International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531

(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Phytotoxic Potentials of some Plants Extracts Against Southern House Mosquito (*Culex quinquefasciatus* SAY)

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Abstract

Six (6) plant species were screened for larvicidal potentials against the larvae of Southern house mosquito, *Culex quinquefasciatus*. The plants included *Hyptis lanceolata, Hyptis suaveolens, Hyptis spicigera, Lantana camara, Cymbopogon citratus* and *Zingiber officinale*. Crude extracts were obtained in different solvents using Soxhlet extractor. Their larviciding activity was investigated by immersion and batches of 20 instar larvae were treated with 62.5, 125, 250, 500, and 1000mg/l of the plant extracts after 96 hours exposure, for 5 days and in three replicates. Extracts that did not cause mortality at 62.5mg/l necessitate the use of higher concentrations (1500 and 2000 mg/l). Control treatment consists of 1ml acetone in distilled water. Results revealed that all the 6 plants exhibited varying larvicidal activity. *L. camara* showed highest percentage mortality (63.20%) at the least concentration of 62.5mg/l. This was followed by *H. lanceolata, H. suaveolens* (57.9%) and *Z. officinale* which caused 57.9% each at this same concentration. Determined L_{50} and L_{95} indicated *L. camara* (56.23 and 223.87mg/l) to be the most potent of the plant extracts. Statistical analysis indicated significant differences ($p<0.05$) in efficacy of concentrations and the various exposure periods. Recommendation was made for the inclusion of these plant extracts in an integrated pest control scheme.

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Keywords: *Culex quinquefasciatus*; instar larvae; *Lantana camara*; crude extract; mortality.

1. Introduction

The Southern house mosquito (*Culex quinquefasciatus*) of the family Culicidae, is a brown colored, medium-sized mosquito found in Tropical and Subtropical regions of the world. It is a vector of many pathogens of humans, domestic and wild animals. The specie is a vector of *Wuchereria bancrofti*; a nematode that causes lymphatic filariasis, avian malaria, and arboviruses including St. Louis encephalitis virus, Western equine encephalitis virus, and West Nile virus[1]. It acts as an intermediate host for the helminthes parasite by harboring the larval stages [2]. It also transmits *Plasmodium relictum*, a malarial parasite of birds, and a principal vector in many endemic areas where they affect the socio-economic status of a nation [3].It is the definitive host for malarial parasite as it harbors the sexual cycle [4].

Cx. quinquefasciatus breeds profusely in dirty water collections, including stagnant drains, cesspools, septic tanks with leaks, burrow pits, and almost all organic polluted water collections. In optimum temperature and humidity, the lifecycle will be completed in seven days, passing through the egg, larval, pupal, and adult stages [5]. Gravid *Cx. quinquefasciatus* females fly during the night to nutrient-rich standing water where they will lay their eggs. They oviposit in waters ranging from waste water areas to bird baths, old tires, or any container that holds water. If the water evaporates before the eggs hatch or the larvae complete their life cycle, they die [6].

The larvae of *Cx. quinquefasciatus* feed on biotic material in the water and require between five to eight days completing their development at 30°C [7]. The larvae progress through four larval instars, and towards the end of the fourth instar they stop eating and molt to the pupal stage. Following 36 hours at 27°C the adults emerge from the pupal stage [7].

Southern house mosquito is a worldwide insect that is vulnerable or dreadful nuisance in transmitting many devastating diseases. Vector borne diseases transmitted by mosquito and other blood-sucking insects to humans and other animals are among the most prevalent of the communicable diseases in Nigeria and other tropical African countries [8]. The development and spread of vectors and diseases transmitted by such mosquitoes are usually aided by man's activities such as the construction of dams, roads, irrigation channels, poor drainages and sanitary conditions which provide breeding sites. The use of synthetic chemicals is the most common practice of mosquito control. These have been established to be expensive, unsafe as they may be hazardous to the food chain; environmentally unfriendly since most are used as aerosols that could deplete the ozone layer and have above all become ineffective due to resistance developed by the insects. However, there is need to seek safe and effective alternative methods of control with an urgent need to explore and utilize naturally occurring plant products for combating mosquito vectors. These have been used traditionally by communities in many parts of the world against the vector and pest species of insects [9].

This research was undertaken to evaluate the phytotoxic/larvicidal efficacy of some locally available plants on Southern house mosquito, *Culex quinquefasciatus*.

2. Hypotheses

- There will be no significant larvicidal effect on the larvae of *Cx. quinquefasciatus* by any of the extracts from *H. lanceolata, H. suaveolens, H. spicigera, L. camara, C. citratus or Z. officinale.*
- There will be no significant difference in the efficacy of the extracts of these plant species.
- There will be no significant difference in the efficacy between varying concentrations of the plant extracts.
- There will be no significant difference in the larvicidal efficacy based on the duration of exposure of larvae to the plant extracts.

3. Materials and Methods

3.1. Collection and Preparation of Plant parts for Extraction

Collection of plant materials was made in February, 2008. *Hyptis suaveolens* was collected along Shika-Giwa road where it was found growing on uncultivated farm borders and roadsides. *H. lanceolata* and *H. spicigera* were collected around the Bomo Lake. *Lantana camara* was collected in the Samaru campus of Ahmadu Bello University (A.B.U.) Zaria Nigeria, where it is grown as an ornamental plant. *Cymbopogon citratus* (Lemon grass) was collected on farmlands in Giwa village, where it is used by farmers to demarcate farmlands. *Zingiber officinale* rhizomes were purchased fresh from the Sabon Gari Market in Zaria. All the collected plants were taken to the Herbarium in the Department of Biological Sciences, A.B.U. Zaria for confirmation of identification and preparation of vouchers. Leaves of *Hyptis* plants, *L. camara* and *C. citratus* and the rhizome of *Z. officinale* were shade-dried for seven days. This was to prevent ultra violet rays from inactivating active ingredients in the plant materials.

3.1.1 Extraction Procedures

The various dry plant materials were pulverized and stored in black polythene bags.

3.2. Maceration or Aqueous Extraction

Maceration was carried out by measuring 20g of each dried materials and putting in 250ml capacity beakers. 100 ml of warm distilled water was then added to each beaker and the contents thoroughly mixed by stirring with a glass rod and proper agitation. The contents were then allowed to stand for 15 minutes, thus allowing suspended materials to settle down. The clear liquids above the solid materials were then decanted and filtered. The solid material remaining at the bottom of each beaker was again covered and mixed with warm distilled water and the procedure repeated. The combined solvent extracts for each plant type were then evaporated in a water bath. The residue was then collected as crude extract and stored.

3.3. Soxhlet Extraction

This involved the use of soxhlet apparatus and an ethanol-acetone organic solvent mixture. 20g of each dried plant material was weighed and placed in a porous thimble and this was in turn placed in the inner tube of the soxhlet apparatus. This apparatus was then fitted to round-bottomed flasks (600ml capacity) containing the

solvent and boiling chips; this was subsequently connected to a reflux condenser. The solvent when heated passed through a tube as vapor and then condensed. The condensed solvent then fell into the thimble and slowly filled the body of the soxhlet to the top of the tube. From this part it then siphoned over the flask, thus removing the portion of the substance extracted from the plant material in thimble. This procedure continued to repeat itself automatically until complete extraction was achieved. This was indicated by the complete coloration of the solvent; which was then evaporated in a water bath. A residue was then left behind in each case, which was the extract in semi solid or paste form. The extracts so obtained were then stored in glass containers and refrigerated before use.

3.4. Collection and Identification of Larvae

Larvae of *Cx. quinquefasciatus* were collected from a pond earlier on identified as a suitable site in Giwa village Zaria, Kaduna Nigeria. These larvae were identified using procedure proposed by [10,11], using keys produced by [12]. The decision to use field collected larvae for this study was based on comparative studies showing that laboratory-reared larvae were more susceptible than those collected from the wild [13,14]. Since the field collected larvae would require higher concentrations of extracts for mortalities to be observed, it then followed that they will give more realistic and reliable figures for concentrations that can be used in the field. These larvae were then placed in plastic breeding pans containing water from the same pond. The pans were then kept in a mosquito-netted cage. After a day or two the older larvae pupated and the adults later emerged. These adults were collected and used for further confirmation of identification.

The third/fourth instar larvae were then removed and used for the larvicidal effect of the extracts. This was in accordance with observation that first instar larvae were not suitable because they were “weak, small, inactive and hardly feed” [15,16]; and that third instar larvae were very active, fed well and their rate of metabolism was high [17,18].

3.5. Preparation of Test Solutions

Test solutions of various concentrations viz: 1000,500,250,125 and 62.5 mg/l were prepared for the six plant extracts. This was done by weighing 250mg of paste and carefully dissolving it in 5ml of acetone. This was then made up to 250ml with distilled water, giving a stock solution initial concentration of 1000mg/l. Serial dilutions were then made to obtain the other four concentrations, using the formula

$$R = \frac{xo}{v};$$

Where R = Required concentration
V = Required volume
O = Concentration of stock
X = Water volume required

3.6. Larvicidal Activity of Plant Extracts

Six glass containers were taken, for each plant extract. In each container 50ml of test solutions of the five different concentrations were introduced, while the sixth contained distilled water with 1ml of acetone, which served as control. Twenty (20) third instar larvae were then placed in each container. Each set up was then observed for mortals after 24 hours for five days. Each treatment was replicated thrice.

3.7. Data Analysis

Mortalities were recorded, converted to percentages and mean percentage mortalities for each plant extract and for different concentrations. The percentage mortalities were then transformed to probits. These values were then regressed on the logarithms of the various concentrations to obtain LC_{50} and LC_{95} for each extract [19]. ANOVA was used to analyze the effects of the various treatments leading to ranking of the plant extracts. The strength of relationship between extract concentration and mortalities was determined using correlation coefficient analysis [20].

4. Results

4.1. Larvicidal Efficacy of Plant Extracts

It has been reported from this study that, the 96 hours post exposure of *Cx. quinquefasciatus* larvae to various concentrations of the named plant extracts: *H. lanceolata, H. suaveolens, H. spicigera, L. camara, C. citrates* and *Z. officinale*. Among all, *L. camara* caused the highest larval mortality of 63.2% at the lowest concentration of 62.5mg/l (Table 1). This was followed by *H. lanceolata, H. suaveolens* and *Z. officinale* which caused 57.9% each at this same concentration. The lowest mortality of 15.8% was recorded for *C. citratus*. This plant extract did not cause any mortality in the 6.25 to 1000mg/l concentrations used in the first 48 hours. This necessitated the use of higher concentrations of 1500 and 2000 mg/l for *C. citratus* and subsequently mortalities were observed and recorded. In all cases, it was also observed that mortalities did not occur instantly on exposure of larvae to test solutions. But the larvae exhibited restless movement for some time before finally settling at the bottom of the container with abnormal wagging of the tail and died slowly.

First mortalities were observed after 3 hours of exposure to 1000 mg/l of *L. camara*, where three larvae became immobilized and confirmed dead when prodded with a pin. Five larvae died in 3 hours after exposure to 1000mg/l of *H. spicigera*, two died in 500 mg/l, while only one death was recorded after 6 hours exposure to 250 mg/ml of the same plant extract. In 2000 mg/l of *C. citratus* only one larva died after 3 hours exposure, while it took 6 hours for one larva to die in 1500 mg/l. In *Z. officinale*, ten larvae died in the first 3 hours of exposure to 1000mg/l and three larvae in 500 mg/l solution. Similarly two larvae died in 1000mg/l of both *H. lanceolata* and *H. suaveolens* on exposure for 6 hours. Figures 7 to 11 show percentage mortalities of the larvae in varying concentrations of the plant extracts and for different exposure periods.

Observations made of rates of development during the course of this study revealed that pupation was attained earlier (faster) in the untreated control solution. This was followed by emergence of adults, especially in the first 24 hours. However, in the other test solutions where pupae emerged, they developed into adults rapidly and were not killed by the plant extracts.

Table 1. Corrected Average (%) Mortalities of *C. quinquefasciatus* in Crude Ethanoic Extracts of Six Plants (96 hours)

		Concentrations (mg/l)						
S/N	Extract source	62.5	125	250	500	1000	1500	2000
1.	*H. lanceolata*	57.9	78.9	89.5	89.5	100	N.A*	N.A*
2.	*H. suaveolens*	57.9	78.9	94.7	100	100	N.A*	N.A*
3.	*H. spicigera*	47.4	78.9	89.5	89.5	100	N.A*	N.A*
4.	*L. camara*	63.2	94.7	94.7	94.7	100	N.A*	N.A*
5.	*C. cirates*	15.8	36.8	47.4	73.7	78.9	100	100
6.	*Z. officinale*	57.9	78.9	78.9	78.9	100	N.A*	N.A*

N.A* = Not Applicable

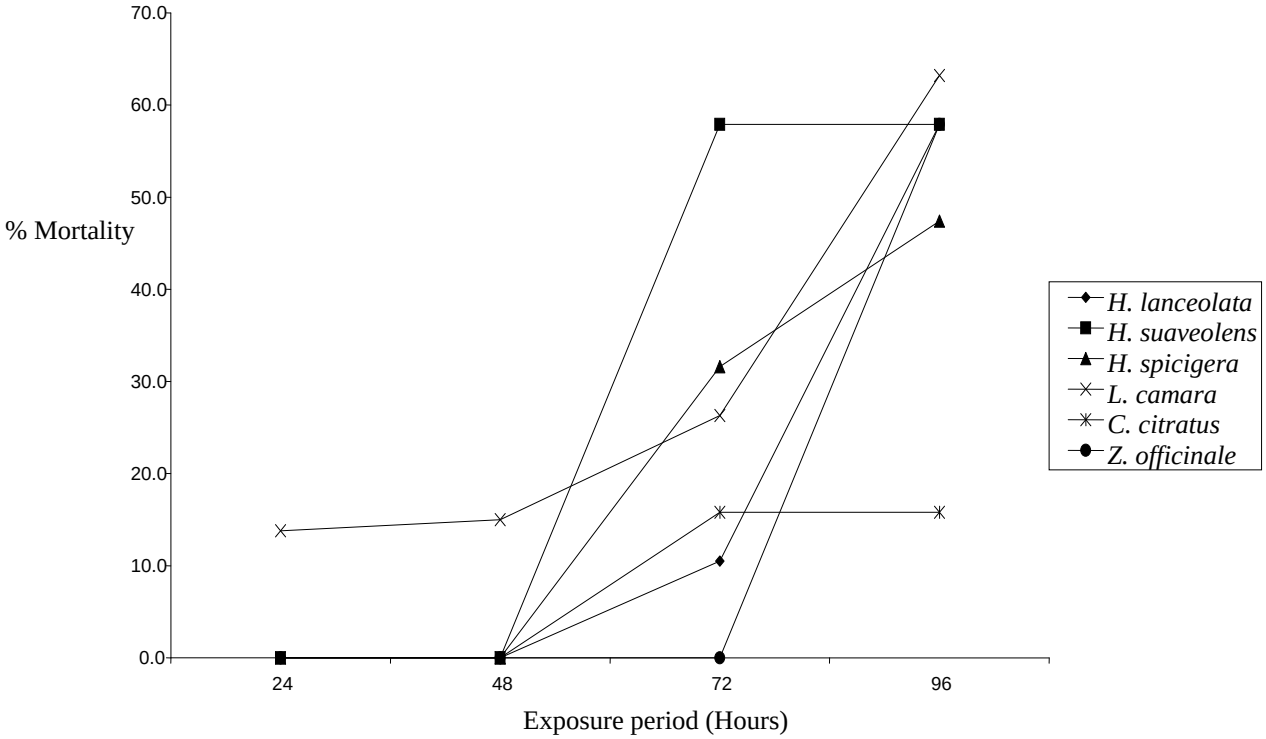

Fig. 1. Percentage mortality in 62.5mg/l at different periods of exposure

4.2 Determination of LC_{50} and LC_{95}

Mortalities were observed and recorded as shown in Table 1. These were used to determine graphically the LC_{50} and LC_{95} values for the six plant species Table 2. The percentage mortalities were transformed into probit values and plotted against log concentration for the determination of LC_{50} and LC_{95} for each of the plant extracts.

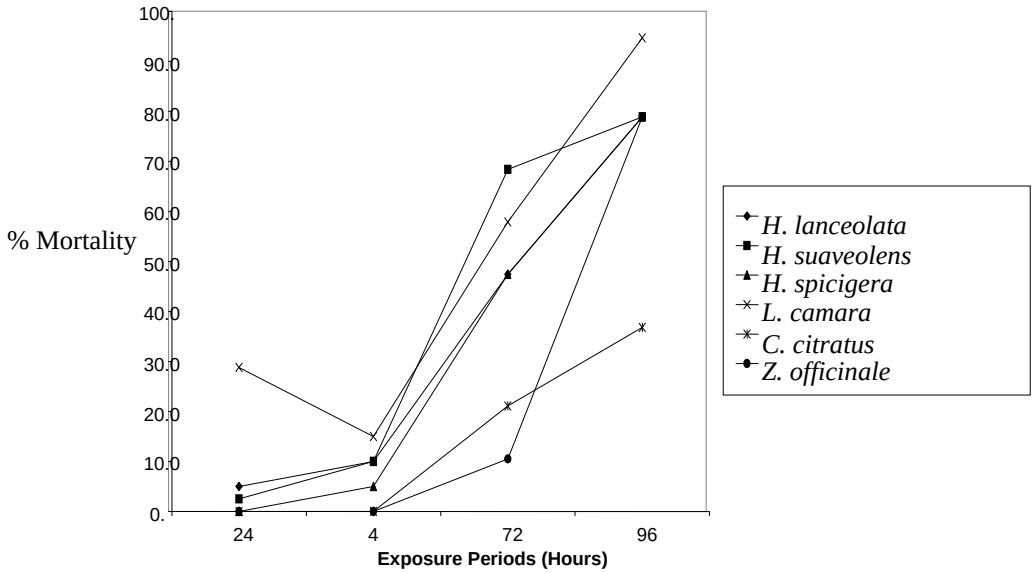

Fig. 2. Percentage Mortality in 125mg/l at different periods of exposure

Table 2. Summary of LC_{50} and LC_{95} values for *Cx. quinquefasciatus* exposed to crude extracts of six plants

S/N	Name of Plant	LC_{50} (mg/l)	LC_{95} (mg/l)	Regression Equation	R^2
1	*H. lanceolata*	70.79	288.40	y=2.6614x + 0.0733	0.9619
2	*H suaveolens*	70.79	316.23	y=2.6694 x + 0.0198	0.9650
3	*H. spicigera*	52.48	190.55	y=2.9856 x – 0.1031	0.9829
4	*L. camara*	56.23	223.87	y=2.7398 x + 0.1984	0.9638
5	*C. citrates*	109.65	436.52	y=2.7288 x – 0.5665	0.9271
6	*Z. officinale*	79.43	354.81	y=2.5754 x + 0.1031	0.9298

4.3. Hypotheses

Four hypotheses were put forward to guide the conduct of this study. All the hypotheses were tested using percentage mortalities observed and ANOVA. The basis for acceptance or rejection of the hypothesis is 0.05 of significance level Table 3.

Table 3. Two factor ANOVA with Replication: Concentration x extract x exposure period

Source of Variation	S.S	Df	MS	F	P-value	F crit
Concentration	40439.29	6	6739.881	9.365991	$8.23x\ 10^{-6*}$	2.420523
Extracts	2568.254	5	513.6508	0.406171	0.3617	2.298431
Exposure period	37983.73	2	18991.87	15.01789	$1.76x10^{-6*}$	3.080387
Interactions	2668.651	10	266.8651	0.211028	0.994897575	1.919467

* Significance difference at P<0.05

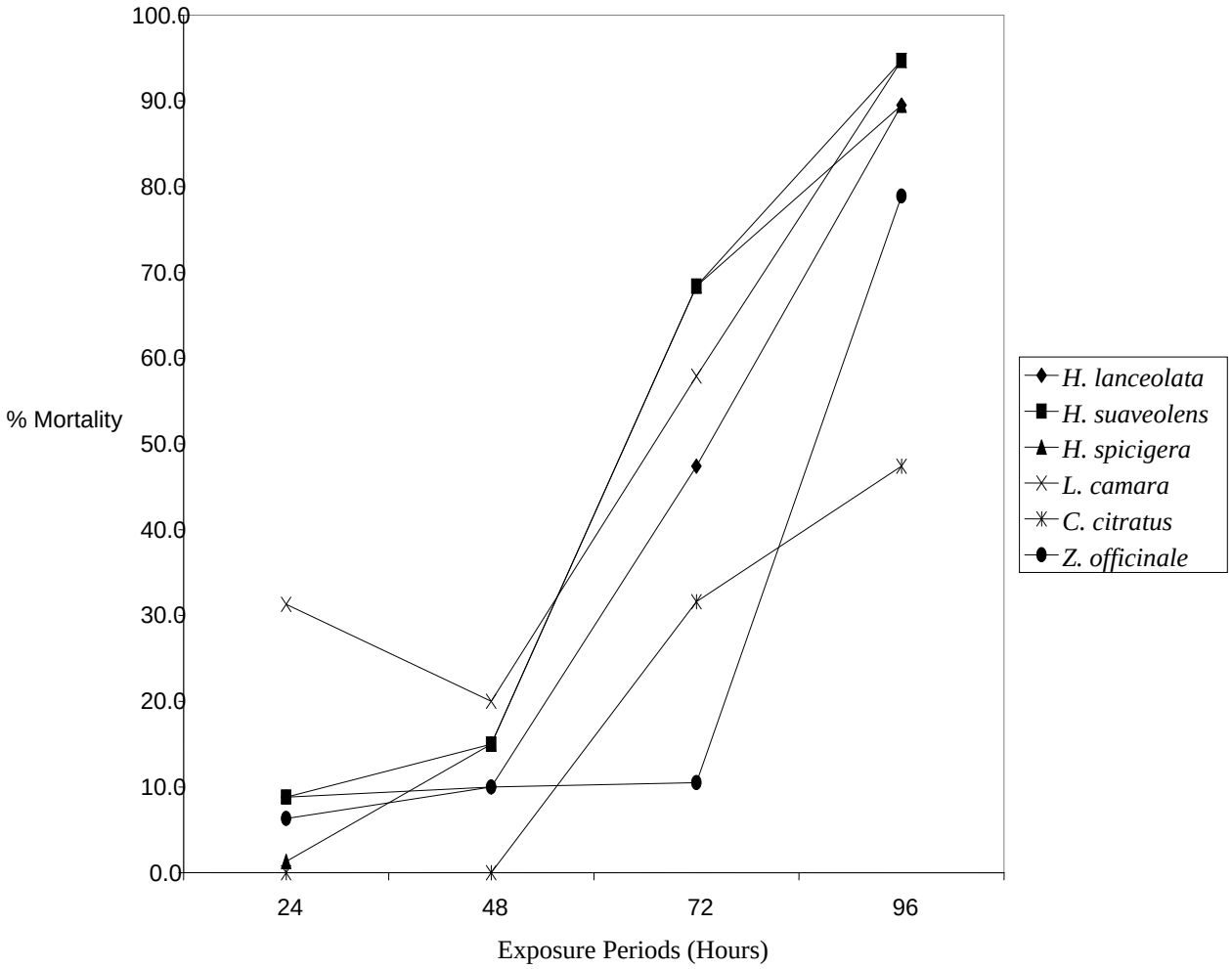

Fig. 3. Percentage Mortality in 250mg/l at different periods of Exposure

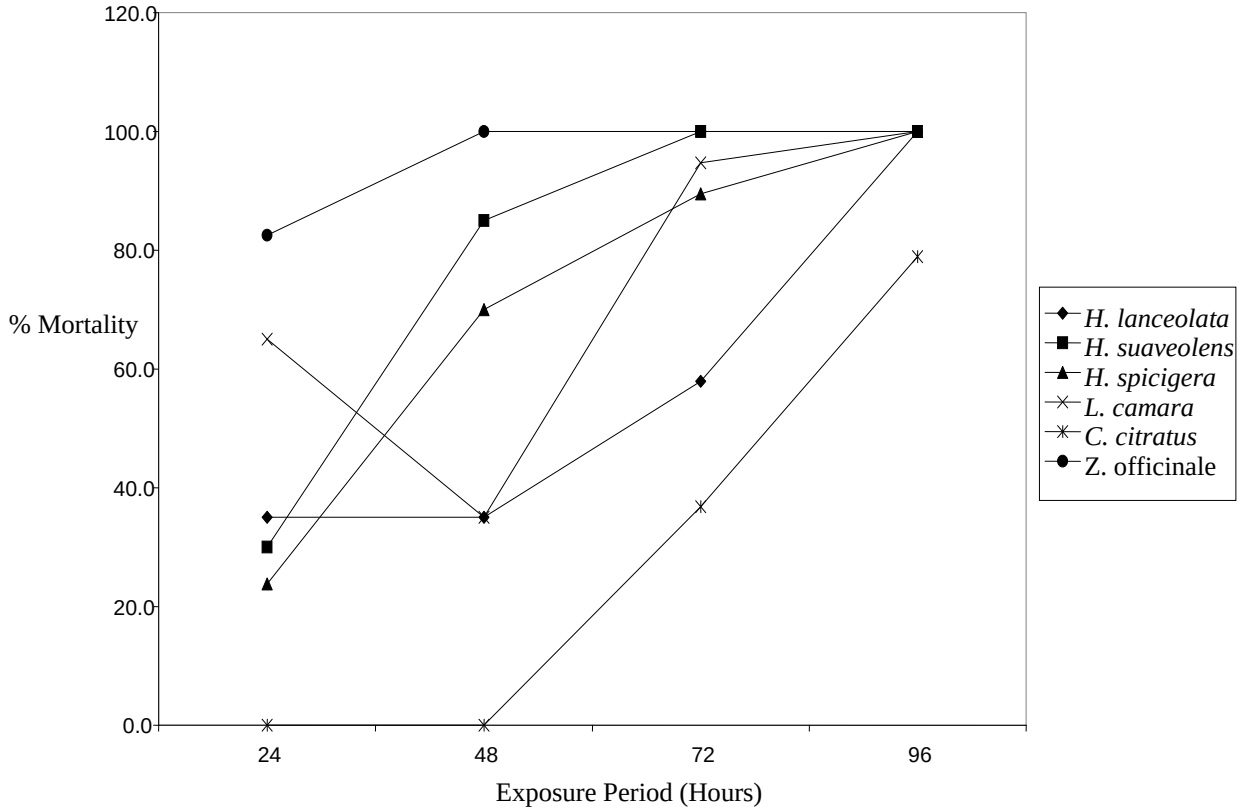

Fig. 4. Percentage Mortality in 1000mg/l at different perios of Exposure

The first hypothesis states that there will be no significant larvicidal effect on the larvae of *Cx. quinquefasciatus* by any of the extracts from *H. lanceolata, H. spicigera, L. camara, C. citratus and Z. officinale.* .

It is obvious from table 1 that all six plant extracts showed varying degrees of potency against the larvae of *Cx. quinquefasciatus*. Table 2 gave further credence to this as it shows the LC_{50} and LC_{95} of the six plants under study. Therefore this null hypothesis is rejected and the alternative hypothesis accepted.

The second hypothesis states that there will be no significant difference in the efficacy of these plant extracts. From the results of the analysis presented in Table 3, a p-value of 0.844 indicates that there is no significant difference in the efficacy of the various extracts (i.e P>0.05). Therefore this null hypothesis is accepted.

The third hypothesis states that there will be no significant difference in the efficacy of these plant extracts among various concentrations. Analysis presented in Table 3 shows a P-value of 8.23×10^{-6}, (i.e $P < 0.05$) and therefore there exists a significant difference in activity between concentrations. Therefore this hypothesis is rejected and alternative hypothesis accepted.

The last hypothesis states that there will be no significant difference in the efficacy of the plant extracts based on the duration of exposure. Table 3 indicates a P-value of 1.76×10^{-6} for this variation (i.e $P<0.05$) therefore

indicating a significant difference; thus, leading to rejection of the null hypothesis and acceptance of the alternative hypothesis.

The results so far obtained in this study have shown that all the plant species screened possessed larvicidal properties to varying degrees on the larvae of the culex mosquito. Table 4.1 which gave average % mortalities indicated *L. camara* (63.3%) at the lowest concentration to be the most potent while the least potent is *C. citratus* (15.8%). However, based on the LC_{50} and LC_{95} determined for the plants (Table 2), *H. spicigera* had the lowest (i.e 52.48 and 190.55 mg/l respectively); thus indicating higher potency than *L. camara* which was assumed more potent when percentage mortalities were considered. This discrepancy may not be unconnected with the fact that while determining LC_{50} and LC_{95} the various concentrations of extracts were taken into consideration. *L. camara* featured as the second most potent with LC_{50} and LC_{95} values of 56.23 and 223.87 mg/l respectively. *C. citratus* with LC_{50} value of 109.65 mg/l and LC_{95} of 436.52 mg/l is the least potent of the six plants screened.

The result analysis indicated significant difference in the larvicidal activity of the six plants as it relates to concentration of extract and exposure period, meaning that concentration of extracts is an important factor in the use of these extracts as larvicides. It is noteworthy that higher mortalities were observed at higher concentrations of all extracts. The same trends have been observed in earlier studies with other plants [21, 14].

5. Discussion

It has been established in this research the extracts *H. lanceolata* and *Z. officinale*, whose concentrations had high positive correlation with mortality, would require higher concentrations for effective utilization, since mortalities would increase in direct proportion with increase in extract concentration. On the other hand *L. camara* and *H. spicigera* whose concentrations had a weak correlation with mortality, but were observed to be most potent at lower concentrations shows that they will be most suitable for use in formulation of larvicides since increase in concentration did not significantly increase mortality. This suggests that they would be more sustainable, since small quantities could be used even over large areas of breeding grounds of this mosquito.

The relevance of exposure period suggests that the active ingredient of the extracts probably acts systemically and therefore requires a time period for slow uptake and distribution before reaching target organs on which effects are exerted. [22] reported that higher plants contain phytochemicals like terpenoids, flavonoids and alkaloids which are active against mollusks and insects. Larval respiratory, circulatory or nervous systems may be points where metabolism is impaired with a resultant [23].

Regarding the morphological abnormalities, several authors recorded similar anomalies, when applied different plant extracts at sub lethal concentrations. Pigmented larvae and pharate pupae were reported after treatment with sesame oil[12]. Albino pupae were recounted after treatment with neem seed kernel extract [24] and nigella oil [25]. Blackish cephalothoraxes were observed after treatment with fenugreek [26] and onion oil [25]. Finally, failure of adult eclosion as well as deformed abdomen and legs were recorded after treatment with fenugreek [26], sesame, nigella, onion oils and garlic [25].

The failure of test solutions to cause pupal mortality suggests that though plant extracts may have a delaying effect on pupation, they had no lethal toxicity on the pupae. However, lower temperatures experienced due to onset of cold season in the course of this study, might have had the effect of further accelerating the rate at which surviving larvae in all the test solutions pupated and adults emerged. This is probably due to a lowering of the rate of metabolism by low temperature and the subsequent non-uptake and assimilation of extract into larval body from test solutions. [27,21] have also observed such prolonged larval and pupal periods while using plant extracts for the control of mosquito larvae.

The chemical constituents or composition of the different plant extracts used in this study have indicated the presence of various probable active ingredients responsible for the observed larval mortalities. Prominent among which are the saponins, a group of plant glycosides that share two common characteristics, viz; they foam in aqueous solutions and cause haemolysis of red blood cells. Triterpenoids and steroidal saponins are the two types of saponins, and both groups are poisonous to insects, bacteria and fungi. Saponins are also insecticidal and their toxicity has been attributed to interference with breathing mechanism [23].

This is probably due to reduction of water surface tension by this surfactant, because of its soapy nature. Reduced surface tension would cause a submerging of larvae and resulting mortality due to absence of oxygen. Other components such as alkaloids are antifeedants with insecticidal properties. Terpenes are both insect repellants and antifeedants [28]. Sesquiterpenes, diterpenes and triterpenes are antifeedants. Hundred percent mortality of *Cx. quinquefasciatus* instars was observed when dipped in aqueous solution of parasterone A a steroid.

However, the high potency of *L. camara* can be attributed to a considerable degree to the presence of 1, 8-cineole [29], an oxygenated monoterpene, which has been isolated as a major component of volatile oils of *Hyptis martiusii* [30]. In another study by [30] obtained high mortalities of *Aedes aegypti* larvae within 24 hours when treated with dosages of as low as 100 mg/l of 1,8-cineole. Further literary evidence of the activity of this monoterpene against ticks and insects is provided by [31]. Conclusively, the high larvicidal potency of *L. camara* is most probably due to the presence of 1, 8-cineole, and the likely synergistic action of other components cannot be overlooked even in *H. spicigera* and the other plant extracts screened. The present study has proved that the plant extracts from 6.25-1000 mg/l applied are highly effective in the control of mosquito larvae.

The prolong developmental periods occurred due to the fact that the plant extracts used have an insect growth regulating activity, which may inhibit insect development. Moreover, the reduction in the larval rates and adult emergences, following exposure to higher ethanolic concentrations of several plant extracts were reported to yield high degree of mortalities, inhibited ovipositing, and larval penetration, together with decreased fertility. [32] reported that neem cake powder applied @ 25, 50 and 100g / m^2 was found to be effective against the larvae of *Cx. quinquefasciatus*.

6. Strengths and Limitations

This research determined the efficacy of the appropriate concentrations, lethal action and LC_{50} and LC_{95} for each of the extracts on the larvae of *Cx. quinquefasciatus*. From the hypothesis, it could be seen that, the extracts have significant phytotoxic efficacy against the larvae of mosquito based on the duration of exposure.

In ability to carry out detailed proximate analysis with sophisticated equipment for all the plants involved during this study, may in turn be a reason in giving out more/most active ingredients in each of the selected plant.

7. Conclusion

From the research conducted, the plant species screened have larvicidal potentials against the *Culex* mosquito. The most promising, however, are *Hyptis spicigera* and *Lantana camara*, which showed the highest levels of toxicity to *Cx. quinquefasciatus*. This has revealed their potent larvicidal activity; although low mortalities were recorded at lower dosages even for these two, there lethality became pronounced with increasing concentration reaching the 100% or acute toxicity level. Conclusively these plants (*H. spicigera* and *L. camara*) could be useful for managing field populations of *Cx. quinquefasciatus* in an integrated vector control programme.

Acknowledgement

The authors wish to express their deepest gratitude to Prof. J. Auta and Dr. E. Kogi of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria; for their guidance and valuable suggestions in the improvement of this research work.

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