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MOSQUITOCIDAL ACTIVITY OF *ARTEMISIA NILAGIRICA* AGAINST FILARIAL VECTOR, *CULEX QUINQUEFASCIATUS* SAY. (DIPTERA: CULICIDAE)

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***Abstract* :**

Mosquitoes are insect vectors responsible for the transmission of parasitic and viral infections to millions of people worldwide, with substantial morbidity and mortality. *Culex* species are known to transmit human pathogens worldwide. *Culex* mosquitoes belong to order dipteral to the culicidae family, a medically and biologically important blood sucking insect that transmits many diseases to humans, causing an epidemic. Chemical insecticides in mosquitoes control results in various harmful effects which includes vector resistance, environment pollution and health hazards. This has necessitated the search for alternative approach for mosquito control pogramme using natural products of plant origin especially plant extract. The Present study was conducted to evaluate the mosquitocidal activity of *Artemisia nilagirica* against *Culex quinquefasciatus*. This study was conducted in the laboratory to evaluate the *Artemisia nilagirica* with different concentration (0.5%, 1% and 2%). The mosquitocidal activity was recorded of 24 hours, under laboratory condition 98% larval mortality was observed in 1st instar larvae of *Culex quinquefasciatus*, after the treatment of *Artemisia nilagirica* at 2% concentration; where as in 3rd and 4th instar larval mortality were 85% and 79% at 2% treatment respectively. The pupal mortality was 94% at 2% concentration treatment. Adult mortality was 91% after the treatment, the adult emergence was 35% drastically reduced after the treatment of *Artemisia nilagirica*. The larval duration was greatly extended up to 4 days after the treatment of *Artemisia nilagirica* at 2% than other concentration. Pupal duration also extended after the treatment of *Artemisia nilagirica*, at 2% concentration Fecundity and egg hatchability also reduced after the treatment of *Artemisia nilagirica* 27%. Adult repellency and Ovipositional deterrency also observed after the treatment of plant extract at 2% concentration adult repellency was 82% and ovipositional deterrency was 95%. *Artemisia nilagirica* at 2% concentration biting deterrency also increased after the treatment of *Artemisia nilagirica* (1% < 2% < 4%). Larval pupal intermediate were very high after the treatment of *Artemisia nilagirica*.

Key words

Culex quinquefasciatus, *Artemisia nilagirica*, larvicidal, pupicidal, adulticidal, pupal duration, adult duration.

1. INTRODUCTION

Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. WHO has declared the mosquitoes as “public enemy number one” Mosquito borne diseases are prevalent in more than 100 countries across the world, in infecting over 700,000.000 people every year globally and 40,000,000 of the Indian population one about 3500 species of mosquitoes have been described worldwide, Relatively few of them are significant vector of the life -threatening diseases. Mosquitoes are serious vectors of important human parasites and microbes. WHO (2010) reported 216 million cases of malaria in the world with an estimated 655,000 malaria deaths. World Malaria report (2011) an estimated 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis. Mosquitoes spread disease to humans, domestic animals and wildlife. *Culex quinquefasciatus* is a vector of lymphatic filariasis which is a widely distributed tropical disease *Wuchereria bancrofti* accounts for approximately 90% of all filariasis cases in the world, followed by *Brugia malayi* and *Brugia timori*. India contributes about 40% of the total global burden of filariasis and accounts for about 50% of the people at risk of infection. 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 insecticidesof botanical origin as a simple and sustainable method of mosquito control. Phytochemicals are botanicals which are naturally occurring insecticides obtained from it control floral resources. Applications of phytochemicals in mosquito control were on use since the 1920s (Shahi *et al.*, 2010), but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. After facing several problems due to injudicious and over application of synthetic insecticides in nature re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the kingdom and an effort to determine its structure and commercial production has been initiated. As the problem of insecticide-resistant mosquitoes to chemical agents are on the rise, natural sources, such as plant sources, are good alternatives to control mosquito vectors. They are harmless to humans and the environment, are target-specific, biodegradable and ecofriendly (Nathan and Kalaivani, 2005). Natural insecticides may contain molecules with mosquitocidal effects.They have different mechanisms of action which reduce the chance of developing resistance in mosquito populations (Okumu *et al*., 2007).

At present phytochemicals make up to one percent of world's pesticide market (Isman, 1997). Therefore in the present study was screened medicinal plant of *Artemisia nilagirica* leaves extract on the larvicidal, pupicidal, adulticidal, pupal duration, adult duration of *Culex quinquefasciatus.* The possible result of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive compounds from indigenous medicinal plant source.

2. MATERIALS AND METHODS

2.1 Collection and preparation of plant extract

The plant material *Artemisia nilagirica* have been collected from the forest of Nilgiri hill. The collected plant material was washed in tap water, cut into small pieces, and air dried. After the plant was completely dry, it have been ground into powder, and then macerated in methanol solvent at room temperature for 3 days, and filtered. The combined filtrate were concentrated to dryness by rotary evaporation at 50□C and kept in a freezer. In preparing test concentrations, the plant extract was volumetrically diluted in methanol solvent.

2.2 Mosquito culture

Mosquito larvae/eggs of *Culex quinquefasciatus* have been collected in an around Ooty. The mosquito colonies were maintained at 27 $\pm$ 2 ^{0}C, 75-85% relative humidity index a14:10 light/dark photo period cycle (Murugan and Jeyabalan, 1999).

2.3 Larvicidal and Pupicidal assays

Larvae tested for the present study was obtained from our laboratory culture. Freshly hatched or moulted larvae were used for the bioassay tests. The required quantity of different plant extract concentrations was mixed thoroughly with 200 ml of rearing water in 500ml plastic troughs. One hundred early fourth instars mosquito larvae were released into each trough. Larvae food consisted of 1g of finely ground dog biscuits per day per trough. Dried coconut midribs were place over water as the substratum for pupation. The plastic trough containing 200 ml of rearing water served as the control. Dead larvae and pupae were removed and counted at 24 h intervals. Observations on larval and pupal mortality were recorded. The experiment was replicated five times. Percentage mortality observed in the control was subtracted from that observed in the treatments (Abbot, 1925). The day from moulting of the larvae to pupation and to adulthood was noted. Fecundity was assessed by counting the number of eggs laid during the life span by control and experimental mosquitoes. The larvae and pupal duration of treated and control individuals were compared and developmental rates were determined.

2.4 Adulticidal assay

Culex quinquefasciatus fresh adults were exposing to filter paper treated with different concentration of plant extract. The paper was kept inside the beaker. Muslin cloth covering the beaker was also treated. Control insects were exposed only to distilled water with water treated paper and muslin cloth. Mortality count was taken after 24h (Sharma *et al.*, 1992).

2.5 Ovicidal assay

Culex quinquefasciatus eggs were released in water. The test extract was added in desired quantities and hatching were observed for one week. The eggs were then exposed to deoxygenated water and the numbers of hatching eggs were recorded. Percentage hatching was compared with the control in which only distilled water was used (Sharma *et al*., 1992).

2.6 Repellency activity

Different concentrations of plant extract are mixed thoroughly with 10ml of goat blood in glass plates. The untreated blood served as the control. Adult females were release into each cage. The number of females landing on the treated blood and untreated blood were record. The repellent index of the plant extract was calculated as described by (Murugan and Jeyabalan, 1999).

2.7 Biting deterrency activity

The percentage protection in relation to dose method was used (WHO, 1996). Blood starved female *Culex quinquefasciatus* (100 nos), 3 - 4 days old, was kept in a net cage (45x30x45 cm^2). The arm of the test person was cleaned with isopropanol. After air drying the arm, a 25 mc^2 area of the dorsal side of the skin was exposed, the remaining portion was covered by rubber gloves. The plant extract was dissolved in water, where distilled water served as control. Different concentration of the plant

oil was applied. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were count over 5 minutes from 6 pm to 6 am. The experiment was conducted five times.

2.8 Statistical analysis

All data was subject to analysis of variance and the treatment mean was separated by Duncan's Multiple Range Test (Duncan, 1955). Statistical analysis was carried out using the (Statistical Package Social Science) SPSS software, version 16.0.

3. RESULTS

The table 1 shows that the effect of *Artemisia nilagirica* on the larval mortality of *Culex quinquefasciatus* after the treatment of at 2% the larval mortality of 1st instar larva was 100%, 2nd instar was 93%, 3rd instar was 85% and the 4th instar was79%. No dead larvae were observed in the negative control (water) *Artemisia nilagirica* produced significant mortality. The table 2 shows that the effect of pupal, adult mortality and adult emergence of *Culex quinquefasciatus* after the treatment of *Artemisia nilagirica*. After the treatment of *Artemisia nilagirica* at 2% of concentration the pupal mortality was 94%, adult mortality was 91% and adult emergence was 35%. The *Artemisia nilagirica* with higher concentrations, was found to be most effective for pupicidal activity against *Culex quinquefasciatus*. The adulticidal efficacy was observed in *Artemisia nilagirica plant extract*.

The presentage of larval mortality and inhibition of adult emergence was significant with the tested plant extract. Then table 3 shows that the effect of adult repellency and ovipositional deterrency of *Culex quinquefasciatus* after the treatment of *Artemisia nilagirica* at 2% concentration the adult repellency was 82% and ovipositional deterrency was 95%. The table 4 shows that the developmental duration of *Culex quinquefasciatus* after the treatment of *Artemisia nilagirica* at 2% concentration the larval duration of 1st instar was 6.4 days, 2nd instar larval duration was 8.8 days, 3rd instar larval duration was 10.6 days and 4th instar larval duration was 11.3 days.

The table 5 shows that the developmental duration of *Culex quinquefasciatus* after the treatment of *Artemisia nilagirica* at 2% concentration the total pupal duration was 9.5 days and total adult duration was 25 days. The *Artemisia nilagirica extract* showed more than 50% pupal mortality. Larval mortality was recorded in *Artemisia nilagirica* . The order of the larvicidal efficacy of plant extract after 24 hours of *Artemisia nilagirica* . The table 6 shows that the effect of *Artemisia nilagirica* on fecundity and egg hatchability of *Culex quinquefasciatus* after the treatment of *Artemisia nilagirica* at 2% concentration the fecundity was 70% and egg hatchability was 27%. The table 7 shows that the effect of larval-pupal intermediate of *Culex quinquefasciatus.* After the treatment of *Artemisia nilagirica* at 2% the larval pupal intermediate was 90%. The table 8 shows that the effect of biting detergency of *Culex quinquefasciatus* after the treatment of *Artemisia nilagirica* at 2% the biting detergency was 84%.

Table 1. Effect of *Artemisia nilagirica* on the larval mortality of *Culex quinquefasciatus*

S. No	Treatment	Concentration (%)	1st Instar	2nd Instar	3rd Instar	4th Instar
1.	Control	00	00^{d}	00^{d}	00^{d}	00^{d}
2.	*Artemisia nilagirica*	0.5	69^{c}	67^{c}	54^{c}	52^{c}
		1	79^{b}	75^{b}	67^{b}	63^{b}
		2	100^{a}	93^{a}	85^{a}	79^{a}

Within a column means followed by the same letters are not significantly different at 5% level by the DMRT

Table 2. Effect of *Artemisia nilagirica* on the pupae and adult of *Culex quinquefasciatus*

S. No	Treatment	Concentration(%)	Pupal mortality(%)	Adult mortality (%)	Adult emergence (%)
1.	Control	00	00^{d}	00^{d}	100^{a}
2.	*Artemisia nilagirica*	0.5	63^{c}	60^{c}	62^{b}
		1	76^{b}	72^{b}	41^{c}
		2	94^{a}	91^{a}	35^{d}

Within a column means followed by the same letters are not significantly different 5% levelby DMRT

Table 3. Effect of *Artemisia nilagirica* on the adult repellency and ovipositional deterrency of *Culex quinquefasciatus*

S. No	Treatment	Concentration(%)	Adult Repellency(%)	Ovipositionaldeterrency (%)
1.	Control	00	00^{d}	00^{d}
2.	*Artemisia nilagirica*	0.5	43^{c}	54^{c}
		1	60^{b}	69^{b}
		2	82^{a}	95^{a}

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

Table 4. Developmental duration of *Culex quinquefasciatus* after the treatment of*Artemisia nilagirica*

S. No	Treatment	Concentration (%)	1st Instar (days)	2nd Instar (days)	3rd Instar (days)	4th Instar (days)
1.	Control	00	2.2^{c}	4.4^{c}	5.4^{c}	6.6^{c}
2.	*Artemisia nilagirica*	0.5	2.5^{c}	4.6^{c}	5.5^{c}	6.8^{c}
		1	4.3^{b}	7.6^{b}	8.6^{b}	9.2^{b}
		2	6.4^{a}	8.8^{a}	10.6^{a}	11.3^{a}

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

Table 5. Pupal and adult duration of *Culex quinquefasciatus* after the treatment of*Artemisia nilagirica*

S. No	Treatment	Concentration (%)	Total pupalduration (days)	Total adultduration (days)
1.	Control	00	5.2^{c}	51^{a}
2.	*Artemisia nilagirica*	0.5	5.5^{c}	43^{b}
		1	7.7^{b}	39^{c}
		2	9.5^{a}	25^{d}

Within a column means followed by the same letters are not significantly different at 5%level by DMRT

Table 6. Effect of *Artemisia nilagirica* on fecundity and egg hatchability of *Culexquinquefasciatus*

S. No	Treatment	Concentration(%)	Fecundity (No. of eggs)	Eggs hatchability (%)
1.	Control	00	206[a]	100[a]
2.	*Artemisia nilagirica*	0.5	176[b]	66[b]
		1	121[c]	43[c]
		2	70[d]	27[d]

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

Table 7. Effect of *Artemisia nilagirica* on Larval-pupal intermediate of *Culex quinquefasciatus*

S. No	Treatment	Concentration(%)	Larval-pupalIntermediate(%)
1.	Control	00	00[d]
2.	*Artemisia nilagirica*	0.5	40[c]
		1	65[b]
		2	90[a]

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

Table 8. Effect of *Artemisia nilagirica* on biting deterrency of *Culex quinquefasciatus*

S. No	Treatment	Concentration(%)	Biting deterrency(%)
1.	Control	00	00[d]
2.	*Artemisia nilagirica*	0.5	35[c]
		1	69[b]
		2	84[a]

Within a column means followed by the same letters are not significantly different at 5%level by DMRT.

4. DISCUSSION

The mosquito-borne diseases can be controlled by either killing or preventing mosquito bite to human beings or also by causing egg, larval and pupal mortality in the breeding centerslike stagnated water, sewage leekage etc. Initially, the mortality of the vector *Culex quinquefasciatus* was achieved by using the synthetic insecticides in the last five decades. Butdue to their physiological resistance development it resulted in environmental hazards. So, there was an urgent need to control the vector naturally because these naturally synthesized products will be easily biodegradable, environmentally safe, low cost and will be morespecific resulting in a drastic reduction in the population of *A. aegypti vector* (Anjali *et al*., 2017; Paoletti and Pimentel, 2000). The phytochemicals extracted from the different aromatic medicinal plant extracts and also from different parts of the plant by using the different solvents have revealed the efficacy of ovicidal, larvicidal and pupicidal activity against the dengue vector (Velu *et al.,* 2015; Alagarmalai *et al*., 2012). In this experiment methanol extract showed the presence of alkaloid, terpenoids, carbohydrate, anthoquinone and coumarins. DCM exhibited the presence of these secondary metabolite's phenols, flavonoids, tannins and protein inaddition to the phytochemicals present in the methanol extract. Whereas for methanol, all other metabolites were presence apart from the secondary metabolites like tannins, saponins and proteins. Plants are the promising botanicals in integrated mosquito management (Kanika Tehri and Naresh Singh, 2015). Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. During the last decadesthe effects of plant extracts were evaluated against different mosquito larvae species (Kovendan *et al.,* 2011; Prabhu *et al*., 2011).

Plant extract have been used traditionally by human communities in many parts of the world against the vectors and insect pests (Muthu *et al.,* 2015). Application of these plant derivatives in mosquito control as an alternative of synthetic insecticides could diminish the environmental hazards and also reduce the overall cost of production (Baskar *et al*., 2017). The phytochemicals derived from plant sources can act as larvicides, insect growth regulators,repellents, ovipositional attractants and also have deterrent actions as being highlighted by many researchers (Rajkumar and Jebanesan, 2007; Pavunraj *et al.*, 2017). Botanical products that are locally available and have major roles in mosquito control would lead to many advantages, like offering ecofriendly and cost-effective means to stimulate efforts to protect the public health against disease spreading mosquito vectors(Senthil *et al.*, 2008 ; Beneli *et al.*, 2016).

A large number of botanically derived plant extract have been reported for their promising mosquitocidal activity against three important mosquito vectors (Beneli, 2015; Kalaivani *et al*., 2012). Plant bioactive components may serve as a suitable alternative to chemical insecticide as they are relatively safe and available everywhere in the world. The efficacy of botanicals however, generally depends on the plant part (Chapagain and Wiesman, 2005), extract concentration, age of plant or location found, solvent used and species of larvae tested (Gupta *et al.,* 1990 ; Babarinde *et al.,* 2011). The solvent used contribute to the variation since it has been shown that the extraction of active biochemical from plants depends upon the polarity of the solvents used (Ghosh *et al*., 2012). Shaalan *et a*l. (2005) reported that screeninginvolves mosquitocidal bioassay guided fractionation to identify highly active fractions and compounds isolated from the crude extract. The crude extract contains a complex mixture of biocidal active compounds. Hence, crude plant extracts have played an important role in this aspect. Several studies have documented the efficacy of plant extracts as the reservoir pool of bioactive toxic agents against mosquito larvae. Further, Tehri and Singh (2015) stated thatthe successful results of preliminary studies on mosquitocidal potential of plant extracts encourage further effort to investigate the bioactive compounds in those extracts that might possess good immature mosquitocidal properties when isolated in pure form. In addition, novel drug delivery system of plant based active substances is the need of the hour.

The current work has shown that the *Artemisia nilagirica* exhibited hundred percent larvicidal activities against 4th instar mosquito larvae of *C. quinquefasciatus*. Our results accounted for greater activity as it was compared with an earlier study by Bosire *et al*. (2014)reported that dichloromethaneand methanol extract of *Millettia usaramensis* showed larvicidalactivity against 4th instar larvae of *Aedes aegypti* with LC_{50} value of 167.0 µg/mL. Earlier, Anitha and Geethapriya (2012) reported that petroleum ether extract of *Lantana camara*, *Tridax procumbens* and *Datura stramonium* showed 100% larval mortality against *Aedes aegypti* at concentration of 1000 µg/mL. (Bagavan *et al.,* 2008) studied the larvicidal activityof hexane, chloroform and ethyl acetate extracts of *P. daemia* against mosquito larvae. They observed that ethyl acetate extract showed 100% mortality against *Anopheles subpictus* and *Cx. tritaeniorhynchus* at 1000 ppm concentration. Balaji *et al. (*2012) also reported that dichloromethane extract of *Roccella montagnei* showed high toxicity against the 3rd instar mosquito larvae of *C. quinquefasciatus*.

Plant extract could be a potential source of bioactive compounds to control mosquitoes. Many plant species have been noted and recognized to have mosquitocidal activity (Bhattacharya and Chandra, 2013). Jagajeevanram *et al,* (2016) reported only 70% death of 4th instar larvae of *Anopheles stephensi* after 72 h exposure to 0.5 % concentration of *C. amada* rhizome methanol extract. Whereas, Porto *et al.* (2017) tested larvicidal activity of several different plants at 0.5 mg/mL concentration against *Aedes aegypti* among which evaluation of larvicidal property of ethanolic extract of *T. indica* leaves was also included and showed no mortality at that concentration. Also, the literature reports that the Menthal species, from India, recorded the LC_{50} value of 42.25 ppm and LC_{90} value of 132.41 ppm against the larvae of *Culex quinquefasciatus* at 24 h exposure (Manimaran *et al.,* 2012). Koliopoulos *et al.* (2010) reported that the LC_{50} values of *Mentha* species, from Central Greece, range d from 47.88 to 74.28 ppm and LC_{90} values ranged from 64.34 to 107.45 ppm against *Culex pipiens* after 48h exposure. The important larvicidal activity observed among the essential oil of *M. pulegium* might be explained by the action or the effect of the major components. In here results are the first in Morocco and this might allow developing an effective low cost and eco-friendly larvicides. The activity of these defensive enzymes in the control and untreated groups was modulated during the normal developmental period (24 h of experiment), thus showing their importance in diverse physiological processes in the development of mosquito larvae, as reported in various insect (Koodalingam *et al.,* 2012; Singh *et al*., 2009).

Lantana camara plant extract was tested for larvicidal and adult knockdown activity against three major mosquito species (Dua *et al.*, 2010). *Tagetes patula* volatile extract has strong mosquitocidal activity with low LC_{50} values 0.0070 mg/cm2 against *Cx. pipiens* (Mansour *et al.*, 2000). Plant-based phytochemicals and plant products are increasingly being used for their efficacy as biocontrol agents for control of insecticide-resistant mosquitoes (Murugan *et al.*, 2005; Senthil *et al.*, 2004). Biopesticides from plant origin have been shown to be more effective on agricultural and medical pests (Senthil *et al.*, 2005; Senthil *et al.*, 2004). Nowadays, plant and microbial sources are increasingly used for vector control programs because they have been shown to have the potential to be effective, more target-specific than chemical insecticides, and ecofriendly (Ibrahim *et al.*, 2017; Senthil *et al.*, 2005).

How-ever there was a gradual overall mortality rate decreased as concentration decreased in the *Artemisia nilagirica*. It was observed that there were significant differences between the low and higher concentrations of the extract and higher mortality at higher concentration. Thisis consistent with the observation of Piyarat *et al*. (1974). Similar results were found by Traboulsi *et al*. (2002) showing the insecticidal activityof four medicinal plants harvested in Lebanon (*Origanum syriacum* L., *Lavandula stoechas* L., ; *Mentha microphylla* K. and *Myrtus communis* L.) against *Cx. pipiens* molestus (LC_{50} varying from 16 to 89 mg/L). Sosan *et al*. (2001) reported larvicidal activities of essential oils of *Ocimum gratissium*, *Cymbopogon citrus*, and *Ageratum conyzoides* against *Aedes aegypti* and achieved 100% of mortality at 120, 200 and 300ppm concentrations, respectively. Previous experiments showed that some plant extract exhibit larvicidal activity properties such as the metabolites of the marine alga, *Caulerpa racemosa* applied to *Cx. pipiens* (Walied *et a*l., 2010), *Tagetes patula* extract against three mosquito species *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Dharmagadda *et al*., 2005) *Origanum syriacum* L, *Lavandula stoechas* L (Traboulsi *et al*., 2002) and *Kelussia odoratissima Mozaffarian* applied to *Cx. pipiens* (Vatandoost *et al*., 2012).

Several factors affect the bioactivity of essential oils including plant species (variety), cultivating conditions, maturation of harvested plants, plant storage, plant preparation and methods of extraction (Park *et al*., 2012 ; Tawatsin *et al*., 2006). In the present investigation, *Artemisia nilagirica* were screened for their larvicical efficacy against *Culex quinquefasciatus*. The seed extract was found to have better larvicidal activity than the leaf extract as corroborated by various reports (Ramos *et al*., 2010; wafa *et al*., 2014). The parts of the plant and type of solvent used for extraction affect the larvicidal activity. It has been reported that non-polar (petroleum ether) extracts of the roots of *Berberis lycium* and leaves of *Hedera nepalensis* are more active against *Aphis craccivora* than that of the polar (aqueous methanol) extracts (Tewary *et al*., 2005). Petroleum ether, carbon tetrachloride, and methanol extracts of fruit (*Azadirachta indica*) and extracts of seed (*Momordica charantia* and *R. communis*) have been evaluated forlarvicidal activity against *Culex quinquefasciatus* (Batabyal *et al*., 2009). In the present study the *Artemisia nilagirica* was found to be more potent. The larvicidal activity of extract of *Leucas aspera* leaf, using hexane as a solvent, was tested for the on the larvae of *Culex quinquefasciatus* and *Aedes aegypti* (Maheswaran *et al*., 2008; Govindarajan, 2011). The leaf extract of *Acalypha indica* with different solvents, viz., benzene, chloroform, ethyl acetate and methanol, were found to have a larvicidal activity and ovicidal an effect on oviposition of *Anopheles stephensi* (Govindarajan, 2011 ; Mullai*et al*., 2008). In the present study, the *Artemisia nilagirica* treatment reduced the larval duration, none the pupal and introverted the adult emergence. Those treated larvae escaped from mortalityshowed reduced longevity. The adult which emerged from treated larvae were morphologically normal but showed a great reduction in fecundity. The same results were mentioned against *Aedes aegypti* (Bishnu *et al*., 2005) some insects (Deore and Khadabadi, 2009). Many reports showed changes in fecundity after treatment with biopesticide *B. thuringiensis* (Aissaoui and Boudjelida, 2014), insect growth regulators (Djeghader *et al*., 2013) and essential oil of plants (Alouani *et al*., 2017).

In the present study the larval stage of *Cx. quinquefasciatus* was recorded as more susceptible among all the developmental stages. This is in conformity with the findings of (Fox *et al*., 2001) where they suggested larvae as more responsive to any physical as well as chemical stresses than other developmental stages. The egg stage was found less susceptible among all developmental stages. Like other insects, eggs of mosquitoes are also covered with shell which differs significantly from the integument of the larva in their structure and biochemical constituents which may add difference in the penetration rate of different insecticides to the body (Soonwera, 2015). In contrast, bioassay against *Ae. aegypti* revealed that the egg stage is more susceptible among all the developmental stages. Variations in some factors like chitin content of the egg shell, egg volume ratio and egg surface density influence the levels of egg resistance to any stress (Farnesi *et al*., 2015) and these factors vary in different species.In addition to these plant deterring oviposition or ovipositional behavior, the volative components may also affect the physical condition of females, reducing their reproductive capacity (Yang *et al*., 2010). For example, Rao *et al*. (1999) found that ovarian development in the red cotton bug, *Dysdercus koenigii* F., was inhibited by *Artemisia annua* L. extract treatment, which caused a reduction in the numbers of oocytes and thus interfered with egg production. *Culex quinquefasciatus* mortality also increased significantly with increasing plant concentration, which is consistent with previous studies with other plant extract or insect targets. Kim *et al. (*2011) reported high mortality of *B. tabaci* with extracts from thyme (*T. vulgaris* L.) and garlic (*Allium sativum* L.). Aslan *et al.* (2004) reported that plant extracted from *Satureja hortensis* L., *Ocimum basilicum* L., and *T. vulgaris* (Lamiacae) were highly toxic to nymphs and adults of *Tetranychus urticae* Koch (Acari and Tetranychidae)and *B. tabaci* adults. Earlier researchers reported ovicidal activity and ovipositional deterrence efficiency of aqueous leaf extracts of against *Ae. aegypti* (Navaneethan *et al*., 2016). Pavela *et al.* (2017) have reported the mosquito larvicidal potential of extract combinations from *Clausena anisata* and *Dysphania ambrosioides* and highlighted the application of ethanomedicines as commercial insecticides. Individual chemical entities present in plant extracts can exhibit various synergistic as well as antagonistic effects. This phenomenon has been documented by systematic studies with binary combination soft the chemical components as well as plant extracts (Pavela, 2015; Benelli *et al*., 2017).

Our results showed that plant extract have significant larvicidal and repellent activities against *C. quinquefasciatus* mosquitoes. The leaf extract of plant with methanol was tested for larvicidal and repellent activities against *C. quinquefacsciatus* (Mullai *et al*., 2008). Insecticidal and repellency effects of *A. digitate* leaves against *An. Gambiae* have been first time studied in

Nigeria (Denloye *et al*., 2006). Many of these components are reported to be toxic and have repellent properties against many insects. Moreover, the repellent properties of several plant extract often appear to be associated with the presence of terpenes such as monoterpenes and sesquiterpenes, which were also identified in the extract (Jaenson *et al*., 2006 ; Kiran and Devi, 2007, Sukumar *et al*., 1991). Nerio *et al.,* (2010) explained that the high repellent activity of plant extract was usually attributable to their major components and to the presence of oxygenates. Most of the chemical insecticides used for controlling the mosquito larvae exhibit negative impact on the natural enemies or predators of mosquito larvae (Lawler *et al*., 2003; 2007). In the present study, the identified plant extract combination is showing less harm to nontarget organism than to the mosquito larvae at lower concentrations. Many of the chemical components present in the promising extracts were known for mosquitocidal and other biological activities which strongly support the mosquitocidal potential illustrated by the results of this study.

5. Conclusion

Artemisia nilagirica are highly toxic even at low dose these plant extract may eventually prove to be useful larvicide of *Culex quinquefasciatus.* Further analysis is required to isolate the active principles and optimum dosages, responsible for larvicidal and adult emergence inhibition activity in *Culex quinquefasciatus*. The extraction of *Artemisia nilagirica* excellent larvicidal and pupicidal activity. The mortality of the larvae increases as the doses of the sample were increased. The fourth instars larvae were less susceptible to the sample than 1st instar larvae. The mortality caused by some neural and muscular disturbance by the presence of variety of active compounds such as cytoxic, diterperiods, lactones and flavariods. Oviposition deterrent and gravid mortality assay, the OAI values also decreased that the gravid and oviposited females were repelled by extracts and the reduced oviposition was due to the greater mortality of adults before they oviposited. All the concentration of plant extract shared promising mosquito repellency properties. When tested against the adult mosquito *Culex quinquefasciatus*, in the biting deterrency result, increasing in the concentration of plant extract from 0.5% to 2% was found to increase the biting deterrency percentage.The product of these plant extract can be well utilized for preparing biocides or phytochemicals from which all the non-target organism can be rescued from harmful vectors. These plants would be eco-friendly and may serve as suitable alternative to synthesis insecticides as they are relatively safe, inexpensive and are readily available in many areas of the world. The result of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant extract source.

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