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Effect of *Berberis Tinctoria* Leaf Extracts On The Filarial Vector, *Culex Quinquefasciatus (Say)* (Diptera: Culicidae)

R. Visalatchi and D. Jeyabalan*

Department of Zoology and Wildlife Biology Government Arts College
Udhagamandalam-643002 The Nilgiris, Tamilnadu, India.
Email: *drjeyabalan@gmail.com

ABSTRACT:

The methanol leaf extracts of *Berberis tinctoria* is highly toxic even at low doses proven to be useful for larvicidal, pupicidal and adulticidal activity against *Culex quinquefasciatus***.** The extraction of *Berberis tinctoria* dose dependent and the mortality of the larvae increases as the doses of the sample were increased. The mortality caused by some neural and muscular disturbance by the presence of variety of active compounds such as cytotoxic diterperiods, lactones and flavonoids in the plants. It was observed that the increase in concentration of extracts increased the potential activity of biocides against *Culex quinquefasciatus.* The decrease in egg hatchability was found to be dose dependent. There was increase in the larval-pupal duration, developmental duration, decrease in fecundity and egg hatchability, biting deterrency, in our study. The extract showed ovipositional deterrence and effective repellence against *Culex quinquefasciatus* at different concentration, with the observation on that maximal egg were laid in lower concentration of the extract and control. In oviposition deterrent and gravid mortality assay, the OAI values also indicated 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, caused by treatment of plant extract which contained of variety of active compounds. All the concentration of plant extracts hared promising mosquito repellency properties when tested against the adult mosquitoes of *Culex quinquefasciatus***.** In the biting deterrency results, increasing in the concentration of plant extracts from 1% to 4% was found to increase the biting deterrency percentage.

KEYWORDS: *Culex quinquefasciatus, Berberis tinctoria*, mortality, biology, repellency

*Corresponding Author:

Dr. D. Jeyabalan

Assistant Professor

Department of Zoology and Wildlife Biology,

Government Arts College

Udhagamandalam-643002

The Nilgiris, Tamilnadu, India.

Email: drjeyabalan@gmail.com

INTRODUCTION:

Mosquitoes are the major vector for the transmission of several communicable diseases like malaria, dengue fever, yellow fever, filariasis, schistosomiasis, Japanese encephalitis, etc., causing millions of deaths every and also cause allergic responses in humans that include local skin and systemic reactions such as angioedema[1,2]. *Culex quinquefasciatus* is a predominant house-resting mosquito in many tropical countries[3]. Breeding in polluted water such as blocked drains, damaged septic tanks, or soak age pools close to human habitations. It is a pan tropical pest and urban vector of *Wuchereria bancrofti* which causes filarial fever[4]. *Culex quinquefasciatus* commonly called the southern house mosquito is the principal vector of lymphatic filariasis caused by *Wuchereria bancrofti* and a potential vector of *Dirofilaria immitis*[5]. It is the one of the most widespread mosquitoes in the world. Lymphatic filariasis is probably the fastest spreading insect-borne disease of humans in the tropics. The disease has a focal distribution, and it is estimated that currently over 2.5 million people are at risk of acquiring filariasis[6]. Both diseases not only cause mortality and morbidity among humans but also cause social, cultural, environmental and economic loss to the society[7].

Chemical insecticides play a major role in efforts to manage mosquito populations. Although chemicals have been used successfully as components of pest management strategies, many of these synthetic insecticides are limited in mosquito control programs. This limitation is largely as a result of environmental contamination and insecticide resistance to DDT and almost all classes of pesticides used for control of disease vectors include pyrethroids, carbamates, and organophosphates[8].

Plants are rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against a number of species including specific target-insects and are eco-friendly. Plant based pesticides are less toxic, delay the development of resistance and are easily biodegradable[9]. Plant based products do not have any hazardous effect on ecosystem. Plant's secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquitoes biodegradable. The phytochemicals derived from plant resources possess a complex of chemicals with unique biological activity. The phytochemicals derived from plant resources can act as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility and smoke toxicity[10].

Many popular practices exist to avoid the deadly disease carried by mosquitoes and nuisance of mosquito bites such as fumigation, burning green leaves on the hut's threshold, mosquito coils, insecticide sprays, and repellents. For example, smoke is a common method of repelling biting mosquitoes that is used throughout the world[11]. Fresh or dried plants are frequently added to fires to enhance the repellent properties of the smoke. Also, this repellency of plant material has been exploited for thousands of years by man, most simply by hanging bruised plants in houses, a practice that is still in wide use throughout the developing countries[12]. Therefore, utilizing homegrown repellents may reduce the need for foreign imports where exchange rate inequalities and transport costs inflate expenditure. Plants have also been used in the form of crude fumigants where plants were burnt to drive away nuisance mosquitoes and later as oil formulations applied to the skin or clothes. Many of the fumigants used produce smoke that is obnoxious to man, and thus was assumed to be repellent to insects. It has also been shown that when applied topically at 2% strength in a base of coconut oil, neem oil provides 100% protection against biting by all Anopheles species during a 12-hour period[13].

Mosquito coils made from dried plants and combustible materials such as sawdust are also a cheap and often an effective method of repelling mosquitoes. As well as being cheap and locally available, natural repellents are normally culturally acceptable and locally known. On the other hand, the use of smoke as a repellent is not desirable due to the respiratory problems it can cause. However, the protection from malaria this practice may provide goes some way to balance out the health risks associated with this method, particularly as the primary use of the fires is for cooking[14]. A possible new, low-technology method to release plant volatiles is thermal expulsion where the plant material is placed on a metal plate over a fire[15]. It appears to provide greater repellency than direct burning of plant material, possibly since it releases different repellent compounds, and may carry a lower risk of causing respiratory disease since less smoke is produced this way.

Oviposition is one of the most important events in the lifecycle of mosquitoes. Therefore, gravid mosquito females show a high degree of preference in selecting oviposition sites (Day, 2016). This preference may be due to the presence of oviposition pheromones or oviposition attractants and repellents in natural habitats[16,17]. Application of oviposition repellent is an effective strategy to control mosquito populations since controlling the egg and larva is easier compared to targeting at the free-flying adult. Essential oils derived from various plants show varied bioactivities against mosquito species. These activities range from toxicity with ovicidal, larvicidal, pupicidal[18,19] to adulticidal activities that include oviposition deterrence and repellent actions[20,21].

The mode of action of the extract as it impacted on the development of the larvae was studied and it was found that there was delay in the development of the larvae to the pupal stage after exposure of the third instar larvae to all the concentrations of the extract and this was especially noted in the lower concentrations. The benefits of larval prolongation is that mosquito larvae numbers are reduced due to the longer periods needed for new generations to complete the mosquito life cycle[22] and many studies have drawn attention to the effects of plant extracts on growth retardation and elongation of developmental periods on mosquito species.

Repellent technologies are similar in regards to their ability to prevent mosquitoes from feeding on susceptible individuals. However, the modes by which these chemistries exert their effects are unique. Biting deterrence may be accomplished by a variety of means including the disruption or interference of proper host-seeking, altering the locomotion of the insect, or causing mortality when in contact with repellent compounds. All of these responses disrupt the ability of the vector to successfully contact and feed on the host, resulting in successful repellency. The act of repelling biting arthropods is not a modern approach. Documented attempts of deterring hematophagous insects date back to antiquity. Among the earliest reports of repellent use are from Herodotus, a Greek historian[23]. His account of communities burning plants to prevent the aggregation of biting flies demonstrates the success of this strategy. Before synthetic chemistry approaches, botanical extracts and mechanical barriers constituted the primary means by which individuals prevented bites from arthropods. Among the most successful plant extracts initially used for the prevention of mosquito bites were citronella, cassia, cedar, lavender, eucalyptus and neem tree oil[24,25].

In fact, many researchers have reported on the effectiveness of plant extracts against mosquito larvae since, they constitute a rich source of bioactive compounds that are target-specific ecological safe, non-development of resistance, reduced number of applications, higher acceptability, and suitability for rural areas[26, 27, 28]. Therefore in the present study I have screened *Berberis tinctoria* leaves extract on the larvicidal, pupicidal, adulticidal, larval, pupal and adult duration, reproductive activity, repellency and biting deterrency of *Culex quinquefasciatus.*

MATERIALS AND METHODS:

Collection and preparation of plant extract

Healthy leaves of *Berberis tinctoria* 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, they have been ground into power, 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 $50^{0}{}_{C}$ and kept in a freezer. In preparing test concentrations, each plant extract was volumetrically diluted in methanol.

Mosquito Culture

Mosquito larvae/eggs of *Culex quinquefasciatus* have been collected in and around Ooty. The mosquito colonies were maintained 27 ± 2^0 C, 75-85% relative humidity index a 4:10 light/dark period cycle[29].

Larvicidal and Pupicidal assays

Larva tested for the present study was obtained from our laboratory culture. Freshly hatched/moulted larvae were used for the bioassay tests. The required of plant extract concentrations were mixed thoroughly with 200ml 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 200ml of rearing water with different solvents served as the control. Dead larvae and pupae was removed and counted at 24hrs 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[30].

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.

Adulticidal assay

Culex quinquefasciatus fresh adults were exposing to filter paper treated with different concentration of plant extracts. The paper was keep inside the beaker. Muslin cloth covering the beaker was also treated. Control insects were exposing only to distilled water with methanol treated paper and muslin cloth. Mortality count was taken after 24hrs[31].

Ovipositional Assay

Different quantities of plant extracts from a stock solution were mixed thoroughly with 200 ml of rearing water in 250 ml glass jars to obtain the concentration desired for the tests with *Culex quinquefasciatus*. The gravid females were given a choice between treated and control jars. During the tests, the groups of females were kept separate for 48hrs in cages measuring 25x 25x30cm. After the eggs were counted the oviposition activity index (OVI) was calculated using the formula:

$$OAI = (Nc-Nt)/ (Nc+Nt) \times 100$$

Where Nc is the number of eggs in the control

Nt is the number of eggs in the treatment

Ovicidal assay

Culex quinquefasciatus eggs were released in water. The test extracts were added in desired quantities and hatching were observed for one week. The eggs were then exposed to deoxygenated water and the number of hatching eggs was recorded. Percentage hatching was compared with the control in which only distilled water with different solvents were used[31].

Repellency Activity

Different concentrations of plant extract were mixed thoroughly with 10ml of goat blood in glass plate. 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 recorded. The repellent index of the plant extract was calculated as previously described[29].

Biting deterrency activity

The percentage protection in relation to dose method was used[32]. Blood starved female *Culex quinquefasciatus* (100 nos), 3-4 days old, was kept in a net cage (45x30x54 **cm** area of the test person was cleaned with isopropanol. After air drying the arm, a 25 mc area of the dorsal side of the skin was exposed, the remaining portion was covered by rubber gloves. The plant extracts were dissolved in methanol, distilled water with methanol solvent served as control. Different concentration of the plant extracts was applied. The control and treated arms was introduced simultaneously into the cage. The numbers of bites was count over 5mints from 6pm to 6am. The experiment was conduct five times. The percentage protection was calculated by using formula:

$$\text{Percentage protection} = \frac{(\text{No. of bites received by control arm}) - (\text{No of bites received by treated arm})}{(\text{No of bites received by control arm})}$$

Statistical analysis

All data was subject to analysis of variance and the treatment mean was separated by Duncan's Multiple Range Test[33]. Statistical analysis was carried out using the (Statistical Package Social Science) SPSS software, version 16.0.

RESULTS:

The locally grown endemic medicinal plant *Berberis tinctoria* was collected and the solvents extracts of their leaves were tested for larvicidal, pupicidal, adulticidal, larval duration, pupal duration, adult duration, reproductive activity, repellency and biting deterrency of *Culex quinquefasciatus*. The assay of the investigated plant species were carried out using different concentration with methanol on *Culex quinquefasciatus*. The plant was more effective at high concentrations, the toxic effect however increased with increase in the concentrations of the extract. A moderate effect of plant extracts were observed at lower concentration but exhibited higher activity as the concentration increased.

Table 1. LC_{50} and LC_{90} values of methanol leaf extracts of *Berberis tinctoria* against larvae of *C. quinquefasciatus*

Mosquito Larval stages	LC_{50} (%)	LC_{90} (%)	95% confidence limit				X^2 (df)	Regression Equation
			LC_{50}		LC_{90}			
			LCL	UCL	LCL	UCL		
1st Instar	1.45	5.97	0.99	2.02	3.80	14.39	6.25(3)	Y=-.335+2.083X
2nd Instar	1.70	6.96	1.47	1.95	5.56	15.36	5.21(3)	Y=-.480+2.090X
3rd Instar	2.36	7.81	1.71	3.34	5.04	18.12	7.18(3)	Y=-.916+2.462X
4th Instar	2.57	8.23	1.91	3.58	5.41	21.99	6.61(3)	Y=-1.040+2.535X

LC_{50}, LC_{90}= Lethal Concentration, LCL= Lower confidence Limit, UCL = Upper confidence Limit, X^2 - Chi-square value, df= degree of freedom, Significant at $P \leq 0.05$, PFOBIT=Intercept + BX (Covariates X are transformed using the base 10.00 logarithm).

The LC_{50} and LC_{90} values of *Berberis tinctoria* with methanolic leaf extract is given in Table - 1). The result reveals that, *Berberis tinctoria* requires less concentration to bring out 50% and 90% mortality of *Culex quinquefasciatus* larval forms and the LC_{50} and LC_{90} values of the experimental plant for 1st instar larva gives the result (1.45; 5.97). It can be clearly understood by this observation that exhibits higher mortality at lower concentration. Similar observation is registered for 2nd, 3rd and 4th instar larval forms. When comparing between the solvent and control, it can be clearly seen that, methanol leaf extract exhibits higher mortality at low concentration than control.

Table 2. Effect of methanol extracts of *Berberis tinctoria* on the pupa and adult of *C. quinquefasciatus*

S.No	Treatment	Concentration (%)	Pupal Mortality (%)	Adult mortality (%)	Adult emergency (%)
1	Control		00^d	00^d	100^a
2	*Berberis tinctoria*	1	25^c	28^c	82^b
		2	36^b	38^b	62^c
		4	58^a	56^a	40^d

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

Plant extracts with methanolic extract of *Berberis tinctoria* was screened for their pupal mortality (%), adult mortality (%) and adult emergence (%), shown in Table 2. It was profound to note that, the plant extract showed promising effect on pupae and adult. It is interesting to note that, even the methanolic extracts showed dose dependent relationship ie., direct relation to pupal mortality (%) and adult mortality (%) and indirect relationship with respect to adult emergence (%). Thus the following trend of mortality percentage was observed in pupae and adult at all stages of the selected plant within the experimental conditions showed remarkable result of dose response of increased mortality with increase in concentration of the extracts in the solvent.

Results of laboratory testing for plant extract of *Berberis tinctoria* was screened for adult repellency (%) and ovipositional deterrency (%). It was very interesting to note that in comparison with all our treated groups, methanolic extract showed the highest adult repellency (%) and ovipositional deterrency (%). In respect to adult repellency (%), *Berberis tinctoria* recorded the least activity among all the concentration (16%, 25% and 32%). Similar observation in respect to concentration gradient was observed for ovipositional deterrency (%) for methanolic extract (Table 3).

Table 3. Effect of methanol extracts on adult repellency and oviposition deterrency of *C. quinquefasciatus*

S.No	Treatment	Concentration (%)	Adult repellency (%)	Ovipositional deterrency (%)
1	Control		00^{d}	00^{d}
2	*Berberis tinctoria*	1	16^{c}	25^{c}
		2	25^{b}	36^{b}
		4	32^{a}	52^{a}

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

After the treatment of *Culex quinquefasciatus*, larvae were assessed for their duration. The plant with methanol extracts of *Berberis tinctoria* was screened in (Table 4). Summatively, it was observed that the larvae took more time to develop into pupae in all the treatments when compared to the untreated group control. At maximum concentration of 4% with all the solvent extracts of plants gave prolonged larval duration in all the instars compared with the control, the total developmental period was observed to be increased with increasing concentration of treatments. Dose – response relationship was determined for plants applied to *Culex quinquefasciatus*. Increase in the concentration of the extracts, increase in the developmental duration, which clearly reveals the dose – response relationship. The duration of larval instars and the total developmental time were prolonged. The possible reason could be a harmonic mimic, on the other hand we are not aware of the exact mechanism to reveal this effect. Hence forth, in our present study the application of plant extracts greatly affected the developmental duration at every concentration, which shows promising efficacy and delay in the growth of *Culex quinquefasciatus* which is a satisfying result for further study of these plants.

Table 4. Larval duration of *C. quinquefasciatus* after the treatment of methanol extracts of *Berberis tinctoria*

S.No	Treatment	Concentration (%)	Total larval duration (days)			
			1st instar	2nd Instar	3rd Instar	4th Instar
1	Control		1.2	1.8	2.4	3.1
2	*Berberis tinctoria*	1	1.5[b]	2.5[c]	2.9[c]	3.4[b]
		2	3.6[a]	3.6[b]	4.2[b]	5.4[a]
		4	3.8[a]	5.0[a]	5.2[a]	6.0[a]

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

The developmental metamorphosis for pupae and adult developmental duration (days) was recorded for the plants with methanol extracts of *Berberis tinctoria* (Table 5). Analysis of methanolic extract with total pupal duration (days) revealed the fact that *Berberis tinctoria* had lower total pupal duration of 6.2 at 4% treatment days. The observed results clarify that treated individuals took prolonged larval and pupal period when compared to control in our test group. Total larval duration increased and total adult duration decreased significantly with increased concentration among our treated individuals of *Culex quinquefasciatus*, exposure to plant extracts to treated groups exposed the gradual increase in pupal duration and decrease in adult longevity.

Table 5. Pupal and adult duration of *C. quinquefasciatus* after the treatment of methanol extracts of *Berberis tinctoria*

S.No	Treatment	Concentration (%)	Total Pupal duration (days)	Total Adult duration (days)
1	Control		3.1[d]	71[a]
2	*Berberis tinctoria*	1	4.2[c]	58[b]
		2	5.0[b]	45[c]
		4	6.2[a]	30[d]

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

Action of methanol extracts of *Berberis tinctoria* on hatching of *Culex quinquefasciatus* eggs freshly laid were obtained from the general stock of mosquitoes and tested for their hatching ability in relation to the different concentrations of methanol extract of plant (Table 6). Percent hatch of eggs placed in control was 100 %, whereas at 1 %, 2% and 4% concentrations of methanol extracts of *Berberis tinctoria* was 75 %, 64 % and 50% respectively, this concentration shows promising decreased level of egg hatchability. It is also observed that the concentration increased the egg hatchability decreased in extracts of solvents within the experimental condition in agreement with methanol extract the level of egg hatchability was highly decreased significantly with increase in concentration (Table 6).

Table 6. Effect of methanol extract on fecundity and egg hatchability of *C. quinquefasciatus*

S.No	Treatment	Concentration (%)	Fecundity(No. of eggs)	Eggs hatchability (%)
1	Control		204[a]	100[a]
2	*Berberis tinctoria*	1	190[b]	75[b]
		2	185[c]	64[c]
		4	182[d]	50[d]

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

The total number of eggs laid in ovitraps containing the given concentration of methanol extracts of plants was always less than the control. Adult fecundity also was marked decreased by the plant extracts treatment. Among the total number of eggs laid in control 204 was eggs were laid but in the treatments when placed with ovitraps with 1%, 2% and 4% methanol extracts of *Berberis tinctoria* with 190, 185, 182 respectively. The plant extracts drastically reduced the fecundity of females and only adults survived, whereas the adult emergence was also significantly low in our treatment groups when compared to the control subjected to experimental conditions.

In our present study, we enumerated the larval-pupal intermediate (%) of *Culex quinquefasciatus* with various increasing concentration (1%, 2% and 4%) of methanolic extracts of *Berberis tinctoria* represented data in table (Table 7), which showed that the larval-pupal intermediate was recorded in experimental concentration 1%, 2% and 4% concentration with values of 15, 23, and 34 respectively.

Table 7. Effect of methanol extract of *Berberis tinctoria* on larval-pupal intermediate of *C. quinquefasciatus*

S.No	Treatment	Concentration (%)	Larval- Pupal Intermediate (%)
1	**Control**		00[d]
2	*Berberis tinctoria*	1	15[c]
		2	23[b]
		4	34[a]

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

Discussion:

Culex quinquefasciatus it breeds profusely in dirty water collection including stagnant drains, cesspools, septic tank with leaks, burrow pit and almost all organic polluted water collection. In optimum temperature and humidity the lifecycle will be completed in seven day passing through the egg, larval, pupal and adult stages. The house mosquito can transmit zoonotic disease that effects humans and wild and domestic animal such as lymphatic filariasis, avian malaria, encephalitis, western equine encephalitis and west Nile fever and may be a vector of a Zika virus, it causes infection through biting during blood meal.

Plant based products offers better solution to ever growing problem created by synthetic insecticides[34]. Unlike chemical larvicide which is based on a single active ingredient, botanical derived larvicide comprises of multiple active compounds which act concertedly. Therefore leaving little chance for the target insects to develop resistance[35]. Plants contain numerous chemicals, some of which are known for their medicinal and pesticidal properties[36]. Thus, in recent years, plant-based insecticides have become more and more popular. Unlike synthetic chemical insecticides, which have an adverse effect on the environment, phytochemical compounds are relatively safe[37]. There are more than 2000 plant species identified for producing secondary metabolites which is valuable in biological pest control and from this number, 344 plant species have been reported to display mosquitocidal activities[38].

Due to indiscriminate use of synthetic chemicals to control the mosquitoes in the natural habitats, they have developed strong resistance to almost all the chemicals that are available today. Moreover, chemical pesticides gradually altered the behavior of non-target organisms. Thus, in this context, the world scientific community intensively searching for the alternative mosquitocidal agent preferably from plants available in nature. Today, the environmental safety of an insecticide is considered to be of important milestone in the field of pest control in general and vector control programme in particular. An insecticide must not cause high mortality in target organisms in order to be acceptable[39].

In the present study experimental results of methanol solvent extracts of *Berberis tinctoria* was accessed and revealed to be more toxic to immature stages of *Culex quinquefasciatus*. The early instar larvae were more vulnerable than the later ones and the pupae, which was not much affected by the solvent. This may be due to the non-feeding behavior of pupae, whereas the bio-pesticide enters the insect system through oral feeding and affects the gut and other organs. These results are also comparable to earlier reports of Murugan *et al.*[40] who have reported that the bio-larvicidal and pupicidal activity of *Acalypha alnifolia* against the I to IV instar larvae and pupae of *C. quinquefasciatus* mosquitoes with LC_{50} value of I instar was 5.388%, II instar was 6.233%, III instar was 6.884%, IV instar was 8.594% and pupae was 10.073%. Further, Prathibha *et al.*[41] have reported the larvicidal efficacy of *Euodia ridleyi* against *C. quinquefasciatus*. Rawani *et al.*[42] established the larvicidal properties of crude extracts of *Carica papaya*, *Murraya paniculata* and *Cleistanthus collinus* against *C. quinquefasciatus* and suggested that the presence of many bioactive principles such as steroids, alkaloids, terpenoids, saponins, etc. which may be responsible for their biocontrol potentiality.

The mosquito larval control using larvicidal agents is a major component in the control of vector borne diseases. Plant as potential larvicides is considered as viable and preferred alternative in the control of the mosquito species at the community level. A large number of plant extracts have been reported to have mosquitocidal or repellent activities against mosquito vectors, but few plant products have shown practical utility for mosquito control[43].

In the present study, all concentrations of plant extracts used exhibited repellency activity against *Culex quinquefasciatus* females. The present study indicates that the methanol extract of plant was more effective in exhibiting a repellency action against the mosquito tested compared to control. Many plant extracts and essential oils manifest repellency activity against different mosquito species. The present results are in accordance with results obtained by Sharma *et al.*[44]

using extracts from the seeds of *Azadirachta indica* against *Anopheles culicifacies* and *Culex quinquefasciatus*. Mansour *et al*.[45] testing different extracts from Nigella sativa seeds against *C. pipens*,.

In the present laboratory experiment observed that as the concentration increased the egg hatchability decreased in extracts of solvent within the experimental condition in agreement with methanol extract the level of egg hatchability was highly decreased significantly with increase in concentration. Similarly Rajkumar *et al*.[46] reported that the extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemical present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion. It is evident from the present study that exposure of eggs of selected mosquitoes. Similar kind of observation was also noted earlier by several workers[46, 47].

In the present work, the larvicidal effect of a local medicinal plant of *Berberis tinctoria* was evaluated against the different larval stages of the most abundant mosquito species *Culex quinquefasciatus*. The treatment with this plant extracts at different concentrations show a toxic effect expressed high mortality to the controls. The search of natural products, specifically the plant extracts that can be used as products in mosquito control. Likewise, lethal concentration of aqueous extract (321.75 ppm) and silver nanoparticles (9.65 ppm) of *Berberis tinctoria* (Nilgiri barberry) leaf which killed 50% of 4th instar *Aedes albopictus* larvae were found to be safe against *Tx. splendens* larvae and *Mesocyclops thermocyclopoides* (copepod) larvae[48].

The present investigation revealed that methanolic leaf extract of *Berberis tinctoria* possesses remarkable ovicidal and development effects against *Culex quinquefasciatus* mosquitoes. The above mentioned plant has mosquito larval control agents. Researches demonstrated that these mosquitoes larvicidal are cost effective to its easily available raw material, while the inorganic insecticides are expensive and environmentally hazardous. Hence, the natural products are novel potent drug generally preferred for control of mosquito due to their environmentally safe, inexpensive and biogradable nature. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plant. Theses extracts are inexpensive, easy to handle and safer products for the control of mosquito in immature and mature stages.

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