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LARVICIDAL EFFECT OF BISHKATALI (*POLYGONUM HYDROPIPER*) LEAF EXTRACT AGAINST *AEDES AEGYPTI* (DIPTERA: CULICIDAE) LARVAE

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ABSTRACT: Extracts of *Polygonum hydropiper* leaves in Methanol was introduced to the 3rd and 4th instar larvae of *Aedes aegypti* with various concentration (.25–1.5 µg/ml). Insecticidal assays data showed significant mortality against the *Aedes aegypti* larvae. The LC50 and LC90 value after 24 hours of exposure was 0.002 and 0.47 µg/ml respectively. As predicted the result showed that the larvicidal activity of *P. hydropiper* on *Ae. aegypti* larvae was dose reliant. The result clearly exhibited that methanol extract of *P. hydropiper,* as a potential biological control agent against *Ae. aegypti* larvae, is eminent.

Key Word: *Polygonum hydropiper*, *Aedes aegypti*, larvicidal activity, vector control, biological control, Bishkatali, LC_{90}

INTRODUCTION

Aedes aegypti (Linn.) is the primary vector of yellow fever, dengue, chikungunya and zika virus (Jayme *et al.* 2019). According to Walter Reed Biosystematics Units *Aedes aegypti* is capable of transmitting 54 species of viruses and 2 species of *Plasmodium* in different parts of the world (WRBU, 2021). In Bangladesh, *Ae. aegypti* is associated with regular outbreak of dengue and dengue haemorrhaegic fever causing a great deal of morbidity and mortality every year (Haider *et al.* 2021).

After the first outbreak of dengue in 1964, known as "Dacca Fever" (Hossain *et al.* 2000) dengue was silent for decades until another major outbreak in 2000 with some cases reported in 1999. Since then, dengue started to spread all over the country with more than 28000 cases and hundreds of deaths reported over the period of January 2000 to December 2014 (Disease Control Directorate 2017). However, the worst outbreak of dengue was in 2019 with a total of 101,354 cases reported from 61 out of 64 districts and which was a 122% increase from the previous year, 2018 (source: DGHS). Chikungunya, another crippling viral disease besides dengue had a sudden outbreak in 2017 with an overwhelming number of cases in Dhaka city. Moreover, the last one of

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the *Aedes* trio, zika virus had been reported and confirmed through RT-PCR from Chittagong in 2014 (Muraduzzaman 2017). Once established these three viruses will be endemic in the cities because of the abundant presence of their primary vectors, *Aedes aegypti* and *Aedes albopictus* (Yuill & Kaye 2015).

It is estimated that dengue fever with all the four serotypes has increased fourfold since 1970 (Deepa *et al.* 2015) as a potential effect of rapid growth of world population and climate change, which favoured the vector habitats. In 1990, about 30% of the world population lived in the regions where the estimated risk of dengue fever transmission was more than 50% (Hales *et al.* 2002). Compare to that the recent statistic shows that 3.9 billion people are at risk of dengue infection (ECDC 2022). Dengue is endemic in 129 countries with 70% cases in Asia (ECDC 2022).

Controlling the spread of these deadly diseases can only be possible by minimizing the population of the vector *Ae. aegypti*, if not eliminating them. Chemical insecticides for mosquito control, with their overuse all over the world, had created a well-known adverse ecological effect, such as, insecticide resistance among the vector species, ecological imbalance due to elimination of many species, health hazards to mammals and other vertebrates and overall environmental pollutions. Therefore, biological control with non-toxic plant materials is a more favourable option to control the vector population.

From ancient times plant materials were used to keep away adult mosquitoes from human habitats, for example, dhoop and dhuna. Compare to that, using extracts of different plant materials as mosquito larvicides is a novel area of study. That being the case, a lot of medicinal plants had already been evaluated as potential mosquito larvicides all over the world in past few decades. However, *Polygonum hydropiper*, (Bishkatali in Bangla) the plant we used in this study was never evaluated for its insecticidal properties before.

Polygonum hydropiper (L.) also known as *Persicaria hydropiper* of Polygonaceae family, is a perennial herbaceous plant with insecticidal properties. Leaf extracts of *P. hydropiper* is traditionally used for headache, toothache, liver enlargement, gastric ulcers, dysentery, loss of appetite, inflammation, neurological disorders and diarrhea (Sharma 2003). *P. hydropiper* plant decoctions are also traditionally used to treat an extensive range of ailments like dyspepsia, menorrhagia, and hemorrhoids as well as skin itching (Chevallier 1996). Various parts of the plant were used as astringent, sedative, antiseptic, respiratory disorders, edema, snake bites and insect bites in the traditional medicine system (Ayaz *et al.* 2016 a). A series of studies showed that *P. hydropiper* has potential drug value as anticholinesterase, antioxidant, phytotoxic, anthelmintic and anti-cancer element (Ayaz *et al.* 2016 b). Lajter *et al.* (2013) proved that *P. hydropiper* has potent cytostatic compounds, including

flavonoids and sesquiterpenes that has a significant preferential antiproliferative properties on Hela cells.

Apart from human medicine *P. hydropiper* extracts were not tested against mosquito larvicidal effect as far as the literature survey of this study could ascertain.

Therefore, the insecticidal potentials of *P. hydropiper* leaf extracts against the larvae of *Aedes aegypti* was evaluated in this prospective study.

MATERIAL AND METHODS

Experiments were conducted in the laboratory of Institute of Epidemiology, Disease Control and Research (IEDCR), Mahakhali, Dhaka, Bangladesh, during the period of July to December 2019.

Aedes aegypti rearing

Aedes aegypti larvae were collected from different areas of Dhaka city to start a colony. After taxonomic identification the colony had been established in an air-conditioned room with a temperature of 22±2˚C and 78±2% RH as well as a fixed photoperiod of 11:13-h light and dark cycles. Larvae were reared in plastic trays and were fed powdered fish food. The amount of food provided was measured as an optimum amount per capita per instar basis (Jannat & Roitberg 2012).

Adults were kept in 30×30×30 inch plexi glass box with an opening of mosquito net. Adults were given 15% sugar solution soaked in cotton pads in a petridish. After 2-3 days females were blood fed from mainly live pigeons (tied up), placed in the cage for 20 minutes. Artificial membrane blood feeding with sheep blood through Glytube device, adopted from Costa-da-Silva *et al.* (2013) was also used occasionally to feed the adult females. The females seemed to prefer black color containers and sugar feeding petridishes as their oviposition sites compare to other containers. For the egg stripes 4" wide filter paper were placed in a round black small bowl with distilled water in such a way that the lower few mm edges of the filter paper is soaked in the water. Eggs stripes were collected 3 days later and were kept in a place away from ants in order to air dry. The stripes were then kept in airtight glass bowl for future use.

The egg stripes from the first generation of the colony were placed to hatch in larval trays. 3rd and 4th instar larvae from this batch were used for the experiment. Plant Extract Bishkatali plants were collected from the surrounding villages of Jahangirnagar University campus. The leaves were washed, dried in the shade and grinded using a commercial grinder. A fine uniformed dust were obtained by using 2 mm pore size sieve, and was stored in an air tight glass jar under laboratory condition. 100 gm of the powder was then extracted in 90%

MeOH (1 litre) using soxhlet apparatus (boiling point range 60˚c-80˚c) for 6 hours (Kamaraj *et al*. 2011). The extract was filtered through a Buchner funnel with Whatman number 1 filter paper. The extract was then concentrated under negative pressure 22-26mm Hg at 45˚c (Rahuman *et al*. 2008). The residue was stored at 4˚c. It was then made up to 1% stock solution with Acetone (Kamaraj *et al*. 2011). From the stock solution 4 different doses were prepared that includes 0.25, 0.50, 1 and 1.5 µg/ml.

Altogether five treatments including a control each having 3 replicates were undertaken. Each replication has 20 early 4th or late 3rd instar larvae of *Ae. aegypti* in 200 ml of water in 250 ml beakers. Mortality was recorded for each treatment in 12 and 24 hours of exposure.

Statistical analysis

Probit analysis was used to calculate the LC50 and LC90 value of the average larval mortality after 12 and 24 hours of exposure, with 95% fiducial limits of upper confidence limit and lower confidence limit. A chi-square test was performed to compare the size of any discrepancies between the expected values of the test samples.

The graph was created in R statistical software (R Core Team 2017). A two-dimensional box plot was generated to compare the variables.

RESULT AND DISCUSSION

All the four concentration of methanol extract of Bishkatali leaf had a significant toxic effect on the late instar larvae of *Ae. aegypti*. The concentration was designed in such a way that the final concentration could achieve 100% mortality within 24 hours of time. In general, the crude extract of plant leaf materials is a combined and complex effect of the active compounds. Although

Table I: Larval Mortality of *Aedes aegypti* after 24 hours of exposure to the methanol leaf extract of Bishkatali

Treatment	Concentration (µg/ml)	Log Concentration (µg/ml)	Mean Mortality After 24 Hours	Probit of Kill
1	0.25	-0.60205999	50.00%	5.06
2	0.5	-0.30103	83.33%	5.95
3	1	0	96.67%	6.88
4	1.5	0.176091259	100.00%	7.37
control	0.00001	-5	11.67%	3.82

The control had insignificant mortality. Due to lack of food the larvae died or preyed upon by the older conspecific.

Table 2: Larvicidal activity (LC50 and LC90) of bishkatali leaf extract in methanol after 24 hours of exposure on *Aedes aegypti* larvae

ANOVA							
	Df	SS	MS	F	Significance F	LC50	LC90
Regression	1	6.014253844	6.014253844	8.56623057	0.061158059	0.002552	0.476064
Residual	3	2.106266156	0.702088719				
Total	4	8.12052					
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*
Intercept	6.46170908	0.434845128	14.85979412	0.0006613	5.077837808	7.845580353	5.077837808
X Variable 1	0.563741247	0.192612706	2.926812356	0.06115806	-0.049238347	1.17672084	-0.049238347

Values were significant at the P<0.05 level. LC50: lethal concentration that kills 50% of the exposed larvae, LC90: lethal concentration that kills 90% of the exposed larvae, UCL: upper confidence limit (95% fiducial limit), LCL: lower confidence limit (95% fiducial limit), df: degrees of freedom

this study did not nail down the most active compound among all the ingredients of leaf extract, the overall goal of 100% mortality was attained at the end, which is, in the first concentration, 0.25 µg/ml 50% mortality was observed after 24 hours, followed by 83.33% and 96.67% mortality respectively in 0.5 µg/ml and 1 µg/ml concentrations. However, the highest mortality, 100% was observed in 1.5 µg/ml concentration after 24 hours of exposure (Table I). Methanol leaf extract of *P. hydropiper* against the 4th instar larvae of *Ae. aegypti* showed LC 50= 0.002 and LC90 = 0.47 with p value 0.0006 respectively (Table II). The data obtained were analyzed using Chi-squared test, comparing experimental and control groups, with a significance level established at $P<0.05$. The control treatments had insignificant amount of larval death after 24 hours (Table I, Figure I). The reason a few larvae died in the control treatments was devoid of food. In one of the control beakers there were missing larvae. The possible reason is cannibalism by the conspecific, which is, in a food stressed environment the stronger conspecific would pray upon the weaker one or the older larvae would pray upon the younger ones (Porretta et al. 2016). The treatments were mixed with 3rd and 4th instar larvae; therefore, cannibalism could happen spontaneously.

Numerous researchers have tested many medicinal plants for their larvicidal/insecticidal properties all over the world. For instance, Sukumar *et al.* (1991) listed and described over 344 plant species in India that showed mosquitocidal activity. Ghosh *et al.* (2012) also worked on different plant materials of about 160 plants in different chemical extraction to find out the larvicidal potentials. Oliveros-Diaz *et al.* (2022) extracted 56 plants of the Columbian Caribbean region to evaluate the potential larvicidal effects against

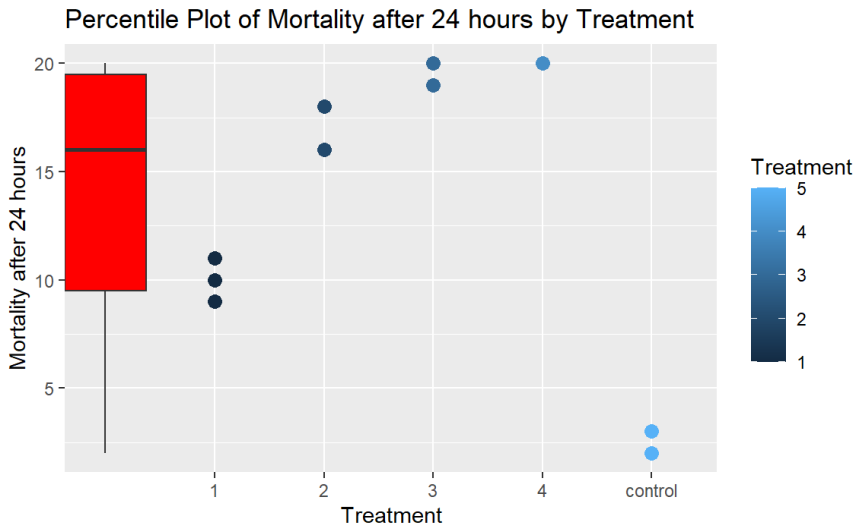

Fig.1: Two-dimensional box plotting showed the mortality rate after 24 hours of exposure to ethanol leaf extract of *P. hydropiper* in various concentration (0.25, 0.5, 1, 1.5µg/ml) on *Aedes aegypti* larvae. The X axis represented Treatment 1= 0.25 µg/ml, Treatment 2 = 0.5 µg/ml, Treatment3 = 1 µg/ml, Treatment 4 = 1.5 µg/ml, Treatment 5 = Control. The Y axis represented the total number of larvae (20 per beaker). 100% mortality was observed in Treatment 4. The mean mortality is the dark line in the box. The X axis represented Treatment 1= 0.25 µg/ml, Treatment 2 = 0.5 µg/ml, Treatment3 = 1 µg/ml, Treatment 4 = 1.5 µg/ml, Treatment 5 = Control. The Y axis represented the total number of larvae (20 per beaker). 100% mortality was observed in Treatment 4. The mean mortality is the dark line in the box.

Ae. Aegypti. Evaluating larvicidal effects using plant extracts is an alternative mosquito control approach, which is gaining momentum in recent years.

As *P. hydropiper* was mostly studied for its medicinal values and chemical compositions to date, information of the toxic effect of bishkatali on insects are scarce. Our literature survey only found out that Khatun *et al.* (2015) reported that the methanol extract of *P. hydropiper* leaves has significant antinociceptive activity against mice. But toxicity against mosquito larvae as a potential larvicide had never been tested.

CONCLUSIONS

The LC50 values of the *Ae. aegypti* larvae were seem to be effective at lower extract concentrations. This proves that the leaf extracts of Bishkatali *P. hydropiper* contains biochemical compounds that inflict appreciable mortality of mosquito vector larvae. Further studies is needed to isolate the chemical constituents of different parts, such as, stem, roots of *P. hydropiper* to find out the various types of potential bioactive compounds.

This is the first report on the larvicidal activity of *P. hydropiper* on the late instar larvae of *Ae. aegypti*. The results that came out of this study had set forth a new

possibility to investigate further on the biochemical compounds of Bishkatali plants along with its insecticidal properties.

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