A PRELIMINARY INVESTIGATION OF TURMERIC EXTRACT AS LARVICIDAL AGENT

Project work by

SHAMNA V S

AB21ZOO040

Under the guidance of

Ms. PARVATHY K R

Assistant Professor

Department of Zoology

St. Teresa's College (Autonomous)

Ernakulam, Kochi

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CERTIFICATE

This to certify that the project work entitled “**A PRELIMINARY INVESTIGATION ON EFFICACY OF TURMERIC EXTRACT AS MOSQUITO LARVICIDAL AGENT”** submitted by **SHAMNA V S**., Reg no: **AB21ZOO040** is a bonafide work done under my guidance and supervision and to the best of my knowledge, this is her original effort.

Ms. Parvathy K. R.	Dr. Soja Louis
Assistant professor.	Head of the Department,
Department of Zoology,	Department of Zoology,
St. Teresa’s College,	St. Teresa’s College,
Ernakulam.	Ernakulam.

EXAMINERS:

1.

2.

DECLARATION

I Shamna V S, hereby declare that the project work entitled, **"A PRELIMINARY INVESTIGATION ON EFFICACY OF TURMERIC 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 is entirely my own.

NAME: SHAMNA V S SIGNATURE

REG.NO : AB21ZOO040

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CONTENTS

ABSTRACT

The use of plant extracts as larvicidal agents is one of the most important approaches of biological mosquito control. The purpose of this study was to assess the effectiveness of Turmeric (Curcuma longa), a well-known culinary spice, in limiting the proliferation of mosquito larvae. Fresh turmeric rhizomes were collected , and crude aqueous extracts were made. Four different extract concentrations were added to the mosquito growing medium. Mosquito larvae exposed to the extract-containing media were examined for behavioral changes and mortality. The results revealed that at the highest concentration of 20%, all larvae died.

INTRODUCTION

Some of the dangerous vectors of deadly pathogens and parasites are arthropods. Theycause serious diseases to humans and animals (Mehlhorn, 2008; Mehlhorn et al., 2012). Among the arthropods, mosquitoes (Diptera: Culicidae) are a major threat to millions of people worldwide, as they are causative agents of diseases like malaria, yellow fever, dengue, West Nile, chikungunya, and filariasis. Major genera of mosquitoes that act as vector for various diseases are Culex (Japanese encephalitis, west Nile, chikungunya, Anopheles (filariasis, malaria) and Aedes (chikungunya, dengue, Yellow fever) (Ghosh,2012). Annually, worldwide 200 million-450 million infections are caused by the Anopheles mosquito that leads towards 2.7 million deaths. In more than 100 rising countries it remains endemic disease(Koech, 2014).In this scenario, vector control is a crucial prevention tool. Typically, microbial control agents, insect growth regulators, and organophosphates are used to manage mosquito larvae. In tropical nations, insecticide-treated bed nets and indoor residual spraying are additional methods used to stop mosquito-borne disease transmission (Lees et al. 2014). However, these substances cause harm to the environment and human health, as well as cause resistance in a variety of vector species (Benelli, 2015). Therefore , eco-friendly tools or natural repellants/extracts have been implemented to enhance control of mosquito vectors.

Utilizing living things to reduce pest numbers and lessen their potential harm is known as biological control.Active poisonous compounds derived from plant extracts have been used as a substitute method of controlling mosquitoes since ancient times. These are safe, readily accessible, reasonably priced, biodegradable, and have broad-spectrum, target-specific activity against many vector mosquito species(Ghosh et al., 2012). Insecticides originating from plants are composed of botanical mixtures of chemical compounds that work together to affect physiological and behavioral processes(Ghosh et al.,2012). . The extracts of *Murraya koenigii, Coriandrum sativam, Ferula asafetida* and *Trigonella foenum graceum* were found to be effective and showed encouraging results against *Aedesaegypti*(Harve and Kammath, 2004) and Culex (Diptera: Culicidae) mosquito larvae(Desai, 2002).

Studies conducted by Murthy and Rani, (2009) showed that two plant extracts out of eight tested, showed toxicity against 3rd instar larvae of yellow fever causing mosquitoe (Aedes aegypti). Their study also proved that extracts of *Delonix regia*(Royal Poinciana) and *Limonia acidissima*(Wood apple)were most active and showed toxicity up to 100 %. In another study by Subramanian et al. (2012) ,the bio-efficacy of Aloe vera leaf extract was assessed against the first to fourth instars larvae of *Aedes aegypti*, under the laboratory conditions. Their investigation exhibited that *A. vera* extract could serve as a potential larvicidal agent. In the study by Kamaraj et al. (2010) the adulticidal, repellent, and larvicidal activity of crude hexane, ethyl acetate, and methanol extracts of eight plants were evaluated against the adult and early fourth instar larvae of two species of mosquito vectors of filariasis. The plant extracts had potential to be used as an ideal eco-friendly approach for the control of these mosquitoes.

Although several studies were conducted to evaluate the larvicidal effects of plant extracts, many locally available plants can be used against vectors of major pathogens.

Curcuma aromatica is a cosmetic herb in South Asia. One of the most important spices used worldwide, importance have been attributed to it, including the ability to repel some kinds of mosquitoes (Ali et al., 2015; Tisgratog et al., 2016). The turmeric plant was used extensively by old Ayurvedics as a natural antiseptic, disinfectant, anti-inflammatory, and painkiller. It was also frequently used to treat skin irritations, promote gut flora, and help with digestion. It has been used for the treatment of various diseases and disorders particularly for urticaria, skin allergy, viral hepatitis, inflammatory conditions of joints, sore throat and for wounds (Chattopadhyay et al., 2004). especiallyin eastern countries, is curcuma longa, or turmeric, which belongs to the Zingiberaceae family (Ravindran, 2007). The tropical perennial herb's subterranean stems yield this vivid yellow-to-orange powder. The main components found in this spice that have been identified are phenolic compounds and terpenoids (Li et al., 2011). Interesting pesticidal qualities of the plant against fungus and insects of agricultural.

In Kerala six mosquito-borne diseases viz., Malaria, Dengue, Chikungunya,Japanese Encephalitis, Wet Nile virus and Lymphatic Filariasis are reported. Hence there is an urgent need to emphasize vector control activities 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 were 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 alternarive measures such as biological control. In spite of the vast availability of many species of medicinal plants, sufficient published information on the application of plant based products in biological control of mosquitoes is not available.

Therefore in the present study, suitability of Turmeric in controlling mosquito larvae(larvicidal properties) will be investigated using different concentrations of crude extract.

REVIEW OF LITERATURE

A study done by Benelli et al. (2016) reviewed the non-insecticide based strategies that have been implemented or are currently being tested.

Another study done by Supavarn et al. (1974) examined the effect of plant extracts from 36 plant species, belonging to 35 genera of 17 families, on 4th instar larvae of *Aedes aegypti*.

Another study was done by Latha et al. (1998), in which Petroleum ether extracts of 41 indigenous plants abundant in different parts of Kerala were studied for the larvicidal activity against the urban mosq***uitoes***, Culex *quinquefasciatus* and the saline water mosquito, Culex siliens. The extracts of 18 plants showed larvicidal activity against the early fourth instar larvae of Cr. siliens. Two plant species Piper longum and Zingiber wighlianum were effective against the early fourth instar larvae o f Cx. *quinquefasciatus* and ex. siliens. Therefore. the plant species P. longllm and Z. wighlianum were identified as potential biopesticides.

In a study by Ghosh et al. (2012) , 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.

In a study done 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 dependant effect of the extracts on mosquito larvae with 5% extract concentration causing 100% mortality within 3days exposure.

In a study done 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 a study by Janelle et al.(2020), *Utricularia macrorhiza* was experimentally evalitated and its larvicidal efficiency for mosquito vectors *Aedes aegypti* and *Aedes albopictus* was also evaluated. 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. The highest larvacidal efficacy was observed in plant cuttings taken from ponds in August.

Another study by Ram et al.(2015), the prey selectivity patterns of *Gambusia affinis* (mosquitofish) preying on larvae of the two Indian major carps (*Catla catla* and *Labeo rohita*) in the presence of varied proportions of alternative prey (rotifers, cladocerans, chironomid and mosquito larvae) were examined under laboratory conditions.

In a study 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.

A study by Kemabonta et al.(2018), evaluated the toxic effects of the essential oils, aqueous and methanolic extracts of Piper nigrum (black pepper) and Curcuma longa (turmeric) extracts on the larvae, pupae and adults of Anopheles gambiae. The insecticidal coil (100% concentration) of P. nigrum and C. longa, showed 76.7% and 70% knockdown action on the adult mosquitoes respectively. Therefore, P. nigrum and C. longa can serve as repellents against A. gambiae, and canbe used in integrated vector management control programs.

In a study conducted by sagnonu et al.(2012),The three curcuminoid components commonly isolated from Curcuma longa, curcumin (1), demethoxycurcumin (2), and bis-demethoxycurcumin (3) were separated and isolated from a commercially available turmeric extract product in high purity and sufficient amounts. Three more derivatives of curcumin, the di-O-demethylcurcumin (4), di-O-methylcurcumin (5) and the di-O-acetylcurcumin (6) were also synthesized and characterized. All six compounds were evaluated for their larvicidal effect against the mosquito Culex pipiens. Curcumin (1) exhibited highly potent larvicidal activity with LC50 value of 19.07 mg L^{-1}. Moreover, di-O-demethylcurcumin (4), was found to be equally active with LC50 value of 12.42 mg L^{-1}. Based on the LC90 values of the two compounds, di-O-demethylcurcumin (4) was the most active of all, resulting in an LC90 value of 29.40 mg L^{-1}, almost half of the LC90 value 61.63 mg L^{-1} found for compound 1. The rest of the compounds were inactive at concentrations even as high as 150 mg L^{-1} indicating a dependence of the larvicidal activity upon the substitution patent and the presence of aromatic hydroxyl and methoxy moieties. These results show for the first time the potential of this valuable natural product regarding its use as vector control agent.

Another study done by Liu et al.(2018)To identify larvicidal compounds from the ethanolic extracts of Curcuma longa root, the active compounds were isolated using activity-guided fractionation with column chromatography and identified based on nuclear magnetic resonance (NMR) and mass spectrometry (MS) data. The dipping method was used to determine the larvicidal activities of each compound against 4th-instar larvae of Culex pipiens pallens. Two compounds were isolated and identified, ar-turmerone and 8-hydroxyl-ar-turmerone. The two compounds exhibited larvicidal activities against the 4th-instar larvae of C. pipiens pallens after 24 hr of treatment with LC50 values of 138.86 and 257.68 ppm, respectively. The larvicidal activities of ar-turmerone and 8-hydroxyl-ar-turmerone against C. pipiens pallens are reported herein for the first time. The elucidation of the structure of these phytochemicals and their insecticidal activities are important for assessing the potential of this plant as a botanical insecticide.

In a study conducted by madhu et al.(2010) and aimed to evaluate the efficacy of extracts from rhizomes of Curcuma aromatica against the larvae of filariasis vector mosquitoes, Culex quinquefasciatus employing standard WHO procedure at Mysore. The soxhlet extraction was carried out using non-polar organic solvent, petroleum ether. The efficacy of petroleum ether extract seemed to be effective with LC50 and LC90 values of 11.42 and 18.00 ppm respectively. Bioassay-guided fractionation through flash chromatography lead to the isolation of two larvicidal compounds namely 9-oxoneoprocurcumenol and neoprocurcumenol. Between the two, 9-oxoneoprocurcumenol exerted significant toxicity ($P < 0.01$) on mosquito larvae with LC50 value of 5.81 ppm and LC90 being 9.99 ppm compared to neoprocurcumenol with 13.69 and 23.92 ppm of LC50 and LC90 values respectively. From the results, C. aromatica could be considered as one of the powerful candidate to bring about useful botanicals so as to prevent the resurgence of mosquito vectors.

A study done by chetri et al.(2022)the study foucus on the larvicidal activity of botanical products in the control of these disease vectors. Azadirachta indica (Neem) and Curcuma longa (Turmeric) are used for the in-silico analysis of their action as larvicides. The larvicidal activity of the test extracts are also studied through larval toxicity tests. A comparative analysis of the results is prepared to understand the effect of Neem and Turmeric in the biocontrol of the target species

METHODOLOGY

1. Collection of plant.

Freshly collected Turmeric(Curcuma longa) rhizomes were collected from a market in Broadway, Ernakulam. The samples were purchased during morning hours at 10.30 AM.

2. Preparation of plant 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.

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 extract s were used for evaluating the efficacy of extract. Experiments were run in duplicate for each concentaration along with a control. Into the first set, 20 % concentration of the extract was transferred. The remaining concentrations of prepared extracts were transfreed to the second, third and fourth sets.

To each petri dish containing the prepared extracts of different concentrations, five mosquito larvae were transferred from the outdoor culture using a dropper. Response of the larvae exposed to the extract were taken after 24 hours

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

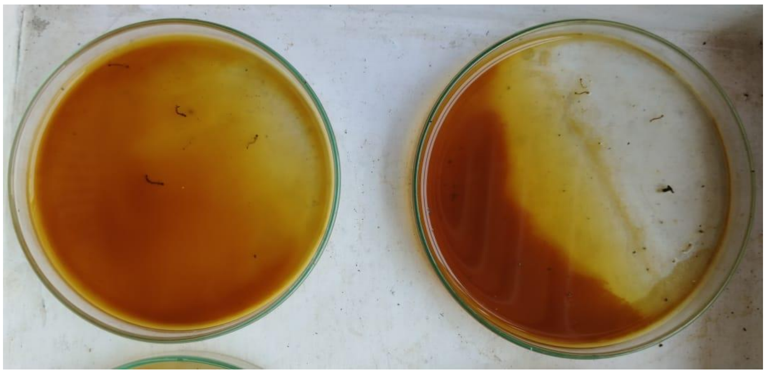
A. Turmeric rhizome B. Turmeric extract C. petridish D. beaker

RESULT

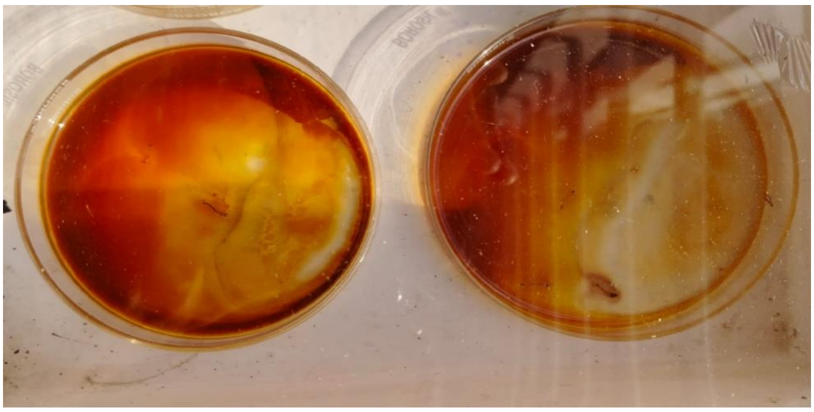
The observations showed that, at 20%, the all the larvae died in both 1st and 2nd plates. At 15%, four larvae died in 1st plate whereas three larvae died in the 2nd plate. At 10%, three larvae died in the 1st plate whereas one larvae died in the 2nd plate. At 5%, all the larvae survived in both 1st and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

Table 2. Mortality observations of mosquito larvae after 24 hours exposure to the extract

	MORTALITY OBSERVATIONS				
EXTRACT CONCENTRATION	**1ST PLATE**		**2ND PLATE**		**% mortality (average)**
	Number of live larvae	**Number of dead larvae**	**Number of live larvae**	**Number of dead larvae**	
20 %	5	5	5	5	100
15%	5	4	5	3	70
10%	5	3	5	1	40
5%	5	0	5	0	0



20% concentration



15 % concentration

10% concentration

5% concentration

DISCUSSION

Because of the negative impacts of antimicrobial drugs on the environment and health issues in humans, there is an increasing need to concentrate on the development of natural insecticides derived from medicinal plants as biocontrol agents.

Reducing mosquito populations is crucial to worldwide public health activities because mosquitoes carry a number of diseases. The goal of larvicides is to kill mosquito larvae in the breeding habitat before they can mature into adults and proliferate. It helps to reduce the quantity of mature mosquitoes in the immediate area.

This study showed that least dose that was required to cause death of mosquito. As a result, it assists in determining the dosage of turmeric extract needed to be administered as a biocontrol agent to kill mosquito larvae. The bulk of the larvae were found to have perished at turmeric extract concentrations of 20% and below. The reason for this is the impact of the biological elements found in the rhizome. The outcome that was seen could potentially be the effect of environmental factors including humidity, heat exposure, and small changes in concentration.

Approximately 50% of the world's population is at risk from mosquito-borne illnesses, with socioeconomically deprived populations being most affected. A number of factors have contributed to the rise and reappearance of diseases carried by mosquitoes, including changes in land use, urbanisation, globalisation, and climate change. In the previous fifty years, for example, the prevalence of dengue fever has increased over thirty times; since 2014, outbreaks of chikungunya, yellow fever, and malaria have all increased in size and frequency. Furthermore, during the 2015–2016 Zika virus (ZIKV) outbreak in Latin America and the Caribbean, hundreds of thousands of individuals became infected, which had a major detrimental macroeconomic impact. Supply-chain interruptions from the 2019 coronavirus illness pandemic are expected to increase the number of malaria- related deaths in sub-Saharan Africa in 2020–2021.

Safe and sustainable methods are desperately needed to lessen the threat of viruses carried by mosquitoes. The effectiveness of common mosquito control strategies, which involve chemical insecticides and environmental management, is only moderate. This is partly because of resistance resulting from behavioural changes and physiological changes, such as insecticide resistance, which causes mosquitoes to alter their blood-feeding schedules in response to bed netts. Unintentional impacts of chemical interventions also extend to non-target insects, such as pollinators.(Wang and others, 2021).

CONCLUSION

According to the current study, extract from turmeric may have a larvicidal impact on mosquito larvae..The use of plant extract in place of artificial pesticides has the potential to drastically alter society.

The study showed that all the larvae died at a maximum concentration of 20%.

It was also hypothesized that some elements, such as high temperatures, humidity, and concentration variations, may cause larval death. Another crucial element that determined the larvicidal property was the larvae's immunity.

It is affordable, easily accessible, non-toxic to the environment, and non-polluting.

Therefore, based on the data, we can say that turmeric works well as a larvicide.

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