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Comparative Analysis on Larvicidal Activity of the Selected Leaf Extracts against *Culex* spp., Mosquito Larvae

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Abstract: Today's research mainly focused on medicinal plants, presence of bioactive compounds and their medicinal properties. These Phytochemicals has great antibacterial activity leading to the larvicidal activities. The study focuses on larvicidal activity of plant extract against *Culex* spp. The plant extracts are used in this experiment are *Gliricidia sepium*, (Jacq.) Kunth ex Walp.: *Ficus elastica*, Roxb.; *Theobroma cacao*, L.; *Murraya konigii*. Sprenge.; etc. The Phytochemicals screening of methanol extract of analyzed by standard methods and shown various phytochemical constituents such as saponins, phenols, alkaloids, protein, tannins, flavonoids, carbohydrates and terpenoides etc., Larvicidal bioassay done and number of mortile larvae was observed, including in the control (0%) during of two days. The plants are easily available and have high content of phytochemical constituents. The study reveals that due to the high presence active principles *Murraya koinigii*, Sprenge.; shows high degree of larvicidal activities among four plants(100%). *Ficus elastica*, Roxb.; and *Giricidia sepium*, (Jacq.) Kunth ex Walp. ; Also shows high mortality rate (>90%). The least larvicidal activity shows by *Theobroma cacao*, L.; although it is More than 50% of mortality rate.

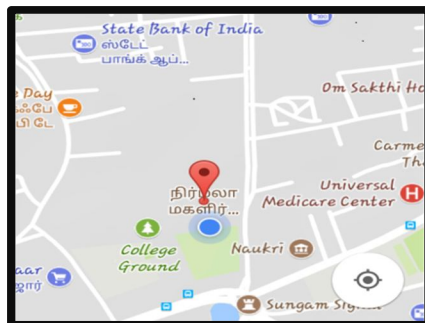
Key words: phytochemical, Larvicidal bioassay, Plant extracts

I. INTRODUCTION

Plants that possess therapeutic properties or exert beneficial pharmacological effect on human body are generally designated as medicinal plants. Recently, the world Health Organization (WHO) estimated that 80% of people worldwide rely on herbal medicines partially for primary health care. Current research trends use variety of plant extracts as alternative larvicides because they contain various phytochemicals. Phytochemicals are the chemicals that present naturally in plants. Now a day these phytochemical become more popular due to their countless medicinal use. These can also be considered as "man friendly medicines" (Sahira banu and Cathrine, 2015). Mosquitoes are small flies that constitute the family Culicidae. Mosquitoes act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever; filariasis etc., WHO has declared the mosquitoes as "public enemy number one". Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population (Anupam Ghosh et al., 2012). Due to pathogenic diseases and serious harms caused by mosquitoes, controlling them has been the primary subject of several new researches over the past few years. The technique in controlling mosquitoes depends on larval stages (egg, larvae, pupae and adult) on target. Mosquitoes control strategies have depended primarily on the use chemical insecticides however, the unfriendly effect of most of the past advocated synthetic chemical insecticides leads the insect pest managers of the world to comb for alternative ways of countering this disease causing insect (Arivoli et al., 2015). The present study includes extracts of larvicidal activity of plant samples such as *Gliricidia sepium*, (Jacq.) Kunth ex Walp.: *Ficus elastica*, Roxb.; *Theobroma cacao*, L.; *Murraya konigii*. Sprenge.; etc. These four plants have many uses like antibacterial activity and medicinal values.

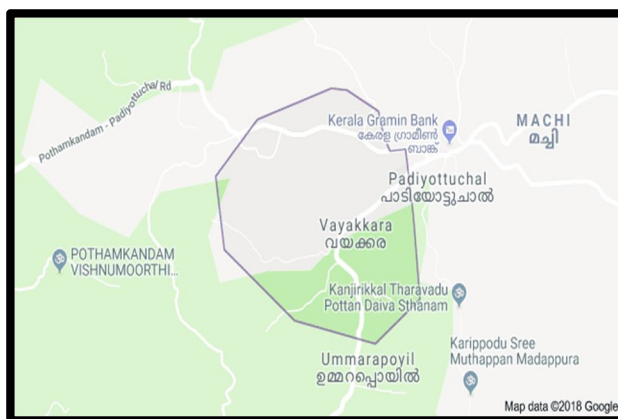
II. MATERIALS AND METHODS

A. Study area -I: (Plate -I)



Tamil Nadu is one of the 28 states in India. Its capital is Chennai (Formerly known as Madras) the largest city. Tamil Nadu lies in the southern most part of the Indian peninsula and is bordered by the union territory of Puduchery and the states of Kerala, Karnataka and Andhra Pradesh. Coimbatore is a place in Tamil nadu from where the plant *Ficus elastica*. Roxb.; is collected.

B. Study area- 2: (Plate- 2)



Kerala is one of the 28 states in India. Its capital is Thiruvananthapuram. It has 600 km of Arabian Sea shoreline to west and Western Ghats to the east. It is bordered by Karnataka to the north and northeast, Tamilnadu to the east and south, the Lakshadweep Sea to the west. Vayakkara is a small village in kannur district in kerala which has a pleasant climate due to the presence of Karnataka forest to the east and Arabian Sea to west. *Murraya konigii*, Spreng., *Theobroma cacao*, L.; *Gliricidia sepium*, (Jacq.) Kunth ex Walp.;

C. Selected samples

Culex sps. L.; Mosquito larvae: (Plate- 3)

Systematic position

Kingdom	:	Animalia
Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Diptera
Family	:	Culicidae
Genus	:	<i>C. sps.</i> L.;



Culex sps. L.; is commonly referred to as the house mosquito. It is a vector for diseases including Japanese encephalitis, Wile nilavirus, St. Louis Encephalitis etc., and also great human nuisance due to biting. Stagnant water mosquitoes tend to lay egg in clumps, called rafts, of 50 to 300 on the surface of standing water at the edges of lakes (Sajal Bhattacharya, et al., 2016).

D. *Murraya konigii*, Sprenge.; - (Plate- 4)

Systematic position

Kingdom	:	Plantae
Division :		Angiosperms
Class	:	Magnoliopsida
Order	:	sapindales
Family	:	Rutaceae
Genus	:	Murraya
Species	:	M. konigii, Sprenge.;



The plant is semi deciduous aromatic shrub or small tree with slender but strong woody stem and branches covered with dark grey bark, leaves are imparipinnate, glabrous, and very strongly aromatic. It is native to Mexico, Nicaragua, Panama, United States of America etc. Fresh leaves, dried leaf powder and essential oil are widely used for flavoring soups, curries etc. It is traditionally used as a whole or in parts as antiemetic, antidiarrheal, febrifuge, blood purifier, antifungal, depressant, anti-inflammatory, body aches, for kidney pain etc., (Vandana Jain *et al.*, 2012).

E. *Theobroma cacao*, L.; - (Plate-5)

systematic position

Kingdom	:	Plantae
Division :		Angiosperms
Class	:	Dicotyledons
Sub class	:	Dilinidae
Order	:	Malvales
Family	:	Sterculiaceae
Genus	:	Theobroma
Species	:	T. cacao, L.;



Theobroma cacao, L.; one of the world's most important perennial crops, is almost exclusively explored for chocolate manufacturing belong to the family sterculiaceae. It is native to deep tropical regions of central and south America. Cacao tree is a shade tolerant, moisture loving, understory rainforest tree. It is a small evergreen tree about 4 to 9 M tall in height. Native to the deep tropical regions of Central and South America. Cocoa is primarily grown for chocolate production. (Marita Briz, 2015).

F. *Gliricidia sepium*, (Jacq.) Kunth ex Walp.; - (Plate-6)

Systematic position

Kingdom	:	Plantae
Division	:	Angiosperm
Class	:	Dicotyledons
Order	:	Fabales
Family	:	Fabaceae
Genus	:	Gliricidia
Species	:	G. sepium, (Jacq.) Kunth ex Walp.;



Gliricidia sepium, (Jacq.) Kunth ex Walp.; is small to medium sized tree ranging height from 2 to 12 m.

bark ranges from grayish- brown to whitish in color and can be deeply fissured at the base of older stem. Native to tropical dry forests in Mexico and Central America.

G. *Ficus elastica*, Roxb.; - (Plate-7)

Systematic position

Kingdom	:	Plantae
Division :		Angiosperms
Class	:	Dicotyledons
Order	:	Rosales
Family	:	Moraceae



Genus : *Ficus*

Species : *F.elastica*, Roxb.;

Often seen as an interior container plant, Rubber Tree has large, 5 to 12-inch-long, thick, glossy evergreen leaves, multiple trunks, and a spreading, irregular canopy. Able to reach 100 feet in height in its native habitat in the jungle but most often seen at about 25 to 40 feet in the landscape. Native to northeast India, Nepal, Bhutan, Burma, China (Yunnan), Malaysia, and Indonesia. Rubber tree is useful as a screen, shade, and a specimen tree. Its coarse texture makes a strong statement in the landscape.

H. Preparation of plant extracts

The leaves were dried for 7-14 days in the shade at the environmental temperatures (27-37°C day time). These dried parts were powdered using pulverizer. 15 grams of each pulverized part were placed in separate flasks; 100 ml of distilled water was added and mixed vigorously. The mixture was kept in shaker system for 48 hours with occasional shaking.

I. Preliminary Phytochemical Analysis, (Raaman, 2006)

The phytochemical screening of methanol extract of analysed by standard methods and shown various phytochemical constituents such as saponins, phenols, alkaloids, protein, tannins, flavonoids, carbohydrates and terpenoids.

II. LARVICIDAL BIOASSAY

Mosquitoes were collected from plant pots with stagnant water which contain kivi fruit parts. It is found that, with fruit parts of kivi fruit and stagnant water contain numerous numbers of larvae within two days. Then enough number of larvae was collected using collecting bottles. 10 larvae of one to fourth instars were placed in petridishes containing 100 ml treatment solution. Each concentration (1ml, 2ml, 3ml, and 4ml) of plant extract is added to the petridish containing 100 ml treatment solution with ten larvae in each petridishes. Then petridish containing the larvae were kept in the growth room maintained at room temperature. The effects of extracts were monitored by counting the number of dead larvae each day up to three days. Number of mortality of larvae was observed, including in the control (0%) during the three days. Statistical analysis done using M.S Excel software

III. RESULTS AND DISCUSSION

Vector control is facing a series threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides. However due to the continuous increase in resistance of mosquitoes to familiar synthetic insecticides, better alternative means are sought. A considerable number of plant derivatives have been screened effective against mosquitoes (Hima, et al., 2014). The research was carried out to investigate the larvicidal activity of methanolic extracts of different plants such as *Murraya konigii*, Spreng.; and *Theobroma cacao*, L.; *Gliricidia sepium*, (Jacq.) Kunth ex Walp.; *Ficus elastica*, Roxb.; After two days *Ficus elastica*, Roxb.; shows high percentage of mortality against mosquito larvae. In 1 ml it shows 46.66% mortality after two days and three days. It shows 56.66%, 96.66%, 100% mortality rate in 2ml, 3ml, and 4ml concentration respectively. Qualitative phytochemical analysis of methanol extracts of *Ficus elastica*, Roxb.; shows the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids, tannins, saponins and terpenoids. Carbohydrates, steroids, tannins are present higher amount in the extract. *Gliricidia sepium*, (Jacq.) Kunth ex Walp.; Methanol extract exhibit 50%, 56.66%, 93.33%, 96.66% death in 1ml, 2ml, 3ml, 4ml concentration respectively. After three days moderate increase in the mortality rate. In qualitative phytochemical analysis of *Gliricidia sepium*, (Jacq.) Kunth ex Walp.; methanol extract shows the presence of carbohydrate, protein, amino acids, glycosides, flavonoids, alkaloids, tannins, terpenoids. Carbohydrates and glycosides are highly present in this extract. Larvicidal activity of *Gliricidia sepium*, (Jacq.) Kunth ex Walp.; leaf extracts on mosquito and its lethal effect on non targeted organism (Jibi Joby mathew, 2015). Here 150, 200, 250 ppm concentration are taken and 70%, 68%, 84%, 93% death are observed. In present study 1ml, 2ml, 3ml, 4ml extract shows 50%, 56.66%, 93%, 97% death are observed. This is comparable to previous reports on larvicidal activity of plant extracts (Kamaraj, et al, 2011). Here methanol extracts of *Annona squamosa* and methanol extracts of *Chrysanthemum* against *Anopheles subpicus*. These extracts also show high level of larvicidal activity *Theobroma cacao*, L.; methanol extract shows least mortality percentage. In 1ml, 2ml, 3ml it shows below 50 % of mortality and in 4ml rapid increases in the mortality percentage and it shows 76.66% of mortality. After three days there is slight increase in the mortality percentage. Tannins, saponins, terpenoids, carbohydrates are moderately present and also shows the minute presence of flavonoids, alkaloids, amino acids, proteins. So due to this moderate presence of phytochemicals, Cacao plant shows least larvicidal activity than other plants.

In the present study “larvicidal activity of selected plant extracts”, *Murraya konigii*, Spreng.; shows maximum larvicidal activity than *Gliricidia sepium*, (Jacq.) Kunth ex Walp.; the plant which undergo more studies related to its larvicidal activity and *Ficus elastica*, Roxb.; a plant also shows high larvicidal capacity also not undergo more studies related to its larvicidal activity. *Theobroma cacao*, L.; also shows larvicidal activity but less than other plants.

The effect of number on death days on larvae found to be minimum in this study. Because when passing number of days, the lethality of larvae are not very much increased.

Table 1: Qualitative analysis of phytochemicals present In the methanolic leaf extracts.

Phytochemicals	<i>Ficus elastica</i>	<i>Gliricidia sepium</i>	<i>Murraya konigii</i>	<i>Theobroma cacao</i>
Carbohydrates	+++	+++	++	++
Proteins	++	++	+++	+
Aminoacids	+	++	++	+
Steroids	+++	-	++	++
Glycosides	+	+++	++	-
Flavonoids	++	++	+	+
Alkaloids	++	++	+	+
Tannins	+++	++	+	++
Saponins	++	-	+	++
Terpenoids	++	++	+++	++

(+++ indicates strongly present, ++ indicates moderately present, + indicates less presence, - indicates absent)

Table 4: Percentage of mortality

Samples	Concentration	Percentage of mortality after 2 days	Percentage of mortality after 3 days
<i>Murraya konigii</i>	1ml	96.66 %	96.66%
	2ml	100%	100%
	3ml	100%	100%
	4ml	100%	100%
<i>Ficus elastica</i>	1ml	46.66 %	46.66%
	2ml	56.66%	56.66%
	3ml	96.67%	96.67%
	4ml	100 %	100%
<i>Gliricidia sepium</i>	1ml	50%	50%
	2ml	56.66%	60%
	3ml	93.3%	93.3%
	4ml	96.67%	96.67%
<i>Theobroma cacao</i>	1ml	3.33%	3.33%
	2ml	6.66%	16.66%
	3ml	23.33%	26.66%
	4ml	76.66%	80%

Chart 1: Larvicidal activity after two days

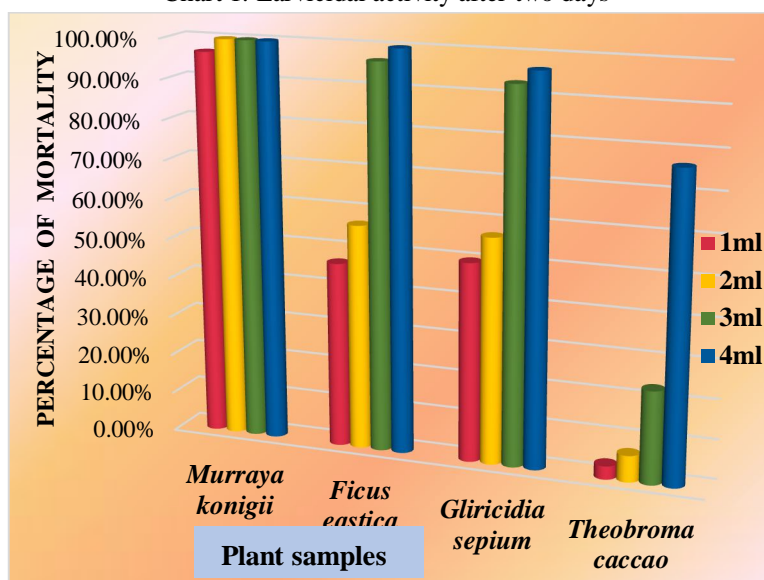


Chart 2: Larvicidal activity after three days

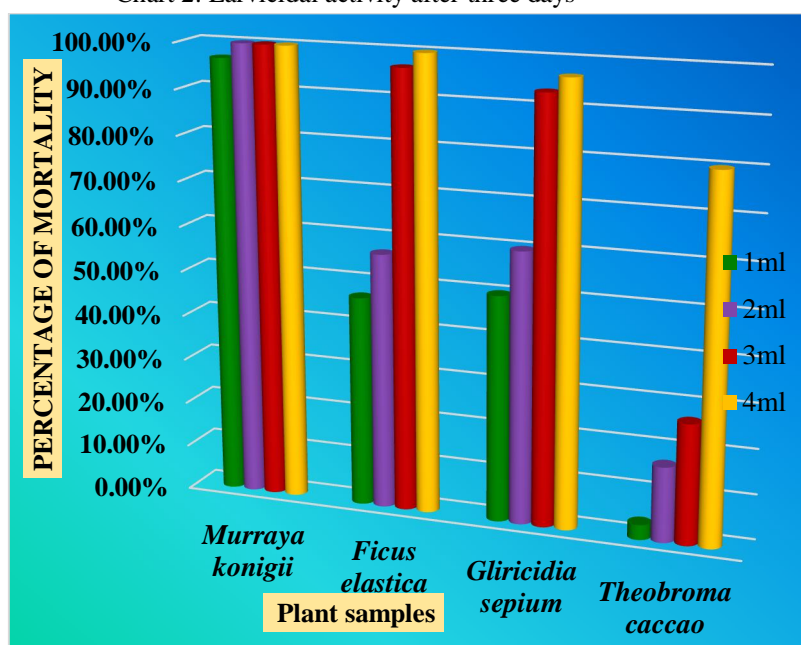


Table 2: Larvicidal activity of plant extract after 2 days

S. No	Samples	Concentration			
		1ml	2ml	3ml	4ml
1	<i>Ficus elastica</i>	4.66±1.15	5.66±0.57	8.33±1.15	8.33±0.57
2	<i>Gliricidia sepium</i>	5±0	5.66±0.57	9.33±1.15	9.66±0.57
3	<i>Murraya koenigii</i>	9.66±0.577	10±0	10±0	10±0
4	<i>Theobroma cacao</i>	0±0	0.66±1.15	2.33±1.52	7.66±2.51

(Mean ± standard deviation)

Table 3: Larvicidal activity of plant extract after 3 days

S. No	Samples	Concentration			
		1ml	2ml	3ml	4ml
1	Ficus elastica	4.66±1.15	5.66±0.57	8.66±0.57	9.33±0.57
2	Gliricidia sepium	5±0	6±0	9.33±1.15	9.66±0.57
3	Murraya koenigii	9.66±0.577	10±0	10±0	10±0
4	Theobroma cacao	0.33±0.57	1.66±0.57	2.66±1.15	8±2

(Mean ± standard deviation)

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