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Original Article

Larvicidal Effects of Extracts of Artemisia Annua Leaves against Anopheline and Culicine Mosquitoes

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Abstract - Mosquitoes are known to vector more diseases than any other arthropod group, affecting millions of people worldwide. The urgent need for novel insecticides that are effective, safe, biodegradable, affordable, and target-specific is highlighted by the problem of insecticide resistance and its consequences on non-target organisms. Plants, which contain a large number of bioactive compounds, may be an alternative source of mosquito control agents. This study was aimed at investigating the larvicidal activity of Artemisia annua against the Anopheline and Culicine mosquito species. Insecticidal susceptibility tests were carried out using the WHO standard method, and mortality was observed after 24, 48 and 72 hours of exposure. Two solvents were used to obtain the plant extract, and the result was compared to the larvicidal activity of Cymbopogan citratus and Azadirachta indica, which are known to be standard plants with larvicidal activity. There was no significant difference in all the comparisons made. The highest larval mortality rate was observed in anophelines exposed to petroleum ether extract of A. annua, with 100% mortality after 72 hours of exposure, followed by Anophelines exposed to aqueous extract of C. citratus, with 97.5% mortality. Then, culicines were exposed to petroleum ether extract of A. annua with 87.5% mortality and aqueous extracts of A. annua, and C. citratus showed 75% mortality on culicine larvae, respectively. This work recommends that A. annua is a safe and natural mosquito larvicide that is just as effective as the commercial chemical product and should be planted around homes, as they could have the potential to repel mosquitoes and reduce mosquito-borne diseases.

Keywords - Artemisia annua, Larvicidal, Mosquitoes, Azadirachta indica, Cymbopogan citratus.

1. Introduction

The Diptera is one of the most diverse insect orders, with a diverse range of vectors [1]. Mosquitoes are well-known disease vectors, carrying the pathogens that cause some of the world's most dangerous diseases [2]. Mosquitoes spread lethal infections and have a substantial role in disease burden, death, poverty and social degeneration in tropical nations [3]. Chemical pesticides such as methoprene, carbamates, malathion, and dichloro-diphenyl-trichloro ethane have been used to control mosquitoes. Synthetic insecticides are costly, hazardous to the environment, harmful to non-target organisms, and some stay in the environment for lengthy periods, posing a risk to public health. In addition, pesticide overuse or misuse has resulted in ineffectiveness [4].

Botanical insecticides are promising since they are effective, biodegradable, environmentally friendly, and affordable. Extracts from leaves, flowers, and roots of plants have been utilized and assessed in many places across the world and have been demonstrated to be new and promising larvicides [5]. Since ancient times, Artemisia annua, a member of the Asteraceae family, has been used to alleviate fever. Artemisinin, a compound found in this plant, is effective against Plasmodium falciparum. Several researches have been conducted to show that Artemisia species are beneficial against insect pests [6].

Mosquitoes are most effectively and cost-effectively eliminated when they are in the larval stage and concentrated at their breeding site, preventing the larvae from maturing into adult mosquitoes [7]. Chemical control technology is subject to the predicament of unstable foreign exchange [1]. Mosquito-borne diseases have had a significant influence on human health, resulting in death and economic losses.

These problems highlight the urgent need for the development of new insecticides which are effective, safe, biodegradable, affordable and target-specific [8]. Because plants are a rich source of bioactive compounds, they may be an alternate supply of mosquito control agents [9]. This research was aimed at assessing the larvicidal effects of Artemisia annua, Cymbopogan citratus and Azadirachta indica leaf extracts on the larvae of Anopheline and Culicine mosquitoes.

2. Materials and Methods

2.1. Collection and Identification of the Plants' Materials

The plant's leaf samples were collected from the Federal College of Forestry Jos, while *they* were collected from Bayero University Kano premises. The plant specimens were authenticated at the herbarium section of the Department of Plant Science, Bayero University Kano, with an accession number of BUKHAN 652.

2.2. Plant Sampling and Authentication

Leaves of A. annua, C. citratus and A. indica were collected from different parts of Jos and Kano states, Nigeria. All selected plant materials were then identified and authenticated by a qualified botanist of the herbarium section of the Department of Plant Science, Bayero University Kano, Nigeria.

Each plant sample was properly washed using tap water and rinsed with distilled water before air drying under shade. Each dried plant sample material was then pulverized separately into powder and stored in clean polythene bags at ambient temperature for further analysis [10].

2.3. Plant Material Extraction

The processed plant materials were separately percolated in a maceration bottle using water and petroleum ether. The powdered plant sample was then allowed to be macerated for one week in the solvent inside the percolator. Each extract was filtered using a pressure suction pump and then evaporated to dryness at 40 ^{0}C using a rotary evaporator. Individual residue produced were allowed to cool, weighed and stored in a refrigerator, until use [11].

2.4. Larval Collection and Identification

2.4.1. Larval Collection

Anopheline larvae were collected from rice fields, and culicine larvae from stagnant water at Auyo local government of Jigawa state. Larvae were collected by dipping method using entomological larval spoons, plastic cups and suitable containers. Larvae were identified by the presence or absence of a siphon and their resting position on the water surface. The larvae were processed at the site, and worms and other insects were removed according to the method described by Cheah et al. [6].

2.4.2. Larval Identification

Morphological features such as the presence or absence of siphons resting position on the water surface contained in taxonomic keys were used to separate the culicine and anopheline larvae [12].

2.4.3. Larval Transportation

All larvae collected were kept in a plastic bucket; each bucket was labeled with the name of the larvae genus, time and date of collection. The buckets were not filled to the brim in other to allow air space for the larvae to breathe. The buckets were well-covered before they were transported to the laboratory [13].

2.4.4. Larval Rearing

Larvae were reared to the fourth instar larval stage. During the period, larvae were kept in the insectary and feeding was done every other day until they developed into pupae. The pupae were transferred into bowls with tap water and placed in a cage until adults emerged. Adults were maintained in cages and provided with sugar solution in a jar with cotton wool, and they were viewed under the microscope for species identification [14].

2.5. Bio-Efficacy Testing

The larvicidal activity of the leaf extracts of A. annua, C. citratus and A. indica were determined by the methods described by Amado et al. [8]. A plastic dropper was used to move twenty 4th instar larvae of Anopheline and Culicine to small test cups (250 ml), respectively.

Each small test cup contained 100 ml tap water to which four known concentrations (100 ppm, 500 ppm, 1000 ppm and 1500 ppm) were added. Each concentration was replicated four times for each of the species. A control containing no plant extract was designed and included in the experiment. All test containers were held at 25^0C-28^0C.

2.6. Determination of Mortality

The mosquito larvae were considered dead if they were unable to move after gentle touching with a needle or glass rod. Moribund larvae were unable to rise to the surface when the water was disturbed. The larval mortality was observed, counted and recorded after 24, 48 and 72 hours of the exposure, respectively [5, 15].

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of introduced larvae}} \text{ X } 100$$

2.7. Statistical Analysis

The statistical tools used in this study include Arithmetic mean to calculate the average number of dead mosquito larvae, and Log probit analysis was used to determine the median Lethal Concentration (LC_{50}) and 90% Lethal Concentration (LC_{90}) of the formulation at 24, 48 and 72 hours of treatment. Student's t-test was used to determine whether there was a significant difference in the mean percentage mortality throughout observation between the three plant extracts with a 95% confidence interval.

3. Results

3.1. Larvicidal Activity of Aqueous and Petroleum Ether Extracts of the Plants on Anopheline Larvae

The percentage mean mortality of anopheline larvae exposed to aqueous and petroleum ether extract of the plants was calculated and the result was presented in Table 1. Mortality of larvae in aqueous extracts of the plants was observed in all the plants at all concentrations, with C. citratus showing the highest percentage mortality of 97.5%.

The mortality of anopheline larvae in petroleum ether extract of the plants was observed. The highest percentage mean mortality of 100% was observed in A. annua.

Table 1. Percentage mean mortality of Anophelines in aqueous and petroleum ether extract of the plants

Concentration (ppm)	*A. annua*				*C. citratus*				*A. indica*		
Aqueous Extracts											
	24hrs	**48hr**	**72hrs**		**24hrs**	**48hrs**	**72hrs**		**24hrs**	**48hrs**	**72hrs**
100	8.75	15	15		27.5	32.5	35		23.75	58.75	65
500	21.5	40	45		46.25	51.25	51.25		18.75	55	68.75
1000	40	58.75	75		60	63.75	65		36.25	72.5	83.75
1500	72.5	85	95		90	95	97.5		38.75	93.75	95
P- value					0.1538	0.2516	0.1459		0.0732	0.0871	0.1459
Petroleum Ether Extracts											
	24hrs	**48hr**	**72hrs**		**24hrs**	**48hrs**	**72hrs**		**24hrs**	**48hrs**	**72hrs**
100	35	45	50		36.25	42.5	42.5		46.25	57.5	58.75
500	50	60	60		50	63.75	63.75		67.5	72.25	73.75
1000	68.75	88.75	90		85	91.25	91.25		81.25	92.5	92.5
1500	98.75	100	100		90	92.5	92.5		90	98.75	98.75
P-value					0.0654	0.0550	0.0691		0.1217	0.9045	0.0896

Legend: Each test cup contained 20 mosquito larvae for all concentrations, and each concentration was replicated four times

3.2. Larvicidal Activity of Aqueous and Petroleum Ether Extracts of the Plants on Culicine Larvae

Table 2 shows the percentage mean mortality of culicines in aqueous and petroleum ether plant extracts. In aqueous extracts, A. annua and C. citratus showed the highest percentage mean mortality of 75%, while in petroleum ether extract, A. annua showed the highest percentage mean mortality of 87.5%.

Table 2. Percentage mean mortality of culicines in aqueous and petroleum ether extract of the plants

Concentration (ppm)	*A. annua*				*C. citratus*				*A. indica*		
Aqueous Extracts											
	24hrs	**48hr**	**72hrs**		**24hrs**	**48hrs**	**72hrs**		**24hrs**	**48hrs**	**72hrs**
100	6.25	10	11.25		8.75	13.75	13.75		7.5	15	17.5
500	13.75	22.5	23.74		26.25	31.25	41.25		8.75	20	26.25
1000	25	35	45		43.75	51.25	58.75		16.25	40	48.75
1500	35	51.25	75		66.25	73.75	75		13.75	53.75	66.25
P- Value					0.9999	0.0550	0.0691		0.1538	0.0609	0.4298
Petroleum Ether Extracts											
	24hrs	**48hr**	**72hrs**		**24hrs**	**48hrs**	**72hrs**		**24hrs**	**48hrs**	**72hrs**
100	10	18.75	21.25		13.75	21.25	21.25		16.25	22.5	27.5
500	18.75	43.75	50		35	40	43.75		37.5	43.75	51.25
1000	40	63.75	66.25		68.75	71.25	71.25		60	70	75
1500	62.5	80	87.5		80	82.5	83.75		55	77	60
P-Value					0.8018	0.9999	0.3124		0.4989	0.6395	0.2846

Legend: Each test cup contained 20 mosquito larvae for all concentrations, and each concentration was replicated four times

3.3. LC_{50} and LC_{90} Values of the Plant Extracts Against Anopheline and Culicine Larvae

Toxicity analyses of the three plant extracts with two different solvents on anopheline larvae show that aqueous A. indica extract had the larvicidal activity with LC50 and LC90 of 109.65 ppm and 47.86 ppm respectively (Table 3). A. indica petroleum ether extract also had the larvicidal activity with LC50 and LC90 of 30.90 ppm and 95.49 ppm using the petroleum ether extracts (Table 4). The toxicity test of the culicine larvae using the aqueous extract shows that C. citratus extract has the highest larvicidal activity. However, while using the petroleum ether extracts against culicine larvae, A. annua showed the highest larvicidal activity with LC50 and LC90 values of 138.38 ppm and 14454.39 ppm (Table 6).

Table 3. LC_{50} and LC_{90} values of aqueous extract of the plants against Anopheline larvae

Plants	Time (hrs)	LC_{50} (ppm)	LC_{90} (ppm)
A. annua	24	1023.9	71413.10
	48	512.86	1621.81
	72	389.05	1621.81
C. citratus	24	371.54	1348.96
	48	275.42	2137.96
	72	1023.9	1659.59
A. indica	24	512.86	67.61
	48	389.05	47.86
	72	109.65	47.86

Table 4. LC_{50} and LC_{90} values of petroleum ether extracts of the plants against Anopheline larvae

Plants	Time (hrs)	LC_{50} (ppm)	LC_{90} (ppm)
A. annua	24	239.88	1202.26
	48	181.97	891.25
	72	147.91	758.58
C. citratus	24	239.88	1905.46
	48	218.78	1258.93
	72	109.65	588.84
A. indica	24	30.90	95.49
	48	102.33	588.84
	72	81.28	426.588

Table 5. LC_{50} and LC_{90} values of aqueous extract of the plants against culicine larvae

Plants	Time (hrs.)	LC_{50}	LC_{90}
A. annua	24	2570.4	112201.85
	48	2137.96	37153.52
	72	912.01	6918.3
C. citratus	24	1071.52	8912.51
	48	776.25	6918.31
	72	616.59	4897.79
A. indica	24	2570.4	112201.85
	48	1862.09	45708.82
	72	977.24	15488.17

Table 6. LC_{50} and LC_{90} values of petroleum ether extract of the plants against culicine larvae

Plants	Time (hrs)	LC_{50}	LC_{90}
A. annua	24	138.38	14454.39
	48	489.78	4073.80
	72	380.19	2630.27
C. citratus	24	537.03	15488
	48	436.52	15488
	72	416.87	15488
A. indica	24	870.96	14454.39
	48	512.86	8128.31
	72	338.84	4073.80

3.4. Dose Response Curve

Figures 1 to 4 show the relationship between, mean percentage mortality, concentration and period of exposure for each of the plant extract against each genus of larvae. It shows how the mortality of larvae increases with an increase in concentrations and time of exposure.

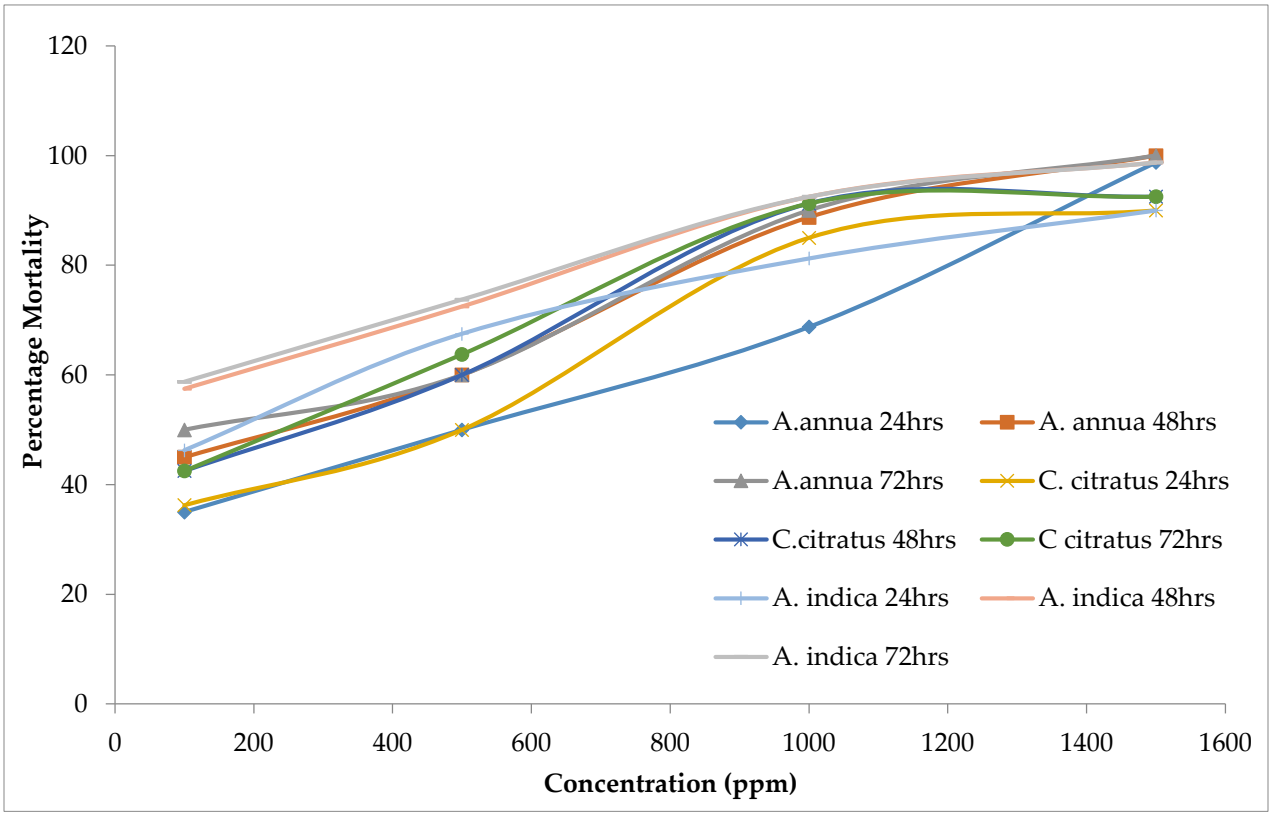

Fig. 1 Percentage mortality of anophelines in aqueous extract of the plants

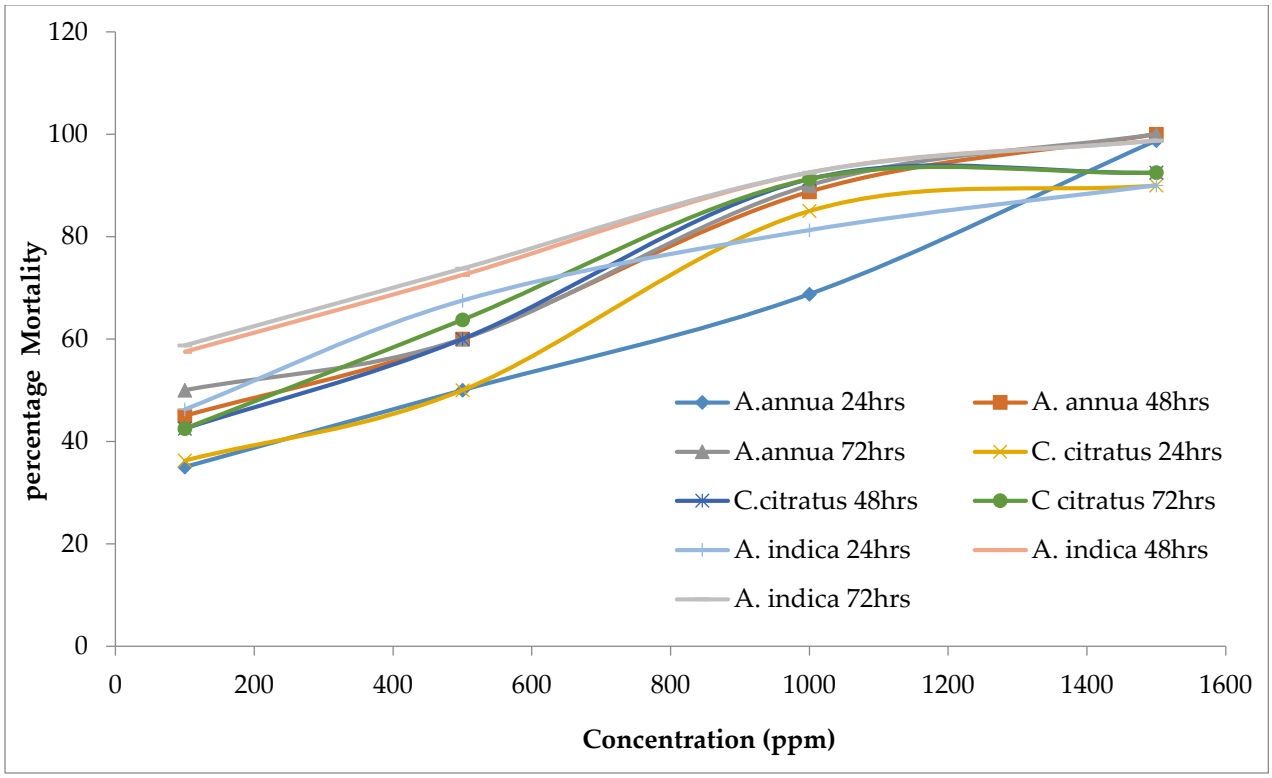

Fig. 2 Percentage mortality of anophelines in petroleum ether extract of the plants

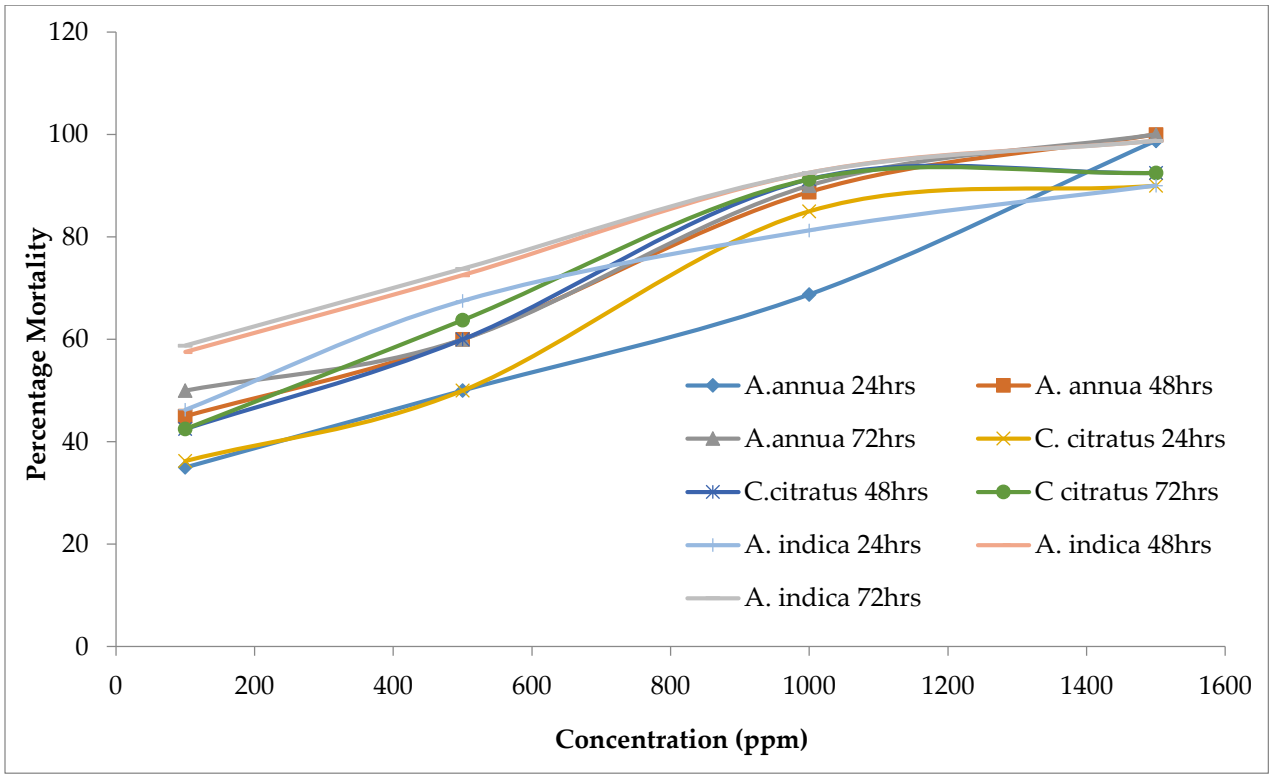

Fig. 3 Percentage mortality of culicines in aqueous extracts of the plants

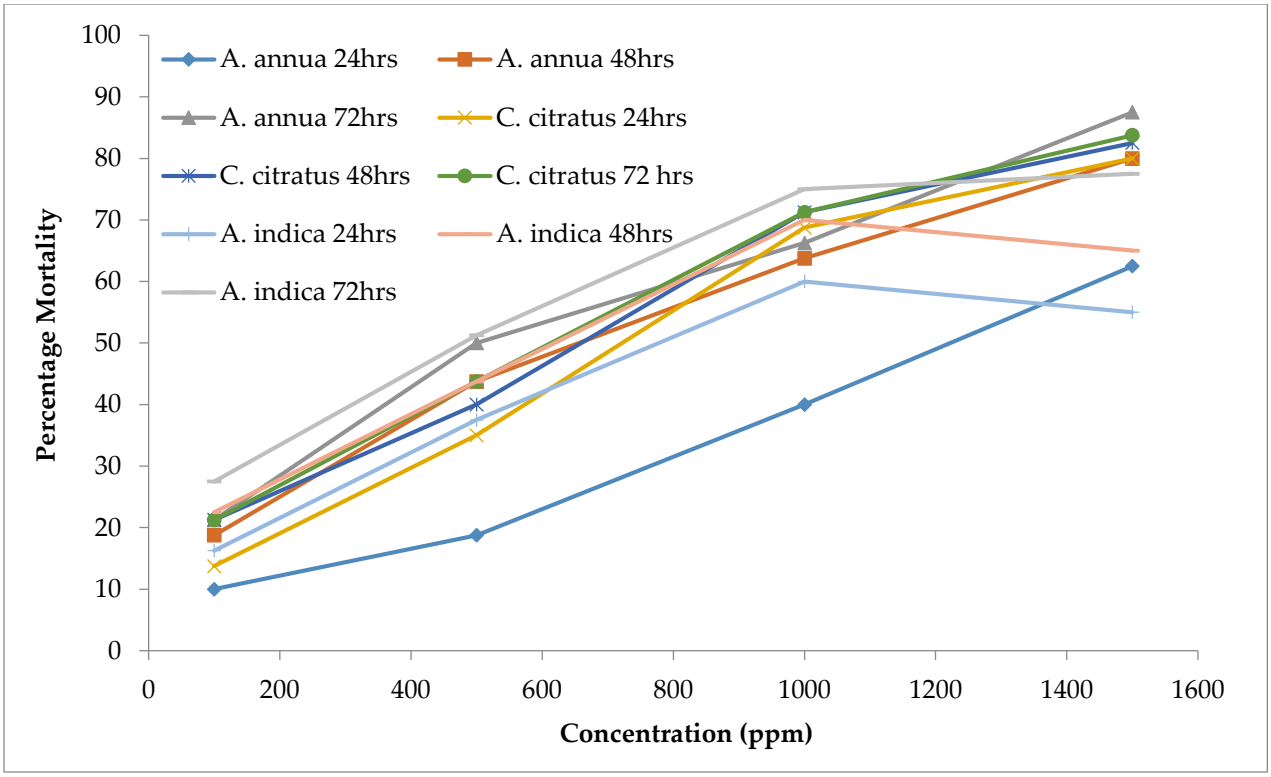

Fig. 4 Percentage mortality of culicines in petroleum ether extract of the plants

4. Discussion

Diseases transmitted by mosquitoes continue to be a major cause of illnesses and death worldwide. Malaria, filariasis, Japanese encephalitis, and dengue fever are the most apparent diseases (parasitic and viral) transmitted by mosquitoes of the genera Anopheles, Culex and Aedes [16]. In this research, parts of three plants (A. annua, C. citratus and A. indica) were extracted using two different solvents (water and petroleum ether) by maceration method because the method of extraction plays a decisive role in the qualitative and quantitative composition of the extracts. Some extraction methods can cause the plant to lose some of its properties [17].

Bio efficacy of the plant extracts was tested against the anopheline and culicine larvae, and the results revealed that all the leave extracts were lethal to 4th instar larvae, especially at higher concentrations; there was no or less mortality in the controls that were set for the study and some of the larvae in the control pupated and developed into adults while those in the extract solutions did not. That shows that the bioactive components in the plants' extracts are responsible for larval mortality, especially at higher concentrations. This result is in line with the result of research titled Insecticidal Activity of A. annua against Anopheles gambiae conducted by Ogbonna et al. [18], where ethanolic extracts of A. annua leaves and seeds showed larvicidal activity on A. gambiae.

A. annua extracted with petroleum ether showed the highest larval mortality of 100% and 87.5% among plants that were extracted with the same solvent and tested on both anophelines and culicines, respectively, probably because the active components of A. annua are extracted better in petroleum ether than in water. Similarly, in research titled Larvicidal effects of A. annua against Aedes aegypti, Alanazi [19] explained that ethanolic extract of the plant caused 100% mortality and aqueous extract caused 84% mortality and concluded that all extracts of A. annua were toxic to the larvae in a dose-dependent manner. C. citratus extracted with water showed the highest larval mortality of 97.5% on anopheline larvae among other plants that were extracted using the same solvent and exposed to the same genus of larvae. This could be because the bioactive components of C. citratus are extracted

well than the other plants in water; this is in line with the findings of Adhikari et al. [20], which explained that the activity of various plant parts varies with respect to solvent and extraction method adopted. It is also in accordance with the result of research by Jong et al. [21].

A higher percentage of mortality was observed on anopheline larvae than on culicine larvae which could be as a result of their breeding habitat. Anopheline larvae breed in fresh waters that are free of pollutants and, therefore, find the plant extracts more toxic than the culicine larvae, which breed in stagnant waters containing pollutants and other toxic substances. Therefore, they are more resistant to the plant extracts.

Based on statistical analysis, the comparison between the mean mortality of larvae in extract of Artemisia annua to the mean mortality of larvae in extracts of Cymbopogan citratus and Azadirachta indica showed no significant difference at all concentrations and hours of exposure. This is because all three plants possess various phytochemical constituents that are responsible for larval mortality. Some of these constituents are common within the plants; therefore, the variation in the percentage mortality of the larvae is a result of the phytochemical constituents present in the plants. This result is similar to the research outcome of Asiry et al. [5].

A. indica has the highest activity on anopheline larvae. This is because the phytochemicals present in A. indica are highly effective on mosquito larvae. According to log probit analysis, the smaller the LC_{50} or LC_{90} values, the higher the activity of the extract. This result is similar to the result of work titled Mosquitoes larvicidal activity of leaf extract of A. indica by Maragathavalli et al. [22], where the result showed methanolic extract of A. indica leaves against Aedes aegypti showed maximum activity at 200 mg compared to 150mg, 100mg and 50 mg. Dua et al. [23] also reported a similar result in the larvicidal activity of neem oil formulation against Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti. Among the aqueous extract of the plants against culicine larvae, C. citratus has the highest activity; it has the lowest LC_{50} and LC_{90} values because the bioactive molecules in lemon grass are well extracted in water and very effective on culicine larvae; this result is comparable to the result presented by Goselle et al. [24], in the larvicidal activity of lemon grass against culex larvae where an extract of dried lemon grass showed highest larval mortality against culicine larvae. Among the petroleum ether extracts of the plants that were tested against culicines, A. annua showed the highest activity which could be as a result of the bioactive molecules of the plant that dissolved well in petroleum ether. Ilahi [25] explained a similar result in the larvicidal activity of different parts of Artemisia vulgaris against C. quinquefasciatus, where the leaves showed higher larval mortality when compared to roots and stems. The result of the dose-response curve presented in this work explains the relationship between mortality of larvae, concentration and period of exposure because mortality increases with an increase in concentration and time of exposure.

5. Conclusion

This study has shown the effectiveness of aqueous and petroleum ether extracts of Artemisia annua leaves on anopheline and culicine mosquitoes. The mean mortality of each species of mosquito is different; lethality varied by type of mosquito and extract used; anopheline larvae were more susceptible to the extracts than the culicine larvae. Among extracts, petroleum ether extract was more toxic to both anophelines and culicines where it caused up to 100% mortality on anopheline larvae and 87% mortality on culicine larvae. Treatment with this plant leaves extract prevented the larvae from developing into pupae and adults, while some larvae in control developed into pupae and adults.

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Appendix

Larval Bioassay