Larvicidal Activity of *Murraya koenigii* against *Aedes* larvae

Project Report

Submitted in partial fulfilment of the requirements for the degree of

Master of Science (Hons.)Zoology

Submitted by:

Shiva Ohri

Registration number: 11614598

Under the guidance of

Dr. Amaninder Kaur

Assistant Professor

SCHOOL OF BIOENGINEERING AND BIOSCIENCES

LOVELY PROFESSIONAL UNIVERSITY

PHAGWARA, PUNJAB-144411

Certification of approval by faculty advisor

To whom may it concern

This is to certify that Registration No. **(11614598)** have completed the project entitled **"Larvicidal Activity of *Murraya koenigii* against *Aedes* mosquito"** was carried out by **Shiva Ohri** under my guidance and supervision. To the best of my knowledge, the present work is the result of their original investigation and study.

No part of the report has ever been submitted for any other degree at any university. The report is fit for submission and partial fulfilment of the conditions for the award of Master of Zoology from Lovely Professional University.

Date: Supervisor signature:

LIST OF CONTENT

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

INTRODUCTION

Mosquitoes belong to family Culicidae. They are midge like-flies and are threat to human life. Species belonging to these genera are *Aedes*, *Anopheles* and *Culex*. Only the female mosquito transmits the disease. *Aedes* is considered most dangerous among all different species of mosquitoes. WHO has declared Mosquitoes as "Public enemy number one"

In India and other nation primary vector of Malaria is *Anopheles*. Among all the malaria endemic countries, India had been declared with 1.5 crore cases of malaria and around 1000 deaths in 2009.

The genera *Aedes* is primary vector for spreading dengue virus. It is an anthropophilic mosquito; it evolved a relationship with humans. There are some distinctive features which help in identification of *Aedes* like black and white markings on their body and legs. Only during day time they are active. Their biting periods are in evening and in early morning. Different species are there of this genera like *A. aegypti, A. albopictus, A. australis, A. polynesiensis, A. rusticus, A. vexans.* Among all these species of mosquitoes, *A. aegypti* is responsible for spreading of Dengue. Only female mosquito transmits the disease. Except Antarctica it is found in all other continents and tropical and subtropical regions. Species like *A. albopictus* is a most invasive disease, German entomologist Johann Wilhelm Meigen in 1818, first described it and named it. Serious diseases are transmitted by some species of these genera like chikngunya, yellow fever, dengue fever and Zika virus. Human lymphatic filiarsis is transmitted by *A. Polynesiensis.*

They cause local and systematic skin reactions such as angioedema, urticaria and also causing allergic reactions (Peng *et al.,* 1999). In Malaysia, *A. aegypti* and *A. albopictus* are considered two main vectors of Dengue fever (Lam, 1993, Rozilawati *et al.*, 2007).

Presence of certain bioactive chemicals in plants may help in control of pests. Many plants are there which help in mosquito control. *Murraya koenigii* is one of the best plants which help in mosquito control.

M. koenigii or *Bergerakoengii* also called as Meethi neem belongs to Rutaceae family. Also known as Curry tree is a sub-tropical and tropical tree native to Sri Lanka and India. The leaves of this tree are used by India and many neighbouring countries in many dishes. In India, it can

be found in different places like Assam, Bengal, Sikkim, and Garhwal. The tree is having different names in area of its distribution. Curry tree is cultivated for its aromatic leaves. Different uses are their of curry leaves and of oil extracted from leaves used for dysentery , blood purifier, tonic, stomachic and also used as flavouring agent in curries and chutneys. Oil is used in perfume and soap industries.

Murraya koenigii show different activities like antifungal activity, anti- inflammatory activity, antioxidant activity, antibacterial effects (Darvekar *et al.,* 2011), cytotoxic activity (Mohan *et al.,* 2013) and antidiarhoeal activity.

It is small deciduous shrub , height up to 6 meters; stem is having dots on it and colour is dark green to yellowish; Leaves are bipinnately compound, exstipulate, is about 30 cm long bearing around 24 leaflets. Flowers are white, funnel shaped, bisexual, sweetly scented, complete, stalked, regular, actinomorphic, pentamerous, and hypogynous. Flowering and fruiting season is December to July. Fruits are 1-1.2 cm in diameter and 1.4-1.6 cm long and are fully ripe, black in colour with shining surface.

Alkaloids are isolated from leaves which are phytoconstituents include koenine, mahanine, girinimbiol, girinimibine 5, koenimbine, O-methyl mahanine, isomahanine, bismahanine, bispyrayafoline 6, O-methyl murrayamine A.

The oil extracted from leaves contain D α-phellandrene, dipentene, D-α-terpinol and caryophyllene, D-α-pinene, D-sabinene. Main constituent which show inhibitory action against A. aegypti are terpinolene and carene.

Antibacterial effects against many species *like B. subtilis, P. vulgaris* and many other is showed by essential oil which is obtained from *Murraya koenigii* leaves. The oil obtained from *M. koenigii* leaves shows anti-fungal activity against many species like *A. niger, C. albicans* and *C. tropicalis.* But the ethanolic extracts of roots don't show anti-fungal activity against *Microsporum canis* and *Cryptococcus neoformnas*. Repellent activity of *Murraya koenigii* is also observed. The petroleum ether and acetone extracts of *Murraya koenigii* leafs has showed the larvicidal activity against mosquito species. *Murraya koenigii* provide 6 hours protection against mosquitoes.

The derivatives of *M. koenigii* plants have good results when used for vector control operation as well as it can also be used for other purposes.

Mahanine, extracted from leafs *of Murraya koenigii* is a carbazole alkaloid has been reported to induce apoptosis in Myeloid Cancer cell.

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Ethanolic extracts *of Murraya koenigii* has been used against 3rd instar larva of *Aedes aegypti* to determine the larvicidal activity after 24 and 48 hours. The hexane extracts was also used to check the LC_{50} value of *Murraya koenigii*. The Phytochemical test of *Murraya koenigii* contains anthrones and flavonoids (Michael *et al.,* 2015).

Petroleum ether and acetone extracts of *Murraya koenigii* leaves has been used against *Aedes aegypti* larva to determine the larvicidal activity with a concentration range of 250ppm – 900ppm (Rahul *et al.,* 2011).

According to Raveen *et al.,* (2015). The hexane extracts has been used from leaves of *Murraya koenigii* to determine the larvicidal activity against different genera like *Aedes*, *Anopheles* and *Culex*. By column chromatography 6 fractions were obtained from hexane extracts. At different concentrations i.e. 20, 50, 75 and 100ppm, the larvicidal activity has been determined against the 6 fractions which were obtained from residues of hexane extract. LC50 values for hexane extract for were 35.06, 27.20 and 42.51ppm for *A. aegypti, C. quinquefasciatus* and *A. stephensi* respectively.

Petroleum ether and acetone extracts of *Murraya koenigii* has been used against third instar larva of *Aedes aegypti* and also used Tenephos and Fenthion which are synthetic larvicides. These were used alone and in combination also. The LC_{50} values of petroleum ether and acetone extracts of plant *Murraya koenigii* were 29.78 and 24.21 respectively (Gauri and Vijayalaxmi, 2004).

Different carbazole alkaloids have been isolated by phytochemical studies on various parts of *M. koenigii*. These alkaloids were girinimbine, murryacine mahanimbine, 3-methylcarbazole and murrayanine murrayafoline-A together with β-sitosterol. The larvicidal activity of chemical constituents and crude extracts which were obtained from isolation has been tested against *Aedes aegypti.* Different extracts from different plants were used against *Aedes aegypti.* For example, hexane extracts from roots, leaves and stem bark, chloroform and methanol extracts of stem bark, leaves and roots. Compounds which were isolated from plant were used against *Aedes aegypti* with LC_{50} values of less than 3mg/ml (Sukari *et al.,* 2013).

According to Nishan and Subramanian (2015). *Murraya koenigii* and *Azadirachta indica* exhibit strong larvicidal activity. The methanolic extracts of two plants have been used against 3rd instar larvae of *Aedes albopictus*. The combined activity was also carried out with plant extracts ratio of 1:1. The different LC_{50} were observed of both the plants and at 3.75mg/ml value different mortality rates were observed. On interaction of both the plants extracts they showed better results.

The plant extracts has been used of different plants *like Murraya koenigii, Coriander sativam, Ferula asafoetida* and *Trigonella foenum graceum* against larvae of two genera of mosquito i.e. *Aedes* and *Culex.* The effective results were found (Goswami *et al.,* 2007).

The plant extract of *Murraya koenigii* has been used, was prepared using the solvent chloroform against $1^{st,}$ 2^{nd}, 3^{rd}, and 4^{th} instar larvae of *Aedes aegypti*. The LC_{50} values were 1.263%, 1.871%, 2.446% and 3.168% respectively. This study showed the presence of alkaloids, saponins, steroids and flavanoids in chloroform extract of leaves of *M. koenigii* (Hima and Manimegalai 2014)

CHAPTER 3

OBJECTIVES

AND

SCOPE OF PRESENT RESEARCH

OBJECTIVE

The main objective of present research is to check the larvicidal effect of *Murraya koenigii* against 3rd or 4th instar larvae of dengue fever mosquito, *Aedes aegypti*. There are many chemicals used every day in the environment which also affect the non- targeted organisms. Mosquitoes have capability to show resistance against these chemicals, so it is very important to eradicate them from the environment.

SCOPE OF PRESENT RESEARCH

The present research deals with the larvicidal activity of *Murraya koenigii* against *Aedes aegypti* mosquito. Daily new pesticides and insecticides are introduced in environment. Not so much work had been done on larvicidal properties *of Murraya koenigii*. Future prospective of studies is to formulate such an effective formulation, which possess relatively more toxicity against *Aedes* mosquito.

CHAPTER 4

MATERIALS AND METHOD

MATERIALS AND METHOD

Collection of Plant Material: Curry plant material was collected from herbal garden of Lovely Professional University.

Fig.1: *Murraya koenigii*

Preparation of Leaf extract: Leaves of curry plant were collected and washed with water. Leaves were kept for shade and sun dry and were crushed in Mortar pestle for powder form. Boiling method was used for preparation of extract.

In Boiling method: 10gm of leaf powder was taken and added in 100ml of distilled water. It was kept for boiling in heating mantle for about half an hour at 80°C. After boiling it was allowed to cool down at room temperature and was filtered with whatman filter paper and kept in refrigerator.

Preparation of Stem extract: Both shade and sundry stem were used for preparation of extract. Boiling method was used for preparation.

In Boling method: 10gm of stem powder was taken in 100ml of distilled water and kept for boiling about 30 minutes at 80°C in heating mantle. After boiling it was cooled down and filtered with whatman filter paper and kept in refrigerator.

Fig.2: Boiling method

Fig.3: boiling extract of sun and shade dry leaves and stem

Collection of Larvae: Larvae of *Aedes* were collected from water sources like water cooler, pot etc. with the help of dipper in plastic bottles and kept in laboratory.

Treatment to Larvae: 3rd and 4th instar larvae of *Aedes* were treated in triplicates with different concentration of leaf and stem extracts of curry plant i.e., 50, 100, 150 and 200ppm. 15 larvae were taken in each beaker having 250ml of de-chlorinated water and extracts of leaves and stem were tested. In one beaker only 15 larvae were taken without adding any extract which was kept as control and examined. Mortality rate was checked after 24, 48 and 72 hours.

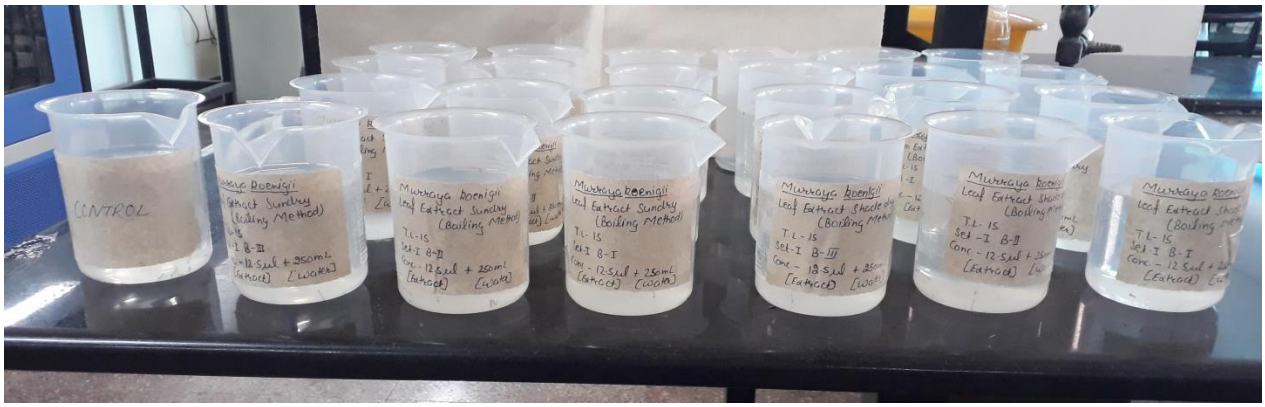

Fig.4: Treatment to larvae

CHAPTER 5

RESULT AND DISCUSSION

RESULT

Table: 1 Mortality rate of *Aedes aegypti* larvae after treatment of sundry leaf extract of *M. koenigii* at 24, 48 and 72hours

Concentration(ppm)	Mortality (%)		
	24hours	48hours	72hours
50	16.67 ±0.57	33.34 ±1.00	53.34 ±1.41
100	26.67 ±0.00	36.67 ±0.70	56.67 ±1.41
150	30.00 ±0.57	43.34 ±0.70	66.67 ±1.73
200	36.67 ±0.70	46.67 ±1.00	73.34 ±1.41
Control(0)	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00

% Percentage, ± S.E.

This table shows mortality rates of *Aedes aegypti* larvae after treatment of sundry leaves extract at different concentration. At 50,100,150 and 200 ppm; mortality rates observed at 72hours were 53.34% ±0.57, 56.67% ±1.41, 66.67% ±1.73 and 73.34% ±1.41 after 72 hours respectively. As the concentration increases mortality rate also increased. On exposure of extracts at different time interval variations were observed which are represented with the help of standard error.

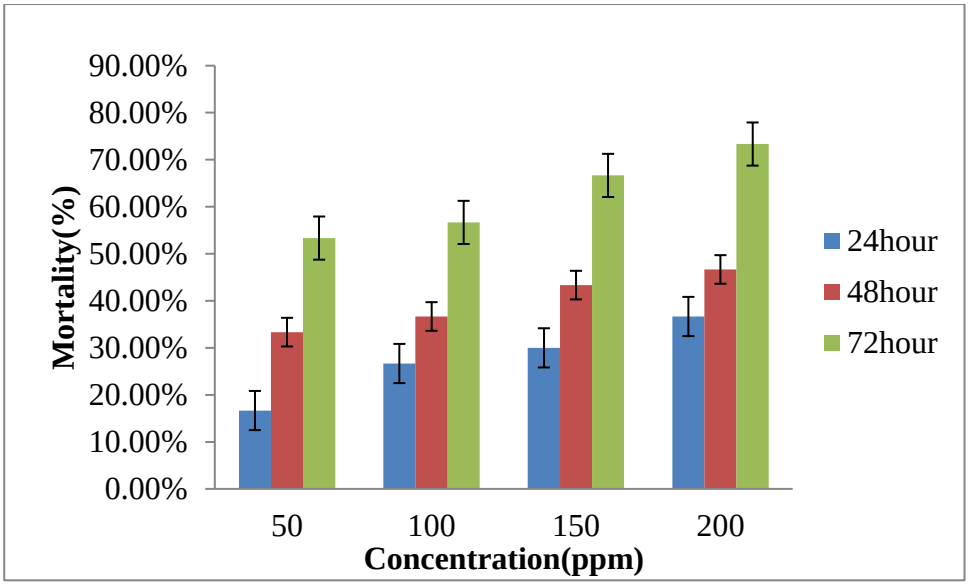

Fig.5: Mortality rate of *Aedes aegypti* larvae at different time interval of sundry leaf extract

This graph depicts about increase in mortality rate as concentration of sundry leaf extract and time increases. At 72hours, 200ppm concentration shows highest mortality rate i.e. 73.34% ±1.41 and lowest mortality was 53.34% ±0.57at 50ppm concentration after 24hours. These bars depict Standard error.

Table: 2 Mortality rates of *Aedes aegypti* larvae after treatment of shade dry leaf extract of *M. koenigii* at 24, 48 and 72hours

Concentration(ppm)	Mortality (%)		
	24hours	48hours	72hours
50	26.67 ±0.00	43.34 ±0.70	60.00 ±0.57
100	33.34 ±1.00	53.34 ±0.70	66.67 ±1.73
150	36.67 ±0.70	60.00 ±0.57	70.00 ±1.41
200	43.34 ±0.70	63.34 ±1.41	76.67 ±1.73
Control(0)	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00

% Percentage, ±S.E.

This table shows the mortality rates of *Aedes aegypti* larvae after treatment of shade dry leaves extract at different concentration. After 72hours mortality rates observed were 60% ±0.57, 66.67% ±1.73, 70% ±1.41 and 76.67% ±1.73 at 50, 100, 150 and 200ppm respectively. Mortality rates increases as the concentration of extract increased. Standard error represents variations on exposure of extracts at different concentrations.

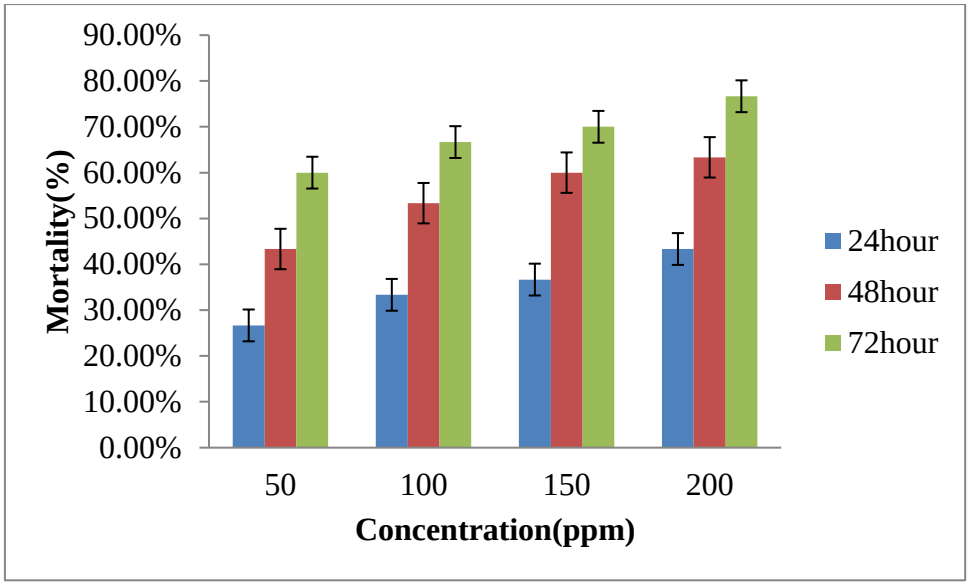

Fig.6: Mortality rate of *Aedes aegypti* larvae at different time interval of shade dry leaf extract

This graph shows that as the concentration and time interval increases mortality rate of larvae also increases. After 72hours mortality rate was 60% ±0.57 at 50ppm concentration but as concentration increased mortality rate also increased i.e. after 72 hours mortality rate was 76.67% ±1.73 at 200ppm concentration. These bars depict Standard error.

Table: 3 Mortality rates of *Aedes aegypti* larvae after treatment of sundry stem extract of *M. koenigii* at 24, 48 and 48hours

Concentration(ppm)	Mortality (%)		
	24hours	**48hours**	**72hours**
50	23.34 ±0.57	36.67 ±0.70	46.67 ±1.00
100	26.67 ±0.00	40.00 ±0.57	50.00 ±1.00
150	33.34 ±1.00	43.34 ±0.70	56.67 ±1.41
200	36.34 ±0.70	50.00 ±1.00	60.00 ±0.57
Control(0)	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00

% Percentage, ±S.E.

This table shows the mortality rate of *Aedes aegypti* larvae after treatment of sundry stem extract at different concentration. At 50, 100, 150 and 200ppm; mortality rates observed at 72hours were 46.67% ±1.00, 50% ±1.00, 56.67% ±1.41 and 60% ±0.57. As the concentration of extract increases the mortality rate also increased. Standard error represents variations on exposure of extracts at different time interval.

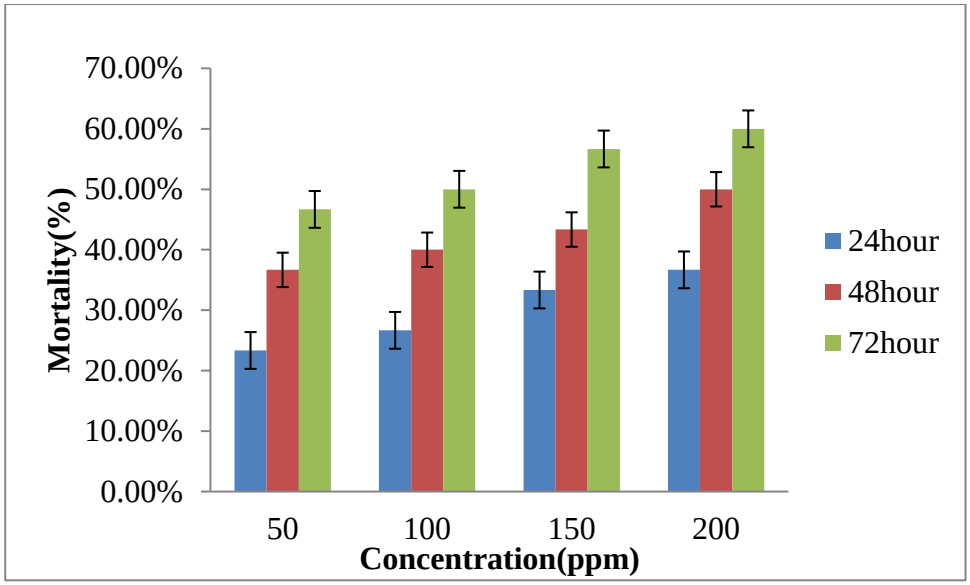

Fig.7: Mortality rate of *Aedes aegypti* larvae at different time interval of sundry stem extract

this graph describes about relationship between mortality rate and concentration of sundry stem extract. After 72hours 46.67% ±1.00 mortality was observed at 50ppm concentration but as concentration increased mortality also increased i.e. at 200ppm 60% ±0.57 of mortality was observed. These bars depict Standard error.

Table: 4 Mortality rates of *Aedes aegypti* larvae after treatment of shade dry stem extract of *M. koenigii* at 24, 48 and 72hours

Concentration(ppm)	Mortality (%)		
	24hours	**48hours**	**72hours**
50	23.34 ± 0.57	43.34 ±0.70	50.00 ±1.00
100	26.67 ±0.00	53.34 ±0.70	56.67 ±1.41
150	33.34 ±1.00	60.00 ±0.57	63.34 ±1.41
200	40.00 ±0.57	63.34 ±1.41	66.67 ±1.73
Control(0)	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00

% Percentage, ±S.E.

This table shows the mortality rates of *Aedes aegypti* larvae after treatment with shade dry stem extract at different concentration. Mortality rates observed at 72 hours were 50% ±1.00, 56.67% ±1.41, 63.34% ±1.41 and 66.67% ±1.73 at 50, 100, 150 and 200 ppm respectively. Mortality rate increases as concentration of extract increased. Variations observed on exposure of extract at different concentrations are represented by Standard error.

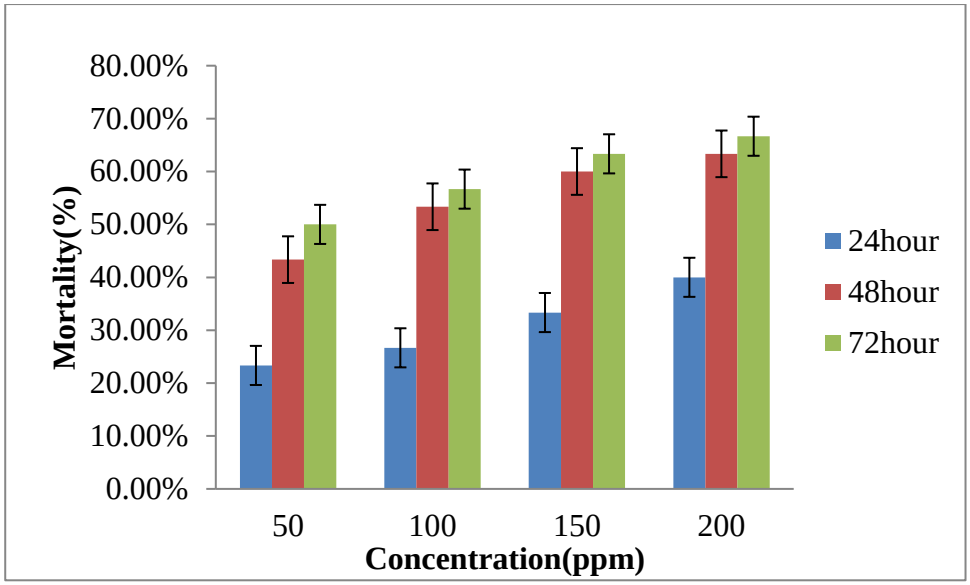

Fig.8: Mortality rate of *Aedes aegypti* larvae at different time interval of shade dry stem extract

This graph depicts about increase in mortality rate as concentration of shade dry stem increases. After 24hours lowest mortality rate was observed i.e. 40% ±0.57 at 200ppm concentration but as time increased mortality rate also increased i.e. 66.67% ±1.73 mortality rate was shown by Aedes larvae at 200ppm concentration after 72hours. These bars depict Standard error.

DISCUSSION

This study is about larvicidal activity of *Murraya koenigii* plant against *Aedes aegypti* larvae. Parts used were Leaves and stem. Shade and sundry leaves and stem extracts were prepared by boiling method. After preparation their effect was checked against *Aedes aegypti* larvae.

The toxicity of shade, sundry leaves and stem was checked on 3rd and 4th instar larvae of *Aedes aegypti* at different time intervals. Various concentrations were used of both the extracts. Good mortality rate was observed in leaves then stem. But as comparison to shade and sundry leaves; good result was in shade dry leaves same results were observed in case of stem; shade dry stem showed better results than sundry stem. Highest mortality i.e. 76.67% was shown by shade dry leaves at 200ppm concentration after 72hours and lowest mortality i.e. 60% was shown by sundry stem extract at 200ppm after 72hours.

The boiling extract of shade and sundry leaves after 72 hours at highest concentration i.e. 200ppm showed 76.67% and 73.34% mortality rate respectively. Another study was conducted by Alvarez et al., (2016) they also checked the mortality rate of third instar larvae of *Aedes aegypti* by using *Murraya koenigii* plant. At 800mg/ml of aqueous extract mortality rate was 2.22% after 24 and 48hours. D.K. Kocher and A.K. Riat (2017) checked larvicidal potential of *Eucalyptus globulus* against *Aedes* and *Culex*. 100% mortality rate was observed on *Aedes* larvae at 100ppm concentration after 3hrs whereas in *Culex*, 100% mortality was shown after 12 hours at 100ppm concentration.

Bio-pesticides should be used rather than using chemical insecticides which are harmful to crops and also to non-targeted insects. Plant based chemicals are safer to environment and also to other organisms. Pests are more prone to develop resistance against synthetic insecticides. So it is good approach to use bio-insecticides. Many phytochemicals are present inside the plant having a great potential to kill mosquito larvae. These results correlate with that of the present report conduct to check the efficacy of *Murraya koenigii* against *Aedes aegypti*.

CHAPTER 6

REFERENCES

REFERENCES

Ajay, S., Rahul, S., Sumit, G., Paras, M., Mishra, A. and Gaurav, A. (2011). Comprehensive review: *Murraya koenigii* Linn. *Asian Journal of Pharmacy and Life Science ISSN*, 2231, 1(4), 4423.

Arivoli, S., Raveen, R. and Samuel, T. (2015). Larvicidal activity of *Murraya koenigii* (L.) Spreng (Rutaceae) hexane leaf extract isolated fractions against *Aedes aegypti* Linnaeus, *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). *Journal of Mosquito Research*, 5(18).

Darvekar, V.M., Patil, V.R., and Choudhari, A.B. (2011). Anti-inflammatory activity of *Murraya koenigii* Spreng on experimental animal. *Journal of Natural Product and Plant Resources,* 1, 65-69.

Das, N.G., Goswami, D. and Rabha, B. (2007). Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *Journal of Vector borne diseases,* 44(2), 145-148.

Harve, G. and Kamath, V. (2004). Larvicidal activity of plant extracts used alone and in combination with known synthetic larvicidal agents against *Aedes aegypti*. *Indian Journal of Experimental Biology*, 42, 1216-1219.

Hima, C.R. and Manimegalai, M. (2014). Studies on the control of mosquito, *Aedes aegypti* (Culicidae: Diptera) using the chloroform leaf extract of *Murraya koenigii* as biocide. *World Journal of Pharmacy and Pharmaceutical Sciences,* 3, 766-773.

Kocher, D.K. and Riat, A.K. (2017). Larvicidal activity of *Eucalyptus globulus* oil against *Aedes* and *Culex* mosquitoes. Journal of Insect Science, 30(1), 5-9.

Lam, S.K., (1993). Two decades of dengue in Malaysia. *Tropical medicine*, 30, 195-200.

Mohan, S., Abdelwahab, S.I., Cheah, S.C., Sukari, M.A., Syam, S., Shamsuddin, N., and Mustafa, M.R. (2013). Apoptosis effect of girinimbine isolated from *Murraya koenigii* on lung cancer cells in vitro. *Evidence- Based Complemetary and Alternative Medicine*, 1.

Nishan, M. and Subramanian, P. (2015). Toxicity activity of *Azadirachta indica* and *Murraya koenigii* against *Aedes albopictus* larvae in laboratory conditions mortality. *Journal of Biodiversity and Environmental Sciences*, 7(3), 27-35.

Noel, Q., Franciso, H. and Michael, A.R. (2016). Larvicidal activity of Ethanolic and Aqueous extracts of *Murraya koenigii* against *Aedes aegypti*. *Journal of Coastal Life Medicine*, 4(2), 143-147.

Peng, Z., Yang, J., Wang, H. and Simons, F.E.R. (1999). Production and characterization of Monoclonal antibodies to two new mosquito *Aedes aegypti* salivary protein. *Insect Biochemistry and Molecular Biology*, 29, 909-914.

Rozilawati, H., Zairi, J. and Adanan, C.R. (2007). Seasonal abundance *of Aedes albopictus* in selected urban and sub urban areas in Penang, Malaysia. *Tropical Biomedicine*, 24, 83.

Sukari, M.A., Noor, H.M., Bakar, N.A., Ismail, I.S., Rahmani, M. and Abdul, A.B. (2013). Larvicidal carbazole alkaloids from *Murraya koenigii* against dengue fever mosquito *Aedes aegypti* Linnaeus. *Asian Journal of Chemistry*, 25(14), 7719-7721.