A PRELIMINARY INVESTIGATION ONEFFICACY OF GINGER EXTRACT AS MOSQUITO LARVICIDAL AGENT



Project work by

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Submitted to St. Teresa's College (Autonomous), Ernakulam Affiliated to Mahatma Gandhi University, Kottayam in partial fulfillment of requirement for Degree of Bachelor of Science in Zoology

CERTIFICATE

This to certify that the project work entitled "A PRELIMINARY INVESTIGATION ONEFFICACY OF GINGER EXTRACT AS MOSQUITO LARVICIDAL AGENT" submittedby SHREYA P., Reg no: AB21ZOO041 is a bonafide work done under my guidance and supervision and to the best of my knowledge, this is her original effort.

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EXAMINERS:

1.

DECLARATION

I Shreya P., hereby declare that the project work entitled, "A PRELIMINARY INVESTIGATION ON EFFICACY OF GINGER EXTRACT AS MOSQUITO LARVICIDAL AGENT" 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 or submitted elsewhere in connection with any other academic course and the opinions furnished in this report are entirely my own.

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ABSTRACT

Out of the several methods of biological control of mosquitoes, the use of plant extract as larvicidal agents is promising. This study was conducted to evaluate the efficacy of a well known culinary spice, Ginger (Zingiber officinale) in controlling the spread of mosquito larvae. Fresh rhizomes of ginger were collected and crude aqueous extracts were prepared. Four different concentrations of the extract were administered into the mosquito rearing medium. The mosquito larvae exposed to the medium containing extracts were observed for behavioral changes and mortality. The results showed that, the larvae showed maximum mortality at 20% concentration of ginger extract for 24 hours whereas at 5% levels no mortalities were observed. Lethal dose of the crude extract for mosquito larvae was estimated following arithmetical (Karber's method) and graphical (Miller Tainter Methods) as 10.5% and 8.85% respectively. More studies using purified extracts and field trials are essential to confirm the mosquito larvicidal potential of ginger and to propose suitable biocontrol measures.

INTRODUCTION

Arthropods are vectors of several deadly pathogens and parasites causing serious diseases to humans and animals (Mehlhorn, 2008; Mehlhorn *et al.*, 2012). Among the arthropods, Mosquitoes (Diptera: Culicidae) represent a significant hazard to millions of people globally, as they are the carriers of diseases such as filariasis, West Nile, malaria, yellow fever, and dengue. Culex (Japanese encephalitis, west Nile, chikungunya), Anopheles (filariasis, malaria), and Aedes (chikungunya, dengue, yellow fever) are the main genera of mosquitoes that act as vectors for a variety of diseases (Ghosh, 2012). Anopheles mosquitoes produce 200 million to 450 million illnesses globally, which results in 2.7 million fatalities annually. It continues to be an endemic disease vector in over 100 developing nations (Koech, 2014).

A vital preventive measure in this situation is vector control. The usual methods for controlling mosquito larvae are organophosphates, microbial control agents, and insect growth regulators. To prevent the spread of mosquito-borne illnesses, other strategies employed in tropical countries include indoor residual spraying and insecticide-treated bed nets (Lees *et al.*, 2014). Yet, these chemicals have negative effects on human health and the environment, and they also make a range of vector species resistant (Benelli, 2015). Consequently, natural extracts or eco-friendly tools have been used to improve mosquito vector control.

Biological control is the use of living things to lower the number of pests and their potential harm.Since ancient times, active toxic chemicals made from plant extracts have been utilized as a replacement for traditional mosquito control methods. These possess broad-spectrum, target-specific efficacy against numerous vector mosquito species and are safe, easily obtainable, affordable, and biodegradable (Ghosh *et al.*, 2012). Plant-based insecticides are made up of botanical concoctions of chemical components that interact to influence behavioral and physiological functions (Ghosh *et al.*, 2012). Other spices like Asafoetida (*Ferula asafetida*), curry leaves (*Murraya koenigii*), coriander (*Coriandrum sativam*) and fenugreek (*Trigonella foenumgraceum*) (Harve and Kammath, 2004) were found to be effective and showed encouraging results against *Aedesaegypti* and Culex (Diptera: Culicidae) mosquito larvae (Desai, 2002).

According to the studies by Murthy and Rani (2009), two of the eight plant extracts examined were poisonous to *Aedes aegypti* mosquito larvae, which cause yellow fever, in their third instar. The most active and most toxic extracts from their study were those of *Limonia acidissima* (Wood apple) and *Delonix regia* (Royal Poinciana), which demonstrated 100% toxicity. In a different investigation conducted in a lab setting, Subramanian *et al.* (2012 \neg) evaluated the bioefficacy of *Aloe vera* leaf extract against *Aedes aegypti* larvae in their first through fourth instars. Their study demonstrated that an extract from *A. vera* might be a useful larvicidal agent for mosquitoes. Eight plant extracts were tested for their adulticidal, larvicidal, and repellant properties against the adult and early fourth instar larvae of two species of mosquitoes that transmit filariasis. The study was conducted by Kamaraj *et al.* (2010). Plant extracts presented a promising environmentally friendly solution for controlling these insects.

Thus from the previous studies on evaluation of the larvicidal effects of plant extracts, it is obvious that many locally available plants can be used against vectors of major pathogens.

Ginger (Zingiber officinale) is a member of a plant family that includes cardamom and turmeric. It is a flowering plant whose rhizome is widely used as a spice and a medicine. Its spicy aroma is mainly due to presence of ketones, especially the gingerols, which appear to be the primary component of ginger studied in much of the health-related scientific research. The rhizome, which is the horizontal stem from which the roots grow, is the main portion of ginger that is consumed. It was originated in Maritime Southeast Asia and was likely domesticated first by Austronesian people. Ginger's current name comes from the Middle English gingivere, but this spice dates back over 3000 years to the Sanskrit word srngaveram, meaning "horn root," based on its appearance. In Greek, it was called *ziggiberis*, and in Latin, *zinziberi*. Interestingly, ginger does not grow in the wild and its actual origins are uncertain. Ginger is used in numerous forms, including fresh, dried, pickled, preserved, crystallized, candied, and powdered or ground. The flavor is somewhat peppery and slightly sweet, with a strong and spicy aroma. Ginger has been purported to exert a variety of powerful therapeutic and preventive effects and has been used for thousands of years for the treatment of hundreds of ailments from colds to cancer. It is found to be effective against several ailments like nausea/vomiting, cancer, asthma, diabetes, platelet aggregation, cardiovascular disease, cholesterol, dementia, ulcers etc.

In Keralasix mosquito-borne diseasesviz. Malaria, Dengue, Chikungunya, Japanese Encephalitis, West Nile virus and Lymphatic Filariasis are reported. Hence there is an urgent need to emphasize vector control activities to prevent the spread of mosquitoes and mosquito borne diseases. At present the mosquito vector control in Kerala is carried out by local authorities using repellant sprays and application of saline water. In most of the households mosquito coils/ mats and liquid devices are the most common personal protective measures used.But these materials have other negative impacts on environement and in humans. So it is better to opt alternative safe measures such as biological control. In spite of the vast availability of many species of medicinal plants in Kerala, reliable published information on the application of plant based products in biological control of mosquitoes is scarce or insufficient.

Therefore, in the present study, suitability of Ginger in controlling mosquito larvae(larvicidal properties) was investigated using different concentrations of crude extract. The specific aims of the project are to 1. Evaluate the mosquito larvicidal potential of crude extract of ginger rhizome 2. Estimate the lethal dose required to cause 50% mortality using the crude extract.

REVIEW OF LITERATURE

Traditionally, vector control methods have concentrated on employing various insecticides to kill mosquitoes. Larvicides, pupicides, and chemical or microbiological ovicides have frequently been employed in conjunction with environmental management aiming at reduction or limination of mosquito breeding grounds. Given the widespread emergence of insecticide resistance and the concerns surrounding environmental harm and effects on non-target organisms, the use of synthetic pesticides must be regulated. The application of pesticides, such as pyrethroids, carbamates, and organophosphates, to control mosquitoes may also be harmful to people's health. Hence there is an increasing demand for innovative, affordable, and effective mosquito control methods.

It has been suggested that bio-control of mosquito vectors can address these problems. Killing the vector, altering its life cycle to increase self-mortality, and creating infertile or disease-transmitting vectors are examples of biocontrol techniques. Additionally, the various stages of vector growth are affected by these bio-control approaches.

In the report by Kamereddine (2012), an overview of the most promising biological control tools for malaria eradication, namely fungi, bacteria, larvivorous fish, parasites, viruses and nematodes were presented. Use of guppy fish as a biocontrol agent is a widely accepted paractice. In the study by Elias et al. (1995), the predation potentiality of a biological control agent, the guppy fish, Poecilia reticulata (= Lebistes reticulatus), was studied over the mosquito larvae under laboratory conditions. The study suggests that this fish could be used, after careful field trial, as a promising and sustainable biological control agent in controlling filariasis and other mosquito-borne diseases. In anotherstudy Ramet al. (2015), reported the prey selectivity patterns of Gambusia affinis (mosquitofish) preying on larvae of the two Indian major carps (*Catlacatla* and *Labeorohita*) in the presence of varied proportions of alternative prey (rotifers, chironomid cladocerans, and mosquito larvae) under laboratory conditions.

The use of medicinal plant extracts for biological control of mosquito is also reported by several workers. Benelli *et al.*(2016) have comprehensively reviewed the non-insecticide based strategies that have been implemented or are currently being tested. They discussed in detail the

non-insecticide based tactics that have been used or are being tested, emphasizing the application of behavioral information about mosquitoes to control strategies.Earlier, Supavarn*et al.* (1974) had examined the effect of extracts from 36 plant species, belonging to 35 genera of 17 families, on 4thinstar larvae of *Aedes aegypti* and reported positive results.

Latha *et al.*(1998), investigated the larvicidal activity of 41 native plant extracts found in abundance throughout Kerala against two types of mosquitoes: *Culex siliens*, a salty water mosquito, and *Culex quinquefasciatus*, an urban mosquito. The early fourth instar larvae of *C. siliens* were susceptible to larvicidal activity from the extracts of eighteen plants. *Zingiber wighlianum* and *Piper longum* were two plant species that showed efficacy against the larvae of *Cx. quinquefasciatus* and *ex. siliens* in their early fourth instar. As a result, it was determined that *P. longllm* and *Z. wighlianum* are possible biopesticides

Ghosh *et al.* (2012)provided a detailed account of 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 the promising advances made in biological control of mosquitoes by plant derived secondary metabolites have been reviewed in this study.

In a study by Azmathullah*et al.* (2011), mosquito larvicidal efficacy and phytochemical screening of aqueous and ethanol extracts of the flowers of *Calotropis procera*, a common weed plant were evaluated. Results revealed a dose and time dependent effect of the extracts on mosquito larvae with 5% extract concentration causing 100% mortality within 3 days of exposure.

In the study by Janelle *et al.*(2020), *Utricularia macrorhiza*was experimentally evaluated for its larvicidal efficiency for mosquito vectors *Aedes aegypti* and *Aedes albopictus*. According to this report, within five days, the presence of *U. macrorhiza* reduced the survival of *Ae. aegypti* and *Ae. albopictus* larvae by 100% and 95%, respectively, compared to controls.

In a study by Rahuman*et al.* (2008), the larvicidal activity of a petroleum ether extract of *Zingiber officinale* Roscoe was evaluated against mosquito species such as *Aedes aegypti* L. and *Culex quinquefasciatus*. Bioassay-guided fractionation led to the isolation of 4-gingerol (1), (6)-

dehydrogingerdione (2) and (6)-dihydrogingerdione (3). The results show that the most effective compound was 4-gingerol.

The essential oils from rhizomes of mango ginger, cassumunar ginger, and English ginger was extracted to evaluate their mosquito larvicidal and repellent activities and the antimicrobial susceptibility against six bacterial strains viz. *Staphylococcus aureus*, Listeria *monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Bacillus subtilis*, and *Lactobacillus casei*(Hazarika *et al.*, 2020).

Kalaivani *et al.*(2012) reported the larvicidal activity of hydrodistillate extracts from *Mentha piperita* L. *Ocimumbasilicum* L. *Curcuma longa* L. and *Zingiber officinale* L. against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae). The results indicated that the mortality rates at 80, 100, 200 and 400 ppm of *M. piperita*, *Z. officinale*, *C. longa* and *O. basilicum* concentrations were highest amongst all concentrations of the crude extracts tested against all the larval instars and pupae of *A. aegypti*.

In a study by Pushpanathan*et al.* (2007), the essential oils extracted by steam distillation from *Zingiber officinalis*was evaluated for larvicidal and repellent activity against the filarial mosquito *Culex quinquefasciatus*. The larval mortality was observed after 24 h of treatmentfor late third instar. The LC₅₀ value was 50.78 ppm. The results clearly reveal that the essential oil of Z. officinalis served as a potential larvicidal and repellent agent against filarial vector *C. quinquefasciatus*.

Govindarajan *et al.* (2016), evaluated the larvicidal and repellent activity of *Zingiber nimmonii* rhizome essential oil (EO) against the malaria vector *Anopheles stephensi*, the dengue vector *Aedes aegypti*, and the lymphatic filariasis vector *Culex quinquefasciatus*. In acute toxicity assays, the EO showed significant toxicity against early third-stage larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, with LC₅₀ values of 41.19, 44.46, and 48.26 µg/ml, respectively. Repellency bioassays at 1.0, 2.0, and 5.0 mg/cm² of *Z. nimmonii* EO gave 100 % protection up to 120, 150, and 180 min. against *An. stephensi*, followed by *Ae. aegypti* (90, 120, and 150 min) and *Cx. quinquefasciatus* (60, 90, and 120 min).

Li *et al.* (2021) worked on the components of *Z. cassumunar* essential oil that show repellent activity against <u>Aedes albopictus</u>. Thelarvicidal and adulticidal activities

of *Z. cassumunar* essential oil against *Ae. Albopictus was also studied*. Bioassay-guided fractionation identified the major active compound of *Z. cassumunar* ... Furthermore, this study provides scientific support for the folk usage of *Z. cassumunar* essential oil as mosquito repellent and indicates that *Z. cassumunar* essential oil and (–)-terpinen-4-ol can be used as plant-derived repellents and insecticides for mosquito control.

Khandagle*et al.* (2011), evaluated the bioactive potential of two commonly occurring plants against mosquitoes. Essential oils extracted by steam distillation from rhizome of *Zingiber officinalis* and leaf and stem of *Achyranthes aspera* were evaluated for larvicidal, attractant/repellent, and oviposition attractant/deterrent activity against two mosquito species *viz. Aedes aegypti* and *Culex quinquefasciatus*. The results reveal that both the oils have control potential against *A. aegypti* and *C. quinquefasciatus*.

METHODOLOGY

1. Collection of plant

Freshly collected Ginger (*Zingiber officinale*) rhizomes werecollected from a market in Broadway, Ernakulam. The samples werepurchased during morning hours at 10.30 AM.

2. Preparation of rhizome extract

The collected plant was washed thoroughly to remove any dirt. After washing, the rhizome was kept for drying for 30 minutes. The rhizome was then ground using mortar and pestle. The ground product was then used for preparing crude extract. Extract was collected in a beaker.

3. Rearing of mosquito larvae

A 25L bucket containing pond water was kept open for a week for allowing growth of larvae. Larval growth was observed within 10 days. (Fig. 1).

4. Experimental administration of prepared extract

a) **20% concentration**

16 ml distilled water was taken in a conical flask to which 4ml extract was added.

b) 15% concentration

17 ml distilled water was taken in a conical flask to which 3 ml extract was added.

c) 10% concentration

18 ml distilled water was taken in a conical flask to which 2 ml extract was added.

d) 5% concentration

19 ml distilled water was taken in a conical flask to which 1 ml extract was added.

5. Experimental setup

Glass petridishes were used for the experimental administration of extract to mosquito larvae. Four different concentrations of extracts were used for evaluating the efficacy of extract. Experiments were run in duplicate for each concentration along with a control. Into the first set,10 ml of extract of 20% concentrationwas transferred. The remaining concentrations of prepared extracts were transferred to the second, third and fourth sets (10 ml each) (Table 1).To each petri dish containing the prepared extracts of different concentrations, five mosquito larvae were carefully transferred from the outdoor culture using a dropper. Response of the larvae exposed to the extract were taken up to 24 hours.

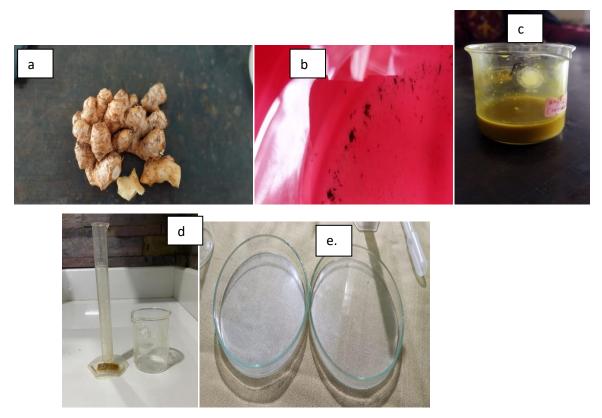


Fig. 1. Experimental set up a. ginger rhizomes b. mosquito culture c. ginger crude extract d. preparation of doses e. petridishes

Crude extract	Volume of ginger	Volume of distilled
concentration	extract (ml)	water (ml)
20%	4	16
15%	3	17
10%	2	18
5%	1	19
Control	-	20

Table 1. Preparation of extracts for experimental exposure.

Estimation of lethal dose (LD₅₀)

The lethal dose which is the amount of extract required to cause mortality of 50% of the group of test organisms (LD₅₀)was estimated using arithmetical methods (Karber's method) and graphical method (Miller and Tainter method)

Karber's method formula :

 $LD_{50} = LD100 - \sum (a \times b) / n$

where,

n= total number of animals in each group

a= the difference between two successive doses of administered extract.

b = the average number of dead animals im two successive doses.

LD100 = Lethal dose causing the 100% death of all test animals.

Graphical method

Lethality of the extract can be determined by the caluculation of LD_{50} ie., the dose that will kill 50% of a particular species, based on the method described by Miller and Tainter (1944). The percentage of animals that had died at each dose is transformed to probit. The percentage dead for 0 and 100 are corrected before the detreminatuion of probits as below :

For 0% dead -100 X (0.25/n)

For 100% dead – 100 X (n- 0.25/n)

The probit values are plotted against log doses and then the dose corresponding to probit five i.e., 50% is found out.

OBSERVATIONS AND RESULTS

The observations showed that, at 20%, the all the larvae died in both 1^{st} and 2^{nd} plates. At 15%, all the larvae died in 1^{st} plate whereas four larvae died in the 2^{nd} plate. At 10%, two larvae died in the 1^{st} plate whereas three larvae died in the 2^{nd} plate. At 5%, all the larvae survived in both 1^{st} and 2^{nd} plate. (Table 1, Fig. 1)

Table 1. Mortality observations of mosquito larvae after 24 hours exposure to the extract

	MORTALITY OBSERVATIONS 1 ST PLATE 2 ND PLATE				
EXTRACT CONCENTRATION	Number of live larvae	Number of dead larvae	Number of live larvae	Number of dead larvae	% mortality (average)
20 %	5	5	5	5	100
15%	5	5	5	4	90
10%	5	2	5	3	50
5%	5	0	5	0	0

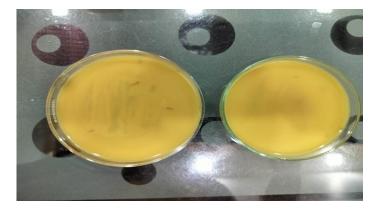
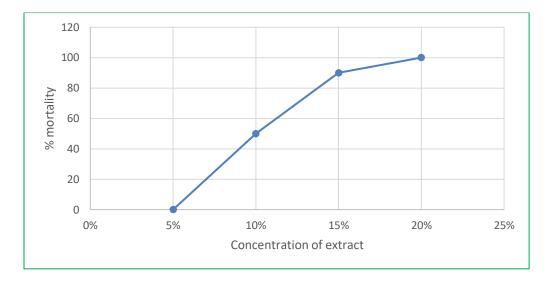


Fig 1. Petri dish containing 20% concentration extract with dead larvae



The mortality pattern observed in the experiment is depicted in Fig 2 .

Fig 2. Graph showing mortality pattern at various doses

As per the arithmetic method (Karber's method), the LD_{50} was worked out as **10.5%**. (Table 2).

Table 2. LD₅₀ value worked out based on Karber's method.

Dose (%)	No. of	Dose	Dead	Interval mean	Product
	animals (n)	difference (a)		(b)	aXb
5	5	0	0	-	-
10	5	5	2.5	1.25	6.25
15	5	5	4.5	3.5	17.5
20	5	5	5	4.75	23.75
L		1	1		1

D50= LD100- $\sum (axb)/n$

20-47.5/5=10.5%

According to the graphical method (Miller and Tainter method), the LD₅₀ was recorded as 8.31%.(Table 3, Fig.3)

Group	Dose (%)	Log dose	% dead	Corrected %	Probits
1	5	0.6989	0	5	3.36
2	10	1	50	50	5.00
3	15	1.1760	90	90	6.28
4	20	1.1030	100	95	6.64

Table 3. Results of the lethal dose of ginger extract

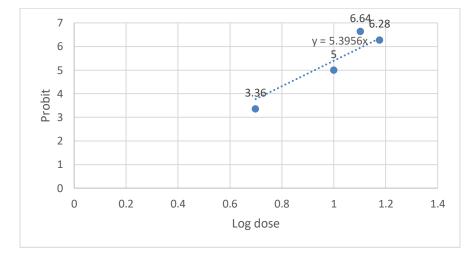


Fig 3. Graph of log dose v/s probits to determine the LD_{50} value of ginger extract.

DISCUSSION

There is a growing need to focus on the development of natural insecticides derived from medicinal plants as biocontrol agents due to the harmful effects of antimicrobial agents on the environment and human health problems.

The goal of mosquito management is to limit the harm that mosquitoes cause to people's health, enjoyment, and economy. Because mosquitoes transmit a variety of diseases, including the Zika virus and malaria, controlling mosquito populations is an essential part of global public health initiatives. Before the larvae in the breeding habitat can develop into adult mosquitoes and spread, they are the target of larvicides. It aids in lowering the number of adult mosquitoes in the surrounding surroundings.

Mosquito-borne diseases pose a threat to around 50% of the global populace, with socioeconomically disadvantaged groups bearing the heaviest impact. A number of factors, including changes in land use, urbanization, globalization, and climate change, have led to the rise and resurgence of diseases spread by mosquitoes. For instance, the incidence of dengue fever has increased over thirty times in the last fifty years, and since 2014, the size and frequency of chikungunya, yellow fever, and malaria outbreaks have all increased. Moreover, hundreds of thousands of people contracted the Zika virus (ZIKV) during the 2015–2016 outbreak in Latin America and the Caribbean, which had a significant negative socioeconomic impact. Moreover, the coronavirus disease pandemic of 2019 is predicted to cause supply-chain disruptions, which would raise the number of malaria-related deaths in sub-Saharan Africa in 2020–2021.

Thus it can be seen that there is a critical need for safe, sustainable approaches to reduce the burden of mosquito-borne pathogens. Common mosquito control strategies with chemical insecticides and environmental management are only moderately effective, in part due to resistance arising from physiological (e.g., insecticide resistance) and behavioral changes (e.g., mosquitoes change their blood-feeding times in response to bed nets). Chemical interventions also have unintended effects on non-target insects, including pollinators(Wang et.al.,2021)

This study showed the least dose that was required to cause death of mosquitoes. Thus it helps in estimating the amount of Ginger extract required for administration as biocontrol agent for killing of mosquito larvae. It was observed that majority of the larvae died at 20% and lower concentrations of ginger extract. It is because of the effect of the biological components present in the rhizome. Many previous authors have reported the efficacy of ginger extracts in mosquito vector control (Rahuman*et al.*, 2008; Hazarika *et al.*, 2020; Pushpanathan*et al.*, 2007).

The larvicidal activities of crude and solvent extracts of *Solanum nigrum* L. leaves against *Culex quinquefasciatus*was studied by Rawani*et al.* (2010) The results of this study indicated that the mortality rates at 0.5% concentration were the highest amongst all concentrations of the crude extracts tested against all the larval instars at 24, 48 and 72 h of exposure. Result of log probit analysis (at 95% confidence level) revealed that lethal concentration LC₅₀ and LC₉₀ values graduallydecreased with the exposure periods in bioassay experiment with the crude plant extract. Similarly, in the present study, using crude extracts of ginger comparable findings on lethal concentrations were obtained.

Laboratory bioassays on insecticidal activity of hexane crude extract derivedfrom four species of ginger were carried out againstthree mosquito larvae vector by Mahardika *et al.* (2017). Results from this study suggested that bioassay guided effective extracts of the tested ginger species are potential larvicidal candidates for controlling mosquito vectors of dengue, chikungunya and filariasis. Even though specific vectors were not selected for the present study, the results confirm that ginger is a potential larvicidal agent even at 10%. However, in a recent study by Ullah *et al.* (2021) extracted oil of *Z. officinale* did not have significant activity againstthe tested mosquito species and suggested further evaluations under filed conditions. Similar studies on larvicidal effects of ginger with promising resultswere carried out recently by Assemie*et al.* (2023) as well.

In a detailed investigation, Bucker *et al.* (2013) have analysed the mosquito larvicidal effect as well as brine shrimp toxicity of ginger rhizome. The toxicity of ginger rhizome to other economically important aquatic species such as fish, particularly fish larvae also need future attention.

According to the study by Chen *et al.* (2010) ginger also has a strong larvicidal impact, with 100% death in 17- and 24-hour observations. Ginger has many bioactive ingredients,

including phenolic and terpene chemicals. Ginger contains phenolic chemicals, including gingerols, shogaols, and paradols. Fresh ginger contains primarily gingerols, including 6-gingerol, 8-gingerol, and 10-gingerol. Various quantities of shogaol, gingerol, and gingerol have the strongest impact on loss of spontaneous movement from 24 to 72 hours. The minimum effective doses of gingerol and shogaol were determined by analyzing the time course of mortality and percentage of loss of spontaneous movements. This study found that gingerol had a greater maximum larvicidal effect and loss of spontaneous movement than shogaol and albendazole. The larvicidal activity of *Zingiber officinale* is attributed to its constituents (Chen *et al.*, 2010).

Another recent study by Hazrika *et al.* (2020) from Assam, India also confirmed that the essential oils from ginger possess good larvicidal, repellant, and antimicrobial activity. Thus considering the larvicidal potential of ginger extract, it is recommended to conduct further studies to extract and purify the active compounds from extract and to identify their efficacy based on bioassays and field trials so as to develop natural effective formulations for biological control of mosquito larvae.

CONCLUSION

1. Many previous studies have proved the potential of use of plant extracts as bio control agents. In Kerala many such plant species are abundantly available ensuring prospects for exploration.

2. The results of this study suggest that ginger extract may have larvicidal properties for mosquito larvae. The use of ginger extract in place of artificial pesticides can be an environmental friendly option to address mosquito vector borne diseases.

3. In this study, the crude extract showed highest larvicidal property at 20% and 15% concentrations and the lowest at 5% concentration.

4. Lethal dose of the crude extract for mosquito larvae was estimated as 10.5% as per arithmetical (Karber's method) method and as 8.85% based on probit analysis (Miller Tainter Methods).

5. Plant extracts are cheap, readily available, non toxic to nature and non polluting. Hence, the use of such natural products should be promoted for use as effective parricides for control of disease vectors.

REFERENCES

1. Azmathullah, N. M., Sheriff, M. A., & Mohideen, A. S. (2011). Phytochemical screening of Calotropis procera flower extracts and their bio-control potential on Culex sp. mosquito larvae and pupae. Int. J. Pharm. Biol. Arch, 2(6), 1718-1721.

2. Assemie, A., & Gemeda, T. (2023). Larvicidal Activities of Allium sativum L. and Zingiber officinale Rosc. Extracts against Filariasis Vectors in Hadiya Zone, Ethiopia. BioMed Research International, 2023

3. Benelli, G., Jeffries, C. L., & Walker, T. (2016). Biological control of mosquito vectors: past, present, and future. Insects, 7(4), 52.

4. Benelli, G. (2015). Research in mosquito control: current challenges for a brighter future. Parasitology Research, 114(8), 2801–2805. doi:10.1007/s00436-015-4586-9

5. Bücker, A., Falcão-Bücker, N. C., Nunez, C. V., Pinheiro, C. C. D. S., & Tadei, W. P. (2013). Evaluation of larvicidal activity and brine shrimp toxicity of rhizome extracts of Zingiber zerumbet (L.) Smith. Revista da Sociedade Brasileira de Medicina Tropical, 46, 377-380.

6. Couret, J., Notarangelo, M., Veera, S., LeClaire-Conway, N., Ginsberg, H. S., & LeBrun, R. L. (2020). Biological control of Aedes mosquito larvae with carnivorous aquatic plant, Utricularia macrorhiza. Parasites & vectors, 13(1), 1-11.

7. Elias, M., Islam, M. S., Kabir, M. H., & Rahman, M. K. (1995). Biological control of mosquito larvae by Guppy fish. Bangladesh Medical Research Council Bulletin, 21(2), 81-86.

8. Govindarajan, M., Rajeswary, M., Arivoli, S., Tennyson, S., & Benelli, G. (2016). Larvicidal and repellent potential of Zingiber nimmonii (J. Graham) Dalzell (Zingiberaceae) essential oil: an eco-friendly tool against malaria, dengue, and lymphatic filariasis mosquito vectors?. Parasitology Research, 115, 1807-1816.

9. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian J Med Res. 2012; 135(23):581-598.

10. Hazarika, H., Boruah, S., Islam, J., Paul, A., & Zaman, M. K. (2020). Antimicrobial, mosquito larvicidal and repellent activity of essential oils isolated from three local species of ginger grown in upper Assam region, India. Current Trends in Pharmaceutical Research, 7(1), 126-142.

11. Koech PK, Mwangi RW. Synergistic repellent activity of plant essential oils against Aedes aegypti on rabbit Skin. International Journal of Mosquito Research. 2014; 1(14):55-59

12. Kumar, R., Muhid, P., Dahms, H. U., Sharma, J., & Hwang, J.S. (2015). Biological mosquito control is affected by alternative prey. Zoological Studies, 54, 1-18.

13. Kamareddine, L. (2012). The biological control of the malaria vector. Toxins, 4(9), 748-767.

14. Kalaivani, K., Senthil-Nathan, S., & Murugesan, A. G. (2012). Biological activity of selected Lamiaceae and Zingiberaceae plant essential oils against the dengue vector Aedes aegypti L.(Diptera: Culicidae). Parasitology research, 110, 1261-1268.

15. Khandagle, A. J., Tare, V. S., Raut, K. D., & Morey, R. A. (2011). Bioactivity of essential oils of Zingiber officinalis and Achyranthes aspera against mosquitoes. Parasitology research, 109, 339-343.

16. Latha, C., Vijayakumar, P. D., Velayudhan, S., & Joseph, A. (1999). Biological activity of indigenous plant extracts as mosquito larvicides.

5. Mehlhorn H (2008) Encyclopedia of parasitology, 3rd edn. Springer, Heidelberg

17. Lees RS, Knols B, Bellini R, Benedict MQ, Bheecarry A, Bossin HC *et al.* (2014) Review: improving our knowledge of male mosquito biology in relation to genetic control programmes. Acta Trop 132S:S2–S11

18. Li, M. X., Ma, Y. P., Zhang, H. X., Sun, H. Z., Su, H. H., Pei, S. J., & Du, Z. Z. (2021). Repellent, larvicidal and adulticidal activities of essential oil from Dai medicinal plant Zingiber cassumunar against Aedes albopictus. Plant Diversity, 43(4), 317-323.

19. Lin, R.J., Chen, C.Y., Chung, L.Y. & Yen, C.M. (2010). Larvicidal activities of ginger (Zingiber officinale) against Angiostrongylus cantonensis. Acta Trop, 115(1-2),69-76.

20. Mehlhorn H, Al-Rasheid KAS, Al-Quraishy S, Abdel-Ghaffar F (2012) Research and increase of expertise in arachno-entomology are urgently needed. Parasitol Res 110:259–265

21. Mahardika, R. W., Ibrahim, H., Nurulhusna, A. H., & Awang, K. (2017). Efficacy of four species of Zingiberaceae extract against vectors of dengue, chikungunya and filariasis. Trop. Biomed, 34, 375-387.

22. Pushpanathan, T., Jebanesan, A., & Govindarajan, M. (2008). The essential oil of Zingiber officinalis Linn (Zingiberaceae) as a mosquito larvicidal and repellent agent against the filarial vector Culex quinquefasciatus Say (Diptera: Culicidae). Parasitology research, 102, 1289-1291

23. Rahuman, A. A., Gopalakrishnan, G., Venkatesan, P., Geetha, K., & Bagavan, A. (2008). Mosquito larvicidal activity of isolated compounds from the rhizome of Zingiber officinale. Phytotherapy Research, 22(8), 1035-1039.

24. Rawani, A., Ghosh, A., & Chandra, G. (2010). Mosquito larvicidal activities of Solanum nigrum L. leaf extract against Culex quinquefasciatus Say. Parasitology Research, 107(5), 1235–1240.Rawani, A., Ghosh, A., & Chandra, G. (2010). Mosquito larvicidal activities of Solanum nigrum L. leaf extract against Culex quinquefasciatus Say. Parasitology Research, 107(5), 1235–1240.

25. Supavarn, P., Knapp, F. W., & Sigafus, R. (1974). Biologically active plant extracts for control of mosquito larvae. Mosquito News, 34(4), 398-402.

26. Ullah, A., Ghramh, H. A., Ahmad, S., Bilal, H., Ahmed, K. S., Ullah, Z., & Amjad, M. (2021). Larvicidal and repellent efficacy of garlic (Allium sativum) and ginger (Zingiber officinale) against the mosquito disease vectors Anopheles stephensi and Aedes aegypti.

27. Wang, G.H., Gamez, S., Raban, R.R., Marshall, J.M., Alphey, L, Li., M, Rasgon, J.L., Akbari & O.S. Combating mosquito-borne diseases using genetic control technologies. Nat Commun, 12, 4388