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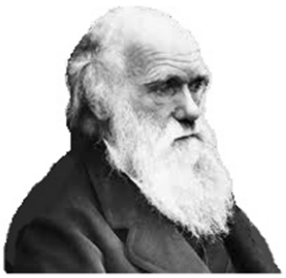

World News of Natural Sciences

An International Scientific Journal

WNOFNS 30(2) (2020) 169-177 EISSN 2543-5426

Evaluation of botanicals against: mosquito larvae to the extracts *Acalypha indica* L.

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ABSTRACT

Acalypha indica is the important medicinal herb found commonly on the waste land and roadsides throughout India. Their various uses in traditional medicine are for curative properties against inflammation, rheumatism, respiratory disorders, fever, bacterial infection and jaundice. The mosquito control potential of common indigenous plant, *Acalypha indica*. Mosquito species used for toxicity bioassay are *Culex quinquefasciatus, Anopheles gambiae* and *Armigeres subalbatus. Acalypha indica* activities of the plant extracts vary according to the species. The mortality rate of 4th stage larva at 5% concentration was significantly higher than the mortality, rates at 1%, 2%, 3% and 4% concentrations of crude plant extract at 12 hours and 24 h exposure. A higher mortality rate was observed at 24 h bioassay than at 12 h. Since these plants are available very easily and cheaply, we can use these extracts in the field.

***Keywords*:** *Culex quinquefasciatus, Anopheles gambiae, Armigeres subalbatus, Acalypha indica*

1. INTRODUCTION

Mosquitoes are the most important group of insects, adversely influencing the health status of dreadful diseases affecting millions of people every year around the world [(1,2)]. They

(Received 10 March 2020; Accepted 01 April 2020; Date of Publication 02 April 2020)

do not transmit parasites and pathogens, but they are also source of allergic reaction that includes local skin and systemic sensitivity [3]. There are currently more than 300 mosquito species in the world grouped in 39 genera and 135 subgenera. Several mosquito species belonging to the genera *Anopheles*, *Culex* and *Aedes* are acting as vectors for many pathogenic organisms causing diseases like Malaria, Filariasis, and Japanese Encephalitis, Dengue fever, Yellow fever, etc.

Two million people, primarily in tropical countries, are being at the risk of dengue [4]. It is considered as the most rapidly spreading mosquito-borne disease with 30 fold rise in global occurrence since the past 5 decades. To prevent mosquito-borne diseases and reduce fatality, it is necessary to stumble on a method to control the mosquito population. *A. aegypti* is generally known as a vector for an arbovirus responsible for dengue and chikungunya, which is endemic to South Asia, the pacific island area, Africa, and America. Mosquito is also a vector of yellow fever in Central and South America and West Africa. Dengue fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. In 1990, almost 30% of the world population, 1.5 billion people, lived in regions wherever the estimated risk of dengue transmission was greater than 50% [5]. In terms of dengue, 2.5 billion people live at risk of infection with one or more of the four serotypes of the virus, which cause an estimated 390 million infections per year [6] and the affected area has increased rapidly in the past 30 years [7].

The incidence of dengue infections, estimate by World Health Organization (WHO), is about 390 million annually of which 96 million are supposed to be manifested clinically [8]. In India, official records of the Union Health Ministry reveal a massive increase in dengue infections every year [9]. Mosquito control represents an important strategy for prevention of disease transmission and epidemic outbreaks. In the present time, use over of chemical insecticides leads mosquitoes to develop the resistance towards chemical insecticides.

Due to environmental concern on the use of synthetic insecticides for vector control and due to existing and further risk of development of widespread insecticide resistance use of environment friendly natural products, such as extracts of plant/plant parts has increased for vector control [10], listed 346 species from 276 genera and 99 families which have been tested against mosquitoes for various effects, such as toxicity, growth inhibition, oviposition deterrence and repellency.

Botanicals are considered as a safe alternative to synthetic pesticides since they are biodegradable and safe for environment, causing low toxicity to humans and non-target organisms [11]. More than 2,000 plants species have already been known to possess chemical factors and metabolites of significance in pest control programs, whilst products of approximate 344 species have been reported to encompass diverse activities against mosquito [12]. Though several plants from different families have been reported for mosquitocidal activity, only a very few botanicals have moved from laboratory to the field use, like neem based insecticides, which may be due to the light and heat instability of phytochemicals compared to synthetic insecticides [13]. A number of such plant products have been used for insect control since primordial time.

They are raditionally used for the treatment of paralysis, swellings, intermittent fevers, asthma, catarrh, anorexia, helminthic infections, inflammations, fever, intestinal worms, cough, bronchitis and dyspepsia [14]. In ancient ayurvedic medicine the plant was also known as "Sweta Arka". It does not require cultivation practices. It is one of the few plants, not consumed by grazing animals. They show antimicrobial activity, insecticidal activity, CNS activity and pregnancy interceptive properties [15-17]. The present study assesses the mosquito control potential of the common indigenous plant, *Acalypha indica*. *Acalypha indica* is the important

medicinal herb found commonly on the waste land and roadsides throughout India. Their various uses in traditional medicine are for curative properties against inflammation, rheumatism, respiratory disorders, fever, bacterial infection and jaundice. The plant is reported to possess vantioxidant, anti-implantation, anti-inflammatory, antidiabetic, central analgesic, antitumour, larvicidal and adult emergence inhibition, antinociceptive and antiasthmatic activity.

Classification of *Acalypha indica*

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Euphorbiales
Family	:	Euphobiaceae
Genus	:	*Acalypha*
Species	:	*indica*

Ecology and Geographical

Distribution

Acalypha indica occurs widely throughout the tropics of the Old World. In Africa it occurs in Nigeria in West Africa and further widely throughout tropical Africa and the Indian Ocean islands. It also occurs in India, South East Asia, and Oceania. It has been introduced to areas of the new world with favorable climates. *A. indica* is an erect, simple or branched, slightly hairy annual herb growing to a height of 40-80 cm. It is commonly known as Muktajhuri, Swetbasanta (Bengali) and Indian acalypha (English).

Medicinal uses

A. indica juice of the leaves and various parts of the plant is used to treat for headaches, stomach infections, muscular pain, intestinal parasites and constipation, wounds and itching, to get relief from piles and bed sores.

2. MATERIALS AND METHODS

Plant material

Plants material was collected from nearby Nagaland India in the month of December, 2019. All plants were authentically identified by the Department of Botany, St. Joseph University Nagaland. Voucher specimens were maintained in our laboratory for further references, *Acalypha indica*.

Processing of plant sample

Mature and healthy leaves of the all the plant were taken. Then leaf materials were ground using mechanical grinder. Dissolved in water this was filtered under Whattman filter paper. These extracts were concentrated and stored for further use.

Mosquito species

Mosquito species used for toxicity bioassay are *Culex quinquefasciatus*, *Anopheles gambiae* and *Armigeres subalbatus.*

Culex quinquefasciatus

Culex quinquefasciatus genus of mosquito is important in that several species serve as a vector of important diseases, such as West Nile virus, Filariasis, Japanese encephalitis, St. Louis encephalitis and avian malaria. The adult mosquito can measure from 4-10 millimeter.

Anopheles gambiae

Anopheles gambiae commonly transmits parasites of the genus Plasmodium, which cause malaria in human in endemic areas. Some species of Anopheles also can serve as the vectors for canine heart worm *Dirofilaria immitis*, the filarisasis – causing species *Wuchereria bancrofti* and *Braugia malayi*. This mosquito is seen during rainy seasons.

Armigeres subalbatus

Armigeres subalbatus are large mosquitoes and produce an immediate allergy reaction in man when it bites. These are large mosquito and size reaches up to 1 cm. The larvae are found in cold climate conditions. They are more active at the time of morning 5 am to 7 am and similarly in the evening 5 pm to 7 pm. They have other hosts like cattle, sheep and other small mammals.

Mosquito larvae

Mosquito larvae have a well-developed head with mouth parts used for feeding, a large thorax and nine segmented abdomens. They have no legs, Except Anopheles all other possess respiratory siphon. It respires through the spiracles. The larvae spend most of their feeding on algae, bacteria, and other microorganisms in the surface micro-layer. They dive below the surface only when they are disturbed. The larvae occur in a wide range of fresh water habitats.

Mortality studies

The bioassay experiments were conducted according to the standard WHO procedure with a slight modification. A total of three trials were carried out against the mosquito larvae for susceptibility test. Larvicidal assays of the extracts were conducted separately using the fourth in star mosquito larvae. Stock solution of the extracts was prepared by dissolving 1 mg of extract in 10 mL of distilled water, for aqueous. And the volume was raised to 100 mL with distilled water. From this, different dilutions of 1%, 2%, 3%, 4%, and 5% concentration were prepared and 10 fourth in star larvae were released in it and mortality was scored after 12 and 24 hours.

Statistical analysis

The statistical tools that were used in this study are the following. The arithmetic mean to get the average number of deaths of mosquito larvae. Analysis of variance (ANOVA), to determine the significant difference on the mortality of mosquito larvae between the control

and the experimental groups and probit analysis to calculate LC_{50} and LC_{90} values to determine lethal concentrations of the plant extracts on mosquito larvae after 12 and 24 hours of treatment.

3. RESULT AND DISCUSSION

Table 1. Toxicity bioassay for larval *Culex quinquefasciatus* against *Acalypha indica*

Timings	Concentration	Mean ± SD	Percentage (%)	F value	LC_{50}	LC_{90}	R value
12 hrs	Control	0.0000 ± 0.00	0	9.100	9.0	11.669	0.92
	1%	0.0000 ± 0.00	0				
	2%	0.7 ± 0.6	5				
	3%	.900 ± 0 .00	9				
	4%	1.8± 0.6	17				
	5%	1.9000± 0.00	21				
24 hrs	Control	0.0000± 0.00	0	17.100	8.1	12.1	1.0
	1%	0.6667± 0.57	7				
	2%	1.0000± 0.00	1				
	3%	1.7± 0 .6.1	17				
	4%	2.0000± 0.00	21				
	5%	2.7± 0.6	25				

Data of larvicidal activity of crude extract of leaves of *Culex quiquefasciatus* against 4th stage *Acalypha indica* at 12 of 24 hours are presented in **Table 1**. At 12 h no mortality was recorded at the lowest of 1% concentration.

The highest of 5% concentration induced 21% mortality and with the LC_{50} of 9.0 mL/L and 11.669 mL/L, respectively. At 24 h the lowest of 1% concentration attained 7% mortality and the highest dose of 5% scored 25% mortality with the LC_{90} 0f 8.1 mL/L and 12.1 mL/L, respectively.

Table 2. Toxicity bioassay for larval *Anopheles gambiae* against *Acalypha indica*

Timings	Concentration	Mean±SD	Percentage %	F value	LC_{50}	LC_{90}	R
12 hrs	Control	0 .00±0.00	0	15.600	8.070	11.289	0.873
	1%	0 .00±0.00	0				
	2%	0.33±0.57	3				
	3%	0.66 ±0.57	6				
	4%	1.00±0.00	10				
	5%	2.00±0.00	20				
24 hrs	Control	0.00±0.00	0	11.800	7.704	11.024	0.869
	1%	0.00±0.00	6				
	2%	0.66±0.57	6				
	3%	0.66±0.57	6				
	4%	1.66±0.57	16				
	5%	2.33±0.57	26				

Data of larvicidal activity of crude extract of leaves of *Acalypha indica* against 4th stage *Anopheles gambiae* at 12 and 24 hours are presented in **Table 2**. At 12 h no mortality was recorded at the lowest of 1% concentration.

The highest of 5% concentration induced 20% mortality with the LC_{50} of 8.070 mL/L and 11.289 mL/L, respectively. At 24 hours, the lowest of 1% concentration attained 6% mortality and the highest dose of 5% scored 26% mortality with the LC_{90} of 7.704 mL/L and 11.024 mL/L, respectively.

Table 3 shows the bioassay efficacy of *Acalypha indica* against 4th stage *Armigeres subalbatus*. At 12 hours exposure, the lowest of 1% attained zero percent mortality and the highest of 5% having 16% with LC_{50} of 9.816 mL/L and 15.734 mL/L. At 24 hours, no mortality was gained at the lowest concentration and thehighest concentration of 5% attained 20% mortality with the LC_{90} of 8.727 mL/L and 12.895 mL/L, respectively.

Table 3. Toxicity bioassay for larval *Armigeres subalbatus* species *Acalypha indica*

Timings	Concentration	Mean±SD	Percentage %	F value	LC_{50}	LC_{90}	R
12 hrs	Control	.0000±.00000	0	8.533	9.816	15.734	0.755
	1%	.0000±.00000	0				
	2%	.6667±.57735	6				
	3%	1.0000±.00000	10				
	4%	1.3333±.57735	13				
	5%	1.6667±.57735	16				
24 hrs	Control	.0000±.00000	0	12.000	8.727	12.895	0.878
	1%	.6667±.57735	0				
	2%	1.0000±.00000	10				
	3%	1.0000±.00000	10				
	4%	1.3333±.57735	13				
	5%	2.0000±.00000	20				

A variety of plant species have been identified to have medicinal properties from ancient times. Some of these medicinal plants have been discovered to have insecticidal properties also. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in the natural product-based mosquito abatement practices because they provide an alternative to synthetic insecticides as they are considered safe, biodegradable, and can often be obtained from local sources. Our present study correlated with many studies [18-21].

4. CONCLOSIONS

In the present study, results of the *Acalypha indica* plant extract against all mosquito larvae, reported in the present study, exhibit the presence of *Acalypha indica* properties.

Acalypha indica activities of the plant extracts vary according to the species. The mortality rate of 4th stage larva at 5% concentration was significantly higher than the mortality, rates at 1%, 2%, 3%, and 4% concentrations of crude plant extract at 12 hours and 24 hours exposure. Higher mortality rate was observed at 24 hours bioassay than 12 h. Since these plants are available very easily and cheaply, we can use these extracts in the field. The mosquito *Acalypha indica* bioassay finding of the present study clearly demonstrated that the percentage of mortality directly proportional to concentration and the mortality rate was highly dose and time dependent activities against mosquito larvae. Since the percentage mortality was observed to increase with the increase of percentage mortality of the treated mosquito larvae, it is supported by the presence of phytochemicals in the plant extracts which have pesticidal activities.

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