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Impact of *Terminalia chebula* Retz. against *Aedes aegypti* L. and non-target aquatic predatory insects

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ABSTRACT

Aedes aegypti Linn is one of the most important mosquito species. The vectors are responsible for causing deadly diseases like dengue and dengue hemorrhagic fever. Several chemical pesticides used to control these dengue vectors caused severe toxic significances on human health and other non-target beneficial insects. Therefore the current investigation has been made to access the bio-efficacy of the crude seed extracts of *T. chebula* against the dengue vector *Ae. aegypti*. The GC-MS analysis of crude seed extracts of *T. chebula* identified nine chemical compounds with major peak area in the 1,2,3-Benzenetriol (61.96%), followed by Tridecanoic acid (09.55%). *Ae. aegypti* larvae showed dose dependent mortality rate was observed between the treatments. Prominent protection rate at greater concentrations of 100 ppm and moderate protection at 75 and 50 ppm was observed in the repellent assay. Lethal concentration (LC_{50} and LC_{90}) of fourth instar larvae of *Ae. aegypti* was observed in 138 and 220 ppm concentration respectively. Similarly, the seed extracts showed 100% adulticidal activity at the concentration of 400 ppm at 30 min of exposure time. Phytochemicals present in the seed extracts of *T. chebula* significantly affects the major portions of the midgut tissues of *Ae. aegypti* at the concentration of 100 ppm. The toxicological evaluation of seed extracts also proved non-toxic towards the *A. bouvieri* and *Tx. splendens* aquatic predatory insects. Hence, the present result suggest that bio-rational plant derived *T. chebula* could be incorporated in the dengue vector control and have no adverse effects on non-target beneficial insects.

1. Introduction

Diseases caused by the mosquito vectors are predominant in more than hundred countries across the nations, infecting over 700,000,000 people per year worldwide. They act as a vector for most of the life frightening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection, etc., in almost all tropical, subtropical countries and many other parts of the nations (Ghosh et al., 2012; Senthilkumar et al., 2009). They are ecologically important components of the aquatic and terrestrial food chain, are the most prominent group of insects in terms of public health status, (Hahn et al., 2001; Thanigaivel et al., 2012) and thus suitable control programs are acceptable. Dengue is the most major human arboviral infection causing nearly half of the world's population at threat (Kraemer et al., 2015). Dengue hemorrhagic fever occurs in Asia, the Americas and some pacific islands. Dengue is endemic in all continents except Europe, and epidemic dengue viruses, causative agents of dengue fever and more severe dengue hemorrhagic fever or dengue shock syndrome infects over 100 million people every year (Thanigaivel et al., 2012).

Aedes aegypti Linn is one of the most important mosquito species, these vectors are responsible for causing deadly diseases like dengue and dengue hemorrhagic fever (Reegan et al., 2015). More than 2.5 billion people in over 100 countries are at risk of constricting dengue alone (WHO, 2014). Mosquito vector control as one of the important measures to reduce diseases spread by mosquitoes (WHO, 2009). The major tool in vector control operation is the application of chemical insecticides such as organochlorine and organophosphate compounds (Ghosh et al., 2012). Synthetic insecticides with a number of originations have been used from the past decade. Organophosphates, such as,

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temephos, have been used as larvicide across the nations since the 1960s (Silva et al., 2004; 2008). Several chemical pesticides enclosing persistent toxic substances (PTS), with persistent organic pollutants (POPs) which caused severe toxic significances such as adverse effects on human health, environment by polluting soil, water and air, and also cause detrimental impact on biodiversity (Senthil-Nathan et al., 2005, 2006; Marcombe et al., 2012). Repeated applications of chemical pesticides leads to toxic effects on beneficial insects, such as pollinators and predacious insects, and contamination of local ecosystems and water sources (Regnault-Roger et al., 2012; Tan et al., 2014; Erland et al., 2015; Govindarajan et al., 2016).

Botanicals are the natural biochemical manufacturer. They produce a different array of diverse phytochemicals which includes alkaloids, terpenes, phenolic compounds, flavonoids and coumarins through their basic mechanisms to decrease pest attacks, both constitutive and inducible, while insects have changed their approaches to overcome these plant and their derivatives defenses (Hunter and Ullman, 1992; Kabir et al., 2013). The application of plant products with marketable value is directly established by the abundant compounds existing in the market and that have endured there in many gears after several years (Senthil-Nathan, 2013; Senthil-Nathan, 2015). Crude extracts of plants could be cheaper and effective, nontoxic to beneficial organisms, biodegradable, and may have different mode of activities and inadequate resistance development in pests (Senthil-Nathan et al., 2009; Cantrell et al., 2012; Kabir et al., 2013). *Terminalia chebula* Retz. belongs to the family *Combretaceae*. It possesses laxative, diuretic, cardio tonic and hypoglycemic properties (Hongbo et al., 2010; Roy et al., 2014). The dried fruit of *T. chebula* is used expansively in the native system of remedy (Ayurvedic) for its homoeostatic, antitussive and cardio tonic activities (Roy et al., 2014). Seeds of *T. chebula* contains major phytochemicals such as tannic acid, chebulinic acid (Lee et al., 2010). Therefore the current investigation has been made to access the bio-efficacy of the crude seed extracts of *T. chebula* against the dengue vector *Ae. aegypti* and their non-target toxicity effects against *Anisops bouvieri* (Hemiptera: Notonectidae) and *Toxorhynchites splendens* (Diptera: Culicidae), sharing the same environmental dwelling of *Ae. aegypti*.

2. Methodology

2.1. Collection of Plant materials

Seeds of *T. chebula* were used as plant material. Fully developed fresh seeds were collected in early morning from in and around Southern Western Ghats, Tirunelveli, India (Fig. 1A). The identification of the plant species were authenticated and deposited with voucher specimen number (1039) in the Sri Paramakalyani Centre for Excellence Environmental Sciences Herbarium (SPKCEESH), Manonmaniam Sundranar University, Alwarkurichi, Tamil Nadu, India. The seeds were washed and shadow dried at room temperature for 7 days until they become brittle, then pulverized to powder.

2.2. Preparation of T. chebula seed crude extract

Hundred gram of powdered seeds of *T. chebula* were dissolved in 500 ml of hexane and the constituents were stirred thoroughly and soaked separately using mini rotary shaker for 48 h to obtain concentrated solution, resulting in active substances being dissolved. The extracts were filtered through muslin cloth followed by filter paper (Whatman Filter No.1) and further concentrated by recovering excess solvents to a thick oily natured substance and stored at 4 °C in air tight sterilized screw capped bottles for further studies.

The residue remaining after the hexane extraction was dried and after the complete solvent removal again subjected into chloroform solvent extraction as per the method described above. The chloroform soluble portion was concentrated using rotary evaporator followed by water bath drying. Likewise, the above dried residue was used for sequential extraction of ethyl acetate, methanol and water.

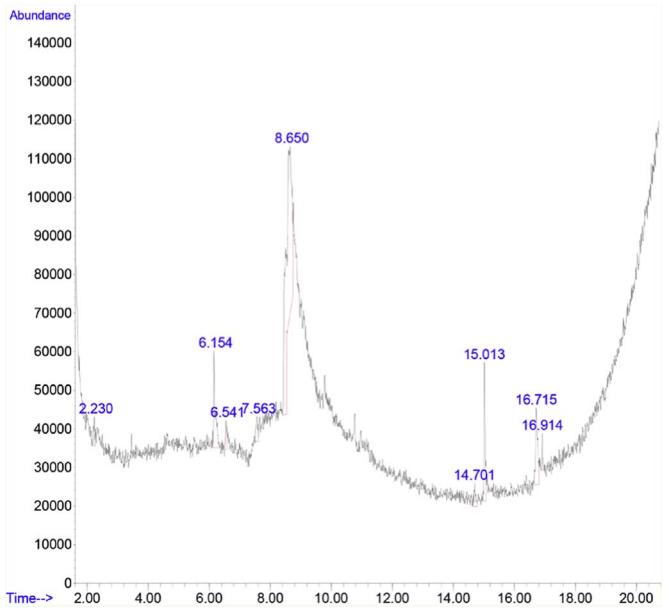

Fig. 1. (A) Morphology of Seeds of *T. chebula* (B) GC-MS chromatogram of methanolic seed extracts of *T. chebula*.

2.3. Mosquito culture

The *Ae. aegypti* culture have been maintained in the Biopesticides and Environmental Toxicology Laboratory (BET Lab), SPK Centre for Excellence in Environmental Sciences, without exposure to pesticides. The mosquito larvae were fed with ground dog biscuits and yeast at 3:1 ratio. The feeding was sustained until the larvae entered the pupal stage. The pupae were collected from the culture trays and moved to plastic containers (9×9 cm) containing 400 mL of water using a small dipper. The plastic jars were kept in a 90×90×90 cm mosquito cage for adult emergence. Mosquito larvae were maintained at 27 ± 2 °C, 75–85% relative humidity, under a photoperiod of 14:10 (light/dark). A 10% sugar solution was provided to newly emerged adults for a period of 3 days before blood feeding. The adult female mosquitoes were allowed to blood feed from a chicken (one chicken per day, exposed on the dorsal side) for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

2.4. Isolation of active compound

The partially purified compound through chromatogram from plant extracts were identified based on spectroscopic analysis, 2 µl of crude and fractions were dissolved in HPLC grade methanol and subjected to GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCMATE II GC-MS (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m×0.25 mm I.D. Analysis conditions were 20 min at 100 °C, 3 min at 235 °C for column temperature, 240 °C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µl) was evaporated in a split less injector at 300 °C. Run time was 22 min. The compounds were identified by gas chromatography coupled with mass spectrometry. The molecular weight, molecular formula and structure of the compounds of test materials were ascertained by interpretation on mass spectrum of GC-MS using the database of National Institute of Standard and Technology (NIST).