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Assessment of *Gluta renghas* L. and *Mangifera indica* L. (Sapindales: Anacardiaceae) extracts on the sublethal effects of dengue vector

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ABSTRACT

Botanical extracts are one of the effective alternative methods for controlling dengue vectors. Methanolic extracts of *Gluta renghas* Linnaeus and *Mangifera indica* Linnaeus were assessed under the field condition for substantial lethal and in the laboratory for sublethal effects on dengue vectors in different seasons (dry and wet), and conditions (shaded and unshaded). The effectiveness of these plant extracts was evaluated for the *Aedes* mosquitoes on the number of eggs, percentage survival of adults, development days to reach adulthood, the number of eggs laid by F1 generation and percentage of hatchability. Significant differences were observed for the number of eggs in both the conditions and weeks of collection ($P < 0.005$) with the least number of eggs recorded by the *Mn. indica* stem treatment in the dry season. Whereas, *Gl. renghas* leave extract had the lowest percentage ($33.51 \pm 2.75\%$) of surviving adults under the dry shaded conditions. Significant effects were noticed for both conditions and weeks on the percentage of *Aedes* survived adults and the time to take to reach adulthood ($P < 0.005$) during both seasons. *Mangifera indica* stem treatment was observed caused prolong in time for emergence to adult stage in dry unshaded conditions (12.42 ± 0.29 days) and significantly reduced the fecundity of the F1 generations in both seasons ($P < 0.005$) with less hatching percentage at $55.67 \pm 4.24\%$ during wet shaded conditions. Overall, both plant extracts performed effectively and better than Abate to control *Aedes* population in the dry season. It can be concluded, that both plant extracts are potential candidates for alternative source of biolarvicides.

Introduction

Vector borne diseases are causing economical, social and health problems in different tropical and subtropical regions of the world (Kamiabi et al., 2013; Rascalou et al., 2012); no regions in the world are safe from vector borne diseases (Ramkumar et al., 2014). Mosquitoes are one of the most important group of insect vectors known for its public health importance due to transmission of malaria, filariasis, encephalitis, yellow fever and dengue fever (Bagavan et al., 2008; Rascalou et al., 2012). Dengue incidence has increased many folds in last few decades with targeting risk to 40% of the world populations (Dias and Moraes, 2014).

Increasing insecticide resistance in mosquitoes is a major cause for the failure of the mosquito control programs (Marcombe et al., 2009). These undeniable facts of insecticides resistance have impelled the concern for the quest of better control techniques and new agents (Palmeira-de-Oliveira et al., 2013) which can control a wide range of vector pests by posing less threat to nontarget organisms (Lalchhandama, 2011). Earlier, the focus was to eliminate the adult stage of mosquito, however the most efficient way is to target the weakest stage of larvae (Govindarajan et al., 2008). One of the most authentic procedures to control the mosquito borne diseases is to eliminate the mosquito vectors by systematic integrated management of their breeding sites through combination of the larvicidal and ecological management (Corbel et al., 2004). However, objectives of the integrated pest management is never been specifically to eliminate the insect pests but to control their number below a threshold level (Haynes, 1988).

Insect growth is interconnected with the developmental time of the different stages of insects that play a dynamic role in their breeding and transmission. Insect growth regulators (IGRs) are insecticides of a special type, which are highly selective and interfere only with the insect metamorphosis, growth and development (Harburguer et al., 2014). IGRs are previously reported for controlling the adult emergence inhibition (Batra et al., 2005; Mulla et al., 2003), female reproduction (Arias and Mulla, 1975), fecundity and fertility of mosquito vectors (Harburguer et al., 2014).

Currently, research on plant extracts became a focus worldwide for the control of mosquito larvae due to its effectiveness. Plant extracts

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and essential oils are rich in bioactive compounds which are non-toxic, readily biodegradable and a better and effective alternate source for the mosquito control which is repeatedly reported by many researchers (Amer and Mehlhorn, 2006; Panneerselvam and Murugan, 2013; Promsiri et al., 2006). Some of the phytochemicals agitate the insects' developmental time by disturbing chemical processes within their bodies resulting in shortening or lengthening of the development time. It also affects the different salient features like adult sterility, maturity, reproduction and other morphological features, which can help in regulating the pest density (Russell, 1977).

Promsiri et al. (2006) examined 112 plants and reported *Mammea siamensis* T. Anders, *Anethum graveolens* Linnaeus and *Annona muricata* Linnaeus had reduced the number of egg and larvae, prolonged the developmental time, growth retardation and increased the mortality in immature stages of *Aedes aegypti* Linnaeus. *Toddalia asiatica* (L.) Lam. and *Aegle marmelos* Correa plants were also confirmed for their larvicidal and smoke repellency against *Ae. aegypti* and affirmed to have significant negative effects on the pest density and egg laying ability of dengue vector (Vineetha and Murugan, 2009). Recently, stem and leaf extracts of *Mangifera indica* Linnaeus, *Anacardium occidentale* Linnaeus, *Melanochyla fasciculiflora* Kochummen and *Gluta renghas* Linnaeus were tested for their larvicidal activities against laboratory and field strains of *Ae. aegypti* and *Aedes albopictus* Skuse and found *Gl. renghas* and *Mn. indica* best against both species with low doses (Zuharah et al., 2015). Similarly, earlier Rahuman et al. (2009) has also reported the methanolic extracts of stem and leaf of *Mn. indica* for its larvicidal activities against filarial vector, *Culex quinquefasciatus* Say.

This study was initiated to investigate and explore the toxic activities of two plant extracts of *Gl. renghas* and *Mn. indica* for their sublethal effects on percentage of surviving adults, days to reach adulthood, number of eggs laid by F1 generation and hatchability rate of the dengue vectors, *Ae. albopictus* and *Ae. aegypti* mosquitoes. Their efficacy in different conditions for both dry and wet seasons in Penang Island, Malaysia was also evaluated.

Material and methods

Plant species

Mature stem and leaves of *Gl. renghas* were collected from Bukit Panchor National Park, Penang (5°10′10.607″N, 100°32′37.291″E) and *Mn. indica* from Teluk Bahang National Park, Penang (5°27′38.56″N, 100°12′18.69″E), Malaysia. The plant species were authenticated by the Botany Department laboratory staff, School of Biological Sciences, Universiti Sains Malaysia (USM).

Plant extracts

Stem and leaves of the plant species were air dried, powdered using milling machine and commercial stainless steel blender (Panasonic: MX-899TM) for a fine powder form. A total of 50 g of powdered sample was extracted using Soxhlet extractor with 1000 mL of methanol was used as solvent. Cellulose thimble was filled with powdered sample (Favorit cellulose extraction thimbles: size 43 × 123 mm) and placed in the extraction tube of Soxhlet apparatus. The solvent was boiled using a heating mantle at methanol's boiling point of 66 °C. The process was repeated three times until the solvent color turned semitransparent due to complete extraction of all plant contents. The entire process was repeated for three times to have adequate crude extracts for the study. The crude extracts were subjected to the evaporation process to remove the excess solvent, using the rotary vacuum evaporator machine under reduced pressure for about 25–30 min at 66 °C with speed of 100 rpm. After the removal of the excess solvent, the crude extract was used to make the stock solution. One gram paste of the crude extract was diluted in 100 mL methanol to prepare the 10,000 mg/L of stock solution. Sequential serial concentrations were made from the stock solution using distilled water.

Sublethal concentrations

Our previous studies have found the LC_{95} values of *Mn. indica* was 1171.95 mg/L for *Ae. albopictus* and 1197.51 mg/L for *Ae. aegypti*. Whereas, *Gl. renghas* exhibited 975.84 for *Ae. albopictus* and 922.69 for *Ae. aegypti* (Yousaf and Zuharah, 2015). Therefore, in this efficacy study, we were rounding the value of 1000 mg/L and 1200 mg/L for *Gl. renghas* and *Mn. indicia* respectively, for the field application. The decided concentration can killed 95% of both *Aedes* populations.

Field collection of Aedes mosquitoes

This study was designed with six treatments tested in the field with a total of 60 replicates; (1) *Mn. indica* stem extract (1200 mg/L), (2) *Mn. indica* leaf extract (1200 mg/L), (3) *Gl. renghas* stem extract (1000 mg/L), (4) *Gl. renghas* leaf extract (1000 mg/L), (5) temephos (Abate) (recommended dose at 111 mg/L) and (6) control (seasoned water only). These six treatments were placed in ten different locations with five locations in shaded areas and five locations in unshaded areas making it 60 ovitraps in total. The positions of the treatments were randomized within groups to avoid preferences by the *Aedes* mosquitoes. Ovitraps placed at Hamna Apartment areas (5°20′53.9″N 100°18′02.8″E), Pulau Pinang, Malaysia, from February 2014 to August 2014. Shaded places were selected inside the human residences and buildings. Ovitraps were placed in corners and under some old furniture and large boxes far from range and access of human beings, in order to avoid any interference. Unshaded areas were selected outside of the human residences in the open and natural environment, under the trees and away from the main passages to avoid any human interaction.

Ovitraps were made using 400 mL tin cans. Tin cans were cleaned, painted with black spray to attract the mosquitoes for egg laying and labeled with the treatment types. Wooden paddles were placed inside the ovitraps as an eggs' laying substrate for *Aedes* mosquitoes. A total of 300 mL treatments solution was poured in the ovitraps which were then refilled on a weekly basis, because of decreasing volume due to evaporation. Wooden paddles were also collected every week, brought back to the laboratory and replaced with the new ones.

Seasons were divided into dry and wet on the basis of rainfall. As described by the Malaysian Meteorological Service, precipitation below than 200 mm per month is considered as dry season, whereas the precipitation >200 mm per month is categorized as a wet season (Malaysian Meteorological Department, 2013).

Laboratory rearing

After bringing back to the laboratory, collected paddles were left to dry for 48 h at room temperature in the laboratory (28 ± 3 °C). After drying process, each paddle was counted for the *Aedes* eggs under light microscope. Due to the mixture of *Aedes* eggs in this field study area which is impossible to be differentiated between *Ae. albopictus* and *Ae. aegypti* at the egg stage, we decided to use *Aedes* term to represent both species throughout the study as our final objectives is to investigate the lethal dose effectiveness of these plant extracts on both species. Dried paddles were placed in plastic container sized 3 in. height × 6 in. width × 9 in. length, filled with 500 mL of seasoned water to check the percentage of hatchability. Emerged larvae were provided with a fine powdered mixture of yeast, beef liver, powdered milk and dog biscuit at a ratio of 1:1:1:2 by weight at 0.01 g daily. Hatched larvae were counted and days took by the different stages of mosquito were also recorded to investigate the sublethal effects of plant extracts.