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MOSQUITOCIDIAL ACTIVITY OF *Musa paradisiaca* Linn root (Banana) EXTRACTS AGAINST HAZARDOUS MOSQUITO VECTOR

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ABSTRACT

Mosquitoes are essential etiological vectors of diseases to humans and domestic animals. The larvicidal and oviposition deterrent activity effects of root extracts of *Musa paradisiaca* was tested on the larvae of the vectors, *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. Phytochemical screening of the extracts was conducted to determine the active toxic compounds. GC-MS analysis data confirmed the identification of the active compound. Various concentrations of the root extracts of *Musa paradisiaca* with different solvents viz., petroleum ether, chloroform, ethyl acetate and ethanol were tested against 1st to 4th -instar larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. All the tested extracts showed moderate to good larvicidal activities. However, the maximum larval mortality was detected in petroleum ether root extract of *M.paradisiaca*. Thus the results suggested that *Petroleum* ether root extract of *M.paradisiaca* act as a very effective mosquitocidal activities and ideal eco-friendly approach for the control of vectors.

INTRODUCTION

Mosquitoes are major vectors responsible for the transmission of diseases. Mosquitoes (Diptera: Culicidae) are the oldest human enemy and represent a significant threat to human health that cause several diseases that afflict millions of people worldwide [1]. Mosquito borne diseases such as malaria, lymphatic filariasis, dengue, yellow fever and Japanese encephalitis contribute significantly to human disease burden and death, in addition to poverty and social delibility in tropical countries [2]. *Ae.aegypti* is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa and the Americas. [3]. About two-thirds of the world's population lives in tropical and subtropical areas infested with dengue vectors, mainly *Aedes aegypti* [4]. *Anopheles stephensi* acts as a vector for *Plasmodium vivax* which is responsible for Malaria. The mosquito *Culex quinquefasciatus* acts as a vector for *Wuchereria bancrofti responsible* for filariasis.

Mosquito control became dangerous due to the indiscriminate uses of synthetic chemical insecticides which causes adverse impact on the environment and disturb ecological balance. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of these insecticides entered into the food chain that causes mutation of genes and these changes become prominent only after a few generations [5].These problems have warranted the need for developing alternative strategies using eco-friendly products. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides [6].

In this regard, India has a rich flora that is widely distributed throughout the country. More than 2000 plants

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species have been known to produce chemical factors and valuable metabolites in the pest control programs [7]. Among the 2000 plants, 344 species have been reported to have a variety of activities against mosquitoes [8]. *M .paradisiaca* plants are rich source of alternative agents for control of mosquitoes due to the presence of bioactive chemicals which act as insecticides, antifeedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimoulting hormones as well as attractants. Phytochemicals are advantageous due to their eco-safety, target- specificity, reduced number of applications, higher acceptability and suitability for rural areas.Plant based pesticides are less toxic, delay the development of resistance because of its new structure and easily biodegradable [9] .Therefore, the present study was carried out to evaluate the mosquitocidal properties of *M .paradisiaca* root extract against the vector mosquito, *Ae. aegypti*. *Anopheles stephensi* and *Culex quinquefasciatus*

MATERIALS AND METHODS

Sample collection and Extraction

The *M. paradisiaca* roots were collected and they were cut into pieces and shadow dried at room temperature. The dried roots were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. 100 g of crushed roots were continuously extracted with Petroleum ether using soxhlet up to 48 h. The extract was filtered and concentrated in rotatory evaporator at 35-40ºC under reduced pressure to obtain a semisolid material, which was then lyophilized to get a powder (28.5%, w/v).

Phytochemical analysis:

Phytochemical tests were carried out on the petroleum ether extract of *M. paradisiaca* using standard procedures to identify the constituents described by Malick and Singh, 1980, Segelman *et al.*, 1969, Harborne,The Banana root was analysed for various phytochemical analysis like alkaloids, tannins, phenolic compounds, flavonoid, anthraquinones, glycosides, steroids, aminoacids, saponins, terpenoid

Gas Chromatography – Mass Spectroscopy (GC-MS) analysis

The *M. paradisiaca* powdered sample (25 g) were soaked and dissolved in 150 ml of petroleum ether, methanol, ethyl acetate, chloroform and ethanol for 24 h. Then the filtrates were collected by evaporated under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbo mas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary colu mn. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Mosquito culture

An. stephensi, *Ae. aegypti* and *Cx. quinquefasciatus* were collected from stagnant water at various places within chennai. They were maintained at 27±2°C; 70-80% relative humidity and a photoperiod of L:D 14:10 (light/dark). Larvae were fed with 3:1 mixture of dog biscuits and yeast powder. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages (23x23x32 cm dimension) for adult emergence. *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* adult mosquitoes were reared in the each glass cages of45x38x38 cm separately covered with a plastic screen, with a glass top and a muslin sleeve for access. The adult colony was provided with 10% sucrose was available at all times. After three days, ovitrap was kept inside the cages and the eggs were collected and transferred to the enamel trays. They were maintained at the same condition.

Larvicidal Assay

Larvicidal activity was evaluated using WHO (1996) method with slight modification. Twenty five number of early1- IV instar mosquito larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were released separately in 500ml capacity of beaker containing 250 ml of water and to treat in various concentrations of plant extracts, and then were distributed in each of the replicates. The experiments were carried out at 25 ± 2°C. The test mosquitoes were a laboratory strain of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus.* The mosquito colonies were maintained at 25 - 28°C and relative humidity 80 - 90% under a photoperiod of 14:10 h (light/night) without exposure to any insecticides and pathogen. During this course, larva were feed on finely ground dog biscuits and yeast powered (1:1) and the adult colony was provide with 10% glucose.

Oviposition deterrent activity

The oviposition deterrent test of Musa root extract was performed using the method of Xue et al[8]. Fifteen gravid female mosquitoes *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were (6 days old, 4 days after blood

feeding) transferred to each mosquito cage (45x38x38 cm) and separately covered a plastic screen, with a glass top and a muslin sleeve for access. A 10% sucrose solution was available at all times. Serial dilutions of root extract of each test tube were made. Enamel bowls containing 100 ml of water treated with root extract to obtain test solutions of 0.01, 0.02, 0.05, 0.07 and 1.0%. Two enamel bowls holding 100 ml of water were placed in the opposite corners of each cage, one treated with the test material and the other with a solvent control (1 ml). The positions of the bowls were alternated between the different replicates so as to nullify any effect on oviposition. Five replicates for each concentration were run, with cages placed side by side for each bio-assay. All experiments were run at ambient temperature (27 ± 2°C) with a relative humidity of 70-80%. After 24 h, the number of eggs laid in treated and control bowls were recorded.

The per cent effective repellency for each root extract concentration was calculated using the following formula:

$$ER (\%) = \frac{NC - NT}{NC} \times 100 (\%)$$

Where, ER = Per cent effective repellency

NC = Number of eggs in control

Statistical analysis

The larvicidal bio-assays and per cent control mortality were calculated using Abbyy's transformation LC_{50} andLC_{90} (lethal concentrations causing 50 and 90 per cent mortality) were calculated using Probit analysis. Datafrom larval mortality was subjected to an analysis of variance. Statistical software SPSS 11.5 was used for data analysis.

RESULTS AND DISCUSSION

Man suffers extensively due to the nuisance of insect populations both in agriculture and health. In agriculture, insects affect directly on growing part of the crop and cause severe damage, resulting in revenue loss. In health point of view, insect vectors especially mosquitoes directly transmit diseases like filarial fever, malaria, dengue fever, chikungunya, etc [10]. A considerable number of plant derivatives have shown to be effective against mosquitoes with a safe manner.Identifying plant based insecticides that are efficient as well as suitable and adaptive to local ecological conditions, biodegradable and have the widespread insecticidal property will obviously work as a new weapon in the arsenal of insecticides and in the future may act as a suitable alternative product to fight against mosquito-borne diseases [11]. *M.paradisiaca* plays a pivotal role in the control and killing mosquitoes from ancient and modern cultures due to their presence of effective phytochemicals. These phytochemicals were detected and identified by using GC-MS and LC- MS analysis. In the present study the petroleum ether root extract of *M.paradisiaca* was subjected to GC-MS and the isolated the active compounds such as 4H-Benzo[f]pyrrolo[1,2-a],[1,Benz[a]anthracene-7- carboxyl cobal tocene,1,1 –diacetyl -octadecanoicacid,docosyle2-Methoxypentylphenyl) pyrimidine,2,4-diamino-5-[Benz (a)anthracene,7-ethoxy Benz(a)anthracene were reported and it possesses larvicidal activity and also antibacterial, anti-carcinogenic and anti-nematicide properties (Table 1).

Nowadays, mosquito control programme is focused more on the elimination of mosquitoes at larval stage with plant extracts. The advantage of targeting mosquito larvae is that they cannot escape from their breeding sites/centers until the adult stage and also to reduce the overall pesticide use in control of adult mosquitoes by aerial application of adulticidal chemicals [12-14]. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product based mosquito abatement practices. The larvicidal activity of the stalks and leaves of *Croton argyrophylloides*, *Croton nepetaefolius*, *Croton sonderianus*, and *Croton zehntneri* aqueous extracts showed 100% mortality at 50 ml against *A. aegypti* [15-17] and it is mainly due to the main components of methyleugenol and alpha-copaene for *C.nepetaefolius* (LC50 of 84 ppm); alpha-pinene and beta-pinene for *C. argyrophylloides* (LC50 of 102 ppm); and alpha-pinene, beta-phelandrene, and transcaryophyllene for *C. sonderianus* (LC50 of 104 ppm) and *C. zehntneri*. Likewise, Suwanneepromsiri *et al* studied fourteen plant extracts; only eight plants were showed 100% larvicidal activity against various types of mosquitoes that supports present results. In the present study, *M.paradisiaca* showed potential larvicidal activity against *Ae. Aegypti*, *An. stephensi* and *Cx. Quinquefasciatus*. While, the microscopic examination of dead larvae showed abnormal stretching of body especially the neck region due to the neurotoxic effect of the root extract of *M.paradisiaca* . However, the highest larvicidal potency depicted on petroleum ether extract than other extracts (Table 2).Therefore ,oviposition deterrent activity carried out on only petroleum ether extract of *M.paradisiaca* root extract

The oviposition is one of the most important events in the life cycle of mosquitoes. Oviposition is prevented when the mosquito life cycle is disrupted and population growth is reduced [18]. The present study shows that the petroleum ether root extracts of *M.paradisiaca* act as oviposition deterrent against *Ae. Aegypti*, *An. stephensi* and *Cx. Quinquefasciatus.* The root extract of *M.paradisiaca* greatly reduced the number of eggs deposited by gravid *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* at several concentrations.

At the highest concentrations the extract reduced egg laying by 98.6% against *Anopheles stephensi*. Lower oviposition deterrent was recorded in *Culex quinquefasciatus* (96.6%) and *Aedes aegypti* (97.5%) respectively (Table 3).

Table 1. GC-MS Analysis of *Musa paradisiaca* L

S.No	Compounds	Retention time	Peak Area	%Peak Area
1	4HBenzo[f]pyrrolo[1,2-a]	41.658	4.478	43.240
2	[1,Benz[a]anthracene7carboxyl	43.404	2.016	19.470
3	cobaltocene,1,1-diacetyl-	43.881	4.070	3.930
4	Octadecanoicacid,docosyle	45.204	444641	0.429
5	1-tripropylsilyloxyundecane	45.347	6.611	6.383
6	2-methoxy-6-(4-pentylphenyl)	45.882	1.152	1.113
7	pyrimidine,2,4-diamino-5-	46.190	3.109	3.002
8	Benz(a)anthracene,7-ethoxy	46.413	1.967	18.990

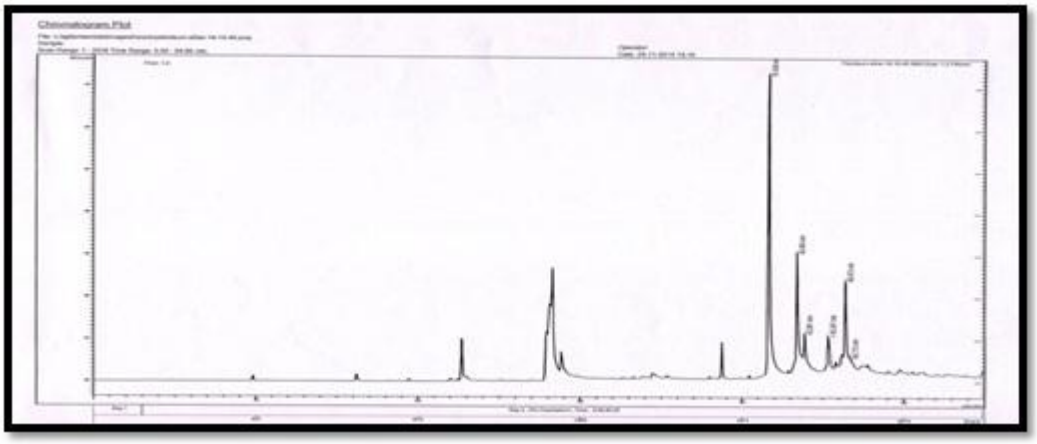
Table 2. Larvicidal activity of root extract of *Musa paradisiaca* L

Larvicidal activity of root extract of *Musa paradisiaca* against *An. stephensi*								
Concentration (mg/10ml)	Ethanol		Chlorofom		Petroleum ether		Ethylacetate	
	Live	dead	live	dead	live	dead	live	dead
0.01	8	2	9	1	6	4	7	3
0.02	7	3	8	2	5	5	6	4
0.03	6	4	8	2	4	6	6	4
0.04	6	4	7	3	4	6	5	5
0.05	5	5	7	3	3	7	5	5
Larvicidal activity of root extract of *Musa paradisiaca* against *Ae. aegypti*								
0.01	7	3	8	2	5	5	6	4
0.02	7	3	8	2	5	5	6	4
0.03	6	4	7	3	4	6	6	4
0.04	5	5	6	4	3	7	5	5
0.05	5	5	6	4	2	8	4	6
Larvicidal activity of root extract of *Musa paradisiaca* against *Cx.quinquefasciatus*								
0.01	7	3	8	2	5	5	6	4
0.02	6	4	7	3	4	6	6	4
0.03	6	4	6	4	4	6	5	5
0.04	5	5	5	5	3	7	4	6
0.05	4	6	5	5	3	7	3	7

Table 3. Oviposition deterrent activity of root extract *Musa paradisiaca* L

Oviposition deterrent activity of root extract of *Musa paradisiaca* against *An. stephensi*			
Concentration%	**treated**	**control**	**Effective repellency%**
0.1	545.5±3.6	756.2+35	16±0.5
0.2	487.6±3.2	763.3±3.6	46.5±0.6
0.5	315.6±3.4	846.4±3.2	64.6±0.5
0.7	98.7±2.4	913.4±3.4	82.4±0.2
1.0	7.8±0.2	997.6±3.2	98.6±0.4
Oviposition deterrent activity of root extract of *Musa paradisiaca* against *Ae. aegypti*			
0.1	523.5±3.3	766.3±3.4	14±0.5
0.2	479.5±3.2	752.3±3.5	45.5±0.4
0.5	308.6±3.3	843.5±3.2	63.5±0.5
0.7	92.5±2.4	902.3+3.43	79.4±0.2
1.0	7.0±0.2	987.6±3.2	97.5±0.4
Oviposition deterrent activity of root extract of *Musa paradisiaca* against *Cx.quinquefasciatus*			
0.1	522.5±3.5	748.2±3.5	15±0.5
0.2	480.5±3.2	761.3±3.5	46.5±0.5
0.5	310.2±3.2	845.4±3.2	66.5±0.5
0.7	94.6±2.5	910.5±3.5	81.4±0.2
1.0	7.2±0.2	978.5±3.2	96.6±0.3

Figure 1.GC-MS Chromatogram of *Musa paradisiaca.L*



CONCLUSION

In conclusion, the findings of the present investigation revealed that petroleum ether root extract of *M. paradisiaca* possess remarkable mosquitocidal activities against *Ae. Aegypti*, *An. stephensi* and *Cx. Quinquefasciatus.* This finding encourages the plant extracts seems to be target specific, effective against mosquitoes and easily available. In future, this study is very helpful to formulate the ecofriendly mosquitocidal strategy based on plant origin insecticides.

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