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SIGNIFICANT DIFFERENCE (HSD) TEST OF MEAN SCORES FOR MOSQUITOES' GENERA

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Additive Factor Effects, Homogeneity, Mosquitoes, Interaction, Two-factor analysis

Abstract

This study investigates the relative abundance of adult mosquitoes in four selected sites in Nigeria Police Academy, Wudil, Kano, Nigeria in June, 2019 via two-factor analysis model. A total of eight hundred and thirty-nine (839) adult mosquitoes involving of 3 genera namely Anopheles, Aedes, and Culex were identified in the four selected sites, and 125 of the mosquitoes were males (14.90%) and 714 were females (85.1%). More so, Anopheles mosquitoes were higher in terms of abundance with a total number of 476samples (56.7%) followed by Culex, 203 (24.20%), and then Aedes, 160 (19.07%). Furthermore, the population of mosquitoes in the girls' hostel is 250 (29.80%), the girls' hostel staircase, 241 (28.72%), the quarter guard, 330 (39.33%), and the clinic, 18 (2.15%). Residual of the ANOVA for the transformed data are normally distributed and homogeneity of variances exist across samples; hence, insect

group means are all equal while, the surveyed site group means are different from the others. More so, no significant differences between groups sample means of girls' hostel and clinic, as well as the girls' hostel staircase and quarter guard; thus, the factor effects are additive and no interaction between factors. Furthermore, significant differences were noticed between groups sample mean of quarter guard and clinic, girls' hostel staircase and clinic, quarter guard and girls' hostel, girls' hostel staircase and girls' hostel; thus, the factor effects are no means additive and interaction between factors exist. Succinctly, no significant differences between group sample means of the insects considered; thus, the factor effects are additive and no interaction between factors

INTRODUCTION

Mosquitoes are flying blood sucking insect vectors that are found in human habitation and in the forest. They possess only a single pair of wings and are cosmopolitan in distribution, though found mostly in the warm humid tropical countries of West and East Africa, South-East Asia, the Caribbean, North and South America, and Europe. Mosquitoes belong to the phylum arthropoda, class insect, order diptera, family culicidae, and the sub-families of *Anopheline* and *Culiine* (Smyth, 1996). There are two main types of mosquitoes, the *Anopheles* and the *Culex*, though other species exists such as *Aedes, Psorphora, mansonia, Haemagogus* and *Toxorhynchus*. Mankind has been plagued by mosquitoes as nuisances and as vectors of mosquito borne diseases for centuries, resulting in inestimable economic losses and indeterminable human suffering. Mosquitoes are readily distinguished from other insects by their conspicuous projecting proboscis and scales on their wing vein (Service, 1993 and Robert, 2004). The female *Anopheles* mosquito is

the known carrier of malaria and they also transmit filariasis. They are easily recognized by the position held when at rest in which the proboscis, head and body are held on a straight line to each other with an angle to the surface it is resting (WHO ,1984). The *Culex* is a carrier of viral encephalitis and of filarial worm. It holds its body parallel to the surface it is resting and its proboscis bent down towards the surface. The *Aedes* are carriers of yellow fever virus, dengue fever and encephalitis. Like the *Culex*, *Aedes* holds its body parallel to the surface with the proboscis bent downwards (WHO, 1984). Mosquitoes are extremely successful organisms due to their ability to adapt to a wide range of habitats. Some studies have shown that mosquitoes may associate with particular vegetation types (Colins and Resh,1989). Certain kinds of emergent vegetation are well known to favour mosquito production (Collins and Resh,1989; deSzalay and Resh,2000; Jiannino and Walton, 2004) whereas expanses of open water are thought to discourage mosquito production (Collins and Resh, 1989). Mosquito larvae colonize a wide range of water bodies, temporary and permanent, highly polluted as well as clean, large or small, stagnant or flowing, and even the smallest accumulations such as water-filled buckets, flower vases, old tyres, bromeliads, hoof prints or leaf axils. Adult mosquitoes vary greatly in their bionomics, *e.g.*in terms of the host-seeking, biting and dispersal behaviour, and strategy for reproduction. (Nobert *et al.*, 2010). Mosquitoes have their mouth-parts specialized for both sucking nectar and blood of man and other animals. They are the most important vectors of public health due to their roles in the transmission of diseases such as Malaria, Lymphatic filariasis and others. Different species of this insect have been identified as the vector transmitting these diseases. *Anopheles (Cellia) gambiae s.s.* Giles (1902) is regarded to be currently in the state of diverging into two different species, the Mopti (M) and Savannah (S) strains, although the two strains are still considered as a single species

(Gentile *et al*. 2001;della Torre *et al*. 2005). *An. gambiae s.s.* is capable of growing very rapidly; under optimal conditions, the development from the egg to the adult may be completed within 6–8 days (De Meillon 1947). This species is one of the most important vectors of malaria in Africa; wherever it occurs it is responsible for intense disease transmission. Apart from its association with high endemic malaria it is also responsible for epidemics and is a vector of periodic bancroftian filariasis as well (Gillies and De Meillon, 1968). Malaria is a life-threatening disease caused and spread by *Plasmodium* parasite and female anopheles mosquitoes, respectively with 92% of malaria cases and 93% of malaria deaths occurring in the African region (WHO, 2018). According to WHO, 2015 the transmission of diseases by mosquitoes is more intense in places where the mosquito life span is longer (so that the parasite has time to complete its development inside the mosquito) and where it prefers to bite humans rather than animals. According to the 2019 World Malaria Report, Nigeria had the highest number of global malaria cases (25 % of global malaria cases) in 2018 and accounted for the highest number of deaths (24 % of global malaria deaths). The mature female anopheles mosquito needs a blood meal for its reproduction. The study was aimed at investigating the abundance of different mosquito species in the selected areas of the academy.

The individual and joint effects of two independent variables on one dependent variable can be verified via the two-way analysis of variance and this tells us about the main effect and the interaction effect. Repeatedly, ANOVA has been used to test equality among several means by comparing variance among groups relative to variance within groups (random error). Additionally, the design gives natural replications that result from overlapped factors. Tests of main effects are tests of one factor averaged over levels of the other factors. Absence of interaction between two factors implies that the additive

effect of one factor is identical across all levels of the other factor. In that situation, tests and interpretation of main factors are straightforward. If interactions exist, one must interpret main effects carefully, because relations among mean levels of one factor differ according to levels of the second factor (Fisher, 1925; Kleinbaum, *et al*., 1988; Fujikoshi, 1993; Zar, 1999; Gelman, 2005; Sit, 2007).

Materials and Methods

Description of Study Area

The study was carried out in four selected sites in some parts of the Nigeria Police Academy Wudil Kano, Nigeria. These sites are Girls' hostel, staircase, Quarter guard (a building used mainly for the detention of offenders in the Academy premises), and the clinic. This study area has a coordinate (latitude 11°49`N and longitude 8°51` E), falls in within the semi-arid Sudan savannah zone of West Africa about 840 kilometers from the edge of the Sahara Desert as shown in Figure 1. Kano has a mean height of about 472.45m above sea level. The temperature of Kano usually ranges between a maximum of 33°C and a minimum of 15.8°C although sometimes during the harmattan it falls down to as low as 10°C and can be as high as 40°c. Kano has two seasonal periods, which consist of four to five months of wet season and a long dry season lasting from October to May. The rainfall in this area ranges between 1016mm and 1524mm with a relative humidity between 60% and 80%. The guinea savannah is divided into two vegetation zone; the northern and southern guinea savannah. The temperature of this area is highly influenced by the Niger Benue trough where heat is trapped.

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Figure 1: Map of Nigeria Police Academy Wudil Kano, Nigeria

Sample collection

In the month of June, mosquitoes were collected indoors between 05:30pm and 06:00am using photo-catalysis inhaled mosquito killer was used accurate results. When using the Raid method mosquitoes were collected on white clothes spread on the floors of the four (4) selected sites: Girls hostel, staircase, quarter guard, and clinic, but when using the Photocatalytic inhaled mosquito killer the machine was connected to a light source either directly to the socket or to a power bank. All knocked down mosquitoes were collected into sample bottles and taken to the laboratory for morphological identification as described by (Amusa, et al., 2013).

Sample identification

Mosquitoes were picked with forceps, dropped on a clean slide and then observed under a light microscope with × 40 objectives. Features such as size of antennae, color and size of the maxillary palps, length of proboscis, colour and shape of abdomen, colour and length of legs, colour of wings etc., were observed and compared. Individual mosquitoes were identified into species level, counted and recorded.

Statistical Analysis Technique

The dataset comprises of three insects (mosquito); Anopheles, Culex, and Aedes being surveyed at different sites (Girls Hostel, Girl's hostel staircase, Quarter Guard, and Clinic) of Nigeria Police Academy, Wudil, Kano State. The insects have male and female as interaction.

A two-way ANOVA test analyzes the effect of the independent variables on the expected outcome along with their relationship to the outcome itself (Yates,1934; Kass, 2011).All these variables are varying independently and normally around a mean. Let Y_{ijk} denote the response random variable in k-th measure for treatment (i,j); $k = 1,\ldots,\mathrm{n}_{\mathrm{ij}}$, $i = 1,\ldots,\mathrm{I}$ indexes the levels of the first factor, and $j = 1,\ldots,\mathrm{J}$ indexes the levels of the second factor, then:

$$Y_{ijk} = \mu_{ij} + \varepsilon_{ij} \qquad (1)$$

Besides the variation explained by the factors, there remains some amount of unexplained variation ε_{ijk} , random error. The mean of the response variable is modeled as a linear combination of the explanatory variables (Gelman, 2005; Gelman and Hill, 2006):

$$\mu_{ij} = \mu + \alpha_i + \beta_j + \nu_{ij} \qquad (2)$$

where μ is the population group means, α_i is the additive main effect of level i from the first factor (i-th row in the contigency table), β_j is the additive main effect of level j from the second factor (j-th column in the contingency table) and ν_{ij} is the non-additive interaction effect of treatment (i,j) from both factors (cell at row i and column j in the contingency table) (Fujikoshi, 1993).

A two-way ANOVA partitions the total sum of squares into within sum of squares and between sum of squares. Carrying out hypothesis test on an appropriate test statistic requires the assumption of normality for the ANOVA model that is; $Y_{ijk} \sim N\left(\mu_{ij}, \sigma^2\right)$ (homoscedasticity) or

equivalently $\varepsilon_{ijk} \sim N\left(0,\sigma^2\right)$ and the homogeneity of variance among groups being compared is expected to be similar across all groups. If one of these assumptions is not met, we might need to transform the data using a logarithmic or exponential transformation to reduce the variability in the data, or you may be able to use a non-parametric alternative.

The mean corresponds to mosquitoes (anopheles, culex, aedes) by gender groups (female, male) that are defined by the combination of the two independent variables surveyed at girls hostel, girls' hostel staircase, quarter guard, and clinics has the following hypotheses adopted at 0.05 level of significance. The dataset was analyzed using R programming language.

Hypothesis (I): Main effect insects

H_o: insect group means are all equal versus

H_A: at least one insect group mean is different from the others

Hypothesis (II): Main effect of site surveyed

H_o: surveyed site group means are all equal versus

H_A: at least one surveyed site group mean is different from the others

Results

A total of eight hundred and thirty nine (839) mosquitoes comprising of 3 genera, Anopheles, Aedes and Culex were identified during the month of June, 2019 in the four selected sites. The population of mosquitoes in girls' hostel is 250 (29.80%); the girls' hostel staircase is 241 (28.72%); the quarter guard 330 (39.33%); and the clinic is 18(2.15%) according to this descriptive statistics mosquitoes are more abundance in the quarter guard followed by the girls' hostel, girls' hostel staircase, and the clinic. Also, Anopheles mosquitoes was higher in terms of abundance with a total number of 476 samples (56.7%)

followed by Culex mosquitoes with a total number of 203 samples (24.20%) and Aedes mosquitoes with a total number of 160 samples, (19.07%). More so, 125 of the mosquitoes were males, (14.90%) and 714 of the mosquitoes were females, (85.10%).

Table 1: Descriptive Statistics

Sites surveyed	Anopheles		Culex		Aedes		Total (mean)
	Male	Female	Male	Female	Male	Female	
Girls hostel	18	170	5	54	0	3	250
Staircase	17	51	8	36	33	96	241
Quarter guard	23	185	14	80	5	23	330
Clinic	1	11	1	5	0	0	18
Total (mean)	59	417	28	175	38	122	839

The F test in table 2 for the main effect of insect with p-value=0.243 indicates that the insects group means are all equal. Also, the F test for the main effect of the sites surveyed with p-value=0.322 indicates that surveyed site group means are all equal.

Table 2: Analysis of Variance (Original data)

Response data	df	SS	MS	F-value	Pr(>F)
Insect	2	7343	3672	1.532	0.243
Sites_surveyed	3	8971	2990	1.248	0.322
Residuals	18	43137	2397		

To validate the inferences made in table 2, the normality and homogeneity of variances assumptions of the model's residual need to be checked.

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Testing Normality Assumption of the Dataset

The visual representation of the residuals is experiential by plotting the histogram of the residuals or standardized residuals; also, the quantile-quantile plot was observed. Shapiro Wilk and JarqueBera tests for normality of the residuals were also verified.

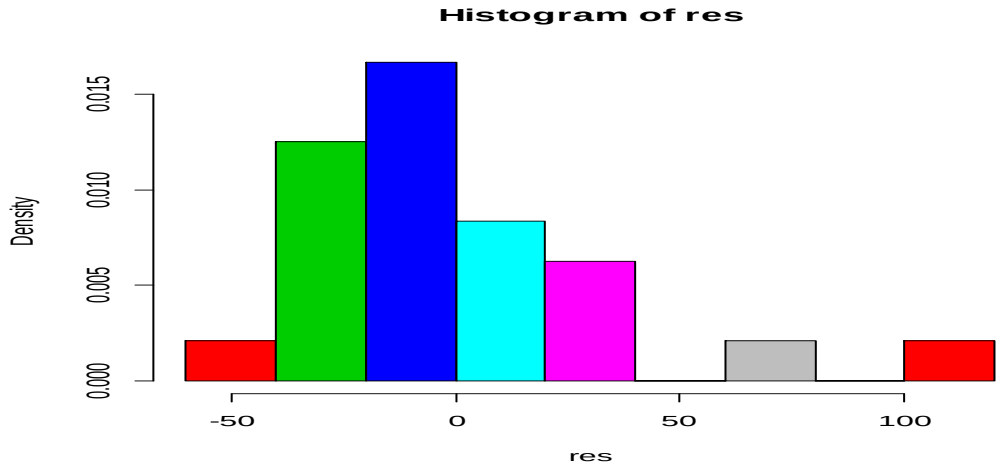

Figure 1: Histogram of the original data

It can be seen in figures 1 above that the residuals of the original data are not distributed symmetrically around the centre of all scores.Figure 2 is the plot of sample quantiles against theoretical quantiles to check whether both sets of quantiles truly come from normal distributions.

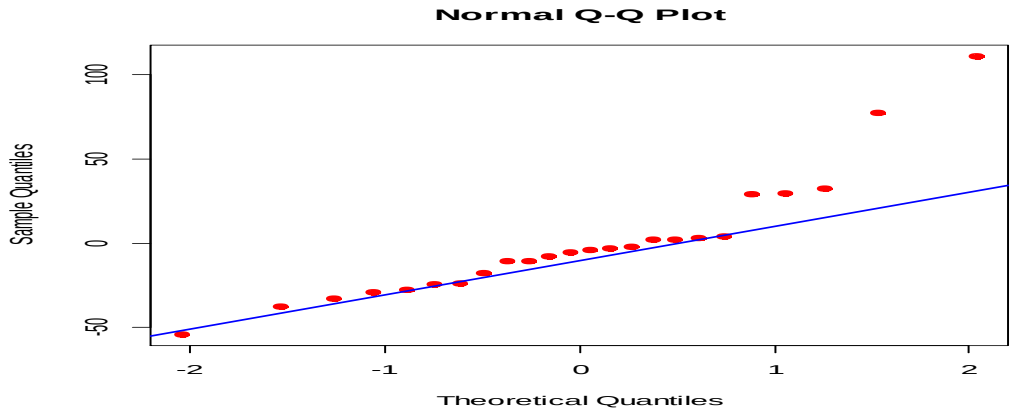

Figure 2: Quantile to quantile plot

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It can be seen that the datasets are not normally distributed since the points seem to deviate markedly away from the straight line with outliers points at the ends of the line, distanced from the bulk of the observations.

Table 2: Normality test (Original data)

Tests	Statistics	P-value
Shapiro-Wilk	0.8738	0.006257
Jarque-Bera	23.7693	6.895e-06

Shapiro-Wilk and Jarque-Bera tests revealed that the residuals are not normally distributed with p-values of 0.006257 and 6.895e-06 which areless than significance level, 0.05.

Testing Homogeneity of Variances Assumption

Bartlett's test is used to test if k samples are from populations with equal variances. The results of table 3 revealed that variances are not equal across groups or samples, since the p-value of 8.009e-05 is less than 0.05 significance level.

Table 3: Homogeneity of Variances test (Original data)

K-squared	Df	p-value
21.5715	3	8.009e-05

The residual of the original data did not satisfy the assumptions of normality and homogeneity of variance; so, the inferences made from table 1 are not valid; as such, logarithmic transformation of the data is required.

The F test on Table 4 for the main effect of insect with p-value=0.0888indicates that the insects group means are all equal. Also, the F test for the main effect of the sites surveyed with p-

value=0.0037indicates that surveyed site group means are all not equal, that is, they are different from one another.

Table 4: Analysis of Variance (Transformed data)

Response data	Df	SS	MS	F-value	Pr(>F)
Insect	2	8.471	4.236	2.779	0.0888
Sites_surveyed	3	29.528	9.843	6.457	0.0037
Residuals	18	27.439	1.524		

To validate the inferences made for the ANOVA in Table 4, we check for normality and homogeneity of variances assumptions of its residuals as done previously.

Table 5: Normality test (Transformed data)

Tests	Statistics	P-value
Shapiro-Wilk	0.9524	0.3054
Jarque-Bera	1.2137	0.5451

Shapiro-Wilk and Jarque-Bera tests revealed that the residuals are normally distributed with p-values of 0.3054 and 0.5451 greater than significance level, 0.05.

Table 6: Homogeneity of Variances test (Transformed data)

K-squared	Df	p-value
1.6624	3	0.3484

The results of table 6 revealed that variances are equal across groups or samples, since the p-value of 0.3848 is greater than significance level, 0.05.

Thus, after the transformation of the data the basic assumptions of ANOVA were satisfied. Hence, the inferences made about Table 4 are

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valid and we conclude that insect group means are all equal; while, the surveyed site group means are different from the others.

The design plot of figure 3 showed that Aedes mosquitoes has low mean of yields and Culex mosquito is just on the overall mean; while Anopheles mosquitos' mean of yields is above the overall mean of the samples. Thus, Anopheles mosquito is more in Polac during the month of June. Furthermore, the quarter guard and girls' hostel staircase have the high means of yields for mosquitoes, while the girls hostel's mean yield is just on the overall mean, the clinics mean yield fall below overall mean of the sample. Thus, the quarter guard and girls' hostel staircase are breeding places for mosquitoes in Polac.

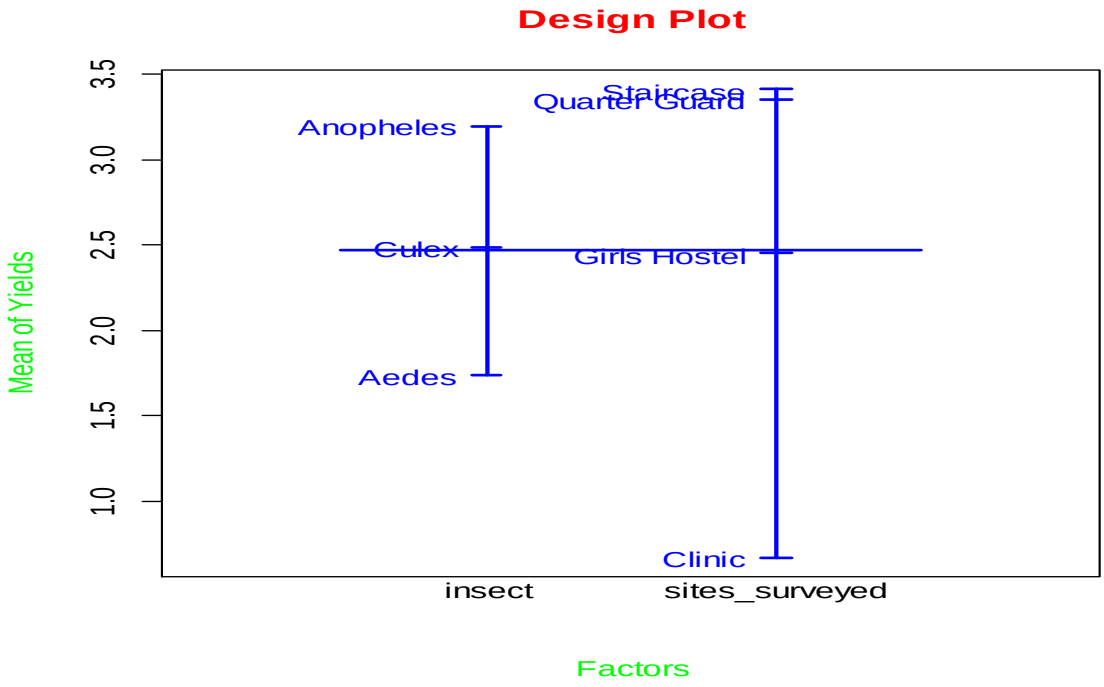

Figure 3: Design plot for mean of yields against the factors

The line that connects the means of Culex and Anopheles are quite parallel; thus, the interaction effects are very small compared to main effects or they are only apparent in a small number of treatments; so, they are probably unimportant and there is no interaction between them. Also, the mean of data for Aedes crisscross the mean of data for Anopheles and Culex; thus, the differences between levels for one

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factor for Aedes will depend on the level of the other factors of Anopheles and Culex mosquitoes and thus has a significant interaction effect.

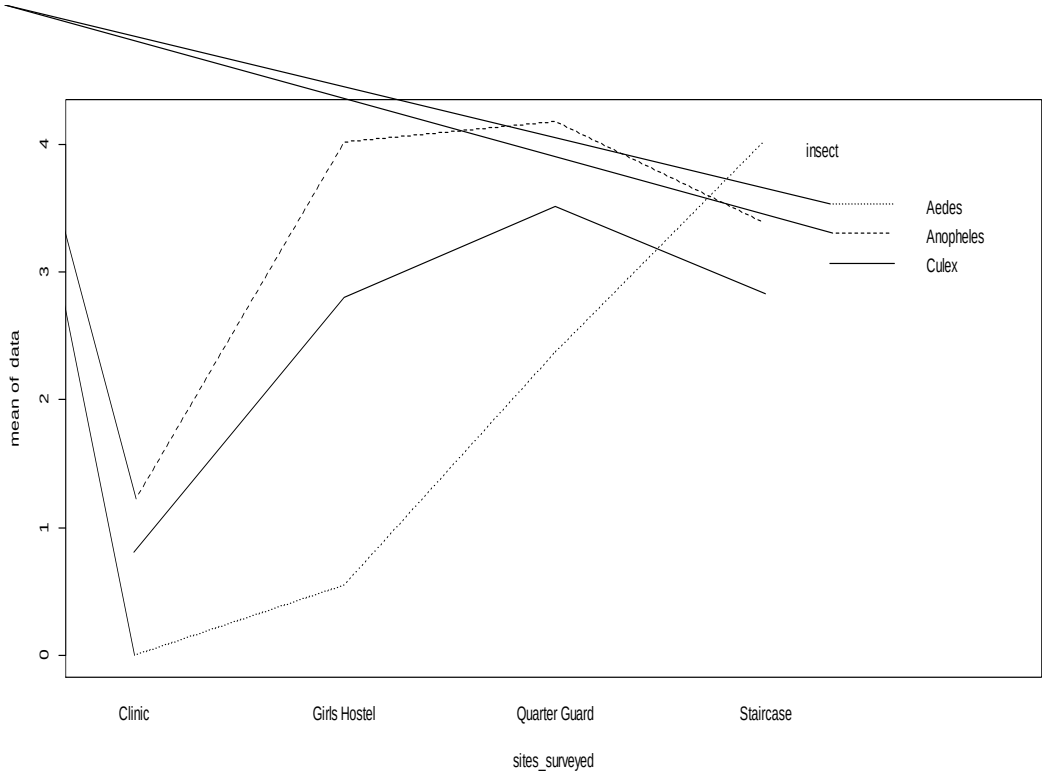

Figure 4: Interaction plot for mean of insects against site surveyed

In figure 5 below, the interaction plotted lines for quarter guard, girls hostel and clinic are close to parallel; thus, the interaction effects will be very small compared to main effects or only apparent in a small number of treatments; so, they are probably unimportant and there is no interaction between them. The girls' hostel staircase interaction plot line crisscross that of girls hostel and quarter guard, and a decrease in the mean of the data was observed; thus, the differences between levels for one factor of the girls' hostel staircase will depend on the level of the other factors of girls hostel and quarter guard. Hence, the interaction effects for girls' hostel staircase are so large and/or pervasive that the main effects cannot be interpreted on their own, so the means of insects do differ at the girls' hostel staircase.

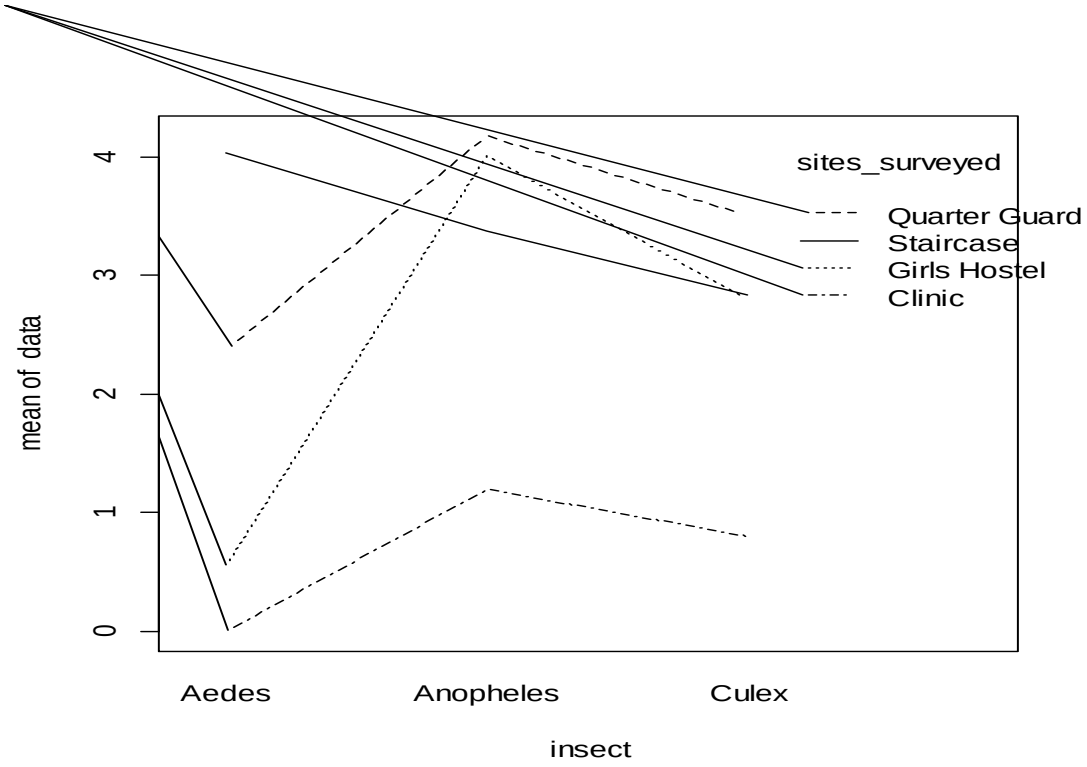

Figure 5: Interaction plot for mean of site surveyed against the insects

Table 7 shows the population means compared by a two sample t-test with pooled standard deviation to know which mean(s) is(are) significantly different from which. It can be seen that, the mean of observation of the girls' hostel is significantly different from the mean of observation of the clinics. Also, the mean of observation of quarter guard is significantly different from the mean of observation of the clinic; but, not significantly different from the mean of the observation of the girls' hostel. More so, the mean of observation of the girl's hostel staircase is significantly different from the mean of observation of the clinics; but, not significantly different from the means of the observation of the girls' hostel and the quarter guard.

Table 7: Fisher's Least Significant Different (LSD) Test

	Clinic	Girls Hostel	Quarter Guard
Girls Hostel	0.0318	-----	-----
Quarter Guard	0.0024	0.2585	-----
Staircase	0.0020	0.2286	0.9378

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The Tukey's honestly significant difference (HSD) test is used to test differences among sample means for significance. It tests all pairwise differences while controlling the probability of making one or more Type I errors; i.e., the probability of rejecting the null hypothesis (no significance difference) given that the null hypothesis is true. This post-hoc analysis will provide greater insight into the differences or similarities between specific groups.

In table 8, it was observed that no significant differences between groups of sample means of the insects considered. This inference was confirmed by figure 6 were the between groups of all insects sample means' plots were seen to have crossed the boundary line to the left.

Table 8: Tukey Honestly Significant Difference (HSD) Test for Insects

Insects	Differences	Lower	upper	p-value adj
Anopheles-Aedes	0.9908037	-0.4801978	2.4618053	0.2254570
Culex-Aedes	0.3426259	-1.1283756	1.8136275	0.8248193
Culex-Anopheles	-0.6481778	-2.1191794	0.8228238	0.5118061

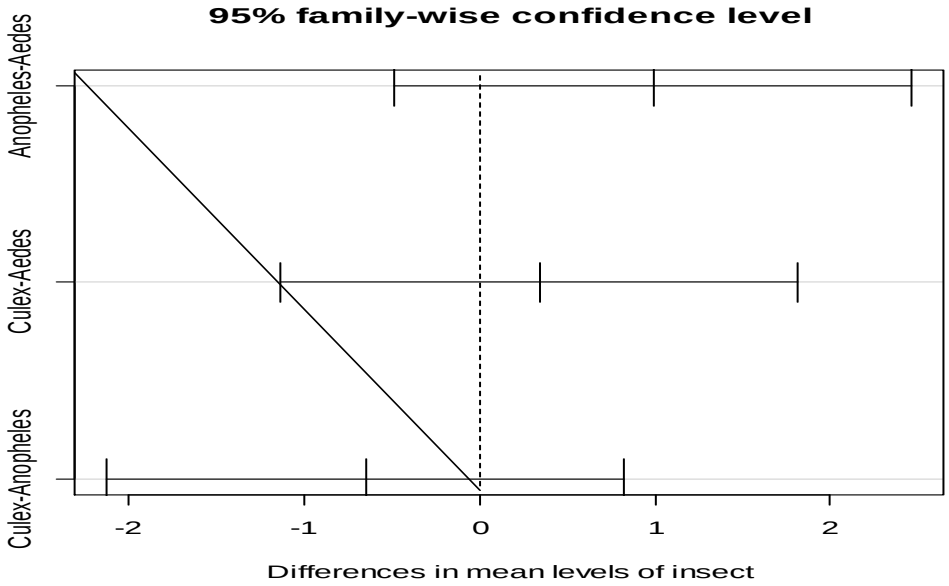

Figure 6: Tukey HSD confidence interval plot of insects

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In table 9, no significant differences between groups sample means of girls' hostel and clinic, as well as the girls' hostel staircase and quarter guard were observed. Succinctly, significant differences between groups sample mean of quarter guard and clinic, girls' hostel staircase and clinic, quarter guard and girls' hostel, girls' hostel staircase and girls' hostel were detected. Moreover, figure 7 below confirmed the inferences made here; and the plots of the 95% confidence interval for the differences in site surveyed sample means for girls' hostel and clinic, as well as the girls' hostel staircase and quarter guard were seen to have crossed the boundary line to the left, while the remaining site surveyed plots did not cross the boundary line and remained at the right side

Table 9: Tukey Honestly Significant Difference (HSD) Test for sites surveyed

Sites surveyed	**differences**	**lower**	**upper**	**p-value adj**
Girls Hostel-Clinic	0.8830529	-0.99795578	2.764062	0.5585873
Quarter Guard-Clinic	2.8230200	0.94201135	4.704029	0.0025197
Staircase-Clinic	3.3460734	1.46506470	5.227082	0.0004649
Quarter Guard-Girls Hostel	1.9399671	0.05895846	3.820976	0.0418850
Staircase-Girls Hostel	2.4630205	0.58201180	4.344029	0.0080968
Staircase-Quarter Guard	0.5230533	-1.35795533	2.404062	0.8598632

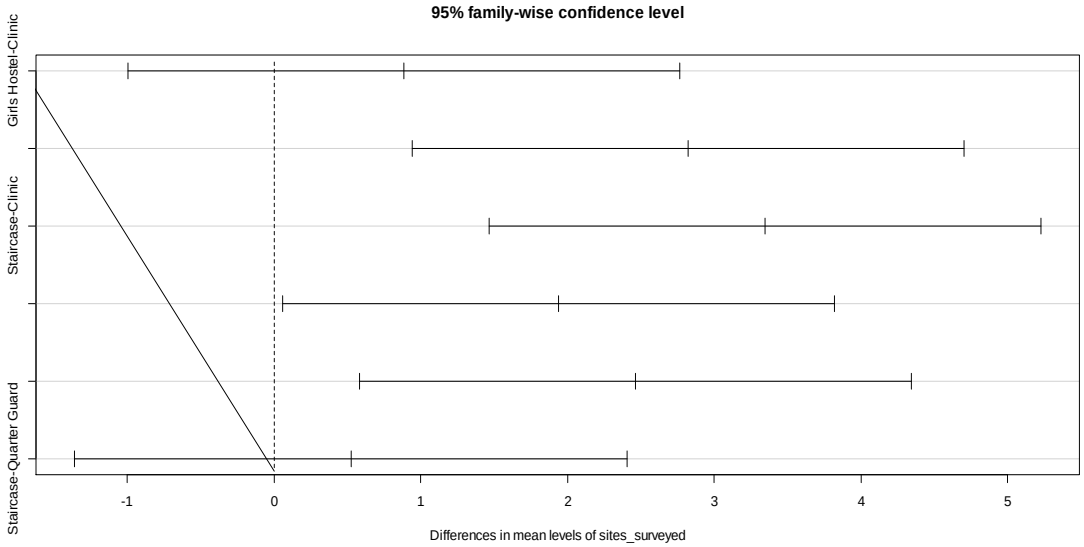

Figure 7: Tukey HSD confidence interval plot of sites surveyed

Discussion

The important malaria vector *Anopheles gambiae*, need temporary sunlit pools, and farming (e.g. rice fields) and forestry activities in Africa have provided extensive additional breeding sites for these mosquitoes (Lehane, M. J. 2005) this confirms why the Anopheles mosquitoes was higher in terms of abundance with a total number of 476 (about 56.7%) samples especially in the Quarter guard where there are more grasses followed by Culex mosquitoes with a total number of 203 samples, about 24.20% and Aedes mosquitoes with a total number of 160 samples, about 19.07%. More so, 125 of the mosquitoes were males, about 14.90% and 714 of the mosquitoes were females, about 85.10%. It was apparent that, the mean of observation of the girls' hostel is significantly different from the mean of observation of the clinics. Also, the mean of observation of quarter guard is significantly different from the mean of observation of the clinic; but, not significantly different from the mean of the observation of the girls' hostel. More so, the mean of observation of the girl's hostel staircase is significantly different from the mean of observation of the clinics; but, not significantly different from the means of the observation of the girls' hostel and the quarter guard. No significant differences between groups sample means of girls' hostel and clinic,

as well asK the girls' hostel staircase and quarter guard; thus, the factor effects are additive and no interaction between factors. Furthermore, significant differences were noticed between groups sample mean of quarter guard and clinic, girls' hostel staircase and clinic, quarter guard and girls' hostel, girls' hostel staircase and girls' hostel; thus, the factor effects are no means additive and interaction between factors exist. Succinctly, no significant differences between group sample means of the insects considered; thus, the factor effects are additive and no interaction between factors.

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