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Bioefficacy of *Cassia fistula* Linn. (Leguminosae) leaf extract against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae)

M. GOVINDARAJAN

Division of Vector Biology, Department of Zoology, Annamalai University, Annamalai Nagar, Tamilnadu (India)

Abstract. – The leaf extract of *Cassia fistula* with different solvens viz, methanol, benzene and acetone were studied for the larvicidal, ovicidal and repellent activity against *Aedes aegypti*. The extract exhibited dose dependent activity and produced significant mortality. The 24 h LC_{50} concentration of the extract against *Aedes aegypti* were observed at 10.69, 18.27 and 23.95 mg/l respectively. Mean percent hatchability of the ovicidal activity was observed 120.00 h after treatment. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. The crude extract of *Cassia fistula* shows significant repellency against *Aedes aegypti*. These results clearly reveal that the crude extract of *Cassia fistula* served as a potential larvicidal, ovicidal and repellent agent against chikungunya vector mosquito, *Aedes aegypti*.

Key Words:

Cassia fistula, Aedes aegypti, Larvicidal activity, Ovicidal activity, Repellent activity.

Introduction

Mosquito spread various vector-borne diseases such as malaria, filariasis, Japanese encephalitis and dengue fever, which are transmitted by the three genera of mosquitoes namely *Anopheles, Culex and Aedes*. 40 million people in India suffer from mosquito borne diseases annually. There are over 3000 mosquito species belonging to 34 genera in the world. Of these, only about 300 transmit human and animal diseases. These diseases devastate Indian economy every year[1]. Dengue, dengue haemorrhagic fever and chikungunya are transmitted by *Aedes aegypti*. The symptoms of the disease are severe pain in the joints and muscles, skin eruptions. However, dengue fever is rarely fatal. The species breed, profusely in rainwater storage containers like cisterns, barrels, pots, etc. Dengue outbreaks are often associated with urban areas due to irregular potable water supply. Dengue fever continues in recurrent epidemic afflicting millions and causing thousands of deaths annually which is transmitted by *Aedes aegypti*. Mosquito are a serious threat to public health through which several dangerous diseases are transmitted in both animals and human beings[2].

Nowadays mosquito coils containing synthetic pyrethroids and other organophosphorus compounds cause so many side effects, such as breathing problem, eye irritation, headache, asthma, itching and sneezing to the users[3]. In addition pests were becoming resistant to chemical treatments. Indoor residual spraying of insecticides stains the walls and leave a long lasting unpleasant odour. These problem have highlighted were need for the development of new strategies for selective mosquito control.

Cassia fistula Linn. (Golden Shower Tree) is a flowering plant in the family Fabaceae, native to southern Asia, from southern Pakistan east through India to Myanmar and south to Sri Lanka. It is a medium-sized tree growing to 10-20 m tall with fast growth. The leaves are deciduous or semi-evergreen, 15-60 cm long, pinnate with 3-8 pairs of leaflets, each leaflet 7-21 cm long and 4-9 cm broad. The flowers are produced in pendulous racemes 20-40 cm long, each flower 4-7 cm diameter with five yellow petals of equal size and shape. The fruit is a legume is 30-60 cm long and 1.5-2.5 cm broad, with a pungent odour and containing several seeds[4].

Corresponding Author: M. Govindarajan, MD; e-mail: drgovind1979@rediffmail.com

Phytochemicals are advantageous due to their eco-safety, target-specificity, and no development of resistance, reduced number of applications, higher acceptability and suitability for rural areas. Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programmes. The plant product of phytochemical, which is used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites. Phytochemicals obtained from the whole plant or specific part of the plant by the extraction with different types of solvent such as aqueous, methanol, chloroform, benzene and acetone, etc., depending on the polarity of the phytochemical. Some phytochemicals act as toxicant (insecticide/larvicide) both against adult as well as larval stages of mosquitoes. While other interfere with growth and growth inhibitor or with reproduction or produce an olfactory stimuli thus acting as repellent or attractant[5,6].

The present investigation was undertaken to study the efficacy of *Cassia fistula* leaf extracts against larvicidal, ovicidal and repellent activities of chikungunya vector, *Aedes aegypti* in the laboratory.

Materials and Methods

Plant Collection

Cassia fistula Linn. (Leguminosae) was collected from Vittaloor, Kumbakonam taluk, Thanjavur District, Tamilnadu, India and authenticated by botanist Dr. V.Venkatesalu, Reader in Botany, Annamalai University, Annamalainagar, Tamil Nadu, India. A herbarium specimen was deposited in the Department of Zoology, Annamalai University, Annamalainagar, Tamil Nadu, India.

Preparation of Plant Extract

The plant leaves were washed with tap water, shade dried at room temperature and powdered by electrical blender (Preethi, India/model-2008). The powder (1.0 kg) was then subjected to extraction in various solvents viz, methanol, benzene and acetone (3.0 l) using Soxhlet apparatus (Hitech, India) (boiling point range 60^0-80^0) for 8 h. The extract was filtered through a Buchner funnel (Borosil, Mumbai, India) with Whatman number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator (Superfit, India/model-PBU-6D). The residue was then made in to a 1% stock solution with acetone. The stock solution for various test concentrations were prepared, which was used for the bioassays.

Test Organisms

The chikungunya vector mosquito, *Aedes aegypti* was reared in the laboratory. The larvae were fed on dog biscuits and yeast power in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for fed with blood meal. Mosquitoes were held at 28 ± 2^0, 70-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

Larvicidal Activity

The Larvicidal activity of the leaf extract of *Cassia fistula* was evaluated as per the method recommended by WHO[7]. Different concentrations of the test samples were used. A 500 ml glass beaker containing 250 ml of tap water. Early third instar of *A. aegypti*[25] were introduced to each of the test solution as well as control. For each experiment six replicates were maintained at a time. The LC_{50} value was calculated after 24 h by probit analysis[8].

Ovicidal Activity

For ovicidal activity the method of Su and Mulla[9] was performed. Preliminary tests indicated that ovicidal activity of *Cassia fistula* leaf extract was influenced by the eggs and concentration of extract. To study this relationship, 100 gravids were placed in a screen cage, where 9 oviposition cups were introduced for oviposition 30 min before the start of the dusk period. Of these 9 cups, 8 were each filled with test solution of 20, 40, 60, 80, 100, 120, 140 and 160 mg/l and one was filled with 100 ml of solvent containing water that served as a control. The eggs were laid (within 4 h, most of the eggs were laid) in the different concentration of leaf extract were collected immediately after oviposist and used in eggs age test after 12-18 h interval after oviposition respectively. Eggs were selected at random and individually transferred to the different concentration of extract for 3 h. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. The

test was replicated six times. The hatch rate was assessed 120 h post treatment by the following formula:

$$\frac{\text{Number of hatched larvae}}{\text{Total no. of eggs}} \times 100$$

Repellent Activity

The minutes of protection in relation to dose method was used[10]. Three day old blood starved female *Aedes aegypti* mosquitoes (100) were kept in net cages (45 cm × 30 cm × 45 cm). The arms of the test person were cleaned with distilled water. After air drying the arm only 25 cm^2 of the dorsal side of the skin in each arm was exposed, the remaining were being covered by rubber gloves. The crude extract was dissolved in ethanol and ethanol served as a control. The concentration of crude extract at 1.0, 2.5 and 5.0 mg/cm^2 was applied. The control and treated arms were introduced simultaneously into the same cage. The time of the test dependent on whether the target mosquitoes day-or night biters. *Aedes aegypti* was tested during the day time from 07.00 to 17.00h. The number of bites was counted over 3 minutes in every 30 minutes intervals. It is make out 30 minutes protection. The experiment was conducted six times. All the experiments were conducted at a temperature of 28 ± 2°C and relative humidity of 80 ± 2%. The percentage of protection was calculated by using the following formula.

$$\text{\% of protection} \frac{\text{Number of bites reccived by control area} - \text{Number of bites reccived by treatment area}}{\text{Number of bites reccived by control arca}} \times 100$$

Statistical Analysis

Statistical evaluation was done using Statistical Package of Social Sciences (SPSS) 13.0 for windows, significance level was set at $p < 0.05$.

Results

The toxicities of the crude extract from *Cassia fistula* to early third instar *Aedes aegypti* larvae was noted, and the LC_{50}, LC_{90}, 95% confidence limits of LCL and UCL and chi-square were also calculated (Table I). The methanol

Table I. Larvicidal activity of leaf extract of *Cassia fistula* against *Aedes aegypti.*

Solvent	Concentration (mg/l)	Larval mortality (%)	LC_{50} (mg/l)	95% confidence limit (mg/l)		LC_{90} (mg/l)	Chi-square
				LCL	*UCL*		
Methanol	25	98.00					
	20	85.33					
	15	71.00	10.69	8.45	12.81	20.47	9.761[a]
	10	47.00					
	05	31.33					
	Control	2.66					
Benzene	50	100.00					
	40	91.66					
	30	79.33	18.27	13.07	22.93	35.67	13.484[a]
	20	55.00					
	10	38.00					
	Control	2.66					
Acetone	50	96.00					
	40	78.66					
	30	54.66	23.95	15.80	31.83	47.13	22.376
	20	41.00					
	10	36.33					
	Control	2.66					

[a]Significant at $p < 0.05$.

Table II. Effect of *Cassia fistula* leaf extract on the hatchability of the eggs of *Aedes aegypti.*

PERCENTAGE OF EGG HATCHABILITY									
	Concentration of extract (mg/l)								
Solvent	*Control*	*20*	*40*	*60*	*80*	*100*	*120*	*140*	*160*
Methanol	100.0	86.3	72.4	59.6	31.3	14.6	NH	NH	NH
Benzene	100.0	91.6	86.3	67.6	41.4	25.3	16.2	NH	NH
Acetone	100.0	98.3	89.6	74.3	55.3	36.4	27.3	19.6	NH

NH – No hatchability (100% mortality).

extract was found to be more effective than the other extract against larvae of *Aedes aegypti* with LC_{50} value of 10.69 mg/l. The chi-square values were significant at $P<0.05$ level. In laboratory test, the oviposition cups treated with different concentration of *Cassia fistula* leaf extract in 100 ml of distilled water received different number of eggs at different concentrations. The solvent containing water served as a control received only small amount of eggs. The different age of eggs *Aedes aegypti* treated with different concentrations of leaf extract caused ovicidal resulting in failure to hatch the eggs (Table II). The crude extract of *Cassia fistula* shows significant repellency against *Aedes aegypti* (Table III). It shows that repellency depends on the strength of the crude extract concentration.

Discussion

Simple crude extracts from plants have been used as insecticides in many countries for centuries[11]. Crude plant extracts often consist of complex mixtures of active compounds. Advances of using complete mixture may act synergistically[12], they may show greater overall bioactivity compared to the individual constituents[13]. The mosquito larvicidal properties of the leaf extract of a herbaceous plant *Ocimum canum* against *Aedes aegypti*. The LC_{50} values for 2nd, 3rd and 4th larvae were 177.82, 229.08 and 331.13 ppm respectively[14]. The egg rafts aged for 0, 4, 8, 12 and 24 hr were exposed to 10 ppm neem suspensions for 36 hrs and the ovicidal activity was only attained in the egg rafts deposited directly (0 hr old) in neem suspensions, not in those with

Table III. Repellent activity of methanol leaf extract of *Cassia fistula* against vector mosquito *Aedes aegypti.*

Solvent	Concentration of leaf extract (mg/cm²)	Mean number of bites received in control	Mean number of bites received in treated	Mean number of hrs of 100% protection	Total % of protection for 10 hrs
Methanol	1.0	51.0 ± 1.0	25.3 ± 1.6	2.45	50.39
	2.5	50.0 ± 1.4	19.5 ± 1.2	4.30	61.00
	5.0	52.0 ± 1.8	16.0 ± 0.8	6.00	69.23
Benzene	1.0	50.0 ± 0.8	29.0 ± 1.2	2.15	42.00
	2.5	51.0 ± 1.8	23.0 ± 1.4	3.45	54.90
	5.0	49.0 ± 0.6	18.0 ± 1.2	5.00	63.26
Acetone	1.0	52.0 ± 1.6	36.0 ± 0.8	2.00	30.76
	2.5	51.0 ± 0.6	31.0 ± 1.6	3.00	39.21
	5.0	50.0 ± 1.8	26.0 ± 1.2	4.30	48.00

Values are mean of six replication ± SD.

ages of 4-24 hr. In this study, the exposure period also played a crucial role in causing toxicity[9]. Methanolic leaf extract of *Cassia fistula* was tested for larvicidal and ovicidal activity against *Culex quinquefasciatus* and *Anopheles stephensi*. The extract was found to be more lethal to the egg and larvae[15]. Repellent activity of *Ferronia elephantum* (Rutaceae) leaf extract against *Aedes aegypti*. The total percentage of protection of *Ferronia elephantum* was 45.8% of 1.0 mg/cm^2 and 59.0% at 2.5 mg/cm^2 for 10 h[16]. Isolation of repellent ingredients from *Lantana camera* (Verbenaceae) flowers and their repellency against *Aedes* mosquitoes. One application of this fraction gave 100% protection for 2 h and may protect 75.8% at 7 h[17]. The finding of the present investigation revealed that the leaf extract of *Cassia fistula* possess remarkable larvicidal, ovicidal and repellent activity against medically important vector mosquitoes. The extract might be used directly as larvicidal and ovicidal agent in small volume aquatic habitats or breeding sites of limited size around human dwellings and also possess good repellent activity. Studies to confirm this hypothesis in field condition are underway in our laboratory.

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