Asian Journal of Innovative Research 1(3) 48-52 (2016)

ISSN 2455 7285

ASIAN JOURNAL OF INNOVATIVE RESEARCH
Available online at http://www.asianjir.com

Received 01 Sep. 2016;
Accepted 10 Oct. 2016
Online October 2016

P. Jegajeevanram,
Research Scholar,
Department of Chemistry, Khadir Mohideen College, Adirampattinam, Tamil Nadu, S. India

*****Corresponding author
Dr. N.M.I. Alhaji,
Associate Professor,
Department of Chemistry,
Khadir Mohideen College,
Adirampattinam,Tamil Nadu, S. India

Research Article **Chemistry**

LARVICIDAL ACTIVITY OF *Curcuma amada* RHIZOME AND *Tagetes erecta* FLOWER AGAINST *Anopheles stephensi*

P. Jegajeevanram[1] and N.M.I. Alhaji[1]*

ABSTRACT

Larvicidal efficacy of the crude extract of *Tagetes erecta* flower and *Mango ginger* rhizome with solvent of methanol was tested against the Larvae of *An. Stephensi*. The mortality rate of all larval instars of *An. Stephensi* tested against 0.5, 1.0, 1.5, 2.0 and 2.5% of each solvent at different intervals such as 12, 24, 48, 60 and 72hrs. LC50 value of *Tagetes erecta* flower and *Mango ginger* rhizome extracts were 4.66 and 4.70 mg/L respectively, after 24 h of exposure but the LC_{50} value of aqueous, methanol and methanolic extract of *Tagetes erecta* flower and *Mango ginger* rhizome extracts were 0.68 and 0.045mg/L respectively, after 72 h of exposure. Among the *Tagetes erecta* flower, *Mango ginger* rhizome extract having strong larvacital activity than aqueous and mehanloic extract. The maximum efficacy was noticed in 2.0 and 2.5% of *Mango ginger* rhizome extract at 60 and 72hrs. The LC_{50} values of *Mango ginger* rhizome extractagainst larvae of *An. stephensi*, were 0.045mg/dl. The potential larvacidal activity of *Tagetes erecta* flower and *Mango ginger*rhizome due to the phytochemicals such as favonoids, terpenoids etc. present in it.

Keywords: *Anopheles stephensi*, Larvicidal, *Tagetes erecta* flower, *Mango ginger*rhizome, Mosquitoes

***Citation*:** P. Jegajeevanram, N.M.I. Alhaji and S. Velavan (2016). Larvicidal activity of *Curcuma amada* rhizome and *Tagetes erecta* flower against *Anopheles stephensi*.. Asian Journal of Innovative Research 1(3): 48-52.

INTRODUCTION

Mosquitoes are among the most important insect pests affecting the health of people and animals. Biting female mosquitoes not only irritate people and animals, but they can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. Annoying populations of mosquitoes can occur anywhere in India because there are habitats favorable for mosquito species almost everywhere in the state. WHO has declared the mosquitoes as “public enemy number one”. Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population (WHO, 2009, 1996).

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale.

Considering these, the application of eco-friedly alternatives such as biological control of vectors has become the central focus of the control programmme. One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems (Ayurveda, Siddha and Unani).Medicinal plants have been investigated and reported to be useful in treatment of cardiovascular diseases, obesity, diabetes, cancer and other chronic diseases. The medicinal value of the chosen plant of *Curcuma amada* rhizome and *Tagetes erecta* flowerhas not been extensively worked out. Therefore, the present study was to investigate the larvicidal activity of *Curcuma amada* rhizome and *Tagetes erecta* flower against *Anopheles stephensi*

MATERIALS AND METHODS

Collection of Plant materials

During the month of February 2014, *Curcuma amada* rhizome and *Tagetes erecta* flowers were collected from various gardens in Keelavandanviduthy Village, Pudukkottai district, Tamil Nadu, India.

Authentication of plants

The plant was identified and carefully examined with the help of region floras. Specimens were further confirmed with reference to herbarium sheets available in the Rapinat Herbarium, St, Joseh's College, Tiruchirappalli, Tamil Nadu, India.

Preparation of extracts

The powdered rhizome and flowers material (20 g) was soaked in 50 ml of 70% Methanol for 12 hours and then filtered through a Whatmann filter paper along with 2 g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1 ml. The extract contains both polar and non-polar phytocomponents.

LARVICIDAL ACTIVITY

Sampling and culture of mosquito larvae

Anopheles stephensi mosquito larvae was collected from ICMR field station at Viruthashalam, Villupuram District, Tamil Nadu. After collecting the larvae, they were transferred to a sterilized plastic container and transported to Harman Research laboratory, Thanjavur. Among the larvae, 4^{th} instar stages were segregated and kept in plastic containers and fed with artificial food i.e. mixture of dog biscuits and dried yeast powder at the ratio of 3:1. All other larval stages and pupae found in the water samples were discarded properly. Colonies were kept free from exposure to pathogen, insecticides or repellents.

Preparation of extracts for experiment

Experiments were carried out with 0.5%, 1%, 1.5%, 2% and 2.5% concentrations of *Tagetes erecta* flowerand *Curcuma amada* rhizome extracts prepared using distilled water.

Larvicidal test

Method for testing larvicidal action of the crude extracts was slightly modified from those of World Health Organization (WHO, 1996). A stock solution was prepared dissolving a known amount of crude extract in water and stored in a refrigerator at $15^{\circ}C$. Twenty healthy late 4^{th} instar larvae were introduced into each testing cup (sterilized plastic cup of 150 ml capacity) containing 100 ml of dechlorinated tap water with stock solution. A measured volume of stock solution was added to obtain the desired concentrations. Experiments were carried out with a series of four concentrations viz. %, 0.2%, 0.3% and 0.4% in triplicates. Each batch of replicates contained one control of 100 ml of water alone and another of 100 ml of water containing a volume of solvent corresponding to the maximum volume of extract tested. As very few larvae succumbed within 12 hours of exposure to the test solutions, mortality was recorded after half hours of exposure, during which no feed was given to the larvae. The mortalities of mosquito larvae were recorded if moribund larvae were incapable of rising to the surface or moving when they were disturbed.

RESULTS

Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be safe. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy availability, economic and

less or no side effects but the disadvantage is that they are the victims of adulteration (Dineshkumar, 2007).

The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. The standardization of crude drugs is important before any work carried out. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The physicochemical test is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken (Tatiya *et al.*, 2012).

Larvicdal activity of *Curcuma amada rhizome* (Mango ginger) and *Tagetes erecta flower* (Marigold)

Data of larvicidal activity of methanolic crude extract of *Tagetes erecta* flower and *Mango ginger* rhizome against *An. stephensi* were presented in table 4.20 to 4.24. The mortality rate of all larval instars of *An. stephensi* at 2.5% concentration of *Mango ginger* rhizome was significantly higher ($P< 0.05$) than the mortality rates at 0.5%, 1%, 1.5%, and 2% concentrations of *Tagetes erecta* flower extract at 24, 48 and 72 h of exposure. Higher mortality rate was also recorded at 72 h bioassay than those at 24 and 48 h. No mortality rate was observed in 12 h of *Tagetes erecta* flower extract. The LC50 values and regression equation and regression coefficient values of *Tagetes erecta* flower extract against *An. stephensi* were presented in table 4.20

The mortality rate of all larval instars of *An. stephensi* at 2 and 2.5% concentration was significantly higher ($P< 0.05$) than the mortality rates at 0.5%, 1% and 1.5% concentrations of *Mango ginger* rhizome extract at 24, 48 and 72 h of exposure. Higher mortality rate was also recorded at 72 h bioassay than those at 24 and 48 h. The LC_{50} values and regression equation and regression coefficient values of *Mango ginger* rhizome extract of against *An. stephensi* were presented in table 4.24.

From table 4.22 revealed that LC_{50} values were gradually decreased from exposure period and there is positive relation between mortality(Y) and concentration(X)having regression coefficient value close to one in each case. LC_{50} value of aqueous, methanol and methanolic extract of *Tagetes erecta flower* and *Curcuma amada rhizome* extracts.were 4.66, and 4.83mg/L respectively, after 24 h of exposure but the LC_{50} value of aqueous extract of *Tagetes erecta flower* and *Curcuma amada rhizome* extracts.were 0.068 and 0.045 mg/L respectively, after 72 h of exposure.

Table : 4.20 Larvacidal activity of Aqueous extract of *Tagetes erecta* flower against larva of *An. stephensi*

Mango ginger rhizome	12 hrs	24 hrs	48 hrs	60 hrs	72 hrs
0.5%	1	1	4	7	7
1%	1	2	4	8	8
1.5%	2	6	7	9	9
2%	2	7	9	10	10
2.5%	3	8	10	10	10
Control	-	-	-	-	-

Values were expressed as death of larva (Out of 10)

Table: 4.21 % of larvacidal activity of Aqueous extract of *Tagetes erecta* flower against larva of *An. stephensi*

***Tagetes erecta* flower**(Aqueous)	12 hrs	24 hrs	48 hrs	60 hrs	72 hrs
0.5%	-	-	-	2	4
1%	-	-	2	4	6
1.5%	-	2	3	5	8
2%	-	2	4	6	10
2.5%	-	2	6	8	10
Control	-	-	-	-	-

Values were expressed as death of larva (Out of 10)

Table : 4.22 Larvacidal activity of methanolic extract of *Mango ginger* rhizome against larva of *An. stephensi*

***Tagetes erecta* flower**(Aqueous)	12 hrs	24 hrs	48 hrs	60 hrs	72 hrs
0.5%	-	-	-	20%	40%
1%	-	-	20%	40%	60%
1.5%	-	20%	30%	50%	80%
2%	-	20%	40%	60%	100%
2.5%	-	20%	60%	80%	100%
Control	-	-	-	-	-

Values were expressed as death of larva (Out of 10)

Table: 4.23 % of larvacidal activity of methanolic extract of and *Mango ginger* rhizome flower against larva of *An. Stephensi*

Mango ginger rhizome	12 hrs	24 hrs	48 hrs	60 hrs	72 hrs
0.5%	10%	10%	40%	70%	70%
1%	10%	20%	40%	80%	80%
1.5%	20%	60%	70%	90%	90%
2%	20%	70%	90%	100%	100%
2.5%	30%	80%	100%	100%	100%
Control	-	-	-	-	-

Values were expressed as death of larva (Out of 10)

Table 4.24: Log-probit analysis and regression analysis of larvicidal activity of methanolic crude extract of *Mango ginger* rhizome and *Tagetes erecta* flower against larva of *An. stephensi*

Extract Hours	LC50	Regression equation	R^2 Value
Tagetes erecta			
24	4.66	Y=12x -6	0.75
48	2.21	Y=28x-12	0.98
60	1.5	Y=28x+8	0.98
72	0.68	Y=32x+28	0.941
Mango ginger			
12	4.83	Y=12x-8	0.900
24	2.59	Y=22x-7	0.916
48	1.31	Y=32x+4	0.955
60	0.045	Y=22x+49	0.945
72	0.045	Y=22x+49	0.945

R= regression coefficient, LC= lethal concentration

DISCUSSION

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites (Sofowara, 1993).

Now a day's vector control becomes problematic due to resistance of mosquitoes to conventional synthetic insecticides. Therefore, it is necessary to look for and find a better insecticide or larvicide, which could provide a safer and long-lasting control against all mosquito species. Botanical insecticides provide an alternative to synthetic insecticides because they are generally considered safe, are biodegradable, and can often be obtained from local sources. In addition, the uses of medicinal plants for mosquito control is likely to generate local employment, reduce dependence enhance public health. Much research has been conducted on plant derived chemicals which are non-toxic to man and domestic animals and serve as useful basis for the development of safer and more selective mosquito insecticides (Sukumar *et al*, 1991).

One of the methods available for the control of mosquitoes is the use of larvicides, among other advantages, use of larvicides controls mosquitoes before they are able to spread and transmit diseases. While other methods like adult spraying may have direct effects like visible protection of populations and may show quick results, larval control has yielded several successes than adult mosquito control (Mathew *et al.*, 2009).

The present investigation revealed that the *Tagetes erecta* flower and *Mango ginger* rhizome extract possess larvicidal activity against *An. stephensi*. Among the *Tagetes erecta* flower and *Mango ginger* rhizome extracts, *Mango ginger* rhizome extractshowed the best result against the mosquito species followed by *Tagetes erecta* flower. The larvacidal activity of methanolic extract of *Mango ginger* rhizome is around 100% at 72 hrs as compared to other extract.

The larvacidal activity of the tested extracts of bark at 48 hr based on LC_{50} values are arranged in a decreasing order as follows: *Mango ginger* rhizome (LC_{50} 1.31 mg/L) >*Tagetes erecta* flower (LC_{50} 2.21 mg/L). The larvacidal activity of the tested extracts of bark at 60 hr based on LC50 values are arranged in a decreasing order as follows: *Mango ginger* rhizome (LC_{50} 0.045 mg/L) >*Tagetes erecta* flower (LC_{50} 1.5 mg/L). The larvacidal activity of the tested extracts of bark at 72 hr based on LC_{50} values are arranged in a decreasing order as follows: *Mango ginger* rhizome (LC_{50} 0.045 mg/L) >*Tagetes erecta* flower (LC_{50} 0.68 mg/L). Prabhu *et al* (2011) studied on the repellent activity of *Moringa olifera* against *An. stephensi* where he recorded the 90.41% repellency at 100% concentration and 23.28% repellency was reduced after the treatment of 20% concentration.

Govindarajan *et al* (2011) from Tamil Nadu (India), reported the repellent activity of *Eratamia coronaria* (*E. coronaria*) and *Caesalpinia pulcherrima* (*C.Pulcherrima*) leaf extract against *Culex quinquefasciatus*(*Cx. quinquefasciatus*), *Aedes aegypti*(*Ae. aegypti*) and *Anopheles stephensi*(*An. stephensi*), the eggs of which lost 100% hatchability due to *E. coronaria*, at concentrations of 250, 200 and 150 ppm, respectively, while *C.Pulcherrima* exerted zero hatchability (100% mortality) aat 375, 300 and225 ppm, respectively against the mosquitoes. *E. coronaria* which was found to have greater repellency than *C.Pulcherrima*, provided 100% protection for 150, 180 and 210 min at 5 mg/cm^2

Previously reported that *Tagetes erecta* flower and *Mango ginger* rhizome having active constituents include tannins, triterpenoid saponins, flavonoids and phytosterols (Bone, 1996; Kapoor, 1990). The potential larvacidal activity of *Tagetes erecta* flower and *Mango*

ginger rhizome due to the presence of this phytochemicals.

Larvicidal efficacy of the crude extract of *Tagetes erecta* flower and *Mango ginger* rhizome with solvent of methanol was tested against the Larvae of *An. Stephensi.* The mortality rate of all larval instars of *An. Stephensi* tested against 0.5, 1.0, 1.5, 2.0 and 2.5% of each solvent at different intervals such as 12, 24, 48, 60 and 72hrs. LC50 value of *Tagetes erecta* flower and *Mango ginger* rhizome extracts were 4.66 and 4.70 mg/L respectively, after 24 h of exposure but the LC_{50} value of aqueous, methanol and methanolic extract of *Tagetes erecta* flower and *Mango ginger* rhizome extracts were 0.68 and 0.045mg/L respectively, after 72 h of exposure. Among the *Tagetes erecta* flower, *Mango ginger* rhizome extract having strong larvacital activity than aqueous and mehanloic extract. Themaximum efficacy was noticed in 2.0 and 2.5% of *Mango ginger* rhizome extract at 60 and 72hrs. The LC_{50} values of *Mango ginger* rhizome extractagainst larvae of *An. stephensi*, were 0.045mg/dl. The potential larvacidal activity of *Tagetes erecta* flower and *Mango ginger*rhizome due to the phytochemicals such as favonoids, terpenoids etc. present in it. These new findings may helpful to be applied in integrated control strategies to gain maximum impact on vector control.Synergestic approaches such as application of mosquito predators with botanica blends and microbial insecticide will provide a better effect in reducing the vector population and the magnitude of epidemiology.

REFERENCES

WHO-World Health Organization. (2009) Malaria Treatment Guidelines Accessed January 9, at http://www.who.int/malaria/docs/TreatmentGuidelines2006.pdf.

WHO -World Health Organization. (1996) Report of the WHO informal consultation on the evaluation on the testing of insecticides, CTD/WHO PES/IC/96.1. Geneva: WHO, 69.

Dineshkumar C. (2007) Pharmacognosy can helpminimize accidental misuse of herbal medicine. *Curr Sci*, 3:1356-1358.

Tatiya A, Surana S, Bhavsar S, Patil D and Patil Y.(2012) Pharmacognostic and preliminary phytochemical investigation of Eulophia herbacea Lindl. Tubers (Orchidaceae). *Asian Pac J Trop Disease*, 2(1):50-55.

Sofowara A (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, *Ibadan, Nigeria*. 289.

Sukumar K, Perich MJ, Boobar LR. (1991) Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc*, **7**: 210-37.

Mathew N, Anitha MG, Bala TS, Sivakumar SM, Narmadha R and Kalyansundaram M. (2009) Larvicidal activity of Saraca indica, Nyctcmthes arbor-tristis, and Clitoria ternatea extracts against three mosquito vector species. *Parasitol Res*, 104: 1017-1025.

Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. (2011) Ovicidal and repellent activities of botanical extracts against Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi (Diptera: Culicidae). *Asian Pac J Trop Biomed*, 1: 43-48.

Bone K. (1996) *Clinical Applications of Ayurvedic and Chinese Herbs*. Warwick, Queensland, Australia. *Phytotherapy Press*, 131-133.

Kapoor LD. (1990) *Handbook of Ayurvedic Medicinal Plants*. Boca Raton, FL. CRC Press, 319-320.

Source of support: Nil;
Conflict of interest: None declared