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Original Article

LARVICIDAL ACTIVITY OF RIPE AND UNRIPE FRUIT PEEL OF *MUSA PARADISIACA* L. AGAINST THE MALARIA VECTOR *ANOPHELES STEPHENSI*

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ABSTRACT

Objective: To evaluate the larvicidal activity of the ripe and unripe fruit peel of *Musa paradisiaca* against the larvae of the malaria vector, *Anopheles stephensi*. There are no published data on the effect of these *Musa paradisiaca* fruit peel on this mosquito, primarily responsible for indigenous malaria.

Methods: The petroleum ether, ethyl acetate, and distilled water extracts of the ripe and unripe peel of *Musa paradisiaca* were tested against the fourth instar larvae of *Anopheles stephensi.* Larvicidal bioassay followed the standard WHO protocol with slight modifications.

Results: The highest larval mortality was found in ethyl acetate ripe peel extracts. The LC_{50} values of *Musa paradisiaca* ripe fruit peel extracts of petroleum ether and ethyl acetate against *Anopheles stephensi* were 3.21, 2.55 mg/ml, while those of unripe fruit peel extracts were 59.82, 48.08 mg/ml, respectively. Distilled water extract showed 14.588 mg/ml for ripe fruit peel and 14.93 mg/ml of unripe fruit peel. The LC_{90} values of *Musa paradisiaca* ripe fruit peel extracts of petroleum ether and ethyl acetate against *Anopheles stephensi* were 4.8, 4.19 mg/ml, while those of unripe fruit peel extracts were 161.1, 122.22 mg/ml, respectively.

Conclusion: *Musa paradisiaca* fruit peels extracts showed promising larvicidal activity. Ripe fruit peels of *Musa paradisiaca,* which is a waste material, can be exploited as an ideal eco-friendly larvicide, which could be used as an alternative for synthetic pesticides.

Keywords: Larvicidal activity, *Musa paradisiaca*, *Anopheles stephensi,* Malarial vector

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INTRODUCTION

Mosquitoes have been designated as the "public enemy number one" by the World Health Organization [1]. They are capable of transmitting diseases more than any other arthropod species, and they affect millions of people around the World. They are a danger to humans and are the primary carrier of life-threatening diseases such as zika, malaria, dengue fever, yellow fever, chikungunya fever, lymphatic filariasis, and Japanese encephalitis [2, 3]. Malaria affects 40 percent of the world's population, with 300 million people afflicted each year and over one million deaths [4]. *Anopheles stephensi* is the primary vector of malaria in India and other West Asian countries [5]. Their native range centers on the Indian subcontinent, from which they are increasingly expanding their geographic distribution [6, 7]. Larvae of the Anopheles species are generally found indistinctly different habitats and are nocturnal, crepuscular in nature, and also transmit the filarial worm causing filariasis [8]. WHO considers the management of *Anopheles stephensi* as a serious challenge to malaria management putting urban dwellers at a substantially higher and potentially novel risk of malaria transmission [9, 10].

Larvicides are used to kill mosquito larvae in the breeding habitat before they develop into adult mosquitos and spread. While larvicides are effective at controlling mosquitos in their breeding sites, they harm beneficial and non-target species [11]. Prolonged use of synthetic pesticides like organophosphates (chlorpyrifos, temephos, and fenthion) and insect growth regulators (diflubenzuron and methoprene) has initiated many detrimental consequences on humans and the environment [3, 12].

Phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides for replacing synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal, and adulticidal properties [13]. Since ancient times, plant components, ingredients, and secondary metabolites of plant origin have been used in pest control [4]. Many studies on plant extracts have been conducted worldwide to screen the larvicidal properties. Certain natural plant compounds are not only a source of new insecticides and insect repellents but are also botanical chemical derivatives that are environmentally friendly than synthetic chemicals [14]. In India, in recent years, much effect has been focused on the exploration of bioactive, chemical compounds from indigenous plants for mosquito control.

Musa paradisiaca is a perennial tree-like herb widely distributed in moist tropics. Due to enriched food value and versatile medicinal value banana is one of the most important fruits and vegetable crops of India. Banana peel is a waste material usually thrown off from the banana industry. Moreover, previous studies on leaves methanol and 95% ethanol extracts of *Musa paradisiaca* against III instar larvae of *Anopheles stephensi* and *Aedes aegypti* showed promising larvicidal activity [15, 16].

Thus, in this particular study, we are making use of the waste material, banana peel. The study assesses the larvicidal ability of extracts of banana fruit ripe and unripe peel against malaria vectors, in light of a growing interest in developing plant-based insecticides as an alternative to chemical insecticides.

MATERIALS AND METHODS

Plant samples

Ripe and unripe fruits of the *Musa paradisiaca* were purchased from the local market. The material was authenticated by Dr. Sheeja T Tharakan, Department of Botany, Vimala College (Autonomous), Thrissur India. Voucher specimens (Vr. No. VCTBHO301) have been deposited in the laboratory of Botany, Vimala College (Autonomous), Thrissur India.

Preparation of crude extract of banana peels

Fresh ripe and unripe peels of banana fruit were manually separated from the whole fruits and chopped into small pieces of approximately 1 cm size using a sharp razer. The chopped material is further dried (7-15) days in the shade at the environmental temperature, 27-37 °C, and was powdered mechanically using a commercial electrical stainless steel blender and extracted with solvents. The crude extract was prepared by dissolving 25g of the dried powder in 250 ml of

solvents namely distilled water, ethyl acetate (Qualigens), petroleum ether (Qualigens), and the filtrate was taken after one week. The total filtrate was concentrated by evaporation on a water bath at a temperature of 40-50 °C to render the thick extracts of petroleum ether, ethyl acetate, and distilled water and later stored in the refrigerator at 5 °C. From the stock solution,0.3125 to 10 mg/ml concentrations were prepared in dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. The control was set up with acetone, dechlorinated tap water, and polysorbate 80.

Larvicidal activity

Test mosquitoes

The mosquito larvae were collected from the stagnant pond water surrounding the campus of our college, Vimala College, Thrissur, and were identified by Dr. Aneesh E. M, Dept. of Zoology, St. Joseph College, Irinjalakkuda as *Anopheles stephensi.* The colonies of mosquitoes were maintained and all the experiments were carried out at 30–35 °C and 60%-80% relative humidity with a photoperiod of 12 h light followed by12 h dark (12L: 12D). Larvae were reared in white plastic trays containing tap water [13]. Dog biscuits were added to each tray for two weeks to feed the mosquitoes to increase their breeding population.

Larvicidal bioassay

The larvicidal bioassay was assessed by the WHO standard protocol [17] with slight modifications. For bioassay test, the larvae were taken in five batches of twenty in 250 ml of water with the desired plant extract concentration (10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 and 0.3125 mg/ml). Polysorbate 80 (Qualigens) is used as an emulsifier at a concentration of 0.05% in the final test solution. The control was set up with acetone, dechlorinated tap water, and polysorbate 80. All the experiments were conducted in triplicate and control was performed at parallel conditions for each series of experiments. No food was provided for the larvae. Larvae were considered dead if they were unarousable within a period, even when gently probed. Larval mortality was recorded after every 3 h of the exposure until 24 h and their mortality rate was evaluated [18].

Statistical analysis

The larvicidal activities of all these extracts were calculated by preparing a concentration versus mortality rate graph. The Statistical analysis of this experimental data was performed using the computer software MS Excel 2003 to find out the LC_{50}, LC_{90} lethal dose, and standard error through probit analysis at n=5 [18].

RESULTS AND DISCUSSION

The environmental safety of an insecticide is considered to be of paramount importance and should not cause mortality on the non-target organism to be acceptable [19]. Mosquito larvae control using plant-based phytochemicals is considered a good alternative to synthetic chemicals. A large number of plant extracts have been reported to have mosquitocidal or repellant activities against mosquito vectors, but very few plant products have shown the practical utility of mosquito control [20].

The present study indicates that the ripe and unripe peel extracts of *Musa paradisiaca* exhibited potent lethality against the third instar larvae of *Anopheles stephensi*. Ripe peel distilled water extracts of *Musa paradisiaca* were found to be more potent and showed 100% mortality from a 1.25 mg/ml concentration. Ethyl acetate and petroleum ether ripe extracts showed 100% mortality at 5 and 10 mg/ml concentrations. Ethyl acetate extract at a lower concentration of 1.25 mg/ml and 2.5 mg/ml killed 20% and 80% larval population when exposed to 24 h. On the other hand, in the unripe peel extracts, no larvicidal activity was observed till 5 mg/ml concentration in petroleum ether and ethyl acetate extracts (fig. 2). Ripe peel distilled water extracts showed 100 % mortality at 10 mg/ml concentration whereas at 5 mg/ml and 2.5 mg/ml showed 80% and 55 % mortality. Ripe peel extracts showed increased lethality than to unripe peel extracts.

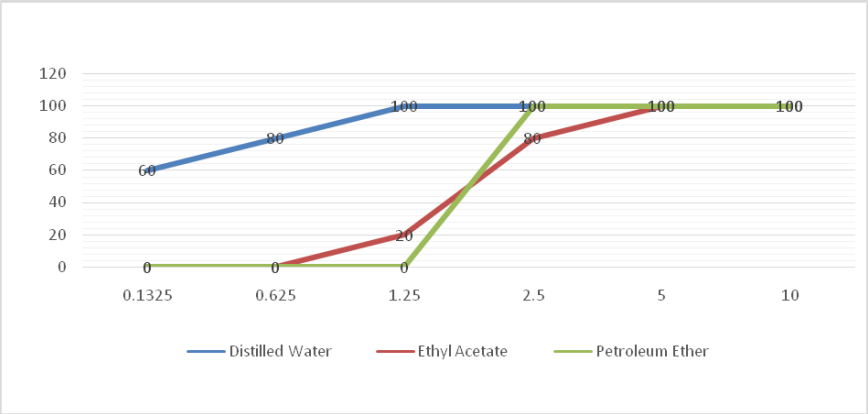

Fig. 1: Mean percentage mortality of ripe extracts at different concentrations against early 3rd instars of *Anopheles stephensi* within 24 h

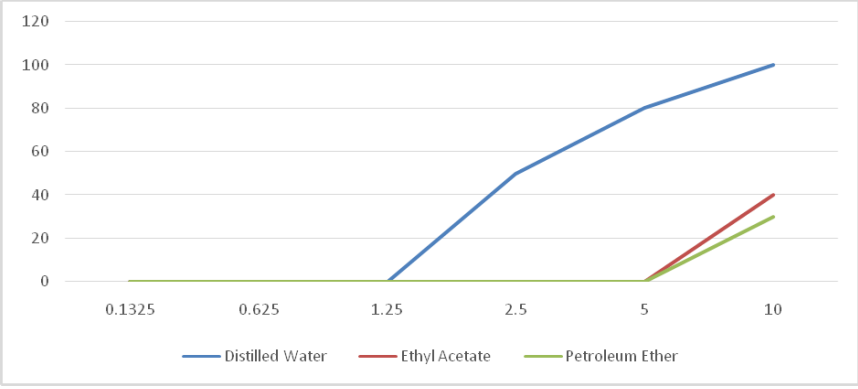

Fig. 2: Mean percentage mortality of unripe extracts at different concentrations against early 3rd instars of *Anopheles stephensi* within 24 h

The LC_{50} values of *Musa paradisiaca* ripe fruit peel extracts of petroleum ether, ethyl acetate, and distilled water against *Anopheles stephensi* were 3.20, 61.2, and 14.58 mg/ml, while those of unripe fruit peel extracts were 59.82, 59.82, and 14.93 mg/ml respectively (table 1). An almost similar result in LC 50 and LC 90 values were observed in the unripe peel extracts of petroleum ether and ethyl acetate. On comparing the LC_{50} and LC_{90} values of both ripe peel extracts, ethyl acetate extract shows the highest mortality percentage. Distilled water extracts of ripe and unripe peel similar results are observed.

Table 1: Larvicidal potential of the ripe and unripe extracts against third instars of *Anopheles stephensi*

Extracts	LC_{50}±SE	LC_{90}±SE
Ripe peel in petroleum ether	3.20+0.815	5.36+0.385
Unripe peel in petroleum ether	59.82+1.79	212.79+1.74
Ripe peel in ethyl acetate	61.2+0.85	88.09+1.14
Unripe peel in ethyl acetate	59.82+0.78	212.79+0.14
Ripe peel in distilled water	14.58+5.75	51.77+1.24
Unripe peel in distilled water	14.93+0.65	44.13+1.73

Control-Nil Mortality, LC50 Lethal Dose 50 concentration which kills 50% of the exposed larval population; LC 90 Concentration which kills 90% of the exposed larval population SE standard error at n=5

Studies of Mary (2018), probit analysis revealed that the ethanolic extract of *Musa acuminata* ripe fruit peel against *Aedes aegypti* exhibited 100 percent mortality at 700 ppm after less than 1 hour of exposure [21]. In another study, aqueous extracts from *Musa paradisiaca* peduncle showed 100% larvicidal property at 0.5 mg/ml concentration within 24 h against *Aedes* mosquito species [22]. Among the seaweed extracts, *Caulerpa racemosa* showed toxicity against 4th instar larvae of *Anopheles stephensi* with an LC_{50} value of 0.0661 µg/ml [23]. The crude dichloromethane extract of *Nyctanthus arbor-tristis* leaves was effective (LC_{50}, 114.56 mg/l; LC_{90}, 367 mg/l) against 4th instar larvae of *Anopheles stephensi* [24]. The study of ethyl acetate extract of *Musa paradisiaca* flowers tested against 4th instar larvae of *Anopheles vagus* showed 65% mortality [25]. Ethanolic extract of *Citrus sinensis*, [26], the methanol crude extract of *Artemisia nilagirica* [27], hexane, chloroform, acetone, methanol, and water extracts of *Morinda citrifolia* leaf [28], methanol extract of the root of *Asparagus racemosus* [29], observed against the larvae of *Anopheles stephensi* suggests the potential use of plant-based phytochemicals to be used as an ideal eco-friendly approach for the control of the vector control programs.

The study has opened up prospects for large-scale extraction of active ingredients of plant origin for effective mosquito control. The present study highlights the conversion of ripe and unripe banana peel produced and dumped as a waste product in municipal landfills, resulting in a major environmental hazard, as a novel product in the larvicidal study. Again this study gains significance as the ripe peels of *Musa paradisiaca*, which are found to be more effective, are perennially available in large quantities.

CONCLUSION

The present study revealed that *Musa paradisiaca* peels have the potential to be included in the formulations of new and safe control products against mosquito vectors. Further studies on the extraction procedure, larvicidal mode of action, their effects on non-target organisms, and formulations for improving their insecticidal potency are to be carried out for their standardization. This can lead to characterizing the active constituents responsible for their larvicidal properties.

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Nil

AUTHORS CONTRIBUTIONS

Manju Madhavan designed the experiment and is involved in the interpretation of data. Shoymol Joy carried out the experimental work, analysis of data, and draft manuscript preparation. The authors went through the final manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest

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