Studies on the Larvicidal and Pupicidal Activities of some Ethnobotanicals from North East Nigeria, against Culicine mosquito

ABSTRACT

Mosquito borne diseases are the major of 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 of 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. Different concentration of the plant extracts was prepared fresh in distilled water for each test. Five replicates comprising 20 larvae each were exposed to 50-200 parts per million (ppm) of extracts at room temperature. The observations were taken 12 hours interval for 36 hours. Larvicidal, pupicidal activities of different extracts of these selected plants, were observed. The results showed that the mortality is concentration dependent (the higher the concentration the higher the mortality). Mortality was recorded all through the exposure of different plant extracts of both methanol and petroleum ether extracts with higher mortality were observed in the methanolic extracts than petroleum ether extracts. The different plant extracts showed high significant differences ($p < 0.05$) to other. *Hyptis suaveolens* proved to be the most effective treatment agent with 20 mean mortality observed at both 150ppm and 200ppm.

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). The transmit several numbers of infectious disease like malaria, filariasis, Dengue, zika virus, Encephalitis, and others, causing millions of deaths every year (Arivoli, 2011; MMCO, 2013). , as also reported by Michigan mosquito control organisation (MMCO, 2013). Eliminating the vectors of the diseases is an important step in the control of mosquito borne diseases. For several decades ago, many synthetic chemical pesticides have been developed and effectively used to eliminate mosquitoes and 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, as 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 application of easily degradable plant compounds is considered to be one

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Comment [IE5]: Was it aqueous extract? Or methanolic extracts?

Comment [IE6]: Where is the conclusion of the abstract?

Comment [IE7]: Citation needed: Add Ito et al., (2015)

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Comment [IE11]: Rephrase to read: In time past, synthetic pesticides…

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Comment [IE13]: Add: See references for citation of the safest methods of control of insect pests (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, chemical derived from plants have projected as weapons of future mosquito control programs as they are shown to be ecological friendly. Moreover, plant based bio-products are mostly non-toxic to humans and other animals and have high degree of biodegradable. Botanical can be used as 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 of some selected plant in the north east Nigeria.

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MATERIALS AND METHODS

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Description of Study Area

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Plants materials were collected from the study areas in June – July 2014 in five state of the north eastern region, Nigeria. The area is situated at latitude 9.082 and longitude 8.6753. It 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.

Comment [IE18]: What plant species? State the names (common and botanical names) of the selected plants you use in the study. It is important you mention the plants.

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Preparation of plant materials

The following plants and their parts used in this larvicidal study are…

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Extract preparation

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EXTRACTION OF ORGANIC MOLECULES

Soxhlet apparatus were used for the plant extraction. The various plants extract for both methanol and petroleum ether extracts were concentrated using water bath which removed the methanol and hexane component leaving behind only the components of the various extracts, which were used for toxicity bioassay (Amusan *et. al.,* 2005).

TOXICITY BIOASSAY

Third instar larvae of Anopheline were 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 millilitre 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 litre 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. Twenty 3rd instars larvae of Anopheline 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 for the various plant extracts and the control (only distilled water).

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Analysis of variance (ANOVA) were used to determine significant difference between the mean values, using Duncan multiple range test.

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RESULT

Biochemical Effects of methanol Extracts on Culicine Development

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The biological activity of the methanol and petroleum ether extracts of north eastern ethno-botanicals against the fourth instar larvae of *Culicine* mosquitoes have been studied. The biological activities included the *larvicidal*, pupal emergency rate, pupal mortality and adult emergence rate. The results of the present study are shown in Table 1, which indicate the biological activities of methanol extracts of ethno-botanicals against the fourth instar larvae of *culicine*. Complete larval mortality (100%) was observed at the higher concentrations (150ppm and 200ppm) of *Hyptis suaveolens* 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. Table 1, also depicts that the neem seed, neem stem, *O. killimanscharikum, Hyptis suaveolens and E. gloublus* showed high percentage of mortalities of 68% or more at 100ppm. Neem leaf extract showed 50% or more lethality 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 *Hyptis suaveolens*, 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 with high amount of adult emergence of 47% and 29% respectively.

Table 1: ***Biochemical Effects of Methanol Extracts on Culicine Development***

Names	Concentrates (PPM)	Larval Mortality %	Pupation %	Pupal Mortality (%)	Larval and Pupal 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; Concentration = ppm

Keys: HS = ***Hyptis suaveolens,*** **OP = orange peels, OK =** ***Occimum kilimanscharikum,*** **EG =** ***Eucarlyptus globulus,*** **NS = neem seed, NST = neem stem, NL = neem leaf.**

Larvicidal Effects of Methanol Extract on 3rdInstar Larvae of *Culicine*

The mean percentage mortality in Figure 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 200 ppm 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 200 ppm 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 50 ppm 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).

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I also suggest you subject the mortality values to regression analysis so you can be able to determine the LD_{50} or LC_{50} of the extracts used.

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Biochemical Mortality Effects of Petroleum Ether Extracts on *Culicine* Development

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The data in Table 2 shows the biochemical effects of petroleum ether extracts of ethno-botanical used against fourth instar larvae of *culicine*. In the table, highest mortality percentage (100%) was recorded at the higher concentrations (150 and 200ppm) of *H. suaveolens* and the lowest mortality percentage (20%) was observed at the lowest concentration (50ppm) of orange peels. The result shows that lowest concentration (50ppm) of the worst performed plant extract (orange peels) proved to be far better than control that showed 0% mortality. The pupation percentage of treated larvae is 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.

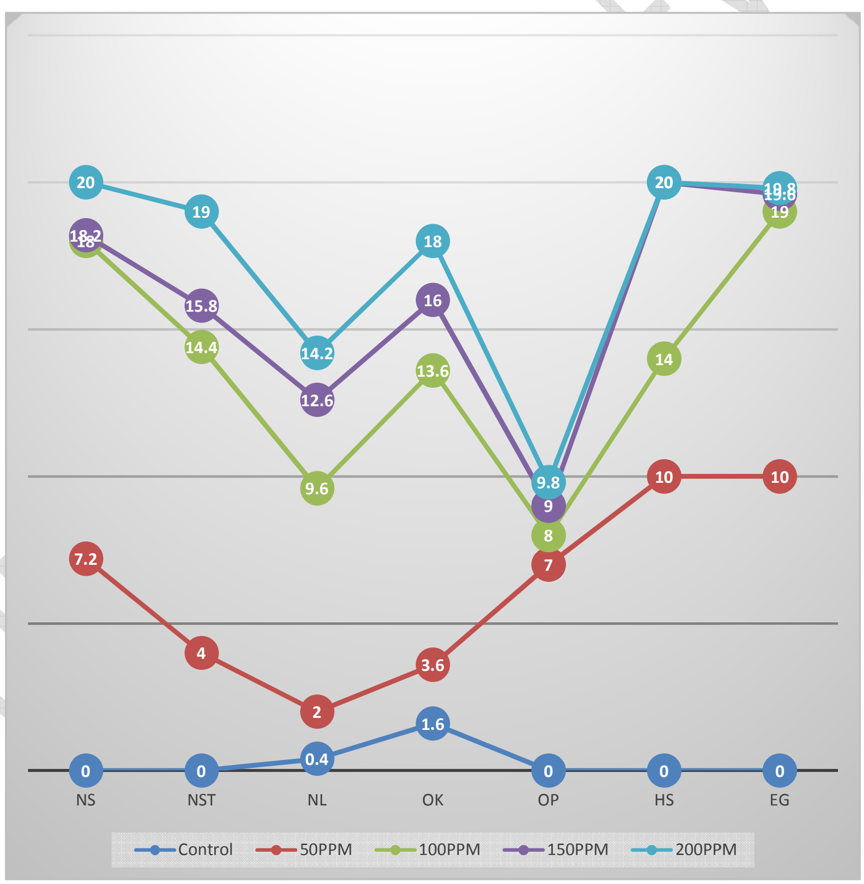

Figure 1: **Larvicidal Effects of methanol Extracts on 3rd Instar Larvae of *Culicine***

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Table 2: Biochemical Effects of Petroleum Ether Extracts on *Culicine* Development

Names	Concentration (PPM)	Larval Mortality %	Pupation %	Pupal Mortality (%)	Larval and Pupal 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;

Keys: Concentration = ppm, HS = *Hyptis suaveolens,* OP = orange peels, OK. = *Occimum kilimanscharikum,* 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 3rd instar larvae. The control column shows no significant differences among the treatment. The doses showed significant differences (p< 0.05) among themselves. The table 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. *Hyptis suaveolens* (20) proved to be the most effective treatment agent followed by neem stem extracts (17.2), *O. kilimanscharikum* (14) and neem seed extracts (15). 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 10 for Neem seed, neem stem, neem leaves, *O. kilimanscharikum,* orange peels, *H. suaveolens* and *E. globulus* respectively, and higher than the 50ppm and 100ppm of the lower concentrations, indicating that the higher the concentration, the higher the mortality.

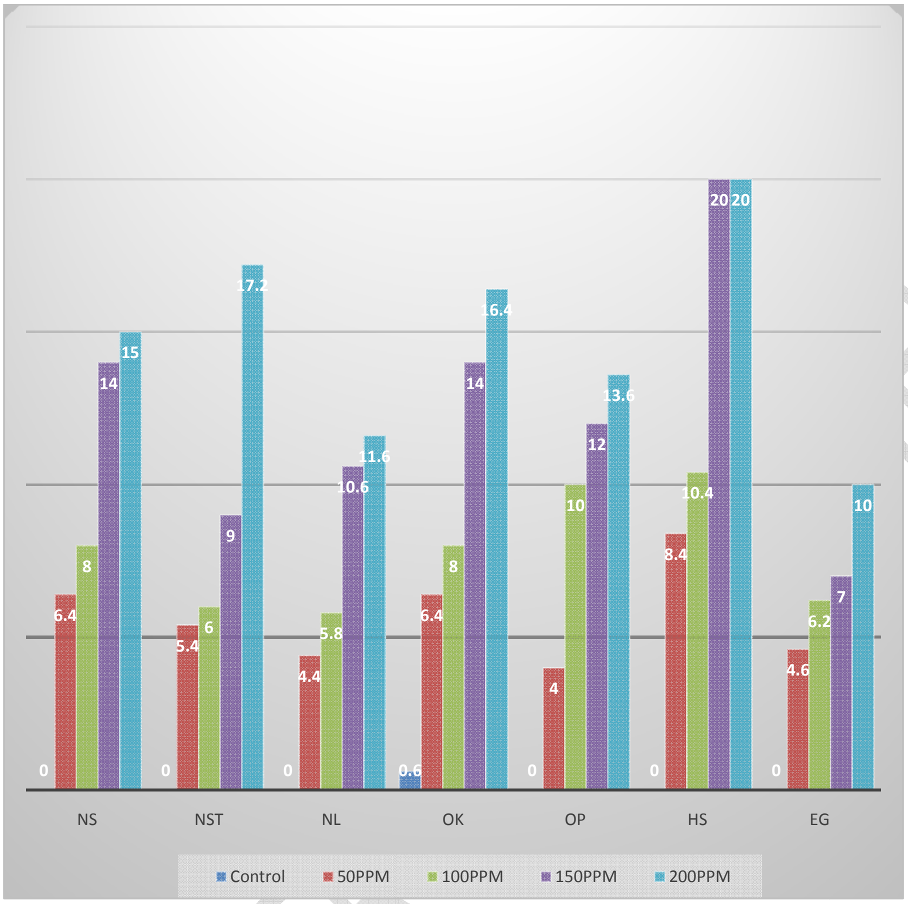

Figure 2: **Larvicidal Effects of Petroleum Ether Extract on 3rd Instar Larvae of *Culicine***

Discusion **Discussion**

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Several diseases are associated to the mosquito-human interaction. Mosquito are the carriers of several diseases that are of major problem to the public health system. The diseases include malaria, arbovirus, encephalitis, dengue fever, chikunguya, west nile virus, yellow fever and zika virus. 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*., 2012). The present study has shown high bioactivity of both methanol and petroleum ether extracts of the different plants. Such result may offer and 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 larvae mortality percent increased as the extract concentration increased in all the different type of plant extracts. The toxicity values of the tested extracts of the different ethnobotanicals on mean value may be arranged in descending order as follows: *Hyptis suaveolen* > 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 the plant extracts in the present study. The pupation percentage decreased as the concentration 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 *culex quinquefasciatus* which also showed the extensionof larvicidal effect to the pupal stage.

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In general, it could be concluded that the ethnobotanicals used in the present study acted as larvicidal, pupicidal and possess emergence inhibition against the culicine adult mosquitoes. 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.

REFERENCE

Comment [IE32]: Follow the journal referencing style.

Amusan A.A.S, Idowu A.B., Arowolo F.S. (2005): Comparative Toxicity effect of bush tea leaves (*Hyptis suaveolens*) and orange peel (Citrus sinensis) oil extract on larvae of the yellow fever mosquito Aedes aegypti. *Tanzanian health research bulletin 7: 174-177*

Arivoli, S. and Samuel, T. (2011). Bioefficiancy of *Citrullus colocynthis*(L) shrad (Cucurbitaceae) whole plants extracts against *Anopheles stephensi,Aedes aegypti* and *culex quiquefasciatus* (*Dipera culicidea*). International journal of current research, 3 (4): 296-304

El-Hag, E. L., Abd-Ei, R., El-nadi, A. h. and Zaitoon, A. A. (2001). Effects of methanolic extracts of neem seeds on egg hatchability and larval development of culexpipiens mosquitoes. *Indian veterinary journal,* 78: 199-201

Egunyomi, A., Gbadamosi, I.T., and Osiname, K. O. (2010). Comparative effectiveness of ethnobotanical mosquito repellents used in Ibadan, Nigerria. *Journal of Applied Biological Sciences,* **36**: pp.2383 -2388

Elumalai, K., Thangarasu, M., Kaliyamoorthy, K. (2015). Mosquito Larvicidal ovicidal and pupicidal activities of Abusprecatorious Linn (Fabaceae) against dengue vector, Aedes aegyti (linn), malarial vector, Anpheles stephensi (Liston), and Filarial vector, C. quinquefasciatus (SAY) (Diptera: Culicidae. *Journal of current Innovation research,*1(3): 73-80

Govnidarajan, M. and Sivakumar, R. (2010). *Adulticidal* and repellent properties of indigenous plant extracts against Culex quinquefaciatus and Aedes aegyti (Dipetraculicideae. Journal of parasitologyresearch, 109(2): 353-367.

Hariral, C. C., Rathy, M. C. and Sajith, U. (2015). Larvicidal efficacy of medicinal plant extracts against the vector mosquito Aedes albopictus. International of mosquito research, 2(2): 80-82

Kovendan, K., MuruganK., Donald, R. B and Vicent, S. (2012). Mosquito larvicidal propertis of *orthosiphon thymiflorus* (Roth) *Sleensen*. (family: *Labiatae*) Against mosquito Vectors, *Anopheles Stephensi, Culex quinquefasciatus and Aede aegypti*. *Asian pacific Journal of tropical Medicine,* ***6(11):***299-305.

Patel, E. K., Gupta, A. and Oswal, R. J. (2012). A review on: Mosquito repellent methods. *International of pharmaceutical, Chemical and Biological Sciences,* **2:** 310-317

Sharma, P., Mohan, L. and Srivastava, C. N., (2006). Growth inhibitory nature of *Artemisera annura* extracts against *Culex quiquefasciatus*(Say). *Journal of Asian pacific parasitic entomology,* 9(4): 389-395

Mandal S. (2012). Mosquito vector management with botanicals–the most effective weapons in controlling mosquito-borne diseases. *Asian Pacific Journal of Tropical Biomedicine, 336.*

Ito E.E and Ighere E.J. (2017). Bio-insecticidal Potency of Five Plant Extracts against Cowpea Weevil, *Callosobruchus maculatus* (F.), on Stored Cowpea, *Vigna unguiculata* (L). *Jordan Journal of Biological Sciences*, 10(4): 317-322.

Ito E.E., Nmor, J.C., Ake J.E.G, and Uteboe K., (2015). Larvicidal Activities of *Pistia stratiotes* (water lettuce) against 3rd and 4th instar larvae of *Aedes aegypti*. *Advances in Research*, 3(6): 589-595.

Ito E.E., and Utebor E.K., (2018). Insecticidal Toxicity of Goat Weed, *Ageratum conyzoides,* Linn. (Asteraceae) on Smoked Fish Weevil, *Dermestes maculatus,* Degeer (Coleoptera: Dermestidae). *Jordan Journal of Biological Sciences*, 11(2): 223 – 229.

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