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Influence of Water Type and Commercial Diets on the Production of *Anopheles gambiae* Giles, under Laboratory Conditions

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Abstract: In the present research we investigated the influence of water resources (tap water, pond water and sprint water...) and nutritional quality on egg hatching and larval development of *An. gambiae* (Diptera: culicidae). Whatever the nutritive quality, the hatching rate was 84 ± 4 in tap water, 89 ± 1.4 in pond water and 91.8 ± 2.2 in spring water. The duration of hatching is 72 hours in tap water and 48 h in spring water. The duration of development (from egg to emergence) is 19.3 ± 1.1 days in tap water, 12.4 ± 0.35 days in pond water and 11.55 ± 0.4 days in spring water. The total death rate was 92% in tap water, 28% in pond water and 22% in spring water. Statistical test shows that the sex ratio at emergence was influenced by the type of water ($\chi^2 = 7.9$; p = 0.02). The female from tap water laid an average of 11 eggs per female whereas female from pond and spring water laid about 150 eggs per female five days after emergence. Physico-chemical analysis shows that temperature of tap water, spring water and pond water were in average 24.5, 27.5 and 25°C, respectively. Chloride concentration was in average 2.49 in tap water, 0.22 in spring water and 0.32 in pond water. Our observations indicated that spring water constituted the excellent medium for *An. gambiae* rearing.

Key words: An. gambiae, breeding, Tetrababy fish food R, Tetramin R, water source

INTRODUCTION

In Cameroon, as elsewhere in the tropical region, malaria is a public health problem. Health data indicates that this disease is responsible for 35 to 45% of death in health units, 42% of morbidity in children of age less than 5, 30% of hospitalisations and 26% of sick leaves (Okala and Fondjo, 2005). Integration of disease treatment with vector control is considered as the most effective means for malaria control (Tawatsin *et al.*, 2001). Research on disease transmission, vector biology and insecticide testing require colonization of vectors species in the laboratory. Laboratory strains provide certain advantages, in both technical and biological context, against the natural field organisms. They are inbred thereby more standardized and will therefore produce more consistent data over time (Aguilar *et al.*, 2005).

A number of recent studies have shown that age at pupation size of various mosquito species may reflect the environmental condition during growth of the larval stages (Reisen *et al.*, 1984; Fish, 1985; Haramis, 1985). For quality production of malaria vectors, for example, it is necessary to adapt anopheline mosquitoes to artificial environments that would have little effect on their natural vitality.

For the laboratory colonization of mosquitoes, optimal and standardized rearing conditions are usually attempted in order to obtain homogenous population with synchronous pupation, high productivity and uniform adult size (Briegel, 1990). Various techniques for the laboratory breeding of *An. stephensis* (Meller, 1962; Shuler and Maryon, 1966; Gerberg *et al.*, 1968) and *An. gambiae* complex (Diop and Molez, 1998; Tchuinkam, 1994; Armstrong and Bransby, 1961) have been reported. Most of these techniques result in low yield due to relatively low percent hatching and higher mortality rates, which however do not reflect those occurring under natural conditions.

Even under natural conditions, there is no clear relationship between environmental habitat parameters affecting larval development and growth and the distribution of larva in the aquatic habitats, because such habitats are constantly subjected to variations of biological and chemical parameters.

In the natural conditions in Cameroon, mosquitoes develop in waters sources that can be spring waters, pond waters and tap waters preserved in the container and abandon outside. Considering the outbreak of the malaria in urban zone, the main objective of this work is to know if the three types of water sources offer the same chance

of survival and development to the developmental stages of *An. gambiae*. We wished also to determine the optimal breeding conditions that are required to obtain a critical population of *An. gambiae* for laboratory test.

MATERIALS AND METHODS

Mosquitoes: Wild larvae (second and third instars) of Anopheles mosquitoes were collected by dipping on field in Yaoundé and carefully taken to the Laboratory at the University of Yaoundé I. They were reared at room temperature (26-30°C) 70-80% RH in the spring water containing Tetramin R and pupae were put into 20 cm cubic cages. At emergence, adults were identified as *An. gambiae* using Gilles and De Meillon (1968), key and put in separate cages. Eggs were collected on paper towels after blood feeding the females adults on rabbit of six months old. Adults were maintained in cages with a 10% glucose solution.

Effects of water source on embryonic development and eggs hatching: Three types of water (tap water, pond water and spring water) were used in order to determine the best rearing medium. One hundred eggs of *An. gambiae* were dispensed into plastic trays (18 cm Ø, 5 cm deep) containing 11 of each type of water. The number of hatched eggs was determined daily by counting first instars larvae observed in each tray until no more hatching occurred. The duration of embryonic development was determined according to Dempster (1961) method; According to this method, it is the time spends by 2/3 of eggs to be hatched for first instars larvae. Each experiment was performed at room temperature (26-30°C, 70-80% RH) with photoperiod LD 12:12.

Effects of water source and food type on larval and nymphal development of An. gambiae: Larvae were fed with 0.3 mg of food per larva. For each type of water, two experiments were conducted; larvae were fed with Tetrababy fish food R (food 1) and Tetramin R (food 2), respectively for the two experiments. At the end of larval development, pupae were counted daily and transfer to a separate jar containing the same type of water used for larval development. Pré-imago duration was determined according to Dempster's method (time spends by 2/3 of number individual to emerge).

Effects of water source and food type on the emergence, sex ratio and fertility of *An. gambiae* females: At emergence, adults were counted and the mosquito's sex determined by visual inspection. Sex ratio was determined

(number of female over the total number of adults that emerged). Subsequently sugar solution (10%) was provided ad libitum. Three days after emergence, females were allowed to blood feed on a rabbit and kept under laboratory condition to lay. Eggs laid by females were counted and the average fecundity of each female was estimated (total number of eggs laid over the total number of female). The total duration of development (from eggs to adults) was determined by considering embryonic development duration, larval development duration and nymphal development duration. Throughout our experiment, mortality rate was determined at each developmental stage (number of death over total number of larvae or pupae at the developmental stage).

Effects of additional proteins and sugars on eggs hatching and larval development of An. gambiae: Six experimental groups were tested. An experimental group consisted of a tray containing 1 L of spring water in which 100 larvae were fed with Tetrababy fish food R as described before with additional proteins of 0.1 mg/larva (ovalbumin of hen egg, soy or crayfish) or sugars (glucose, galactose, fructose) was added. For the control, 0.4 mg/larva of Tetrababy fish food R was used. Hatching rate, embryonic development duration, larval stages development duration, pupae development duration, sex ratio, fertility of females and mortality at each developmental stage were measured as described above.

Physico chemical analysis of water: Physico chemical analysis of water was studied in the hydrobiology laboratory, Faculty of Science, University of Yaoundé I.

Temperature is measured with the aid of a mercury thermometer; pH with a pH meter, conductivity with a conductimeter; suspended solid, turbidity, colour NH₄⁺, PO₄⁺, with a spectrophotometer. Dissolve oxygen according to the winkler method, BOD₅ according to the respirometry method, chlorine was determine by titration.

Statistical analysis: The different means were compared with SPSS statistical program, version 10.1, for windows, using non parametric Z test of Wilcoxon (1945), χ^2 of Kruskal Wallis (1952) test and U-test of Mann Whitney (1947) (p<0.05).

RESULTS

Eggs of *An. gambiae* dispensed in spring water and pond water hatch after 48 h; whereas those preserved in tap water hatch in 72 h (Table 2). The χ^2 test comparing the difference between mean of the duration of embryonic development is highly significant ($\chi^2 = 23$; p = 0.01)

Table 1: Hatching rate of An gambiae eggs in: Tap water, Pond water and Spring water

	Tap water	Tap water	Pond water	Pond water	Spring water	Spring water	Comparison
Parameters	tetrababy fish	tetramin baby	tetrababy fish	tetramin baby	tetrababy fish	tetramin baby	test
Average of hatching rate	82.5±2.8	85.8±4.6	90.3±1.03	87.8±1.1	90.8±1.6	92.8±0.8	$\chi^2=8.3$; p = 0.02**
Comparison test	$\chi^2 = 0.34$; p = 0.5	56*	$\chi^2 = 1.72$; p = 0.18	*	$\chi^2 = 0.35$; p = 0.5	55*	

^{*}Not Significant, **Significant

Table 2: Embryonic development duration and other development stages duration (number of hours for the developmental stages and the number of days for the total duration) of An. gambiae in: Tap water, Pond water and Spring water

Parameters	Tap water tetrababy fish	Tap water tetramin baby	Pond water tetrababy fish	Pond water tetramin baby	Spring water tetrababy fish	Spring water tetramin baby	Comparison test
Embryonic development	72±0	72±0	48±0	48±0	48±0	48±0	$\chi^2 = 23$; p = 0.01***
Comparison test	$\chi^2 = 0; p = 1*$	$\chi^2 = 0; p = 1*$	$\chi^2 = 0; p = 1*$				
Larvae duration	366±46.2	330±38.2	267±6	216±12	198±22.7	204±18.9	$\chi^2 = 18.3; p = 0.01***$
Comparison test	$\chi^2 = 0.1$; p = 0.76*		$\chi^2 = 2$; p = 0.15*	$\chi^2 = 0.1$; p = 0.75*			
Nymphs duration	60±6.9	60±12	24±0	24±0	24±0	24±0	$\chi^2 = 22.1 \text{ p} = 0.01***$
Comparison test	$\chi^2 = 0.11$; $p = 0$	0.73 *	$\chi^2 = 0$; p = 1*		$\chi^2 = 0$; $p = 1*$		•
Total duration	19.3±1.1	19.3±1.3	12.8±0.3	12±0.4	11.3±0.5	11.8 ± 0.3	$\chi^2 = 17.9$; p = 0.01 ***
Comparison test	$\chi^2 = 0.02$; p = 0	.88*	$\chi^2 = 2.02$; p = 0.15	5*	$\chi^2 = 0$; p = 1*		

^{*}Not significant, **Significant, ***Highly significant

(Table 2). Then, it is clear that embryo take more time to hatch in the tap water than in the other rearing water.

Of the 100 eggs dispensed in each tray, the number that successful hatch varied from 80% in tap water to 93% in the spring water (Table 1). The χ^2 test comparing hatching rates in the three breeding medium is highly significant ($\chi^2 = 8.3$; p = 0.02). The effect of water type on eggs hatching rate or the development of embryo was significantly obvious with tap water compare to other water source (Table 1). The tap water is less propitious to the hatching and or the development of embryo of *An. gambiae* eggs than in the two other rearing settings. No significant difference is recorded as concern the hatching rate in the various breeding settings (spring water, pond water and the tap water) (Table 1).

The effect of type water on larval development showed that the duration after hatching to the pupate stage varied from one medium to another. Larvae spend about 198 hours in the spring water, 216 h in the pond water and 366 h in the tap water (Table 2). The χ^2 test comparing the duration of larval development in the three medium is highly significant. This duration is shorter in the spring water whereas, it is more longer in the tap water (p<0.01; $\chi^2 = 18.3$) (Table 2).

The larval death rate varied from 3.08% in the spring water to 39% in the tap water (Table 3). The larval death rate varied significantly following the type of water used or breeding ($\chi^2 = 20.2$; p<0.01). The mortality of larvae is higher in the tap water (Table 3). The spring water is more suitable for survival and development of larvae of *An. gambiae*.

The duration of nymphal development varied: 24 h in spring water to 60 h in tap water (Table 2); the χ^2 test comparing the duration of nymphal development is highly significant ($\chi^2 = 22.1$; p<0.01) (Table 2). The tap water is

less favourable for the development of nymphs; this type of water extends the duration of the development of nymphs.

The death rate of nymphs varied from 2.1% in the spring water to 26.04% in the tap water (Table 3). The comparison of the death rates of nymphs in different medium is highly significant ($\chi^2 = 9.7$; p = 0.01) (Table 3). The tap water is less suitable for nymphs' survival as compared to the result obtained with spring water and the pond water.

Finally, the total duration of larval and nymphal development of *An. gambiae* varied from 11.25 days in the spring water to 19.2 days in the tap water (Table 2). Comparative analysis of these durations gives a highly meaningful difference ($\chi^2 = 17.9$; p = 0.01) (Table 2). In the spring water the development of *An. gambiae* was faster than the development in the tap water. In the same way, the total mortality rate of larvae and nymphs is higher in the tap water (93.75%) and very few in the spring water (19.5%). A difference by χ^2 test is highly significant ($\chi^2 = 17.9$; p = 0.01) (Table 3).

Using Tetramin R or Tetrababy fish R during rearing does not affect significantly the duration of the development of larvae and nymph of *An. gambiae* in our conditions (Table 2). The two types of food used during the rearing of these mosquitoes were quite suitable; there were no significant difference obtained.

In order to determine if the characteristic of the breeding site can influence adults' development and females' fertility, the number of adults that emerged and number of eggs laid by females were counted. The average numbers of adults (both male and female) obtained from tap water (7.3 ± 0.5) differed significantly from the number of adults obtained from spring water (76 ± 1.1) ($\chi^2=17.9$; p=0.01) (Table 4). Sex ratio is more

Table 3: Mortality rate of the various developmental stages of An gambiae in: Tap water, Pond water and Spring water

Parameters	Tap water tetrababy fish	Tap water tetramin baby	Pond water tetrababy fish	Pond water tetramin baby	Spring water tetrababy fish	Spring water tetramin baby	Comparison test
Larvae mortality rate	39±14.6	38.1±8.6	5.1±0.9	4±0.6	4.2±0.9	3.1±0.9	$\chi^2 = 20.2; p = 0.01***$
Comparison test	$\chi^2 = 0.1$; p = 0	.77*	$\chi^2 = 0.3$; p = 0.56*	•	$\chi^2 = 5.3$; p = 0.	02**	, , , , , , , , , , , , , , , , , , ,
Nymphs mortality rate	26.1±9.4	22.1±5.2	6.1±0.4	2.3 ± 1.6	4.3±1	2.1 ± 1	$\chi^2 = 9.7$; p = 0.01***
Comparison test	$\chi^2 = 0.33$; p =	0. 56*	$\chi^2 = 2.1$; p = 0.14*	•	$\chi^2 = 3$; p = 0.08	3**	
Adults mortality rate	31.4±11	34.2 ± 6.8	23.6±3.2	23.7±6	8.5±0.02	9 ± 0.1	$\chi^2 = 12.2$; p = 0.01 ***
Comparison test	$\chi^2 = 0.02$; p =	0.88*	$\chi^2 = 0$; p = 1*		$\chi^2 = 5.5$; p = 0.	02**	
Total mortality rate	93.8±2.6	91.8±2.2	30.8±1.3	27.3±1	27±1.1	19.5±1	$\chi^2 = 17.9$; p = 0.01 ***
Comparison test	$\chi^2 = 0.53$; p =	0.47 *	$\chi^2 = 3.1$; p = 0.07*	•	$\chi^2 = 5.4$; p = 0.	02**	

^{*}Not Significant, **Significant, ***Highly significant

Table 4: Number of adults obtained in average and number of eggs laid per female of An gambiae in: Tap water, Pond water and Spring water

Parameters	Tap water tetrababy fish	Tap water tetramin baby	Pond water tetrababy fish	Pond water tetramin baby	Spring water tetrababy fish	Spring water tetramin baby	Comparison test
Adults number	6.3±2.6	8.3±2.2	69.3±1.3	72.8±1	73±1.1	80.5±1	$\chi^2 = 17.9$; p = 0.01 ***
Comparison test	$\chi^2 = 0.53$; p = 0	$\chi^2 = 0.53$; p = 0.47 *		$\chi^2 = 3.1$; p = 0.79*		02**	
Males number	3.8±1.8	5.5±1.8	35±1.8	41.8±2.9	32.8±2.3	38.3 ± 2.4	$\chi^2 = 15.7$; $p = 0.01***$
Comparison test	$\chi^2 = 0.77$; p = 0.77*		$\chi^2 = 2.58$; p = 0.11*		$\chi^2 = 2.13$; p = 0.14*		
Females number	2.5 ± 0.9	2.8 ± 0.5	34.3 ± 0.9	31±2	40.3±1.9	42.3 ± 2.4	$\chi^2 = 19.4$; p = 0.01 ***
Comparison test	$\chi^2 = 0.38$; p = 0	$\gamma^2 = 0.38$; p = 0.54*		$\chi^2 = 1.3$; p = 0.0.25*).39*	
Eggs number	31.3±5.6	26.5±5.5	5019.3±463.3	4523±342.2	5771.5±674.8	5580.3±409.1	$\chi^2 = 16.8$; p = 0.01 ***
Comparison test	$\chi^2 = 0.2$; p = 0.66*		$\chi^2 = 0.75$; p = 0.38*		$\chi^2 = 0.1$; $p = 0.77$ *		
Sex ratio	0.9±0.4	0.6 ± 0.1	0.98 ± 0.1	0.8 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	$\chi^2 = 7.9$; $p = 0.02**$
Comparison test	$\chi^2 = 0.02$; p = 0	.88*	$\chi^2 = 2.1$; p = 0.15	*	$\chi^2 = 0.1$; $p = 0$.	77*	

^{*}Not significant, **Significant, ***Highly significant

higher in the spring water than in the others types of water (Table 4). Thus, more females than male were obtained from spring water than from other rearing medium. The fertility (or fecundity) of females obtained from larvae raised in the spring water and the pond water is significantly higher than the fertility of females raised in the tap water (($\chi^2 = 16.8$; p = 0.01). The number of eggs laid per female from the pond water is 146.5, 143.4 eggs per female was laid by females reared in the spring water and 12.1 eggs per female was laid by females reared in the tap water (Table 4). The fertility of An. gambiae female breed in tap water is negatively affected, thus the rearing medium influenced the fertility of females of this mosquitoes. Food type did not seem to influence the level of emergence, the sex ratio and the number of eggs laid by anopheles. These observations suggest that the 2 types of food used have good nutrients that are optimal for An. gambiae larvae breeding.

Physical and chemical characteristics of rearing waters are represented in Table 5. Temperature was significantly higher in spring water (range: 26.4-29°C) than that recorded in tap water (22.8-25.7°C) and pond water (24.9-25.2°C). The concentration of chlorine is high in tap water (2.3-2.63 mg L^{-1}), low in pond water (0.29-0.35 mg L^{-1}) and very low in spring water (0.19-0.25 mg L^{-1}). The conductivity, the turbidity, the BOD $_{\scriptscriptstyle 5}$ (Biochemical oxygen Demand), the total hardness, the colour and the suspended solids varies slightly between tap water and spring water (Table 5).

The development of *An. gambiae* larvae has been followed thereafter in the spring water containing the Tetramin baby R and additional of proteins and sugars.

Table 5: Physicochemical characters of Tap, Pond and spring water

Variables	Pond water	Spring water	Tap water
T (°C)	24.9-25.2	26.4-29	22.8-25.7
pH	6.59-7.06	5.05-6.1	6.76-7.43
Conductivity µs cm ⁻¹	121-313	60-109	71-143.3
Turbidity (FTU)	29-142	1-16	2-19
Color	183-1220	5-20	15-60
$SS (mg L^{-1})$	19-106	0-4	4-9
$NH_{4}^{+}(mg\ L^{-1})$	2.07-3.24	0-0.06	0-0.25
$PO_4^+ (mg L^{-1})$	5.06-5.80	2.05-2.10	2.30-2.70
Dissolve oxygen			
(mg L^{-1})	0.8-4	0.4-1.90	0.9-3.10
BOD ₅	35-50	7-9	3-6
Total chlorine (mg L-1)	0.29-0.35	0.19-0.25	2.30-2.63
Total hardness (mg L ⁻¹)	5-6	0.5-1.2	0

SS: Suspended Solid, BOD5: Biological Oxygen Demand

Eggs hatch after 54 h in the presence of additional proteins and approximately after 60 h in the presence of additional sugars. The hatching rate varied between 80-86 and 82-87%, respectively in the presence of additional proteins and additional sugars. From the first instars larvae to pupae stage, the development take about 144 and 166 h on average, respectively when protein and sugar were added. The duration of larval development is significantly shorter when proteins used as food supplement, compared to the duration recorded when sugar was food supplement. On average, the death rate of larvae was 2.79, 2.79, 2.06, 2.95, 1.92 and 3.27%, respectively with additional food such as ovalbumin of hen egg, soy, crayfish, glucose, galactose and fructose. The mortality rate of nymphs, adults and the total mortality is higher when the additional food in the breeding milieu is of proteins or sugars. The number of adults varied between 70 and 78, the number of male and

Table 6: Influence of additional proteins and sugars on various development stages of An. gambiae in laboratory in spring water

Parameters	Control	Ovalbumin of hen eggs	Soy	Crayfish	Glucose	Galactose	Fructose
Hatching rate	91±0.57	86.50±1.70	88.50±1.32	80.75±1.25	87.25±1.10	88.75±1.10	82.25±2.65
Hatching duration	60 ± 6.92	54 ± 6.00	54.00±6.00	54.00±6.00	66.00±6.00	54.00±6.00	72.00 ± 0.00
larval stage duration	198±12.92	144 ± 13.84	138±12.92	144±13.84	162±18	168 ± 25.84	174±19.84
Nymphal Duration	42 ± 6.00	36 ± 6.92	42.00±6.00	30.00 ± 6.00	42.00±6.00	42.00 ± 6.00	48.00±0.00
Total duration	12.5 ± 0.28	9.75 ± 0.25	9.75 ± 0.47	9.50 ± 0.28	11.25 ± 0.25	11.25 ± 0.62	12.00 ± 0.40
Mortality rate of larvae	2.2 ± 1.83	2.79 ± 0.56	2.79 ± 0.53	2.06 ± 0.29	2.95 ± 0.42	1.92 ± 0.62	3.27 ± 0.82
Mortality rate of nymph	2.03 ± 0.71	5.53±0.99	4.42±1.66	2.68 ± 0.56	4.87 ± 0.65	5.14 ± 0.69	2.69 ± 0.71
Mortality rate of adults	7.36 ± 0.86	10.35 ± 2.05	10.26 ± 0.97	9.62 ± 1.18	8.86 ± 1.42	9.29 ± 0.78	8.04 ± 0.76
Total mortality rate	14 ± 2.04	27.50 ± 1.84	24.50±1.25	27.50±1.25	26.75±1.10	22.00 ± 0.70	30.00±3.10
Number of adult	81.50±0.64	72.50 ± 1.84	75.50 ± 1.25	72.50 ± 1.25	73.25±1.10	78.00 ± 0.70	70.00±3.10
Number of male	37.75±2.286	34.25 ± 1.25	38.25±1.43	36.00±1.58	35.25 ± 0.85	38.50±0.64	36.75±1.97
Number of female	43.75±1.75	38.25 ± 0.62	37.25±1.93	36.50 ± 0.86	38.00 ± 1.73	39.50±0.64	33.25±2.39
sex ratio	1.17 ± 0.11	1.11 ± 0.02	0.97 ± 0.08	1.01 ± 0.06	1.08 ± 0.07	1.02 ± 0.02	0.90 ± 0.08
Number of eggs	131.50±9.28	132.32±8.14	123.16±14.06	101.33±12.02	127.28±7.99	142.19±7.99	111.5±5.39

Proteins: Ovalbumin of hen eggs, soy and crayfish, Sugars: Glucose, galactose and fructose

female varied between 34 and 38, the number of eggs laid by female varied between 101 and 142, respectively when proteins and sugars were added for larval breeding. These results reveal that the additional proteins are more helpful for the development of *An. gambiae* than the additional sugars (Table 6).

DISCUSSION

Depending on the environmental conditions the development of embryo, eggs hatching and larval development showed a considerable flexibility in the hatching rate and duration of larval development in contrast of the constant duration of the pupal development period of An. gambiae as described before us by Timmermann and Briegel (1993). In this study we observed that the rhythm of hatching is 1.5 times higher in the spring water and pond water than in the tap water. The development of An. gambiae in our experimental condition took 11.25 days in the spring water, 12.75 days in the pond water and 19.25 days in the tap water. However it has been documented that the developmental cycle of An. gambiae spend 12 to 16 days (Holstein, 1952; Diop and Molez, 1998). So, the abnormally long period of time obtain in tap water can be due to the presence of a chemical product which act as a toxic or a growth retardation factor that may caused the physiological alteration of mosquitoes. The results yet obtain confirmed the work of Barbosa et al. (1972). The chemical analysis of the composition of rearing water shows that the tap water is very rich in chloride. The consequence of this is the increase of its concentration in larvae cells. With regards to regulation of organic solute concentration, it has been showed that the high levels of inorganic salts impair enzyme function in many biochemical pathways (Somero and Yancey, 1997; Rodier, 1978). For example, high inorganic salts levels perturbing serine hydrolase function in the serine catabolic pathway (Burton, 1991)

and inhibited trehalase activity, enzyme that hydrolyses the disaccharide trehalose to glucose molecules (Patrick and Bradley, 2000).

Survival times are very sensitive for the effects of almost any physiological parameter. Larvae may merely accumulate in water containing toxic compounds because they develop slowly (Ye-Ebiyo *et al.*, 2003). This justify the small number of adults obtained from tap water (6.25) compared to the number obtained from spring water (80.5) and pond water (72.25), those results are correlated to the death rates observed in rearing medium.

The higher rate mortality of the larvae reared in the tap water is due to the characteristics of this medium, neither on the food quality nor its quantity. Lyimo *et al.* (1991) and Eugene *et al.* (1968) achieved the best rearing of anopheles out puts in a 27°C medium. Conditions that tap water do not fulfil.

In tap water, a higher rate of mortality was observed among female, than male. This suggest that they are more affected by the metabolic disturb caused by inorganic salts (Briegel et al., 2001). Under these condition larvae of An. gambiae spend more time for their development and this affected the body size of adults. It is well known that body sizes of females influence directly the reproductive level of many insects (Karino et al., 2004). Moreover eggs laid are inhibited by highly saline substrates (Schmidtmann et al., 2000). This can justify the ditch observe between the number of eggs laid per female from tap water (28 eggs average) and the number of eggs laid per female from spring water (5675 eggs average) and pond water (4771 eggs average).

Generally, well water and spring water are of higher temperature and of low levels of dissolve oxygen so, they constituted predilected medium for *An. gambiae* rearing. The tap water proves to be disconductive for the mosquitoes breeding, because of the relatively higher chlorine concentrations, furthermore this water is cold and saturated in gas.

CONCLUSIONS

The present study has examined the rearing conditions of *An. gambiae*. The findings showed that chemical characteristic of the rearing medium strongly affects larval development duration, the number of adults and the eggs laying of females. Spring water is a more suitable medium for the rearing of this vector. However tap water and pond water sources can also be use, but they differ on the rearing yield. The two types of food used bring out similar results in the three types of water tested. These foods can be recommended therefore like basic food for *An. gambiae* rearing.

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