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## Mosquitoes' larvicidal activity of *Phoenix dactylifera* Linn extracts against *Aedes aegypti*

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### Abstract

*Aedes aegypti* mosquitoes are inducers of viruses such as DENV and ZIKV when they bite a person, which could lead to clinical symptoms like fever, headache, and rashes. This contemporary study aspired to examine the insecticidal potency of different extracts of the seed of *Phoenix dactylifera* on larvae of *Aedes aegypti*. The cold maceration process was used for the extraction of an accurately weighed 100 g of the finely powdered seed of *P. dactylifera* plant using hexane, petroleum ether, and methanol with strong handshaking for 48 hours. Methanolic extract of *P. dactylifera* showed 52% mortality at 1000 ppm and 20% dead at 500 and 250 ppm, respectively, and showed no larvicidal potency at 125 ppm against the fourth instar larvae of *Ae. aegypti*. Whereas hexane and petroleum ether seed plant extracts were found to be equally less effective against the fourth instar larvae of *Ae. aegypti* species, both showed 20% and 12% at 1000 and 500 ppm, respectively. The various concentrations of *P. dactylifera* extracts of hexane, petroleum ether, and methanol induced the larvicidal potency (LC<sub>50</sub>) after 24 h against IV instar larvae of *Ae. aegypti* were 2049.229, 2049.229, and 984.488 ppm, respectively. It was concluded that the seed extracts of *P. dactylifera* are potential candidates to be utilized as novel plant insecticides in curbing the spread of DENV and ZIKV disease vectors.

**Keywords:** *Aedes aegypti*, *Phoenix dactylifera*, extract, larvicidal, mosquitoes, lethal dose

### Introduction

Dengue virus (DENV) and Zika virus (ZIKV), belonging to the family of Flaviviridae, which are infected by *Aedes aegypti* and *Aedes albopictus*, have become one of the major threats among vector-borne diseases, especially in tropical areas [1, 2, 3]. Global travelers increase vector-borne disease transmission as a result of the effects of global warming and increased urbanization [3]. The dengue virus is majorly generated by *Ae. aegypti* also ZIKV is caused by mosquitoes like *Ae. aegypti*, *Aedes africanus*, *Aedes apicoargenteus*, *Aedes vitattus*, *Aedes luteocephalus*, and *Aedes furcifer* [4, 5].

These viruses, DENV and ZIKV, might be induced when a vector, such as an *Ae. aegypti* mosquito, bites a person, which could lead to clinical symptoms like fever, headache, and rashes. However, when the disease progresses to dengue haemorrhagic fever and dengue shock syndrome, it can cause plasma leakage, coagulopathy, and the inability of many organs to function [6, 7].

It is estimated that 500,000 of the 500,000 infected cases require hospitalization for dengue infection, with 5% dying each year [8]. Only in the year 2010, it was recorded that 390 million individuals were infected with dengue viruses, of which clinically evidenced that 96 million people were severe dengue, leading to 21,000 deaths worldwide [9].

Furthermore, several outbreaks of ZIKV have occurred in many African countries in Gabon, Cape Verde, Guinea-Bissau, and Angola in 2007, 2015, and 2016; a respective year of over 7000 suspected cases was reported. 80 per cent of cases of ZIKV Infection recorded were human asymptomatic [10].

For dengue and Zika viruses, there are no current treatments for younger children. The dengue vaccine produced has not been approved for children under the age of eight due to the safety and efficacy of CYD-TDV-Dengvaxia®, a dengue vaccine. It is even only licensed to 20 countries for Zika [9, 11, 12]. Biological control, long-lasting insecticidal nets, choice of resistant diversities, and chemical control by terrestrial, air, or systemic methods are currently methods

of controlling disease vectors such as *Ae. aegypti* and *Ae. albopictus* [13]. Negative consequences had been noted whenever conventional synthetic pesticides like carbamates, organochlorines and organophosphates were dispersed in the environment causing serious toxicity to human health, contaminated agricultural products, and development of resistance to commonly used insecticides [14]. Due to the toxicity of synthetic pesticides on humans, the management of breeding sites is now a widely applicable method for controlling the vectors as a complementary measure. This systemic way of reducing the number of larvae and nymphs of mosquito have resulted in larvicidal treatments, which required constant insecticide application [15, 16].

Recently, there has been an enormous effort to improve the attributes of plant extracts and isolated phytochemicals as insecticidal [10]. Several reports have shown that essential oils extracted from herbs such as *Lantana camara*, *Ocimum tenuiflorum*, *Taraxacum officinale*, *Saussurea lappa*, and *Ocimum kilimandscharicum*, *Nigella sativa*, and *Hyssopus officinalis* proved to be insecticidal agents owing to the presence of secondary metabolites [17, 18, 19].

The plant date palm (*Phoenix dactylifera* Linn) has been reported to contain secondary metabolites such as alkaloids, tannins, terpenoids, flavonoids, phenols, amino acids and carbohydrates which could serve as agents of larvicidal [20]. Source of energy and nutrients that comprise the substantial diet balance required for the body, and dietary fibres derived from many plants that have therapeutic importance in preventing obesity, diabetes, hypertension, coronary heart disease, and hyperlipidemia [21]. Also, this plant has shown to have anti-inflammatory, anti-bacterial, antifungal, antiviral, anti-ulcerative, anticancer, antioxidant, hepato-protective, and anti-mutagenic potentials due to the presence of phytochemical constituents [22, 23].

However, great work needs to be carried out on mosquito control. This contemporary study aspired to examine the insecticidal potency of different extracts of the seed of *P. dactylifera* on larvae of *Ae. aegypti*.

## Materials and Methods

### Plant Collection

The collected *P. dactylifera* seeds were acquired from the Federal College of Dental Technology and Therapy (FCDT & T), Enugu, in October 2021. The recognition of the seed part of the plant was done by a plant taxonomist Mr. Alfred Ozioko from the Bio-resources Development and Conservation Programme (BDGP), Nsukka, Enugu State, Nigeria. The collected plant seed was made waterless at room temperature ( $25 \pm 2$  °C) for two weeks. The dried seeds of *P. dactylifera* were fine-grained into fine particles operated with an electric-powered grinder and then later with muslin cloth of particle size of 0.4 mm. The fine-powdered plant material was kept in a non-transparent container and stored in a refrigerator at a temperature below 4 °C before it was collected for extractions.

### Preparation of Plant Extract

The cold maceration process was used for the extraction of accurately weighed 100 g of the finely powdered seed of *P. dactylifera* plant using hexane, petroleum ether, and methanol with strong handshaking for 48 hours in the Research Laboratory of the School of FCDT & T, Enugu State. The extracts suspended were filtrated using Buchner funnel was incorporated into Whatman® No. 1 filter paper of particle

size of 24 cm. A rotary flash evaporator (RE300-ROTAFL0, England) was employed for the concentration of the crude extracts of the seed of the plant and kept at ( $40 \pm 5$  °C) in an air-tight bottle and preserved in a refrigerator below the temperature of -4 °C until further use. All procedures were strictly followed according to Ajaegbu *et al.*, 2022 [24].

### Sources of mosquito larvae

All the larvae of *Ae. aegypti* employed for this research were obtained from National Arbovirus and Vectors Research Centre in Enugu, and all tests were performed against laboratory standards. The rearing of the *Ae. aegypti* larvae were carried out using distilled water and populated in the laboratory of the School of Preliminary Studies, FCDT&T, Enugu State. The reared larvae of *Ae. aegypti* was rid of vulnerability to insecticidal and pathogens, and the larvae were fed on larval food (grower-chicken feed & fish in a proportion of 3:1). The water that was supplied in culture beaker was conscientiously replaced every day in an alternating manner before IV instar larvae were habituated for bioassay and adult *Ae. aegypti* mosquitoes were enriched with a 10% sugar mixture for 5 days. Matured *Ae. aegypti* mosquitoes were periodically fed with Guinea pig blood. Mosquitoes were kept at  $26 \pm 3$  °C,  $80 \pm 4\%$  RH, and 12: 12 (L: D) h photoperiod cycles [25].

### Larvicidal Bioassay

The plant's susceptibility to *Ae. aegypti* larval IV instar was tested using the standard WHO procedure with some modifications [26]. All of the bioassays were performed in a temperature-controlled room ( $26 \pm 2$  °C) with  $81 \pm 2\%$  relative humidity. The stock solutions of each of the plant extracts were prepared by dissolving accurately weighed 1g of the powdered seed of *P. dactylifera* plant in 2 ml of Tween 80 (an emulsifier) then further increased up to 100 ml of distilled water volumetrically as stock solutions. To obtain four concentrations (1000, 500, 250, and 125 ppm), a serial dilution method was employed for each of the plant extracts, which gave larvae mortality in 48 hours. To ascertain the activity profiles, mosquito larvae were subjected to concentrations of a broad range of the three extracts. All the experiments (extracts) were carried out three times with an equal quantity of controls that were set up simultaneously against larvae of *Ae. aegypti* mosquito species. The two controls were set up. The first was a negative control, which was a mixture of 1ml of Tween 80 in 99 ml of tap water, and the other was a positive control with a daksh insecticide (Dichlorvos 100% EC weight/volume) with a concentration of 2500 ppm (recommended concentration). The insecticide used as a positive control was gotten from the local market at Awka market, Anambra State, Nigeria. Groups of 25 ( $n = 25$ ) early IV instar larvae were transferred through droppers to each 250 ml beaker, containing 100 ml of the test extracts with various concentrations, which gave larval mortality after 24 hours of post-treatment for the tests and controls. Each dose was run four times on different days with the controls, and no food was supplied to the larvae in the test and control groups. The percentage mortality at each concentration was expressed from the dead larvae. If the bioassay tests showed > 20% negative control mortality, the experiments were discarded and repeated. However, during the experiment when bioassay when larvae were unresponsive to gentle prodding with a fine needle, they were considered dead and the observed was corrected by Abbott's formula where

negative control mortality ranged from 5-20%, and (Abbott 1925) [27].

### Statistical evaluation

The percentage of larval mortality data was subdued to Probit evaluation for calculating LC<sub>50</sub>, LC<sub>90</sub> mortality of larvae 24 h post-exposure and other statistics at 95% confidence (limits of upper confidence limit, lower confidence limit), slope and chi-square values, and the mean was calculated using the Student Newman Keuls (SNK) test significantly ( $p=95\%$ ) were calculated using the SPSS 23.0 (Statistical Package of Social Sciences) software.  $p>0.05$  results were considered statistically significant [28].

### Results

The results of the larvicidal activity of *P. dactylifera* are

presented in Table 1. Methanolic extract of *P. dactylifera* showed 52% mortality at 1000 ppm and 20% dead at 500 and 250 ppm, respectively, and showed no larvicidal potency at 125 ppm against the fourth instar larvae of *Ae. aegypti*. Whereas hexane and petroleum ether seed plant extracts were found to be equally less effective against the fourth instar larvae of *Ae. aegypti* species, both showed 20% and 12% at 1000 and 500 ppm, respectively. The various concentrations of *P. dactylifera* extracts of hexane, petroleum ether, and methanol induced the larvicidal potency (LC<sub>50</sub>) after 24 h against IV instar larvae of *Ae. aegypti* were 2049.229, 2049.229, and 984.488 ppm, respectively. On the other hand, the larvicidal activity (LC<sub>90</sub>) of the plant seed extracts of hexane, petroleum ether, and methanol was 9108.3, 9108.31, and 4122.79 ppm, respectively, against IV instar larvae after 24 h of post-exposure (Table 1).

**Table 1:** *Phoenix dactylifera* seed solvent extracts against larval *Aedes aegypti*, 24 h post-exposure

Plant Extract	Conc (ppm)	% Mortality (Mean $\pm$ SD)	LC <sub>50</sub> (UCL-LCL) (ppm)	LC <sub>90</sub> (UCL-LCL) (ppm)	Slope $\pm$ SE	$\chi^2$
Hexane	125	0 $\pm$ 0 <sup>a</sup>	2049.229 (99386.48-1151.66)	9108.31 (22270320.55-2432.64)	2.372 $\pm$ 0.935	1.372
	250	0 $\pm$ 0 <sup>a</sup>				
	500	12 $\pm$ 2.0 <sup>b</sup>				
	1000	20 $\pm$ 2.0 <sup>c</sup>				
	F-value	144.0*				
Petroleum Ether	125	0 $\pm$ 0 <sup>a</sup>	2049.229 (99386.48-1151.66)	9108.31 (22270320.55-2432.64)	2.372 $\pm$ 0.935	1.372
	250	0 $\pm$ 0 <sup>a</sup>				
	500	12 $\pm$ 2.65 <sup>b</sup>				
	1000	20 $\pm$ 3.0 <sup>c</sup>				
	F-value	72.0*				
Methanol	125	0 $\pm$ 0 <sup>a</sup>	984.488 (2260.49-678.96)	4122.79 (35671.98-1928.76)	2.06 $\pm$ 0.522	3.583
	250	20 $\pm$ 3.67 <sup>b</sup>				
	500	20 $\pm$ 1.0 <sup>b</sup>				
	1000	52 $\pm$ 2.0 <sup>c</sup>				
	F-value	326.59*				

Means within a product followed by the same letter do not differ significantly at  $p=0.05$  (Student-Newman-Keuls's test); \* $p<0.05$ ; LC<sub>50</sub> and LC<sub>90</sub>: Lethal concentrations able to kill 50 and 90% of larvae, respectively; ppm: Parts per million; LCL: Lower confidence limit; UCL: Upper confidence limit; (-): No confidence limit estimated;  $\chi^2$ : Chi-square; Number of replicates: 4.

### Discussion

Curbing and reducing dengue and Zika vector-borne diseases is a vital tool via mosquito larval controlling agents in using plant extracts as possible larvicides measure have been regarded another as a workable and preferred choice in the control of mosquito species at various community levels. A large number of plant extracts have been reported to have mosquitocidal or repellent activities against mosquito vectors, but few plant products have shown practical utility for mosquito control [29].

The results in our study are affirmative with previous reports that showed plant extracts possess secondary metabolites and have insecticidal activity. According to Bouda *et al.* (2001), oil from *L. camara* caused 100 per cent mortality in *Sitophilus zeamais* at a concentration of 0.5% (v/w) [30], with leaf and bark extracts of *Cryptomeria japonica* showed that it has strong larvicidal activity against *Ae. aegypti* [31]. Similarly, research conducted on the fruit extract of *Thevetia neriifolia* and the leaf extracts of *Calotropis procera* and *Solanum macrocarpon* had moderate (about 56%) larvicidal activity after 48 hours [32].

This present study revealed that methanolic extract of the seed part of *P. dactylifera* showed greater larvicidal activity of 52% and 20% at concentrations of 1000 and 250 ppm, respectively, against the IV instar larvae of *Ae. aegypti* in contrast to the extracts of hexane and petroleum ether of *P. dactylifera*, which showed similar larvicidal activity of 20%

and 12% at concentrations of 1000 and 500 ppm, correspondingly, against the fourth instar larvae of *Ae. aegypti*.

Our results are consistent with those reported by Oluah and Ezeabiakwa (2011) [33]. The extracts of leaf of *L. camara* using aqueous and methanolic extractions of concentrations (0.3, 0.6, 0.9, and 1.2 g/l). After 24-hour exposure showed percentage mortality rates in larvae treated with 0.3 to 1.2 g/L of ethanolic *L. camara* leaf extract ranged from 91.66 to 96.66% found very effective when compared with aqueous extract counterpart [33].

Likewise, Kamaraj *et al* showed the larvicidal activity of the bark *A. squamosal* against the larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* (LC<sub>50</sub> = 93.80, 104.94 mg/ml), leaf ethyl acetate extract of *C. indicum* against the larvae of *An. subpictus* and *Cx. tritaeniorhynchus* (39.98, 42.29 mg/ml) and leaf acetone extract of *T. procumbens* against the larvae of *An. subpictus* and *Cx. tritaeniorhynchus* (51.57 mg/l, 69.16 mg/l) [34].

It is also in agreement with researches conducted by Nnamani *et al.* (2008) [35], Nayak and Trajani (2014) [36] and Chandrasekaran *et al.* (2019) [37]; they reported that volatile oil and leaf methanol extract of *Vitex negundo* and *Vitex trifolia* have strong bio-control potential against *Cx. quinquefasciatus* and *Ae. aegypti* [35, 36, 37].

The research was conducted on mature fruits and leaves from *Toddalia asiatica* using hexane, acetone, and methanolic



solvent extraction against Dengue carrier (*Ae. aegypti*), and the filarial vector (*Cx. quinquefasciatus*). Extracts of hexane manifested highest larvicidal activity compared to acetone, and methanol extracts against these mosquito Dengue vectors (*Ae. aegypti*) disease with LC<sub>50</sub> (37.23, 50.69, and 125.55 ug/ml) and filarial transmitter (*Cx. quinquefasciatus*) (33.23, 82.20, and 215.19 ug/ml). Hexane, acetone, and methanol extracts of leaves also showed potency against *Ae. aegypti* with LC<sub>50</sub> values of 133.80, 177.20, and 79.48 and against *Cx. quinquefasciatus* with LC<sub>50</sub> values of 164.53, 175.28, and 87.87 ppm, respectively [38]. The purpose of this work was to study the larvicidal activity of *Cassia occidentalis* (Linn.) against the larvae of *Cx. quinquefasciatus* [38].

Similarly, Deepak *et al* 2014 reported the efficacy of petroleum ether and N-butanol extract of *C. occidentalis* (Linn.) at various concentrations tested on third instar larvae of *Cx. quinquefasciatus*. Their work proclaimed that petroleum ether and N-butanol extract of *C. occidentalis* (Linn.) completely have a mortality effect on the larvae of *Cx. quinquefasciatus* at 200 and 300 ug/ml and deduced that larvae of the most transmitters that cause deadly destructive diseases such as filariasis, dengue, yellow fever, malaria, Japanese encephalitis, chikungunya which are considered harmful to the population in tropic and subtropical regions can be terminated with the use of extract from secondary metabolites [39].

Moreover, it has been established the presence of tannins, flavonoids, anthocyanins, leuco-anthocyanins, and triterpenes in n-hexane, dichloromethane, and methanol extracts of *Elaeis oleifera* and *Launaea taraxacifolia* and their further evaluation against the 3rd stage larvae of two genotypes of *An. gambiae* mosquito. Ahousesou *et al.* (2017) [15] revealed that the hydro-methanolic of *E. oleifera* and *L. taraxacifolia* extracts were found to be the most active on the two larval origins, with LC<sub>50</sub> of (448.01 ppm and 182.68 ppm) for 24 hours and (51.38 and 135.13 ppm) for 48 hours exposure for the Kisumu strain and (4199.63 ppm and 157.36 ppm) for 24 hours and (1456.44 and 116.88 ppm) for 48 hours exposure for wild larvae [15].

Furthermore, similar studies carried out by Govindarajan (2010) showed a larvicidal efficacy of the methanolic extract of *Ficus benghalensis* on larvae of *Cx. quinquefascia*, *Ae. aegypti*, and *Anopheles stephensi* compared to the solvents acetone and benzene [40]. The crude extracts studied presently gave varying degree of larvicidal potential, which is attributable to the phytoconstituents in *P. dactylifera*.

## Conclusions

The seed extract of *P. dactylifera* exhibited remarkable larvicidal activity against *Ae. aegypti* larvae, with harmful effects on the larvae. Its consequential toxic effects were an additional symbol that indicated larvicidal activity of the plant is concentration-dependent. The seed extracts of *P. dactylifera* are potential candidates to be utilized as a novel plant insecticide in curbing the spread of DENV and ZIKV disease vectors. In addition, because different extracts of the seed of *P. dactylifera* have proven to be an insecticidal activity that can be effortlessly, and universally cultivated, it is, therefore, a precious replacement for the effective and eco-friendly control of vector-borne infection for both advanced communities.

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