"LARVICIDAL ACTIVITY OF LEAF EXTRACTS AGAINST ANOPHELES MOSQUITO LARVAE"

Dissertation submitted to Mahatma Gandhi University

In partial fulfillment of the requirements for the award of the degree of

BACHELOR OF SCIENCE IN ZOOLOGY

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BHARATA MATA COLLEGE

THRIKKAKARA

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Date: **Submitted by,**

Teacher in charge: **SHIFNA C F**

Examiner: **Reg no :170021037733**

DEPARTMENT OF ZOOLOGY
BHARATA MATA COLLEGE
THRIKKAKARA

Date: .05. 2020

CERTIFICATE

This is to certify that the project entitled LARVICIDAL ACTIVITY OF LEAF EXTRACTS AGAINST ANOPHELES MOSQUITO is a bonafide work done by Shifna C F with Register No: 170021037733 during 2019-20 in partial fulfilment 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

DECLARATION

I do hereby declare that the work embodied in the dissertation entitled **"Larvicidal activitiy of leaf extracts against anopheles mosquito larvae",** submitted to Mahatma Gandhi University, Kottayam in partial fulfillment for the award of Bachelor of Science in Zoology is a record of bonafide dissertation done by me under the supervision of **Dr. Sherin Antony**, Associate professor of the 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

Date:

Signature of the candidate

Name: SHIFNA C F

REG NO: 170021037733

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SHIFNA C F

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ABSTRACT

Background Insecticide resistance carries the potential to undermine the efficacy of insecticide based malaria vector control strategies. Therefore, there is an urgent need for new insecticidal compounds. This study was designed to determine the larvicidal effect of leaf extract(Neem, Adhatoda and Aloe vera)against the larvae of anopheles mosquitoes. Anopheles larvae were obtained using a deeper from stagnant water and taken to our lab to further analysis. Samples of Neem, adhatoda and aloe vera were collected. The concentration taken is 0.5mg/ml, 1.0mg/ml, 2.0mg/ml. Mortality of Anopheles larvae depends on these extracts and increase with time of exposure and concentration of extract.2mg/ml recorded the highest mortality rate of 24hours of exposure for anopheles larvae.while 0.5mg/ml recorded minimum mortality after 48hour of exposure. The study demonstrated the potency neem, adhatoda and aloe vera in managing the larvae and thus contributes as an affordable way to control anopheles larvae of mosquitoes.

Keywords : neem, adhatoda, aloevera, anopheles mosquito

INTRODUCTION

Mosquitoes are an ancient group of insects, which have persisted for millions of years. Human malaria is transmitted only by females of the genus Anopheles. Of approximately 430 species of Anopheles, only 30-40 transmit malaria in nature (National Centre for Infectious Diseases (NCID) (2004). The diseases spreading vectors are: Culex, the ordinary mosquitoes found in houses, carrier of encephalitis and filariasis in tropical and sub-tropical climates, with life cycle of 10-14 days. Aedes is responsible for Yellow fever, Dengu fever, Encephalitis, etc., with life cycle of about 10 days to one month that affect millions of people worldwide (WHO,1996). In general, Cx. quinquefasciatus breeds in water polluted with organic debris such as rotting vegetation, breeding in pit latrines and blocked drains and ditches. It is very clear that over two billion people in tropical countries are at risk from mosquito borne diseases such as dengue fever, haemorrhagic fever, malaria and filariasis. The search for effective vaccines against these diseases is still in progress. Cx. quinquefasciatus (Say.) acts as a vector for filariasis in India. Human filariasis is a major public health hazard and remains a challenging socioeconomic problem in many of the tropical countries (WHO, 1975). Lymphatic filarasis caused by Wuchereia bancrofti and transmitted by mosquito Cx. quinquefasciatus is found to be more endemic in the India and its subcontinent. It is reported that Cx .quinquefasciatus infects more than 100 million individuals worldwide annually (National Centre for Infectious Diseases (NCID) (2004). W. bancrofti is the most predominant filarial nematode, which is usually characterized by progressive debilitating swelling at the extremities, scrotum or breast (elephantiasis) in an infected individual (WHO, 1996).

One of the approaches for control of mosquito-borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding centres of vectors. Plant products have been used traditionally by human communities and application of easily degradable plant

compounds is considered to be one of the safest methods of control of insect pests and vectors (Alkofahi et al. 1989). Many medicinally important plant extracts have been studied for their efficacy as mosquito agent against different species of vector mosquitoes (Nandita et al. 2008).

About 2.5 billion people worldwide are at risk of contracting dengue fever, dengue hemorrhagic fever, and dengue shock syndrome (WHO 2010), with potentially serious implications due to absence of neither effective drug nor vaccine against these diseases. Physical and chemical methods are the only feasible alternatives to controlling the mosquito-borne diseases. Physical approaches are barrier to infection of humans by infected mosquitoes, achieved through repellents and insecticide-treated bed nets. However, resistance to pyrethroids present a real and immediate challenge to efficacy of these intervention methods (Etang et al. 2004). Mosquitocidal factors that interrupt vector ecology are ovicidal, larvicidal, pupicidal, and adulticidal and include organochlorides, organophosphates, and synthetic pyrethroids (WHO 2010, Panneerselvam et al. 2012). However, successive changes in insecticide regiments has now resulted in multiple resistance among vector populations in Sub-Saharan Africa (Chandre et al. 2000, Enayati and Hemingway 2010) and India (Govindarajan 2011), including in the larvae (Diabate et al. 2003, Amy et al. 2005, Wirth et al. 2005).

Not only can medicinal plant extracts be effective but also they may greatly reduce the risk of adverse ecological effects and do not induce pesticide resistance in mosquitoes. Therefore, the present study had been carried out to evaluate the larvicidal activity with Adhatoda vasica leaf extracts along with silver nanoparticles against the filarial vector Culex quinquefasciatus (Say). Development of resistance by pests and vectors against the botanicals has not been reported (Sharma et al. 1995) because botanical insecticides are generally pest specific, readily biodegradable, target specificity, lower bioaccumulation and lack toxicity to higher animals (Sharma et al. 1989). Many medicinally important plant extracts have been studied for their efficacy as mosquito agent against different species of vector mosquitoes (Saxena et al. 1993). More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. Members of the plant families-Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various types of larval, adulticidal or repellent activities against different species of mosquitoes (Shaalan et al. 2005).

According to the latest WHO statistics, the parasitic disease caused by mosquitoes infects from 300 to 500 million persons per year in the world and kills more than a million and a half each year, mainly African Children. Control measures used against mosquitoes include elimination of breeding sites, application of surface films of oil to clog the breathing tubes of wrigglers and the use of larvicides. Many strains of the mosquito are resistance to conventional insecticides. From the foregoing account, it is very clear that the larvicidal activity of Silver Nanoparticle Synthezed by the leaf extracts of A. vasica against Cx. quinquefasciatus is essentially unstudied. Therefore, the present study was conducted to evaluate the larvicidalactivity of crud extract of A. vasica along with mixture of Silver nanoparticle against the larvae of Cx. quinquefasciatus.

Understanding the biology and behavior of Anopheles mosquitoes can help understand how malaria is transmitted, and can aid in designing appropriate control strategies. Factors affecting a mosquito's facility to transmit malaria include its innate susceptibility to Plasmodium, its host choice and its longevity. Factors that should be taken into consideration when designing a control program include the susceptibility of malaria vectors to insecticides and the preferred feeding and resting location of adult mosquitoes.

The search for herbal preparations that do not produce any adverse effects on the non target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control (Redwane et al., 2002). As a part of our search for the biodiversity resource available in India for natural products with utilizable bioactivity, we have assayed larvicidal potential of the extracts of Azadirachta indica, Aloe barbadensis miller and Justicia adathoda against Aedes aegypti and Anopheles stephensi larvae. These are widely distributed in many parts of India.

AIM AND OBJECTIVE

AIM

To compare the larvicidal activity of three leaf extracts, Neem (*azadirachta indica)*, Aloe vera (*aloe barbadensis miller*), Adathoda (*justicia adathoda)* against mosquitoe larvae.

OBJECTIVE

To find out the larvicidal activity of leaf extracts

To find natural way to prevent mosquitoe growth and mosquitoe borne diseases.

To reduce use of artificial larvicides.

REVIEW OF LITERATURE

Larvicidal activity of plant products against mosquitoes

The use of biologically active plant materials with anti-larval properties has attracted considerable interest of scientists all over the world. Because of their biodegradable nature and being relatively safer to human beings and non target organisms in the environment, extensive survey of the flora was undertaken to search for potential plant extracts which could be used in the management of agricultural and household pests. Moreover, investigations on the insecticidal properties of plant extracts have gained great impetus because of imposition of restrictions on the use of chemical pesticides for insect control.

Saxena and Sumithra (1988) noted that the leaf extracts of Ipomoea carnea fistulosa significantly increased the average larval and pupal development periods of the mosquito, C. quinquefasciatus and also resulted in increased larval and pupal mortali **Evans** and Raj (1988) observed that the crushed aqueous extracts of Quassia amara, T. neriifolia, Anacardium occidentale, Carica papaya, Hevea brasilionsis and Nerium indicum showed larvicidal activity when tested against C. quinquefasciatus.

Thangam and Kathiresan (1988) reported that the acetone extracts of 12 sea weeds showed larvicidal activity against the fourth instar larvae of A. stephensi and C. quinquefasciatus. Growth inhibitory properties and toxicity of Melia volkensii fruit extract fractions against mosquito larvae had been reported for A. arabiensis (Mwangi and Mukiama, 1988), A. aegypti (Mwangi and Rembold, 1988) and C. quinquefasciatus (Al-Sharbok et al., 1991).

Evans and Raj (1991) studied the larvicidal efficacy of Quassin against C. quinquefasciatus. Crushed aqueous extracts of leaf, wood, bark and flowers of Quassia amara showed antilarval activity against C. quinquefasciatus. Quassin has been identified to be the antilarval principle present in this plant and was effective against mosquito larvae at a concentration of 6 ppm. Jalees et al. (1993) investigated the crude ethanolic extract of C. sativa, to determine its insecticidal properties against the larvae of An. Stephensi, C. quinquefasciatus and Ae. aegypti.

Achary et al. (1993) evaluated the efficacy of Iopmoea carnea to control the larvae of C. quinquefasciatus. Mwaiko and Savaeli (1994) carried out tests on lemon peel oil extract as a mosquito larvicide. The oil was found to be toxic to larvae, pupae and eggs of C. quinquefasciatus. Mohsen et al. (1995) found that the crude ethanolic extracts of the weed. I.

cylindrical exerted moderate larvicidal and oviposition deterring activities against C. quinquefasciatus and produced 100% mortality against third instar larvae at 1500 ppm. The leaf extract of Vitex negundo, Nerium oleander and seed extract of Syzigium jambolanum exhibited larvicidal activity against C. quinquefasciatus and A. stepensi larvae (Pushpalatha and Muthukrishnan, 1995).

Mittal et al. (1995) determined the efficacy of six neem products against fourth instar larvae of An. stephensi, C. quinquefasciatus and Ae. aegypti. These neem products showed poor larvicidal activity within 24 h exposure, but some proved to have good insect growth regular activity. Larvae of An. Stephensi were generally the most susceptible to these neem products while Ae. aegypti were the least.

Bowers et al. (1995) obtained organosoluble extracts from 55 Turkish mecidinal plants and tested them understandardized condition for biological activity against third instar larvae of mosquitoes, A. aegypti and A. gambiae. Eight extracts demonstrated significant larvicidal activity with A. gambiae being more susceptible than A. aegypti in all cases. Macedo et al. (1997) tested 83 plants in the state of Minus Gerais, Brazil for the larvicidal activity against the mosquito A. fluviatilis. The ethanol extract of Tagetes minuta showed active results whereas extracts of Achyryrocline satureoides, Gnaphalium spicatum, Senecio brasiliensis, Trixis vanthieri, T. patula and Vernonia ammophila showed less active.

Thangam and Kathiresan (1996) investigated the sea weeds, sea grasses and mangrove plants for their larvicidal, skin and smoke repellent activities against mosquito species. Some of them were effective in killing the larvae or repelling adult female mosquitoes. Leaves of Excoecaria agallaha and Acanthus ilicifolius were found to show smoke repellent activity. Isolation and identification of active compounds from the effective samples would be useful in synthesizing mosquito larvicides or repellents on a large scale.

Karmegam et al. (1997) investigated bioassay studies of laboratory colonized larvae and field collected larvae of C. quinquefasciatus with 5 concentrations of petroleum ether extracts of 12 plants collected and showed that the extracts of Argemone mexicana, Jatropha curcus, Pergularia extensa and Withania somnifera had acute toxicity. Batra et al. (1998) examined

neem oil water emulsion was used in mosquito 'breeding' habitats to determine its larvicidal effect on immatures of different mosquito species. Sharma et al. (1998) reported larvicidal activity of Gliricidia sepium against mosquito larval of A. stephensi, A. aegypti and C. quinquefasciatus. Crude ethanol extracts of dried leaves, fresh leaves, dried petioles and stem bark of G. sepium against third instar larvae of A. stephensi, Ae. aegypti and C. quinquefasciatus. Okorie and Lawal (1998) tested the larvicidal properties of ethanolic extracts of fruits of P. guineense (African black pepper) on larvae of Aedes aegypti (L) at different concentrations.

The extracts of Cryptotaemia Canadensis, both chloroform and water were bioassayed against fourth instar larvae of C. pipiens at concentrations between 5 and 50 ppm (Eckenbach et al., 1999). The larvicidal activity of petroleum ether extract of root of Glycosmis pentaphylla on the juveniles of C. quinquefasciatus, A. stephensi and A. aegypti was determined (Latha and Ammini, 1999). Al-Dakhil and Morsy (1999) tested the larvicidal actions of three ethanol extracts of peel oils of lemon, grape fruit and naval orange against the early fourth instar larvae of C. pipiens and the resulting pupal stages. Pushpalatha and Muthukrishnan (1999) studied the efficacy of two tropical plant extracts for the control of mosquitoes. The larvicidal activity of seed and leaf extracts of Calophyllum inophyllum and leaf extracts of Rhinacanthus nasutus on the juveniles of Culex quinquefasciatus, Anopheles stephensi and Aedes aegypti was determined. Ethyl acetate soluble fractions of C. inophyllum and a petroleum ether fraction of R. nasutus extracts showed very high larvicidal activity.

Thomas and Callaghan (1999) reported the use of garlic and lemon peel extracts as Culex pipiens larvicides. Both garlic and lemon were toxic to mosquitoes. Latha et al. (1999) evaluated petroleum ether extracts of 41 indigenous plants abundant in different parts of Kerala, India, were studied for larvicidal activity against Culex quinquefasciatus and C. sitiens. The extracts of 18 plants showed larvicidal activity against the fourth instar larvae of C. sitiens. Thyagaraj (1999) reported the effect of neem based insecticides against the mosquito (Culex quinquefasciatus) larvae.

Sagar et al. (1999) reported the bioactivity of ethanol extract of Karanja seed coat against mosquitoes. Treatment of A. aegypti and C. quinquefasciatus larvae with ethanol extract of P.

glabra seed coat significantly increased the larval mortality and development period in proportion to increases in the extract concentrations Aedes proved more sensitive to the effect of extract in terms of mortality than Culex. Markouk et al. (2000) studied the larvicidal properties of 16 extracts of four Moroccan medicinal plants, C. procera, Cotula cinerea, S. sodomaeum and S. elaeagnifolium were tested against A. labranchiae mosquito larvae.

Dwivedi et al. (2000) examined the acetone extracts of leaves of four plants, Jasminum arbores, Eucalyptus rudis, Bignomia carpreolata and Acacia nilotica were against A. aegypti. The extracts proved to be potent larvicides causing complete developmental arrest of fourth instar larvae. Obutour and Onajobi (2000) evaluated the aqueous and n-butanol fractions of methanolic extracts of Raphia hookori fruit mesocarp for cytotoxic properties. The n-butanol fraction exhibited greater lytotoxity than the aqueous fraction when tested against C. quinquefasciatus.

Ignacimuthu (2000) studied the larvicidal activity of 50 medicinal plants collected from Western Ghats. Results revealed the Streblus aspera, Vitex negunda, Phyllanthus deblis, L. aspera, Evolvulus aljinoides and Chloroxylon swietenia showed strong larvicidal activity against the filarial vector C. quinquefasciatus. Crude acetone extract of seeds of A. squamosa (L) against third and fourth instar larvae of laboratory strain of C. quinquefasciatus indicated a dose dependent larvicidal and pupicidal activity.

Venkatachalam and Jebanesan (2000) tested the plant parts of 156 plants, extracted with different solvents. The results indicated that the plant extracts acted as general toxicants and specifically as larvicides, insect growth regulators, repellents and adulticides. Rahuman and Venkatesan (2000) assessed the larvicidal activity of ethyl acetate, acetone, methanol and petroleum ether extract of five indigenous plants, namely Acacia arabica, D. alba, Morinda citrifolia, Mukia scabrella and Zingiber officinale against the fourth instar larvae of C. quinquefasciatus.

Nicolescu et al. (2000) studied the effects of 82 species of plant extracts against larvae and adults of mosquitoes, A. aegypti and A. atropareus. These plant extracts acted as toxicants, growth, developmental and reproduction inhibitors and repellents. Lee (2000) found methanol fruit extract of Piper longum active against larvae of C. pipiens pallens at 10 mg/ml after 24 h. A

piperidine alkaloid, piperenon alanine was responsible for this activity with 24 h median lethal dose (LD50) value of 21 mg/litre. Ansari et al. (2000) tested the oil of M. piperita for larvicidal activity against A. aegypti, A. stephensi and C. quinquefasciatus. Of the three species tested C. quinquefasciatus was most susceptible followed by A. aegypti and A. stephensi.

Rahuman et al. (2000) examined the bioassay guided fractionation of the acetone extract of Feronia limonia dried leaves afforded a potent mosquito larvicide, which was identified as n-hexadecanoic acid and found to be effective against fourth instar larvae of C. quinquefasciatus, A. stephensi and A. aegypti. Namrata et al. (2000) studied the larvicidal effects of essential oil extracted from the leaves of four plants namely Tagetes erecta, Ociumum sanctum, Mentha piperita and Murraya koenigii were evalued against Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti. Ciccia et al. (2000) studied the insecticidal activity of 11 extracts from nine South American medicinal plants using the A. aegypti larvicidal assay. Eight of the 11 plant extracts studied showed toxicity against the A. aegypti larvae. Mehra and Hiradhar (2000) studied the crude acetone extract of seeds of Annona squamosa Linnaeus tested against third and fourth instar larvae and pupae of a laboratory strain of C. quinquefasciatus. Latha and Ammini (2000) reported the leaves and tubes of Curcuma raktakanda as the mosquito larvicide against the early fourth instar larvae of C. quinquefasciatus, C. sitiens, A. aegypti and A. stephensi. The petroleum ether extract of the leaves and tuber exhibited toxicity towards all the test species. Huang et al. (2000) examined a preliminary study on the toxicity of insecticidal plant against the mosquito larvae of Culex pipiens pallens.

Momin and Nair (2001) analysed the methanolic extract of Apium graveolens seeds for bioactive compounds and isolated the mosquitocidal, nematicidal and antifungal compounds, sedanolide (1), senkyun-olide-N (2) and senkyunolide – J (3). Crude aqueous extracts of dried fruit pericarp, flower, root and stem of solenostemma argel were tested for larvicidal activity against the third instar larvae of the mosquito, C. quinquefasciatus. Extracts of the fruit pericarp was most effective (El-kamali, 2001). The larvicidal activity of the plant, Hydrocotyle javanica against C. quinquefasciatus was evaluated using petroleum ether, benzone, chloroform,

ethylacetato and methanol (Venkatachalam and Jebanesan, 2001). Redwane et al. (2002) studied the efficacy of extracts and fractions of Quecuslusitania infectoria galls against second and fourth instar larvae of C. pipiens. The methanolic, chloroform and ether extracts of Euphorbia tirucalli latex and stem bark were evaluated for larvicidal activity against C. quinquefasciatus.

Vahitha et al. (2002) reported larvicidal efficacy of leaf extracts of Pavonia zeylanica and Acacia ferruginea was tested against the late third instar larvae of C. quinquefasciatus. Jaybala and Rajkumar (2003) determined the larvicidal activity of plant extracts of D. metal (seed), Cinnamomum zeylanicum (leaf), Baliospermum montanum (seed) in A. stephensi.

Prabhakar and Jebanesan (2004) tested the larvicidal efficacies of extracts of five of Cuccubitacious plants, Momordica charantia, Trichosanthes anguina, Luffa acutangula, Benincasa cerifera and Citrullus vulgaris against the late third larval stage group of C. quinquefasciatus. Rajkumar and Jebanesan (2004) evaluated the toxicity of the plant Moschosma polystachum on C. quinquefasciatus. The crude leaf extract and active compound octacosane showed negligible mortality against early third instar larvae of C. quinquefasciatus. Cheng et al. (2004) assessed the chemical position of leaf essential oils from eight provenances of indigenous Cinnamon (Cinnamomum osmophloeum Kanch) were compared. The larvicidal activities of leaf essential oils and their consitutents from the five chemotypes of indigenous cinnamon trees were evalued by mosquito larvicidal assay.

Chapagain and Wiesman (2005) reported the larvicidal activity of the fruit mesocarp extract of B. aegyptiaca and its saponin fractions against A. aegypti. Fruit mesocarp extract of B. aegyptiaca was tested against the laboratory-reared third instars larvae and compared with their LC50 values. Govindarajan et al. (2005) examined the larvicidal effect of extra cellular secondary metabolites of different fungi against the mosquito, C. quinquefasciatus. The culture filtrates of five different fungi (A. flavus, A. parasiticus, Penicillium falicum, Fusarium vasinfectum and Trichoderma viride were tested for the larvicidal activity against third instar larvae of mosquito vector.

Nath et al. (2006) studied the methanol extracts of 19 indigenous plants as mosquito larvicide. Among these, pericarp of Zanthoxylum limonella was found to have the most promising larvicidal properties against A. albopictus and C. quinquefasciatus. Das et al. (2007) studied the larvicidal activity of methanol and ethanol extracts of five aromatic plant species against Ae. Albopictus and C. quinquefasciatus larvae varied according to plant species. Methanol extract of Aristolochia saccata roots was found to be the most effective against Ae. albopictus larvae.

Mustafa and Al-khazraji (2008) investigated the effects of the extracts of eight plant species collected from Ninavah governorate on the second instar stage of Culex pipiens molestus Forskal. Three out of the eight plant extracts Azadirachta excelsa Jack. Cleome glaucescens Dc. and Quercus infectoria caused 100% mortality of larvae at a concentration of 200 □g/ml after 3 days of treatment. Mustafa (2005) reported that the seeds extract of Pimpinella anisum L. has high toxicity to second instar larvae of C. pipiens molestus. Maheswaran et al. (2008) studied that larvicidal activity of Leucas aspera against the larvae of Culex quinquefasciatus and Aedes aegypti. The hexane extract of Leucas aspera showed highest larvicidal activity against the two vector mosquitoes followed by chloroform and ethanol.

Cheng et al. (2008) examined the mosquito larvicidal activities of methanolic extracts from different plant parts of red heartwood-type Cryptomeria japonica against the fourth-instar larvae of Aedes aegypti and Aedes albopictus. Results showed that the larval mortality of the methanolic extracts of C. japonica heartwood, back and leaf extracts did not exceed 50.0% indicating no significant toxicity to A. aegypti and A. albopictus, while the sapwood caused 100% larval mortality in 24 h. Sathishkumar and Maneemegalai (2008) investigated the larvicidal effect of Lantana camara extract against early third and fourth instar larvae of Aedes aegypti and Culex quinquefasciatus. Extracts from leaves, flowers and roots of plants and oils were found to have mosquito larvicidal activity.

Senthil Kumar et al. (2009) used the powders of neem seed, leaf, gum, flower, bark and root for studying antipupational, adulticidal and adult repellency properties against A. stephensi. Rahuman et al. (2000) undertook bioassay studies using the acetone extract of Feronia limmia leaves. This extract served as a potent mosquito larvicide and was effective against fourth-

instar larvae of C. quinquefasciatus, A. stephensi and A. aegypti. Row et al. (2009) examined the essential oil and methanolic and aqueous extracts of Piper betle L. for their antimicrobial activity, mosquito larvicidal activity, antioxidant property and mushroom grosinase inhibition. The methanolic and aqueous extracts showed strong activity against the yeasts: C. albicans and M. Pachydermatis.

Deore and Khadabadi (2009) revealed that the laboratory studies carried out to ascertain that larvicidal properties of Chlorophytum borivilianum sant. and Fernand belonging to family. Liliceae is a very well known plant for its aphrodisiac as well as immunomodulatory properties (Oudhia, 2001). Roots of the plant are used both in Ayurveda and Unani system to treat oligospermia, arthritis, diabetes and dysuria (Wealth of India, 1996). Anticancer (Arif, 2005), immunomodulatory (Singh et al., 2004), anti- diabetic (Govindarajan et al., 2005), antistess (Gopalkrishna and Patil, 2006), aphrodisiac (Thakur et al., 2006), antimicrobial (Deore and Khadabadi, 2007) and anti inflammatory (Deore et al., 2008) activities of root extracts have been evaluated. Roots of this plant contain carbohydrates, phenolic compounds, saponins and alkaloids (Deore et al., 2008). Samidurai et al. (2009) studied larvicidal, ovicidal and repellent activities of Pemphis acidula Forst (Lythraceae) against Filarial and Dengue vector mosquitoes. The larval mortality was observed after 24 h exposure.

Dua et al. (2009) observed that larvicidal activity of neem oil formulation against mosquitoes. Kihampa et al. (2009) examined the larvicidal activity of seventeen Tanzanian plant species against the malaria vector. Anopheles gambiae S.S. Giles larvae. The crude extracts from the leaves, stems and root barks of the investigated plants were obtained by solvent extraction and then bio-assayed following WHO protocols. Aina et al. (2009) studied the efficacy of some plant extracts on An. gambiae mosquito larvae. The ethanolic and aqueous extracts of the fruits of Physalis angulata, Xylopia aethiopica and seed of Piper guineense schum and Jatropha curcas Linn. were tested on the second instar larvae of A. gambiae (L) at varying concentrations. Borah et al. (2010) studied the larvicidal efficacy of nature fruits and leaves of Toddalia asiatica against the larvae of A. aegypti and C. quinquefasciatus.The hexane, acetone and methanol extracts of

nature fruits and leaves were investigated to establish its bio-control potentiality under laboratory condition against fourth instars larvae of A. aegypti and C. quinquefasciatus.

Patil et al. (2010) studied the larvicidal activity of crude chloroform, dichloromethane and methanol extracts of the leaves and roots of the six Indian plants, Aegle marmelos L., Balanites aegyptica L., Calotropis gigantica L., Murraya koenigii L., Nyctanthes arbor-tristis L. and Plumbago zeylanica L. were tested against the early fourth instar larvae of Aedes aegypti L. and Anopheles stephensi. The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects. Tomass et al. (2011) studied that larvicidal effects of Jatropha curcas L. against Anopheles arabiensis. The LC50 and LC90 values of Jatrophas curcus crude methanol leaf extract were found respectively.

Mehdi et al. (2011) assessed the larvicidal and IGR properties of leaf extract of Cassia fistula and Saraca indica. The early fourth instar larvae of mosquito were exposed to acetone leaf extracts of Saraca indica and Cassia fistula. Leaf extracts showed moderate to high larvicidal activity. Zewdneh et al. (2011) observed that larvicidal effects Jatropha curcas L. against Anopheles arabiensis.

Adediwura et al. (2011) investigated the larvicidal activities of the petroleum ether, chloroform fractions and methanol extract of Buchholzia coriacea seed as potential agent in vector control for malaria. The extract and fractions of B. coriacea tested showed that the chloroform fraction was more effective against the third and fourth instar larvae of A. gambiae than the petroleum ether fraction and methanol extract. Manilal et al. (2011) assessed the laboratory and field evaluation of some chemical and biological larvicides against Culex spp. Tomaphos Bti and Phyriproxyfen.

Maragathavalli et al. (2012) evaluated the mosquito larvicidal activity of leaf extract of neem. Larvicidal effect on third and fourth instar larvae of Aedes aegypti and Culex quinquefasciatus were tested. Rabha et al. (2012) reported the aqueous solutions obtained during the stream distillation of medicinal and aromatic plants. The hydrolates of four plants Zanthoxylum limonella, Zingiber officinale, Curcuma longa and Cymbopogon citrates were evaluated for their

larvicidal activity against two laboratory reared mosquito species Aedes albopictus and Culex quinquefasciatus.

Poonguzhali and Nisha (2012) studied the larvicidal activity of two seaweeds, Ulva fasciata and Grateloupia lithophila against mosquito vector Culex quinquefasciatus. Increasing documentation of negative environmental and health impact of synthetic insecticides and increasingly stringent environment regulation of pesticides have resulted in the development and use of bioinsect management products for controlling mosquitoes and pest. Of the two algae screened G. lithophila was found to effective against the larva Culex in all the three extracts methanol, acetone and benzene.

Anstrom et al. (2012) evaluated mosquitocidal properties of natural product compounds isolated from Chinese herbs and synthetic analogs of curcumin and their ability to inhibit binding of cholesterol of Ae. Aegypti sterol carrier protein – 2 in vitro. Kumar et al. (2012) determined the effectiveness of seaweed (Sargassum wightii) extract combined with Bacillus thuringiensis val. Israclensis for the control of Anopheles sundaicus Liston, a malaria vector that occurs in the coastal areas of Peninsular India. Nour et al. (2012) reported larvicidal activity of extracts from different parts of neem (Azadirachta indica) against Aedes aegypti mosquitoes. The toxic effect of different solvents (acetone, chloroform, ethanol) extracts from different parts (bark, leaf, root and seed) of neem, Azadirachta indica against A. aegypti larvae.

Illahi et al. (2012) examined larvicidal activities of different parts of Melia azedarach Linn. against Culex quinquefasciatus. Various concentrations (50, 100, 500, 1000, 1500 and 2000 ppm) of aqueous extracts of leaves, fruits and bark of Melia azedarach were tested for larvicidal activity against Culex quinquefasciatus. There occurred a continuous increase in mortality of third and fourth instar larvae with increase in concentration of the extracts.

Sakthivadivel et al. (2012) studied the evaluation of toxicity of plant extracts against vector of lymphatic filariasis, C. quinquefasciatus. The toxic effects of petroleum ether leaf extracts of plants viz., Argemone mexicana, Clausena dentala, Cipadessa baccifera, Dodonaea angustifolia and Melia dubia were evaluated under laboratory conditions individual an in combination against third instar and fourth instar larvae of Cx. Quinquefasciatus.Illahi and Ullah (2013)

reported the larvicidal activity of different parts ofArtemisia vulgaris Linn. Against C.quinquefasciatus. The third and fourth instar larvae of C.quinquefasciatus were exposed for 24 hrs to various concentrations (50, 100, 500, 1000 and 1500 ppm) of methanol extracts of different parts of Artemisia vulgaris.

MATERIALS AND METHODS

Collection and preparation of extracts of plant leaves

Neem (Azadirachta indica) was collected during the month of january 2020. The leaf of neem was washed thoroughly and it is cut into small pieces and used for further studies. The extraction of Azadirachta indica was carried out by washing the leaf .It was then grinded. After that, filter the extract if any solid materials found.The leaf of Aloe vera (Aloe barbadensis miller) was collected during the month of january 2020.The Aloe gel was taken and grind well for further studies (there is no need for drying). After grinding the gel is collected in a petridish. After that sieve the gel to avoid the solid particle.The leaf of adhatoda (justicia adhatoda) was collected during the month of january 2020. The leaf is crushed to make powder for further experimental analysis. These crushed particles are sieved to remove the unwanted large solid particles. These sieved powder was collected in a petridish for further studies

Collection of mosquitoe larvae

The mosquitoe larvae was collected from my locality, moist areas around house.Water in a tyre was kept in a shaded area for the mosquito to came and lay eggs check the water content frequently to make sure that the mosquitoe lay their eggs. Make aure that there is no overflow of water takes place due to rain or any water falls to avoid the escape of larvae through water. After two or three days the eggs will changed into larvae and it can be collected from the water container and stored in a bottle. And it is used for futher analysis of larvicidal activities of various spices. Anopheles is collected from my region. And the further experiment was based on the larvae of anopheles.

Larvicidal bioassay

All larvae were reared and bioassays conducted under standard insectory conditions. The larvae collected was treated for the larvicidal activity of three leaf extracts, Azadirachta indica, Aloe barbadensis miller, Justicia adhatoda. The extracts of each samples at various concentration in different petridishes to test separately. 0.5mg/ml, 1mg/ml, 2mg/ml of extracts where taken in different petridishs and distilled water at 1ml added quickly and 5 larvae are added to all petridish each applied for the bioassay experiment where conducted in triplicate and control were performed at parallel condition in each series of experiments. The larvae was not feeded at that time. Larvae mortality was recorded at 1h exposure by Azadirachta indica and Justicia adhatoda. Aloe vera shows slighter larvicidal activity within 3h.

Larvae	Leaf (extract)	Sample mg/ml	Mortality rate (hrs)
Anopheles	Neem (Azadirachta indica)	0.5mg/ml 1mg/ml 2mg/ml	24 hr-10%, 48 hr-20% 24 hr-20%, 48 hr-35% 24 hr-30%, 48hr-45%
Anopheles	Aloe vera (Aloe barbadensis miller)	0.5mg/ml 1mg/ml 2mg/ml	24 hr, 48 hr-no change 24 hr, 48 hr-no change 24 hr-no change, 48

			hr-10%
Anopheles	Adhatoda (justicia adhatoda)	0.5mg/ml 1mg/ml 2mg/ml	24hr-no change, 48hr-no change 24 hr-10%, 48 hr-20% 24hr-15%, 48hr-35%

RESULT AND OBSERVATION

The results obtained on the mortality of anopheles mosquito larvae treated with different concentrations of aqeous extract of Neem (Azadirachta indica), Aloe vera (Aloe barbadensis miller), Adhatoda (justicia adhatoda), are presented below.

Larvicidal activity of Neem against larvae of anopheles mosquito at different concentrations.

Concentrations	Number of larvae introduced	Number of larvae died
0.5mg/ml	5	4
1mg/ml	5	4
2mg/ml	5	5

Larvicidal activity of Aloe vera against anopheles mosquito larvae at different concentrations.

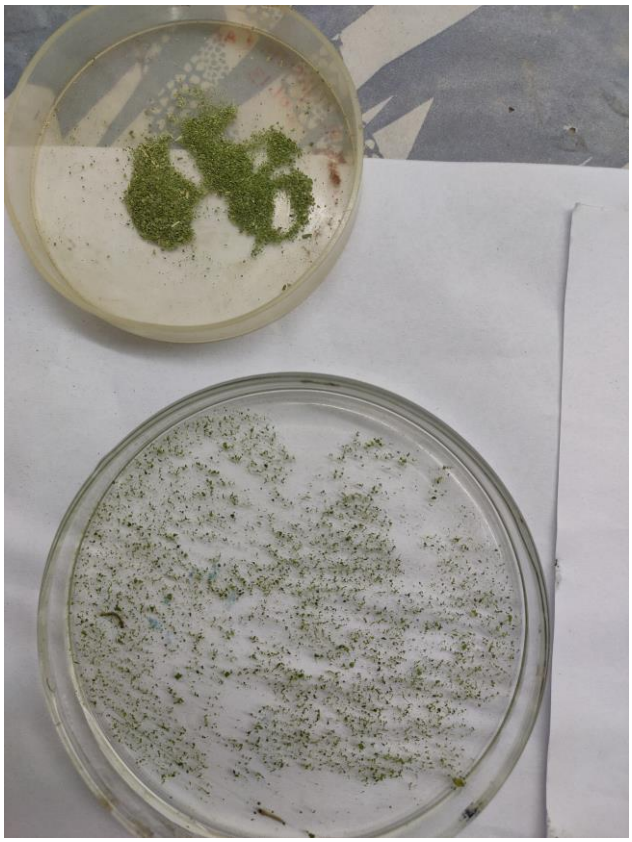
Concentrations	Number of larvae introduced	Number of larvae died
0.5mg/ml	5	0
1mg/ml	5	0
2mg/ml	5	1

Larvicidal activity of Adhatoda against larvae of anopheles mosquito at different concentrations.

Concentrations	Number of larvae introduced	Number of larvae died

0.5mg/ml	5	0
1mg/ml	5	2
2mg/ml	5	4

Larvicidal effect is shown greatly by Neem and adathoda and slightly by Aloe vera.



Anopheles mosquito

DISCUSSION

Mosquitoes are a serious threat to public health through which several dangerous diseases are transmitted in both animals and human beings. The cosmo-tropical mosquito Aedes aegypti serves as the most important domestic vector of urban yellow fever and dengue. Vector control is a global problem. Management of mosquitoes has been going on over a period of several

decades to find a suitable alternative method to replace the chemical control method because of concern about environment pollution and ecological preservation. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. Best strategy has to be integrated with the biological control.Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. Now a days mosquito control is mostly directed against larvae and only against adult when necessary. This is because the fight against adult is temporary, unsatisfactory and polluting the environment, while larval treatment is more localized in time and space resulting in less-dangerous outcomes. Larval control can be an effective tool to the low mobility of larval mosquitoes, especially where the principal breeding habitats are manmade and can be easily identified. Recent studies stimulated the investigation of insecticidal properties of plant derived materials or botanicals and concluded that they are environmentally safe, degradable and target specific botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programmes.

The larvicidal activity of Allium sativum against the IV instar larvae was so much effective. It has the larvicidal action even at a very low concentration. Similar observations were reported by Amonkar and Reeves in early 1970 in USA. They reported that the extracted oil and crude methanolic extract of garlic at very low concentrations could control larvae of 5 different mosquito species. The lethal concentration 50 and 90 value of Pongamia pinnata taken for study against the IV instar larvae was 0.8943 and 1.1694ppm, where as crude solvent of leaf, bark and flower extracts of P.pinnata showed only moderate larvicidal effects after 24 hours of exposure as was observed by Abdul Rahuman in 2008. He also observed the larvicidal effect of M. Indica against culex quinquefasciatus which has moderate effect at 1000ppm. Similar observations were made in our study against Aedes aegypti. Therefore the M.indica exhibit a moderate larvicidal effect on both the species. In 1995 Ambrose used lower concentration of neemoil on the 3rd and 4th instar larvae of culex. quinquefasciatus and showed that they were having LC50 values of 0.99ppm and 1.20ppm respectively and for the de-oiled neem cake it was 0.55ppm and 0.72ppm.The neem seed kernal extracts are effective against mosquitoes were prepared with hexane, ethylether, acetone, ethanol and methane. From this study Tonk et al.,

in 2006 studied that the most potent larvicide is 0.71% with hexane extract. Okumu et al., 2007 indicated that the larvicidal property of neem oil has higher persistency in 11pm and lasted for 8 days. In the present study the neem oil extraction process is very simple and easy method.

The neem oil is proved to be a larvicide, where the LC50 & LC90 value was 0.9673ppm and 1.2119ppm. This observation is in par with observation made by above said researchers but the species of mosquito differs.The toxic effect of Ocimum Sanctum extract against the early fourth instar larvae of Aedes aegypti was least and this is in par with the observation made by Senthilnathan (2006), where he used many plant extract against fourth instar larvae of culex quinquefasciatus.

Thus from our study it reveals that plant extract serves as an effective, alternative, ecofriendly, biodegradable larvicide in controlling the mosquitoes before they disperse and transmit disease.

The results of this study indicate the plant-based compounds such as Azadirachtin (compounds present in the Meliaceae plant family seed) may be an effective alternative to conventional synthetic insecticides for the control of Culex pipiens. Undoubtedly, plant derived toxicants are valuable sources of potential insecticides. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future (Mordue and Blackwell, 1993). The results of this study will contribute to a great reduction in the application of synthetic insecticides, which in turn will increase the opportunity for natural control of various medicinally important pests by botanical pesticides. Since these are often active against a limited number of species including specific target insects, less expensive, easily biodegradable to non-toxic products, and potentially suitable for use in mosquito control programme (Alkofahi et al., 1989), they could lead to development of new classes of possible safer insect control agents. Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack (Berenbaum, 1988; Murugan et al., 1996; Senthil Nathan et al., 2005a).

The intensive use of pesticides produces side effects on many beneficial insects and also poses both acute and chronic effects to the milieu (Abudulai et al., 2001). Recently, bio-pesticides with plant origins are given for use against several insect species especially disease- transmitted

vectors, based on the fact that compounds of plant origin are safer in usage, without phytotoxic properties; also leave no scum in the environment (Schmutterer, 1990; Senthil Nathan et al., 2004, 2005a, d). The work published by Khan et al., (2007) microscopically demonstrated that the decrease in fecundity of Bactocera cucurbitae and Bactocera dorsalis exposed to neem compound was due to the block of ovarian development.

Likewise, mixing of a commercial formulation of neem in the adult diet caused reduction in the fecundity of C. capitata by interfering with oogenesis (Di Ilio et al., 1999). The block in the ovarian activity of C. capitata, resulting from neem compound, was verified by histological observation (Di Ilio et al., 1999). Results from the study of Lucantoni et al., (2006) clearly indicated that the neem treated female mosquito, A. stephensi, displayed a delay in oocyte development in the vitellogenesis. As discussed by Weathersbee III and Tang (2002), the disruption of reproductive capability could lead to substantial population decline over time. Furthermore, Dhar et al., (1996) revealed that exposure to neem extract suppressed rather than inhibited oviposition in mosquitoes. The present study clearly proved the efficacy of Azadirachtin on larvae, pupae, and adult of Culex pipiens. Further studies such as mode of action and synergism with the biocides under field condition are needed.

CONCLUSION

On the basis of above results, we can conclude that Azadirachta indica has a paramount larvicidal importance. But the use of plants for larvae control offers a safer alternative too. A vast physiological problems and health effects can be reduce this way. The combination of the extract of Azadirachta indica and Justicia adhatoda also have 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.No mortality and other abnormalities were noticed on non targeted organisms and further studies are needed to investigate the chemical structure of active principles which are responsible for larvicidal activity.

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