C., Kulwichit, W., Tantawichien, T., Thisyakorn, U., & Thisyakorn, C. (2002). Dengue infection: a global concern. *Journal of the Medical Association of Thailand= Chotmaihet thangphaet*, 85, S25-33.

26. Pavela, R. (2007). Possibilities of botanical insecticide exploitation in plant protection. *Pest Technology,* 1(1), 47-52.

27. Pitasawat, B., Champakaew, D., Choochote, W., Jitpakdi, A., Chaithong, U., Kanjanapothi, D., Rattanachanpichai, E., Tippawangkosol, P., Riyong, D., Tuetun, B., & Chaiyasit, D. (2007). Aromatic plant-derived essential oil: an alternative larvicide for mosquito control. *Fitoterapia*, 78(3), 205–210.

28. Prakash, Anil & Mohapatra, Pradyumna & Bhattacharyya, Dibya & Mahanta, Jagadish. (2003). Resurgence of malaria in north-east India. *Journal. Assam Science Society*. 43. 57-62.

29. Service, M. W. (2008). Medical Entomology for Students. United Kingdom: Cambridge University Press.

30. Shepard, D. S., Halasa, Y. A., Tyagi, B. K., Adhish, S. V., Nandan, D., Karthiga, K. S., Chellaswamy, V., Gaba, M., Arora, N. K., & The Inclen Study Group (2014). Economic and disease burden of dengue illness in India. *The American journal of tropical medicine and hygiene*, 91(6), 1235–1242.

31. Sivagnaname, N., & Kalyanasundaram, M. (2004). Laboratory evaluation of methanolic extract of *Atlantia monophylla* (Family: Rutaceae) against immature stages of mosquitoes and non-target organisms. *Memorias do Instituto Oswaldo Cruz,* 99(1), 115–118.

32. Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Limited. Ibadan, Nigeria, 1-153.

33. Taha, A. K., Osman, H. E., & Sidahmed, O. A. A. (2011). Larvicidal effects of some plant extracts against *Anopheles arabiensis* Patton larvae (Diptera: Culicidae). *Journal of Science and Technology,* 12. 67 - 74.

34. Trease, G.E. and Evans, W.C. (1989) Pharmacognosy: A Physician's Guide to Herbal Medicine, 13th Edition, Baillière Tindall, London.

35. Vikas, K., Pankajkumar, S. Y., Singh, U. P., Bhat, H. R., & Zaman, M. K. (2009). Pharmacognostical and phytochemical study on the leaves of *Paederia foetida* linn. *International Journal of PharmTech Research*, 1(3), 918-920.

36. Wiederholm, T. (1983). Chironomidae of the Holarctic region. Keys and diagnoses. Part 1-Larvae. *Entomol Scand Suppl*, 9, 1-457.

37. Willcox, M., Burford, G., & Bodeker, G. (2004). An overview of ethnobotanical studies on plants used for the treatment of malaria. Traditional Medicinal Plants and Malaria, 187, 197.

38. Yang, Y. C., Lee, S. G., Lee, H. K., Kim, M. K., Lee, S. H., & Lee, H. S. (2002). A piperidine amide extracted from *Piper longum L.* fruit shows activity against *Aedes aegypti* mosquito larvae. *Journal of agricultural and food chemistry*, 50(13), 3765-3767.