Evaluation of dry seed extracts of some forest tree species against *Aedes aegypti* larvae

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**ABSTRACT**

An experiment was conducted to know the larvicidal activities of dry seed extract of some important forest tree species against the larvae of dengue vector *Aedes aegypti* (L.) under laboratory condition for round the year. On this purpose late 3rd instar larvae of *Aedes aegypti* (L.) were exposed to different concentrations (5%, 4%, 3%, 2% and 1%) of dry seed extract of three forest plant species for 0 to 72 hours. The test plants were, *Swietenia mahagoni*, *Anacardium occidentale* and *Terminalia catappa*. After 24 hours of exposure maximum mortality was observed in dry seed extract of *Anacardium occidentale* (85%), followed by *Terminalia catappa* (70%) and *Swietenia mahagoni* (35%). Among the dry seed extracts minimum LC₅₀ value after 24 hours exposure was observed in dry seed extract of *Anacardium occidentale* (2.384), followed by *Terminalia catappa* (3.020) and *Swietenia mahagoni* (6.78). The minimum LC₉₀ values after 24 hours of exposure was observed in dry seed extract of *Anacardium occidentale* (5.80), followed by *Terminalia catappa* (13) and dry seed extract of *Swietenia mahagoni* (16.75). Lowest LC₉₅ values was also recorded in dry seed extract of *Anacardium occidentale* (7.46), then in *Terminalia catappa* (21.428) and in *Swietenia mahagoni* (21.706) after 24 hours of contract of the larvae. From the above information, it is clear that dry seed extract of some forest plants might contain certain phytochemicals that can be used as larvicide that is an environmentally friendly approach.

**Key Words:** *Aedes aegypti*, dengue, dry seed extract, larvicide and mosquito.

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I. Introduction

Dengue hemorrhagic fever is a great health problem in the whole world. Due to environmental conditions, dengue is a severe concern in Southeast Asia (Mangali et al. 2019). It is a viral disease which spread quickly in contact with the mosquito. In the globe, every year, half a million cases of dengue hemorrhagic fever and 100 million cases of dengue fever occur. About 90% of dengue hemorrhagic fever infects children those old are less than 15 years. In the present time, dengue is...
endemic in more than hundreds of countries of the world. At the same time, dengue is a global concern with a steady increase in the number of countries reporting the disease in the Asia Pacific region, approximately seventy five percent of the global population exposed to dengue. (WHO, 2012 and Malaviye et al. 2004). Like other Southeast Asian countries, Bangladesh is situated both in the tropical and sub-tropical regions and it has become a suitable zone for the dengue vector. Before 2000, only sporadic dengue cases were reported from Dhaka and other parts of the country (Russell et al. 1966 and Amin et al. 2000). A sudden outbreak in 2000 occurred in the country, where more than 5,500 cases and about 100 deaths were reported and made a serious public health concern throughout the country. During the dengue outbreaks (2000-2017) in Bangladesh, both vectors such as Aedes aegypti and Aedes albopictus were identified (Chowdhury et al. 2000 and Breiman et al. 2003).

Dengue virus and chikungunya virus, these two viruses expand by the Aedes mosquitoes, are very concerning. They have made an overload in morbidity and mortality with an inadequate distribution of wealth under the infectious disease control operational plan of the Health, Population, and nutrition sector program in Bangladesh and other Asian countries. (Sharmin et al., 2010 and Guzman et al., 2013). When the virus has appeared in Bangladesh, soon they are supposed to stay in the habitat as the vector is always present and will cause increased public health problems. There are no protective vaccines against the causal agents and the culprits of this menace, albeit the rational management procedure to ameliorate the progression is through the effective management of the mosquito vector Aedes aegypti that belongs to the family Culicidae of the order Diptera (Autran et al., 2009). As the disease is a contagious and severe loss caused by it, managing dengue has been the preliminary subject of several new types of research over the past few years (Invest and Lucas, 2008). The technique in controlling mosquitoes depends on the larval stages on target. Mosquito control includes targeting the adult mosquito by spraying chemical insecticides or killing the mosquito larvae before they emerge into adults via synthetic larvicides or botanical extracts as an alternative larvicide (Tiwary et al., 2007). However, the use of synthetic larvicides creates threats both to human health and to the ecosystem.

When any chemical pesticides are sprayed in the environment, some may stay on for a very long time. (Mathivanan et al., 2000). Due to the application of synthetic larvicides, natural biological control systems become disrupt and sometimes resistance development occurs. This phenomenon has triggered and urged alternative techniques using natural products such as botanicals or biopesticides. Present research trends, use of dry seed extracts of some forest tree species as an alternative larvicide because they contain various phytochemicals that are specific in killing mosquito larvae without harming other organisms and the environment (Hedlin et al., 1997 and Arnason et al., 1989). The use of these organic substances results in the lower level of resistance among pest and insect populations. (Brito et al., 2011). Because of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of dry seed extract of some forest tree species which are available in Bangladesh such as Swietenia mahagoni, Anacardium occidentale and Terminalia catappa against the medically important vector mosquito larvae of Aedes aegypti.

II. Materials and Methods
The research study was done to know larvicidal activity of dry seed extracts of 3 indigenous forest plants against late 3rd instar larvae of Dengue vector mosquito Aedes aegypti (L) under laboratory conditions (air temperature 22°-35°C, water temperature 25°-35°C and relative humidity 65%-90%) round the year in the Medical Entomology Laboratory, Department of Zoology Jahangirnagar University, Savar, Dhaka from January 2009 to December 2010.

Required materials
This research work needed different types of equipment and other materials such as rearing cage, sweep net, mosquito net, earthen bowl, plastic cup, ovitrap, petri dish, dropper, brush, pipette, cotton, glucose tube, tap water, test plants, Cerelac®, yeast powder, glucose, pigeon (for blood feeding the adult female mosquitoes), glass beaker, measuring cylinder, magnifying glass, hygrometer, thermometer, funnel, plastic cup, filter paper, conical flask, mortar-pestle etc.
Rearing of *Aedes aegypti* (L.)

**Egg collection:** Wild eggs of *Aedes aegypti* mosquito were collected by placing ovitraps in different areas of Jahangirnagar University campus for the rearing purpose. A long strip of filter paper wrapped inside a black colored glass jar to make Ovitraps. A little amount of water was kept in the bottom of glass jar so that some portion of the filter paper became wetted and moistened. *Aedes* mosquito laid eggs on the moist surface of the filter paper. The egg strips were dried in the air for 1-2 days after collecting the eggs. After that the egg strip was placed in normal tap water for hatching.

**Rearing of the larvae:** In normal laboratory conditions the hatched larvae of the mosquito were reared. They were kept in an earthen jar and as larval food, daily Cerelac® baby food and yeast granules were provided. The bowls were kept in mosquito rearing cages to prevent egg-laying by other mosquito species.

**Rearing of pupae:** The larvae became pupae after 4th larval molt. When pupation starts, the pupae were separated from the larvae using a dropper and kept in a previously water filled plastic bowl. Then the pupae were kept in mosquito rearing cages for the emergence of adult mosquitoes.

**Rearing of adult:** In the mosquito-rearing cage adult mosquitoes emerged from the pupae. As food for adults, 10% glucose solution was supplied daily. The male mosquitoes took only the glucose feed throughout their lifetime. For the first two or three days of emergence, the female also took only the sugar feed. From the third day of emergence, the female took blood meal and the glucose meal throughout her life. The blood meals of pigeon were supplied to the females for the autogenous development. For about half an hour to one hour, the pigeon was kept tight on the cage’s roof, which allowed the females to suck blood to their full content. After a blood meal females oviposit two to three days later. For laying eggs *Aedes aegypti* preferred clean water. The egg rafts were transferred after oviposition to an earthen bowl filled with water for hatching and then the bowl was kept within a mosquito rearing cage. The experimental larvae were lab reared F1 generation.

**Process of extraction**

Fresh seeds of the forest tree species of *Swietenia mahagoni*, *Anacardium occidentale* and *Terminalia catappa* were collected from the Jahangirnagar University campus and surrounding areas. Fresh seeds were dried in the sun. After drying it was crushed and powdered with the help of mortar pester, grinder and blender. Then it was in powdery form. The powder of the dry seeds was weighted with the help of electric balance for different concentrations (5%, 4%, 3%, 2% and 1%). Dry seed extract was made by mixing the powder of the dry seeds (weighed 100gm) in a beaker filled with 1900ml of distilled water. Then the total amount of solution was 2000 ml (w/v) and it was left for 24 hours for extraction to settle down. The filter paper filtered the solution after successful extraction. This was the stock solution (concentration 5%).

**Dose preparation for the experiment**

The 5% concentrated stock solution was prepared. After that the stock solution was diluted to various concentrations (4%, 3%, 2%, and 1%). 400 ml of stock solution was mixed with 100ml of distilled water to make 4% concentration. For 3% concentration, 300 ml of stock solution was mixed with 200ml of distilled water. Such as 2% and 1% concentration were made by adding 200 ml and 100 ml of stock solution respectively.

**Bioassay**

Bioassay of *Swietenia mehagoni*, *Anacardium occidentale*, and *Terminalia catappa* were carried out in the laboratory against late 3rd in star larvae of mosquito *Aedes aegypti* (L.). For each test plant dry seeds were used in five different concentrations (5%, 4%, 3%, 2% and 1%) and 5 replications were maintained for each concentration. For control five replications were also maintained. Each replication 100 ml solution was taken in a plastic cup and 20 larvae were exposed. To prevent contamination the plastic cups were covered with fine mosquito net, and then the cups were kept undisturbed. Larval mortality was recorded after 0, 24, 48 and 72 hours of exposure. The percentage of mortality was calculated by using the following formula.

\[
\text{Mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100
\]
Statistical analysis
The data were analyzed using Probit Analysis Program Version 1.5, which was developed by the 'Ecological Monitoring Research Division', Environmental Monitoring Systems Laboratory, U. S. Environmental Protection Agency (EPA), Cincinnati, Ohio 45268. This system is used to determine LC50, LC90 and LC95 values. To know the larvicidal effects of various seed extract, LC values were determined. For multiple group comparisons, differences of means among groups were compared using one way analysis of variance (ANOVA). DMRT (Duncan Multiple Range Test) was done using SPSS (Statistical Package for Social Science) program (version 12). Graphical representations were done using Microsoft Office Excel 2010.

III. Results and Discussion
Toxic effect of dry seed extract of Swietenia mahagoni
The dry seed extract of Swietenia mahagoni was effective against the late 3rd instar larvae of Aedes aegypti (L.). After 24 hours of exposure, maximum percentage of mortality (35%) observed at 5% concentration, which followed by 20%, 15%, 5% and 0% at 4%, 3%, 2% and 1% concentration respectively. After 48 hours of exposure maximum percentage of mortality (80%) observed at 5% concentration, at 4% concentration percent mortality was 65%, which followed by 35%, 20% and 10% at 3%, 2% and 1% concentration respectively. Maximum mortality of all the concentrations occurred after 72 hours of exposure, in which 95% larvae killed at 5% concentration. At 4%, 3%, 2% and 1% concentration, percent mortality was 80%, 50%, 30% and 20% respectively (Figure 01).

Figure 01. The effect of different concentration of dry seed extract of Swietenia mahagoni on larvae of Aedes aegypti (L.) at different time points

Probit analysis revealed the LC50, LC90 and LC95 values of the dry seed extract of Swietenia mahagoni with a 95% confidence limit. LC50 value after 24 hours of exposure was 6.718, where after 48 and 72 hours, it was 3.217 and 2.357, respectively. LC90 value after 24 hours of exposure was 16.753 where after 48 and 72 hours, it was 8.104 and 5.899, respectively. LC95 value after 24 hours of exposure was 21.706, where after 48 and 72 hours, it was 10.530 and 7.651, respectively (Figure 02).
Figure 02. The estimated LC values of dry seed extract of *Swietenia mahagoni* at different time points

**Toxic effect of dry seed extract of *Anacardium occidentale***

The dry seed extract of *Anacardium occidentale* was very effective against late 3\textsuperscript{rd} instars larvae of *Aedes aegypti* (L.). After 24 hours of exposure, maximum percentage of mortality (85\%) observed at 5\% concentration, which followed by 70\%, 60\%, 50\% and 40\% at 4\%, 3\%, 2\% and 1\% concentration respectively. After 48 hours of exposure maximum percentage of mortality (95\%) observed at 5\% concentration, at 4\% concentration percent mortality was 80\%, which followed by 70\%, 60\% and 50\% at 3\%, 2\% and 1\% concentration respectively. Maximum mortality of all the concentrations occurred after 72 hours of exposure, in which 100\% larvae killed at 5\% concentration. At 4\%, 3\%, 2\% and 1\% concentration, percent mortality was 90\%, 80\%, 70\% and 55\% respectively (Figure 03).

Figure 03. The toxic effect of different concentration of dry seed extract of *Anacardium occidentale* on larvae of *Aedes aegypti* (L.) at different time points
Probit analysis revealed the LC$_{50}$, LC$_{90}$ and LC$_{95}$ values of the dry seed extract of *Anacardium occidentale* with 95% confidence limit. LC$_{50}$ value after 24 hours of exposure was 2.384, where after 48 and 72 hours it was 2.629 and 1.422, respectively. LC$_{90}$ value after 24 hours of exposure was 5.804, where after 48 and 72 hours it was 4.397 and 3.644, respectively. LC$_{95}$ value after 24 hours of exposure was 7.469, where after 48 and 72 hours, it was 5.599 and 4.758, respectively (Figure 04).

**Figure 04. The estimated LC values of dry seed extract of *Anacardium occidentale* at different time points**

**Toxic effect of dry seed extract of *Terminalia catappa***

The dry seed extract of *Terminalia catappa* was effective against the late 3rd instar larvae of *Aedes aegypti* (L.). After 24 hours of exposure, maximum percent of mortality (70) observed at 5% concentration, which followed by 60%, 45%, 35% and 20% at 4%, 3%, 2% and 1% concentration respectively. After 48 hours of exposure maximum percent of mortality (80%) observed at 5% concentration, at 4% concentration percent mortality was 65%, which followed by 60%, 45% and 30% at 3%, 2% and 1% concentration respectively. Maximum mortality of all the concentrations occurred after 72 hours of exposure, in which 90% larvae killed at 5% concentration. At 4%, 3%, 2% and 1% concentration, percent mortality was 75%, 65%, 55% and 40% respectively (Figure 05).

**Figure 05. The effect of different concentration of dry seed extract of *Terminalia catappa* on larvae of *Aedes aegypti* (L.) at different time points**
Probit analysis revealed the LC\textsubscript{50}, LC\textsubscript{90} and LC\textsubscript{95} values of the dry seed extract of \textit{Terminalia catappa} with 95% confidence limit. LC\textsubscript{50} value after 24 hours of exposure is 3.007, where after 48 and 72 hours it was 2.128 and 1.552, respectively. LC\textsubscript{90} value after 24 hours of exposure was 13.887, where after 48 and 72 hours, it was 10.829 and 7.503, respectively. LC\textsubscript{95} value after 24 hours of exposure was 21.428, where after 48 and 72 hours, it was 17.174 and 11.727, respectively (Figure 06).

![Figure 06. The estimated LC values of dry seed extract of \textit{Terminalia catappa} at different time points](chart)

Chemical pesticides are used as major control practice of mosquitoes all over the world including Bangladesh. Different types of formulations such as bait, fumigants and oil-based suspensions of organ chlorine, organophosphate, and carbonated compounds are used. The unjustified use of these synthetic chemical pesticides produced too many serious problems. Most common are genetic resistance by insect pest, toxic residues, increasing costs of application and storage, and environmental pollution, including hazards for handling the pesticides (Arifuzzahan, 2001). For the above reasons, mosquito killing nowadays is disturbed due to conventional insecticides because these have caused their own problems, such as adverse effects on the biodiversity and the immergence of pesticide resistance in some mosquitoes. This problem stimulated a search for safer alternative.

In today’s world, plant seed extracts are one of the safer alternative methods of mosquito control, especially the extracts of certain forest seed plants. Vigneshwaran and Lalita (2017) studied the Antibacterial activity of seed extracts of \textit{Swietenia mahagoni} and found that the ethanolic extract and ethyl acetate extract of \textit{Swietenia mahagoni} seed at different doses (40, 60, 80mg/ml) level have significant anti-microbial activity. The plant can be considered as low cost, potent, herbal medicine for good anti-microbial activity. Tohfa et al. (2000) reported that crude seed extract of \textit{Anacardium occidentale, Swietenia mahagoni} and \textit{Terminalia catappa}, could kill more than 70% of larvae dengue vector \textit{Aedes aegypti} after 24 hours of exposure. Minimum LC\textsubscript{50} value was recorded in fresh seed extract of \textit{Anacardium occidentale} (1.44) followed by \textit{Swietenia mahagoni} (3.11) and \textit{Terminalia catappa} after 24 hours of exposure at 5% concentration.

Yasotha et al. (2020) studied the phytochemical and antimicrobial potential of seed and bark extracts of \textit{Swietenia mahagoni} (L.). They observed phytocompounds including alkaloids, terpenoids, tannins, and glycosides are major active constituents in the seed and bark of \textit{Swietenia mahagoni}. Very potential antimicrobial activity against the standard strains was showed by the seed and bark extracts. The fungicidal action was good in seed extracts and bacterial activity was significant in bark extracts.
The leaf and shoot powder of *Anacardium occidentale* L. contained very high secondary metabolites, fruits and other parts that have shown diverse applications. The secondary metabolites are found in *Anacardium* plants have effective antioxidant and antimicrobial effects (Salehi et al., 2019). As an alternative to control *Aedes aegypti*, larvicidal activity of *Anacardium occidentale* and its toxicity in *Rattus norvegicus* was studied by Guissoni et al. (2013). They found that the lethal concentrations LC$_{50}$ and LC$_{90}$ of *Anacardium occidentale* were, respectively, 6.55 and 10.98 ppm. These botanicals performed larvicidal potential against *Aedes aegypti* and no sign of toxicity were evident in the parameters analyzed.

Phytochemical analysis of *Terminalia catappa* leaf on n-hexane fraction revealed steroid, terpenoid, saponin, and flavonoid compounds, while ethyl acetate and water-ethanol fraction contained tannin, saponin, and flavonoid compounds. The primary test exhibited that water-ethanol fraction possessed the highest larvicidal activity on *Aedes aegypti* with larval death of 96.67% at 2000 ppm concentration (Redo et al., 2019). *Terminalia catappa* leaves in different media such as aqueous, ethanol and acetone extracts against the larvae and pupae of *Aedes aegypti* mosquito were examined. Pupae and newly molted 3rd instar larvae of *Aedes aegypti* mosquitoes were exposed for up to three days to dilution 2, 4, 6, 8 and 10% of aqueous extracts and 100, 200, 300, 400 and 500ppm of ethanol and acetone extract of leaves. All the extracts showed larval mortality except aqueous extract, and other extracts showed pupal mortality. However, larval mortality was greatest with the ethanol extract followed by acetone and aqueous extract. Maximum pupal mortality was observed in acetone extract followed by ethanolic extract. Based on Probit analysis, the LC$_{50}$ values of aqueous, ethanol and acetone extract of *Terminalia catappa* for the 3rd instar larvae was found to be 5%, 166.0 ppm and 177.8 ppm and for the pupae, it was 169.8 ppm and 161.4 ppm for ethanolic and acetone extract respectively (Unnikrishnan, 2014). Torres et al. (2015) studied characterization and bioassay for larvicidal activity of *Anacardium occidentale* shell waste fractions against dengue vector *Aedes aegypti* and found that the hexane fraction gave the strongest activity among the fractions with an LC$_{50}$ of 4.01 mg/L and LC$_{90}$ of 11.29 mg/L highly comparable to the commercial larvicide, which exhibited an LC$_{50}$ of 1.71 mg/L and LC$_{90}$ of 8.41 mg/L. The dichloromethane fraction exhibited 9.70 mg/L LC$_{50}$ and 18.44 mg/L LC$_{90}$. The remarkable toxicity effects exhibited by these fractions indicate their potential to provide core structures from which sustainable and environmentally safe plant-based larvicidal agents can be synthesized.

From above, it was observed that phytochemicals have a major role in mosquito control programme. The presence of Corchorin in *Corchorus capsularis*, Anacardin in *Anacardium occidentale*, Azadiractin in *Azadiracta indica* and Momordenol and Petroselinic acid in *Momordica charantia* might be the reason for their larvicidal effect against dengue vector *Aedes aegypti* (L.)

**IV. Conclusion**
Mosquito control is mostly based on larvea control because fighting against adult mosquitoes is temporary and unsatisfactory. Adult control processes also pollute the environment. The purpose of mosquito control larvicide application is more effective because it is applied in specific breeding zones and comparatively less dangerous. In case of common and small breeding grounds of *Aedes aegypti* such as plastic containers, discarded tires, bottles, pots and pans that can hold rainwater, larvicide especially spraying botanicals, is an easy way to control the larvae of *Aedes aegypti* may be an effective control tool due to no alternative place to move. Seeds of *Swietenia mahagoni*, *Anacardium occidentale* and *Terminalia catappa* are available in Bangladesh because these are planted in the home yard as agroforestry species. The larvicidal characteristics of the seeds of these plants can be utilized while planning alternative vector control strategies based on integrated vector control measures through community based approaches. However, further studies on identifying the active ingredients of those seeds and their mode of action and field trials are needed to recommend the dry seed extract of these forest plant species as a larvicide of dengue vector used to combat and protect from *Aedes aegypti* mosquito in a control program.
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References


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