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COMPARATIVE PERFORMANCE OF DIFFERENT SAMPLING METHODS USED FOR MOSQUITO CATCHES IN IKEDURU L.G.A., IMO STATE, NIGERIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mosquitoes are obnoxious and notorious blood-sucking insects that devastate human population through malaria attack. This study was carried out in Ikeduru Local Government Area of Imo State, Nigeria in year 2020 with the aim of identifying the most effective method of mosquito sampling. Collection sites were randomly selected based on areas where human activities that aid the breeding of mosquitoes were observed. Mosquitoes were collected using four methods for the adult stage and one method for the larval stage. The methods applied and compared were the pyrethrum knock down/ spray sheet mosquito sampling method, the human bait/ human landing catch technique, the sweep net collection method, the mosquito net-light trap technique and the dipper technique. Sampled mosquitoes were identified using standard methods. The results of the comparative performance evaluations of the different mosquito sampling methods revealed that sweep net and pyrethrum knock down/ spray sheets had the highest occurrence of mosquito species while the dipper mosquito sampling method showed the least performance. This implies that the most effective way for mosquito vector catch from their breeding sites is through the use of sweep net and pyrethrum knock down/ spray sheets. The two effective sampling techniques permit mosquitoes to be picked intact without dismembering them for easy identification.

Keywords: Mosquitoes; human population; malaria; breeding sites; sampling techniques.

1. INTRODUCTION

Mosquitoes are important vectors transmitting both human and animal diseases [1,2,3,4,5]. Different mosquito species transmit different disease-causing organisms and their activities are influenced by environmental factors [1,5]. Some species of mosquitoes do not bite humans, but are inadvertently agents of zoonotic diseases [1,4]. It is evident that there are many ways mosquitoes can cause problems to human beings and therefore, it is pertinent to develop techniques and strategies that will facilitate mosquito capturing with the aim of reducing their abundance in the environment.

Mosquito sampling is an important public health practice all over the world especially in the tropics where environmental factors favour rapid breeding of

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the obnoxious insects. However, preference for any sampling method depends on both its field efficiency and the characteristics of local vector population. This point in case is incontrovertible. The use of an accurate mosquito trapping method is biologically necessary, because studies have reported significant differences in capture efficiencies between methods [6,7,8,9,10]. The same authors have listed the human landing catch technique and use of miniature light trap (CDC-LT) as important techniques used for mosquito sampling. Other sampling methods such as resting boxes, clay pots, pit shelter and bed nets traps have been evaluated under different epidemiological settings in Africa with different degrees of success [11]. This is to mention but a few. The use of window exit traps, BG-sentinels traps and ifakara tents trap design in mosquito sampling have been reported [12,13,14]. In the present study, the mosquito sampling methods considered are the pyrethrum knock down/the spray sheet technique, the human bait trap/ human landing catch (as gold standard method), the sweep net method, the mosquito light trap method and dipper method. Therefore, this study is aimed at sampling adult and larva mosquitoes in Ikeduru L.G.A. of Imo State, Nigeria using different sampling techniques in order to compare and establish the sampling efficiency of each technique.

2. MATERIALS AND METHODS

2.1 Study Area

Ikeduru Local Government Area of Imo State, Nigeria, the study area has already been described by Nzewuihe et al. [5]. Briefly, the place is located between latitude 5°45ˈN and 6°58ˈE and longitude 5°34ˈ N and 7°4ˈ E in the western part of Imo State, in South-East Nigeria (Fig. 1). It has eight months of rainfall (April to November) and four months of dry season (November to March). The average annual rainfall is 2000 mm while the mean daily temperature varies from 23.5°C in the rainy season to 31.1°C in the dry season. The residents of Ikeduru are primarily farmers, traders, civil servants and merchants.

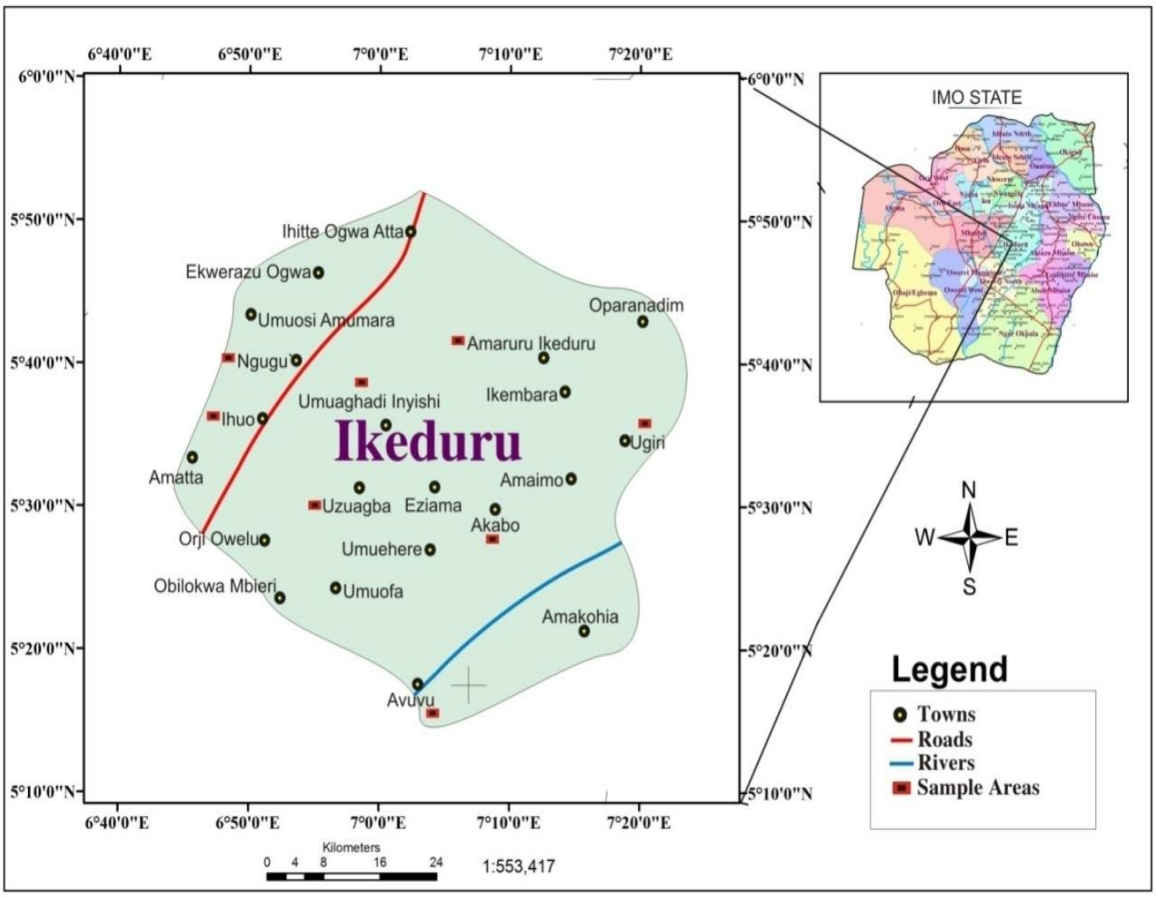

Fig 1. Map Representing Ikeduru L.G.A. and the Sampled Locations in the Study
Source: Nzewuihe et al. [5]

2.2 Sampling Methods

Mosquitoes were collected using four methods for the adult stage and one method for the larval stage [15]. The adult stage mosquitoes collected were isolated into vials labeled according to their breeding sites. The larvae were preserved in vials containing 35% isopropyl alcohol and a drop of glycerin [16] and taken to the laboratory and identified following Hopkins [17] and Gillet [18] while the larvae that could not be identified were allowed to emerge into adult inside mosquitoes rearing cage box before identification according to the standard procedure for rearing mosquitoes adopted from the manual of the animal rearing and quarantine unit at International Centre of Insect Physiological and Ecology [19]. Collection sites were randomly selected based on areas where human activities that aid breeding of mosquitoes were observed. The abundance and distribution of mosquito species in various habitats according to different sampling methods were recorded. The different mosquito sampling methods and procedures applied in this study are briefly described as follow.

The Pyrethrum knock down method/ spray sheet collection method: In the spray sheet collection, a five-man spray team was constituted in order to get catches as much as possible. Each man stood in the room and sprayed the inside and outside of the house, around the eaves (openings) and the inner walls that divide the room from the rest of the house, putting up barriers for spray round all possible exist. Spraying was synchronized so that the eaves of air space above each wall were sprayed simultaneously from inside and outside. In each location, five rooms were randomly chosen and the collection was performed between the hours of 6.00 am to 8.00 am. The floor surfaces of the room, as well as the beds, were completely covered with white sheets. The windows and doors were closed and the eaves were blocked to prevent mosquitoes from escaping. Then the aerosol (Rambo containing 0.25% Transfluthrin and 0.20% Permethrin as active ingredients) was used to spray the room and around the eaves outside the room, then the room was closed for 15 minutes. After 15 minutes, the mosquitoes were collected into labelled petri dish using a pair of forceps and the sheets were carefully retrieved from the floor, starting from the door by lifting them at the four corners. The collected mosquitoes were then taken to the laboratory for identification.

The human bait/ human landing catch method: In each location, two adult volunteers were used to carry out the mosquito collections. The volunteers were trained on mosquito sampling protocols using the human bait. Legs and arms of the volunteers were exposed for the duration of the experiment. Any mosquito which perched on the exposed body part was caught before it fed, by inverting a small glass tube over it. All tubes containing mosquitoes were labelled to indicate date and time of capture. The catch was performed at dusk between 17.00 to 19.00 hours as most species have biting peak after sunset. The catch was performed by two immunized adult volunteers who exposed and caught the mosquitoes as they attempted to bite. After collections, mosquitoes were taken to the Research Laboratory of the Department of Biology, Alvan Ikoku Federal College of Education, Imo State, Nigeria for identification.

The sweep net collection method: Sixteen sweep net traps were used to collect mosquitoes from indiscriminate refuse dump site, cassava and maize processing sites, and septic tanks. The mosquitoes were isolated into labelled vials and taken to the laboratory for identification as explained in the human bait/ human landing catch method.

Mosquito net-light trap method: Sixteen mosquito net-light traps were used. Each mosquito net-light trap method was hung; white sheet was sprayed under it, a tray with dry ice baited with carbon iv oxide sachet and a rechargeable lamp were incorporated. Dry ice has a very nice feature of sublimation, as it breaks down; it turns directly into carbon iv oxide gas rather than a liquid. In this technique, brightness of light attracted mosquitoes at night. Captured mosquitoes were identified as already described [20].

Dipper method: Dipping was used to sample immature mosquitoes as reported by Service [6]. The dipper method was done once in a month in twelve different houses in the various sampled communities.

2.3 Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 17.0 was used to run data analysis. Data were subjected to analysis of variance (ANOVA) and significantly different means were separated using least significant difference (LSD) at $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

Table 1 shows the result of the pyrethrum knock down/ spray sheet mosquito sampling method. Mosquito species captured using this technique included *Culex quinquefasciatus, Aedes aegypti*, *Anopheles gambiae*, *Aedes africanus*, *Culex vittatus*, *Aedes albopictus*, *Aedes luteocephatus* and *Aedes taylori*. Significant differences ($P < 0.05$) occurred in the number of mosquitoes captured in the various

locations/ towns of the Local Government Area. Table 2 shows performance of the human bait/ human landing mosquito catch technique. A total of 2064 mosquitoes were collected and identified. Mosquito species identified included *Aedes aegypti, Aedes albopictus, Aedes taylori, Aedes africanus, Aedes luteocephalus, Culex quinquefaciatus, Anopheles gambiae and Culex vittatus* with significant variations ($P < 0.05$) in the number sampled in each town. Table 3 summarizes the performance of the sweep net mosquito sampling method. A total of 4977 mosquitoes were captured and the species included *Culex quinquefasciatus, Aedes aegypti, Anopheles gambiae*, *Aedes africanus*, *Culex vittetus*, *Aedes albopictus*, *Aedes luteocephatus* and *Aedes taylori*. There were significant differences in the number of mosquitoes captured in the different locations of the place. Table 4 presents the performance of mosquito net-light trap used in sampling mosquitoes. A total of 1408 mosquitoes were caught and the species included *Culex quinquefasciatus, Aedes albopictus, Aedes aegypti, Culex vittatus and Aedes luteocephalus*. Their occurrences varied significantly ($P < 0.05$) in the various towns of the L.G.A. Table 5 shows the performance of the dipper mosquito sampling methods for immature stages. A total of 850 larvae of mosquito were caught and species analysis included *Aedes aegypti, Anopheles gambiae*, *Aedes africanus*, *Aedes albopictus* and *Aedes luteocephatus*. The number of mosquito larvae captured differed significantly among the towns of the Local Government Area. Table 6 summarizes the comparative performance of the different sampling methods used to capture various mosquito species in the study. From the results, the human bait sampling method caught 2064 (16.94%) mosquitoes, the pyrethrum knock down/ spray sheets sampling method caught 3548 (29.12%), the mosquito net-light trap sampling method captured 1408 (11.56%), the sweep net method caught 4312 (35.40%) while the dipper method captured 850 (6.98%) mosquitoes. In the distribution of mosquito species captured using the different methods, sweep net method and pyrethrum knock down method/ spray sheet collection method had the highest occurrence of mosquito species- 4312 (35.40%) and 3548 (29.12%), respectively, while the dipper method captured the least 850 (6.98%).

The different sampling methods used to catch mosquitoes revealed that in the study, *Culex quinquefasciatus* and *Anopheles gambiae* captured using the pyrethrum knock down/ spray sheet sampling method is attributable to the fact that the species are highly anthropophilic and can only be found indoors while the *Aedes albopictus* and more *Aedes africanus* captured (which are outdoor mosquitoes) were collected as indoor mosquitoes because residents planted ornamented crops and carried out their cassava processing business near human habitation. Some settlements near streams or rivers also made concerned mosquito species to be collected indoors using the spray method. They are very active at night and are usually attracted to smelly feet. The *Culex quinquefasciatus* and *Anopheles gambiae* are primary vectors of several arboviral diseases particularly the West Nile Virus, lymphatic filariasis, elephantiasis and they are very important transmitters of the most dangerous malaria parasite to human beings [1]. It was also revealed that the human bait/ human landing catch technique collected more *Aedes species*, especially the *Aedes aegypti*, than other species identified in the study locations. This is due to the fact that, the *Aedes species* are outdoor feeders and mostly feed late in the afternoon or even in full sunlight. The *Aedes* species transmit yellow fever, dengue fever, chikungunya, heartworm, encephalis, Ross river virus and West Nile virus [1,4] and therefore this species is capable of causing serious public health problems. The results revealed that sweep net method captured the highest mosquito species. This can be explained on account of the inhabitants planting ornamented crops, carrying out their cassava processing business close to their houses, dumping refuse and broken plastics and metal buckets close to their houses and some settlements living close to stream or river. This association is in agreement with the reports of Battle et al. [21]. The results further indicated that the mosquito net-light trap captured low number of mosquito species. This observation agrees with the findings of Govella et al. [13]. The low catch by the net-light trap sampling method was probably because the light trap was set-up outdoors and predominant species in the community are highly anthropophilic and are less influenced by light, and large types of vegetation in the community might have covered the brightness of the light thereby interfering with attractions. It was observed from the results that more *Culex quinquefasciatus* were collected using the mosquito net-light trap, probably, other species are not significantly attracted to the light. The results also indicated that the dipper method captured the least mosquito species and this is also supported by literature. The least catch in the dipper method was probably because water collections in exposed broken plastics or containers were out door and might have lacked the right PH level. Other factors of consideration include lack of exposure of stored water to sunlight, hardness of the water, temperature, chemical composition and presence of bacteria fauna which influence species of mosquito to breed in the exposed water [22,23,24].

Table 1. Adult mosquitoes identified in the different towns in Ikeduru L.G.A. using the pyrethrum knock down/ spray sheet method

Name of town	No. of houses sampled	Total No. of mosquitoes*	Mosquitoes species identified
Amaruru	5.00	318.00[d]	*Aedes aegypti* (13), *Aedes africanus* (85), *Aedes albopictus* (120), *Aedes luteocephalus* (6), *Anopheles gambiae* (18) and *Culex quinquefasciatus* (76).
Inyishi	5.00	706.00[a]	*Aedes aegypti* (78), *Aedes africanus* (192), *Aedes luteocephalus* (21), *Aedes taylori* (61), *Aedes albopictus* (152), *Anopheles gambiae* (43), *Culex quinquefasciatus* (124) and *Culex vittetus* (35).
Iho	5.00	476.00[c]	*Aedes aegypti* (62), *Aedes africanus* (138), *Aedes albopictus* (129), *Aedes luteocephalus* (18), *Anopheles gambiae* (28), *Culex quinquefasciatus* (95) and *Culex vittatus* (6).
Ngugu	5.00	455.00[c]	*Aedes aegypti* (51), *Aedes africanus* (136), *Aedes taylori* (23), *Aedes albopictus (*130),*Culex quinquefasciatus* (88) and *Anopheles gambiae* (27).
Uzoagba	5.00	440.00[c]	*Aedes aegypti* (46), *Aedes africanus* (142), *Aedes albopictus* (115), *Anopheles gambiae* (32) and *Culex quinquefasciatus* (105).
Akabo	5.00	594.00[b]	*Aedes aegypti* (65), *Aedes africanus* (149*), Aedes albopictus* (133),*Culex quinquefasciatus* (115), *Anopheles gambiae* (36), *Aedes taylori* (41), *Aedes luteocephatus* (34) *and Culex vittetus* (21).
Ugiri	5.00	305.00[d]	*Aedes aegypti* (34), *Aedes africanus* (123), *Aedes albopictus* (88), *Anophele gambiae* (21), and *Culex quinquefasciatus* (39).
Avuvu	5.00	254.00[d]	*Aedes aegypti* (28), *Aedes africanus* (109), *Aedes albopictus* (72), *Anopheles gambiae* (15) and *Culex quinquefasciatus* (30).
Total	40.00	3548.00	

**Mean values in the column with same letter are not significantly different by LSD ($\alpha = 0.05$)*

Table 2. Adult mosquitoes caught in the towns of Ikeduru L.G.A. using the human bait/ human landing sampling method

Name of town	Duration (Hours)	No. of human bait	Total No. of mosquitoes captured*	Mosquitoes species identified
Amaruru	96.00	4.00	180.00[d]	*Aedes aegypti* (77), *Aedes albopictus* (30), *Aedes africanus* (52) *Anopheles gambiae* (17) and *Culex quinquefasciatus* (4).
Inyishi	96.00	4.00	507.00[a]	*Aedes aegypti* (102), *Aedes luteocephatus* (72), *Aedes africanus* (72), *Aedes albopictus* (62), *Anopheles gambiae* (37), *Culex quinquefasciatus* (42), *Culex vittatus* (22) and *Aedes taylori* (98).
Iho	96.00	4.00	253.00[c]	*Aedes aegypti* (85), *Culex quinquefasciatus* (10), *Aedes luteocephatus* (14), *Aedes africanus* (67*), Aedes albopictus* (46), *Anopheles gambiae* (31).
Ngugu	96.00	4.00	277.00[c]	*Aedes aegypti* (65), *Culex quinquefasciatus* (26), *Aedes taylori* (56), *Aedes africanus* (59), *Aedes albopictus* (43), *Anopheles gambiae* (15) and *Culex vittatus* (13).

Name of town	Duration (Hours)	No. of human bait	Total No. of mosquitoes captured*	Mosquitoes species identified
Uzoagba	96.00	4.00	182.00[d]	*Aedes aegypti* (79), *Aedes albopictus* (51) and *Aedes africanus* (52).
Akabo	96.00	4.00	404.00[b]	*Aedes aegypti* (89), *Aedes albopictus* (57), *Aedes africanus* (68), *Anopheles gambiae* (32), *Aedes taylori (*41), *Culex vittatus (*21). *Aedes luteocephatus* (68) and *Culex quinquefasciatus* (28).
Ugiri	96.00	4.00	126.00[d]	*Aedes aegypti* (62), *Aedes africanus* (23) and *Aedes albopictus* (41).
Avuvu	96.00	4.00	135.00[d]	*Aedes aegypti* (53), *Aedes albopictus* (34) and *Aedes africanus* (48).
Total	768.00	32.00	2064.00	

**Mean values in the column with same letter are not significantly different by LSD (α = 0.05)*

Table 3. Adult mosquitoes captured and identified in different towns using the sweep net mosquito sampling method

Name of town	No. of net-light trap	No. of mosquitoes captured*	Mosquitoes species identified
Amaruru	2.00	353.00	*Culex quinquefasciatus* (146), *Aedes aegypti* (86), *Aedes africanus* (67) and *Anopheles gambiae* (54).
Inyishi	2.00	1068.00[a]	*Culex quinquefasciatus* (225), *Culex vittetus* (56), *Aedes aegypti* (205), *Anopheles gambiae* (142), *Aedes africanus* (105), *Aedes albopictus* (135), *Aedes luteocephatus* (134) and *Aedes taylori* (66).
Iho	2.00	700.00[c]	*Culex quinquefasciatus* (192), *Anopheles gambiae* (89), *Aedes aegypti* (143), *Aedes africanus* (102), *Aedes albopictus* (102), *Aedes taylori* (31), *Aedes luteocephetus* (41).
Ngugu	2.00	767.00[c]	*Culex quinquefasciatus* (188), *Aedes aegypti* (162), *Anopheles gambiae* (75), *Culex vittetus (33), Aedes albopictus* (86), *Aedes luteocephatus* (101), *Aedes africanus* (86), *Aedes taylori* (36).
Uzoagba	2.00	612.00[d]	*Culex quinquefasciatus* (176), *Aedes aegypti* (151), *Aedes albopictus* (40), *Culex vittatus* (21), *Anopheles gambiae* (34), *Aedes luteocephatus* (98), *Aedes africanus* (92).
Akabo	2.00	844.00[b]	*Culex qunquefasciatus* (179), *Aedes aegypti* (162), *Culex vittetus* (46), *Aedes africanus* (113), *Anopheles gambiae* (81), *Aedes luteocephetus* (62), *Aedes taylori* (79), *Aedes albopictus* (122).
Ugiri	2.00	335.00[e]	*Culex quinquefasciatus* (96), *Aedes aegypti* (125), *Aedes albopictus* (46), *Anopheles gambiae* (25), *Aedes africanus* (43).
Avuvu	2.00	298.00[e]	*Culex quinquefasciatus* (76), *Aedes aegypti* (116), *Aedes africanus* (57), *Aedes albopictus* (33), *Anopheles gambiae* (16).
Total	16.00	4977.00	

**Mean values in the column with same letter are not significantly different by LSD (α = 0.05)*

Table 4. Adult mosquitoes captured and identified in different towns using the mosquito net- light trap sampling method

Name of town	No. of mosquitoes net-light trap	No. of mosquitoes captured*	Mosquitoes species identified
Amaruru	2.00	122.00[c]	*Culex quinquefasciatus* (96), *Aedes albopictus* (26).
Inyishi	2.00	394.00[a]	*Culex quinquefasciatus* (210), *Aedes albopictus* (72), *Culex vittetus* (43), *Aedes aegypti* (32), *Aedes luteocephalus* (37).
Iho	2.00	219.00[b]	*Culex quinquefasciatus* (154), *Aedes albopictus* (43), *Aedes aegypti* (22).
Ngugu	2.00	160.00[c]	*Culex quinquefasciatus* (115), *Culex vittetus* (21), *Aedes aegypti* (18), *Aedes luteocephalus* (6).
Uzoagba	2.00	152.00[c]	*Culex quinquefasciatus* (121), *Aedes albopictus* (31).
Akabo	2.00	241.00[b]	*Culex quinquefasciatus* (105), *Aedes albopictus* (62), *Culex vittetus* (18), *Aedes aegypti* (35), *Aedes luteocephatus* (21).
Ugiri	2.00	58.00[d]	*Culex quinquefasciatus* (43), *Aedes albopictus* (15).
Avuvu	2.00	62.00[d]	*Culex quinquefasciatus* (36), *Aedes albopictus* (17), *Aedes aegypti* (9).
Total	16.00	1408.00	

**Mean values in the column with same letter are not significantly different by LSD ($\alpha = 0.05$)*

Table 5. Larva mosquitoes captured and identified in different towns using dipper sampling method

Name of town	No. of times mosquito larvae were collected	No. of larva mosquitoes captured*	Mosquitoes species identified
Amaruru	12.00	30.00[e]	*Aedes aegypti* (14), *Anopheles gambiae* (16).
Inyishi	12.00	231.00[a]	*Aedes aegypti* (72), *Aedes albopictus* (36), *Anopheles gambiae* (41), *Aedes africanus* (82).
Iho	12.00	102.00[c]	*Aedes aegypti* (56), *Aedes albopictus* (25*), Aedes africanus* (21).
Ngugu	12.00	110.00[c]	*Aedes aegypti* (36), *Aedes albopictus* (29), *Aedes africanus* (45).
Uzoagba	12.00	87.00[cd]	*Aedes aegypti* (41), *Aedes africanus (29), Anopheles gambiae* (17).
Akabo	12.00	170.00[b]	*Aedes aegypti* (61), *Aedes albopictus* (17), *Aedes africanus* (63), *Anopheles gambiae* (29).
Ugiri	12.00	64.00[cde]	*Aedes aegypti* (34), *Anopheles gambiae* (13), *Aedes luteocephatus* (17).
Avuvu	12.00	56.00[de]	*Aedes aegypti* (21), *Anopheles gambiae* (17), *Aedes albopictus* (12), *Aedes luteocephatus* (6).
Total	96.00	850.00	

**Mean values in the column with same letter(s) are not significantly different by LSD (α = 0.05)*

Table 6. Comparative performance of the different sampling methods used in catching mosquitoes species in Ikeduru L.G.A., Imo State, Nigeria

Mosquitoes Species	Human bait	Spray	Mosquitoes light trap	Sweeping net	Dipper	Total
Aedes aegypti	612.00[a]	377.00[d]	116.00[c]	1150.00[a]	335.00[a]	2590.00
Aedes africanus	441.00[b]	1074.00[a]	0.00[e]	0.00[f]	240.00[b]	1755.00
Aedes taylori	195.00[d]	125.00[f]	0.00[e]	212.00[d]	0.00[d]	532.00
Aedes albopictus	364.00[c]	939.00[b]	266.00[b]	564.00[b]	119.00[c]	2252.00
Aedes luteocephatus	154.00[de]	79.00[f]	64.00[d]	436.00[c]	23.00[d]	756.00
Anopheles gambiae	132.00[e]	220.00[e]	0.00[e]	516.00[b]	133.00[c]	1001.00
Culex quinquefasciatus	110.00[de]	672.00[c]	880.00[a]	1278.00[a]	0.00[d]	2940.00
Culex vittatus	56.00[f]	62.00[f]	82.00[cd]	156.00[e]	0.00[d]	356.00
Total	2064.00	3548.00	1408.00	4312.00	850.00	12182.00

Mean values in a column with same letter(s) are not significantly different by LSD (α = 0.05)

4. CONCLUSION

In conclusion, the comparative performance of the different mosquito sampling methods investigated in the present study revealed that the sweep net method and pyrethrum knock down/ spray sheets method had the highest occurrence of mosquito species while the dipper mosquito sampling method showed the least performance. This implies that the most effective way for mosquito vector catch from their breeding sites is through the use of sweep net and pyrethrum knock down/ spray sheets. The two efficient sampling techniques will allow mosquitoes to be picked intact without dismembering them for easy identification. The assertion here is supported by Mgbemena et al. [4].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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