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Article

Larvicidal efficacy of selected medicinal plants against the filarial vector mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract: The present research was conducted to assess the mosquito larvicidal potential of selected medicinal plants using an effective but simple method. Aqueous extracts of roots of three selected medicinal plants viz. *Derris scandens*, *Rubia cordifolia* and *Saussurea lappa* were evaluated for their mosquito larvicidal potential against the 3rd instar larvae of *C. quinquefasciatus* Say (1823) under laboratory settings. Aqueous extracts of these plants at seven different concentrations (1, 25, 50, 100, 150, 200 and 300 ppm) exhibited considerable mortality of the 3rd instar larvae after 24 and 48 h exposure. Among the plants, *D. scandens* root extract exhibited the highest toxicity inducing 100% larval mortality after 24 h exposure at 250 ppm concentration, followed by the root extracts of *R. cordifolia* and *S. lappa* inducing 98.4% and 87.8% larval mortality, respectively. Overall, extracts of all the plants exhibited a strong positive correlation between the concentration of extracts and larval mortality ($p<0.001$) with a correlation coefficient of more than 0.90. The LC_{50} and LC_{90} values after 24 h contact demonstrated *D. scandens* as the most toxic with the lowest LC_{50} and LC_{90} values (LC_{50}=78.20 ppm, LC_{90}=147.33 ppm) followed by *R. cordifolia* (LC_{50}= 89.32 ppm, LC_{90}=204.09 ppm) and *S. lappa* (LC_{50}=112.29 ppm, LC_{90}=248.72 ppm), respectively. Our results clearly indicated that all the plants' aqueous extracts showed considerable larvicidal potential against the 3rd instar larvae of *C. quinquefasciatus*. To conclude, the application of aqueous extracts from these plants to larval habitats may efficiently control *C. quinquefasciatus* mosquitoes, hence, can be recommended as a potential alternative to chemical insecticides against these vectors.

Keywords: *Derris scandens*; *Rubia cordifolia*; *Saussurea lappa*; *Culex quinquefasciatus*; larvicidal potential; aqueous extract

1. Introduction

Mosquitoes are responsible for the transmission of a number of infectious diseases, such as dengue fever, yellow fever, malaria, filariasis and many types of encephalitis. The southern house mosquito, *Culex quinquefasciatus* Say is a cosmopolitan species. It is a major vector of numerous pathogens, responsible for the transmission of two flaviviruses (West Nile virus and St. Louis encephalitis virus) and the phlebovirus Rift Valley fever virus, filarial worms and avian malarial parasites. In India and Southeast Asia, it is the principal vector of *Wuchereria bancrofti*, a nematode that causes lymphatic filariasis (LF), commonly known as elephantiasis. LF is one of the important causes of acute and chronic morbidity worldwide, infecting 51 million people as of 2018 and 863 million people in 47 countries are at risk of infection (World Health Organization, 2022). The Global

Programme to Eliminate Lymphatic Filariasis (GPELF) carried out mass drug administration (MDA) to more than 923 million people since 2000. However, the target of elimination of LF by the GPELF in 2020 was not successful by then, hence, another target was set to achieve the goal by 2030 (Rahman & Helder, 2021; World Health Organization, 2020).

Conventional synthetic insecticides, viz., organochlorines, organophosphates, pyrethroids and carbamates are being used from several decades to control mosquitoes because of their quick action. Indiscriminate, repeated and large-scale application resulted in decreased efficacy of these insecticides and unintentional artificial selection of resistant mutants within the vector population and development of resistance (Albrieu Llinás *et al*., 2010; Chen *et al*., 2013; Geetha & Shetty, 2018; Mulyatno *et al*., 2012; Sayono *et al*., 2019). Besides the high cost of insecticides, unwanted action on beneficial non-target organisms, and growing public concern over ecological imbalance manifested by environmental pollution have fostered the interest in alternative approaches for control of mosquitoes which would be environment-friendly and specific in their action (Bayen, 2012; Heckman, 1993; Madhu *et al*., 2010; Sutthanont *et al*., 2010). Plant-derived insecticides are such an alternative source and a large number of research works on phytochemicals against vector mosquitoes revealed that they could be used as an alternative to synthetic chemical pesticides (Han *et al*., 2013; Jang *et al*., 2002; Pelah *et al*., 2002; Perumalsamy *et al*., 2009).

Plants possess a variety of bioactive mosquito larvicidal phytoconstituents such as steroids, tannins, terpenes, saponins, etc. These phytochemicals are specific in action, ecofriendly in nature, less toxic to human health and rapidly biodegradable (Ghosh *et al*., 2012; Isman, 2006; Joseph *et al*., 2004). Consequently, consideration has been progressively diverted toward insecticides of plant origin for mosquito control. Phytochemicals are not only being used as general toxicants against mosquito larvae, but are also being used as their growth and reproduction inhibitors as well as repellants and oviposition deterrents (Grainge and Ahmed, 1988).

The present study aimed to determine the larvicidal potential of three medicinal plants viz. *D. scandens* Roxb., *R. cordifolia* Linn., and *S. lappa* Falc. against the 3rd instar larvae of *C. quinquefasciatus* under laboratory settings.

2. Materials and Methods

The present study was carried out to evaluate the larvicidal efficacy of aqueous root extracts of *D. scandens*, *R. cordifolia* and *S. lappa* against the 3rd larval instar of *C. quinquefasciatus* Say under laboratory settings at 24-32^{0}C air temperature, 22-30^{0}C water temperature and 65-92% relative humidity.

2.1. Collection and processing of plant materials

Roots of the medicinal plants viz. *D. scandens*, *R. cordifolia* and *S. lappa* were collected from different local markets of Dhaka, Bangladesh, kept in polyethylene bags and transported to the Entomology laboratory of the Zoology department at Jahangirnagar University. Plant species were identified using available taxonomic keys (Kapoor, 2017; Khare, 2007) and online resources. The plant materials were washed thoroughly with tap water to remove the dusts and other impurities and dried at room temperature for 15-20 days. The dried materials were powdered by using an electric blender. Then the powdered materials were sieved through a kitchen strainer, put in glass sealed cans and finally kept in a refrigerator for extraction.

2.2. Extract preparation

The powdered plant materials were taken out from the refrigerator and subjected to an extraction process using distilled water. The aqueous plant extracts were prepared following the standard protocol used in the Ayurvedic medicine described elsewhere (Rahman *et al*., 2009). For each plant species, about 5 grams of powder was mixed with 100 ml of distilled water in a 250 ml sterilized conical flask. The mixture was reduced to 40 ml by boiling and filtered through Whatman® filter papers which was referred to as collection I. The residue was mixed again with 100 ml water and heated till the volume was reduced to 40 ml and filtered which was collection II. The two filtrates were mixed together and heated gently to get the final volume of 20 ml. Thus 20 ml of 25% (w/v) stock solution known as 'Kwath' was prepared and kept in a freezer until used for mosquito bioassay experiments.

2.3. Mosquito collection and rearing

Wild populations of mosquito larvae were collected from suitable breeding places in Savar area, Bangladesh using a long handled dipper. The larvae were collected in the early morning (7:00 am-9:00 am), put in plastic jars along with water from the larval habitat, covered with fine netting, transported to the laboratory. The larvae were washed with distilled water in earthen pots. The larvae of *C. quinquefasciatus* were identified following

suitable taxonomic keys (Barraud, 1924; Bram, 1967). Finally, the larvae were reared in the laboratory and the larvae of F_2 generation were used for the experiment. Dried yeast and biscuit powder were used as a larval diet during rearing and bioassay experiments.

2.4. Bioassay procedure

Bioassays were carried out using the 3rd larval instars of *C. quinquefasciatus* exposed to concentrations of 1, 25, 50, 100, 150, 200 and 250 ppm plant extracts prepared from the stock solution. Twenty larvae were exposed to a 250 ml beaker containing 100 ml solution of each concentration A control using only 100 ml distilled water was also maintained with each concentration. Five replicates were conducted simultaneously for each concentration and control. Yeast powder, Cerelac baby food and cotton soaked with 10% glucose solution were provided as larval food. The beakers were covered with a fine mosquito net so that other insects could not lay eggs. Larvae showing no sign of movement even after being gently touched with a glass rod were considered dead (Langat *et al*., 2012). Larval mortality was recorded after 24 and 48 h treatment and corrected using the following formula (Abbott, 1925):

$$\text{Corrected mortality (\%)} = \frac{\text{\% mortality in treatment-\% mortality in control}}{\text{100-\% mortality in control}} \times 100$$

2.5. Statistical analysis

Data from bioassay experiments were analysed using the statistical software IBM SPSS. Percentage mortality of the larvae, LC_{50} and LC_{90} values, intercepts, slopes, Chi-square and p values along with other parameters were calculated using Probit analysis. Graphical representations were performed using Microsoft excel 365.

3. Results and Discussion

The extracts of all medicinal plants in our study revealed potent larvicidal activity against the 3rd instar larvae of *C. quinquefasciatus*. However, larvicidal activities varied with the plant species, extract concentration and exposure time. The percentage of dead larvae at 24 and 48h after exposure are shown in Figure 1. Overall, the *D. scandens* root extract showed the highest toxicity as it induced 100% larval mortality after 24 h exposure at 250 ppm concentration, followed by the root extracts of *R. cordifolia* and *S. lappa* inducing 98.4% and 87.8% larval mortality, respectively (Figure 1). However, the extracts of *R. cordifolia* at 250 ppm concentration exhibited 100% larval mortality after 48 h exposure. In general, all of the tested plant extracts exhibited a strong positive correlation between the concentration of extracts and larval mortality ($p<0.001$) with a correlation coefficient more than 0.90 (Figure 2). This pattern of positive correlation between concentration and larval mortality is comparable with previously published literature showing that increased concentration of aqueous extracts of the root of *Derris elliptica* (Sayono *et al*., 2019), chloroform extracts of the root of *Derris indica* (Mondal *et al*., 2011), aqueous dried leaf extracts of *Cinnamomum tamala*, *Aloe vera* and *Ocimum basilicum*, aqueous dried fruit extracts of *Datura alba and Zingiber officinale* (Iqbal *et al*., 2018), different solvent extracts of the root of *Annona reticulata* (Mallick & Chandra, 2015) and aqueous rhizome extracts of *Curcuma longa*, *C. zedoaria* and *Z. officnale* (Rahman *et al*., 2009) exhibited increased larval mortality of *C. quinquefasciatus*.

The LC_{50} and LC_{90} values of all the plant extracts were determined at different concentrations. The *D. scandens* root extract caused the highest larval mortality with the lowest LC_{50} values (24 h=78.20 ppm and 48 h= 67.29 ppm) followed by *R. cordifolia* (24 h=89.32 ppm and 48 h= 68.92 ppm) and *S. lappa* (24 h=112.29 ppm and 48 h= 91.45 ppm) (Table 1). Similarly, the LC_{90} values were also lowest for *D. scandens* (24 h=188.52 ppm and 48 h= 160.82 ppm*)* followed by *R. cordifolia* (24 h=204.09 ppm and 48 h=175.51 ppm) and *S. lappa* (24 h=248.72 ppm and 48 h=219.79 ppm*)*. In a previous study, the effective larvicidal potential of plant extracts based on 48 h exposure was classified into three levels viz. high (<50 ppm), moderate (<100 ppm) and low (<750 ppm) (Sayono *et al*., 2020). According to this classification, all the plants showed moderate larvicidal activity (Table 1).

Although there is no previous report concerning the mosquito larvicidal activity of *D. scandens*, solvent extracted phytochemicals from this plant exhibited insecticidal activities against stored-product insect pests viz. *Sitophilus oryzae*, *Rhyzopertha dominica*, *Callosobruchus chinensis* and *Tribolium castaneum* (Hymavathi *et al*., 2011). However, another species *D. elliptica* exhibited higher larvicidal efficacy compared to our results, against the laboratory reared 3rd instar larvae of *Aedes aegypti* manifested by lower LC_{50} values of 34.94 and 6.46 ppm after 24 and 48 h exposure time, respectively (Sayono *et al*., 2020). Additionally, *D. elliptica* root extracts exhibited larvicidal efficacy against the temephos-resistant *Aedes aegypti* larvae (Sayono *et al*., 2019). It was reported that Alizarin, a compound isolated from methanolic extracts of the root of *R. cordifolia* exhibited

strong mosquitocidal activity with LC_{50} values of 0.81 and 1.31 ppm against the 3rd instar larvae of *C. quinquefasciatus* and *A. aegypti*, respectively (Gandhi *et al*., 2016).

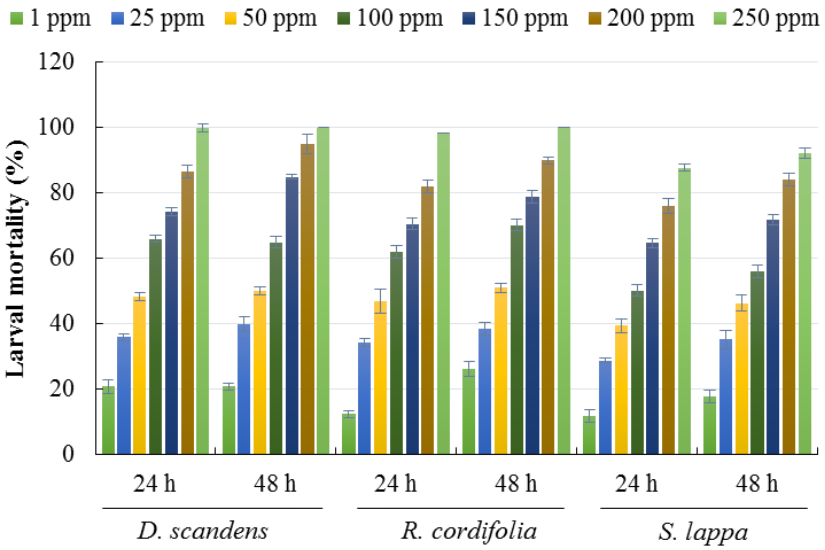

Figure 1. Toxicity of aqueous extracts of different plants against the 3rd instar larvae of *C. quinquefasciatus* after 24 and 48 h of exposure. Values shown are mean of five replicates. Y-error bar represents the respective standard error of means.

Table 1. Probit analysis showing larvicidal efficacy of different plant extracts against the 3rd instar larvae of *C. quinquefasciatus.*

Plant species	Exposure time	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Intercept±SE	Slope±SE	χ^2
			LL	UL		LL	UL			
S. lappa	24 h	112.29	82.48	146.60	248.72	201.16	341.41	1.05±0.07	0.01±0.00	30.28*
	48 h	91.45	59.57	124.89	219.79	174.59	311.16	0.91±0.07	0.01±0.00	35.51*
R. cordifolia	24 h	89.32	56.25	124.70	204.09	159.80	298.67	0.99±0.07	0.01±0.00	44.74*
	48 h	68.92	37.05	100.21	175.51	135.82	259.90	0.82±0.07	0.01±0.00	42.71*
D. scandens	24 h	78.20	46.55	110.59	188.52	147.33	274.84	0.90±0.07	0.01±0.00	40.40*
	48 h	67.29	43.30	92.08	160.82	128.49	221.81	0.92±0.07	0.01±0.00	32.40*

LC_{50}-concentration of plant extract that is lethal to 50% of the exposed larvae, LC_{90}-concentration of plant extract that is lethal to 90% of the exposed larvae, LL- lower confidence limit, UL- upper confidence limit, χ^2- chi-square, df-degrees of freedom, *Significant at $p<0.05$ level.

Another study described *S. lappa* essential oil as a potent larvicide against the 3rd instar larvae of *Aedes albopictus* with an LC_{50} value of 12.41 ppm after 24 h exposure. Two major compounds isolated from the essential oil (costunolide and dehydrocostus lactone) also caused significant larval mortality of this mosquito with LC50 values of 3.26 and 2.34 ppm, respectively (Liu *et al*., 2012). The underlying reason behind the higher efficacy of *R. cordifolia* and *S. lappa* compared to our results might be differences in extraction procedures.

In general, organic solvent-based extraction is a popular method for evaluating the mosquitocidal activity of plant extracts (Gandhi *et al*., 2016; Pavela, 2009; Zoubiri & Baaliouamer, 2014). In the present study, a simple and economical aqueous extraction method was used for the preparation of plant extracts. It is evident from our results that medicinal plants have strong larvicidal efficacy against *C. quinquefasciatus.* Previous literature reported that the plants used in our study are rich in bioactive constituents which possess insecticidal properties (Iqbal *et al*., 2018; Mallick & Chandra, 2015; Sayono *et al*., 2019) which could be the underlying cause for the high mortality of *C. quinquefasciatus* larvae. Therefore, these plants in aqueous form might be effective larvicidal agents against *C. quinquefasciatus* larvae, hence, might be recommended as a better alternative to synthetic chemical insecticides for the control of *C. quinquefasciatus* mosquitoes. Nevertheless, further research

on the larvicidal potential of aqueous and organic solvent extracts of these plants are required against all stages of *C. quinquefasciatus* and other mosquito species. Furthermore, isolation and identification of active ingredients responsible for mosquitocidal efficacy are needed.

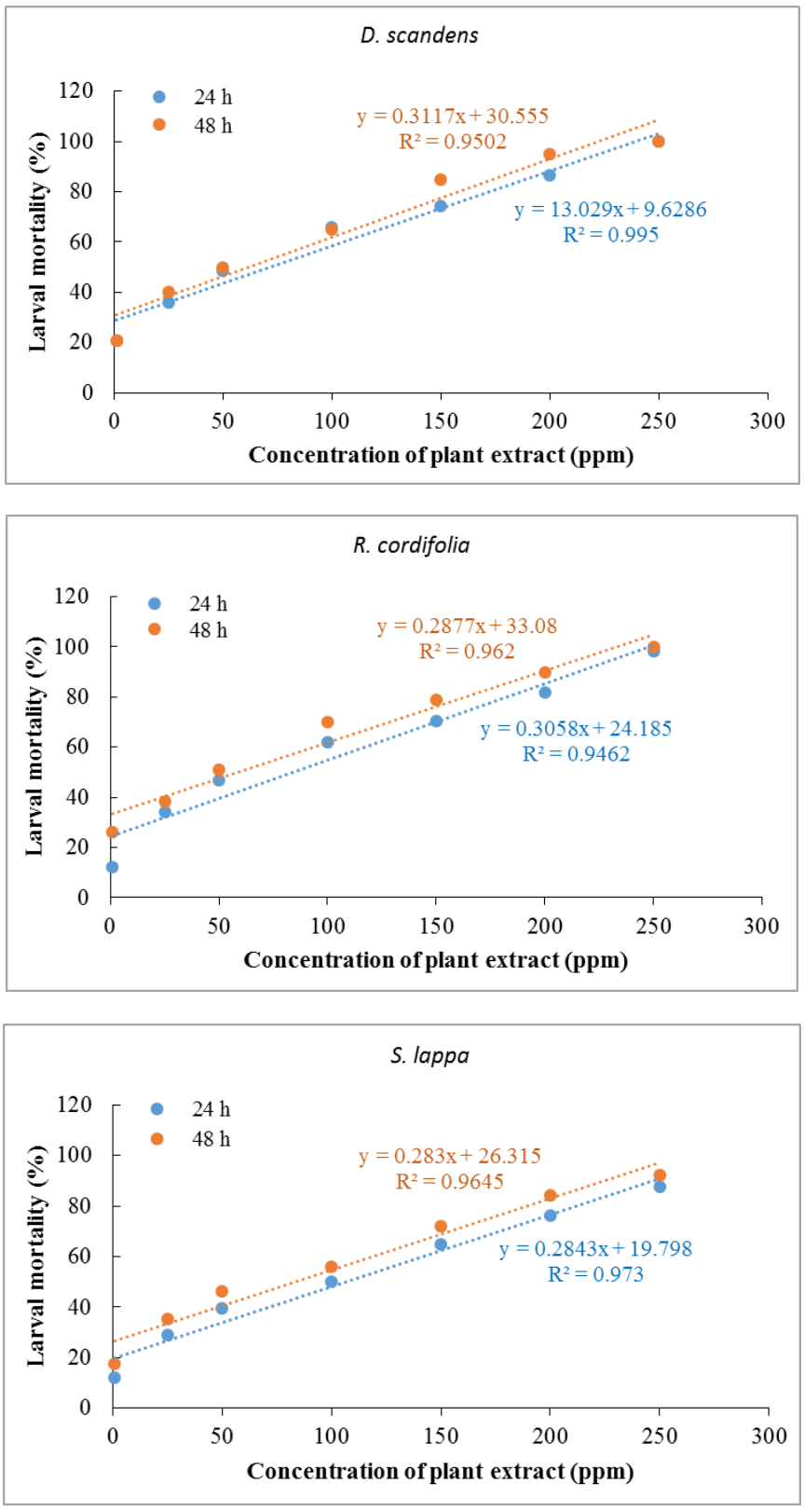

Figure 2. Diagrams showing correlation coefficient (R) and linear regression equation (Y) between concentration of plant extract and mortality of 3rd instar larvae of *C. quinquefasciatus* after 24 and 48 h of exposure.

4. Conclusions

The present study revealed the larvicidal potential of aqueous extracts of roots of three medicinal plants viz. *D. scandens*, *R. cordifolia* and *S. lappa* against the 3rd instar larvae of *C. quinquefasciatus* under laboratory conditions. All the plant extracts exerted potent larvicidal efficacy and are promising candidates for the development of botanical insecticides. Further studies are needed for the identification of the active ingredients of these plants as well as their mode of action.

Data availability

All the relevant data generated or analysed are provided in the published article. The datasets generated during and/or analysed during the current study are available from the corresponding author on valid request.

Conflict of interest

None to declare.

Authors' contribution

Khondoker Md Zulfiker Rahman: conceptualization, methodology, data collection, analysis, manuscript writing, manuscript reviewing and editing; Mohammad Abdur Razzak: data collection, manuscript reviewing and editing. All authors have read and approved the final manuscript.

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