LARVICIDAL EFFECTS OF PLANT EXTRACTS ON MOSQUITO LARVAE

Project work by

KANTIPUDI GOURI SREEPRIYA
Reg No: AB19ZOO011

Under the guidance of
Dr. MEERA JAN ABRAHAM, Associate Professor,
Department of Zoology, St. Teresa's College
(Autonomous), Ernakulam
Kochi- 682011

Submitted to St. Teresa's college (Autonomous) Ernakulam
Affiliated to Mahatma Gandhi University, Kottayam in partial
Fulfilment of requirement for the degree of Bachelor of science
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CERTIFICATE

This is to certify that the project report entitled "**LARVICIDAL EFFECTS OF PLANT EXTRACTS ON MOSQUITO LARVAE",** submitted by **Ms. KANTIPUDI GOURI SREEPRIYA** Reg No: **AB19ZOO011** in partial fulfillment of the request of Bachelor of Science degree of Mahatma Gandhi University, Kottayam, is a bonafide work done under my guidance and supervision and to the best of my knowledge, this is her original effort.

Dr. Meera Jan Abraham
Associate Professor
Department of Zoology
St. Teresa's College
Ernakulam

Dr. Soja Louis
Head of Department
Department of Zoology
St. Teresa's College
Ernakulam

EXAMINERS

1)

2)

DECLARATION

I, hereby declare that this project work entitled, "**LARVICIDAL EFFECTS OF PLANT EXTRACTS ON MOSQUITO LARVAE",** is submitted to St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam in partial fulfilment of the requirements of Bachelor of Science degree in Zoology. This work has not been undertaken nor submitted elsewhere in connection with any other academic course and the opinions furnished in the report is entirely my own.

KANTIPUDI GOURI SREEPRIYA Signature

Reg No: AB19ZOO011

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KANTIPUDI GOURI SREEPRIYA

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ABSTRACT

Chemical control methods using synthetic insecticides had been favored for so long because of their speedy action and ease of application. However, it has now been realized that due to several reasons known, chemical insecticides and larvicides can no longer be used for vector control in the same scale as before from the point of view that environmental pollution has been taking place exponentially. Biologically active plant extracts are therefore used to be studied for their potential efficacy to minimize the extent of pollution and to reduce the cost.

Extracts derived from seven plant species, *Curcuma longa, Citrus lemon, Coleus barbatus, Azadirachta indica, Piper nigrum, Zingiber officinale, Allium sativum*, were evaluated for efficacy against larvae of mosquitoes using larvicidal bioassays. Plant extracts were extracted from fresh parts of plants and different concentrations of extract was prepared for each of the plant species. Insecticidal and repellent activity of extracts against larvae form of mosquitoes, collected from small pools located in and around Ernakulam were examined via bioassay method.

Results of current study on insecticidal and repellent activity of mentioned plant extracts against the larvae show considerable effects. Concentration of undiluted extract had the highest insecticidal and repellent activity against the larvae for all of the extracts and extracts of Neem and Pepper were seen more potent than the other plant extracts taken.
The highest larvicidal bioassay was established from *Azabirchta indica* (neem) and *Piper nigrum* (pepper).

The study affords some information regarding the action of different plant extracts and their potential in preventing the growth of mosquito larvae and therefore can be used in mosquito control. The study also shows that these plant extracts can be considered as good replacements for chemical pesticides but more experiments are needed for this purpose. Study on insecticidal activity of these plant extracts in the field condition can be considered as a subject for next experiments.

INTRODUCTION

Mosquitoes are members of a group of almost 3,600 species of small flies within the family Culicidae (from the Latin culex meaning “gnat”) The word “mosquito” (formed by mosca and diminutive -ito) is Spanish and Portuguese for “little fly”. Mosquitoes have a slender segmented body, one pair of wings, one pair of halteres, 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. These larvae are important food sources for many freshwater animals, such as dragonfly nymphs, many fish, and some birds such as ducks. 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, which contains protein and iron needed to produce eggs. 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 parasitic diseases such as malaria and filariasis, and arboviral diseases such as yellow fever, Chikungunya, West Nile, dengue fever, and Zika. By transmitting diseases, mosquitoes cause the deaths of more people than any other animal taxon: over 700,000 each year. It has been claimed that almost half of the people who have ever lived have died of mosquito-vectored disease, but this claim is disputed, with more conservative estimates placing the death toll closer to 5% of all humans. Mosquitoes cannot live or function properly when the air temperature is below 10 degrees Celsius (50 degrees Fahrenheit). They are mostly active at 15–25 degrees Celsius (60–80 degrees Fahrenheit). Mosquito control manages the population of mosquitoes to reduce their damage to human health, economies, and enjoyment. Mosquito control is a vital public-health practice throughout the world and especially in the tropics because mosquitoes spread many diseases, such as malaria and the Zika virus. An obvious method for the control mosquito- borne diseases is the use of insecticides, and many synthetic agents has been developed and employed in the field with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. It has also provoked undesirable effects, including toxicity to non-target organisms, and fostered environmental and human health concerns. These problems have highlighted the need for the development of new strategies for selective mosquito larval control. Plant extracts or essential oils from plants may be alternative source of mosquito larval control agents, as they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. Materials used for the experiment are lemon, ginger, garlic, pepper, neem, Tulsi, Mexican mint and lemon grass oil. The Citrus lemon is a species of small evergreen trees in the flowering plant family Rutaceae, native to Asia, primarily Northeast India, Northern Myanmar or China. It is a plant origin insecticide as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of citrus lemon. *Zingiber officinale* is a flowering plant whose rhizome, Ginger root or Ginger, is widely used as a spice and a folk medicine. Ginger has a greater larvicidal effect than *Allium sativum*, which is a species of bulbous flowering plant in the genus Allium. Garlic is a potent mosquito ovicide and larvicide. *Curcuma long*a is a flowering plant of the Ginger family, *Zingiberaceae*, the rhizome of which are used in cooking. Anti-parasitic effect of curcumin, which is one of the active compounds in *Curcuma longa,* has been observed to be dose dependent with greatest effects. *Piper nigrum* is a flowering vine in the family Piperaceae, cultivated for its fruit, known as a peppercorn, which is usually dried and used as a spice and seasoning. Black pepper shows potential as a larvicide for the control of certain malaria vector species. *Azadirachta indica*,is one of two species in the genus *Azadirachta*, and it is native to the Indian subcontinent and most of the countries in Africa.

Neem is an effective larvicide against mosquito larvae. It is highly toxic to mosquito larvae and inhibits the development of pupae. Coleus barbatus is a semi-succulent perennial plant in the family *Lamiaceae* with a pungent oregano-like flavor and odor. It is an excellent option for keeping mosquitoes away. It has the greatest larvicidal effect.

Objective: To find eco-friendly larvicides to tackle the growing population of mosquitoes.

This has an added benefit of reducing the population in the environment.

REVIEW OF LITERATURE

Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbitaceous plants, *Citrullus colocynthis, Coccinia indica, Cucumis sativus, Momordica charantia*, and *Trichosanthes anguina*, were tested against the early fourth instar larvae of *Aedes aegypti L*. and *Culex quinquefasciatus*. The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in petroleum ether extract of *Citrullus colocynthis*, methanol extracts of *Coccinia indica, Cucumis sativus, Momordica charantia*, and acetone extract of *Trichosanthes anguina* against the larvae of *Aedes aegypti L* (LC50 = 74.57, 309.46, 492.73, 199.14, and 554.20 ppm) and against *Culex quinquefasciatus* (LC50 = 88.24, 377.69, 623.80, 207.61, and 842.34 ppm), respectively. The petroleum ether extract of *Citrullus colocynthis* and methanol extract of *Momordica charantia* were more effective than the other extracts. This is an ideal eco-friendly approach for the control of the dengue vector, *Aedes aegypti*, and the lymphatic filariasis vector, *Culex quinquefasciatus*. (Rahuman & Venkatesan, 2008.)

The efficacy of methanol, benzene and ethyl it's solvent extract of leaf of *E.coronaria* and *C.pulcherrima* were tested against the early 3rd larvae of *Anopheles subpictus* and *Cx.tritaeniorhyncus*. the data where recorded and statistical data regarding the LC 50, LC90, Chi-square and 95% confidence limits were calculated, among three solvents tested the methanolic extract of *E. coronaria* and *C. pulcherrima* showed highest larvicidal activity against *Anopheles subpictus* and *Culex tritaeniorhynchus*. The LC 50 values were 86.47 (159.59) and 113.26 (207.73) ppm for *Anopheles subpictus* and 131.53 (245.37) and 165.28 (299.45) ppm for *Culex tritae-niorhynchus* respectively. No mortality was observed in control. The chi-square values were significant at $p < 0.05$ level. (Govindarajan et al., 2012)

Mosquito species of *Anopheles, Aedes* and *Culex* are major vectors for malaria, dengue, zika, chikungunya, filariasis and Japanese encephalitis diseases. *Bacillus thuringiensis subsp. israelensis* (Bti) is spore-forming bacterium having worldwide distribution produces proteins which are toxic to these mosquito larvae. The local isolate, Bti ISPC-12 showed high toxicity to mosquito larvae of *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*. The genes encoding insecticidal toxin proteins have been expressed in the E. coli expression system for molecular and structural studies. Sustained-release formulation using spore-crystal powder of Bti

ISPC-12 has been developed. The spore crystal powder (active ingredient) and formulation has been found to be safe to mammals. Field studies carried out in Anushaktinagar, Mumbai and RRCAT, Indore townships demonstrated the efficacy against mosquito larvae. Thus Bti ISPC-12 based formulation is a potent biopesticide for the management of mosquito population. (Kinkar & Makde, 2021)

Plant-based repellents have been used for generations in traditional practice as a personal protection measure against host-seeking mosquitoes. Knowledge on traditional repellent plants obtained through ethnobotanical studies is a valuable resource for the development of new natural products. Recently, commercial repellent products containing plant-based ingredients have gained increasing popularity among consumers, as these are commonly perceived as safe in comparison to long-established synthetic repellents although this is sometimes a misconception. To date insufficient studies have followed standard WHO Pesticide Evaluation Scheme guidelines for repellent testing. There is a need for further standardized studies in order to better evaluate repellent compounds and develop new products that offer high repellency as well as good consumer safety. This paper presents a summary of recent information on testing, efficacy and safety of plant-based repellents as well as promising new developments in the field. (Maia & Moore, 2011)

An insecticide containing Azadirachtina tree (*Azadirachta indica*) extract, was tested against *Culex pipiens* mosquito larvae and pupae in the east of the Republic of Algeria under laboratory conditions. First, after treatment of larval stage, LC50 and LC90 values for Azadirachtin were 0.35 and 1.28 mg/L in direct effect and 0.3-0.99 mg/l in indirect effect, respectively. Second, after treatment of the pupal stage, the LC50 andLC90 in direct effect were measured as0.42 -1.24mg/l and in indirect effect was 0.39mg/l-1.14mg/l respectively. Mosquito adult fecundity were markedly decreased and sterility was increased by the Azadirachtin after treatment of the fourth instar and pupal stage. The treatment also prolonged the duration of the larval stage. The results show that the Azadirachtin is promising as a larvicidal agent against *Culex pipiens*, naturally occurring biopesticide could be an alternative for chemical pesticides. (Alouani, Rehimi & Soltani, 2009)

Mosquitoes transmit serious human diseases, causing millions of deaths every year and the development of resistance to chemical insecticides resulting in rebounding vectorial capacity. Plants may be alternative sources of mosquito control agents. This study assessed the role of larvicidal activities of hexane, chloroform, ethyl acetate, acetone, and methanol dried leaf and bark extracts of *Annona squamosa L*., *Chrysanthemum indicum L*., and *Tridax procumbens L.* against the fourth instar larvae of malaria vector, *Anopheles subpictus Grassi* and Japanese encephalitis vector, *Culex tritaeniorhynchus Giles*. Larvicidal activities of three medicinal plant extracts were studied in the range of 4.69 to 1000 mg/l in the laboratory bioassays against early 4th instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus*. The mortality data were subjected to probit analysis to determine the lethal concentrations (LC50 and LC90) to kill 50 and 90 percent of the treated larvae of the respective species. All plant extracts showed moderate effects after 24 h of exposure; however, the highest toxic effect of bark methanol extract of *Annona squamosa*, leaf ethyl acetate extract of *Chrysanthemum indicum* and leaf acetone extract of *Tridax procumbens* against the larvae of *Anopheles subpictus* (LC 50 = 93.80, 39.98 and 51.57 mg/l) and bark methanol extract of *squamosa*, leaf methanol extract of *Chrysanthemum indicum* and leaf ethyl acetate extract of *Tridax procumbens* against the larvae of *Culex tritaeniorhynchus* (LC50 =104.94, 42.29 and 69.16 mg/l) respectively. This data suggests that the bark ethyl acetate and methanol extract of *Annona squamosa*, leaf ethyl acetate and methanol extract of *Chrysanthemum indicum*, acetone and ethyl acetate extract of *Tridax procumbens* have the potential to be used as an ecofriendly approach for the control of the *Anopheles subpictus*, and *Culex tritaeniorhynchus*. (Kamaraj et al.,2011)

Mosquitoes act as a vector for most of the life-threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile Virus infection, etc. Under the Integrated Mosquito Management (IMM), emphasis was given on the application of alternative strategies in mosquito control. The continuous application of synthetic insecticides causes development of resistance in vector species, biological magnification of toxic substances through the food chain and adverse effects on environmental quality and non-target organisms including human health. Application of active toxic agents from plant extracts as an alternative mosquito control strategy was available from ancient times. These are non-toxic, easily available at affordable prices, biodegradable and show broad spectrum target-specific activities against different species of vector mosquitoes. In this study, the current state of knowledge on phytochemical sources and mosquitocidal activity, their mechanism of action on target population, variation of their larvicidal activity according to mosquito species, instar specificity, polarity of solvents used during extraction, nature of active ingredient and promising advances made in biological control of mosquitoes by plant derived secondary metabolites have been reviewed. (Gosh, Chowdhury & Chandra, 2012).

In order to develop an environment-friendly botanical mosquito larvicide alternative to the chemical larvicides, extracts were made from the leaves of *Hyptis suaveolens*, *Lantana camara*, *Nerium oleander*, and *Tecoma stans* with three organic solvents such as methanol (ME), chloroform (CH), and petroleum ether (PE) using a Soxhlet extractor. The plant extracts were screened for larvicidal activity individually and in combination against the larvae of *Aedes aegypti* and *Culex quinquefasciatus* as per WHO protocol. Among the extracts, the maximum larvicidal activity was shown by the PE extract of L. camara (LC50 10.63 mg/L) followed by the PE extract of *Tecoma stans* (LC50 19.26 mg/L), ME extract of *Nerium oleander* (LC50 35.82 mg/L), and PE extract of *Hyptis suaveolens* (LC50 38.39 mg/L) against *Culex quinquefasciatus*. In the case of *Aedes aegypti*, the PE extract of *Tecoma stans* showed maximum activity with LC50 value of 55.41 mg/L followed by *Hyptis suaveolens* (LC50 64.49 mg/L), PE extract of *Lantana camara* (LC50 74.93 mg/L), and ME extract of *Nerium oleander* (LC50 84.09). A blend of these four extracts resulted in a combination with corresponding LC50 values of 4.32 and 7.19 mg/L against *Culex quinquefasciatus* and Aedes *aegypti*. The predator safety factors were 12.55 and 20.88 for *Gambusia affinis* with respect to *Aedes* and *Culex* larvae for the extract combination. Chemical constituents in extracts were also identified by FT-IR and GC-MS data. The present investigations suggest the possible use of this blend of botanical extracts as an ideal ecofriendly, larvicide against *Aedes aegypti* and *Culex quinquefasciatus* larvae (Hari & Mathew, 2018).

They examined the chemical composition of garlic and asafoetida essential oils and their individual and combined toxicity against larvae of *Culex pipiens Linnaeus* and *Culex restuans Theobald* (Diptera: *Culicidae*). The effect of the two essential oils on egg hatch was also examined. Ten and 12 compounds, respectively, were identified in garlic and asafoetida essential oils. Allyl disulfide (49.13%) and diallyl trisulfide (31.08%) were the most abundant compounds in garlic essential oil accounting for 80.2% of the total oil. In contrast, (E)-sec-butyl propenyl disulfide (30.03%), (Z)-sec-butyl propenyl disulfide (24.32%), and disulfide, methyl 1-(methylthio) propyl (21.87%) were the most abundant compounds in asafoetida essential oil. Allyl disulfide accounted for 7.38% of the total oil in asafoetida essential oil and was one of only three compounds found in both oils. For both mosquito species, garlic essential oil was more toxic than asafoetida essential oil with Cx. restuans (LC50: garlic = 2.7 ppm; asafoetida = 10.1 ppm) being more sensitive than

Culex pipiens (LC50: garlic = 7.5 ppm; asafoetida = 13.5 ppm). When combined, the two essential oils had antagonistic effects. The majority of *Culex* egg rafts exposed to garlic (73.1%) or asafoetida (55.8%) essential oils failed to hatch and larvae of the few that did hatch mostly died as first instars. Allyl disulfide exhibited strong ovicidal and larvicidal activity suggesting its important contribution to the overall toxicity of the two essential oils. Thus, garlic and asafoetida essential oils are potent mosquito ovicides and larvicides but if used jointly, they could undermine vector control programs. (Muturi, Ramirez & Rooney, 2018)

This study was conducted to determine the larvicidal effect of aqueous extract of garlic against the 4th instars of culex and anopheles mosquito larvae. Anopheles and culex mosquito larvae were obtained using a deeper from stagnant water in the fadama at kofar Kade along illela road, Sokoto and taken to the Physiology laboratory of biological sciences, Usmanu Danfodiyo University Sokoto for further analysis. Fresh samples of garlic were obtained from central market, Sokoto state and were taken to the physiology laboratory for processing. The concentration of the extract used was 0.5mg/ml, 2.0mg/ml and 3.0mg/ml were obtained by weighing 0.5mg, 2.0mg, and 3.0mg in 10ml of water. Mortality of *Culex* and *Anopheles* depends on the garlic extract and increase with time of exposure and concentration of the extract. 3.0mg/ml recorded the highest mortality rate of 3 hours of exposure for both *Culex* and *Anopheles* at a mean of 10.00 each while 0.5mg/ml recorded minimum mortality after 1 hour of exposure for both anopheles and culex with mean of 2.33 and 3.67 respectively. The study demonstrated the potency of garlic (*Allium sativum*) in managing the larvae and thus contributes as an affordable way to control *Anopheles* and *Culex* larvae of mosquito. (Kasim, Ann & Yahaya,2019)

METHODOLOGY

While mosquitoes spend most of their life in the air, newly born mosquitoes spend their time under water. They are known as mosquito larvae. Mosquito larvae are just one stage of a mosquito's life. In order to rear mosquito larvae, firstly a location was chosen and filled a bucket with water and placed it in open spaces. Mosquito larvae were also collected from wells and reared in plastic and enamel trays in tap water.

Plant materials such as *Curcuma longa, Citrus lemon, Coleus barbatus*, *Azadirachta indica, Piper nigrum, Zingiber officinale* and *Allium sativum* were collected from in and around Kochi.

Plant materials

Fresh leaves of *Azadirachta indica* (Neem), *Coleus barbatus* (Mexican mint), fruits of *Citrus limon* (Lemon), roots of *Zingiber officinale* (Ginger), *Curcuma longa* (Turmeric), bulbs of *Allium sativum* (Garlic), seeds of *Piper nigrum* (Pepper) were collected from different areas of Ernakulam. A larvicide is a type of insecticide used to control mosquitoes indoors and outdoors around your home. They work by killing mosquito larvae before they can grow into adults. Some formulations are activated when ingested by the mosquitoes, and some formulations work when they come into contact with the larvae.

Turmeric

Turmeric is a flowering plant, *Curcuma longa*, of the ginger family, *Zingiberaceae*, the rhizomes of which are used in cooking. Insect control properties of turmeric pertains to the powder, the plant extracts, the essential oil, and certain bioactive constituents of the plant. Its products have been found active as insect repellents and insecticidal agents. The presence of curcumin in turmeric is what makes it beneficial in so many ways. It helps in soothing the itching. The strong aroma of turmeric also acts as a mosquito repellent.

Pepper Black

Pepper is a flowering vine in the family *Piperaceae*, cultivated for its fruit, known as a peppercorn, which is usually dried and used as a spice and seasoning. The fruit is a drupe which is about 5 mm in diameter, dark red, and contains a stone which encloses a single pepper seed.

Garlic

Garlic is a species of bulbous flowering plant in the genus *Allium*. Its close relatives include the onion, shallot, leek, chive, Welsh onion and Chinese onion. Garlic makes a powerful natural insect repellent. Garlic can be used to repel a variety of crawling and flying insects, including mosquitoes," according to Patrick Parker, SavATree Plant Health Care Program Director. One treatment with garlic is effective for 2 weeks and can repel insects for up to one month. The sulfurs contained within the garlic extract have been shown to be effective against a wide range of insects, including mosquitoes, and the lingering odor can deter mosquitoes from the area for weeks. It is thought that garlic may be an alternative mosquito repellent for humans as well.

Ginger

Ginger is a flowering plant whose rhizome, ginger root or ginger, is widely used as a spice and a folk medicine. It is a herbaceous perennial which grows annual pseudostems about one meter tall bearing narrow leaf blades. Ginger extracts have proven to be beneficial in offering medicinal, insecticidal and antioxidant values to humans, animals and plants. Research shows that ginger (*Zingiber officinale*, USDA zones 9-12) — especially its extracted oils — can repel certain mosquito species.

Neem

Azadirachta indica, commonly known as neem or Indian lilac, is a tree in the mahogany family *Meliaceae*. It is native to the Indian subcontinent. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. Neem is of great importance and has promising larvicidal activity against important vectors of malaria, filaria, dengue, dengue haemorrhagic fever, yellow fever and chikungunya. The larvae of a number of mosquito species (including Aedes and Anopheles) are sensitive to neem.

They stop feeding and die within 24 hours after treatment. If neem derivatives are used alone, relatively high concentrations are required to obtain high mortality.

Mexican mint

Coleus amboinicus, synonym *Plectranthus amboinicus*, is a semi-succulent perennial plant in the family Lamiaceae with a pungent oregano-like flavor and odor. The pungent nature of mint deters bugs from making your home their home. Pests like ants, mosquitos, and mice will avoid mint plants whenever possible, and it can also help with other menaces like roaches, spiders, and flies.

Lemon

The lemon is a species of small evergreen trees in the flowering plant family *Rutaceae*, native to Asia, primarily Northeast India, Northern Myanmar or China. Citrus plants, as well as their crushed leaves and extracts made from them, naturally repel mosquitoes. Oranges, lemons, lavender, basil and catnip naturally produce oils that repel mosquitoes and are generally pleasant to the nose – unless you're of the feline persuasion.

Preparation of plant extracts

The leaves (100 g) of each plant (Neem, Mexican mint) and fruits (100g) of Lemon, Garlic, Ginger, Turmeric, Pepper were made into a paste mechanically using a commercial electrical stainless steel blender by mixing it with 100ml water each. From the paste formed, the liquid extract of each material was extracted using a strainer. For each plant extract, at first, 100 grams of the plant material was mixed with 100 ml of water and blended to make a paste. The solution thus formed was used as the stock solution (100g/100ml). Stock solution is the solution that is of the highest concentration. All the other solutions to perform the serial dilution was prepared from the stock solution.

Fig 1: extract of lemon

Fig 2: extract of garlic

Fig 3: extract of ginger

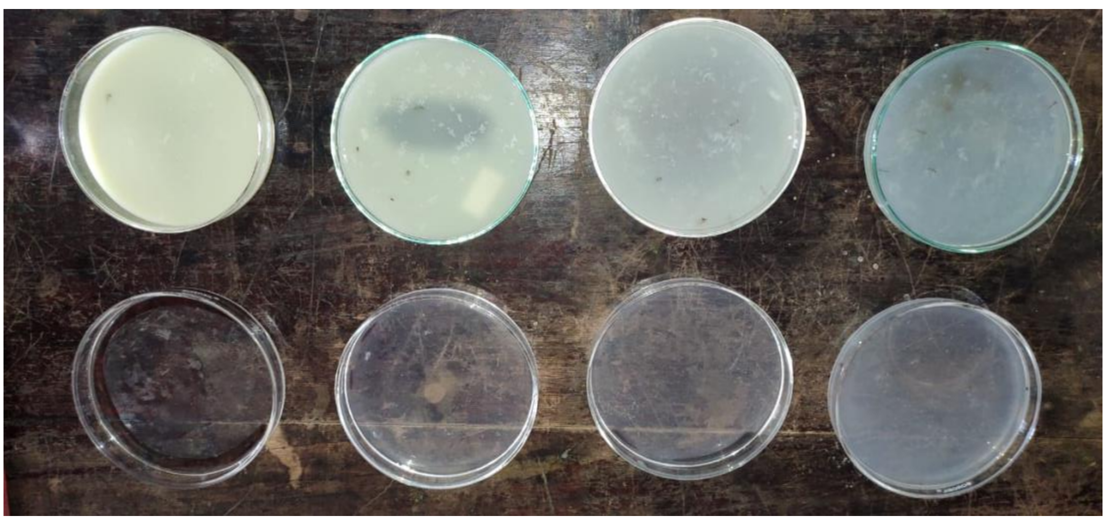

Fig 4: introduction of larvae in different concentration gradients of lemon extract

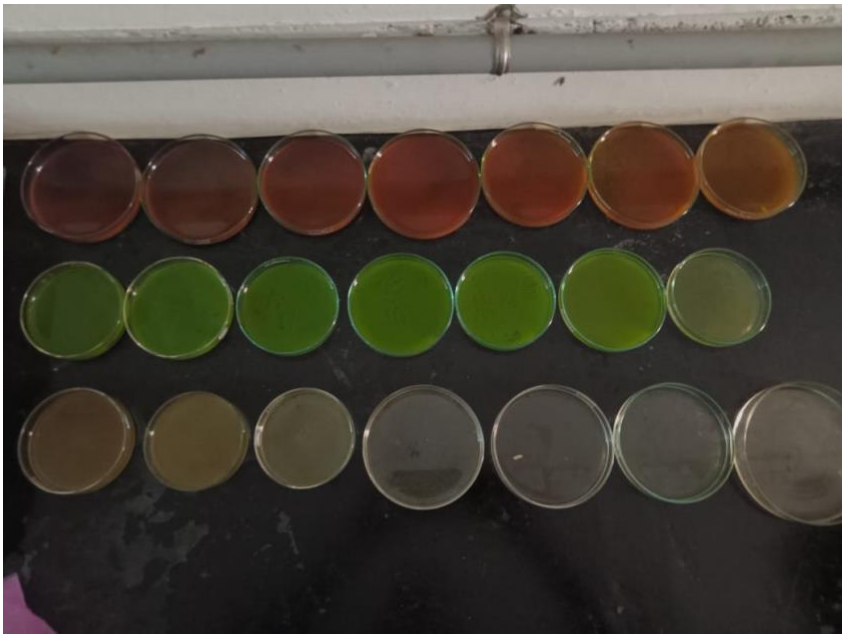

Fig 5: from top to bottom ginger, neem and pepper respectively

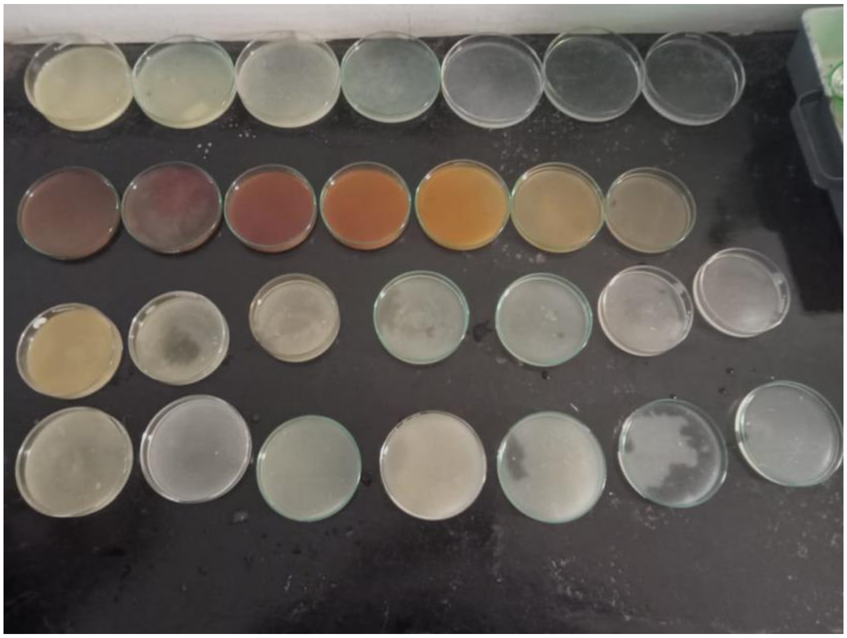

Fig: From top to bottom, garlic, turmeric, lemon and Mexican mint respectively

Larvicidal bioassay

A minimum of 5 larvae/ concentration were used for all the experiments. These experiments were repeated 3 times with 7 plant extracts in total. The percentage of mortality was calculated by using the formula:

Percentage of mortality = (No. of dead larvae/ No. of larvae introduced) x100

OBSERVATIONS AND RESULTS

Results are given in the table 1 & 2

Table 1: Percentage of Mortality of mosquito larvae after 17 hours of exposure

SL NO:	PLANT EXTRACTS	1gm/1ml percentage of mortality (%)	0.5gm/ml percentage of mortality (%)	0.25gm/ml percentage of mortality (%)	0.12gm/ml percentage of mortality (%)	0.06gm/ml percentage of mortality (%)	0.03gm/ml percentage of mortality (%)	0.01gm/ml percentage of mortality (%)
1	*Curcuma longa*	100	100	100	100	100	100	60
2	*Citrus lemon*	100	100	100	100	0	0	0
3	*Coleus barbatus*	100	100	100	100	0	0	0
4	*Azabirchta indica*	100	100	100	100	100	100	100
5	*Piper nigrum*	100	100	100	100	100	100	100
6	*Zingiber officinale*	100	100	100	100	100	80	60
7	*Allium sativum*	100	100	100	100	80	60	20

Table 2: Percentage of Mortality of mosquito larvae after 24 hours of exposure,

SL NO :	PLANT EXTRACTS	1gm/1ml percentage of mortality (%)	0.50gm/ml percentage of mortality (%)	0.25gm/ml percentage of mortality (%)	0.12gm/ml percentage of mortality (%)	0.06gm/ml percentage of mortality (%)	0.03gm/ml percentage of mortality (%)	0.01gm/ml percentage of mortality (%)
1	*Curcuma longa*	100	100	100	100	100	100	100
2	*Citrus lemon*	100	100	100	100	100	100	100
3	*Coleus barbatus*	100	100	100	100	100	60	60
4	*Azabirchta indica*	100	100	100	100	100	100	100
5	*Piper nigrum*	100	100	100	100	100	100	100
6	*Zingiber officinale*	100	100	100	100	100	100	60
7	*Allium sativum*	100	100	100	100	100	100	80

DISCUSSION

Mosquitoes are the major vector for many diseases. Mosquito vectored diseases include protozoan diseases, i.e., malaria, filarial diseases such as dog heartworm, and viruses such as dengue, encephalitis and yellow fever. In India, malaria caused due to the transmission of plasmodium by the female *Anopheles* mosquito is one of the most important causes of direct or indirect infant, child and adult mortality with approximately two to three million new cases arising every year. *Anopheles culicifacies* is the main vector of malaria, and India contributes 77 per cent of the total malaria in Southeast Asia. *Culex* is huge genus of mosquitoes that is a principle vector for transmission of diseases such as Japanese encephalitis, Lymphatic filariasis, West Nile fever etc. *Culex tritaeniorhynchus* and *Culex vishnui* are primary vectors of Japanese encephalitis (JE) virus, with a distribution throughout Southeast Asia and South. Seasonal outbreaks of acute encephalitis syndrome (AES) among children have been reported in the India causing high morbidity and mortality.

Though larvicides play a vital role in controlling mosquitoes in their breeding sites, these also show a negative impact in areas of beneficial and non-target organisms. In view of an increasing interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of the extracts from the locally available plants against medically important species of malaria vector, *Anopheles* and Japanese encephalitis vector, *Culex*.

Our data suggest that the Pepper and Neem have the potential to be used as an eco-friendly approach for the control of the mosquito larvae.

The activity of plant extracts is often attributed to the complex mixture of active compounds. Seven different extracts were tested against mosquito larvae and 100 percent larval mortality was observed in extract of *Piper nigrum* and *Azadirachta indica* in all concentrations.

All plant extracts showed moderate toxic effect on *the larvae* after 24 h of exposure; however, the highest mortality was found in extract of *Piper nigrum* and *Azadirachta indica.*

CONCLUSION

Based on current study, it was concluded that the use of plant extracts is an alternative to synthetic insecticide as they are cheap, easily available and relatively safe to the natural enemies and other non-target species. Therefore, it is recommended to use different plant extracts for the control of mosquito larvae in open spaces.

From the table 1 & 2, it can be observed that extracts of seven plants had a significant mortality on the mosquito larvae. In all of the plant extracts, the highest effect occurred at a concentration of 1gm/ml while the smallest effect was at 0.15625gm/ml. The increased concentration led to increased larval mortality. The extract of Pepper and Neem showed the highest effect on the larvae. The smallest effect was shown by the extracts of Lemon and Mexican mint.

Repellent activity of Neem extract against mosquito larvae was different for different concentrations and its efficacy was more at the higher concentrations; and highest repellent activity was seen in undiluted extract and it gradually decreased with decrease in concentration (diluted extracts). Same results were observed for Pepper extracts and highest repellent activity was seen in undiluted extract.

Comparison between results of the seven plant species shows that in the same concentrations, repellent activity of Neem and Pepper was higher in all concentrations and this was common for all of the treatments. In the whole of concentrations, Neem and Pepper were more potent than other extracts.

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