American Journal of Zoology
2021; 4(1): 9-13
http://www.sciencepublishinggroup.com/j/ajz
doi: 10.11648/j.ajz.20210401.12

Impact of the Technique of Mosquito Egg Impregnation on the Emergence Rate in the Insectarium

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To cite this article:

Andre Sominahouin, Sebastien Koudenoukpo, Germain Gil Padonou, Razacki Osse, Constantin Adoha, Casimir Kpanou, Boulais Yovogan, Albert Salako, Esdras Odjo, Filemon Tokponon, Martin Codjo Akogbeto. Impact of the Technique of Mosquito Egg Impregnation on the Emergence Rate in the Insectarium. *American Journal of Zoology*. Vol. 4, No. 1, 2021, pp. 9-13. doi: 10.11648/j.ajz.20210401.12

Received: September 19, 2020; **Accepted**: September 29, 2020; **Published**: March 17, 2021

Abstract: The breeding of the main vectors is essential for the conduct of many operational research programs. The right option of the technique of watering malaria vector larvae helps entomologists, insectarium managers and entomology technicians to produce high-quality Anopheles mosquitoes in the laboratory for different research and operational objectives. Evaporation of water in the tank causes suffering to the embryos stuck to the wall of the tank and due to lack of immersion, these embryos eventually die. We have experimented with a technique that consists in keeping the eggs in the center of the tank and preventing them from migrating towards the wall with a rectangular opening paper. The aim of this study is to understand the impact of the An. gambiae egg impregnation technique on the harvest rate in the insectarium. The hatching rate for all the eggs put in water in the paper trays is of the order of 83% to 99%. The results of paperless trays are necessarily worse than those of paper trays. In addition, the risk of mortality of larvae in the pupal stage is twenty (20) times lower with the paper tray than with the paperless tray. They therefore constitute basic indications to avoid a number of false steps from the outset.

Keywords: Breeding, Insectarium, *Anopheles gambiae*, Malaria, Paper

1. Background

Malaria is a disease for which Benin pays a heavy price and whose major vector is female Anopheles [1]. To combat this disease, the WHO recommends indoor residual spraying and the use of long-lasting insecticidal nets (LLINs). These control tools are only effective for a given period of time and should therefore be monitored. Thus, evaluation of the effectiveness of these tools requires a vector population as biological material. In order to make it available, vector populations are raised under controlled conditions at the insectarium [9].

The rearing of the main vectors is essential for the conduct of many operational research programs, (experimental infections, insecticide tests, etc.) [2, 3]. There are four stages in the life cycle of mosquitoes: egg, larva, pupa and adult. The time taken for each stage to develop depends on water temperature and other factors [4, 5]. The correct technique of watering the larvae of malaria vectors helps entomologists, insectarium managers, and entomology technicians to produce high-quality Anopheles mosquitoes in the laboratory and in the field for various operational research purposes. Mass breeding of mosquitoes requires a large number of eggs from which an experimental population would be collected [7]. Studies on the viability of Anopheles eggs have shown that eggs hatch within 2 days after laying under optimal environmental conditions (27°C±2, 80%±10 relative humidity). However, changes in environmental conditions

have been shown to affect egg viability and delay hatching in mosquitoes [8]. Mosquito breeding is therefore complex and demanding for several reasons. The basic materials and methods of mosquito rearing are similar to those described many years ago [6].

One of the methods of rearing mosquitoes in the insectarium is by watering the nests. Once in the water, the eggs will stick to the wall of the tank. The evaporation of water in the tank causes suffering to the embryos stuck to the wall of the tank. Due to the lack of immersion, these embryos eventually die. As a result, the quantity of larvae of the first generation (F1) is in clear decrease in the tanks. To remedy this, a technique that keeps the eggs in water in the center of the tank would be necessary in order to have more first generation lava (F1) after hatching of the eggs. With this in mind, we have experimented with a technique that consists in keeping the eggs in the center of the tank and preventing them from migrating towards the wall with a paper in which we have created a rectangular opening.

The objective of the study is to compare the two methods of egg impregnation in order to improve the larvae production technique and consequently the quality of the mosquitoes used for the different tests in the laboratory.

2. Material and Method

The present study was conducted from June to August 2020 at the insectarium annex of the Centre de Recherche Entomologique de Cotonou in southern Benin, West African country.

For this experiment, the following material was used:

a. 2 clean bins (plastic utensils, volume 3L) + 2 mosquito nets
b. 1 dissecting forceps
c. 1 clean scalpel.
d. 1 clean A4 paper with rectangle opening (specify size of opening)
e. distilled or dechlorinated water

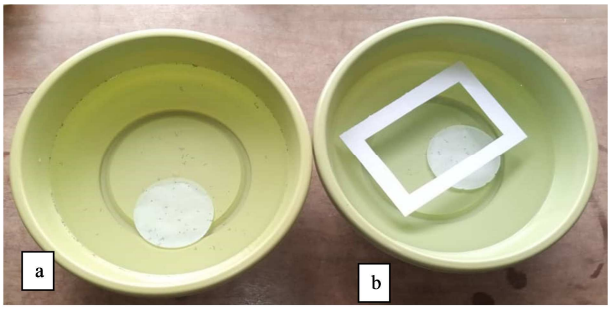

Figure 1. *Trays containing An. gambiae s.l. eggs without (a) and with (b) rectangular paper.*

Both bins were filled with 3L of water each and labeled. An A4 paper with a rectangular opening was placed in one tray, the second one being paperless (Figure 1). Using pliers, the adult mosquitoes were removed from the filter paper (egg-laying) which was then cut in two pieces by diameter with the scalpel. A part of the filter paper was placed in the A4 paper tray, taking care to insert it into the rectangle and then the second part into the other A4 paperless tray. A pinch of food was added to the trays which were then covered with the mosquito netting (Figure 2). The trays were placed on a support at room temperature of the insectarium. After 24 to 48 hours, first generation larvae were observed. Egg hatching, development of emerged larvae, larval survival and adult emergence were recorded.

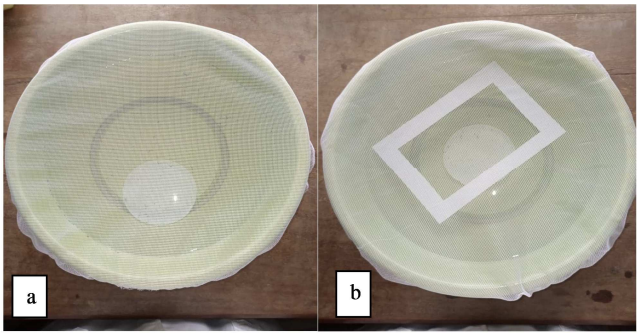

Figure 2. *Anopheles gambiae larvae tray (a) without paper and (b) with rectangular paper.*

For this purpose, the following indicators were calculated:

$$\text{Hatching rate}=\frac{\text{Number of larvae L1}}{\text{Number of eggs laid in water}}x\ 100$$

$$\text{Pupal stage arrival rate}=\frac{\text{Number of pupae harvested}}{\text{Number of larvaer}}\ x100$$

$$\text{Emergence rate}=\frac{\text{Number of adults harvested}}{\text{Number of nymphs}}\ x\ 100$$

3. Results

1. Influence of techniques of egg impregnation of An. gambiae Kisumu s.l. without rectangle paper on the pre-imaginary development in the insectarium.

The following results compare hatching rates, pupal stage arrival rate and emergence rate of *An. gambiae* Kisumu (Tables 1, 2 and 3).

This study involved a total of 2000 eggs of *An. gambiae* Kisumu laid in each type of tank. A total of 301 wild females. Of these eggs, 1650 or 82.50% were hatched in the paperless tank while 1950 or 98.75% were hatched in the paper tank (Table 1).

The first experiment shows that the floating eggs migrate towards the wall of the tray and if they adhere. It can then be estimated, based on the performance obtained, that the hatching rate for all the eggs laid in the paper trays is, on the order of 82% to 99%, although the results of the paperless trays are necessarily worse than those of the paper trays. Table 1 shows that the paper tray allows more larvae hatching than the paperless tray ($p<0.0001$).

Table 1. *Hatching rates of An. gambiae Kisumu obtained according to bins.*

	Eggs hatched	Number of larvae hatched	Hatching rate (%)	P-value
Paperless Tray	2000	1650	82.5	<0.0001
Tray with paper	2000	1975	98.75	

p-value: p-value of the chi-square test to compare proportions.

The risk of mortality of larvae in the pupal stage is twenty (20) times lower (1/0.05) with the Paper Tray than with the Paperless Tray (p<0.001) (Table 2).

Table 2. *Pupal stage arrival rate of An. gambiae Kisumu according to trays.*

	Total Larvae	Number of Deaths	Pupation Rate	RR	IC-95% [RR]	P (Fisher)
Paperless tray	1000	485	70.60	1	-	<0.001
Tray with paper	1975	50	97.46	0.05	0.03 -0.07	

RR: Risk Ratio; 95% CI-95% [RR]: 95% confidence interval; P (Fisher): P-value of the Fisher test

Also, Table 3 shows that larvae of An. gambiae are about 3 times more likely to reach the adult stage in the paper tray (p<0.001).

Table 3. *Emergence rate of An. gambiae Kisumu larvae in the tanks.*

	Number of nymphs	Number of adults	Emergence rate	OR	IC-95%[OR]	P (Fisher)
Paperless Tray	515	480	6.796	1	-	<0.001
Tray with paper	1950	1900	2.564	2.77	[1.77 – 4.32]	

OR: Odds ratio; 95% CI-95% [OR]: 95% confidence interval; P (Fisher): P-value of the Fisher test

4. Discussion

The present experimental study was carried out under laboratory conditions (Insectarium) from June to August 2020 using samples of *Anopheles gambiae* Kisumu.

It compares two techniques of Anopheles gambiae s.l. larvae watering in the insectarium. The first one is the usual one and the second one consists in keeping the eggs in the center of the tank and preventing them from migrating towards the wall of the tank thanks to a rectangular paper [1].

The hatching rate performance for all the eggs laid in the trays is in the range of 82.5% to 99%, but the results of paperless trays are inevitably worse than those of paper trays. The other proportions of unhatched eggs could be explained by the viability of the eggs and the lack of immersion of the larvae that would stick to the walls of the trays. The results of this work show that there was a significant difference between the average number of eggs laid, the number of larvae reaching the pupal stage and the number of adults in the experimental trays.

The results of the paperless rectangle technique show that the majority of L1 larvae fail to emerge at the end of the experiment. These results show that the chance of larvae to emerge is about three times (2.77) greater in the paper tray than in the paperless tray. This pattern is similar to that of studies by Khan and others, in which the egg hatch and larval emergence rates of *An. gambiae* decreased with increasing storage time of eggs at 16°C.

Our results are contrary to those of Mazigo et al, 2019, who showed that increasing egg storage time had negative consequences on larval survival [11]. This means that larval mortality was inversely proportional to egg storage time. Higher larval mortalities were recorded in eggs stored for 15 days.

Eggs laid and the resulting larvae are as viable in the paper tray as the paperless tray. This result is different from that obtained by Amadou (2008) who found that the reduction in the number of eggs laid as a result of *P. falciparum* infection in *An. arabiensis* and the M and S molecular forms of *An. gambiae* s.s. is not certain when the infection reaches the sporozoite stage [1, 12]. It would be recommended to continue these studies under field conditions with emphasis on the dynamics of *P. falciparum* populations in relation to that of vector populations in different epidemiological situations in the districts where IRS is implemented.

Our results are similar to those of previous studies on delayed larval development of *An. gambiae* and *Aedes aegypti*, with a longer egg storage life [13, 14]. In addition, reduced locomotor and feeding activities were reported in the diapause-terminated larvae [15]. Several studies have reported a strong modification of gene expression patterns and regulatory pathways during diapause [16, 17]. This change in gene expression patterns may result in a lack of normal physiological development. The delay in larval development observed in larvae from eggs stored in this study could be due to the impact of the slides and changes in gene expression patterns, as previously reported in various studies [10].

5. Conclusion

Mosquito breeding has made considerable progress in recent years, thanks mainly to research on insecticides. Only the technique with rectangular paper can be used instead of the usual (paperless) one. This allows a high percentage of emergence within an acceptable time. The second causes either a significant mortality or a lengthening of the pre-imaginal period, which congests the insectarium. The

quantity of L1 larvae of An. gambiae in the tray with rectangular paper is four times higher than the quantity of L1 larvae in the tray without paper. Half a cage of adult mosquitoes is obtained from the paperless tray, while in the rectangular paper tray two cages of 600 to 1000 mosquitoes each can be used.

The breeding of *An. gambiae* s.l. can be carried out in the laboratory without too much difficulty. The technical details that we give are not the only ones that can give satisfaction. They are therefore basic indications to avoid, from the very beginning, a number of false steps.

Ethics Approval and Consent to Participate

The protocol of this study was evaluated and approved by the Institutional Ethical Committee of CREC (IECC) (Grant No IORG005698). Prior to their involvement in this study, voluntary mosquito collectors have been trained and gave their consent. They were checked up, taken care in case of confirmed malaria case and, vaccinated against yellow fever.

Consent for Publication

Not applicable.

Availability of Data and Materials

The data used and/or analysed in this study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

This study was financially supported by the US President's Malaria Initiative (PMI) through the United States Agency for International Development (USAID) Africa Indoor Residual Spraying Project (AIRS).

Authors' Contributions

AS, SK and MCA conceived the study. AS, MCA have participated in the design of the study. Entomologic data was collected by AS, SK and laboratory analysis was carried out by AS, MCA. AS drafted the manuscript.

Statistical data analysis by AS and MCA critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Authors' Information

We are grateful to the President's Malaria Initiative which supported financially this study. We would also like to acknowledge Martin Akogbéto who provided technical support to the study and critically revised the manuscript.

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