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EVALUATION OF THE EFFICACY OF THREE PLANT EXTRACTS AS POTENT MOSQUITO LARVICIDES, PUPICIDES, AND ADULTICIDES

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ABSTRACT

Mosquitoes are the major cause of several diseases all over the world. Plants have a variety of small organic molecules which are secondary metabolites, and act as natural insecticides This study was planned to evaluate the insecticidal effect of *Citrus clementina Hort***.** peel and whole fruits of *Mimusops elengi L.* and *Ricinus communis L.* on the larvae, pupae & adult stages of *Culex quinquefasciatus*. The tested plant parts were collected fresh and their efficacy was confirmed at different concentrations. The crude extract of three plant parts in different graded concentrations, i.e., 0.1, 0.2, 0.3, 0.4, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml, and 1 ml, showed significant larval mortality at $P > 0.05$. 100 % larval mortality was observed at 1 ml concentration of peel extract of *C. clementina* followed by 93.33% (fruit extract of *M. elengi*) and 86.67 % (fruit extract of *R. communis*) against 2nd instar larvae of *Cx. quinquefasciatus*. The lowest LC_{50} value calculated was 0.30 ml, 0.21 ml, and 0.27 ml of peel extract of *C. clementina* and crude fruit extract of *R. communis* and *M. elengi* respectively. The crude extracts of both fruits and peel also showed pupicidal and adulticidal activity with LC_{50} values of 4.23 ml, 0.22 ml, and 8.44 ml; 0.21 ml, 3.98 ml, and 0.42 ml, respectively. In the laboratory, qualitative phytochemical tests are also carried out. The result of the phytochemical analysis indicates the presence of secondary metabolites such as flavonoid, phenolic compound, phlobatannin, tannins, and protein in the crude extract of the tested plant parts of three plants. Observation under a compound microscope was done to find out the potential cause of the death of mosquito larvae and pupae. From the results, it can be concluded that the crude extracts of tested plant extracts have an excellent potential to be used as an ideal eco-friendly approach to control the different life forms of *Cx. quinquefasciatus.*

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INTRODUCTION

Mosquitoes are the principal vector of many vector-borne diseases affecting human beings and animals, in addition to nuisance (Panneerselvam *et al*. 2012). It is the most important single group of insects in terms of public health problems, which transmit several diseases, such as malaria, filariasis, dengue, Japanese encephalitis, chikungunya, and yellow fever, causing millions of deaths every year in almost all tropical and subtropical countries and many other parts of the world (Ghosh *et al*. 2012). *Culex quinquefasciatus*, generally known as the indoor domestic mosquito, is a night-biter. It transmits zoonotic diseases such as lymphatic filariasis, avian malaria, St. Louis encephalitis, Western equine encephalitis, and West Nile fever that distress humans, wild animals, and domestic animals. Presently 863 million people in 47 countries worldwide remain threatened by lymphatic filariasis and require preventive chemotherapy to stop the spread of this parasitic infection (WHO, 2022).

In India alone, 25 million people harbor microfilaria (mf), and 19 million people suffer from filarial complaint manifestations (NICD, 1990; Maheswaran *et al*., 2008). *Culex quinquefasciatus* is capable of transmitting the Ross River virus, Alfay, Almpiwar. Coriparta, dengue, Japanese encephalitis virus (Ruben *et al*. 1994), reticulo - endothelialis virus (Holder *et al*., 1999). In addition, *Cx. quinquefasciatus* is a laboratory host for a variety of other arboviruses, including Murray Valley encephalitis (Weinstein *et al*, 1997). The approach to combat different mosquito-borne diseases largely relies on interruption of the disease transmission cycle by either targeting the mosquito larvae at breeding sites through spraying of stagnant water or by killing/repelling the adult mosquitoes using different insecticides (Corbel *et al*. 2004; Joseph *et al*, 2004). The use of synthetic insecticides has generated some environmental problems, such as the expansion of resistant insect strains, ecological disparity, and harm to other non-target organisms (Maheswaran *et al*., 2008). Synthetic insecticides have been used repeatedly to control mosquitoes, disrupting natural biological control mechanisms and causing resurgences in mosquito populations (Das *et al*. 2007). Phytochemicals are botanicals that are obtained from floral resources and can act as natural insecticides (Arivoli *et al*. 2016). They are responsible for the color and organoleptic properties of the plant (Yadav *et al* 2017). Botanicals are secondary metabolites that serve as means of defense mechanism for plants to withstand the continuous selection pressure from herbivore predators and other environmental factors (Roy *et al*. 2016). Plants have a vast repository of secondary metabolites such as steroids, alkaloids, terpenes, phenolic compounds, etc. that are either singly or in combination responsible for their insecticidal properties (Bekele, 2018). Insecticidal effects of plant extracts can vary according to plant parts used, plant species, the species of mosquito tested, and geographical position (Ghosh *et al*., 2012). Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their larvicidal, pupicidal, adulticidal, repellent, and ovicidal properties (Panneerselvam *et al*., 2012). Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water and, thus, are easy to deal with them in this habitat (Amer *et al*. 2006). Another important aspect of these plant-based insecticides is that they are environmentally safe and biodegradable, and there is no fear of the development of resistance among target species. There is a need for an alternate source with minimum risk to the environment and human health, and this study intends to identify potential plant-based mosquito larvicides against the mosquito vectors *Cx. quinquefasciatus*. The present piece of work was done to evaluate the larvicidal, pupicidal, and adulticidal activities of extracts of seeds of two plants namely *Mimusops elengi* L. and *Ricinus communis* L., and peel extract of *Citrus climentina* Hort. Against *Culex quinquefasciatus* Say. The study also includes a qualitative phytochemical analysis of the tested plant extracts to determine the nature of the active ingredient responsible for their insecticidal properties. A microscopic study of dead larvae was also done to define the possible mode of action of the tested plant parts.

MATERIAL AND METHODS

Study area: This study was conducted in the laboratory of Parasitology, Vector Biology, and Nanotechnology, Department of Zoology, University of Gour Banga, Malda. The period of the study was from February 2022 to August 2022.

Collection of plant material and authentication: Fresh *Citrus climentina* were collected from the market nearby. Fresh fruits of *Mimusops elengi* (Sapotaceae), *Ricinus communis* (Euphorbiaceae) were collected between April and May 2022, from premises of University of Gour Banga, Malda (25.0119°N 88.1433°E), India. The plants were authenticated by Dr. Monoranjan Chowdhury, Professor, Department of Botany, University of North Bengal, Darjeeling district, India. All the collected samples were washed twice with water to remove the adhering dusts and other associated animals.

Collection and maintenance of larvae: Larvae of all the instars of *Culex quinquefasciatus* were collected from drains surrounding the university locality in the plastic bucket (5 l). They were brought into the laboratory and were transferred into the plastic trays (45×07×30 cm) containing 500 ml of water from their natural habitat. The larvae were maintained 25℃-27℃, 75-85% related humidity and 14L:10D photoperiod.

Maintenance of pupae and adults: The pupae were collected from the trays and transferred to plastic containers containing 500 ml of water with the help of dropper. The plastic jars were kept in a 45×34×45 cm mosquito cage for adult emergence. A 10% glucose solution was provided for their nutrition.

Crude extract preparation: The collected fresh fruit samples and peel of *C. climentina* were washed with tap water to remove any dirt and then grinded using a mechanical grinder and the extract was squeezed out by passing it through a cotton cloth with mesh size of 20 µm. The extract was collected and different concentration gradient was prepared. The left-over extract was stored at 4℃ for future use (Rawani *et al*., 2009).

Larvicidal bioassay: 1st, 2nd, 3rd and 4th larval instar of *Culex quinquefasciatus* were used for this experiment following the methodology of WHO, 2005. Twenty-five larvae of each instar were taken into beaker containing 100 ml of water. Required concentrations (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0%) of the crude extracts of tested plant parts were added to each beaker following a low to high gradation of concentration. A control set up was also there for each instar containing 100ml of tap water only. The larval mortality was observed for up to 24 hours. The larvae were declared dead when they didn't show any movement when probed with a needle in the siphon or cervical region. The experiments were replicated thrice at three different days. The percentage mortality observed (%M) was corrected using Abbott's (1925) formula during the observation of the larvicidal potentiality of the plant extract (WHO, 2005; Subramanium *et al*., 2012; Ullah *et al*., 2018).

Pupicidal bioassay: Pupae were taken into five glass beakers (100 ml capacity containing 100 ml tap water) using a pipette. Five different concentrations of the fruit extracts (0.5, 0.8, 1, 2 ,3 ml) taken up for the experiment. A control was also run simultaneously which comprised of water only. The experiment was carried out in a glassbeaker for 24 h at room temperature and after 24 hours the mortality rate was recorded and assessed. The pupal mortality in each concentration and control was recorded after 24 hours of exposure (WHO, 1975).

Adulticidal bioassay: *Cx. quinquefasciatus* adult mosquitoes were selected for this bioassay.0.5, 1, 2 and 3 ml fruit and peel extracts were collected in a petridish. Then the extracts were impregnated on filter papers. Then the filter papers were dried at room temperature. A cotton bed soaked with 10% glucose solution was prepared and attached with cellotape on net. A 500 ml beaker was taken for this experiment. The soaked filter paper was placed at the bottom of the beaker. For each concentration 25 mosquitoes were taken. The mosquitoes were transferred from the cage to the beaker and the top of the beaker was surrounded by the cotton bed. The set up was held for 24 hours and after that the percent mortality of the adult mosquitoes were counted (Saini *et al*. 1986).

Corrected mortality =

$$\frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae/pupae}}{\text{Number of larvae /pupae introduced}} \times 100$$

Qualitative phytochemical analysis: The peel of *C. climentina*, fruits of *M.*elengi and *R. communis* were collected and then washed properly with tap water. The plant parts were then shade dried at room temperature for two weeks and grinded to powder. The resultant crude fine powder was used for phytochemical investigations according to Trease and Evans, 1989, Choudhury *et al*. 2012, Mallick *et al*. 2016 &Rawani 2022.

Examination of dead larvae: The treated and untreated dead larvae were examined in a compound microscopic 10 x view image to determine the possible cause of the death of the larvae.

Statistical analysis: The percentage of larval mortality (M %) was corrected using Abbott's formula. Statistical analysis of the experimental data was performed using the computer software Stat plus 2009 and MS EXCEL 2013.

RESULTS

In the present study, the details of the plants such as common name, morphological description, and medicinal uses were given in Table 1. The result of larvicidal activity against all the instars of *Cx. quinquefasciatus* in different concentrations ranges from 0.1 to 1 % of crude extract of different plant parts of *M. elengi, R. communis*, and *C. clementina* presented in Table 2.

Table 1. Description of the plants tested for their mosquitocidal activity in this study

Plants	Common name	Systematic position (Order: Family)	Parts used	Morphological description	Medicinal properties
Mimusops elengi	Spanish Cherry (English) Bakul (Bengali)	Ericales: Sapotaceae	Fruit	grows from15-30 metres, Bark: dark grey Leaves: simple and spiral Flowers: star shaped, small, white Fruit and seed: berry, yellow ovoid, ellipsoid, reddish brown when ripe, seed one, (Khatri et al. 2014).	It has a long history of being used in Indian traditional medicine (Niranjan et al. 2009). The various extracts of the plant (bark, fruit, leaves, seed and flowers) have been reported to be cardio tonic, alexipharmic, stomachic, hypotensive, antibacterial, anthelmintic, anti-gastric ulcers, teeth cleaner and antimalarial activities (Ganu et al. 2011).
Ricinus communis	Castor oil plant	Malpighiales: Euphorbiaceae	Fruit	Fast- growing, grows around 12 metres, Leaves: alternate, curved, large, Flowers: monoecious, large Fruit: blunt, greenish, deeply grooved, tricoccus, seeds are ovoid in shape. (Polvèche, 1996; Ivan, 1998).	*Ricinus communis* possess wound healing activity due to active constituent of castor oil.Various medicinal properties of *R. communis* are well-documented in the literature such as hepatoprotective, anti-inflammatory, diuretic, anticancer, antiasthmatic, antibacterial, antidiabetic, insecticidal, hypoglycemic and free radical scavenging (Visen et al. 1992; Ilavarasan et al. 2005; Nath et al. 2011; Sawhney et al. 1978; Sharma et al. 1990; Shokeen et al. 2008).
Citrus clementina	Clementines	Rutales: Rutaceae	Peel	*Citrus clementina* hort. is a two to three-meter-high tree, bearing highly fragrant leaves and flowers. The leaves are dark green, 3-4 cm long, glistening and lanceolate. As for the flowers, they are white and joined in clusters. Flattened at the poles, the fruit has a spherical shape as well as a thin, orange, and slightly adherent peel. The pulp is juicy, fragrant and practically seedless (Hamdani, 2018; Robin, 2011).	The clementine fruit is widely consumed as a natural source of antioxidant compounds such as vitamin C, vitamin B, pectin, carotenoids, etc. (Ghasemi *et al.,* 2009; Mahrouz *et al.,* 2002). The Clementine leaves have antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and nutritional properties (Al-Gendy *et al.,* 2017; Bissim *et al.,* 2020; Butelli *et al.,* 2019; Menichini *et al.,* 2011; Rao and Gajula, 2016; Singh *et al.,* 2020).

Table 2. Mean larval mortality of larvae of *Cx. Quinquefasciatus* mosquitoes at different concentration of crude extracts of fruits of *Mimusops elengi, Ricinus communis* and peel extract of *Citrus climentina*

Larval instar	Concentration (%)	Mortality rate (%) (Mean ± Standard Error)after 24 hours		
		Mimusops elengi	***Ricinus communis***	***Citrus climentina***
1st instar	0.1	30.00±0.58	41.33±0.88	16.00±1.15
	0.2	40.00±0.58	46.67±0.33	36.00±0.58
	0.3	46.67±0.67	50.00±0.88	38.00±1.15
	0.4	56.67±0.33	60.00 ±0.58	56.00±1.15
	0.5	66.00±1.15	66.67±0.67	66.00±1.52
	0.6	69.33±0.33	73.33±0.33	77.67±1.45
	0.7	73.33±0.58	80.00±0.58	80.33±0.33
	0.8	83.33±0.33	83.00±0.58	90.00±0.58
	0.9	86.00±0.88	86.67±0.88	91.67±0.58
	1	93.33±0.67	90.00±0.58	99.33±0.33
	Control	0.00	0.00	0.00
2nd instar	0.1	30.00±0.58	36.67±0.88	15.00±1.15
	0.2	40.00±0.58	46.67±0.88	34.00±0.88
	0.3	43.33±0.67	53.33±0.33	44.00±1.15
	0.4	49.33±0.88	60.00±1.15	54.00±1.15
	0.5	50.00±0.58	66.67±0.88	64.00±1.52
	0.6	66.67±0.88	70.11±1.15	71.33±0.88
	0.7	70.00±0.58	73.33±0.33	80.00±0.58
	0.8	83.33±0.88	80.00±0.58	89.00±0.58
	0.9	90.00±0.58	83.33±0.67	96.33±1.20
	1	93.33±0.33	86.67±0.33	100.00±0.00
	Control	0.00	0.00	0.00
3rd instar	0.1	20.00±1.15	33.33±0.33	22.00±1.15
	0.2	23.33±0.67	43.33±0.88	29.67±0.88
	0.3	30.00±0.58	53.33±0.67	40.00±0.88
	0.4	46.67±0.67	70.00±1.15	49.67±0.88
	0.5	56.67±0.88	76.67±0.33	62.33±1.45
	0.6	66.67±0.33	80.67±0.88	75.00±1.15
	0.7	73.33±0.33	83.33±0.33	82.00±1.15
	0.8	80.00±1.15	86.67±0.67	90.00±0.58
	0.9	83.33±0.88	89.67±0.88	94.00±0.58
	1	90.00±0.58	90.00±1.15	97.00±0.58
	Control	0.00	0.00	0.00
4th instar	0.1	6.67±3.33	6.67±0.33	12.67±1.76
	0.2	13.33±0.33	10.00±0.88	16.67±0.88
	0.3	16.33±0.33	16.67±0.88	25.00±1.15
	0.4	26.67±0.33	23.33±0.67	36.00±0.58
	0.5	33.33±0.33	30.00±1.15	40.00±0.58
	0.6	40.00±1.15	33.33±0.67	51.00±0.58
	0.7	50.00±1.45	36.67±0.33	61.00±0.58
	0.8	60.00±0.58	46.67±0.33	66.00±0.58
	0.9	66.67±0.67	53.33±0.33	71.00±0.58
	1	73.33±1.45	63.33±0.33	76.00±0.58
	Control	0.00	0.00	0.00

Among all the larval instar, the highest mortality was observed in 2nd instar larvae that showed 100 % mortality in peel crude extract of *C. clementina* followed by 93.33 % in fruit crude extract of *M. elengi* but the fruit crude extract of *R. communis* showed the highest mortality of 3rd instar larvae (90 %) after 24 hours of exposure. Table 3 presented the mean pupal percentage mortality of crude fruit extracts of *M. elengi, R. communis*, and peel extract of *C. clementina* against *Culex quenquefasiatus* pupae.

Table 3. Mean pupal mortality of pupa of *Cx. quinquefasciatus* mosquitoes at different concentration of crude extracts of fruits of *Mimusops elengi, Ricinus communis* and peel extract of *Citrus climentina*

Concentration (%)	Mortality rate (%) (Mean ± Standard Error) after 24 hours		
	Mimusops elengi	*Ricinus communis*	*Citrus climentina*
0.5	6.67±0.33	6.66±0.33	10.00±0.88
0.8	13.33±0.33	10.00±0.00	16.67±0.33
1	23.33±0.33	16.67±0.33	26.67±0.33
2	30.00±1.15	20.00±1.15	33.33±0.33
3	40.00±1.15	30.00±1.15	43.33±0.33
Control	0.00	0.00	0.00

Values are the mean of 3 (n=3±SE).

Table 4. Percentage mortality of adults of *Cx. quinquefasciatus* mosquitoes at different concentration of crude extracts of fruits of *Mimusops elengi, Ricinus communis* and peel extract of *Citrus climentina*

Concentration (%)	*Mimusops elengi*	*Ricinus communis*	*Citrus climentina*
0.5	81.00±0.58	75.33±0.33	61.00±0.58
1	90.67±0.33	85.67±0.67	80.67±0.67
2	99.33±0.33	91.00±0.58	97.67±0.33
3	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Table 5. LC_{50} and LC_{90} values for larvicidal, pupicidal and adulticidal activity of tested plant parts

Larval instar	*Mimusops elengi*		*Ricinus communis*		*Citrus climentina*	
	LC_{50} (ml/100ml)	LC_{90} (ml/100ml)	LC_{50} (ml/100ml)	LC_{90} (ml/100ml)	LC_{50} (ml/100ml)	LC_{90} (ml/100ml)
1st	0.27	1.40	0.21	1.52	0.30	0.93
2nd	0.30	1.56	0.23	1.86	0.31	0.92
3rd	0.38	1.41	0.21	1.06	0.31	0.99
4th	0.67	2.34	0.89	4.02	0.54	2.23
Pupicidal activity	4.23	30.29	8.44	95.57	3.98	36.11
Adulticidal activity	0.22	0.81	0.21	1.28	0.42	1.25

Table 6. Result of qualitative phytochemical analysis of the crude extract of the tested plants

Phytochemicals	*Mimusops elengi*	*Ricinus communis*	*Citrus climentina*
Alkaloids	++	++	--
Anthraquinones	--	--	--
Flavonoids	++	++	++
Glycoside	--	--	--
Phenolic compounds	++	++	++
Phlobatannin	++	++	++
Proteins	++	++	++
Saponins	--	--	++
Steroids	--	--	++
Tannins	++	++	++
Terpenoids	++	--	++
Carbohydrate	++	--	--

++ Presence of the compound; -- Absence of the compound

Here highest pupal mortality was observed in 3 ml concentration of peel crude extract of *C. clementina* (43.33 %) followed by crude fruit extract of *M. elengi* (40 %) and *R. communis* (30 %) after 24 hours of exposure. Table 4 showed the 100 % mortality of adults of *Culex quinquefasciatus* in the crude extract of the fruit of *M. elengi*, *R. communis*, and peel extract of *C. clementina* after 2h h of contact at 3 ml concentration. $LC_{50}$and LC_{90} values of the larvicidal activity, pupicidal activity, and adulticidal activity of crude fruit extracts of *M. elengi*, *R. communis,* and peel extract of *C. clementina* were presented in Table 5. The results of the qualitative phytochemical analysis of all three tested plant parts are presented in Table 6. Among 12 phytochemicals, flavonoid, phenolic compound, phlobatannin, tannins, and protein were present while anthraquinone and glycoside were absent in all three plant-tested parts. The larvae of the studied mosquito species underwent a general behavioral change, and it was noticed that they slowly stopped moving after a few hours of treatment. The microscopic examination of dead larvae showed damage to the cuticle as well as the gut of mosquito larvae in the fruit extract of *M. elengi* (Figure B), while in the case of *R. communis*-treated larvae, there was blockage of spiracles as well as damage to the cuticle (Figure C). Figures D and E showed that the gut is fully destroyed and degenerated, and the external cuticle also starts to degenerate in the peel extract of *C. Clementina*-treated larvae.

Figure A shows the live, untreated larvae, which supports the view of the efficacy of all three tested plant parts. The light microscopic (4x) view of the treated *Cx. quinquefasciatus* pupa after 24 hours of exposure showed a damaged and degenerated cuticle (Figure F). However, further research is needed to prove the above view.

DISCUSSION

Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or the development of newer insecticides. Plant extract exerts myriads of biological activity on pests including larvicidal, pupicidal, repellent, insect growth regulator, etc.

Fig. A. Normal untreated larva

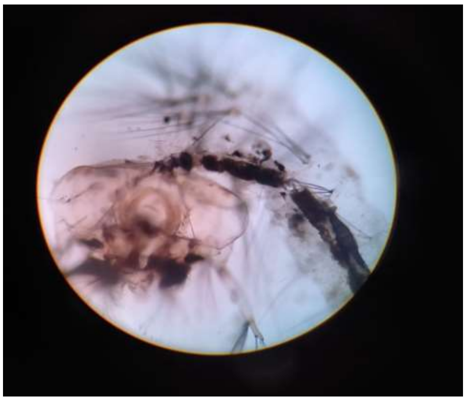

Fig. B. Treated larvae of *M.elengi*

Fig. C. Treated larvae of *R. communis*

Fig. D. Treated larvae of *C. clementina*

Fig. E. Treated larvae of *C. clementina*

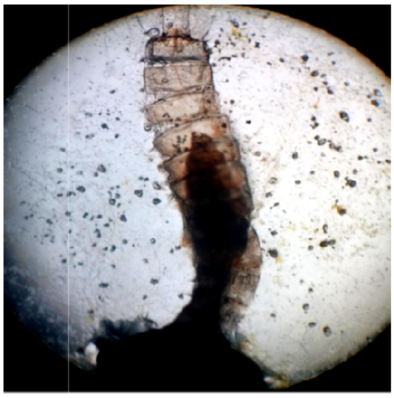

Fig. F. Treated pupa of *C. clementina*

This can be due to various phytochemicals present in the plants might be acting synergistically to produce such responses. Botanical pesticides are biodegradable and rarely develop resistance against pests because of the synergistic activity of complex biomolecules thus moderating the long-term environmental effects of synthetic pesticide use (Maurya *et al*. 2012). Phytoextracts are emerging as potential mosquito control agents, with low-cost, easy-to-administer, and risk-free properties as compared to isolated or synthesized biopesticides, and can be used successfully in mosquito management (Rahuman and Venktesan 2008). Nowadays, the mosquito control program is focused more on the elimination of mosquitoes at the larval stage with plant extract. *C. climentina*, *M. elengi*, and *R. communis* have the potential to be employed in vector control programs because of the abundance of phytochemicals it contains. A considerable number of plant derivatives have shown to be effective against mosquitoes in a safe manner. Euphorbiaceae and Sapotaceae species are commonly used in traditional medicine and have been reported to possess various biological activities. Vimala *et al*. 2020 studied that an aqueous extract of *M. elengi* seeds showed an LC50 value of 18.75 µg/ml after 24 h of exposure against *Cx. quinquefasciatus*. Rawani *et al*., 2013 studied the efficacy of crude and chloroform: methanol (1:1, v/v) extracts of fresh, mature, green berries of *S. nigrum* against *Cx. quinquefasciatus*. The mortality rate at 3 % concentration was higher (P <0.05) than all other concentrations of the crude extract tested for larval mortality. The highest mortality observed was 86.70% 1st instar larvae after 72 h of exposure and the LC_{50} value calculated was 2ml after 24 h of exposure. In the present study, after 24 h of the exposure period, the larvicidal activity for the crude extract of *Mimusopselengi* fruits showed LC_{50} value is 0.27 ml against the 1^{st}instar larvae of *Cx. quinquefasciatus*. Crude extract of seeds of *Carica papaya* was tested 3rd larval instars of *Cx. quinquefasciatus* and *An. stephensi* by Rawani *et al*, 2012 and 0.5% concentration showed significantly higher (p < 0.05) mortality rates than the other concentrations such as 0.1%, 0.2%, 0.3%, and 0.4% concentrations of crude plant extract after 24, 48, and 72 hours of exposure. Here LC_{50}value calculated was 0.2 ml after 24 h of exposure. In the present study, at 24 h of the exposure period, the larvicidal activity for the crude extract of *R.communis* fruits showed lowestLC_{50} value is 0.21 ml against the 3rd instar larvae of *Cx. quinquefasciatus*. Mandal, 2010 studied the larvicidal and adult emergence inhibition activities of castor seed ethanol extract against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. albopictus*. LC_{50} for *Cx. quinquefasciatus* was lowest while that for *Ae. albopictus* was the highest, in the order *Cx. quinquefasciatus* (7.10 ppm) <*An. stephensi* (11.64 ppm) <*Ae. albopictus* (16.84 ppm). Mallick *et al*., 2016 studied the different concentrations of aqueous fruit peel extracts of *Citrus limetta* against *Cx. quinquefasciatus* mosquito species. Aqueous extract showed good larvicidal activity with very low concentrations against 1^{st} to 4^{th}instars larvae. The lowest LC_{50} value calculated was 0.05 ml against both 1st and 2nd instar larvae of *Cx. quinquefasciatus* after 24 h of exposure. The efficacy of peel and leaf extracts of *Citrus sinensis* was tested against larvae of *Culex quinquefasciatus* & the results indicated that the different concentrations of peel and leaf extracts have significant larvicidal effects against *Culex quinquefasciatus*. The 2%, 3%, and 4% extracts gave 100% mortality but extracts with 4% concentrations gave mortality in the least days (Sattar & Iqbal, 2016). While in the present study low concentrations (0.1 to 1 ml concentrations of peel extracts) were used and showed 100% mortality at 1 ml concentration of C. clementina peel extract against 2^{nd} instar larvae after 24 h of exposure. In the present investigation *R. communis*, *M. elengi*, and *C. climentina* plant extracts were screened for their larvicidal efficacy against *Cx. quinquefasciatus* and it is observed that all the tested three plant parts showed good efficacy against all the larval stages of the mosquito *Cx. quinquefasciatus*. The highest mortality 100 %, 93.33 %, and 86.67 % were observed in crude peel extract of *C. clementina,* fruit extracts of *M. elengi*, and *R. communis* against 2nd instar larvae of *Cx. quinquefasciatus* respectively in 1 ml concentration. The crude extracts of both fruits and peel also showed pupicidal activity and adulticidal activity with LC_{50} values of 4.23 ml, 0.22 ml, and 8.44 ml; 0.21 ml, 3.98 ml, and 0.42 ml respectively.

The qualitative phytochemical analysis indicates the presence of several phytochemicals such as flavonoid, phenolic compound, phlobatannin, tannins, and protein in crude peel extract of *C. clementina*, fruit extracts of *M. elengi*, and *R. communis*. Among the three plant parts, the peel extract of *C. clementina* showed better results. The microscopic studies of dead larvae and pupae also support the potency of these three plant extracts against *Cx. quinquefasciatus*.

CONCLUSION

In recent years, eco-friendly and plant-based insecticides have gained renewed importance. These are safe to use in aquatic ecosystems due to their target specificity and do not cause resistance to target species. The present study revealed that the peel extract of *C. climentina* and fruit extract of *M. elengi* and *R. communis* fruit extract could be used as a natural mosquito larvicide, pupicide, and adulticide against the *Culex quinquefasciatus***.** It will be cost-effective as it works at a very low dose rate; and it is indigenously available, easily biodegradable, and safe in comparison to synthetic insecticides instead which are harmful to the environment and also toxic. The results reported in this study open the possibility of further investigations on the nature and structure of active ingredients responsible for mosquitocidal activities.

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