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The Novel Prokopack Aspirator Versus Popular Pyrethrum Spray Catch: Assessment of the Composition and Abundance of *Anopheles gambiae sensu lato* in Nasarawa State, Central Nigeria

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ABSTRACT

The continuous need for complimentary, substitutionary, as well as alternative techniques in malaria vectors surveillance cannot be over emphasized. Also, the studies on complementary and or substitutionary sampling techniques in sub-Saharan Africa is lacking to a very large extent. Thus, this study compared the novel prokopack aspirator versus the popular pyrethrum spray catch in relation to the composition and abundance of *Anopheles gambiae sensu lato* in Nasarawa State, Central Nigeria. Mosquitoes were collected at day time from 0600 to 0900 hours using the novel Prokopack Aspirator and Pyrethrum Spray Catch (PSC) techniques. Sixty randomly selected houses were surveyed quarterly, thirty houses for each sampling technique employed. Adult mosquitoes collected were sorted and morphologically identified using standard identification keys in the laboratory. Two thousand eight hundred and forty-three (2,843) mosquitoes were collected in which differences between the anopheline group (2,774, 97.57%) and the culicine 69 (2.43%) varied significantly (χ^2 = 2,573.7, df = 1, P < 0.0001). A significant difference (χ^2 = 10,659, df = 4, P < 0.0001) was observed in relation to the abundance between five mosquito species recorded in which *An. gambiae s. l.* was the most dominant (2,770, 97.43%). Over 60% (1,650) of the pooled female *Anopheles gambiae* s. l. collected were from Prokopack Aspirator collection, whereas PSC only yielded 39.78% (1,131) individuals and differences varied significantly (χ^2 = 118.73, df = 1, P < 0.0001) between the two sampling techniques. The number of female *An. gambiae s. l.* caught positively associated with the number of people that slept indoors the previous night prior to collection (t = 4.7677, df = 294, P < 0.0001, r = 0.27). In conclusion, the high mosquitoes catch obtained from Prokopack Aspirator possibly suggests its recommendation for consideration for wide usage in entomological surveillance to either compliment or substitute the PSC technique.

Keywords: Malaria Vectors Surveillance; Prokopack Aspirator; Pyrethrum Spray Catch; Nasarawa State; Central Nigeria

Abbreviations: IRS: Indoor Residual Spraying; LGA: Local Government Area; PAC: Prokopack Aspirator Catch; PMI: President's Malaria Initiative; PSC: Pyrethrum Spray Catch; USAID: United States Agency for International Development; WHO: World Health Organization

Introduction

About half of the world's population at risk of malaria [1], hence, the need to develop tools that are efficacious in order to mitigate biological threats to malaria [2]. The outcome of the Global Technical Strategy for Malaria 2016–2030 (GTS) morbidity milestone is still disturbing due to global deviation from the track by 40% with malaria case incidence of 59 cases per 1000 population at risk instead of the expected 35 cases per 1000 [3]. Malaria is responsible for huge economic losses based on an estimated budget of US$ 6.8 billion in 2020, rising to US$ 9.3 billion per year by 2025 and US$ 10.3 billion per year by 2030 [4] for a possible successful eradication. Of all malaria deaths globally, Africa disproportionately accounts for 93% [3]. World Health Organization (WHO) [1] revealed that the African region in the year 2020 was faced with malaria cases and deaths at 95% and 96%, respectively. In Nigeria, *Anopheles stephensi* was first recorded in the year 2020 [5] which may likely be an additional public health problem to overcome. A study in western Kenya found a new cryptic *Anopheles* species involved in the transmission of human which clearly underscores the fact that it is a very critical necessity to understand malaria vector species composition and their bionomic characteristics in the Africa region so as to develop a robust effective and efficient vector control interventions that will reduce malaria transmission [6].

Countries can achieve effective health interventions and assess the impact of their malaria control programmes only through strong malaria surveillance systems. This is the continuous and systematic collection, analysis and interpretation of malaria-related data, and the use of such data in the planning, implementation and evaluation of public health practice termed Evidence-Based [1]. Recent entomological surveillance by Nigerian Institute for Medical Research (NIMR) reported the presence of *Anopheles stephensi* in Gombe State, North East Nigeria [7] which is the main malaria vector in Asia [8] and categorized invasive malaria vector in Kenya, East Africa [9,10]. The use of a novel and existing protocols is key in the fight against malaria. Pyrethrum Spray Catch (PSC) which is an existing or popular technique targets the indoor resting adult mosquitoes using pyrethroid [11].

Battery-powered Prokopack Aspirator Model 1419 was developed to collect mosquitoes such as *Aedes aegypti*, anophelines, and *Culex* of all physiological stages and both sexes directly from their resting sites, allowing better estimations of species diversity, abundance, sex ratio, age structure, and physiological status [12] which is an indication of novelty and complementarity. The novel sampling methods for monitoring outdoor *Anopheles* mosquitoes in Eritrea by Charlwood, et al. [13] confirmed the superiority and efficiency of Prokopack Aspirator over other methods in outdoor mosquitoes survey.

Findings of Ombugadu, et al. [14] showed that associations between mosquitoes and the number of indoor sleepers positively correlated. Lwetoijera, et al. [15] similarly demonstrated in their study in rural Southern Tanzania that the density of mosquitoes increased as the number of people sleeping within the rooms increased. In a recent study in Morogoro and Dodoma in Tanzania, it was also found that a very high population of mosquitoes were collected from areas with a lot of indoor sleepers from the previous night [16].

The studies on complementary and or substitutionary sampling techniques in sub-Saharan Africa is lacking to a very large extent. There is a need for effective and affordable mosquito sampling methods. Pyrethroid knockdown collection is expensive, time, insecticide and personnel consuming, subject to inter-operator and location heterogeneity, and impractical in many urban environments [12,17]. The composition of *An. gambiae s. l.* in Nassarawa Eggon Local Government Area (LGA) in relation to Prokopack Aspirator and Pyrethrum Spray Catch collections is unknown. To this end, this study compared the effectiveness of Prokopack Aspirator and Pyrethrum Spray Catch in relation to the composition and abundance of *Anopheles gambiae sensu lato* in Nasarawa State, Central Nigeria. assessing key malaria transmission and insecticide resistance indices in *An. gambiae s. l.* in Nassarawa Eggon LGA, Nasarawa State, Central Nigeria.

Materials and Methods

Study Site

The study was carried out in Nassarawa Eggon LGA (Latitude 8° 54.275´ N, Longitude 8° 23.660´ E), Nasarawa State with an elevation of 445 metres (1,460 feet). It has an area of 1,208 km2 and a population of 149,129 at the 2006 census. The area is characterized by two seasons, wet and dry in the tropical savannah climate that is suitable for the cultivation of different varieties of crops. It has a mean temperature of 15.6 ℃ and 26.7 ℃ with an annual rainfall between 1317 mm and 1450 mm from April to October [18]. According to a Geographic Reconnaissance (GR) survey by Ojo, et al. [19], the average number of persons per household (HH) in the area is seven (7) individuals across the 309 towns and villages. The total number of HouseHolds (HH) in the area is 213,507 in which less than five (5) years of age are 42,976 and the number of pregnant women is 7,948. Also, the average number of children less than five (5) years of age per hundred (100) HH is one hundred and eighteen (118) while the average number of pregnant women per hundred (100) HH is twenty-two (22).

Ethical Consent

An advocacy visit was made to the paramount Chief of Nassarawa Eggon LGA and the heads of villages prior to the commencement of the survey to adequately inform them of the importance of the research. They, in turn, consented and granted full permission and informed their subjects to support the study by making their houses available. Also, ethical consent was sought from heads of households selected a day before the sampling day in which they favourably accepted.

Duration of the Study

The longitudinal study lasted for two years, from June 2016 to May 2018.

Sample Collections

Mosquito samples were collected quarterly (early and late wet seasons as well as early and late dry seasons) indoors [20] using the Prokopack Aspirator and Pyrethrum Spray Catch techniques, respectively. A total of sixty (60) houses that met the WHO criteria (rooms without eaves) for mosquito day catch were randomly selected from four villages that are far apart and marked using the Global Positioning System (GPS). Thirty (30) houses were selected for each collection technique pooled from two villages, fifteen (15) houses from each village. The distance between the randomly selected houses was at least 200 metres apart. Selected houses were revisited throughout this study (i.e. each house was visited eight times within the study period). Rooms were selected a day prior to collection and heads of households and occupants of the selected rooms were informed to keep doors and windows closed during the morning hours until mosquitoes were collected. Collection of adult mosquitoes was done during the day time between 6:00 am and 9:00 am.

Indoor *Anopheles gambiae* Sampling by Prokopack Aspirator

The Prokopack Aspirator collection was as described by Vazquez-Prokopec, et al. [17]. The Prokopack Aspirator (Plate 1a) is a battery powered equipment for insects' collection [17]. The Prokopack Aspirator operation is simple (Plate 1b). A headlamp was worn on the head so as to successfully move around selected rooms without hitches in the course of collection. Prior to the commencement of the operation in the room, all windows and doors were closed. The battery in the outer compartment of the backpack was attached very carefully to the power cord (red indicates positive polarity and black indicates negative polarity) of the Prokopack Aspirator (Plate 1c). The Prokopack Aspirator was turned on with a rotary switch located by the handle. The Prokopack Aspiratior was held with the hand and moved around on wall and ceiling surfaces, underneath the bed and tables, to aspirate all the endophilic mosquitoes [17]. After collections were made in each room at an average of eight minutes, the lid on the collection cup was properly covered before turning off the unit. Afterwards, cotton wool well soaked with chloroform was used to knockdown mosquitoes for a period of five minutes and were transferred into a well labeled petri dish and transported to the Insectary of the Department of Zoology, University of Jos for sorting, morphological identification and molecular processing which cut across characterization of *Anopheles gambiae s. l.* siblings, sporozoite screening, blood meal source, and Sanger sequencing to determine their population structure in the area.

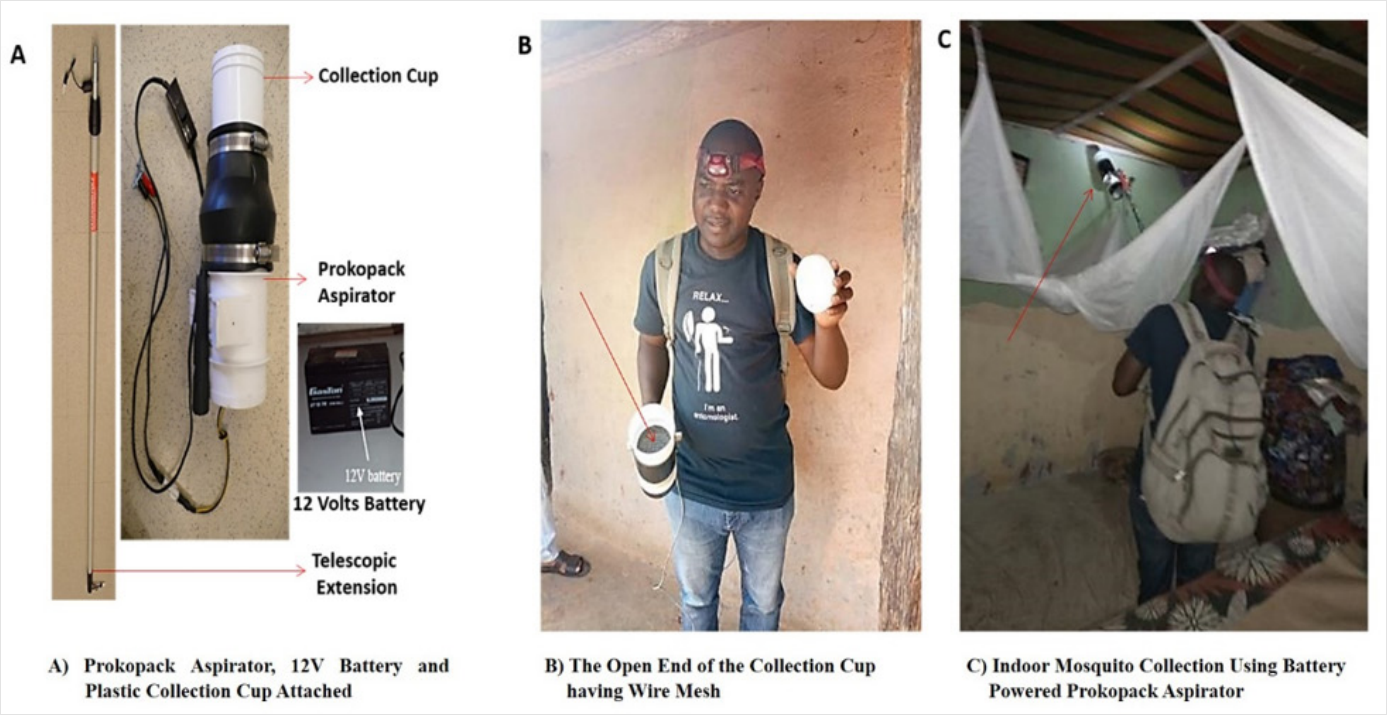

Plate 1: Prokopack aspirator set-up and operation.

Indoor *Anopheles Gambiae* s. l. sampling by Pyrethrum Spray Catch

The pyrethrum spray catch (PSC) operation was carried out as described by Williams [21]. Firstly, before spraying, all animals indoors were removed, all food covered and small furniture removed from the room the collection was to take place. The operation was carried out very carefully with a headlamp that was worn on the head to lighten the room. All windows and doors were closed. White cotton sheets were spread underneath the tables, laid to cover the floor completely and all flat surfaces followed by careful spraying of the room with Raid insecticide which contains pyrethroids (these include Deltamethrin, D-allethrin and Tetramethrin). The spraying was done in a clockwise direction towards the ceiling until the room was filled with a fine mist. The sprayed room was rapidly exited, closed the door and waited for 10 minutes in order to knockdown the mosquitoes. Beginning from the room's entrance, the corners of the sheet were lifted and the sheet was taken outside. All knocked down mosquitoes were collected in the daylight with forceps and placed in a well labeled petri-dish, on top of a layer of damp cotton wool and filter paper. Mosquitoes collected in each house were stored in separate petri-dishes appropriately labeled (collection date and hour, village, household number/ name of head of household) and transported to the Insectary of the Department of Zoology, University of Jos for sorting, morphological identification and molecular processing.'

Morphological Identification

Mosquitoes were sorted out and morphologically identified based on their visible features with the aid of a dissecting microscope and identification keys [22-26]. Identified *An. gambiae s. l.* was preserved dry singly in Eppendorf tubes containing silica gel and tissue paper barrier for molecular processing.

Statistical Analysis

Data obtained was analyzed using R Console software version 4.0.2. Pearson's Chi-square ($\chi 2$) test was used to compare proportions between mosquito groups, species, and sampling techniques, respectively. Associations between mosquito abundance and number of occupants that slept indoors the previous night was analyzed using Pearson's product-moment correlation test. The level of significance was set at $P < 0.05$.

Results

Composition of Indoor Resting Mosquitoes in Nassarawa Eggon LGA, Nasarawa State, Obtained from Two Sampling Techniques

A total of 2,843 mosquitoes were collected in this study in relation to the two indoor sampling techniques employed, out of which the anopheline group was dominant 2774 (97.57%) over the culicines 69 (2.43%) as shown in Table 1. Therefore, there was a very high significant difference ($\chi 2 = 2573.7$, df = 1, $P < 0.0001$) between the population of the two mosquito groups. Figure 1 shows the order of dominance of five mosquito species recorded as follows: *An. gambiae s. l.* 2770 (97.43%) > *Cx. quinquefasciatus* 67 (2.36%) > *An. coustani* 3 (0.11%) > *Ae. aegypti* 2 (0.07%) > *An. rufipes* 1 (0.04%). Thus, there was a very high significant difference ($\chi 2 = 10659$, df = 4, $P < 0.0001$) in the abundance of indoor resting mosquito species. Within the anopheline group, the population of indoor resting *An. gambiae s. l.* was the highest, 2770 (99.86%), while the proportion of An. coustani 3 (0.11%) and *An. rufipes* 1 (0.34%) was very low (Table 1). Hence, the variation in abundance between anopheline species showed a very high significant difference ($\chi 2 = 5524$, df = 2, $P < 0.0001$). Similarly, the population of indoor resting *Cx. quinquefasciatus* was higher (67, 97.10%) than *Ae. aegypti* (2, 2.90%) within the culicine group showing a very high significant difference ($\chi 2 = 61.232$, df = 1, $P < 0.0001$) in abundance between culicine species. The pooled number of indoor resting female mosquitoes was more (2776, 97.64%) than males (67, 2.36%). The abundance of indoor resting females was significantly higher ($\chi 2 = 2581.3$, df = 1, $P < 0.0001$) than that of male mosquitoes. The female anopheline was higher (99.06%) than males (0.94%), while the situation was reversed among the culicine group in which males (41, 59.42%) were more than females (28, 40.58%) as shown in Table 1.

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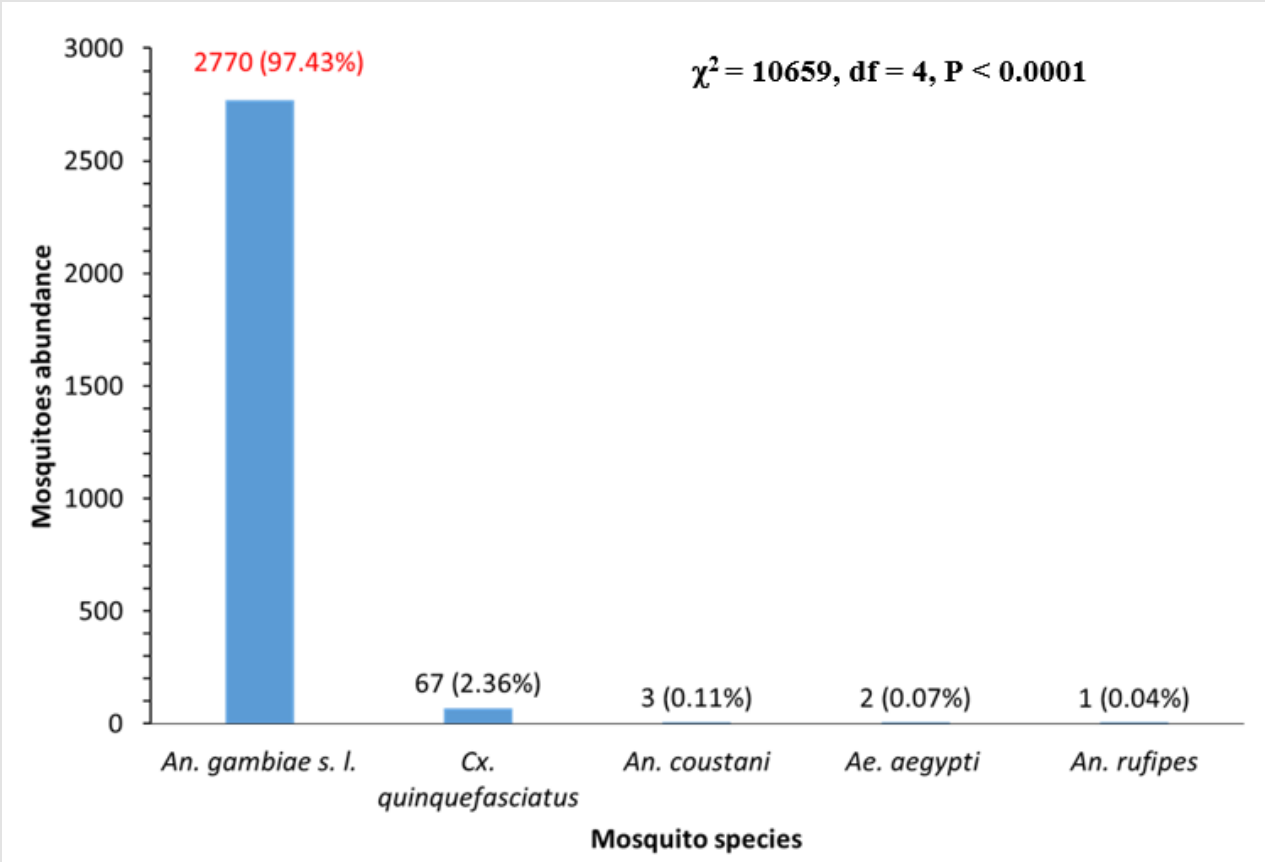

Figure 1: Order of Dominance of Five Mosquito Species Collected in Nassarawa Eggon LGA, Nasarawa State, Nigeria.

Table 1: Checklist of Mosquitoes Collected in Nassarawa Eggon LGA, Nasarawa State, Using Two Sampling Techniques.

Mosquito Group	Species	Techniques				Subtotal		Total (%)
		PAC		PSC				
		Female	Male	Female	Male	Female	Male	
Anopheline	*An. coustani*	0	0	3	0	3	0	3 (0.11)
	An. gambiae	1649	26	1095	0	2744	26	2770 (99.86)
	An. rufipes	1	0	0	0	1	0	1 (0.34)
	Subtotal (%)	1650	26	1098	0	2748 (99.06)	26 (0.94)	2774 (97.57)
Culicine	*Ae. aegypti*	2	0	0	0	2	0	2 (2.90)
	Cx. quinquefasciatus	10	24	16	17	26	41	67 (97.10)
	Subtotal (%)	12	24	16	17	28 (40.58)	41 (59.42)	69 (2.43)
	Total (%)	1662	50	1114	17	2776 (97.64)	67 (2.36)	
	Grand total (%)	1712 (60.22)		1131 (39.78)				2843

Note: PAC: Prokopack Aspirator Collection; PSC: Pyrethrum Spray Catch

Mosquitoes Abundance in Relation to the Two Sampling Techniques Employed

Prokopack Aspirator collection (PAC) had 60.22% (1712) of pooled mosquito species collected whereas PSC only had 39.78% (1131) individuals, as shown in Table 1. Therefore, the abundance of pooled mosquitoes in relation to the two sampling techniques showed a significant difference (χ 2 = 118.73, df = 1, P < 0.0001).

Abundance of Female *Anopheles* Mosquitoes in Relation to Sampling Techniques

The abundance of female *Anopheles* mosquitoes between PAC and PSC techniques was 1650 (60.04%) and 1098 (39.96%), respectively (Table 1). Thus, there was a significant difference (χ 2 = 110.88, df = 1, P < 0.0001) in the mean abundance of female *Anopheles* mosquitoes in relation to the two sampling techniques. Also, PAC had more female *An. gambiae* 1649 (60.09%) than PSC 1095 (39.91%) technique. Hence, the abundance of female *An. gambiae* in relation to the two sampling techniques significantly varied (χ 2 = 111.85, df = 1, P < 0.0001).

Association Between Abundance of Female *Anopheles gambiae* and Household Occupants Prior to Mosquito Collections

There was a relatively strong positive association between the number of female *An. gambiae* and the number of people that slept indoors the previous night (t = 4.7677, df = 294, P < 0.0001, r = 0.27, Figure 2).

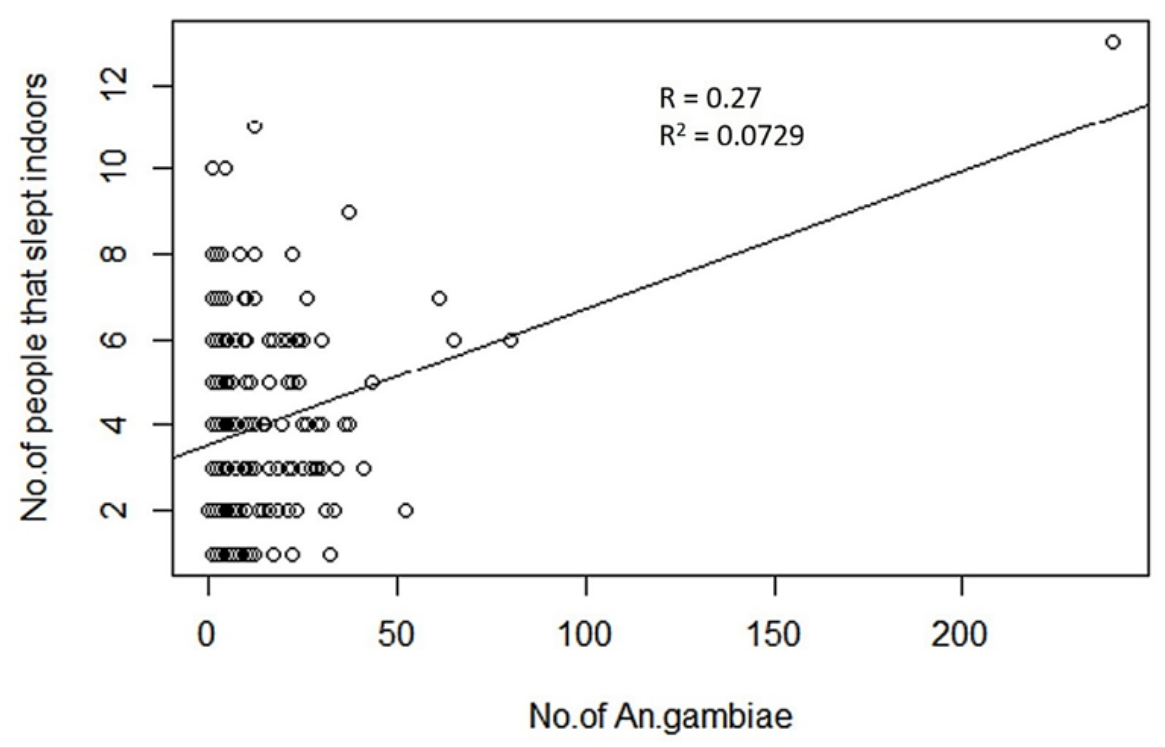

Figure 2: The Relationship Between Indoor Resting Female *Anopheles gambiae* Abundance and the Number of People Who Slept in the Houses the Previous Night Prior to Collection.

Discussion

Composition and Distribution of Mosquitoes in Nassarawa Eggon LGA

The high mosquito population and diverse species recorded in Nassarawa Eggon LGA of Nasarawa State, Nigeria suggests that the lowland nature of the area, which is characterized by high temperature and relative humidity as well as the presence of generally hilly and rocky terrains, with numerous rivers and streams in the study area which empties into the Benue River greatly supports mosquitoes breeding success all year round. The occurrence of mosquitoes despite the pilot study of the Indoor Residual Spraying (IRS) in 2012 under the President's Malaria Initiative (PMI) funded by the United States Agency for International Development (USAID) [27,28] as well as the IRS repeat cycle in 2013 [29] is quite alarming and worrisome. The diverse mosquito species recorded in this study agree with the

studies by Chuntar, et al. [30], who found six *Anopheles* species in some selected eco-settings of Nasarawa State. The number of anopheline mosquitoes recorded is in line with the finding of Ombugadu, et al. [31], who reported *Anopheles gambiae* as the most dominant over culicines collected in a peri-urban area.

Abundance of Female *Anopheles* mosquitoes in Relation to Sampling Techniques

The remarkable variation in abundance of *Anopheles* between sampling techniques in favour of the PAC suggests that it is a highly efficient mosquito collection equipment. This observation aligns with recent findings by Ombugadu, et al. [14,32], who conducted a mosquito survey in student Hostels of the Federal University of Lafia, Nasarawa State, using a Prokopack Aspirator; the equipment proved efficient in mosquito collection. Earlier studies by Maia, et al. [33] comparing the CDC Backpack aspirator and the PAC in the sampling of mosquitoes support the assertion about the effectivity of PAC over the CDC backpack aspirator. Current research on novel sampling methods for monitoring mosquitoes by Charlwood, et al. [13] explored techniques including odour baited trap, a novel tent-trap, human landing collection and PAC. Again, in that study, the Prokopack Aspirator was revealed to be most effective in mosquito collection. Kakilla, et al. [34-36], have also adopted the use of PAC to survey malaria vector species. This observation may be due to its simple and user-friendly nature, as it can be easily carried and manoeuvred, especially for an efficient field collection. In contrast, the CDC light traps and PSC demonstrated some efficiency during a study on collection of mosquitoes for entomological indices in Western Kenya as reported by Abong'o, et al. [37]. A similar opinion from Onyango [38] suggests that the efficiency of the Prokopack Aspirator in collecting anopheline mosquitoes is second to the light traps.

Mosquitoes Abundance in Relation to Number of Indoor Occupants

The strong positive association between several *Anopheles* mosquitoes and indoor occupants observed may be due to the high concentration of volatiles such as carbon dioxide (CO_2). These findings tally with that of McCann, et al. [39], in a study on elucidating the variation in adult *Anopheles* indoor resting abundance, the relative effects of larval habitat proximity and insecticide treated bed net use established that the number of *An. gambiae s. s*. and *An. funestus* was positively associated with the number of occupants in the room; however, the number of *An. arabiensis* decreased with an increasing number of people sleeping in the room. A fairly similar study by Obembe, et al. [40] also observed an association between indoor occupants and the abundance of indoor resting *Anopheles* mosquitoes. Nevertheless, Obembe and others focused on the differential behaviour of endophilic *Anopheles* mosquitoes in rooms occupied by tobacco smokers and non-smokers in two Nigerian villages, where a reduced mosquito endophily was observed in the habitations of tobacco smokers compared to the non-smokers. The current finding is congruent with previous studies by Kirby, et al. [41], who also proved that mosquito numbers increased per additional person in the house. Likewise, Animut, et al. [42] have also opined that the mean number of *Anopheles* mosquitoes resting in houses where greater than or equal to five occupants slept the previous night was significantly higher than in those with less than or equal to four occupants. This, again, emphasizes the strong positive association between the number of *Anopheles* mosquitoes and the number of indoor occupants in the room.

Conclusion

This study shows that the novel Prokopack Aspirator is very efficient and eco-friendly equipment that can complement or substitute already popular pyrethrum spray collection, which is laborious. The dominant malaria vector in the Nassarawa Eggon area is *An. gambiae sensu lato*. The number of malaria vectors correspondingly rises for every additional increase in the number of indoor occupants. The Prokopack Aspirator is hereby recommended for consideration for wide usage in entomological surveillance based on its efficiency and eco-friendliness with less workforce. Also, the number of indoor occupants in the study area should always be low (at most 4 persons per room) to reduce the concentration of volatiles that easily attract mosquitoes to human host.

Declarations

Conflict of Interest

All the authors declared no conflict for the publication of this article.

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