“COMPARATIVE ANALYSIS OF LARVICIDAL ACTIVITY OF THREE DIFFERENT FRUIT EXTRACTS AGAINST AEDES AEGYPTI”

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CERTIFICATE

This is to certify that the project work entitled **"COMPARATIVE ANALYSIS OF LARVICIDAL ACTIVITY OF THREE DIFFERENT FRUIT EXTRACTS AGAINST AEDES AEGYPTI"** is a bonafide work done by **RAHEEMA U A** with Reg. no 170021037729 during 2019 – 2020 in partial fulfillment of the requirement for the award of the Bachelor Degree of Science in Zoology of M G University Kottayam.

Head of the Department

Dr. Priyalakshmi G

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Lastly, I owe my friends for their moral support, encouragement and co-operation throughout my work.

DECLARATION

I do hereby declare that the work embodied in the dissertation entitled "**COMPARATIVE ANALYSIS OF LARVICIDAL ACTIVITY OF THREE DIFFERENT FRUIT EXTRACTS AGAINST AEDES AEGYPTI"**, submitted to Mahatma Gandhi University, Kottayam in partial fulfillment for the award of Bachelor of Science in Zoology, is record of bonafide dissertation done by me under the supervision of **Dr. SHERIN ANTONY**, Assistant professor, Department of Zoology, Bharata Mata College, Thrikkakara, and that no part of this work has been submitted for the award of any other degree/diploma/associate ship/fellowship or any other similar title to any candidate of any university.

Place: Thrikkakara Signature of Candidate

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ABSTRACT

Mosquitoes transmit serious human diseases, causing millions of deaths every year and the development of resistance to chemical insecticides resulting in rebounding vectorial capacity. And it cause adverse environmental effects in addition to high cost. Plants may be alternative sources of mosquito control agents. *Aedes aegypti,* yellow fever causing mosquito is a vector for transmitting several tropical fevers. It spreads dengue, chikungunya, zika fever, and other diseases. Currently no vaccines or drugs are developed to prevent dengue, zika, and chikungunya. The plan of present study was to analyse the larvicidal efficacy of fruit extracts of *Carica papaya; Citrullus lanatus; and Mangifera indica* against the larvae of Aedes aegypti. In the present study, larvicidal efficacy of different concentrations of *Carica papaya; Mangifera indica*; and *Citrullus lanatus* were evaluated for studying the mortality of *Aedes aegypti*. Present study revealed that fruit extract of *Carica papaya* shows much more effect in larvicidal activity of *Aedes aegypti*. Comparatively less activity showed by extract of *Citrullus lanatus* and no or little activity by extract of *Mangifera indica*. Different concentrations shows different activities. Higher concentrations shows relative high larvicidal activity. Combinations of extracts shows much more larvicidal activity. Of these, combination of *Carica papaya* and *Citrullus lanatus* shows better larvicidal activity, and other two combinations (*Citrullus lanatus + Mangifera indica* ; and *Carica papaya+ Mangifera indica*) showed less activity, it may due to the resistant larvicidal activity of *Mangifera indica*. As there is no vaccine for dengue , the only primary way to control the disease causing agent by natural ways. Our study revealed that fruit extract of *Carica papaya* and its combination with extract of *Citrullus lanatus* is a well ,powerful and cost effective biopesticide for the control of mosquitoes.

INTRODUCTION

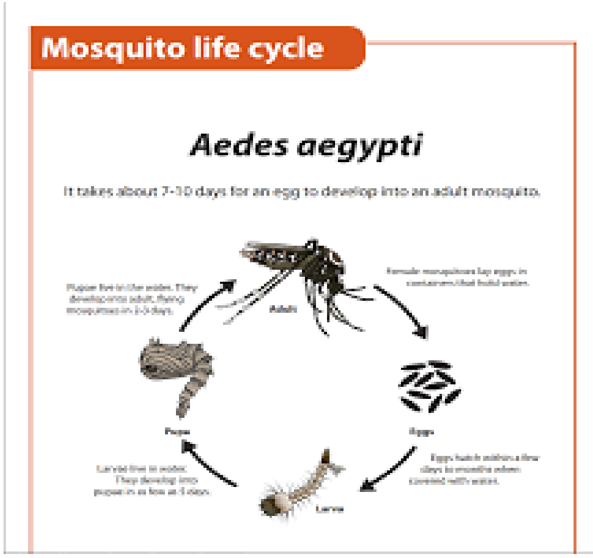
Mosquitoes comprise a group of about 3,500 species of small insects that are flies (order Diptera). Within Diptera they constitute the family **Culicidae.** Mosquitoes have a slender segmented body, one pair of wings, one pair of halters, three pairs of long hair-like legs, and elongated mouthparts. The mosquito life cycle consists of egg, larva, pupa, and adult stages. Eggs are laid on the water surface; they hatch into motile larvae that feed on aquatic algae and organic material. The adult females of most species have tube-like mouthparts (called a proboscis) that can pierce the skin of a host and feed on blood. Thousands of mosquito species feed on the blood of various hosts — vertebrates, including mammals, birds, reptiles, amphibians, and some fish; along with some invertebrates, primarily other arthropods. The mosquito's saliva is transferred to the host during the bite, and can cause an itchy rash. In addition, many species can ingest pathogens while biting, and transmit them to future hosts. In this way, mosquitoes are important vectors of diseases such as malaria, yellow fever, Chikungunya, West Nile, dengue fever, filariasis, Zika and other arboviruses. By transmitting diseases, mosquitoes cause the deaths of more people than any other animal taxon: over 700,000 each year.

Like all flies, mosquitoes go through four stages in their life cycles: egg, larva, pupa, and adult or imago. The first three stages—egg, larva, and pupa—are largely aquatic. Each of the stages typically lasts 5 to 14 days, depending on the species and the ambient temperature, but there are important exceptions. Mosquitoes living in regions where some seasons are freezing or waterless spend part of the year in *diapause*; they delay their development, typically for months, and carry on with life only when there is enough water or warmth for their needs. The eggs of some species of *Aedes* remain unharmed in diapause if they dry out, and hatch later when they are covered by water. Eggs hatch to become larvae, which grow until they are able to change into pupae. The adult mosquito emerges from the mature pupa as it floats at the water surface. Bloodsucking mosquitoes, depending on species, sex, and weather conditions, have potential adult lifespans ranging from as short as a week to as long as several months.

Aedes aegypti, the **yellow fever mosquito**, is a mosquito that can spread dengue fever, chikungunya, Zika fever, Mayaro and yellow fever viruses, and other disease agents. The mosquito can be recognized by white markings on its legs and a marking in the form of a lyre on the upper surface of its thorax. This mosquito originated in Africa but is now found in tropical, subtropical and temperate regions throughout the world. Only the female bites for blood, which she needs to mature her eggs. To find a host, these mosquitoes are attracted to chemical compounds emitted by mammals, including ammonia, carbon dioxide, lactic acid, and octenol. *Aedes aegypti* mosquitoes most commonly feed at dusk and dawn, indoors, in shady areas, or when the weather is cloudy, "they can bite and spread infection all year long and at any time of day. The yellow fever mosquito's distribution has increased in the past two to three decades worldwide, and it is considered to be among the most widespread mosquito species. Life stages of *Aedes aegypti* also includes egg, larva, pupa, and adult.

EGG

- Adult, female mosquitoes lay their eggs on the inner, wet walls of containers with water, above the waterline.
- Mosquitoes generally lay 100 eggs at a time.
- Eggs are very hardy; they stick to the walls of a container like glue and can survive drying out for up to 8 months— even over the winter in the southern United States.
- It only takes a very small amount of water to attract a female mosquito. Bowls, cups, fountains, tires, barrels, vases and any other container storing water makes for a great "nursery."



LARVA

- Larvae emerge from mosquito eggs, but only after the water level rises to cover the eggs. This means that rainwater or humans adding water to containers with eggs will trigger the larvae to emerge.
- Larvae feed on microorganisms in the water. After molting three times, the larva becomes a pupa.

PUPA

- Pupae will develop until the body of the newly formed adult flying mosquito emerges from the pupal skin and leaves the water.

ADULT

- After adult mosquitoes emerge: male mosquitoes feed on nectar from flowers and female mosquitoes feed on humans and animals for blood to produce eggs.
- After feeding, female mosquitoes will look for water sources to lay more eggs.
- Aedes aegypti only flies a few blocks during its life.
- Unlike other mosquito species, Aedes aegypti mosquitoes prefer to bite people.
- Aedes aegypti mosquitoes prefer to live near people. They can be found inside homes, buildings, and businesses where window and door screens are not used or doors are left propped open.

Members of the genus *Aedes* are known vectors for numerous viral infections. The two most prominent species that transmit viruses are *A. aegypti* and *A. albopictus*, which transmit the viruses that cause dengue fever, yellow fever, West Nile fever, chikungunya, eastern equine encephalitis, and Zika virus, along with many other, less notable diseases. Infections with these viruses are typically accompanied by a fever, and in some cases, encephalitis, which can lead to death. A vaccine to provide protection from yellow fever exists, and measures to prevent mosquito bites include insecticides such as DDT, mosquito traps, insect repellents, and mosquito nets.

Personal protection from mosquito bites is currently the most important way to prevent transmission of these diseases (Fradin et al., 1998). To prevent the proliferation of this mosquito borne diseases and to improve the quality of environment and public health, mosquito control is essential.

Biological control or "biocontrol" is the use of natural enemies to manage mosquito populations. There are several types of biological control including the direct introduction of parasites, pathogens and predators to target mosquitoes. Effective biocontrol agents include predatory fish that feed on mosquito larvae such as mosquitofish (*Gambusia affinis*) and some cyprinids (carps and minnows) and killifish. Tilapia also consume mosquito larvae. Direct introduction of tilapia and mosquitofish into ecosystems around the world have had disastrous consequences. However, utilizing a controlled system via aquaponics provides the mosquito control without the adverse effects to the ecosystem. Other predators include dragonfly (fly) naiads, which consume mosquito larvae in the breeding waters, adult dragonflies, which eat adult mosquitoes, and some species of lizard and gecko.

A **larvicide** (alternatively **larvacide**) is an insecticide that is specifically targeted against the larval life stage of an insect. Their most common use is against mosquitoes. Larvicides may be contact poisons, stomach poisons, growth regulators, or (increasingly) biological control agents.

Larviciding is successful way of reducing mosquito densities in their breeding places before they emerge into adults. Pesticides are indeed very effective in its use. However, the use of chemical insecticides are often toxic to both human and non-target animals. The intensive use of chemical insecticides led to the development of resistant insect populations, resulting in reduced control, environmental pollution resulting in bio-amplification in food chain and contamination (Hag et al., 1999).

Plants have the major advantage of still being the most effective and cheaper alternative green measure for the control of arthropods of public health importance (Rawani et al., 2009; Halder et al., 2011; Banerjee et al., 2012) [11, 8, 7]. Natural products of plant origin are safe to use than the synthetic insecticides (Kishore et al., 2011).

Carica papaya is one of the sole plant extracts used that have better larvicidal activity. Carica papaya, the sole species in the genus Carica of the plant family Caricaceae is widely cultivated. Papaya is a large tree-like plant, with a single stem growing from 5 to 10 meters tall with spiral leaves. It is used as remedy against a variety of diseases (Mello et al., 2008 and Munoz et al., 2000). The papaya is a small, sparsely branched tree, usually with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit were borne. The fruit is a large berry about 15–45 cm (5.9–17.7 in) long and 10–30 cm (3.9–11.8 in) in diameter. It is ripe when it feels soft (as soft as a ripe avocado or softer), and its skin has attained an amber to orange hue. The stem, bark and seed extracts have bactericidal activities (Emeruwa, 1982). The root infusion is used for syphilis in Africa and also used as analgesic. Leaf of papaya smoked for asthma relief in various remote areas.

Mangifera indica, commonly known as mango, is a species of flowering plant in the sumac and poison ivy family Anacardiaceae. It is native to the Indian subcontinent where it is indigenous. Hundreds of cultivated varieties have been introduced to other warm regions of the world. It is a large fruit-tree, capable of growing to a height and crown width of about 30 metres (100 ft) and trunk circumference of more than 3.7 metres (12 ft). Mangiferin (a pharmacologically active hydroxylated xanthone C-glycoside) is extracted from mango at high concentrations from the young leaves (172 g/kg), bark (107 g/kg), and from old leaves (94 g/kg). In Ayurveda, it is used in a Rasayana formula sometimes with other mild sours and shatavari (*Asparagus racemosus*) and guduchi (*Tinospora cordifolia*). In traditional medicine, varied properties are attributed to different parts of the mango tree.

Citrullus lanatus is a plant species in the family Cucurbitaceae, a vine-like flowering plant originating in West Africa. It is a highly cultivated fruit worldwide, having more than 1000 varieties. Citrullus is a scrambling and trailing vine in the flowering plant family Cucurbitaceae. There is evidence from seeds in Pharaoh tombs of watermelon cultivation in Ancient Egypt. large edible fruit, which is a berry with a hard rind and no internal divisions, and is botanically called a *pepo*. The sweet, juicy flesh is usually deep red to pink, with many black seeds, although seedless varieties exist. The watermelon is an annual that has a prostrate or climbing habit. Stems are up to 3 m long and new growth has yellow or brown hairs. Leaves are 60 to 200 mm long and 40 to 150 mm wide. These usually have three lobes which are themselves lobed or doubly lobed. Plants have both male and female flowers on 40-mm-long hairy stalks. These are yellow, and greenish on the back. The *citrullus lanatus* is a large annual plant with long, weak, trailing or climbing stems which are five-angled (five-sided) and up to 3 m (10 ft) long. Young growth is densely woolly with yellowish-brown hairs which disappear as the plant ages. The leaves are large, coarse, hairy pinnately-lobed and alternate; they get stiff and rough when old. The plant has branching tendrils. The white to yellow flowers grow singly in the leaf axils and the corolla is white or yellow inside and greenish-yellow on the outside.

The main objective of this study was to test the larvicidal activity of fruit extract of Carica papaya(papaya), Citrullus lanatus(water melon), and Mangifera indica(mango). And also the combining efficacy of these fruit extracts by two each other (Carica papaya + Citrullus lanatus; Carica papaya + Mangifera indica; and Citrullus lanatus + Mangifera indica). To comparatively analyse the larvicidal activity of these fruit extracts.

AIM

- To analyse the larvicidal activities of extracts of three different fruit species (*Carica papaya; Citrullus lanatus*; *Mangifera indica*) against the larvae of *Aedes aegypti* and the mortality rate of *Aedes aegypti.* And also to analyse the larvicidal efficacy of fruit extracts when used in combination (*Carica papaya+ Citrullus lanatus; Carica papaya+Mangifera indica; Citrullus lanatus+Mangifera indica*) against Aedes larvae at different concentrations.

OBJECTIVES

- To find out an efficient biolarvicide- to control the growth of dengue causing mosquito,Aedes aegypti.
- To minimize the use of various harmful chemical insecticides.
- To analyse the larvicidal efficacy of three fruit extracts.
- To compare the larvicidal efficacy of three fruit extracts when used in combination (Carica papaya; Citrullus lanatus; Mangifera indica).

REVIEW OF LITERATURE

Literature on various aspects of the utilization of botanicals in the management of selected culicine larvae pertaining to the present investigation is reviewed.

Use of larvicides, dates back to as early as 1899, when Sir Ronald Rossapplied kerosene on anopheline larval breeding sites in Sierra Leone, West Africa was an approach with great potential for malaria control (Bockarie et al.,1999;Kileen et al., 2002). Toxicity of phytochemicals to mosquitoes was first reported by Campbell et al. (1933). They have also evaluated the relative toxicity of nicotine, nabasine, methylanabasine and lupine for culicine mosquito larvae.

Watson (1941) reported the effect of antimalarial oil against mosquito larvae. Toxicity of rotenone extracted from indigenous Derris roots to mosquito larvae was investigated by Ameen et al. (1983)

Toxicity of natural essential oils to mosquitoes, Aedes aegypti L. and Culex fatigans Wiedemann (syn. Culex quinquefasciatus Say) was investigated by Sharma et al. (1994). Perich et al. (1994) demonstrated the toxicity of extracts of three species of Tagetes against adults and larvae of yellow fever mosquito and Anopheles stephensi Liston. (Diptera: Culicidae). Sharma and Saxena (1994) studied the phytotoxicological potential of Tagetes erectes L. extract on aquatic stages of An. stephensi. Das et al. (1996) studied the toxicity of some alkaloids on the larvae of Culex quinquefasciatus Say. Azmi et al. (1998) evaluated the toxicity of neem leaf extract (NLX) and compared the same with that of malathion (57 E.C.) against late third instar larvae of Cx. fatigans (wild strain) by the WHO method.

El Hag et al. (1999) reported the toxic and growth-retarding effects of three plant extracts (Azadirachta indica A. Juss, Rhazya stricta Decne and Syzygium aromaticum L. Merrill and Perry) on Culex pipiens larvae (Diptera: Culicidae). Differential toxicity of leaf litter to dipteran has been worked out by David et al.(2000). Anyaele and Amusan (2003) highlighted the toxicity of hexanolic extract of Dennettia tripetala G. Baxer to the larvae of Ae. aegypti. Toxicity of the latex of Nerium indicum Mill and Euphorbia royleana Boiss to Cx. quinquefasciatus larvae was assessed by Srivastava et al. (2003). The toxic effects of neem products (A. indica) on Ae. aegypti larvae were investigated by Ndione et al. (2007).

BIOLOGICAL STUDIES

Botanicals that are tested against the life stages of the mosquitoes have a profound effect on the developmental period, growth, adult emergence, fecundity, fertility and egg hatchability. Several studies have been carried out by various researchers to assess the impact of plant products in the life stages of mosquitoes.

Maradufu et al. (1978) reported the isolation of (5E)-ocimenone, a mosquito larvicide, from Tagetes minuta L. Effects of certain essential oils on mortality and metamorphosis of Ae. aegypti were reported by Osmani and Sighamony (1980). Arnason et al. (1981) analysed the mosquito larvicidal activity of polyacetylenes from certain species of the family Asteraceae. Some components and their properties of the neem tree (A. indica) and their use in pest control in developing countries were assessed by Schmutterer (1981). Kalyanasundaram and Babu (1982) described the biologically active extract of the plant, Cleome viscose L. as a mosquito larvicide. Saxena and Yadav (1983) evaluated the extract of Oligochaeta ramose Roxb. Wagenitz (Family:Compositae) to suppress the population of the yellow fever and dengue vector Ae. aegypti (Diptera: Culicidae).

Studies on the larvicidal properties of Nerium indicum Mill. (Apocynaceae) leaves were carried out by Chavan and Nikam (1983). More than 2,000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes (Ahmed et al., 1983). Zebitz (1984) suggested that azadirachtin acts as an antiecdysteroid and thus kills the larvae by inhibiting their growth. Effects of Melia azadirachta L. extracts on many insects have been reported by various investigators (Saxena et al., 1984; Schmidt et al., 1998; Juan et al., 2000; Carpinella et al., 2003; Nathan and Saehoon, 2005). The effect of crude methanolic extracts of Ajuga spp. on postembryonic development of different mosquito species was observed by Marcard et al. (1986).

Laboratory studies of some plant extracts as mosquito larvicides were reported by Qureshi et al. (1986). Indigenous plant oils were evaluated for their larvicidal potential against An. stephensi (Kumar and Dutta, 1987). Evaluation of Melia volkensii Gurke extract fractions as mosquito larvicides was done by Mwangi and Mukiama (1988). Growth-inhibiting and larvicidal effects of M. volkensii extracts on Ae. aegypti larvae were suggested by Mwangi and Rembold (1988).

Influence of several plant extracts on the oviposition behaviour of Aedes fluviatilis (Lutz) (Diptera: Culicidae) in the laboratory was reported by Consoli et al. (1989). Mosquito larvicidal and ovipositional activity of Descurania sophia (L.) Webb ex Prantl extract was studied by Mohsen et al. (1990). Jackson et al. (1990) evaluated the larvicidal effects of sorghum (Sorghum bicolor (L.) Moench) seedling extracts upon Cx. pipiens larvae. Chockalingam et al. (1990) analysed the larvicidal activity of different plant products against mosquito larvae.

Monzon et al. (1994) observed the larvicidal potential of five Philippine plants against Ae. aegypti and Cx. quinquefasciatus. Effect of neem oil on mosquito larvae was observed by Sinniah et al. (1994). Control of mosquito breeding has also been demonstrated in the field in some confined habitats using indigenous methods of application of neem oil in water and neem oil coated wooden scraps (Nagpal et al., 1995; Batra et al., 1998). Sharma (1996) assessed the ovicidal and growth disrupting activity of Sphaeranthus indicus Linn extract against the filariasis vector, Cx. quinquefasciatus. Dhar et al. (1996) demonstrated the effect of neem oil volatiles on the gonotropic cycle and inhibition of oviposition in An. stephensi and Anopheles culicifacies Giles. Ee and Lee (1997) studied the larvicidal activity of some plants belonging to the Sarawak province. Limonoids from Citrus reticulate Blanco and their moult-inhibiting activity against Cx.quinquefasciatus larvae were described by Jeyaprakasha et al. (1997). Indigenous plant extracts as larvicidalagents against Cx. quinquifasciatus was investigated by Karmegam et al. (1997).

Fruit extracts of Melia azadirachta Linn. exhibit a variety of effects in insects such as antifeedant, growth retardation, reduced fecundity, moulting disorders, morphogenetic defects and changes in behaviour (Schmidt et al., 1998; Gajmer et al., 2002; Banchio et al., 2003; Wandscheer et al., 2004). Moawed (1998) compared the combined action of some plant extracts against Cx. pipiens larvae and their physiological impact.

Nicharat et al. (1999) analysed the larvicidal effects of cashew nut (Anacardium occidentale L.) shell extracts on mosquito larvae. Palakulk et al. (1999) studied the larvicidal activity of Thai Ka-lum-pak sa-lad dai Euphorbia antiquorum Linn. against Aedes, Culex, Anopheles and Mansonia larvae in laboratory conditions. Larvicidal, adulticidal and repellent effects of Kaempferia galanga Linn (Family:Zingiberaceae) was found out by Choochote et al. (1999). Olagbemiro et al. (1999) investigated the production of (5R, 6S)-6-acetoxy-5- hexadecanolide, the mosquito oviposition pheromone, from the seed oil of the summer Cyprus plant, Kochia scorparia L. Roth (Chenopodiaceae). Murugan and Jeyabalan (1999) observed that Lecus aspera (Wild) Link, Ocimum sanctum

Linn, A. indica, Allium sativum L. and Curcuma longa Linnaeus had a strong larvicidal, antiemergence, adult repellency and antireproductive activity against An. stephensi.
Mosquito larvicidal activity of pipermonaline, a piperidine alkaloid derived from long pepper, Piper longum L. was described by Lee (2000). Evaluation of some Moroccan medicinal plant extracts for larvicidal activity was reported by Markouk et al. (2000). Ee et al. (2000) analysed potential larvicides from Malaysian plants. Insecticidal activity of some South American medicinal plants against Aedes larvae was evaluated by Ciccia et al. (2000).

Comparative sensitivity of larval mosquito to vegetable polyphenols versus conventional insecticides was described by Rey et al. (2001). Effects of plant extract on fecundity and fertility of mosquitoes has been reported by Muthukrishnan and Pushpalatha (2001). The use of commercial saponin extracted from Quillaja saponaria Molina bark as a natural larvicidal agent against Ae. aegypti and Cx. pipiens was reported by Pelah et al. (2002). Yang et al. (2002) implied that a piperidine amide extracted from Piper longum L. fruit shows activity against Ae. aegypti mosquito larvae. The larvicidal activity of leguminous seeds and grains against Ae. aegypti and Culex pipiens pallens (Coquillett) was confirmed by Jang et al. (2002). Goniothalamin, extracted from Bryonopsis laciniosa L. was found to be a potent mosquito larvicide by Kabir et al. (2003). The larvicidal activity of essential oils from

Brazilian plants against Ae. aegypti was tested by Cavalcanti et al. (2004). The ovicidal activity of Moschosma polystachyum Linn. (Lamiaceae) leaf extract against Cx. quinquefasciatus was tested by Rajkumar and Jebanesan (2004). Chapagain and Wiesman (2005a and 2005b) elucidated the larvicidal activity of the fruit mesocarp extract of Balanites aegyptiaca (L.) Delile and its saponin fractions against Ae. aegypti.

Larvicidal activity of Tagetes patula L. essential oil against three mosquito species was reported by Dharmagadda et al. (2005). Nathan et al. (2006a) reported the efficacy of Melia azedirach L. extract on the malarial vector An. stephensi. Chaithong et al. (2006) investigated the larvicidal effect of pepper plants on Ae. Aegypti. From the seed extract of Sterculia guttata Roxb an effective mosquito larvicide was isolated by Katade et al. (2006). Promsiri et al. (2006) investigated the effects of the extracts of hundred and twelve medicinal plant species of which eight out of fourteen plant species showed 100 percent mosquito larvae mortality and the LC50 values were less than 100 µg/mL.

Evaluation of larvicidal activity of the leaf extract of a weed plant, Agerantina adenophora (Spreng.) King and H. Rob against two important species of mosquitoes, Ae. aegypti and Cx. quinquifasciatus, was reported by Mohan and Ramaswamy (2007).

Tiwary et al. (2007) analysed the chemical composition and larvicidal activities of the essential oil of Zanthoxylum armatum DC (Rutaceae) against Ae. aegypti, Cx. quinquefasciatus and An. stephensi. Among the three mosquito species tested Cx. quinquefasciatus was the most sensitive followed by Ae. Aegypti and An. stephensi with LC50 values of 49 ppm, 54 ppm and 58 ppm. Larvicidal activities of some Brazilian medicinal plants against Ae. Aegypti was assessed by Omena et al. (2007).

Kaushik and Saini (2008) highlighted the larvicidal activity of the leaf extract of Millingtonia hortensis L.f. (Begnoniaceae) against An. stephensi, Cx. quinquefasciatus and Ae. aegypti. Kempraj and Bhat (2008) investigated the ovicidal and larvicidal activities of the essential oils extracted from Cyperus giganteus Vahl and Cyperus rotundus Linn. against Aedes albopictus (Skuse). Larvicidal effect of Lantana camara Linn. against Ae.

aegypti and Cx. quinquefasciatus was reported by Kumar and Maneemegalai (2008). Larvicidal activity of Leucus aspera (Wild.) against the larvae of Cx. quinquefascistus and Ae. aegypti was recorded by Maheswaran et al. (2008). Rahuman et al. (2008) identified the mosquito larvicidal activity of oleic and linoleic acids isolated from Citrullus colocynthis (Linn). Larvicidal activity of neem and karanja oil cakes against mosquito vectors, Cx. quinquefasciatus, Ae. aegypti and An. stephensi was observed by Shanmugasundaram et al. (2008). Anees (2008) found out the larvicidal activity of Ocimum sanctum Linn. (Labiatae) against Ae. aegypti and Cx. Quinquefasciatus

Vincent et al. (2009) assessed that methanol extract of Citrus limon (L.) Burm. F. elicited hundred percent ovicidal activity at 3 % concentration against Ae. aegypti eggs. Sulaiman et al. (2009) evaluated the efficacy of bifenthrin and hexane extract of Acorus calamus Linn. against the fourth instar of Ae. aegypti L. with LC50 and LC90 values of 0.4418 and 11.3935 ppm and Ae. albopictus (Skuse) with a higher LC50 and LC90 values of 21.2555 ppm and 36.1061 ppm, respectively. There was a significant difference on the effect of A. calamus extract on both the species of Aedes larvae ($P< 0.05$).

Row and Ho (2009) investigated the antimicrobial activity, mosquito larvicidal activity, and antioxidant property and tyrosinase inhibition of Piper betle L. Larvicidal activity of a neem tree extract (Azadirachtin) against mosquito larvae in the Republic of Algeria was observed by Alouani et al. (2009) Larvicidal and emergence inhibitory activities of NeemAzal T/S 1.2 percent EC against vectors of malaria, filariasis and dengue were studied by Gunasekaran et al. (2009). Ramos et al. (2009) observed the potential of laticifer fluids in inhibiting Ae. aegypti larval development.

Larvicidal efficacy of seed oils of Pterocorpus santalinoides DC and tropical Manihot species against Ae. aegypti and effects on aquatic fauna was observed by Adeleke et al. (2009). Egg hatchability and larvicidal activity of Swertia chirata Buch.-Hams.ex.Wall. against Ae. aegypti L. and Cx. quinquefasciatus was investigated by Balaraju et al. (2009). Evaluation of larvicidal effects of essential oils of some local plants against Anopheles arabiensis Patton and Ae.aegypti Linnaeus (Diptera:Culicidae) in Ethiopia was observed by Massebo et al. (2009).

Borah et al. (2010) investigated the hexane, acetone and methanol extracts of mature fruits and leaves from Toddalia asiatica (Linn). Lam to establish its bio-control potentiality under laboratory condition against fourth instar of dengue vector, Ae.aegypti. Hexane extract of fruits of T. asiatica showed highest larvicidal activity against both mosquito vectors. LC50 value of hexane, acetone and methanol extracts of fruits against Ae. aegypti were 37.23, 50.69 and 125.55 ppm. .Chemical composition and larvicidal activity against Ae. aegypti larvae of essential oils from four Guarea species was analysed by Magalhaes et al. (2010). Screening of the weed plant species Croton bonplandianum Baill. for larvicidal activity of Ae. aegypti was done by Jeeshna et al. (2010).

MANAGEMENT STRATEGIES

Studies on the effect of botanical derivatives on mosquito vectors of disease indicated that biopesticides are good alternatives for synthetic chemical pesticides. Different methods have been designed in the management of mosquitoes.

Bowers et al. (1995) identified the activity of Turkish medicinalplants against mosquitoes Ae. aegypti and An. gambiae. Isolation of the insecticidal components of Tagetes minuta L. (Compositae) against mosquito larvae and adults was reported by Perich et al. (1995). Insecticidal fatty acids and triglycerides from Dirca palustris L. was assessed by Ranaweera (1996). Evaluation of some wild herb extracts for the control of mosquitoes was carried out by El Hag et al. (1996).

The potential of botanical essential oils for insect pest control was reported by Roger (1997). Rohani et al. (1997) evaluated the adulticidal properties of some Malaysian plants on vector mosquitoes. Chemical methods for the control of vectors and pests of public importance were evaluated by Chavasse and Yap (1997). Thorsell et al. (1998) suggested the efficacy of plant extracts and oils as mosquito repellents). Ramsewak et al. (1999) reported that three biologically active compounds from Murraya koenigii L. Sprengel possess mosquitocidal and antimicrobial properties, exhibiting topoisomerase 1 and 11 inhibition activities. Palsson and Jenson (1999) studied the plant products that are used as mosquito repellents in GuineaBissau.

Bindra et al. (2000) reported the use of essential oils containing preparation for human protection against mosquitoes. Bioactivity of some medicinal plants against chosen insect pests/vectors has been highlighted by Tare (2000). Effect of Feronia limonia L. on mosquito larvae was assessed by Rahuman et al. (2000). Repellent activity of constituents identified in Foeniculum vulgare Mill. fruit against Ae. aegypti was described by Ahn et al. (2002). Comparative efficacy of insect repellents against mosquito bites was observed by Fradin and Day (2002).

Bioactivity of selected plant essential oils against the yellow fever mosquito Ae. aegypti larvae was studied by Cheng et al. (2003). Choochote et al. (2004) found out the potential of crude seed extract of celery, Apium graveolens L., against the mosquito Ae. aegypti. The adulticidal potency with LD50 and LD95 values of 6.6 and 66.4 mg/cm2 and repellency against Ae.aegypti females with ED50 and ED95 values of 2.03 and 28.12 mg/cm2 respectively.

Rongsriyam et al. (2006) carried out a preliminary study to verify the efficacy of tablets prepared from the crude extract of Rhinacanthus nasutus L. (local Thai plant) against Ae. aegypti larvae. Efficacy of natural product from Clerodendron inerme L. against dengue vector Ae. aegypti was reported by Patil et al. (2006). Efficacy of botanical extracts from Callitris glaucophylla (Joys Thomps. and L. A. S. Johnson) against Ae. aegypti (Skuse) mosquitoes was observed by Shaalan et al. (2006).

The repellent activity of Piper aduncum Linn (Piperaceae) essential oil against Ae. aegypti using human volunteers was analysed by Misni et al. (2008). Hardin and Jackson (2009) described the applications of natural products in the control of mosquito-transmitted diseases. Insecticidal activities of leaf and twig essential oils from Clausena excavata Burm.f. against Ae. aegypti and Ae. albopictus larvae were documented by Cheng et al. (2009). The LC50 values of leaf and twig essential oils against fourth instar larvae of Ae.aegypti and Ae.albopictus were 37.1-40 μg mL-1 and 41.1-41.2 μg mL-1 .

METHODS AND METHODOLOGY

COLLECTION AND PREPARATION OF EXTRACT OF PLANT MATERIALS

The fruit of *Citrullus lanatus* was collected during the month of January 2020. The fruit was washed thoroughly and dried in shade. The shade fruit was cut open to take the mesocarp part of the fruit and used for further studies. Extraction of mesocarp of *Citrullus lanatus* was carried out by washing the mesocarp and allowed to dry at room temperature. The mesocarp was cut into small pieces for easier grinding. Then a few pieces were subjected to grinding in the grinder. After grinding collect the extract in a petridish. Repeat this with remaining pieces for more extract. After that filter the extract if any solid materials are found.

The fruit of *Mangifera indica* was collected during the month of January 2020. The fruit was washed thoroughly and dried in shade. The shaded fruit was cut open to take the mesocarp part of the fruit and used for further studies. Extraction of mesocarp of *Mangifera indica* was carried out by washing the mesocarp and allowed to dry at room temperature. The mesocarp was cut into small pieces for easier grinding. Then a few pieces were subjected to grinding in the grinder. After grinding collect the extract in a petridish. Repeat this with remaining pieces for more extract. After that filter the extract if any solid materials are found.

The fruit of *Carica papaya* was collected during the month of January 2020. The fruit was washed thoroughly and dried in shade. The shade fruit was cut open to take the mesocarp part of the fruit and used for further studies. Extraction of mesocarp of *Carica papaya* was carried out by washing the mesocarp and allowed to dry at room temperature. The mesocarp was cut into small pieces for easier grinding. Then a few pieces were subjected to grinding in the grinder. After grinding collect the extract in a petridish. Repeat this with remaining pieces for more extract. After that filter the extract if any solid materials are found.

COLLECTION OF MOSQUITO LARVAE

The water in a tyre was kept in a shaded area for the mosquito to come and lay eggs. Check the water content if there any larval movement can seen after two days under shaded region. Make sure that no overflow of water take place due to rain or any water falls to avoid escape of larvae through water. After two days larvae can be collected from the water container. And collect it into a bottle. And used for further analysis of larvicidal activity of various fruit extracts. Larvae of *Aedes aegyptii* was collected from my region. And further experiment was based on the larvae of *Aedes aegyptii.*

LARVICIDAL BIOASSAY

The larvae collected was tested for the larvicidal activity of three plant fruit extracts of *Mangifera indica*, *Carica papaya*, *Citrullus lanatus* and the combinations of *Mangifera indica* and *Carica papaya*; *Mangifera indica* and *Citrullus lanatus*; *Carica papaya* and *Citrullus lanatus*. The larvicidal bioassay followed by World Health Organization (WHO) standard protocol with slight modifications. Aqueous extact of each sample at various concentrations in different petridishes to test separately. 0.5g,1g,1.5g,2g,2.5g of extracts were taken in different petridishes. And distilled water at 1ml quality added and 5 larvae are added to all petridishes each applied for the bioassay experiment. All the experiments were conducted in triplicate and control were performed at parallel condition in each series of experiments. No food was provided for the larvae. Larvae was considered dead if they were unrousable within a period of time. Larval mortality was recorded at 48h exposure by *Carica papaya* and slighter larvicidal activity was shown by *Citrullus lanatus*. *Mangifera indica* shows no larvicidal activity.

STATISTICAL ANALYSIS

Statistical analysis of the experimental data was prepared.

Table.1

LARVAE	SAMPLE	CONCENTRATION(G)	MORTALITY(%)		
			24h	48h	72h
Aedes aegypti	Citrullus lanatus	0.5	0	0	20
		1	0	0	20
		1.5	0	20	40
		2	0	20	40
		2.5	0	20	40
	Carica papaya	0.5	20	20	40
		1	40	40	60
		1.5	40	40	60
		2	80	80	100
		2.5	80	80	100
	Mangifera indica	0.5	0	0	0
		1	0	0	0
		1.5	0	0	0
		2	0	0	0
		2.5	0	20	20

RESULT AND OBSERVATIONS

Larvicidal activity of *Mangifera indica* against larvae of Aedes aegypti at different concentrations.

Table2.

Conc. Of Mangifera indica	No .of larvae introduced	No. of larvae died
0.5	5	0
1	5	0
1.5	5	0
2	5	0
2.5	5	1

Larvicidal activity of *Carica papaya* against larvae of Aedes aegypti at different cocentratios.

Table3.

Conc. Of Carica papaya(g)	No. of larvae introduced	No. of larvae died
0.5	5	2
1	5	3
1.5	5	3
2	5	5
2.5	5	5

Larvicidal activity of *Citrullus lanatus* against larvae of Aedes aegypti at different cocentrations.

Table4.

Conc. Of Citrullus lanatus(g)	No. of larvae introduced	No. of larvae died
0.5	5	1
1	5	1
1.5	5	2
2	5	2
2.5	5	2

Larvicidal activity of combinations of *Carica papaya* and *Mangifera indica* against larvae of Aedes aegypti at different concentrations.

Table5.

Conc. Of Carica papaya+ Mangifera indica(g)	No. of larvae introduced	No. of larvae died
0.5	5	2
1	5	3
1.5	5	3
2	5	5
2.5	5	5

Larvicidal activity of combinations of *Carica papaya* and *Citrullus lanatus* against larvae of Aedes aegypti at different concentrations.

Table6.

Conc. Of Carica papaya+ Citrullus lanatus(g)	No. of larvae introduced	No. of larvae died
0.5	5	5
1	5	5
1.5	5	5
2	5	5
2.5	5	5

Larvicidal activity of combinations of *Citrullus lanatus* and *Mangifera indica* against larvae of Aedes aegypti at different concentrations.

Table7.

Conc. Of Citrullus lanatus + Mangifera indica	No. of larvae introduced	No. of larvae died
0.5	5	0
1	5	0
1.5	5	1
2	5	1
2.5	5	2

Larvicidal activity is shown greatly by extract of *Carica papaya* and slightly by *Citrullus lanatus* and *Mangifera indica* does not show any larvicidal activity comparably. In combination treatment, combinations of *Carica papaya* and *Citrullus lanatus* shows maximum larvicidal activity in the experiment (analysis). Combination of *Carica papaya* and *Mangifera indica* also shows considerable larvicidal activity while the combination of *Mangifera indica* and *Citrullus lanatus* shows less or no larvicidal activity but it is slightly more than when treated with *Mangifera indica* alone.

DISCUSSION

Mosquitoes not only cause nuisance by their bites but also transmit a number of diseases than any other group of arthropods and affect more than 700 million people worldwide annually, including arboviruses responsible for yellow fever, dengue hemorrhagic fever, epidemic polyarthritis, several forms of encephalitis and bancroftianfilariasis (Kazembe and makusha, 2012) [6] and pathogens which continue to have devastating effect on human beings (Maheswaran et al., 2008) [5]. Dengue Fever (DF) is transmitted by *Aedes aegypti* while*, Ae. albopictus* is considered as maintance vector in Southeast Asia (CDC 2001).

Among the approximately 4000 known mosquito species less than 10% are regarded as efficient vectors of pathogenic agents of infectious diseases having high impact,both direct and indirect, on human welfare and health. Mosquito transmitted diseases remain a major cause of the loss of human life worldwide with more than 700 million peoples suffering from these diseases annually (Taubes 1997). Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however no part of the world is free from vector-borne diseases(Fradin and Day 2002).

Personal protection from mosquito bites is currently the most important way to prevent transmission of these disease (Fradin, 1998) [15]. To prevent proliferation of this mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. Rational control of mosquitoes lies in personal protection and community education as the most economical method in eradicating breeding sites and application of eco-friendly larvicides for the control of mosquito larvae (Certin et al. 2004). Synthetic insecticides are no doubt having quick actions but due to their adverse effects to the environment received wide public concern (St leger et al. 1996), like insecticide resistance (Severini et al. 1993), environmental pollution, toxic hazards to human and other non-target organisms (Forget 1989). To mitigate these problems, a major emphasis has recently been explored which includes the use of natural plant based products as larvicides which can provide an alternate to synthetic chemical insecticides (Junwei et al. 2006)

The larvicidal activity of selected parts of various plants shows mortality of *Aedes aegypti.* Bowers et al. (1995) carried out tests on organo soluble extracts from 55 Turkish medicinal plants and tested them under standardized conditions for biological activity against third instar larvae of mosquitoes, Ae. aegypti and An. Gambiae. Park et al. (2002) found the larvicidal activity of fruits of Piper nigrum against third instar larvae of Ae. aegypti. Bansal et al. (2011) found that larvicidal potential of the extracts from different parts viz. green and red fruits, seeds, fruits without seeds, leaves and roots of Withania somnifera in different solvents were evaluated against third and fourth instar larvae of Ae. aegypti.

The present study revealed the effectiveness of different plant extracts in larvicidal activity of *Aedes aegypti.* In the present study, the larvicidal activity of Carica papaya, Mangifera indica, Citrullus lanatus against the larvae of Aedes aegypti was tested. In the individual test, fruit extract of Carica papaya shows highest larvicidal activity in 72 hours. In 24 hour, it shows less activity and in low concentration. Thus it can reveal that the larvicidal activity exceeds as the concentration and time exposure increases. It is followed by the fruit extract of Citrullus lanatus, it shows no effect in first 24 hour. In 72 hour, it shows less activity. Thus larvicidal activity of Citrullus lanatus is less as combared to effect of Carica papaya. The extract of Mangifera indica shows the least larvicidal activity when combared to other two extracts. It shows no effect in low concentration. But it shows no or less larvicidal effect in 2.5g concentration even in 72 hour exposure.

Also studied the larvicidal effect when in used in combination. The combinations of *Carica papaya and Citrullus lanatus; Carica papaya and Mangifera indica;* and *Citrullus lanatus and Mangifera indica* were used to check the larvicidal activity. The activity was highest in combinations of *Carica papaya and Citrullus lanatus*. It shows maximum effect in 72 hour exposure to Aedes. It is followed by Carica papaya alone within 72 hours. The combination of Citrullus lanatus and Mangifera indica; and combination of Carica papaya and Mangifera indica shows resistant to the larvicidal activity due to the presence of extract of Mangifera indica. Whereas other extracts of Citrullus lanatus and combinations of Citrullus lanatus and Mangifera indica; and combination of Carica papaya and Mangifera indica shows slight larvicidal activity within 78hours. Percent mortality was found to increase upon exposure to higher concentrations.

In the present study Carica papaya showed maximum larvicidal activity compared with other extracts in the study. Carica papaya shows synergetic effect when combined with other extracts of Mangifera indica and Citrullus lanatus. Whereas Mangifera indica shows less or no larvicidal activity alone and also when combined with other extracts.

Our data suggest that the Carica papaya fruit extract have the potential to be used as an ecofriendly approach for the control of Aedes aegypti.

CONCLUSION

Our findings showed that fruit extracts of Carica papaya can be developed as ecofriendly larvicide. Synthetic chemicals which are widely used to control mosquitos could be obtained easily at a very low cost, but they cause serious health hazards to humans and other aquatic organisms when it reaches water bodies. Use of plants for larvae control offers a safer alternative. The combination of the extract of Carica papaya and *Citrulus lanatus* also has a significant larvicidal activity. By using these extracts as an alternative, we can surely save money, health effects and we can have safer products for the control of mosquito larvae. Also our results open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts.

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