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Capsicum Chinesis (Hot Pepper) Powder Larvicidal Activity on Mosquitoes Larvae in Lafia Local Government Area, Nasarawa State, Nigeria

Ombugadu A[1*]
Micah EM[1]
Adejoh VA[1]
Odey SA[1]
Njila HL[2]
Nkup CD[1]
Pam VA[1]
Echor B[2]
Pam DD[4]
Ahmed HO[1]
Aimankhu OS[1]
Uzoigwe NR[1]
Deme GG[5]
Yohanna JA[4]
Samuel MD[1]
Dogo KS[1]
Musa M[6]

[1]Department of Zoology, Faculty of Science, Federal University of Lafia, Lafia, Nasarawa State, Nigeria
[2]Department of Science Laboratory Technology, Faculty of Natural Sciences, University of Jos, Plateau State, Nigeria
[3]Department of Biology, College of Arts, Science and Technology, Kurgwi, Plateau State, Nigeria
4Department of Zoology, Faculty of Natural Sciences, University of Jos, Jos, Plateau State, Nigeria
[5]State Key Laboratory of Ecology and Conservation, Institute of Zoology, Chinese Academy of Science, Beijing 100101, PR China
[6]Department of Botany, Faculty of Science, Federal University of Lafia, Lafia, Nasarawa State, Nigeria

Abstract

Mosquitoes spread more diseases than other known arthropod and have shown resistance to conventional insecticides despite several control efforts, prompting the need to explore alternative control measures such as the use of bio-larvicides which are environmentally friendly. To this end, a study of larvicidal efficacy of the powder of *Capsicum chinensis* on mosquito larvae in Lafia Local Government Area, Nasarawa State, Nigeria was carried out through collection of wild field mosquito larvae from May to July, 2018. Fresh *Capsicum chinensis* fruits were collected from farmlands and dried under room temperature and further processed to fine powder from which varying concentrations were used against the larvae. The larvae were exposed at 24, 48 and 72 hours respectively. *Anopheles gambiae* larvae were susceptible (100% mortality) to the various concentrations of the powder at the end of the 72 hours exposure period while *Culex quinquefasciatus* were resistant (0% mortality). There was a very high significant difference ($P<0.0001$) in mortality rate of *An. gambiae* larvae in relation to concentrations while there was no significant difference ($P=1$) in mortality rate of *Cx. Quinquefasciatus* across concentrations. LD_{50} and LD_{90} values for *An. gambiae* at 24 hours were 2.4mg/ml and 0.10mg/ml respectively; 0.1mg/ml and 0.26mg/ml respectively at 48 hours and 0.00mg/ml and 0.01/ml respectively ay 72 hours. This study shows that *Capsicum chinensis* is a promising bio-larvicide for controlling *An. gambiae.* The results also showed that *Cx. Quinquefasciatus* may possibly require higher multiple doses of Capsicum to influence mortality.

Keywords

Capsicum; Mosquito larvae; Lafia

Introduction

Mosquitoes are considered insects of public health importance due to their ability to transmit a variety of diseases including West Nile, dengue, yellow fever, lymphatic filariasis (elephantiasis) and malaria which is the leading cause of morbidity and mortality in Nigeria [1]. Malaria, which causes about 1.2 million deaths (majorly among pregnant women and children under five years old) is caused by *Anopheles gambiae* so. l. [2]. Despite several efforts in controlling this vector, the medical and economic burdens caused by it continue to grow [3]. Currently, mosquito control strategies are fixated on the use of synthetic insecticides as constituents for Long Lasting Insecticide Treated bed Nets (LLINs) and Indoor Residual Sprays (IRS) as methods recommended by the World Health Organization (WHO) [4]. Unfortunately, the success recorded by this strategy in terms of reduction of morbidity and mortality [5] is short-lived as mosquito resistance to insecticide has increased through time [6-11]. The failure in current control measures and the growing insecticide resistance is necessitating the search for newer and more effective control strategies [12].

The use of botanicals (plant based products) is one of the best alternatives to synthetic insecticides [13,14]. This is because they offer a more environmentally friendly method of mosquito control [15] in that they have very weak adverse effect on non-target subjects [16]

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***Corresponding author:**
Ombugadu A
Department of Zoology
Faculty of Science
Federal University of Lafia
Lafia, Nasarawa State, Nigeria
E-mail: akwash24@gmail.com

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and are easily biodegradable [17]. Consequently, several plant species have been employed, worldwide, to control mosquito population.

Capsicum chinensis (hot pepper) has both medicinal and insecticidal value and is used traditionally as medicine for treatment of various illnesses [18] including asthma, pneumonia, diarrhea, cramps, indigestion and toothache and it has been reported to possess repellent activity against insect pests of stored grains [19]. Extracts of *Capsicum species* have been proven as repellants to some species of some stored product beetles such as *Sitophilus zeamais* Motschulsky (Coleopteran: Curculinidae) and *Tribalism Castaneum* (Herbs') (Coleopteran: Tenebriouidae) [20]. There are reports of using capsicum as bio pesticides against Alfalfa weevil larvae *hyper brunneipennis* [21]. The toxicity of *Capsicum* species against insects is thought to be the effects of secondary metabolites such as alkaloids, siphoning and falconoid compounds [22]. Against this backdrop, this study was carried out to determine the larvicidal efficacy of the powder of *Capsicum chinensis* on mosquito larvae in Lafia Local Government Area, Nasarawa State, Nigeria.

Materials and Methods

Study area

The study was carried out in Lafia Metropolis with the following coordinate's 8°29′30″N 8°31′0″E/8.49167°N 8.51667°E. It is the capital and the largest town in Nasarawa State.

Collection of sample plant

Fresh ripe fruits of *Capsicum* were collected from cultivated farmlands on 5th April, 2018 along Agyaragu Tofa road in Lafia, Nasarawa State. Taxonomic identification of the plant was carried out at the Department of Botany Laboratory of Federal University of Love by Mr. Markus Musa. The plant samples were rinsed in clean water to remove dirt/debris, sliced into parts, then air dried under room temperature at the Department of Zoology Laboratory, Federal University of Lafia for 3 weeks. The dried plant was ground into fine powder in an electric grinder [23] then weighed on a weighing balance to get 20mg, 40mg, 60mg, 80mg and 100mg doses respectively.

Mosquito larvae collection

First and second in star larvae of *Anopheles* and *Culex* mosquitoes were collected from different breeding sites in Lafia using standard dippers and identified using identification keys by Gillies and de Mellion [24] Burges and Cowan [25]. The larvae were kept in containers and allowed to adapt for 24 hours at room temperature before carrying out the test.

Preparation of bioassay

The bioassay was prepared according to the recommended protocol of World Health Organization [26] for susceptibility of the larvae of *Anopheles gambiae* and *Culex quique fasciatus* exposed to several doses (20mg, 40mg, 60mg, 80mg, and 100mg) of the powder of *Capsicum chinensis.* Four replicates were kept for each concentration, with two controls for each experiment, and in each replicate 25 first and second in star larvae were released into disposable 250 ml bowls containing 100 ml of distilled water. Control (0mg) was prepared as 100 ml distilled water only. The knockdown rate of larvae was recorded after 5mins, 15mins, 20mins, 30mins and 60mins respectively. Larval mortality was recorded in 24, 48 and 72 hours exposure period. Mortality was confirmed by gentle prick on the abdomen of the larvae with a needle [26,27].

Test analysis

The interpretation of the mortality rate of Anopheles gambiae and *Culex quinquefasciatus* larvae is as described by WHO, (2013).

1. Mortality rate between 98 – 100 % within the diagnostic time indicates susceptibility.
2. Mortality rate between 80 – 97 % suggest possible resistance.
3. Mortality rate < 80 % indicates resistance.

Percentage mortality

Unmoved and moribund larvae were recorded as dead.

$$\%\ Mortality = \frac{No.\ of\ Dead\ Larvae}{No.\ of\ Larvae\ introduced} x \frac{100}{1}$$

Determination of LD_{50} and LD_{90}

The mortality data were further subjected to profit analysis for estimating lethal dose values (LD_{50} and LD_{90}) using Finney method [28]. The 24, 48 and 72 hours LD_{50} and LD_{90} were determined through Microsoft excel regression analysis using the regression equation ($Y = a + bx$).

Data analysis

Data obtained was analyzed using R Console version 3.2.2. Pearson's Chi Square test was used to compare mortality rate of mosquito's larvae in relation to concentrations of *C. chinensis* powder. P values < 0.05 was considered statistical significant.

Results

Mortality rate of mosquitoes larvae in relation to concentrations of *Capsicum chinensis* powder and exposure periods

Anopheles gambiae: *An. gambiae* larvae at 24 hours exposure period had highest mortality rate of 92% at 20mg/ml, 80mg/ml and 100mg/ml concentrations and no mortality (0.00%) was observed in control 0mg/ml as shown in Table 1. Therefore, there was a very high significant difference (χ^2 = 91.442, df = 5, P < 0.0001) in mortality rate of *An. gambiae* larvae in relation to concentrations of *C.* powder.

At 48 hours exposure period mortality rate of *An. gambiae* larvae was highest 98% at 60mg/ml while no mortality 0% was recorded in the control 0mg/ml (Table 1). Thus, mortality rate of *An. gambiae* larvae in relation to concentrations of *C.* powder showed a very high significant difference (χ^2 = 96.835, df = 5, P < 0.0001).

An. gambiae larvae mortality rate at 72 hours exposure period was 100% for all concentrations with the exception of control 0mg/ml which had 0% (Table 1). Hence, mortality rate of *An. gambiae* larvae in relation to concentrations of *C.* powder showed a very high significant difference (χ^2 = 100, df = 5, P < 0.0001).

Culex quinquefasciatus: No mortality was observed at 24 hours, 48 hours and 72 hours exposure period respectively in the larvae of *Cx. Quinque fasciatus* in relation to varying concentrations of *C.* Powder (Table 1). **Lethal dose (LD_{50} and LD_{90}) of *Capsicum***

Lethal dose (LD_{50} and LD_{90}) of *Capsicum chinensis* Powder that exhibits larvicidal activity against An. gambiae larvae in relation to exposure periods

The lethal dose of *C.* That will exhibit larvicidal activity against 50% and 90% respectively of *An. gambiae* larvae at 24 hours exposure period is 2.14mg/ml and 0.10mg/ml (Table 2).

Table 2 also shows that the lethal dose of *C.* required at 48 hours exposure period to exhibit larvicidal activity against 50% and 90% respectively of the *An. gambiae* larvae is 0.01mg/ml and 0.26mg/ml.

After 72 hours exposure period, the lethal dose of *C. chinensis* required to exhibit larvicidal activity against 50% and 90% respectively of the An. gambiae larvae is 13.80mg/ml and 30.19mg/ml (Table 2).

Resistance/susceptibility profiles of mosquitoes

Species	Time (Hours)	% Mortality across concentrations (mg/ml)						χ^2	df	P value
		0	20	40	60	80	100			
An. gambiae	24	0	92	90	91	92	92	91.442	5	0.0001*
	48	0	97	96	98	96	97	96.832	5	0.0001*
	72	0	100	100	100	100	100	100	5	0.0001*
Cx. quinquefasciatus	24	0	0	0	0	0	0	0	5	1^{ns}
	48	0	0	0	0	0	0	0	5	1^{ns}
	72	0	0	0	0	0	0	0	5	1^{ns}

*: Significant

^{Ns}: Not significant

Table 1: Mortality rate of mosquitoes larvae in relation to concentrations of *Capsicum powder* and exposure periods

Time (Hours)	Lethal dose (mg/ml)	
	LD_{50}	LD_{90}
24	2.14	0.1
48	0.01	0.26
72	0	0.01

Table 2: Lethal dose (LD_{50} and LD_{90}) of *Capsicum chinensis* Powder that Exhibits Larvicidal Activity against *An. gambiae* Larvae in Relation to Exposure Periods

larvae exposed to *C.* powder in relation to World Health Organization Indices

At 24 hour exposure period *An. gambiae* showed possible resistance across all concentrations mortality rate range from 91% to 92% while *Cx. quinquesfaciatus* were absolutely resistant having 0% mortality rate.

At 72 hours exposure period, *An. Gambiae* were susceptible while *Cx. quinquesfaciatus* were absolutely resistant.

Discussion

The present investigation revealed that the powder of *C. chinensis* showed promising larvicidal efficacy against *An. gambiae* but may not be against *Cx. quinquefasciatus.* This is in agreement with the studies by Abok *et al.* [14] and Dalis [29] who recorded that the leaf extracts of *H. suaveolens* were potent against the larvae of *An. gambiae.* However, this result did not conform to that of Madhumathy *et al.* [30] in which the ethanol extract of *C. annum* proved to be more effective on *Cx. quinquefasciatus* than *An. stephensi* in India. This could be due to the difference in species of *Capsicum* (*C.* and *C. annum*) and extracts (powder and ethanol extracts) used in the respective studies.

The highest knockdown rate was observed at 100mg/ml dose of *Capsicum chinensis* Powder used against *An. gambiae* larvae which possibly suggest that the highest concentrations get to the target site of the larvae very fast to initiate larvicidal efficacy of *C. chinensis* powder. This is in accordance with the finding by Abok *et al.* [14] in a study on *Hyptis suaveolens* extract exhibits larvicidal activity against *Anopheles gambiae* larvae at the highest concentration. Knockdown was not recorded however for *Cx. quinquefasciatus* across all doses of *C. chinensis* Powder which possibly suggests them as tolerant to *C. chinensis* powder and affirms their survival in polluted water bodies.

The results obtained after 24, 48 and 72 hours exposure time in relation to *An. gambiae* demonstrated progressive increase in percentage mortality as concentrations increased. This buttresses Finney [28] assertion that larvicidal efficacy increased with an increase in concentration. The findings are in agreement with studies by Odey [31] who investigated the larvicidal potency of the leaf powder of *Millettia aboensis* on the larval stages of *An. gambiae* and *Cx. quinquefasciatus* and Kholhring [32] who investigated the mosquitocidal activity of *M. pachycarpa* on the larvae and eggs of *Ae. Aegypti*, recording peak mortality at the highest concentration after total exposure time. Chukwujekwu *et al.* [33] who investigated the Anti-plasmodia diterpenoids from the leaves of *H. suaveolens* also recorded similar results. This result also coincides with findings of Olotuah [24] who conducted a laboratory evaluation of pesticidal activities of *H. suaveolens* against stored product pests *Sitophilusoryzae*, *Sitophilus zeamais and Callosobruchus maculatus* and recorded peak mortality at the highest concentration (100 mg/ml) after total exposure period.

The 100% mortality recorded in *An. gambiae* larvaein relation to the treatments after 72 hours exposure period possibly suggests time is a factor that should be considered so as to achieve a good susceptibility profile. This agrees with the findings by Abok *et al.* and Odey [14,31] who showed that exposure duration played a major role in determining the resistance/susceptibility profiles in mosquitoes.

After 24 hour exposure period, the larvae of *An. gambiae* showed possible resistance to the powder of *C. chinensis* across all doses. At 48 hour exposure period, they showed possible resistance at 20mg/ml, 40mg/ml, and 80mg/ml and 100mg/ml doses of the plant powder but were susceptible to the plant powder at 60mg/ml. However, the larvae were susceptible to all doses after 72 hour exposure period. The resistance/susceptibility profiles recorded in relation to exposure periods was similar to the finding of Abok *et al.* [14].

The powder was shown to be more effective after 72 hours exposure time, with LD_{50} of 13.80mg/ml and LD90 30.19mg/ml. A possible reason for susceptibility recorded may be that the plant powder used up the dissolved oxygen available in the water, making it difficult for the mosquito species to survive [14] and also from the activity of capsaicin in the plant powder, which has significant lethal and anti-feedant effect on mosquito larvae [30].

Generally, *An. gambiae* larvae were more susceptible to all doses while resistance was observed in *Cx. quinquefasciatus* larvae.

Consequently, the finding by Meenakshi and Jayaprakash [34] showed that *Anopheles* larvae were more susceptible to the leaf extract of *Rhizophora mucronata* than *Aedes* larvae. Also, Kemabonta et al. [35] reported mortality of *An. gambiae* in relation to the insecticidal activity of essential oils for both *P. nigrum* (black pepper) and *C. longa* (tumeric) to be a 100.0%. Furthermore, the habitat of

the *Culex* larvae suggests a possible reason for their resistance as they thrive in dirty, toxic and polluted water unlike *Anopheles* mosquitoes that prefer clean and clear water [25].

Conclusion

The result of this study has indicated that *Capsicum chinensis* possesses larvicidal properties against larvae of *An. gambiae* while *Cx. Quinquefasciatus* are resistant in relation to varying concentrations. The mortality rate of *An. gambiae* showed an increase as concentration increased.

The LD_{50} and LD_{90} values obtained indicate that the powder of *C.* is most effective against the mosquito larvae after 72 hours of exposure to the powder and hence supports its traditional application as an insecticide.

Results from the research showed that the larvae of *An. gambiae* were susceptible to the powder of *Capsicum chinensis,* in contrast to the larvae of *Cx. quinquefasciatus* which were resistant to all doses of the powder treatment administered.

Powder of *C. chinensis.* can be applied to mosquito breeding sites in multiple of doses so as measure to control the vector populations, particularly *An. gambiae.*

Finally, large scale cultivation of *C. chinensis* and further studies on the isolation of its active ingredients for should be carried out.

References

1. Braack L, Hunt R, Koekemoer LL, Gericke A, Munhenga G, et al. Biting Behavior of African Malaria Vectors: 1. Where Do the Main Vector Species Bite on The Human Body? Parasit. Vectors. 2015 Feb:8: 76.
2. Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C. et al. Averting a Malaria Disaster: Will Insecticide Resistance Derail Malaria Control? Lancet. 2016 Apr 387: 17851788.
3. Bhatt S, Weiss D J, Cameron E, Bisanzio D, Mappin B, et al. The Effect of Malaria Control on Plasmodium falciparum in Africa Between 2000 and 2015. Nature. 2015 Oct;526:207211.
4. Badolo A, Traore A, Jones C M, Sanou A, Flood L, et al. Three Years of Insecticide Resistance Monitoring in Anopheles gambiae in Burkina Faso: Resistance on the Rise? 2012 Jul;1:232.
5. Oyewole IO, Kolawole, AA, Adeogun OC, Awololaaa S, et al Susceptibility pattern of Anopheles mosquito to different classes of insecticides in selected communities in Ila-Orangun, Southwest Nigeria. International Journal of Mosquito Research. 2018 Nov;5(1):106-111.
6. Radhika D, Ramathilaga A, Sathesh P. C. and Murugesan, A. G. Evaluation of Larvicidal Activity of Soil Microbial Isolates (Bacillus and Acinetobactor sp.) against Aedes aegypti (Diptera: Culicidae) - The Vector of Chikungunya and Dengue. Proc Int Acad Ecol Environ Sci. 2011;1(3-4):169-178.
7. Abok JI, Ombugadu A, Angbalaga AG. Hyptis suaveolens Extract exhibits larvacidal activity against Anopheles gambiae larvae. Tropical Journal of Natura Product Research. 2018 Nov;2(5):245-249.
8. George DR, Finn RD, Graham KM, Sparagano OA. Present and Future Potential of Plant-Derived Products to Control Arthropods of Veterinary and Medical Significance. Parasit. &Vectors. 2014 Jan;7: 28.
9. Irungu LW, Mwangi RW. Effects of a Biologically Active Fraction from Melia volkensii on Culex quinquefasciatus. Inst Appl Sci. 1995 Feb;2:159-162.
10. Pavela R, Govindarajan M. The Essential Oil from Zanthoxylum monophyllum a Potential Mosquito Larvicide with Low Toxicity to the Non-Target Fish Gambusia affinis. J Pest Sci. 2017 Jan;90: 369378.
11. Pavela, R. Essential Oils from Foeniculum vulgare Miller as a Safe Environmental Insecticide against the Aphid Myzus persicae Sulzer. Environ. Sci. Pollut. Res. Int, (2018), Apr, 25: 1090410910.
12. Grubben GJH, El Tahir IM. Capsicum annuum L. In: Grubben, G. J. H and Denton, O. A. (Eds.). PROTA 2: Vegetables/ Légumes. [CD Rom]. PROTA, Wageningen, the Netherlands. 2004.
13. Al-Doghairi MA, Hag EE. Effect of Several Bio-pesticides on Alfalfa Weevil Larvae, Hyper brunneipennis. Pakistan Journal of Biological Science. 2003 Oct;6:77781.
14. Bouchelta A, Boughdad A, Blenzar A. Biocide Effects of Alkaloids, Saponins and Flavonoids extracted from Capsicum frutescens L. (Solanaceae) on Bemisia tabaci (Gennidus) (Homoptera: Aleyrodidae). Biotechnol AgronSoc Environ. 2005;94: 2569.
15. Raghavendra K , Singh SP, Sarala KS. Dash AP. Laboratory Studies on Mosquito Larvicidal Efficacy of Aqueous and Hexane Extracts of Dried Fruit of Solanumnigrum Linn. Indian J Med Res. 2009 Jul;130:74-77
16. Gillies MT, de meillon B. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region). Publications of the South African Institute for Medical Research. 1968;54:1-343.
17. Burgess NRH, Cowan GOA Colour Atlas of Medical Entomology. 1993;Pp 143.
18. Finney D J. Probit Analysis, III edn. London: Cambridge University Press. 1971;25325.
19. Dalis DY. Larvicidal Potency of Hyptis suaveolens Leaf Extract against Malaria Vector, Anopheles gambiae. M. Sc.Thesis submitted to the Department of Zoology, University of Jos (Unpublished data). 2016.
20. Madhumathy AP, Aivazi AA, Vijayan VA. Larvicidal efficacy of Capsicum annum against Anopheles stephensi and Culex quinquefasciatus. J Vet Borne Dis. 2007 Sep;44:223226.
21. Odey SA. Bio-Efficacy of the Leaf Extract and Powder of Millettia aboensis Against Larval Stages of Anopheles gambiae and Culex quinquefasciatus in Lafia, Nasarawa State. M. Sc. Thesis submitted to the Department of Zoology, Federal University of Lafia (Unpublished data). 2017.
22. Kholhring L. Mosquitocidal activity of Millettia pachycarpa on the larvae and eggs of Aedes aegypti. Scholars Research Library. Annals of Biological Research. 2011 Jan;2(3):217-222.
23. Chukwujekwu JC, Smith P, Combes PH, Mulholl , Vanstanden J. Antiplasmodial diterpenoids from the leaves of Hyptis suaveolens. Journalof Ethnopharmacology. 2013 Nov;102(2):295-297.
24. Olotuah O. F. Laboratory Evaluation of Pesticidal Activities of Hyptis suaveolens in Pest Management. International Journal of Agricultural Research. 2013;8:101-106.
25. Meenakshi SV, Jayaprakash K. Mosquito larvicidal efficacy of leaf extract from mangrove plant Rhizophora mucronata (Family: Rhizophoraceae) against Anopheles and Aedes species. Journal of Pharmacognosy and Phytochemistry, 2014;3(1):78-83.
26. Awolola TS, Adeogun A, Olakiigbe AK, Oyeniyi YA, Okoh, et al Pyrethroids resistance intensity and resistance mechanisms in Anopheles gambiae from malaria vector surveillance sites in Nigeria. PLoS ONE. 2018 Dec;13(12): e0205230.
27. Ho SH, Ma Y, Tan HTW. Halid H. Repellency of Some Plant Extracts to the Stored Products Beetles, Tribolium castaneum (Herbist) and Sitophilus zeamais Motsch. Proceedings of the Symposium on Pest Management for Stored Food and Feed. Southeast Asian Regional Centre for Tropical Biology. 1997;BIOTROP59: 20915.
28. Kemabonta, K. A, Odediran, O. I. and Ajelara, K. O. The Insecticidal Efficacy of the Extracts of Piper nigrum (Black Pepper) and Curcuma longa (Turmeric) in the Control of Anopheles gambiae Giles (Dip, Culicidae). Jordan Journal of Biological Sciences. 2018;11(2):195-200.
29. Lynd A, Gonahasa S, Staedke SG. Oruni A, Maiteki-Sebuguzi,C, etal. Evaluation in Uganda Project (LLINEUP): a cross-sectional survey of species diversity and insecticide resistance in 48 districts of

Uganda. Parasites Vectors. 2019;12(94):1-10.

30. Mwansat GS, Nanvyat N, Ombugadu A, Mafuyai MJ, Lapang et al. Insecticide susceptibility status, resistance intensity and mechanism of pyrethroids resistance in An. gambiae s.l. mosquitoes from six Local Government Areas in Plateau State Nigeria. Abstract of the 50th Annual Conference of Entomological Society of Nigeria (ESN) Yola 2019. ESN24/MVE07. P 66.
31. Oduola AO, Abba E, Adelaja O, Ande AT, Yoriyo, etal. Widespread Report of Multiple Insecticide Resistance in Anopheles gambiae s.l. Mosquitoes in Eight Communities in Southern Gombe, North-Eastern Nigeria. Journal of Arthropod-Borne Dis. 2019 Mar:13(1):5061.
32. Olotuah OF. Laboratory Evaluation of Pesticidal Activities of Hyptis suaveolens in Pest Management. International Journal of Agricultural Research. 2013 8:101-106.
33. Wendy CV, Goddard J, Harrison B. Identification Guide to Adult Mosquitoes in Mississippi. Accessed 2nd January 2018. 2012.
34. WHO World malaria report 2019, 232Pp. ISBN 978-92-4-156572-1
35. World Health Organization Susceptibility Test Manual Instructions for Determining the Susceptibility or Resistance of Mosquito Larvae to Insecticides. (2013)WHO/VBC/75.583.