International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2017; 4(2): 142-147

Received: 20-01-2017
Accepted: 21-02-2017

A Rajesh
Department of Biotechnology, Thanthai Hans Roever College-Perambalur, Tamil Nadu, India

Dr. Mohamed Shamsudin
Dean & Head, Department of Zoology, Jamal Mohamed College- Tiruchirappalli, Tamil Nadu, India

Correspondence
A Rajesh
Department of Biotechnology, Thanthai Hans Roever College-Perambalur, Tamil Nadu, India

Evaluation of ovicidal and larvicidal potential of *Kalanchoe pinnata* leaf extracts against filarial mosquito vector, *Culex quinquefasciatus*

A Rajesh and Dr. Mohamed Shamsudin

Abstract
Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases. Vector borne diseases are major sources of illness and death worldwide. Mosquitoes are primary vectors for many dreadful and fatal diseases. *Culex quinquefasciatus* is the principal vector of lymphatic/bancroftian filariasis and also a potential vector of several arboviruses. To prevent proliferation of mosquito-borne diseases and to improve quality of the environment and public health, mosquito control is essential. Plants enriched with phytochemicals are reported to possess insecticidal properties particularly mosquitocidal. Botanical origin may have the potential to controlling mosquito successfully nowadays. Therefore, in the present investigation was undertaken to evaluate ovicidal and larvicidal activities of crude acetone, benzene, ethyl acetate, petroleum ether and aqueous extracts of *Kalanchoe pinnata* leaf were assayed for their toxicity against filarial vector mosquito, *Culex quinquefasciatus*. The rates of mortality were directly proportional to various concentrations ranging from 50-300 ppm for eggs and 100-500 ppm for fourth instar larvae of target vector mosquitoes under the laboratory conditions. The ovicidal and larvicidal activities were observed after 24 hours exposure. The mortality (eggs) was observed 100% at 250 ppm of the acetone extract, followed by benzene, ethyl acetate, petroleum ether and aqueous leaf extract exerting 100% mortality at the concentration of 300 ppm. The control eggs showed 100% hatchability. The results revealed that the acetone leaf extract of *Kalanchoe pinnata* showed the highest larvicidal (LC_{50} and LC_{90}) activity than the other extracts. The highest larvicidal effect observed in the leaf acetone extract of *Kalanchoe pinnata* against *Culex quinquefasciatus* with the LC_{50} and LC_{90} values 199.86 and 387.70ppm respectively. This is the first report on the mosquito ovicidal and larvicidal activities of the reported *Kalanchoe pinnata* leaf.

Keywords: Kalanchoe pinnata, Botanical insecticides, ovicidal, Larvicidal, Culex quinquefasciatus

1. Introduction
Arthropods are dangerous vectors of deadly pathogens and parasites, which may spread as epidemics or pandemics in the increasing world population of humans and animals [1]. The vector-borne diseases caused by mosquito are one of the major health problems in most of the countries. In particular, mosquitoes (Diptera: Culicidae) present an immense threat to millions of people worldwide, since they act as vectors for devastating pathogens and parasites, including malaria, filariasis, yellow fever, dengue, chikungunya and Zika virus disease [2,3]. *Culex* mosquito is probably the most abundant house mosquito in towns and cities of the tropical countries. *Culex* mosquitoes develop in standing water, such as polluted ponds, marshes, tanks, street gutters, tin cans, barrels, ornamental ponds, puddles, creeks, ditches, etc. [4]. The Southern house mosquito, *Culex quinquefasciatus* acts as an important "urban bridge vector" which bridges different reservoir/amplifier hosts to humans because of its encounter with different vertebrates. It also creates an ecological bridge between urban, periurban and rural areas owing to its presence and adaptability in diverse ecological niches. They emerged as a smart vector because of the adaptive fitness, ecological plasticity, invasive behavior, host specificity and high reproductive potential along with expanded immune gene repertoire property at the genetic level. This mosquito possesses the necessary potential to initiate and facilitate the disease transmission by establishing an effective vector-host transmission cycle for diverse pathogens in different environments [5].

A wide variety of sites, mostly characterized by colored, foul water with high nutrient values and low dissolved oxygen content, such as pumping and irrigation wells, canals, wastewater treatment, ponds, sewage overflows, rain pools, rice paddy fields, fish ponds, septic tanks, drains, cesspools, agricultural trenches, vegetable farms, etc. generally are preferred as the breeding sites by this mosquito [6,7]. *Culex quinquefasciatus* is the principal vector of bancroftian filariasis and a potential vector of *Dirofilaria immitis,* West Nile virus (WNV) and Rift Valley fever virus, avian pox and protozoa like *Plasmodium relictum* that causes bird malaria [5]**.** Additionally, it can transmit Japanese Encephalitis virus (JEV), St. Louis encephalitis virus (SLEV), Reticulo-endotheliosis virus, Murray Valley encephalitis and Reovirus Type 3 [8].

Lymphatic filariasis is endemic in 81 countries in tropical and subtropical regions of Asia, Africa, Central and South America and Pacific island nations, with more than 120 million people infected in 73 countries worldwide and continues to be a worsening problem, especially in Africa and the Indian subcontinent [9]. An estimated 25 million have genital disease and 15 million have lymph edema or elephantiasis caused by *Wucheraria bancrofti* or *Brugia malayi* [10]. Lymphatic filariasis is caused mainly by *Wucheraria bancrofti* and transmitted by *Culex quinquefasciatus* [11].Mosquito bites may also cause allergic responses including local skin reactions and systemic reactions such as urticaria and angioedema [12]. Evidently this cosmopolitan mosquito is a potential vector of many important pathogens causing to public health. Mosquitoes transmit serious human diseases, causing million of deaths every year.

To prevent proliferation of mosquito and control the diseases to improve quality of environment and public health is essential. In this scenario, vector control is a crucial prevention tool. The use of synthetic pesticides began in the 1930's and became widespread after World War II. Ever since, mosquito control has relied upon synthetic insecticides with very few different types being discovered in terms of mode of action and targeted receptors. These products have been a continuing source of concern due to the emergence of widespread resistance in targeted species, effects on non-target species, human health and issues associated with environmental persistence and bioaccumulation. The researchers look for alternative approaches to promoting the adoption of effective and transparent mosquito management strategies that focus on monitoring and surveillance, source reduction, eco-friendly and least-toxic larval control. These factors have resulted in an urge to look for environment safety, biodegradable and target specific insecticides against mosquito species [13]. India is a varietal emporium of endemic and exotic flora enriched with phytochemicals having pharmacognostical and toxicological properties. The toxicological properties of phytochemicals reflect the potential of plants as a source of insecticidal agents. Prospection for new larvicidal molecules based on rich plant biodiversity is appreciable as compounds of plant derivatives are safer to use and leaves no residue in the environment [14].

In the current era, research is focused on natural products to combat these disease transmitting vectors and a recent emphasis has been placed on plant material and various reports on the use of natural plant products against mosquito vectors have been documented [15]. Using natural products of plant origin (botanical derivatives) is an alternative and recent approach for mosquito control. Despite their toxicity to pests, they are readily degradable and usually lack toxicity to higher animals [16, 17].There is provocative interest in research for mosquitocidal compound from natural sources. Control of mosquitoes by using chemical insecticides for long time, repeated use and house hold spray to develop target resistance [18]. Therefore, in the present study, the crude leaf extracts of plant species, *Kalanchoe pinnata* belonging to Crassulaceae family were tested for their ovicidal and larvicidal efficacy against *Culex quinquefasciatus*. To date, there is no report about the *Kalanchoe pinnata* extract against the mosquitoes. We decided to investigate the potential use of this plant extract against *Cx. quinquefasciatus*, since this Plant is found naturally throughout the country. In the present paper, an attempt has been made to a step forward to observe rational impact of *Kalanchoe pinnata* leaf extracts on *Cx. quinquefasciatus* due to their ovicidal and larvicidal activity. To best of our knowledge, this is the first report on mosquito ovicidal and larvicidal activities of the plant *Kalanchoe pinnata*.

2. Materials and Methods

2.1 Plant collection and extraction

Mature fresh and healthy leaves of *Kalanchoe pinnata* from Perambalur district, Tamil Nadu, India, was brought to the laboratory. Taxonomical identity was confirmed by the voucher specimen (Herbarium) have been deposited in the ABS botanical garden in Karipatty, Salem District, and Tamil Nadu. Mr. A. Balasubramnian. (Consultant central siddha research)Executive Director ABS botanical garden, Salem, authenticated the plant as *Kalanchoe pinnata (LAM.)PERS.* (Family-Crassulaceae). The leaf washed with dechlorinated tap water, cut into small pieces and shade dried at room temperature. Dried leaf of *Kalanchoe pinnata was* powdered with the aid of an electric blender. The powdered leaf was individually extracted with different solvents *viz.,* acetone, benzene, petroleum ether, and ethyl acetate and aqueous by using soxhlet apparatus [19]. The crude leaf extracts were filtered through a Buchner funnel with whatman number one filter paper and were then evaporated to dryness in a rotary vacuum evaporator to obtain crude extracts of *Kalanchoe pinnata* leaf. One per cent stock solution from the crude extracts of plant was prepared by adding adequate volume of respective solvent and was refrigerated at 4ºC until for ovicidal and larvicidal bioassay.

2.2 Test mosquitoes

Culex egg raft collected from various places in Perambalur district, Tamil Nadu, India was transported to the laboratory where, the egg raft of mosquitoes were transferred to enamel larval trays until adult emergence. After emergence, the adult mosquitoes were identified up to species level and confirmed before rearing. The characteristic features are the absence of a pale band on the proboscis and the presence of basal pale bands on the terga [20]. Cyclic generations of *Culex quinquefasciatus* were maintained separately in mosquito cages in an insectary with a mean room temperature of 27 ± 2 °C and a relative humidity of 70-80%, photoperiod of 14:10 hours light: dark. The adult mosquitoes were fed on ten per cent glucose solution in water fora period of three days before

blood feeding. The adult female mosquitoes were allowed to feed on the blood meals by rabbit (a rabbit per day) for two days to ensure a adequate blood feeding for five days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside a mosquito cage for adult emergence [10].

2.3 Ovicidal activity

The method of Su & Mulla [21] was followed to test the ovicidal activity. The stored leaf extracts were diluted in the respective solvent with water to achieve different concentrations (50, 100, 150, 200, 250 and 300 ppm). The freshly laid egg raft containing 100 eggs. Ahead of treatment eggs of the *Cx. quinquefasciatus* was counted individually with the help of hand lens. Freshly laid eggs (0-6 h) of this mosquito species (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to respective solvents in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each concentration was replicated six times. The hatch rate was assessed 24 hours post treatment by the following formula.

$$\text{Percentage of egg mortality} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs in egg raft}} \times 100$$

2.4 Larvicidal bioassay

Standard WHO [22] protocol with minor modifications was adopted for the study. The tests were conducted in glass beakers. *Culex quinquefasciatus* larvae particularly fourth instar larvae from laboratory colonized mosquitoes of F1 generation were used for the study. The required test concentrations and quantity of test solution was prepared by serially diluting one percent stock solution of each crude extract. Twenty healthy larvae were collected with a pasture pipette and released into each 250 ml glass beaker containing 200 ml of dechlorinated water with various test concentration (100-500 ppm). Mortality was observed 24 hours after treatment. A total of five trials with five replicates per trial for each concentration was carried out. Controls were run simultaneously. Treated control was prepared by the addition of respective solvent (1ml) with dechlorinated water (199ml). Distilled water served as untreated control. The beakers were covered with muslin cloth to avoid entry of any foreign material. In between the experiment, no food was ceded to larvae. At the end of 24 hours period to observed the mortality was recorded. There is no sign of any movement even after mild touch with a glass rod and dead larvae are to be counted as described in the WHO technique report series [23]. The percentage of crude mortality was corrected by Abbot's formula and 95% of lower confidence limit (LCL) and upper confidence limit (UCL) were calculated by probit analysis [24, 25].

2.5 Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating $LC_{50}$and LC_{90}(lethal concentration) values and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), and Chi-square values were calculated using the SPSS(Statistical Package Social Science) 12.0 software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

Results of the ovicidal and larvicidal effects of *Kalanchoe pinnata* leaf crude extracts against *Culex quinquefasciatus* are presented in Table 1, 2 and Figure 1, 2. The extracts showed a dose-dependent toxicity to *Culex quinquefasciatus* eggs and larvae. Among the extracts tested, the crude acetone extract of medicinal plantleaves was found to be effective with hundred percent mortality of eggs at 250 ppm and the LC_{50} and LC_{90} values of fourth instar larvae were 199.86 and 387.70 ppm respectively. The finding of the present investigation revealed that the leaf extracts of *Kalanchoe pinnata* possess remarkable ovicidal and larvicidal activity against medically important vector mosquitoes and this is the low cost and ideal eco-friendly approach for the control of mosquitoes.

3.1 Ovicidal activity of *Kalanchoe pinnata* plant leaf extracts against filarial vector, *Culex quinquefasciatus*

The ovicidal activity was determined against mosquito species, *Culex quinquefasciatus* to various concentrations ranging from 50-300 ppm under the laboratory conditions. The water, acetone, benzene, petroleum ether and ethyl acetate extracts of *Kalanchoe pinnata* was found to be more effective against filarial vector. Among the four tested solvents and the water extracts, acetone crude extract was found to be most effective for ovicidal activity against the mosquito species. No mortality was recorded in the control. The extract of acetone exerted 100% mortality at 250 ppm and the other extract of benzene, petroleum ether, ethyl acetate and aqueous exerted 100% mortality at 300 ppm against the vector *Culex quinquefasciatus*. From the result (Table1 and Figure1) the plant extract contains effective ovicidal bioactive principles which may be needed for further purification to obtained natural product and used as insecticidal properties.

Table 1: Ovicidal activity of *Kalanchoe pinnata* plant leaf extracts against *Culex quinquefasciatus*

Selected solvent	Percentage of egg hatchability						
	Concentration ppm						
	Control	50ppm	100ppm	150ppm	200ppm	250ppm	300ppm
Acetone	100.0±0.0	70.0±0.4	59.4±1.5	37.8±0.7	11.8±0.6	NH	NH
Benzene	100.0±0.0	73.8±0.8	62.0±1.2	39.6±0.9	21.6±0.6	10.5±0.6	NH
Ethyl acetate	100.0±0.0	77.5±1.0	64.0±0.1	41.3±1.7	25.6±0.3	12.3±1.3	NH
Pet. Ether	100.0±0.0	73.7±1.7	57.6±1.3	44.1±0.9	30.4±1.9	19.3±0.9	NH
Aqueous	100.0±0.0	81.2±1.3	64.2±0.6	45.1±1.0	29.3±1.6	17.5±1.3	NH

NH: No hatchability (100% Mortality), each value: Represents mean of six values

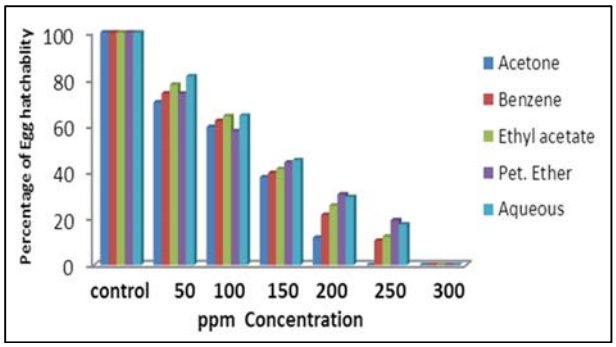

Fig 1: Ovicidal activity of *Kalanchoe pinnata* leaf extracts against filarial vector, *Culex quinquefasciatus*

3.2 Larvicidal activity of *Kalanchoe pinnata* plant leaf extracts against *Culex quinquefasciatus*

The larvicidal activity of acetone, benzene, petroleum ether, ethyl acetate and aqueous extracts of *Kalanchoe pinnata* leaf against *Culex quinquefasciatus* larvae revealed that the acetone extract indicates the higher mortality rates compared to the other (aqueous, solvent) extracts. Table 2 and Figure 2indicates the mortality of 4th instar larvae of the mosquito, *Culex quinquefasciatus*. When the larvae were treated with different solvent extracts, the larvae were at first restless then they were sluggish and coiled and finally death occurred. The highest larvicidal potentials with LC_{50} and LC_{90} values of *K. pinnata* leaf extracts were depicted after 24 hours. LC50 values were 199.86, 220.35, 237.34, 255.46 and 277.47 ppm; and LC90 values were 387.70, 415.98, 488.95, 480.01 and 507.53 ppm.

Table 2: Larvicidal activity of *Kalanchoe pinnata* plant leaf extracts against the vector, *Culex quinquefasciatus*

Selected solvent	Larval mortality percentage ± SD					LC_{50}	LC_{90}	X^2
	100ppm	200ppm	300ppm	400ppm	500ppm	(LCL-UCL)	(LCL-UCL)	
Acetone	36.2±0.8	55.61±1.4	72.4±1.2	87.03±1.6	98.8±1.2	199.86 (129.25-261.55)	387.7 (315.32-539.87)	20.6
Benzene	31.2±1.0	48.9±1.4	68.8±1.6	84.1±1.2	97.2±0.8	220.35 (160.74-275.52)	415.98 (347.56-546.52)	15.89
Ethyl acetate	27.6±1.4	48.1±1.2	63.7±2.0	81.1±1.8	93.4±1.0	237.34 (179.14-292.73)	488.95 (317.52-583.54)	14.29
Pet. Ether	25.3±1.2	44.4±1.4	59.6±0.8	77.4±2.0	90.2±1.6	255.46 (199.16-312.03)	480.01 (405.18-621.44)	13.26
Aqueous	21.4±1.4	40.2±1.8	54.1±0.8	72.5±1.2	88.3±1.6	277.47 (225.85-331.65)	507.53 (432.81-644.15)	11.22

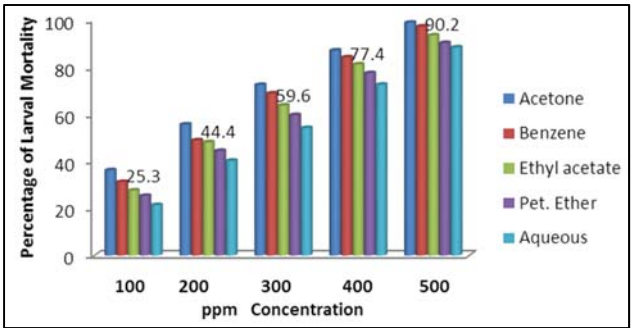

Fig 2: Larvicidal activity of *Kalanchoe pinnata* leaf extracts against filarial vector, *Culex quinquefasciatus*

The 95% confidence limits LC_{50} and LC_{90} (LCL – UCL) were also calculated the results of larvicidal activity clearly indicate that the percentage of mortality being directly proportional to the concentration of the extract. This proves that concentration plays important role in larvicidal activity. Chi-square values were significant at $P<0.05$ level, each test included a control group with for each individual concentration. No mortality was recorded in the control. From the results it can be concluded the crude extract of *Kalanchoe pinnata* leaf was a excellent potential for controlling *Culex quinquefasciatus* mosquitoes and to isolated compound of the plant consider as botanical insecticides.

4. Discussion

Mosquitoes are potent vectors for the transmission of various pathogens and spread to a variety of diseases in humans and other animals. The latex of *Calotropis procera* was found effective for the control of mosquito larvae as indicated by the rate of mortalities [35]. Extract of *Citrullus vulgaris* has been reported to have caused high mortalities in larvae of *Anopheles stephensi* [26]. During the last three decades, pest control methods were directed to the use of insecticides of plant origin. This trend appeared as a result of the accumulated side effects and environmental contamination from a long term extensive application of toxic synthetic insecticides. An example of these botanical insecticides was those derived from the neem tree (*Azadirachtaindica*) and tried against more than 400 species of pests [27].The growing resistance of *Ae. aegypti* populations to the current commercial pesticides has hampered the efforts to control dengue vector effectively. In addition, other serious problems such as high environmental and human toxicity and low biodegradability have been created by the continuous use of synthetic pesticides. Hence, there has been an increasing interest in the development of alternative methods of mosquito control which are less hazardous to humans and other living organisms. In this regard, plant derived compounds have emerged as good candidates, not only as new effective tools in vector management but also as environmentally safer agents. Larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranging from 38 to 48 mg/L. The crude extract had strong repellent action against three species of mosquitoes as it provided 100% protection against *An. Stephensi* for 180 minutes followed by *Ae. aegypti* (150 min) and *Cx. quinquefasciatus* (120 min) respectively [28].

The larvicidal activity of Petroleum ether, Chloroform, Ethyl acetate and Methanol extracts of *J. adhatoda* leaf against *Cx.*

quinquefasciatus larvae reveals that the methanol extract indicates the higher mortality rates compared to the other solvent extracts. The highest larvicidal potency with LC_{50} and LC_{90} was depicted after 24 hours was 126.21, 134.41, 140.92, 146.55 ppm and 385.38, 397.45, 407.17, 410.98 ppm. While, after 48 hours the LC_{50} and LC_{90} indicated 75.39, 78.19, 84.50, 91.37 ppm and 207.12, 259.67, 323.86, 369.88 ppm [10]. Plant extracts as alternative larvicide because they contain various phytochemical that are specific in killing mosquito larvae without harming other organisms and the environment. Botanical derivatives have drawn attention as potential insect control agents targeting only larval stages in the mosquito control programme in the last three decades [29]. Insecticides of plant origin become a priority to carried out and assess the role of mosquitocidal activities against the species *Cx. quinquefasciatus*.

The result of larvicidal activity of the plant showed that bark methanolic extract (58.67 ±1.63) has more % mortality than the leaf extracts (56 ±2.83). LC_{50} value for the bark extract was 316.24±9.5 ppm and for the leaf extract value was 547.46±45.16 ppm. Similarly 95% confidence LFL-UFL Lower for the bark extract was 302.2-329.11 ppm and 476.47-600.14 ppm for the leaves extract. The study showed that the bark extract has more potent larvicidal activity than that of the leaves extract [30]. The ovicidal activity was determined against two mosquito species to various concentrations ranging from 60-300 ppm under the laboratory conditions. The petroleum ether, chloroform, ethyl acetate and methanol extracts of *J. adhatoda* was found to be more effective against *Cx. quinquefasciatus* than *Ae. aegypti.* Among four tested solvents methanol and ethyl acetate crude extract was found to be most effective for ovicidal activity against the mosquito species. The extract of methanol and Petroleum ether exerted 100% mortality at 240 ppm against Cx. *quinquefasciatus* and *Ae. aegypti* respectively. From the results it can be concluded the crude extract of *J. adhatoda* was a possible for controlling *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes [10].

Medicinal plants are used in traditional treatments to cure a variety of diseases and act as biocontrol agents. In the last few decades there has been an exponential growth in the field of herbal medicine and its natural products have been a source of drugs for centuries [31]. Mosquito risk has become more acute in recent time and the death of millions of people every year due to mosquito-borne diseases has resulted in the loss of socioeconomic wealth in many countries. The control of mosquito by chemical substance is not safe at present because of insecticide resistance by vectors and environmental imbalance. Application of chemical or synthetic insecticides leads to deleterious effects in the long term; hence it does not provide absolute results. That is why alternative mosquito control method is needed [32]. The extract which is obtained from plant parts like leaves, root, flower, bark, seed, and fruits in their crude extracts has been used as conventional larvicide [33]. The secondary compounds of plants are vast repository of compounds with a wide range of biological activities. Tennyson [34] studied the larvicidal activity of twenty-five plant extracts against the larvae of *Culex quinquefasciatus*.

Prevention of mosquito population is very important because they transmit a variety of diseases by acting as primary vectors for the pathogens that cause severe infections. Chemical control measures have a number of impaired health risks by affecting the natural environment. Therefore environment friendly control measures of mosquitoes are important by means of that plant extracts having insecticidal potential. Extracts of plants, tree parts, herbs, shrubs and fruits has been used effectively against mosquito larvae. Biologically active plant extracts are mostly used for the control of various species of mosquitoes [35]. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on plant source. In view of the increased interest in developing plant-based insecticides as an alternative to chemical insecticides, this study was undertaken to assess the ovicidal and larvicidal properties of *Kalanchoe pinnata* leaf extracts against the filarial vector mosquito, *Culex quinquefasciatus*.

5. Conclusion

The present investigation revealed that different extract of *Kalanchoe pinnata* possesses remarkable ovicidal and larvicidal, activity against filarial vector mosquito. These extracts might be used directly as ovicidal and larvicidal agent in small volume aquatic habitats or breeding sites of limited size around human dwellings. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants. The plant extracts contain effective ovicidal and larvicidal bioactive compounds which may be needed for further purification to obtained natural product of ovicidal and larvicidal drug to control of mosquito species. These extracts are inexpensive, easy to handle and safer products for the control of mosquito in immature stage.

6. References

1. Mehlhorn H, Al-Rasheid KA, Al-Quraishy S, Abdel-Ghaffar F. Research and increase of expertise in arachno-entomology are urgently needed. Parasitol Res 2012; 110:259-265.
2. WHO A global brief on vector-borne diseases. Geneva, Switzerland 2014.
3. WHO Zika virus, Fact sheet, World Health Organization. Geneva, Switzerland. 2016.
4. Samuel T, Jayakumar M, William SJ. *Culex mosquito*: An overview. In: Defeating the public enemy, the mosquito 2007. A real challenge, Loyola College, Chennai, India.
5. Bhattacharya S, Basu P. The southern house mosquito, *Culex quinquefasciatus*: profile of a smart vector. J Entomol Zoo Stud. 2016; 4:73-81.
6. Matthys BN, Goran EK, Koné M, Koudou BG, Vounatsou P *et al*. Urban agricultural land use and characterization of mosquito larval habitats in a medium-sized town of Côte 'Ivoire. J Vector Ecol. 2006; 31:319-333.
7. Opoku AA, Ansa-Asare OD, AmoakoJ. The occurrences and habitat characteristics of mosquitoes in Accra, Ghana. West African J Appl Ecol. 2007; 11:81-86.
8. Sakthivadivel M, Saravanan T, Tenzin G, Jayakumar M, Raveen R *et al*. Laboratory Evaluation of Two Meliaceae Species as Larvicides Against *Culex quinquefasciatus* Say (Diptera: Culicidae). Vector Biol J. 2016; 1(2):2-10.
9. Ottesen EA, Duke BO, Karam M, Behbehani K. Strategies and tools for the control/ elimination of lymphatic filariasis. Bull World Health Organ. 1997; 75:491-503.

10. Jayapriya G, Gricilda Shoba F. Larvicidal, ovicidal, adulticidal and repellent activity of *Justicia adhatoda Linn* (acanthaceae) against *Aedes aegypti linn* and *Culex quinquefasciatus say*. Int J Recent Sci Res, 2014; 5(12):2321-2327.
11. World Health Organization. Global programme to eliminate lymphatic filariasis: progress report on mass drug administration. Weekly epidemiological record. 2010; 35(86):377-388.
12. Peng Z, Beckett AN, Engler RJ, Hoffman DR, Ott NL, Simons FE. Immune responses to mosquito saliva in 14 individuals with acute systemic allergic reactions to mosquito bites.J Allergy ClinImmunol. 2004; 114:1189-1194.
13. Govindarajan M. Larvicidal activity of *Cassia fistula* flower against *Culex tritaeniorhynchus Giles, Aedes albopictus Skuse* and *Anopheles subpictus Grassi* (Diptera: Culicidae). Int J Pure App Zoo. 2013; 1:117-121.
14. Unninathan AR, Unnikrishnan G. Larvicidal bioassay of five tropical plants against *Aedesaegypti*. W J Pharma Res. 2015; 4:2436-2446.
15. Kishore N, Mishra BB, Tiwari VK, Tripathi V. Areview on natural products with mosquitocidal potentials. In: Opportunity, challenge and scope of natural products in medicinal chemistry. Research Signpost. 2011.
16. Al-Zanbagi NA, Barrett J, Banaja AA. Laboratory evaluation of the molluscicidal properties of some Saudi Arabian euphorbiales against *Biomphalaria pfeifferi*. Acta Trop. 2001; 78:23-29.
17. Bowers WS. Biorational approaches for insect control. Korean J Appl Entomol. 1992; 31:289-303.
18. Das. NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. J Vector Borne Dis. 2007; 44(2):145-148.
19. Vogel AL. Text Book of Practical-Organic Chemistry. The English Language Book Society and Langman, London. 5th edition. 1978.
20. Reuben R, Tewari SC, Hiriyan J, Akiyama. Illustrated keys to species of Culex associated with Japanese encephalitis in Southeast Asia (Diptera: Culicidae). Mosquito systematic. Am Mosq Control Assoc. 1994; 26(2):75-96
21. Su T, Mulla MS. Ovicidal activity of neem products (azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J Am Mosq Control Assoc. 1998; 14(2):204-209.
22. WHO. Guidelines for laboratory and field testing of mosquito larvicides, Geneva. 2005.
23. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization [Online]:http://whqlibdoc.who.int/hq/2005
24. Abbott WS. A method of computing the effectiveness of an insecticide. 1925. J Am Mosq Control Assoc. 1987; 3:302-303.
25. Finney DJ. Probit analysis. Cambridge University Press. United Kingdom. 3rd edition. 1971, 68-72.
26. Mullai K, Jebanesan A, Pushpanthan T. Mosquitocidal and repellent activity of the leaf extract of *Citrullus vulgaris* (Cucurbitaceae) against the malarial vector, *Anopheles stephensi* list on (dipteraculicidae). Eur Rev Med Pharmacol Sci. 2008; 12:1-7.
27. Schumutterer H, Ascher KRS, Isman MB, Jacobson M, Ketkar CM, Kraus W *et al*,. The Neem Tree (*Azadirachtaindica*) and other Meliaceous plants. VCH Verlag, Weinheim, Germany, 1995, 990.
28. Govindarajan M. Larvicidal and repellent activities of *Sida acuta Burm.* F. (Family: Malvaceae) against three important vector mosquitoes. Asian Pac J Trop Med. 2010; 3:691-695.
29. Rawani A, Ghosh A, Chandra G.. Mosquito larvicidal potential of four common medicinal plants of India. Indian J Med Res. 2014; 140(1):102-108.
30. Wankhade MS, Mulani RM. Larvicidal Activity and Phytochemical Investigation of *Albiziajulibrissin Durazz.* Methanolic Leaf and Bark Extract against *Aedes aegypti* Linn. Larvae. Ijppr. Human; 2016; 6(3):172-179.
31. Hariprasad PS, Ramakrishnan N. GC-MS analysis of *Rumex vesicarius* L. Int J Drug Dev& Res. 2011; 3(2):272-279.
32. Pavela R. Larvicidal activities of some Euro-Asiatic plants against *Culex quinquefasciatus Say* (Diptera: Culicidae). J Biopest. 2008; 1:81-85.
33. Vinayagam A, Senthilkumar N, Umamaheswari A. Larvicidal activity of medicinal plant extract against malaria vector *Anopheles stephensi*. Res J Parasitol. 2008; 3:50-58.
34. Tennyson S, Ravindran KJ, Arivoli S. Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pac J Trop Biomed. 2012; 2(2):S1130–S1134.
35. Anjum SI, Hussain S, Attaullah M, Khan U, Khattak B, Fouad H. Evaluation of the larvicidal potential of *Calotropis procera* plant extract against *Culex pipiens.* Int J Mosq Res. 2016; 3(6):01-05.