LarvicidalActivities of some Ethnobotanicals from North East Nigeria, against Culicine mosquitoes

ABSTRACT

Mosquito borne diseases are the major cause of economic loss due to high morbidity and mortality in Africa. Elimination of culicine vectors using ethnobotanical extracts is one of the best methods for controlling mosquito-borne diseases. The methanolic and petroleum ether extracts of five plants, *Azadirachtaindica*(neem),*Hyptissuaveolens*(bush tea), *Eucarlyptus globulus* (pole wire), *Citrus senensis* (orange), and *Ocimumkilimanscharicum*(bush scent leaf), were investigated for their effectiveness in control of subfamilies *Culicidae*mosquitoes' larvae from June 2017 to October 2017. The results showed that the mortality is concentration dependent. Mortality was recorded for both methanol and petroleum ether extracts.Higher mortalities were observed in the methanolic extracts than petroleum ether extracts. The different plant extracts showed high significant differences ($p < 0.05$) to each other. *Hyptissuaveolens* proved to be the most effective treatment agent with 100% mortality observed at both 150ppm and 200ppm. The present study has demonstrated larvicidal effects, and the effects were a extended pupae emerged from the different treatment which led to the low adult emergence as compared to the control.

Key words: Larvicidal,Pupicidal,Ethnobotanicals, Culicine mosquito, Methanol, petroleum ether

INTRODUCTION

Mosquitoes are the most important insect group in terms of public health importance(Ito et al., 2015). They transmit several numbers of infectious disease like malaria, filariasis, Dengue, Zika virus, Encephalitis, and others, causing millions of deaths every year (Arivoli, 2011), as also reported by Michigan mosquito control organization (MMCO, 2013).Eliminating the vectors of the diseases is an important step in the control ofdiseases. In time past synthetic pesticides have been developed and effectively used to eliminate mosquitoesand other storage pests (Ito and Utebor, 2018). However, the management of these disease vectors using chemical pesticides has partially failed, due to their efficiency in attaining physiological resistance (Elumalai, *et al.,* 2015). In addition, the application of such chemicals has resulted in long-term harmful effects on non-target organism and other environmental component (Patel, *et al*., 2012). Most of the mosquito control programs target larval stage in breeding sites, sincse adulticides may only reduce the adult population temporary (El-Hag, *et al*., 2001). The conventional chemical method employed for this purpose includes insecticides, insect's growth regulators (IGRs), juvenile hormone compounds (Hariral*et al.,* 2015). In addition, botanical products have been used traditionally by human communities and its application is easy.They are highly biodegradable and are considered as the safest methods for insects pest control (Ito and Ighere, 2017) and vectors (Ito *et al,* 2015) These plants are rich source of novel natural substances that can be used to develop environmental safe methods for insect control (Mandal, 2012).In recent time, chemicals derived fromplants, fungus and nanoparticles from biological originshave been projected as weapons of future mosquito control programs as they are shown to be ecological friendly, biodegradable, cheaper, target specific and low toxicity to non-target organisms (Vivekanandhan*et al.,*2018a, b, c, d e). Moreover, plant-basedproducts are mostly non-toxic to humans and other animals and have a high degree of biodegradable. Botanical can be used as an alternative to synthetic insecticides or along with other insecticides vector control programs. In view of the increasing interest in developing plant-based insecticides as an alternative to chemical insecticides the present study was undertaken to assess the larvicidal potential of the methanol and petroleum ether extracts and their extended effects on the pupae that emerged from the treated larvae.

MATERIALS AND METHODS

Description ofStudy Area

Plants [*Azadirachtaindica*(neem),*Hyptissuaveolens*(bush tea), *Eucarlyptus globulus* (pole wire), *Citrus senensis* (orange), and *Ocimumkilimanscharicum*(bush scent leaf)] material were collected from the study areas in June – July 2014 in five states of the north eastern region, Nigeria. The area is situated at latitude 9.082 and longitude 8.6753 andit is about 840KM from the age of the Sahara Desert and 475.5 meters above sea level. It falls within the Sudan savannah zone. The area is large and shares boundary with two other Nigerian geopolitical zones (north central and north western region) and also shares boundary with two (2) countries, including Cameroon, Chad.

Selection of Plant Material

For this study, a survey was carried out and five (5) plant species were selected from the states of North-eastern region (Adamawa, Borno, Gombe and Taraba) of Nigeria, and their organic molecules were extracted and tested against larval and adult mosquitoes. Twenty people were interview from each Local Government Area, for the types of plants and parts of plants that were in use against mosquitoes in the localities. Three local government areas from each state, one from each geopolitical zone were covered. Plant selection in the study area, were based on interviewing members of the community to specify the indigenous plant species known for their use in the regular control of mosquitoes and other insects in their localities. This species of plants was selected because of their popularity among the local and were ranked following the application of weighted criteria as described by Kweka*etal*., (2008). The plants were further selected by combining the ethno botanical leads and chemotaxonomic evidence (popularity of plants already used as insecticides by local people and documented evidence of insecticidal constituents in the family to which the candidate species belongs.

Collection of Plant Materials

Fresh leaves, fruit peels and whole plants were collected in study area and identified at the Federal University Lokoja herbarium. The plants materials were shade dried, pounded into powdered form using mortar and pestle and stored in air tied polytene bags for soxhlet extraction*.* The extraction of oil with methanol and petroleum ether were done using soxhlet extractor.

3.4 Extraction of crude extracts

Clean boiling flasks (250ml) were dried in the Oven at 110^{0}c for about 30 minutes. Then transferred into desiccators and allowed to cool. About hundred grams each of the pulverized and air-dried plants powder weighed separately into extractor. The flask was filled with 200ml of Methanol, then the extractor plugged tightly with cotton wool. The soxhlet apparatus were then assembled to allow for reflux for about 6hours. After six hours, the thimbles containing the sample were removed with care and the methanol and petroleum ether drained into the various containers for re-use. The various plants extract for both methanol and petroleum ether extracts were concentrated using water bath which removes the methanol and petroleum ether component living behind only the components of the various extracts, which were used for toxicity bioassay (Amusan*et. al.,* 2005).

TOXICITY BIOASSAY

Third instar larvaewere collected from the rice fields and some natural water bodies in Yola, Adamawa State, Nigeria. Larvicidal bioassay was also carried out in insectary prepared for the course of this study in Yola, Adamawa State.One milliliter of various plant extracts was measured and emulsified with 3 drops of Tween $_{-}^{+}$80 from a needle tip. The emulsified was made up to 1 liter with distilled water to form 1000ppm stock solutions. For all the stock solutions, serial concentration was prepared. The ranges start from 50ppm, 100ppm, 150ppm, 200ppm. From each concentration, 250ml of all extracts was measured and introduce into separate labeled 500ml of specimen bottles. Twenty3rdinstars larvae of Culicine mosquitoes were introduced to each beaker. Each treatment had five replicates. Mortality served as the end point of the test and result were used to determine the potential efficacy of the various plant extracts. Larva was considered death if there was no moving or no response to gentle probing with a fine glass rod three times, 10 second each. Mortalities were recorded at after 36 hours post treatment for the various plant extracts and the control (only distilled water).

Analysis of variance (ANOVA) was used to determine significant differences between the mortality mean values,using Duncan multiple range test. Then LC50 and LC90 values were obtained through use of probit analysis.

RESULT

Culicine Mosquito Species found during the study

Data shown in **Table 1,** reveals that 1000 mosquito samples identified, were belong to five genera, *Aedes*500(50%), *Culex*350(350%), *Mansoni africanus*146(14.6%) and *Toxorhynchites* 4(0.4%). Mosquito abundance in relation to species showed, that Aedes aegypti 500(30.9%) proved to be the most abundant genus in study area, followed by *Culex*quinquefasiatus 231(23.1%). The least genus observed, was *Toxorhynchites* 4(0.4%)

Effects of Methanol Extracts on Culicine Development

The biological activity of the methanol and petroleum ether extracts of north eastern ethno-botanicals against the third instar larvae of *Culicine* mosquitoes have been studied. The biological activities included the *larvicidal*, pupal emergency rate, pupal mortality and adult emergence rate. Table 2, shows the biological activities of methanol extracts of ethno-botanicals against the 3rdinstar larvae of *culicine*. Complete larval mortality (100%) was observed at the higher concentrations (150ppm and 200ppm) of *Hyptissuaveolens* and neem seed extracts (200ppm). The Table also depicts that mortality increased with increase in concentration from 50 -200ppm. The result showed that *H.suaveolens*proved to be the most effective treatment agent used during the experiment and no pupation (0.0%) was observed.

The effects of methanol extracts were also extended to the pupal stage, and therefore the adult emergence was also affected in all concentrations used, as compared to the control groups. The neem seed, neem stem, *O.killimanscharikum, Hyptissuaveolens and E. gloublus were* showed high percentage of mortalities of 68% or more at 100ppm (Table 2). Neem leaf extract showed 71% at 150ppm. The least among the plant extracts was the orange peels that showed 50% lethality only at the highest concentration (200ppm). A remarkable reduction in adult emergence was observed, at 200ppm of *Hyptissuaveolens*, Neem seeds, *E. globulus,* neem stem and O. *kilimanscharikum,* with percentage emergence of 0%, 0%, 1%, 5%, and 10%respectively. Orange peels and neem leaf proved to be less effective adult emergence with47% and 29% respectively.

Table 1: Mosquito Species Abundance in Yola

Mosquito species	Number (%)	Total number (%)
Aedes aegypti	309(30.9)	500(50)
Aedes albopictus	191(19.1)	
Culex quiquefasciatus	231(23.1)	350(35)
Culex fatigans	119(11.9)	
Mansoni africanus	100(10)	146(14.6)
Toxorhynchites	4(0.4)	4(0.4)

Key: %= percentage

Table 2: ***Methanol Extracts on Culicine Development***

Plants	Conc. Levels (PPM)	Larval Mortality %	Pupation %	Larvicidal extended effects (%)	Total mortality %	Adult Emergency %
NS	50	36	64	4	40	60
	100	90	10	3	93	7
	150	93	7	3	96	4
	200	100	0	0	0	0
	Control	0	100	0	0	100
NST	50	20	80	6	26	74
	100	72	28	4	76	24

	150	81	19	0	81	19
	200	95	5	0	95	5
	Control	5	95	0	5	95
NL	50	10	90	0	10	90
	100	48	52	0	48	52
	150	67	33	0	67	33
	200	71	29	0	71	29
	Control	2	98	0	2	98
OK	50	18	82	3	21	79
	100	68	32	0	68	32
	150	79	21	0	79	21
	200	90	10	0	90	10
	Control	8	92	0	8	92
OP	50	35	65	0	35	65
	100	40	60	0	40	60
	150	45	55	3	48	52
	200	50	50	3	53	47
	Control	0	0	8	8	92
HS	50	50	50	8	58	42
	100	70	30	4	74	24
	150	100	0	0	100	0
	200	100	0	0	100	0
	Control	0	100	0	0	100
EG	50	48	52	8	56	44
	100	95	5	3	98	2
	150	98	2	1	99	1
	200	99	1	0	99	1
	Control	0	100	0	0	100

No. of larvae tested = 100; Replication =5; Conc. Level = ppm, ppm= part per million - = all animaldead
Keys:HS = *Hyptissuaveolens,* OP = orange peels, OK = *Occimumkilimanscharikum,*EG = *Eucarlyptus globulus,* NS = neem seed, NST = neem stem,NL = neem leaf.

Larvicidal Effects of Methanol Extract on 3rd Instar Larvae of *Culicine*

The mean percentage mortality inFigure 1 shows significant differences among the treatment agents within the group. The control showed no significant difference among the group. The treated larvae showed significant differences ($P < 0.05$) among all the treatment doses.

The results, showed that, the lower the concentration, the lower the mortality and the higher the concentration the higher the mortality. All the larvae treated with 50 to 200ppm doses showed significant differences ($P < 0.05$) among all the treatment agents used during the experiment. The Neem seed extract (20) and *H. suaveolens* (20) showed highest effectiveness with 100% mortality at 200ppm followed by means of *E. globulus* (19.8), neem stem extract (19) and *O. kilimanscharicum*(18). *H. suaveolens* (10) and*E. globulus* (10) proved to be the most effective treatment agents used at 50ppm dose, followed by neem seed (7.2) extract and orange peels (7.0) extracts, while neem leaves (2.0) proved to be the most ineffective treatment agent. *E. gloublus* (19) extracts and neem seed (18) prove to be the most effective treatment agents used at 100ppm followed by Neem stem (14.4) extracts, *H. suaveolens* (14.0) and *O. kilimanscharicum* (13.6).

Effects ofPetroleum Ether Extracts on *Culicine* Development

Table 3 shows the biological effects of petroleum ether extracts of ethno-botanical used against third instar larvae of *culicine*. The highest mortality percentage (100%) was recorded at the higher concentration levels (150 and 200ppm) of *H. suaveolens* and the lowest mortality percentage (20%) was observed at the lowest concentration levels (50ppm) of orange peels. The pupation percentage of treated larvae are far much lower than the pupation of the untreated larvae. The pupation is concentration dependent. The total larval and pupal mortality percent were 50%, 60%, 100% and 100%at 50, 100, 150 and 200ppm of *H. suaveolens* respectively, as compared to 0.00% for the non-treated group.

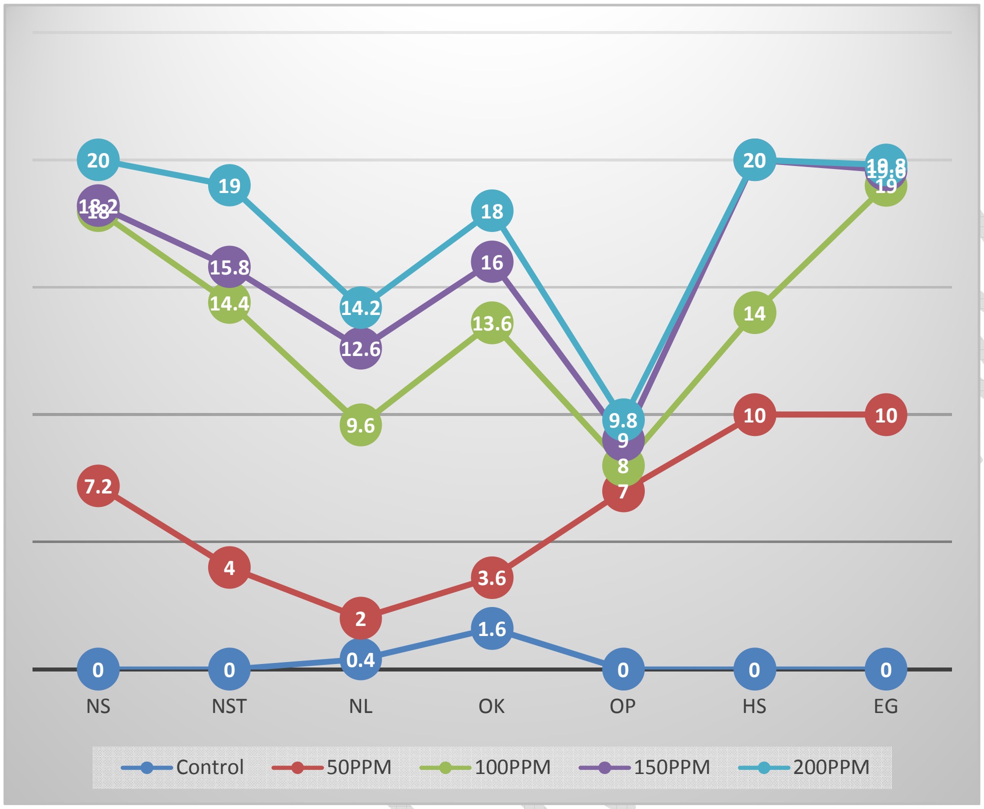

Figure 1: **Larvicidal Effects of methanol Extracts on 3rd Instar Larvae of *Culicine***

Table 3: Effects of Petroleum Ether Extracts on *Culicine* Development

Names	Conc. levels (PPM)	Larval Mortality %	Pupation %	Larvicidal extended effects (%)	Total mortality %	Adult Emergency %
NS	50	32	78	0	32	68
	100	40	60	0	40	60
	150	70	30	5	75	25
	200	76	24	6	82	18
	Control	0	100	0	0	100
NST	50	27	73	1	28	72
	100	30	69	2	32	68
	150	45	55	3	48	52
	200	86	14	5	91	9
	Control	0	100	0	0	100
NL	50	22	78	1	23	77
	100	29	71	0	29	71
	150	49	51	3	52	48
	200	58	42	1	59	41

	Control	2.0	98	0	2.	98
OK	50	34	66	4	38	62
	100	40	60	5	45	55
	150	70	30	6	76	24
	200	82	18	10	92	8
	Control	3	97	0	3	97
OP	50	20	80	0	20	80
	100	50	50	4	54	46
	150	60	40	0	60	40
	200	70	30	0	70	30
	Control	0	100	0	0	100
HS	50	42	58	8	50	50
	100	50	50	10	60	40
	150	100	0	0	100	0
	200	100	0	0	100	0
	Control	0	100	00	0	100
EG	50	23	77	0	23	77
	100	31	69	5	36	64
	150	35	65	7	42	58
	200	50	50	10	60	40
	Control	0	100	0	0	100

No. of larvae tested = 100; Replication =5; Conc. Level = ppm, ppm= part per million - = all animal dead

Keys:HS = *Hyptissuaveolens,* OP = orange peels, OK = *Occimumkilimanscharikum,*EG = *Eucarlyptus globulus,* NS = neem seed, NST = neem stem,NL = neem leaf.

Larvicidal Effects of Petroleum ether Extract on 3rd Instar Larvae of *Culicine*

The data presented in figure 2, are the mean values of mortality of *culicine*mosquitoes larvae due to the effects of various of plant extracts of petroleum ether that were tested against the 3rdinstar larvae. The control column shows no significant differences among the treatment. The doses showed significant differences ($p< 0.05$) among themselves. The figure reveals that 50ppm showed significant difference ($p< 0.05$) among the different extracts and had the lowest means of mortality when compared to other doses used during the experiment, with *H. suaveolens*(8.4) being the most effective treatment agent at this dose. Treatments of 100ppm showed significant difference ($p < 0.01$) within themselves and *H. suaveolens* (10.4) had the highest mean mortality and neem leaf (5.8) had the least mortality mean at the dose. Treatment of150ppm showed significant difference ($p< 0.001$) within themselves and same as 200ppm dose. *Hyptissuaveolens* (20) proved to be the most effective treatment agent followed by neem stem extracts (17.2), neem seed extracts (15) and *O. kilimanscharikum*(14) The figure also depicts that the mean values of 200ppm had the highest mortality means of 15, 17.2, 11.6, 16.4, 13.6, 20 and 10for Neem seed, neem stem, neem leaves, *O. kilimanscharikum,* orange peels, *H. suaveolens* and *E. globulus* respectively, and higher than lower concentration levels.

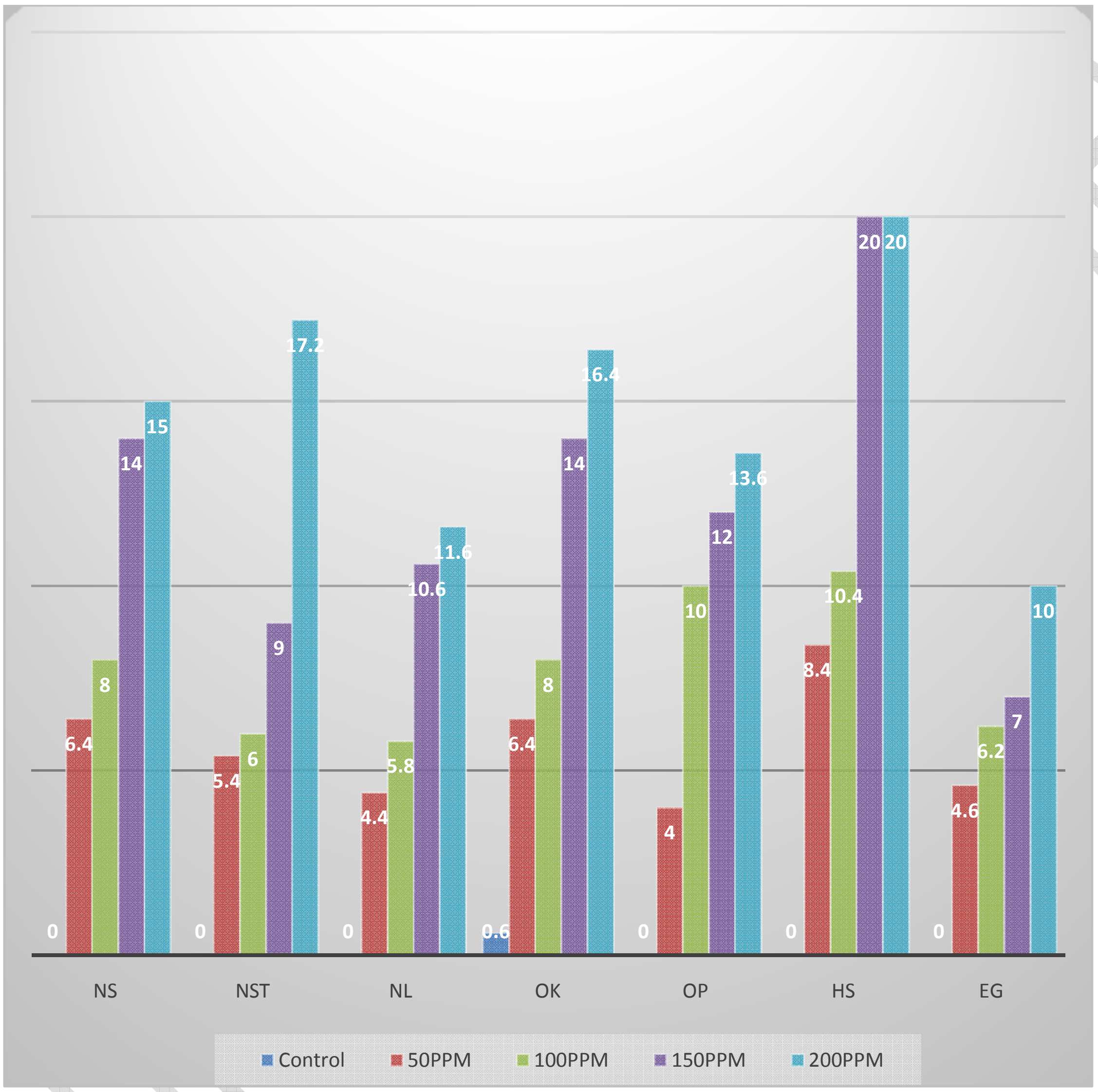

Figure 2: **Larvicidal Effects of Petroleum Ether Extract on 3rdInstar Larvae of *Culicine***

Effects of solvent used in extraction of extracts on *culicine*3rdinstar larvae

The result of the probit analysis shows various degree of effectiveness of some plant extracts used against 3rdinstar larvae of *Culicine* mosquitoes. LC50 of petroleum ether extracts 100.25, 115.53, 145.88, 68.44, 114.55, 46.79, and 175.07 of neem seed, neem stem, neem leaf,*Ocimumkilimanscharicum,* orange peels, *Hyptissuaveolens* and *E. gloublus* respectively, and when these were compared with LC50 of methanol extracts 45.91, 68.75, 126.56, 38.52, 141.73. 42.05 and 37.32, the result proved that methanol extracts were more effective than petroleum ether extracts **(Figure 3)**. The figure also showed that *E. globulus* (37.32) of methanol extracts had the lowest concentration (LC50) that killed 50% of the 3rdinstar larvae of *culicine* mosquitoes, followed by *Ocimumkillimanscharikum*(38.52), *Hyptissuaveolens (*42.05), and neem seed (45.95) of the same methanol extracts, while orange peels (141.73) proved to be the most ineffective treatment agents of the extracts. The result also showed that *E. gloublus* (93.12) and*Hyptissuaveolens (*107.15) of petroleum ether, killed 90% of 3rdinstar larvae of *culicine*. The *E. globulus* of methanol extract showed that 37.32ppm killed 50% and 93.12ppm killed and 90% of 3rdinstar larvae of *culicine* larvae that were exposed to it, and this proved that methanol extracts are far better than petroleum ether extracts (175.07 and 676.27ppm) of LC50 and LC90 respectively. Neem leaf (1985.93ppm) and orange peels (526.18ppm) of methanol extracts are the only extracts that showed toxicity effects against 3rdinstar of culicine when compared to their counterpart of petroleum ether extracts of neem leaf (530.05ppm) and orange peels (387.89ppm). All the extracts of both methanol and petroleum ether showed high degree of effectiveness, when compared with control that showed 0.0% mortality of 3rdinstar larvae of *culicine*.

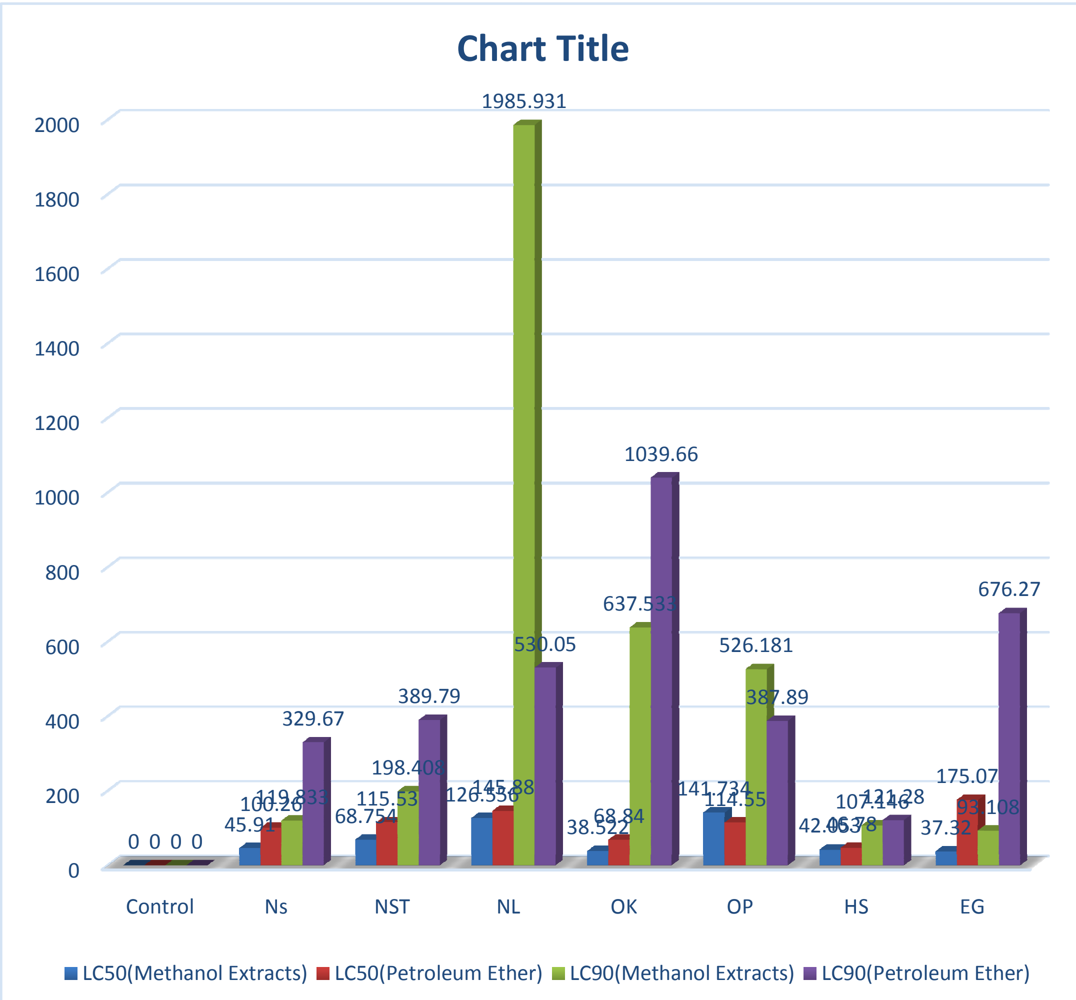

Figure 3: Effects of solvent used in extraction of plants extracts on *culicine*3rdinstar larvae

Discussion

Several diseases are associated with the mosquito-human interaction. Mosquito are the carriers of several diseases that are of major problem in the public health system. The diseases include malaria, arbovirus, encephalitis, dengue fever, hikunguya, west Nile virus, yellow fever, zika virus and so on. These diseases produce significant morbidity in humans and livestock worldwide. The plants tested in this present study are well known to be eco-friendly and are non-toxic to non-targeted species. These plants are grown all over the north eastern state of Nigeria and some have been used as herbal medicine in the region. Moreover, it is proved that these plants have been used as mosquito repellent, through burning of smoke or hanging them inside the rooms. The plants extracts are less expensive and highly efficacious for the control of mosquito rather than chemical compound (Kovendan*et al*., 2012Vivekanandhan et al.,2018a). The present study has shown high bioactivity of both methanol and petroleum ether extracts of the different plants. Such result may offer opportunity for developing alternative to rather expensive and environmental hazardous organic insecticides.

Toxicity of the tested plant extract against 3rdinstar larvae varied according to the type of plants or the plant part and solvents used for the extraction and also the extracts concentration used. The larval mortality percent increased as the extract concentration increased in all the different plant extracts. The toxicity values of the tested extracts of the different ethnobotanicals on mean value may be arranged in descending order as follows:*Hyptissuaveolen*> Neem seed > E. globulus > neem stem >*O. kilimanscharicum*> neem leaves > orange peels. These results agree, to some extent with the previous mentioned suggestions of Egunyomi*et al*. (2010) and Govnidarajan.(2010). Extracts from several other plant species were tested on different mosquito species. The activity of plant extracts on larval mortality of culicine mosquitoes agreed with the result obtained by several researcher (Patel *et al*., 2012;Kovendan*et al.,* 2012 and Ito *et al.,* 2015).*H. suaveolens* proved to be the most effective treatment agent with 100% mortality recorded at 150ppm and 200ppm, followed by neem seed that showed 100% mortality at 200ppm.

A remarkable decrease in pupation percent was induced by all plant extracts in the present study. The pupation percentage decreased as the concentration level of the plant extract increased. Moreover, the pupation rate depended on the type of plant species used. The present study showed that the toxic effect of all the ethnobotanical used had been extended to pupae. In addition, ethnobotanicals induced reduction of the adult emergence. The reduction was found to be concentration dependent. This result is comparable to earlier result of Sharma *et al.* (2006) who also used petroleum ether extract of *A. annura* against *An. stephensi* and *culexquinquefasciatus*which also showed the extensionof the larvicidal effect to the pupal stage.

The results have shown that all the effect of the treatment agents of methanol and petroleum ether extracts used against culicine are significantly different from each other. The LC50 methanol extracts (37.32, 38.52, 42.05, 45.91, 68.75, and 126.56ppm) of E. globulus, O.kilimanscharicum, H. suaveolens, neem seed neem stem and neem leaf respectively, showed higher larvicidal effect than their counterpart petroleum ether extracts with LC50 (175.07, 68.44, 46.79, 100.25, 115.53, 145.88ppm).The LC50 (114.5ppm) petroleum ether extractsa against culicine mosquito larvae proved to be better than its counterpart of methanol extracts with LC50 (141.73ppm). The 90 values of methanol extracts (93.11, 107.12, 119.83, 198.42, 637.53ppm) of E. globulus, H. suaveolens, neem seed, neem stem, O. kilimanscharicum respectively, are more effective than their counterpart petroleum ether extracts with LC90 (676.27, 121.28, 329.67, 389.72, 1039.66ppm). this agrees with report of Naphtali et al., 2018 and Ngwamah et al., 2018, Dixon 2015 that showed methanol extracts as the best agents against many mosquito species than some of the extracts from other solvents. This may be attributed to high polarity effect of the methanol as reported by Ngwamah et al (2018). In the case of neem leave (198593ppm) and orange peals (526.18ppm) of methanol extracts showed to be less effective against 3rd instar larvae of culicine mosquitoes as compared to their counterpart petroleum ether extracts with LC90 (530.05) of neem leaf and orange peals (387.89). this contrary to the findings of Kadri et al (2006), Dixon and Jeena 2017 that reported that higher larvicidal effect of methanol extracts against larval mosquitoes than the petroleum ether extracts, but is in agreement with the report of Komalamisra etal., (2006).

Conclusion

In general, it could be concluded that the crude extractsfrom all the plant material used during this study have larvicidal potential and some of the plant extracts have demonstrated extension of larvicidal effect to the pupal stage, where some pupae later died after pupation. Thislet to the low adult emergence as compared to the negative control. Furthermore, the result of the present study may contribute to the reduction in the application of synthetic chemical pesticides, which will increase the opportunity of natural control of various medically important pesticides by botanical insecticides.

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