The Insemination Rates of some Anopheline and Culicine Populations in the Makurdi Area of Benue State, North Central Nigeria.

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Abstract- Studies on the insemination rates of mosquito populations in Makurdi, Benue State, Nigeria were carried out using four mosquito infested localities: High-level, Wurukum, North- bank and Wadata, over a 12 month period. A total of 4,320 adult female mosquitoes, comprising anopheline and culicine species were identified and dissected using standard procedures. Of these, 1,040 (24.1%) were *Anopheles gambiae* sensu lato (s.l.); 641 (14.8%) were *Aonopheles funestus* Giles and 2,418 (56.0%) of the mosquitoes were *Culex quinquefasciatus* Say while 221 (5.1%) were tagged 'unidentified' species of *Anopheles* mosquitoes. Chi-square statistic showed a significant difference between the mosquito species and their abundance ($P < 0.05$). The overall mosquito population was found to be highly inseminated (88.9%) which varied significantly ($\chi^2 test,\ P < 0.05$) across the localities with *Culex quinquefasciatus* having the highest insemination rate of 51.2%, followed by *Anopheles gambiae* s.l. which had insemination rate of 21.6%, while 11.6% of the *Anopheles funestus* were inseminated. Meanwhile, the 'unidentified' *Anopheles* species had the lowest insemination rate of 4.5%. There was a significant difference between the insemination rates and the mosquito species ($\chi^2 test,\ P < 0.05$). In terms of localities, the order of insemination was: Wadata (99.3%) > North-bank (97.7%) >High-level (91.1%) > Wurukum (70.5%) respectively. There was also a significant difference between the insemination rates of the mosquito species across the localities from where they were caught ($\chi^2 test,\ P < 0.05$).The findings indicate that the residents of Makurdi are potentially prone to mosquito bites and that *Anopheles gambiae*, *Anopheles funestus* and *Culex quinquefasciatus* were the major mosquito vectors in the study area. This work may provide an entomological baseline data that is required for evaluation and implementation of vector control interventions in Makurdi.

Index Terms- *Anopheles*, *Culex*, Mosquitoes, Insemination, Makurdi, Nigeria.

I. INTRODUCTION

There have been several reports on the effects of insemination on the circadian flight activity of mosquito species (Jones and Gubbins 1977&1978, Gomuiski, 1990). Bill (2003) defines insemination rate as 'the percentage of female mosquitoes that are carrying spermatozoa in their spermathecae at a given period'.

It has been reported that after insemination the first peak of activity of a mosquito population is greatly reduced and the secondary phase of activity is enhanced (Jones and Gubbins, 1978).

Jones and Gubbins (1977&1978) also reported that once inseminated, the flight and biting activity of female mosquitoes would change, shifting the peak of activity from dusk to a later time in the night. They reasoned that the behavioural changes observed in the inseminated females were a direct consequence of the transference of the male accessory gland substance called Matrone that is activated by insemination. Gomuiski (1990) opined that the changes in behavior due to insemination could be particularly important in a species such as *Anopheles gambiae* in which mating and feeding take place at entirely different sites.

Under normal conditions, female *Anopheles gambiae* are inseminated only once in their life time and usually 2-3 days after emergence (Goma, 1963) and that further insemination is suppressed by the action of the matrone, which quickly makes the female refractory to subsequent matings. However, it is believed that this mechanism may fail if the female mates with another male before the matrone takes effect in the female (Graig, 1967).

When female *Anopheles. gambiae* and other species are inseminated, they normally receive mating plugs which block the entrances of the spermathecal ducts, mechanically preventing insemination (Giglioli and Manson, 1966). Nevertheless, a number of mosquito species including *Aedes aegypti* and several *Culex* species, exhibit a degree of polyandry prior to the first gonotrophic cycle (Gomuiski, 1990). The present study therefore, aims at determining the insemination rates of some anopheline and culicine mosquitoes in Makurdi with a view to establishing their flight and biting potential which would further help in the designing of mosquito control programmes in the study area.

II. MATERIALS AND METHODS

Study Area

Makurdi is the capital of Benue State and is located in the middle belt, North of Central Nigeria. It is situated between longitude 8°35'E and 8°41'E and latitude 7°45'N and 9°52'N, characterized by undulating rolling plain with irregular river valleys and ridges with steep slopes. According to the federal republic of Nigeria official gazette of 2006 population census, published in 2010, Makurdi had the population of 297,398 people (comprising 157, 295 males and 140,103 females); and the town is placed 106.4m above sea level (National Meteorological Agency, 2011).

Makurdi is an urban setting which lies within the Benue trough, intersected by the river Benue which is a major source of water, with other net-works of streams, standing pools, over filled and blocked drainages. Over grown bushes and fields, even around residential homes and offices are easily noticeable in Makurdi which provide suitable breeding sites for mosquitoes throughout the wet season (April-October) and dry season (November-March).There is also characteristic high temperature in Makurdi, (30^{O}C-39^{O}C), which aids in the speedy development and hatching of mosquito eggs. It is suspected that temperature may have an impact on transmission of vector diseases in the selected localities (High-level, Wurukum, North-bank and Wadata) throughout the year.

The above localities were selected for mosquito sample collection because they are the most populated parts of Makurdi town and they have more breeding sites for mosquitoes in the area; they also have a closer proximity to river Benue in the study area (Fig. 1).

Other detailed geographical and regional indices of the study area have been provided by Udo (1981) and Nyagba (1995).

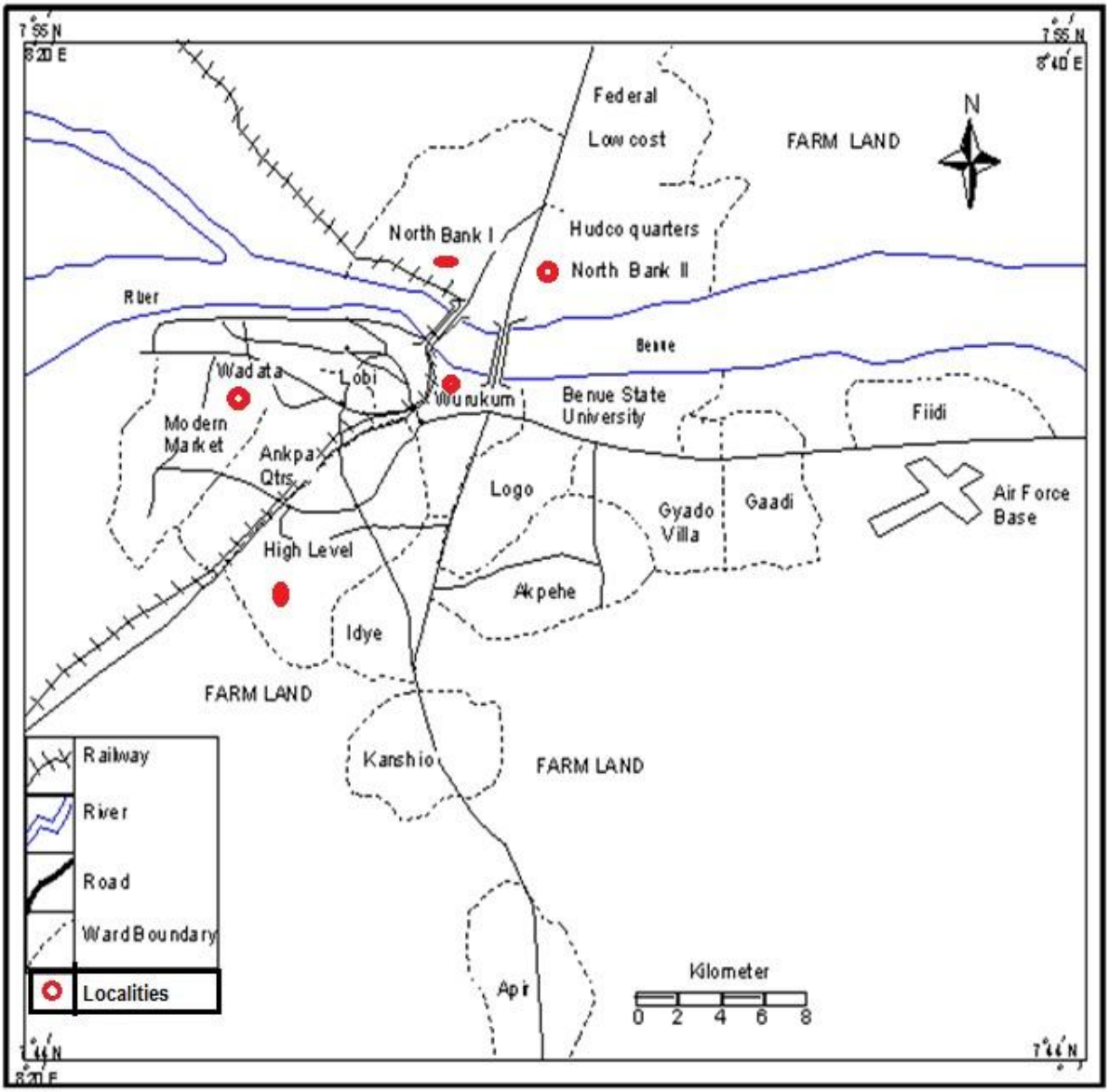

Fig.1. Map of Makurdi town Showing the Study Localities.
(Source: *Benue State Ministry of Lands and Survey, Makurdi, 2012)*

Ethical Consideration and Collection of Mosquito Samples

Mosquito samples were collected from a total of forty (40) households, ten (10) from each locality in the study area using a randomised design.

Verbal informed consent was obtained from the head of each of the randomly selected households before their houses were accessed for mosquito collection in all the study localities. All mosquito samples were collected using standard procedures as provided by WHO (1975). Sampling units were randomly selected from the four localities and due to the present security challenges in Nigeria, the mosquito samples were collected with the help of "fly boys" who were recruited from the respective study localities.

The mosquitoes were collected from 6 am to 9 am and 6 pm to 9 pm from living rooms in the study localities, either alive or dead. These periods for sample collection were chosen because previous studies have shown that most mosquitoes enter houses to feed at early hours of the night and struggle to go out in the early hours of the day to rest outdoors (Laumann, 2010; Service, 2012).

The mosquitoes were collected from dark corners, walls, ceilings, clothing and other objects inside living rooms using mouth-aspirators (sucking tubes) with the help of battery-operated torch-lights (Service, 1976; Dandalo, 2007); pyrethrum spray collection (PSC) was also used for mosquito collection, which involved the laying of white cloth on the floor and on surfaces of immovable furniture in the houses. The houses were then sprayed using BAYGON (1.0% propoxum, 0.1% imiprothrin and 98% propellant/solvent) as described by Dandalo (2007). After 10 minutes, the cloth was removed and inspected outdoors for knocked down mosquitoes. Window trap method was also used where applicable: The trap consisted of a cage made of 1 ft^3 framework of wire which was covered with mosquito netting. A narrow entrance funnel of ¼ in diameter was made at one end and a string was tied from its narrow end to the four corners of the trap to support the funnel (Service, 1976; Dandalo, 2007).The window traps were now installed in the houses and inspected on daily basis for mosquito collection. The suitability of the sampling methods was determined based on the nature of the houses to be sampled. The mosquito specimens collected from the different capture methods were sorted out separately using forceps and kept in holding tubes, inside cooling boxes, and carried to the laboratory on the same day or the following day for characterization, identification, dissection and examination using methods as in Ungureanu, (1972); WHO (1975); Goodman *et al.* (2003); Aigbodion and Nnoka, (2008) and Abeyasingha *et al.* (2009). Those mosquito samples that could not be processed on the same day were refrigerated and dissected on the following day according to the methods of Ungureanu (1972).

Even though, the mosquito population for this study was only drawn from indoor-resting mosquitoes, which were expected to be only females, some male mosquitoes were also caught along with the females. Male mosquitoes were therefore, distinguished from the females using key morphological features as described by Service (2012).

Identification of Mosquito Samples

Dissecting microscope was used for detailed observation and identification of the mosquitoes with particular reference to the head, thorax, wings and hind-legs according to Gilles and Coetzee, (1987) and Coetzee (1989). Morphological characteristics such as length of maxillary palps, wing spots, leg shape, mouthparts and abdominal end model as presented by Coetzee (2000), Oguoma *et al.* (2010) and Service (2012) were used to identify the *Anopheles* species that co-exist in Makurdi. Observations of the morphological features were made at $\times 40$ magnification of the microscope.

Preparation of Mosquitoes for Dissection

Live blood fed mosquitoes were killed with chloroform, ether or carbon (IV) oxide while unfed mosquitoes were collected in a test tube and while at the bottom, the end of the tube was rubbed sharply against the palm of the hand to stun the mosquitoes according to the WHO standard of 1975.

After immobilization, each mosquito was placed on a slide and held by one wing while the legs were being removed one at a time and after wards, the other wing was pulled off.

The mosquito was then placed on a fresh dry slide and arranged in a more suitable position for dissection of the stomach/abdominal region and salivary glands as described by WHO (1975) and as adopted by Abeyasingha *et al.* (2009). A mosquito dissection CD titled: 'Mosquitoes and Malaria' (1988): courtesy of the Nigerian Institute of Medical Research (NIMR), was also used as a guide during the dissection session.

Extraction of Spermathecae for Determination of Insemination Rate

The intention here was to identify the fertilized and unfertilized females and establish the insemination rate among the mosquito population sampled as described by WHO (1975). The seventh abdominal segment of the mosquitoes were teased with the dissecting needle under a dissecting microscope ($\times 6$) to isolate the tiny spermathecae. These were isolated and transferred to another slide with a drop of normal saline to avoid drying up of the specimen.

A cover slip was then placed on the slide and viewed with a $\times 40$ objective of a dissecting Olympus inverted microscope. A gentle pressure was applied to the cover slip with the dissecting needle and the spermathecae (depicted in Fig. 1) were crushed to view for spermatozoa. The thread-like spermatozoa were seen to exhibit a rotational movement in an inseminated female mosquito while no such movements were observed in those female mosquitoes that were not inseminated.

Statistical Analyses of Data

The Predictive Analytical Software (PASW) Version 18 was used in running Chi-square statistic on the data collected. Significant levels were measured at 95% confidence level with significant differences considered at $p < 0.05$.

The Chi-square statistic was used to test for homogeneity across sample localities so as to determine whether or not the nature of the sample localities affected the distribution of data across them.

III. RESULTS

A total of 3,841 (88.9%) out of the 4,320 mosquitoes dissected in this study were inseminated (carrying spermatozoa in their spermathecae) while 479 (11.1%) were not inseminated. Statistically, there was a significant difference $(\chi^2 test,\ p < 0.05)$ between the inseminated and non-inseminated mosquitoes dissected. The rate of insemination also varied significantly $(\chi^2 test,\ p < 0.05)$ across the four localities (Table 1).

It was found that mosquitoes from Wadata locality had the highest insemination rate of 99.3% while Wurukum area had the least insemination rate of 70.5%. Specifically, of the 1,128 mosquitoes dissected from High-level locality, 1,028 (91.1%) were inseminated, 401 (35.5%) of which were *Anopheles* species while 627 (55.6%) were *Culex quinquefasciatus*.

At Wurukum, 841 (70.5%) out of the 1,193 mosquitoes dissected were inseminated; 20 (1.7%) were 'unidentified' *Anopheles* species, 148(12.4%) were *Anopheles gambiae* s.l; 161(13.5%) were *Anopheles funestus* while 512 (42.9%) were *Culex quinquefasciatus* respectively.

The insemination rates were significantly different $(\chi^2 test,\ p < 0.05)$ between *Culex quinquefasciatus* and all the *Anopheles* species from the four localities. There was also a significant difference $(\chi^2 test,\ p < 0.05)$ between the percentage insemination within *Anopheles gambiae* complex and *Anopheles funestus* mosquitoes.

In the North-bank locality, 70 (8.4%) of the 'unidentified' *Anopheles* species were inseminated, 119 (14.3%) of the *Anopheles gambiae* were inseminated, 105 (12.6%) of the *Anopheles funestus* were inseminated while of the 526 *Culex quinquefasciatus* dissected from this locality, 521(62.5%) were inseminated.

Both nulliparous and parous mosquitoes showed insemination across the two genera of mosquitoes dissected.

In the Wadata locality, a total of 604 (51.8%) out of the 610 *Anopheles* species dissected were inseminated whereas 553 (47.5%) out of the 555 *Culex quinquefasciatus* dissected were inseminated.

Table 1. Parity Rate of the Mosquitoes Dissected from different Localities in Makurdi

Study Locality	Number Dissected	Number Parous (%)	Number Nulliparous (%)
High-Level	1,128	1,121(99.4)	7(0.6)
Wurukum	1,193	1,128(94.5)	65(5.4)
North- Bank	834	626(75.1)	208(24.9)
Wadata	1,165	1,114(95.6)	51(4.4)
Total	**4,320**	**3,989 (92.3)**	**331 (7.7)**

Parous vs nulliparous: $\chi^2 = 3097.445,\ d.f = 1,\ P = 3.841$

Table 2: Insemination Rates of *Anopheles* and *Culex* Mosquitoes from four Study Localities in Makurdi.

	Mosquito species/Insemination Rates (%)					
Study Locality	Number Dissected	Unidentified *Anopheles spp.*	*Anopheles gambiae*	*Anopheles funestus*	*Culex quinquefasciatus*	Total Number Inseminated (%)
High-Level	1,128	52(4.6)	239(21.2)	110(9.7)	627(55.6)	1,028(91.1)
Wurukum	1,193	20(1.7)	148(12.4)	161(13.5)	512(42.9)	841(70.5)
North-Bank	834	70(8.4)	119(14.3)	105(12.6)	521(62.5)	815(97.7)
Wadata	1,165	51(4.4)	426(36.6)	127(10.9)	553(47.5)	1,157(99.3)
Total	**4,320**	**193(4.5)**	**932(21.6)**	**503(11.6)**	**2,213(51.2)**	**3,841(88.9)**

(a) Species: $\chi^2 = 2465.952,\ d.f = 3,\ P = 7.815$ (b) Locality: $\chi^2 = 81.873,\ d.f = 3,\ P = 7.815$

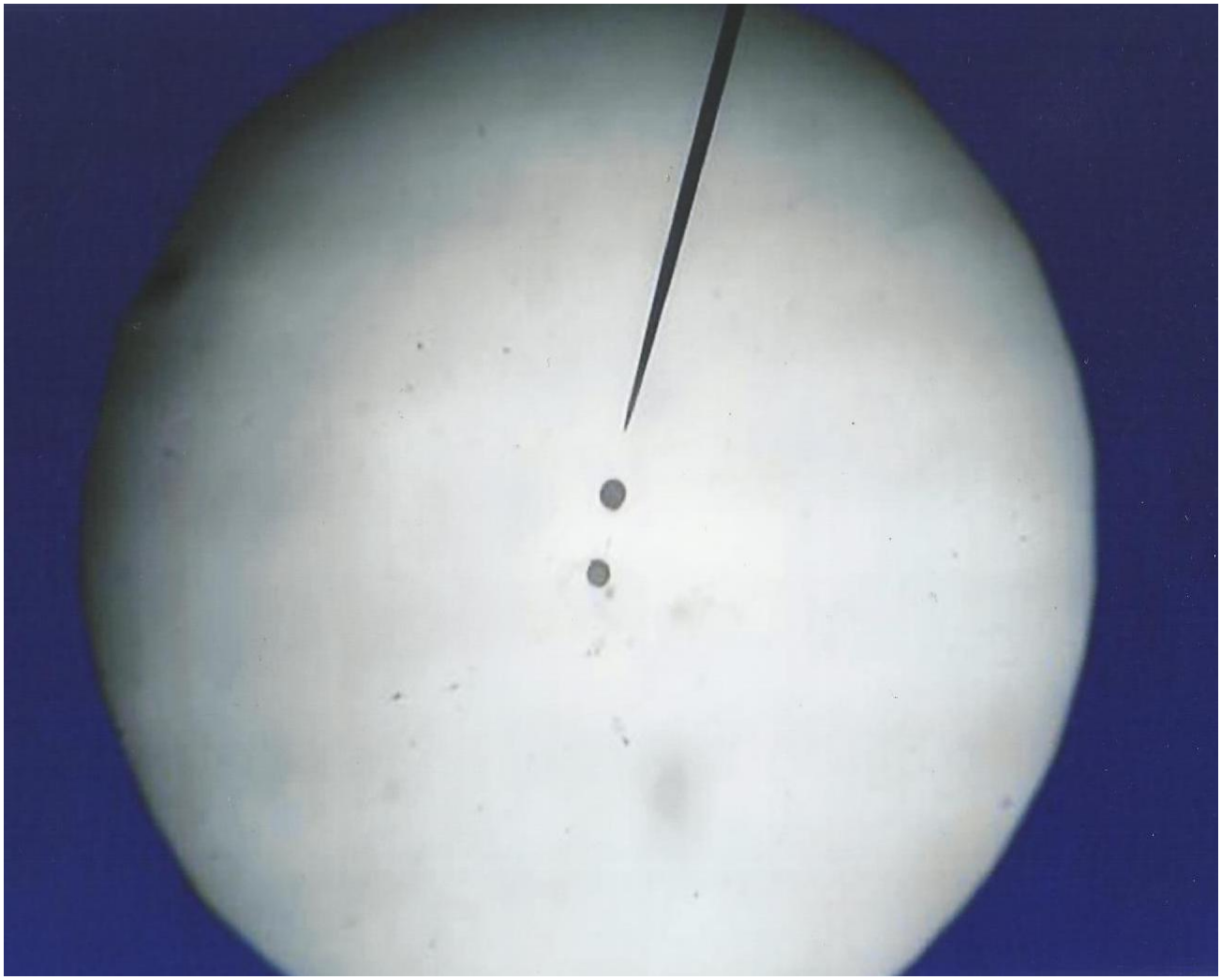

Fig. 1. A Photomicrograph showing two spermathecae from a dissected *Culex quinquefasciatus.*

IV. DISCUSSION

The overall insemination rate in this study was found to be 88.9%, with the highest insemination rate of 99.3% occurring at Wadata locality. Wurukum locality had the least insemination rate of 70.5% while North-bank and High-level localities had 97.7% and 91.1% insemination rates respectively. The proportion of female mosquitoes inseminated in this study were 21.6% in *Anopheles gambiae*, 11.6% in *Anopheles funestus*, 4.5% in 'unidentified *Anopheles* species and 51.2% in *Culex quinquefasciatus* respectively.

Similar insemination rates have been reported elsewhere by various authors; Goma (1963) observed insemination rates of 44.7%-66.1% in *Anopheles gambiae* complex. Ibrahim (1994) also found high insemination rates of 73% in *Anopheles gambiae* and 90% in *Anopheles funestus* in the Jos area. Similarly, Inyama *et al*. (2003) had the proportion of female mosquitoes inseminated in parts of Plateau State as: 73.8% in *Anopheles gambiae*, 73.9% in *Anopheles funestus*, 83.2% in 'unidentified' *Anopheles* species and 97.9% in *Culex quinquefasciatus* respectively.

The high insemination rates of the mosquitoes reported in the present study, irrespective of the species, imply that their activity in terms of biting and flight would be greatly enhanced at night than during the dusk. This is in accordance with the findings of Jones and Gubbins, (1977 and 1978) who reported that once inseminated, the flight and biting activity of female mosquitoes would change, shifting the peak of activity from dusk to a later time in the night. They reasoned that the behavioural changes observed in the inseminated females was a direct consequence of the transference of the accessory gland substance called Matrone that is activated by insemination.

This may explain the writer's observation that mosquito populations in this study area were higher during the night catches than the morning catches. This also translates to increased biting potential at night by these species in the study area.

It has already been confirmed that insemination in female mosquitoes stimulates oviposition, modifies biting activity, increases the rate of blood meal digestion and possibly enhances longevity (Jones and Gubbins, 1977, 1978). Therefore, if the findings of Jones and Gubbins are accepted then, the high

insemination rates across the four localities surveyed in the present study show that the mosquitoes would have higher oviposition potentials, implying increase in vector populations in the study area. Similarly, their biting behavior would increase, indicating more chances of parasite acquisition and subsequent transmission. The inseminated female mosquitoes in the study area would also live longer than the non-inseminated females (Jones and Gubbins, 1977, 1978) thus, having more gonotrophic cycles to enhance transmission. Gomuiski (1990) opined that the changes in behavior due to insemination could be particularly important in a species such as *Anopheles gambiae* in which mating and feeding take place at entirely different sites. This is indicated in the present study because the population of *Anopheles gambiae* that was inseminated (982/4320, 21.6% was higher than that of *Anopheles funestus* (503/4320, 11.6%). Although *Culex quinquefasciatus* had an overall higher insemination rate of 51.2% (2213/4320), this could be attributed to the numerous breeding sites that were abundant in the study area, in favour of this species hence their increased population and insemination.

Both nulliparous and parous females showed insemination across the two genera of mosquitoes dissected in this study from all the four localities. *Culex quinquefasciatus* females were seen with 2-3 spermathecae as against the single spermatheca that was observed in *Anopheles* females. This agrees with the findings of Service (2012) who stated that *Anopheles* mosquitoes have only 1 spermatheca while *Culex* mosquitoes have a minimum of 2 and a maximum of 3 spermathacae. Inyama *et al.* (2003) stated that high insemination rate in a mosquito population would mean high parity rate. Therefore, the high parity observed in this study and the subsequent high infection may be due to the age of the mosquitoes and changing biting habits of the different mosquito species.

V. CONCLUSION

The overall insemination rate in the mosquito population dissected was found to be 88.9%. This is the percentage of females that had undergone mating with the male mosquitoes before they were caught and dissected. If the findings of Jones and Gubbins (1977 & 1978) that 'inseminated female mosquitoes have increased flight and biting activities at night' are acceptable, then the high insemination rate of 88.9% obtained in the present study would translate to increased biting potential at night by the mosquito species in the Makurdi area. It is therefore, recommended that the breeding sites of the mosquito species in this area should be cleared or eliminated to prevent them from building up their populations in the various localities. Living rooms should be well secured with door and window nettings to prevent mosquitoes from entering houses to bite, especially at night. Hence the use of Insecticide Treated bed Nets (ITBNs), insecticidal sprays, effective mosquito repellent creams, screening of windows and doors, wearing of long sleeves and other personal protection practices against mosquito bites should be employed by the inhabitants of Makurdi.

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