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Insecticidal activities of the ethanolic extract of citrus fruit seeds for the control of *Culex* mosquitoes

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Abstract

Mosquitoes are among the significant vectors of public health concern. *Culex* mosquitoes have been implicated in spreading disease pathogens that inflicted both animal and man. The Hazards caused by using synthetic insecticide in controlling this vector cannot be overlooked. Thus, there is need to find alternative control method that is harmless and environment friendly. This research aimed to study the insecticidal activities of extract from four *Citrus* species seeds in the control of larval and adult stages of *Culex* mosquitoes. Different concentrations (1.0, 1.5, 2.0, 2.5, and 3.0%) of the extract was prepared using extraction solvent (ethanol) The highest percentage mortality of *Culex* larval was observed from application of 3.0% concentration of *C. paradisi* seeds extract within the period of exposure, 35.00%, 58.33%, 80.00%, and 100.00% larval mortality was recorded respectively. There is a significant difference ($p < 0.05$) comparing control and all the concentrations of *Citrus* seeds extract. Larvicidal potency of 3.0% seed extract concentration of *C. sinensis*, *C. aurantium*, *C. aurantifolia*, and *C. paradisi* are not statistically different ($p > 0.05$). The adulticidal potency of the seed oil varies with *Citrus* species. *Citrus sinesis* seed oil gave higher percentage of adult *Culex* mortality in all the period of exposure. Statistically, the adulticidal potency of 2.5% concentration of all the *Citrus* seed oil are not different significantly ($p > 0.05$), expect the potency of *C. aurantium* seed extract. The lethal concentration requires for 50% (LC_{50}) and 90% (LC_{90}) of *Citrus* seeds oil extract varies with respect to period of exposure. Extract from *Citrus* seeds should be adopted for the control and management of insect vectors and it should be incorporated to integrated vector management (IVM) program.

Article highlights

- The study established the insecticidal potency of *Citrus* seeds extract in the management of vectors of public health importance.
- The results of the study revealed the usefulness of *Citrus* fruits (seeds) waste products which can serve as important raw materials in the production of botanical or plant-based insecticide.
- The findings from the study revealed the more effective seed extract from four types of *Citrus* toxic to both larval and adult stages of *Culex* mosquito.

Keywords *Citrus* · *Culex* · Larvicidal · Adulticidal · And potency

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1 Introduction

Culex mosquitoes are vector of numbers of parasites and virus diseases that afflicted human and animals. Mosquitoes from this genus *Culex* are major vector of avian malaria, filariasis and arbovirus [1]. They are widely distributed across the world with exception of temperate zone of the world. Implication of *Culex* mosquitoes in diseases transmission call for its management, most especially in the tropical region of the world where they are more abundant. Therefore, management of this insect vector required a long-term control to reduce the menace of vector-borne diseases that afflicted human and animal [1, 2]. The use of intervention approaches is one of the most effective control measures. Adulticides, larvicides, and topical repellents among other interventions are used to interrupt vector-host interactions or contact. The life cycle of the parasitic pathogens transmitted by *Culex* mosquitoes is disrupted when the link between mosquito vector and human hosts are broken [2].

Management of insect-vector with synthetic insecticides has been reported as a success in the past due to their quick effect and ease of application [3, 4]. The use of synthetic pesticides or insecticides has shown to have a cognate effect on non-targeted organisms, altering their circumstances by interfering with their normal processes, and in some cases, causing the death of non-targeted organisms [5–7]. Over 200,000 people died every year as a result of synthetic chemicals exposure and misuse [8, 9]. Aside from the environmental issues linked with the usage of synthetic insecticides, there is also the issue of resurgence insect vector and resistance to insecticides [10–12]. To overcome these problems, it is necessary to sort for alternative method of control management of this insect vector. Plants derivatives have been proved by studies throughout time to efficiently control mosquitoes while posing no risk to the environment [6, 13].

Several researchers have proved the insecticidal efficiency of plant components in various studies, and these plants components are referred to as botanical insecticides. Larvicidal potency of the *Thymus* plant extract against *Culex. pipiens pallens*, *Cx. quinquefasciatus*, and *Cx. pipiens* have been reported [14–16]. Larvicidal activities of aromatic plants *Satureja* species against *Cx. Pipiens* had been observed [17]. *Pelargonium roseum* extract toxicity against Cx. *pipiens* was also reported [18]. *Cinnamomum osmophloeum* and *Carum copticum* extract had larvicidal activity against *Cx. quinquefasciatus* and *Cx. pipiens*, respectively [19, 20]. High toxicity activities of *Cinnamomum verum*, *Citrus aurantifolia*, *Cuminum cyminum*, *Syzygium aromaticum*, *Laurus nobilis*, *Lippia berlandieri* and *Pimpinella anisum* extracts against *Cx. quinquefasciatus* larval and pupal were also reported [21]. *Citrus* seeds and peels contain a variety of chemicals that have been proven to be effective against insects [22]. The dried peels of some *Citrus* fruits have been used in the management of pests of storage importance in a variety of methods [23]. The use of crude extract product of peels and seeds of some of *Citrus* fruits in the control of vectors of public health particularly vectors of parasitic diseases have recently gain recognition [24, 25]. Thus, this study is designed to evaluate the use of ethanolic extracts from four *Citrus* seeds species in the management of *Culex* mosquitoes.

2 Materials and methods

2.1 Study area

The research was conducted at the department of Biology laboratory, Federal University of Technology Akure, Ondo State. The State is located between 5° 45′ and 7° 52′ north latitude and 4° 20′ and 6° 05′ east longitude. It has a land area of around 15,500 square kilometres.

2.2 Collection of plant materials

Freshly harvested *Citrus* fruits (*Citrus sinensis*, *Citrus limon*, *Citrus paradisi* and *Citrus aurantium*) were purchased from commercial market and identified at the department of crop science, Federal University of Technology Akure, Ondo State.

2.3 Plant extract preparation

Before peeling, freshly obtained *Citrus* fruits were rinsed with distilled water. The *Citrus* fruit's seeds were carefully separated from the pulp. The seeds were air-dried separately for 21 days at ambient temperature (28 ± 3 °C). The dried seeds were ground into powdered materials using a commercial electric blender and stored in air-tight 250 ml transparent plastic containers. The powdered ingredients were prepared in large enough quantities (400 g) to yield up to 500 ml of extract from each *Citrus* species. Extraction the seed oil was carried out using a Soxhlet extractor using ethanol as the solvent. One hundred (100) grams of the powder was placed into the Soxhlet apparatus (2L) and 500 ml of ethanol was added and mixed. The mixture was heated until *Citrus* volatile oil was exhaustively extracted at a temperature range between 60 and 80° C for 6 h following the method described by Vogel [26] with slight modification. The extract and solvent were separated using rotary evaporator. The extracted oil was exposed at temperature (28–30° C) for the remaining ethanol to evaporate.

2.4 Collection of mosquitoes

Culex mosquito eggs/larva were collected from a slow-moving stream and allowed to hatch in the laboratory collection container. The eggs/larva were identified using the morphological keys described by Williams [27] with the use of an Olympus stereo-dissecting microscope. Before the adults emerged, the larvae were fed baker yeast and dog biscuits in the laboratory until they reached the 4th instar stage. The adult mosquitoes emerged from the pupal phases, which were transported to a rearing cage. The adult *Culex* mosquitoes were fed with a 10% sucrose solution.

2.5 Preparation of *Citrus* seeds extract concentrations

The different concentrations of the *Citrus* seed extract were prepared by adding 0.1 ml of the extract to 9.9 ml of distilled water to make 1% concentration of the extract as described by Simon-Oke and Akeju [24]. The following different concentrations 5%, 10%, 15%, 20%, and 25% concentrations of the extract were prepared as follows; 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml of the extract were dissolved in 9.5 ml, 9.0 ml, 8.5 ml, 8.0 ml and 7.5 ml of the distilled water respectively with three (3) replicates for each concentration, while 10 ml of distilled was used as the control (0%).

2.6 Larvicidal bioassay

The larvicidal bioassay was performed using a similar method described by WHO [28], with minor modifications. Twenty (20) 3rd/4th instar larvae from the culture were individually introduced into the various concentrations of extracted oils of the *Citrus* seeds, as well as a control group containing distilled water. All of the concentrations that were examined were replicated three times. An aliquot of 5 ml of the different concentrations was added to 245 ml of distilled water, while the control contains 250 ml of water. Larvae mortality was recorded after 12, 24, 36, and 48 h, the number of dead larvae was counted and recorded using the formula below:

$$\%\,\text{Larval mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

$$\%\text{Corrected mortality} = \frac{\%\text{test mortality} - \%\text{control mortality}}{100 - \%\text{control mortality}} \times 100$$

2.7 Adulticidal bioassay

The method for mosquito adulticidal test described by WHO [29] and Simon-Oke and Akeju [24] with slightly modified was adopted in this study. Twenty (20) adults were placed in a 1500 ml container with perforated top. An aliquot of 5 ml of 5%, 10%, 15%, 20%, and 25% of the *Cirtus* seeds extracts was soaked in cotton wools and placed in the containers containing adult *Culex* mosquitoes. This experiment was replicated three times. The number of dead mosquitoes was recorded after 30, 60, 90 and 120, 180 min. Percentage mortality was calculated using the formula below:

$$\% \text{ Adult mortality} = \frac{\text{Number of dead adults}}{\text{Number of adults introduced}} \times 100$$

2.8 Data analysis

The results of the adulticidal and larvicidal bioassays were subjected to one-way analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) was used to separate the means. For bioassays where percentage mortalities were the values reported, Probit analysis was used to derive lethal concentrations (LC) required to induce 50% and 90% (LC_{50} and LC_{90}) population mortality.

3 Results

3.1 Larvicidal potency of *Citrus* seed extract on *Culex* mosquitoes larval

The larvicidal effect of *Citrus* seed extract on larval of *Culex* mosquitoes is presented in Table 1. The larvicidal effect of the extract was time and concentration-dependent. The mortality of *Culex* mosquitoes larval increased as the period of exposure and concentration increases. After 12 h of application, 1.0% concentration of *C. sinesis* and *C. aurantium* seed oil evoked the highest larval mortality (20%) followed by *C. paradisi* (13%) and *C. aurantifolia* (10%). The effectiveness of 1.0% concentration of all the *Citrus* seed extracts, 1.5%, 2.0% and 2.5% of *C. aurantifolia* and 1.5% concentration of *C. paradisi* were not different significantly (p > 0.05) after 12 h of application. The highest larval mortality was recorded after

Table 1 Larvicidal effect of some *Citrus* seeds extract on *Culex* mosquitoes larval

Citrus seed extracted oil	Conc. (%)	Mortality (%)			
		12 h	24 h	36 h	48 h
C. sinesis	1.0	20.00 ± 2.89^{bcd}	55.00 ± 17.56^{bc}	66.67 ± 4.41^{bcd}	90.00 ± 2.89^{b}
C. aurantium		20.00 ± 2.89^{bcd}	55.00 ± 2.89^{bc}	66.67 ± 4.41^{bcd}	90.00 ± 2.89^{b}
C. aurantifolia		10.00 ± 5.77^{ab}	31.67 ± 4.41^{b}	61.67 ± 4.41^{b}	90.00 ± 5.77^{b}
C. paradisi		13.33 ± 3.33^{abc}	38.33 ± 4.44^{bc}	70.00 ± 5.77^{bcd}	90.00 ± 2.89^{b}
C. sinesis	1.5	30.00 ± 5.77^{cde}	55.00 ± 2.89^{bc}	71.67 ± 6.01^{bcd}	93.33 ± 3.33^{b}
C. aurantium		30.00 ± 5.77^{cde}	55.00 ± 2.89^{bc}	71.67 ± 6.01^{bcd}	93.33 ± 3.33^{b}
C. aurantifolia		16.67 ± 6.01^{abcd}	40.00 ± 10.00^{bc}	65.00 ± 2.89^{bc}	91.67 ± 6.01^{b}
C. paradisi		25.00 ± 2.89^{bcde}	46.67 ± 6.01^{bc}	73.33 ± 6.01^{bcd}	95.00 ± 2.89^{b}
C. sinesis	2.0	35.00 ± 2.89^{de}	55.00 ± 2.89^{bc}	76.67 ± 3.33^{bcd}	95.00 ± 2.89^{b}
C. aurantium		35.00 ± 2.89^{de}	55.00 ± 17.56^{bc}	76.67 ± 10.14^{bcd}	95.00 ± 2.89^{b}
C. aurantifolia		16.67 ± 3.33^{abcd}	41.67 ± 8.33^{bc}	65.00 ± 7.64^{bc}	95.00 ± 2.89^{b}
C. paradisi		30.00 ± 2.89^{cde}	50.00 ± 5.77^{bc}	76.67 ± 6.01^{bcd}	95.00 ± 2.89^{b}
C. sinesis	2.5	35.00 ± 7.63^{de}	56.67 ± 8.82^{bc}	81.67 ± 4.41^{cd}	95.00 ± 2.89^{b}
C. aurantium		35.00 ± 7.64^{de}	56.67 ± 8.82^{bc}	76.67 ± 3.33^{bcd}	95.00 ± 2.89^{b}
C. aurantifolia		21.67 ± 3.33^{bcde}	43.33 ± 3.33^{bc}	75.00 ± 2.89^{bcd}	95.00 ± 2.89^{b}
C. paradisi		31.67 ± 6.09^{cde}	58.33 ± 4.41^{c}	78.33 ± 7.27^{bcd}	96.67 ± 1.67^{b}
C. sinesis	3.0	40.00 ± 11.55^{e}	63.33 ± 3.33^{c}	83.33 ± 6.01^{d}	96.67 ± 3.33^{b}
C. aurantium		40.00 ± 11.54^{e}	63.33 ± 3.33^{c}	81.67 ± 4.41^{cd}	96.67 ± 3.33^{b}
C. aurantifolia		33.33 ± 6.01^{de}	48.33 ± 4.41^{bc}	75.00 ± 2.89^{bcd}	95.00 ± 2.89^{b}
C. paradisi		35.00 ± 2.89^{de}	58.33 ± 6.01^{c}	80.00 ± 2.89^{cd}	100.00 ± 0.00^{b}
Control	0.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Means followed by the same letter in superscript along the column are not significantly different ($p > 0.05$) from one another using Duncan's New Multiple Range Test (DNMRT)

12 and 24 h of exposure from 3.0% concentration of *C. sinesis* and *C. aurantium*, 40% and 63.33% mortality were recorded respectively; this larvicidal effectiveness was not statistically difference ($p > 0.05$) from 1.5%, 2.0%, 2.5% concentration of *C. sinesis*, *C. aurantium, C. paradisi*, and 2.5%, 3.0% concentration of *C. aurantifolia*. *Citrus sinesis* 30% concentration evoked the highest larval mortality of 83.33% after 36 h of exposure, this value was not significantly difference ($p > 0.05$) from larval mortality evoked by other concentrations of *Citrus* seeds extract except for 1.0% and 1.5% concentration of *C. aurantifolia* seed extract. After 48 h of exposure, all the *Citrus* seed extract concentrations evoked 90% larval mortality and above which was significantly difference ($p < 0.05$) from the control.

3.2 Lethal concentration of *Citrus* seed extract needed to achieve 50% and 90% mortality of *Culex* mosquitoes larval

The concentration of *Citrus* seed oil needed to obtained 50% (LC_{50}) and 90% (LC_{90}) mortality response of *Culex* mosquitoes larval is presented in Table 2. Estimated lethal concentration (LC) to require to evoked 50% and 90% *Culex* larval

Table 2 Lethal concentration (LC_{50} and LC_{90}) of the larvicidal potency of *Citrus* seed extract on *Culex* mosquito

Citrus seeds extract	Period of exposure (hours)	Intercept ± S.E	Lethal concentration (%)		R^2	Sig.
			LC_{50} (LCL–UCL)	LC_{90} (LCL–UCL)		
C. sinesis	12	4.21 ± 0.16	4.84 (2.34–10.01)	62.73 (30.32–129.77)	0.94	0.95
	24	5.04 ± 0.44	0.81 (0.11–5.86)	11.81 (6.36–18.53)	0.90	0.93
	36	5.40 ± 0.17	0.45 (0.21–0.97)	5.60 (2.64–11.89)	0.99	1.00
	48	6.29 ± 0.27	0.06 (0.02–0.20)	0.97 (0.29–3.27)	0.87	1.00
C. aurantifolia	12	3.69 ± 0.13	6.89 (3.77–12.58)	45.98 (25.18–83.96)	0.76	0.69
	24	4.55 ± 0.22	3.56 (1.32–9.56)	30.32 (18.50–35.67)	0.86	0.98
	36	5.25 ± 0.22	0.50 (0.19–1.35)	16.38 (6.11–43.89)	0.78	0.99
	48	6.29 ± 0.32	0.03 (0.01–0.15)	0.99 (0.24–4.17)	0.69	1.00
C. aurantium	12	4.21 ± 0.16	4.84 (2.34–10.01)	62.73 (30.32–129.77)	0.94	0.95
	24	5.04 ± 0.44	0.81 (0.11–5.86)	11.81 (6.36–18.53)	0.90	0.93
	36	5.40 ± 0.17	0.45 (0.21–0.97)	5.60 (2.64–11.89)	0.99	1.00
	48	6.29 ± 0.27	0.06 (0.02–0.20)	0.97 (0.29–3.27)	0.87	1.00
C. paradisi	12	3.97 ± 0.13	5.07 (2.80–9.19)	38.58 (21.31–69.85)	0.98	0.87
	24	4.71 ± 0.16	1.83 (0.89–3.77)	25.81 (12.55–53.09)	0.91	0.99
	36	5.52 ± 0.29	0.17 (0.05–0.63)	13.63 (3.70–50.16)	0.99	1.00
	48	6.32 ± 0.24	0.09 (0.03–0.27)	0.94 (0.33–2.70)	0.75	0.99

R^2 = Statistical measure of mortality proportion in regression model

S. E. = Standard error

LC_{50} = Lethal concentration at which 50% population response

LC_{90} = Lethal concentration at which 90% population response

LCL = Lower confidence limit

UCL = Upper confidence limit

mortality varies with respect to the different *Citrus* seeds extract. The seed extract of *C. sinesis* and *C. aurantium* has the lowest estimated lethal concentration of 4.84% to evoked 50% (LC_{50}) *Culex* larval mortality within the population of 20 larval for 12 h. However, seed extract of *C. aurantifolia* has the lowest estimated lethal concentration of 0.03% to evoked 50% mortality (LC_{50}) in population of 20 *Culex* larval for the exposure period of 48 h. The lowest lethal concentration of 38.58% and 0.95% concentration was estimated from the effectiveness of C. paradisi to evoked 90% mortality (LC90) in population of 20 *Culex* mosquitoes larval for 12 and 48 h exposure period respectively.

3.3 Adulticidal potency of *Citrus* seed extracted on against of adult *Culex* mosquitoes

The adulticidal activities of *Citrus* seed extract on *Culex* mosquitoes is represented in Table 3. The adulticidal potency of the *Citrus* seed extract was concentration and time depended. There is no significant difference ($p > 0.05$) in *Culex* mosquito mortality recorded from the use of 0.5% seed extract concentration of *C. sinesis*, *C. aurantifolia*, *C. aurantium*, and 1.0% seed extract of *C. aurantifolia*, *C. aurantium,* and control after 30 min of application. The highest mortality (75%) of adult *Culex* mosquitoes after 30 min of application was observed from 2.5% concentration of *C. sinesis* seeds extract; this value was different significantly ($p < 0.0$) from 2.5% concentration of others *Citrus* seeds extract. *Culex* mortality recorded from 1.0%, 1.5% and 2.0% concentration of *C. sinesis, C. aurantifolia*, and *C. paradisi* are not significantly difference ($p > 0.05$) after 60 min of application with the highest percentage mortality of 53.33% recorded from 2.0% concentration of *C. sinesis* and *C. aurantifolia* seed extracts. The 2.5% concentration of *C. sinesis* seed extract of evoked the highest *Culex* mosquito mortality (91.67%) after 60 min of application, this was significantly higher compare to the mortality recorded from the application of 2.5% *C. aurantifolia*, *C. aurantium* and *C. paradisi*. After 90 min of *Citrus* seed extract adulticidal assay application, the mortality (90%) observed from the application of 2.0% *C. aurantifolia* seed extract was higher compared to others *Citrus* seeds extract; however, this value was not statistically difference ($p > 0.05$) from mortality of adult *Culex* mosquito observed from *C. sinesis* (80%), *C. aurantium* (71.67%), and C. paradisi (81.67%). The adult mortality of 93.33% and 100% was observed from 2.5% concentration of *C. sinesis* after 90 min and 120 min respectively. There is not significant difference ($p > 0.05$) in percentage mortality recorded from 2.5% concentration of

Table 3 Adulticidal effect of *Citrus* species seeds extract on *Culex* mosquitoes

Citrus seed oil extracted	Conc. (%)	Mortality (%)			
		30 min	60 min	90 min	120 min
C. sinesis	0.5	10.00 ± 5.77^{abc}	41.67 ± 10.14^{cdefg}	66.67 ± 4.41^{cd}	90.00 ± 5.77^{cde}
C. aurantium		0.00 ± 0.00^{a}	15.00 ± 2.89^{ab}	50.00 ± 2.89^{b}	78.33 ± 1.67^{b}
C. aurantifolia		8.33 ± 3.33^{ab}	33.33 ± 8.82^{bcd}	70.00 ± 5.77^{cde}	91.67 ± 1.67^{cdef}
C. paradisi		20.00 ± 5.77^{bcde}	36.67 ± 8.82^{cde}	71.67 ± 6.01^{cdef}	91.67 ± 4.41^{cdef}
C. sinesis	1.0	18.33 ± 3.33^{bcd}	43.33 ± 4.41^{cdefg}	73.33 ± 6.67^{def}	93.33 ± 1.67^{cdef}
C. aurantium		5.00 ± 2.89^{a}	25.00 ± 5.00^{bc}	55.00 ± 2.89^{bc}	85.00 ± 2.89^{bc}
C. aurantifolia		10.00 ± 2.89^{abc}	38.33 ± 9.28^{cdef}	75.00 ± 8.66^{def}	95.00 ± 2.89^{def}
C. paradisi		23.33 ± 6.01^{def}	45.00 ± 5.77^{cdefg}	71.67 ± 1.67^{cdef}	95.00 ± 5.00^{def}
C. sinesis	1.5	25.00 ± 2.89^{def}	51.67 ± 4.41^{defgh}	78.33 ± 11.67^{defg}	96.67 ± 3.33^{def}
C. aurantium		20.00 ± 2.89^{bcde}	45.00 ± 2.89^{cdefg}	70.00 ± 5.77^{cde}	88.33 ± 1.67^{cd}
C. aurantifolia		18.33 ± 1.67^{bcd}	40.00 ± 5.77^{cdefg}	86.67 ± 6.01^{efg}	98.33 ± 1.67^{ef}
C. paradisi		28.33 ± 1.67^{def}	50.00 ± 5.77^{defgh}	80.00 ± 5.77^{defg}	95.00 ± 2.89^{def}
C. sinesis	2.0	28.33 ± 6.01^{def}	53.33 ± 3.33^{defgh}	80.00 ± 2.89^{defg}	98.33 ± 1.67^{ef}
C. aurantium		30.00 ± 2.89^{def}	55.00 ± 5.77^{efgh}	71.67 ± 4.41^{cdef}	90.00 ± 2.89^{cde}
C. aurantifolia		21.67 ± 3.33^{cde}	53.33 ± 11.67^{defgh}	90.00 ± 2.89^{fg}	100.00 ± 0.00^{f}
C. paradisi		31.67 ± 7.27^{ef}	51.67 ± 1.67^{defgh}	81.67 ± 4.41^{defg}	100.00 ± 0.00^{f}
C. sinesis	2.5	75.00 ± 2.98^{g}	91.67 ± 6.01^{i}	93.33 ± 4.41^{g}	100.00 ± 0.00^{f}
C. aurantium		31.67 ± 3.33^{ef}	60.00 ± 5.77^{gh}	76.67 ± 4.41^{defg}	90.00 ± 2.89^{cde}
C. aurantifolia		25.00 ± 2.89^{def}	58.33 ± 4.41^{fgh}	90.00 ± 2.89^{fg}	100.00 ± 0.00^{f}
C. paradisi		35.00 ± 2.89^{f}	66.67 ± 4.41^{h}	88.33 ± 6.01^{efg}	100.00 ± 0.00^{f}
Control	0.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Means followed by the same letter in superscript along the column are not significantly different ($p > 0.05$) from one another using Duncan's New Multiple Range Test (DNMRT) all the *Citrus* seed extracts after 90 min. *C. sinesis*, *C. aurantifolia* and *C. paradisi* 2.5% seed extract evoked 100% mortality after 120 min of application.

3.4 Lethal concentration of *Citrus* seed extract required to achieve 50% and 90% mortality of adult *Culex* mosquitoes

Table 4 show the estimation of *Citrus* seed extracted needed to achieve 50% (LC_{50}) and 90% (LC_{90}) population mortality of adult *Culex* mosquitoes with respect to time required. There is a sharp decrease in estimated concentration of *Citrus* seed extracts required to LC_{50} and LC_{90} as the time of exposure decreases. About 2.35%, 3.22%, 10.68%, and 11.16% concentration of *C. sinesis*, *C. aurantium*, *C. paradisi*, and *C. aurantifolia* is required respectively to achieve 50% (LC_{50}) population mortality of adult *Culex* mosquitoes within 30 min of application. The estimated concentration of *C. paradisi* (951.67%) and *C. aurantifolia* (174.25%) seed extract needed in 30 min to achieve 90% (LC_{90}) mortality of adult *Culex* mosquitoes is extremely high compared to concentration of *C. sinesis* (9.11%) and *C. aurantium* (8.64%) seed extract to achieve the

Table 4 Lethal concentration (LC) of the adulticidal potency of *Citrus* seed extract on *Culex* mosquito

Citrus seed extract	Time (mins)	Intercept ± S.E	Lethal concentration (%)		R^2	Sig.
			LC_{50} (LCL–UCL)	LC_{90} (LCL–UCL)		
C. sinesis	30	4.19 ± 0.09	2.34 (1.57–3.45)	9.11 (6.14–13.49)	0.68	0.02
	60	5.03 ± 0.11	0.98 (0.59–1.60)	7.14 (4.37–11.66)	0.46	0.14
	90	5.69 ± 0.16	0.26 (0.13–0.55)	3.23 (1.54–6.77)	0.71	0.97
	120	6.59 ± 0.28	0.04 (0.01–0.13)	0.54 (0.15–1.87)	0.93	0.96
C. aurantifolia	30	3.87 ± 0.19	11.16 (4.56–27.28)	174.25 (71.27–426.0)	0.99	0.85
	60	4.76 ± 0.20	1.85 (0.75–4.58)	50.49 (20.44–124.7)	0.83	0.82
	90	5.83 ± 0.17	0.21 (0.10–0.46)	2.33 (1.06–5.10)	0.98	0.99
	120	6.67 ± 0.34	0.02 (0.004–0.08)	0.40 (0.08–1.85)	1.00	0.96
C. aurantium	30	3.47 ± 0.08	3.22 (2.39–4.52)	8.64 (4.02–18.63)	0.85	0.26
	60	4.48 ± 0.10	1.83 (1.19–2.82)	8.60 (6.11–12.67)	0.99	0.83
	90	5.27 ± 0.17	0.57 (0.27–1.23)	8.33 (5.42–12.82)	0.98	0.96
	120	6.03 ± 0.29	0.04 (0.01–0.17)	2.60 (0.57–8.30)	0.98	1.00
C. paradisi	30	4.32 ± 0.29	10.68 (2.90–39.33)	951.67 (258.5–3503)	0.99	0.99
	60	4.89 ± 0.19	1.30 (0.55–3.08)	30.79 (13.02–72.83)	0.83	0.90
	90	5.72 ± 0.24	0.14 (0.05–0.41)	4.83 (1.62–14.42)	0.85	0.99
	120	6.58 ± 0.51	0.002 (0.00–0.019)	0.31 (0.03–3.08)	1.00	0.92

R^2 = Statistical measure of mortality proportion in regression model

S. E. = Standard error

LC_{50} = Lethal concentration at which 50% population response

LC_{90} = Lethal concentration at which 90% population response

LCL = Lower confidence limit

UCL = Upper confidence limit

same mortality for the same time of exposure. The lethal concentration of C. sinesis seeds extracts need to for 120 min to achieved 50% and 90% adult mosquito mortality were 0.04% and 0.54% respectively; this estimated concentration is slightly higher when compared with *C. aurantifolia* (0.02% and 0.40%) seed extracts concentration required to achieve the same mortality of *Culex* mosquito. *C. paradisi* seed extract lethal concentration required to evoked 50% mortality of adult *Culex* mosquitoes were 1.30%, 0.14%, and 0.002% for the exposure period of 60 min, 90 min, and 120 min respectively.

4 Discussion

Plant-based insecticide is the safest insecticide for the control of insect vector. This is due to their less toxic to human and environment compared to synthetic insecticides. Though, synthetic insecticides are most preferred because of their fast and quick action in eliminating vectors of parasitic diseases, but the long-term use of synthetic insecticides has been reported to be toxic to human health, contribute to insect resistance, destabilization of ecosystem and many more [30, 31]. Extract from four *Citrus* seed (*C. sinesis*, *C. aurantium*, *C. aurantifolia*, and *C. paradisi*) were evaluated in this study to show adulticidal and larvicidal potency in the control of *Culex* species mosquitoes. All the *Citrus* seed extracts are effective adulticides and larvicides, though the effectiveness varies based on the period of exposure, *Citrus* species seed, adult and larval of *Culex* species mosquitoes used in this study. Larval and adult *Culex* mosquitoes exposed to higher concentration of *Citrus* seed extract gave the range between 90 to 100% mortality. This finding was in agreement with the report of Sanei-Dehkordi et al. [32] and Sarma et al. [33].

C. sinesis and *C. aurantium* are more effective as larvicidal than adulticidal in controlling of *Culex* mosquitoes. Mohamed et al. [34] reported the larvicidal activities of *C. sinesis* and some other plants against *Culex pipiens* larval; the study stated out the importance of phytochemical contents from plant that induced larvicidal activities and adult mosquito repellent potency against *Cx. pipiens*. In a study carried out by Simon-Oke and Akeju [24], extract from peels and seeds were used to control adult *Anopheles* mosquitoes. The insecticidal activities of *Citrus* seeds extract in this study was in agreement with the result of Simon-Oke and Akeju [24]. The result of larvicidal study of *C. sinesis* extract reported by Sattar et al. [35] showed that the mortality responses of *Cx quinquefasciatus* larval to *Citrus* seed extract is concentration and time depended. The result of this study is similar to the findings of Sattar et al. [35]. Larvicidal potency of *C. sinesis* essential extract and its synergetic effect when combine with other plant extracts against *Musa domestica* and *Anopheles stephensi* has been reported by Chauhan et al. [36], it was confirmed in their study that extracted oil from *C. sinesis* have high potency when combine with other plant extracts for vector control. Insecticidal and repellent activity of *C. aurantium* essential oil against *Aedes aegypti*, the main vector of arbovirus has been reported by Leyva et al. [37]; the result of the study showed the effectiveness of oil extracted from *C. aurantium* by causing inhibition of adult mosquito emergency when the mosquito larval was exposed to LC_{90} of the extracted oil. However, the oil is less effective as adulticidal and repellent at a low concentration, but with higher concentration the *C. aurantium* oil perform excellently as adulticidal. The result of this present study was in agreement with the report of Leyva et al. [37].

The result of this study revealed that *C. paradisi* and *C. aurantifolia* are more potent in the control of adult *Culex* mosquitoes and less potent for the control of larval stage. However, the potency of all the *Citrus* seed extracts was found to be concentration dependent. Sarma et al. [33] reported that *C. aurantifolia* oil possess more ovicidal activity than larvicidal activity, but the larvicidal effect increase rapidly as the concentration and time of exposure increases. The findings of the study by Sarma et al. [33] collaborated outcome of the larvicidal effect of *C. aurantifolia* in this present study.

5 Conclusion

Larvicidal and adulticidal potency of *Citrus* seeds extract against larval and adult *Culex* mosquitoes has been established in this research. The toxicity effect of all the four *Citrus* seed extract are concentration and time dependent. Among the four *Citrus* fruits seed investigated, *C. sinesis* seed extract is more effective as larvicidal and adulticidal, while *Citrus paradisi* is more effective as adulticidal than larvicidal. These *Citrus* fruits seeds extract which otherwise regarded as waste could serve as promising plant-based insecticides for the control of insects of public health importance.

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Author contributions IAS, YOL and AVA design the research work, involved in field and laboratory work. AVA carried out statistical analysis. Interpretation of the analysis result was done by YOL. YOL write the first draft of the manuscript. IAS and AVA review the manuscript. All author read and approved the final manuscript.

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Data availability All analysed data involved in this study are included in this manuscript.

Declarations

Ethics approval and consent to participate Not applicable.

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