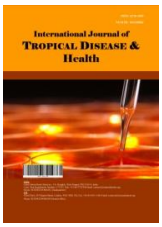

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Effectiveness of Three Fruit Seed Extracts as Larvicide against Three Major Mosquito Vectors *Aedes aegypti* Linnaeus, *Culex quinquefasciatus* Say and *Anopheles gambiae* Giles (Diptera: Culicidae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors LY, KMO and ENN designed the study. Author LY performed the statistical analysis. Authors LY and ENN wrote the protocol and wrote the first draft of the manuscript. Authors LY, TKK, SEE and GAA managed the analyses of the study. Authors LY and KMO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The CH_2Cl_2-MeOH (30:70 v/v) extracts of the seeds of *Mangifera indica* (Mango), *Persea americana* (Avocado) and *Dacryodes edulis* (African plum) were evaluated for potential mosquito larvicidal activity against 3rd and 4th instar larvae of *Aedes aegypti*, *Culex quinquefasciatus* and

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Anopheles gambiae. Extracts were diluted with 1 mL of methanol and concentrations ranging from 1000 to 125 mg/L in 4 replicates each, were prepared in the volume of 100 mL in the plastic cups (250 mL). A volume of 1 mL of methanol added to 99 mL of tap water was prepared as negative control and Bi-one (1000 mg/L) constituted a positive control. In each test solution, 25 larvae of each mosquito species were separately transferred and larval mortality was recorded after 24 h post-treatment. As results, the three plant seed extracts applied at 1000 mg/L caused for at least 79% mortality of each mosquito species larvae assessed. The seed extract of *P. americana* (LC_{50} of 98.31, 129.24 and 136.26 mg/L, respectively against *An. gambiae, Ae. aegypti* and *Cx. quiquefasciatus* larvae) was the most potent followed by *D. edulis* (LC_{50} of 176.87 mg/L for *An. gambiae*, 198.68 mg/L for *Ae. aegypti* and 201.70 mg/L for *Cx. quinquefasciatus*) and *M. indica* (LC_{50} of 258.98 mg/L for *An. gambiae*, 297.35 mg/L for *Ae. aegypti* and 435.45 mg/L for *Cx. quinquefasciatus*). Globally, all the seed extracts were more toxic against *An. gambiae* larvae compared to other mosquito species and need further exploration for the development of a new botanical larvicide to reduce mosquito densities.

Keywords: Larvicidal; CH_2Cl_2-MeOH seed extracts; mosquito species; Anopheles gambiae.

1. INTRODUCTION

Mosquitoes are regarded as the most serious insect pests of public health importance due to their role in vectoring various pathogens such as protozoa, nematodes and arboviruses causing diseases to human and animals [1]. In African countries, mosquito-borne diseases like yellow fever, dengue fever, malaria and lymphatic filariasis are prevalent resulting in millions of deaths every year [2].

Lymphatic filariasis (LF), a neglected tropical disease and a serious health threat in Africa is principally caused by the filarial nematodes *Wuchereria bancrofti*, *Brugia timori* and *B. malayi*. Although species belonging to five mosquito genera i.e. *Ochlerotatus, Culex*, *Aedes, Anopheles* and *Mansonia*, are involved in the transmission of that filariasis, *Culex quinquefasciatus* is its principal vector in tropical and subtropical regions [3,4]. In 2019, an estimated 339.3 million people in 32 countries of WHO African Region were infected by the LF and required mass drug administration [5]. In Cameroon, Nana-Djeunga et al. [6] reported that LF infection is distributed nationwide.

Arboviruses causing yellow fever, dengue, chikungunya, infections mainly transmitted through the bites of tiger mosquito *Aedes aegypti*, are common in Africa. In Cameroon, increasing prevalence of dengue infection has been reported during the last decade [7,8,9,10,11]. In view of scarce availability of dengue vaccine, vector control remains the important tool to prevent and control this disease. Despite the availability of Yellow Fever (YF) vaccine, it remains a major public health problem in Sub-Saharan Africa. Entomological studies conducted in Cameroon and Democratic Republic of Congo have implicated *Ae. aegypti* in the infection, transmission and dissemination of YF [12].

Malaria, transmitted through the bites of *Anopheles* spp, still remains the major public health problem particularly in sub-Saharan Africa. In 2018, WHO African region contributed to 94% of estimated malaria deaths globally of which 67% deaths occurred in children below 5 years [13]. In Cameroon, approximately 6,228,154 malaria cases and about 11,192 deaths were reported in 2018 [13].

Mosquito vector control options include chemical and biological control methods. The most commonly used control method involves application of synthetic chemical insecticides such as pyrethroids, organophosphates, organochlorines, pyrroles carbamates and phenyl pyrazole against mosquitoes [14]. This method, though effective, suffers from the disadvantages of the developing resistance in the target species against these chemicals and the persistence of their residue in the environment which is harmful to the human being and also non-target organisms [15,16]. This, calls for the search of alternative substitute for the chemical insecticides. Botanicals / plant products could be a potential alternative in this regard because of their target specificity, eco-friendly nature, biodegradability and cost-effectiveness. Besides, plant products contain a battery of phytochemicals that limits the development of resistance mosquito vectors [17]. Therefore, various parts such as leaf, bark, stem, flower, fruit, seed and root of the potential plants have

been investigated for their insecticidal properties against many insect pests. These plant parts may contained alkaloids, flavonoids, saponins, tannins, and phenolic compounds that possess insecticidal properties [18]. Thousands of tons of avocado, mango and African plum fruits are produced yearly and their seeds are generally discarded, thereby generating, waste of environmental concern [19].

Persea americana Mill. (Lauraceae) commonly called avocado pear is a fruit tree native to Central America. This plant is widely cultivated in subtropical zones. Orange pigment from its seeds is used as a natural colorant [20,21]. The seed extracts are used in traditional medicine to manage hypertension and in the treatment of dysentery, diarrhea, intestinal parasites, skin infection and tooth ache [22,23]. Extracts have been reported to have larvicidal properties against *An. stephensi*, *Ae. aegypti, Cx. quinquefasciatus* mosquito larvae [24,25,26].

Dacryodes edulis (G. Don) H. J. Lam (Burseraceae), also known as safou or African plum, is an evergreen tree indigenous to and largely distributed in Gulf of Guinea and Central African regions [27]. As medicinal plant, it is used to treat leprosy, dysentery, anaemia, and also possesses antioxidant, antibacterial and antidrepanocytary properties [28-30]. Aqueous, ethanol and hexane extracts of the leaves and seeds of *D. edulis* have been found to possess good larvicidal activity against the larvae of *Ae. vittatus, An. gambiae* and *Cx. quinquefasciatus* mosquito species [31,32].

Originated from South-East Asia, *Mangifera indica* L. (Anacardiaceae), the mango is a tree largely grown in tropical and subtropical regions for its fruits [33]. Tons of mango fruits are industrially processed or locally consumed for its pericarp and seeds are discarded and consequently generate a considerable amount of waste in the environment. Acetone, chloroform, and methanol leaf extracts of *M. indica* exhibited a moderate larvicidal activity against *Cx. quinquefasciatus* larvae [34]. *M. indica* crude extract caused substantial larvicidal activity against *Ae. aegypti* larvae [35].

The present study aimed to evaluate the larvicidal efficacy of the seed extract of three common edible fruits in Cameroon against the major sub-Saharan Africa mosquito vectors including *Ae. aegypti, Cx. quinquefasciatus* and *An. gambiae*.

2. MATERIALS AND METHODS

2.1 Plant Material Collection

The ripe Avocado, African plum and Mango fruits were purchased from Melen market in the Mfoundi Division, region of Centre, Cameroon in May, June and October 2019, respectively. Seeds of each plant were removed from the fruits, cleaned with tap water, chopped in small pieces and grinded in the electric blender. The paste, thus obtained was dried in the oven set at 60°C for 24 h to obtain a dry seed powder. Each plant seed powder were packaged in black plastics and stored in the refrigerator until needed for extraction.

2.2 Extraction of the Plant Seeds

The extraction of the plant seeds was carried out in the laboratory of Phytochemistry, Institute of Medical research and Medicinal Plant Studies of Yaounde in Cameroon. Each dried seed powder was separately soaked in the mixture of CH_2Cl_2-MeOH solvent in proportion (30:70 v/v) for 72 h in which it was manually stirred twice a day. After 3 days of maceration, the macerate was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator and each dried plant seed extract was kept in dark glass bottles in the refrigerator until their uses for phytochemical screening tests and mosquito larvae tests. For each plant seed powder, extraction yield was calculated using the following formula.

$$\text{Extraction yield (\%)} = \frac{\text{Weight of plant seed extract obtained (g)}}{\text{Weight of plant seed powder used (g)}} \times 100$$

2.3 Phytochemical Screening

Seeds of all the three plants were investigated for the presence of phytochemicals likely having insecticidal activity such as alkaloids, flavonoids, phenolic compounds, saponins, tannins, terpenoids and steroids following Harbone [36] in the laboratory of Phytochemistry, IMPM, Yaounde, Cameroon.

2.4 Mosquito Species Collection and Rearing

The larvae of the three mosquito species (*An. gambiae, Ae. aegypti* and *Cx. quinquefasciatus*) were collected from their natural breeding sites in the Yaounde town. In the laboratory, mosquito larvae were separated in sub-families wise

(Culicinae and Anophelinae) based on the morphological identification keys adopted by Azari-Hamidian and Harbach [37] and Gillies and Coetzee [38].

The larvae were fed with biscuits and crayfish (3:1) till they turn into pupae. After pupation species wise, pupae were transferred into 30 cm × 30 cm × 35 cm mosquito cages for emergence. Three days after emergence, mosquitoes were allowed to feed on a rabbit restrained in a wire cage. A bowl lined with filter paper containing tap water was provided in each cage for oviposition. Eggs of each mosquito species were transferred in to buckets containing well water for hatching. Third and fourth instar larvae of that 1st generation were used for the larvicidal test.

2.5 Larvicidal Bioassays

The standard procedure method described by WHO [39] was followed to carry out the larvicidal activity of the extract of the plant seeds against the 3rd and 4th instar larvae *of An. gambiae, Ae. aegypti and Cx. quinquefasciatus* in the laboratory. Indeed, plant extracts were previously diluted with 1 mL of an emulsifier Tween-40 and 4 different concentrations of 1000, 500, 250 and 125 mg/L were prepared in the total volume of 100 mL of tap water in the plastic cups (250 mL) and each concentration was repeated 4 times. The volume of 1 mL of methanol was added to 99 mL of tap water constituted a negative control while the commercial insecticide Bi-one (Dichlovos 49%) tested at the recommended concentration of 1000 mg/L was used as positive control. In each concentration solution test and controls (negative and positive controls), 25 larvae of each mosquito species were separately transferred and larval mortality was monitored after 24 h post treatment. Is declared as dead, when mosquito larval did not longer move even pinched with an entomological needle. The mortality percentages were calculated and then corrected using Abbott [40] formula below if the mortality in the negative control is comprised between 5 and 20%.

> Mortality (%) = Number of dead larvae in test/controls / Total number of larvae used x 100
>
> Corrected mortality (%) = (Number of dead larvae in control -Number of dead larvae in test)/ (100 - Number of dead larvae in control) × 100

2.6 Statistical Analyses

The corrected larval mortality was subjected to the Analysis of Variance (ANOVA) using the Statistical Package for the Social Science (SPSS version 16.0). For means comparison, Tukey test at P= 0.05 was deployed and Probit analysis [41] was performed to determine the concentrations that caused 50% (LC_{50}) and 95% (LC_{95}) mortality of mosquito larvae.

3. RESULTS

3.1 Extraction Yield

From *D. edulis* (360 g), *P. americana* (300 g) and *M. indica* (270 g) plant seed powders macerated in CH_2Cl_2-MeOH solvent (30:70 v/v), extraction yields of 9.41, 7.01 and 11.32%, respectively were obtained (Table 1). However, the extraction yield of *M. indica* (11.32%) was slightly high compared to *D. edulis* and *P. americana* yields.

Table 1. Phytochemical screening results of CH_2Cl_2-MeOH seed extracts

Phytochemical components	Plant species		
	Dacryodes edulis	***Persea americana***	***Mangifera indica***
Alkaloids	+++	+	+
Flavonoids	++	+	++
Phenolic compounds	+	++	-
Saponins	++	+++	+
Tannins	++	+	-
Steroids	-	+	-

-=absent; +=present at low concentration; ++=present at moderate concentration; +++= present at high concentration

3.2 Phytochemical Screening

High concentrations of alkaloids in *D. edulis*; that of saponins in *P. americana* and flavonoids in *M. indica* was detected in phytochemical screening of the plant seed extracts. In general, *M. indica* seed extract was relatively poor in various phytochemicals (Table 1).

Table 2 presents the mortality percent and LC_{50} and LC_{95} (mg/L) values of Plant seed extracts against larvae of *An. gambiae* 24 h post-treatment. Globally, the three plant seed extracts exhibited a significant (P<0.001) larvicidal activity and that efficacy augmented with the increasing concentration of the plant seed extracts. Treated with *P. americana* seed extract, the mortality of *An. gambiae* larvae ranged significantly ($F_{(5, 17)}$ = 747.34; P<0.001) from 63% at 125 mg/L to 100% at 1000 mg/L. With the seed extract of *M. indica*, a moderate larval mortality of *An. gambiae* varying significantly ($F_{(5, 17)}$ = 377.70; P<0.001) from 21% (at 125 mg/L) to 90% at 1000 mg/L) was recoded. Larval mortality of *An. gambiae* ranged significantly ($F_{(5, 17)}$ = 545.76; P<0.001) from 39% (at 125 mg/L) to 100% (at 1000 mg/L) when tested with *D. edulis* seed extract.

After 24 h post-exposure of *An. gambiae* larvae to plant seed extracts, *P. americana* (LC_{50} = 98.31 mg/L and LC_{95} = 379.89 mg/L) was revealed as the most effective against *An. gambiae* larvae compared to *D. edulis* (LC_{50} = 176.87 mg/L and LC_{95} = 781.44 mg/L) and *M. indica* (LC_{50} = 258.98 mg/L and LC_{95} = 1338.45 mg/L

Results of mortality percent, LC_{50} and LC_{95} values of *Ae. aegypti* larvae exposed for 24 h to the seed extracts of *P. americana, M. indica* and *D. edulis* are presented in Table 3. All the plant seed extracts assessed caused a significant larvicidal activity against *Ae. aegypti* larvae and that activity varied with the increasing concentration of each plant seed extracts tested. Tested at the lowest concentration of 125 mg/L, *P. americana* seed extract caused 53% mortality of *Ae. aegypti* and significantly ($F_{(5, 17)}$ = 588.52; P<0.001) reached 100% mortality when applied at the highest dose of 1000 mg/L. The seed extract of *M. indica* caused also a moderate mortality of *Ae. aegypti* larvae varying significantly ($F_{(5, 17)}$ = 209.06; P<0.001) from 16% at the lowest dose (125 mg/L) to 85% at the highest dose (1000 mg/L). *D. edulis* seed extract exhibited also a high larvicidal activity against *Ae. aegypti* larvae and that efficacy ranged significantly ($F_{(5, 17)}$ = 307.04; P<0.001) from 32% at 125 mg/L to 97% at 1000 mg/L.

As against *An. gambiae*, the seed extract of *P. Americana* (LC_{50} = 129.24 mg/L and LC_{95} = 588.52 mg/L) was found to be the most effective against *Ae. aegypti* larvae compared to *D. edulis* (LC_{50} = 198.68 mg/L and LC_{95} = 977.79 mg/L) and *M. indica* (LC_{50} = 297.34 mg/L and LC_{95} = 1739.77 mg/L) seed extracts.

Table 4 presents the mortality rate of *Cx. quinquefasciatus* larvae exposed for 24 h to the seed extracts of *P. americana, M. indica* and *D. edulis*. In general, the three plant seed extracts tested showed a significant toxicity against the larvae of *Cx. quinquefasciatus* and that activity significantly increased with the increasing concentration of each plant seed extract.

The mortality of *Cx. quinquefasciatus* larvae ranged significantly ($F_{(5, 17)}$ = 321.24; P<0.001) from 47% (at 125 mg/L) to 93% (at 1000 mg/L) when exposed to the seed extract of *P. americana*. After application of the seed extract of *M. indica,* mortality of *Cx. quinquefascaitus* larvae varied significantly ($F_{(5, 17)}$ = 269.30; P<0.001) from 8% (at 125 mg/L) to 79% (at 1000 mg/L). Tested with the seed extract of D. edulis, the larval mortality *of Cx. quinquefasciatus* ranging significantly ($F_{(5, 17)}$ = 539.17; P<0.001) from 33% (at 125 mg/L) to 90% (at 1000 mg/L) was registered after 24 h post-treatment.

Among the three plant seed extracts tested, the seed extract of *P. americana* (LC_{50} = 136.26 mg/L and LC_{95} = 1394.20 mg/L) was revealed as the most potent against *Cx. quiquefasciatus* larvae compared to *D. edulis* (LC_{50} = 201.70 mg/L and LC_{95} = 1393.46 mg/L) and *M. indica* (LC_{50} = 435.45 mg/L and LC_{95} = 2208.24 mg/L) seed extracts.3

Table 2. Mortality percentage of mosquito larvae and LC_{50} as well as LC_{95} (mg/L) values of *Persea americana, Mangifera indica* and *Dacryodes edulis* CH_2Cl_2-MeOH seed extracts after 24 h post-exposure against *Anopheles gambiae* larvae in the laboratory (26±3°C, 74±4% R.H.)

Plant species	Conc (mg/L)	% mortality	Slope±SE	R^2	LC_{50} (CI at 95%)	CL_{95} (CI at 95%)	χ^2
Persea americana	0	0.00±0.00d					
	125	63.00±2.51c					
	250	85.00±1.91b	2.80±0.20	0.62	98.31	379.89	17.33^{ns}
	500	98.00±1.15a			(85.71-109.67)	(340.35-436.10)	
	1000	100.0±0.00a					
	Bi-one (1000 mg/L)	100.0±0.00a					
	$F_{(5, 17)}$	747.34***					
Mangifera indica	0	0.00±0.00f					
	125	21.00±2.51e					
	250	52.00±2.82d	2.30±0.11	0.79	258.98	1338.45	17.88^{ns}
	500	75.00±02.51c			(240.59-277.71)	(1159.43-1587.89)	
	1000	90.00±1.15b					
	Bi-one (1000 mg/L)	100.0±0.00a					
	$F_{(5, 17)}$	377.70***					
Dacryodes edulis	0	0.00±0.00e					
	125	39.00±1.91d					
	250	61.00±3.00c	2.54±0.13	0.85	176.87	781.44	30.88**
	500	84.00±1.63b			(154.10-198.72)	(646.25-1008.17)	
	1000	100.0±0.00a					
	Bi-one (1000 mg/L)	100.0±0.00a					
	$F_{(5, 17)}$	545.76***					

*Mean of mortality percent ± standard error within a column followed by the same letter did not differ significantly according to Tukey test at P= 0.05; ^{ns}P>0.05; **P<0.01; **: p<0.001; CI= Confidence interval; SE= Standard error; R^2=Coefficient of determination; χ^2 = Chi-square; Number of replicates: 4*

Table 3. Mortality percentage of mosquito larvae and LC_{50} as well as LC_{95} (mg/L) values of *Persea americana, Mangifera indica* and *Dacryodes edulis* CH_2Cl_2-MeOH seed extracts after 24 h post-exposure against *Aedes aegypti* larvae in the laboratory (26±3°C, 74±4% R.H.)

Plant species	Conc (mg/L)	% mortality	Slope±SE	R^2	LC_{50} (CI)	CL_{95} (CI)	χ^2
Persea americana	0	0.00±0.00d					
	125	53.00±1.91c					
	250	70.00±2.58b	2.49±0.15	0.78	129.24	588.52	30.14**
	500	93.00±1.91a			(107.36-149.02)	(488.36-762.00)	
	1000	100.0±0.00a					
	Bi-one (1000 mg/L)	100.0±0.00a					
	F(5, 17)	588.52***					
Mangifera indica	0	0.00±0.00f					
	125	16.00±1.63e					
	250	52.00±2.82d	2.14±0.11	0.75	297.34	1739.77	44.90***
	500	68.00±4.32c			(255.37-342.72)	(1289.66-2677.62)	
	1000	85.00±3.00b					
	Bi-one (1000 mg/L)	100.0±0.00a					
	F(5, 17)	209.06***					
Dacryodes edulis	0	0.00±0.00e					
	125	32.00±2.30d					
	250	61.00±3.00c	2.37±0.12	0.82	198.68	977.79	32.42**
	500	79.00±3.00b			(172.61-224.20)	(792.20-1298.81)	
	1000	97.00±1.91a					
	Bi-one (1000 mg/L)	100.0±0.00a					
	F(5, 17)	307.04***					

*Mean of mortality percent ± standard error within a column followed by the same letter did not differ significantly according to tukey test at p= 0.05; **p<0.01; **p<0.001; ci= confidence interval; se= standard error; r^2=coefficient of determination; χ^2 = chi-square; number of replicates: 4*

Table 4. Mortality percentage of mosquito larvae and LC_{50} as well as LC_{95} (mg/L) values of *Persea americana, Mangifera indica* and *Dacryodes edulis* CH_2Cl_2-MeOH seed extracts after 24 h post-exposure against *Culex quinquefaciatus* larvae in the laboratory (26±3°C, 74±4% R.H.)

Plant species	Conc (mg/L)	% mortality	Slope±SE	R^2	LC_{50} (CI)	CL_{95} (CI)	χ^2
Persea americana	0	0.00±0.00e					
	125	47.00±2.51d					
	250	68.00±2.82c	1.73±0.11	0.76	136.26	1394.20	22.13ns
	500	84.00±1.63b			(110.24-160.50)	(943.33-1711.29)	
	1000	93.00±2.51ab					
	Bi-one (1000 mg/L)	100.0±0.00a					
	F(5, 17)	321.24***					
Mangifera indica	0	0.0±0.00e					
	125	8.00±01.63e					
	250	33.00±3.00d	2.33±0.11	0.88	435.45	2208.24	26.79*
	500	55.00±2.51c			(392.99-484.95)	(1736.65-3024.92)	
	1000	79.00±3.41b					
	Bi-one (1000 mg/L)	100.0±0.00a					
	F(5, 17)	269.30***					
Dacryodes edulis	0	0.00±0.00f					
	125	33.00±1.91e					
	250	58.00±2.00d	1.96±0.11	0.78	201.70	1393.46	11.85ns
	500	80.00±2.30c			(182.94-220.16)	(1174.40-1717.72)	
	1000	90.00±1.15b					
	Bi-one (1000 mg/L)	100.0±0.00a					
	F(5, 17)	539.17***					

*Mean of mortality percent ± standard error within a column followed by the same letter did not differ significantly according to Tukey test at P= 0.05; $^{ns}P>0.05$; *P<0.05; ***: p<0.001; CI= Confidence interval; SE= Standard error; R^2=Coefficient of determination; χ^2 = Chi-square; Number of replices:4*

4. DISCUSSION

Seeds of numerous wild or domestic edible fruits are commonly discarded after its pulps locally consumed or industrially processed. Worldwide, fruit seed wastes (mango, avocado, etc.) are produced in hundreds of thousands tons yearly and their use as medicine, animal food supplements or insecticides may reduce the environmental disposal problems [19]. Indeed, several previous studies reported the insecticidal efficacy of diverse fruit seed extracts and oils against insect pests and particularly against mosquito species. Study carried out on seed ethanolic extracts of 21 Brazilian plants revealed only three plant seed extracts including *Myracrodruon urundeuva*, *Piptadenia moniliformis* and *Luetzelburgia auriculata* having significant insecticidal property against immature stages of *Ae. aegypti* [42]. *Methanol seed extracts of Momordica charantia, Azadirachta indica,* and *Ricinus communis*, were found toxic against *Anopheles stephensi larvae with* LC_{50} values of 87.00, 15.25 and 54.95 ppm, respectively recorded [43]. Seed extracts of rough lemon (*Citrus jambhiri*) and lemon (*Citrus limon*) were revealed being toxic against *Aedes albopictus* with lowest LC_{50} values of 119.99 and 137.25 ppm respectively registered [44]. The ethanol, chloroform and acetone seed extracts of *Datura stramonium* were found highly effective in the control of rice weevil, *Sitophilus oryzae* [45]. The hexane of *Zanthoxylum heitzii* seed extract played a significant insecticidal efficacy against *An. gambiae* s.s [46]. Acetone, chloroform, and methanol leaf extracts of *M. indica* exhibited a moderate larvicidal activity against *Cx. quinquefasciatus* larvae [34] *M. indica* crude extract caused substantial activity against *Ae. aegypti* larvae [35]. Against other insect pests, *Tagetes erecta, Cynodon dactylon* and *Azadirachta indica* seed extracts showed high direct contact toxicity towards red flour beetles *Tribolium castaneum* [47]. From Bangladesh, seed extracts of *Aphanamixis polystachya* exhibited a strong insecticidal and repellent effects as well as moderate feeding deterrent activity against *Tribolium castaneum* [48]. *Lansium domesticum, Annona muricata, A. squamosa,* and *Sandoricum koetjape* ethanolic seed extracts significantly inhibited the growth of the polyphagous lepidopteran pest *Spodoptera litura* larvae [49]. The crude seed extracts of *Annona squamosa* was found also toxic and possessed antifeedant effect against larvae of cabbage moths, *Plutella xylostella* and *Trichoplusia ni* [50,51].

Those seed extracts might contain diverse bioactive compounds acting singly or in synergic on mosquito eggs larvae, pupae and adults. Most of these discarded fruit seeds might contain diverse phytochemical constituents such as polyphenols, alkaloids, tannins, steroids, flavonoids, etc., having insecticidal proprieties. Like in this present study, *P. Americana* and *D. edulis* seed extracts were rich in alkaloids, flavonoids, tannins, saponins and phenolic compounds and consequently exhibited a high dose-dependent larvicidal efficacy against *Cx. quinquefasciatus*, *An. gambiae* and *Ae. aegypti* larvae. Similarly, Leite et al. [52] detected condensed tannins, flavonoids, triterpenes and alkaloids in methanol seed extract of avocado and these phytochemicals were responsible of high mortality of *Ae. aegypti* larvae with LC_{50} value of 8.87 $mg.mL^{-1}$. The phytochemical β-sitosterol present in the petroleum ether extract of *Abutilon indicum* was reported to be the responsible of high larvicidal activity against *An. stephensi, Ae. aegypti*, and *Cx. quinquefasciatus* [53]. The phytochemical screening of *Annona squamosa and A. muricata* seed extracts revealed that they are rich in flavonoid and alkaloids and consequently induced high mortality ranging from 0.5 to 1% against larvae and from 1 to 5% against adults of *Ae. albopictus* and *Cx. quinquefasciatus* [54]. Dabas et al. [25] reported the richness of avocado seeds in phenolic compounds, playing a role in the toxic effect against insects. The phytochemical screening of ethanol seed extract of *P. americana* conducted by Torres et al. [26] showed the presence of alkaloids, tannins, saponins, unsaturated steroids and triterpenoids, flavonoids, fats and oils and also exhibited high larvicidal toxicity against *Ae. aegypti* larvae with LC_{50} of 16.48 mg/L and LC_{90} of 45.77 mg/L. Some plant components such as the lipids and fatty acids detected in the seed of *D. edulis* contain linoleic acid and linolenic acid, unsaturated fatty acids oleic acid having larvicidal properties against mosquito larvae [28,55]. Study conducted by Torres et al. [26] revealed hexane seed extract of *P. americana* as the most toxic with LC_{50} of 9.82 mg/L and LC_{90} of 22.19 mg/L compared to ethanol seed extract of the plant fruit with LC_{50} of 16.48 mg/L and LC_{90} of 45.77 mg/L on *Ae. aegypti* larvae. Those authors attributed the highest efficacy of that seed extracts to their richness in alkaloids, tannins, saponins, unsaturated steroids and triterpenoids, flavonoids, steroids, terpenoids, essential oils, fats and oils and phenolic compounds previously reported to for their insecticidal properties [56]. In

fact, inhalation, ingestion or cuticle absorption of plant secondary metabolites by mosquito larvae may create basic metabolic, biochemical, physiological and behavioral dysfunctions in the insect systems [57]. According to Rattan et al. [58], botanical secondary metabolites once in contact or ingurgitate by mosquito larvae may penetrate in the insect system to cause a serial physiological dysfunction such as the inhibition of neurotransmitter synthesis, receptor function or pathway enzymes transduction. For the authors, some phytochemical compounds can inhibit GABA-gated chloride channel, acetylecholinestrase and cellular respiration leading to molecular events disruption causing behavior and memory alteration followed by the weakening and the death of the insect.

Coefficient of determination (R^2) values for the 3 plant seed extracts against larvae of mosquito species were ≥0.6, confirming the effectiveness of our 3 plant seed extracts tested. Indeed, according to Faraway [59], regression analysis model using result data from the biological experiments are favorably attributed to the efficacy of the products if R^2 ≥0.6.

5. CONCLUSION

The three plant seed extracts tested in this present study exhibited a high larvicidal property against *An. gambiae, Ae. aegypti* and *Cx. quinquefacsiatus* larvae. Overall, the seed extract of *P. Americana* was the most effective against the three mosquito species larvae evaluated. Among the three mosquito species investigated, larvae of *An. gambiae* were the most sensible to all plant seed extracts tested. Thus, the three plant seed extracts and especially *P. americana* seed extract should be considered as a candidate of the new botanical mosquito larvicide to control mosquito larvae in their breeding sites around the buildings.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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