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STUDY THE USE OF LARVIVOROUS FISH AGAINST LARVAE OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE) IN LABORATORY CONDITIONS IN DOGBO DISTRICT IN SOUTH-WESTERN BENIN, WEST AFRICA

Nazaire Aïzoun[1*], Habib Tamegnon[1], Eloi Honvoh[1], Thierry Agblonon[2] and Daniel Chougourou[3]

[1]Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET), Normal High School of Technical Teaching (ENSET) of Lokossa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) of Abomey, P. O. Box 133 Lokossa, Cotonou, Benin.
[2]Laboratory of Hydrobiology and Aquaculture, Faculty of Agronomic Sciences, University of Abomey-Calavi (UAC), Cotonou, Bénin.
[3]Department of Environment Genius, Polytechnic School of Abomey-Calavi (EPAC), University of Abomey-Calavi (UAC), Cotonou, Benin.

***Corresponding Author: Nazaire Aïzoun**
Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET), Normal High School of Technical Teaching (ENSET) of Lokossa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) of Abomey, P. O. Box 133 Lokossa, Cotonou, Benin.

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ABSTRACT

Background: The use of chemical insecticides causes important damages to environment and human health and there is a need to search for alternative solutions. **Objective:** The current study aimed to investigate on the use of larvivorous fish against larvae of *Aedes aegypti* in laboratory conditions in Dogbo district in south-western Benin, West Africa. **Methodology:** Larvae of *Aedes aegypti* mosquitoes were collected from breeding sites using the dipping method from August to November 2023 during the small rainy season in Dogbo district. Adult *Aedes aegypti* from these breeding sites were previously identified. Alive *Orechromis niloticus* fishes were bought immediately once catched by fishers of Ganvié location in So-Ava district and carried by car from Ganvié location to the Laboratory. Laboratory evaluation for larvivorous efficacy was conducted. **Results:** The results showed that larvivorous fishes ate more larvae of *Aedes aegypti* when they were unfed than when they were fed. The results obtained after the introduction of unfed larvivorous fishes in glass jars showed high larva-eating capacity of these fishes. **Conclusion:** Biological control against larvae of *Aedes aegypti* using larvivorous fish has given good results in laboratory conditions in the current study. Biological control also preserves or respects environment.

KEYWORDS: Larvivorous fish, larvae of *Aedes aegypti*, biological control, laboratory conditions, Benin.

INTRODUCTION

Mosquitoes transmit numerous human and animal pathogens and chemical insecticides are widely employed in their control. However the success of control programs is now threatened as the repeated exposure of mosquito populations to chemical insecticides has led to the selection of mutations conferring an increased resistance to these insecticides.[1] So, the efficacy of these insecticides is influenced by the mosquitoes' history of past exposure. Long-term exposure to a toxicant will eventually select for mutations conferring a level of resistance to that toxicant and indeed, insecticide-resistant populations of mosquitoes are now threatening the success of control programmes. Extensive research efforts are aimed at elucidating the molecular basis of this resistance in order to facilitate the management of insecticide resistance in the field. Less attention has been paid to the short-term effect of exposure to insecticides or other xenobiotics on the mosquitoes' tolerance to insecticides and yet this could also have a significant impact on the efficacy of mosquito control. For example, it was shown that *Aedes aegypti* larvae exposed to the herbicide atrazine become more tolerant to the organophosphate temephos.[2] Similarly, exposure of *Aedes albopictus* larvae to benzothiazole (a major leachate compound of automobile tires) and pentachlorophenol (a wood-protecting agent) can increase their tolerance to insecticides such as carbaryl, rotenone and temephos.[3-4] This increase in tolerance in *Ae. albopictus* is correlated with an induction of cytochrome P450 activity. This enzyme family, together with the carboxylesterases and glutathione transferases, play a central role in the detoxification and in the metabolism of insecticides and other xenobiotics.[5-6]

The researches have to explore several alternative avenues of controlling the bites of adult *Aedes aegypti*,

and one particular approach that appears to be gaining attention is an environmental management strategy that aims to reduce adult vector population by targeting their aquatic immature stages (i.e., mosquito eggs, larvae and pupae).

Among the biological control agents of mosquitoes, fishes are the most extensively used species in several countries since the beginning of the twentieth century.[7] Certain exotic fishes such as *Poecilia reticulata*, *Gambusia affinis* and *Oreochromis mossambicus* have been used in various ecological conditions in India for mosquito control.[8-10] Use of these exotic fish has raised environmental concerns in view of their suspected adverse effects on local aquatic fauna.[11] Consequently, fish fauna surveys and evaluation of larvivorous potential of native fishes have been of high research priority in the area of biological control of vectors of disease.

The goal of the current study was to evaluate the eating capacity of the larvivorous fish against larvae of *Aedes aegypti* in laboratory conditions in Benin, West Africa in a context where integrated vector control is necessary.

MATERIALS AND METHODS

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-western Benin and the study was carried out more precisely in Dogbo district (Fig. 1). The southern borders of this district are Lokossa and Bopa districts. The northern border is Djakotomey district. The eastern border is Lalo district and the western border of Dogbo district is Togo republic. Dogbo district covered 475 km^2 and belongs to geographic region of ADJA. The choice of the study site took into account the economic activities of populations, their usual protection practices against mosquito bites, and peasant practices to control farming pests. We took these factors into account to study the use of larvivorous fish against larvae of *Aedes aegypti* in laboratory conditions in Dogbo district in south-western Benin. Couffo has a climate with four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August). The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.

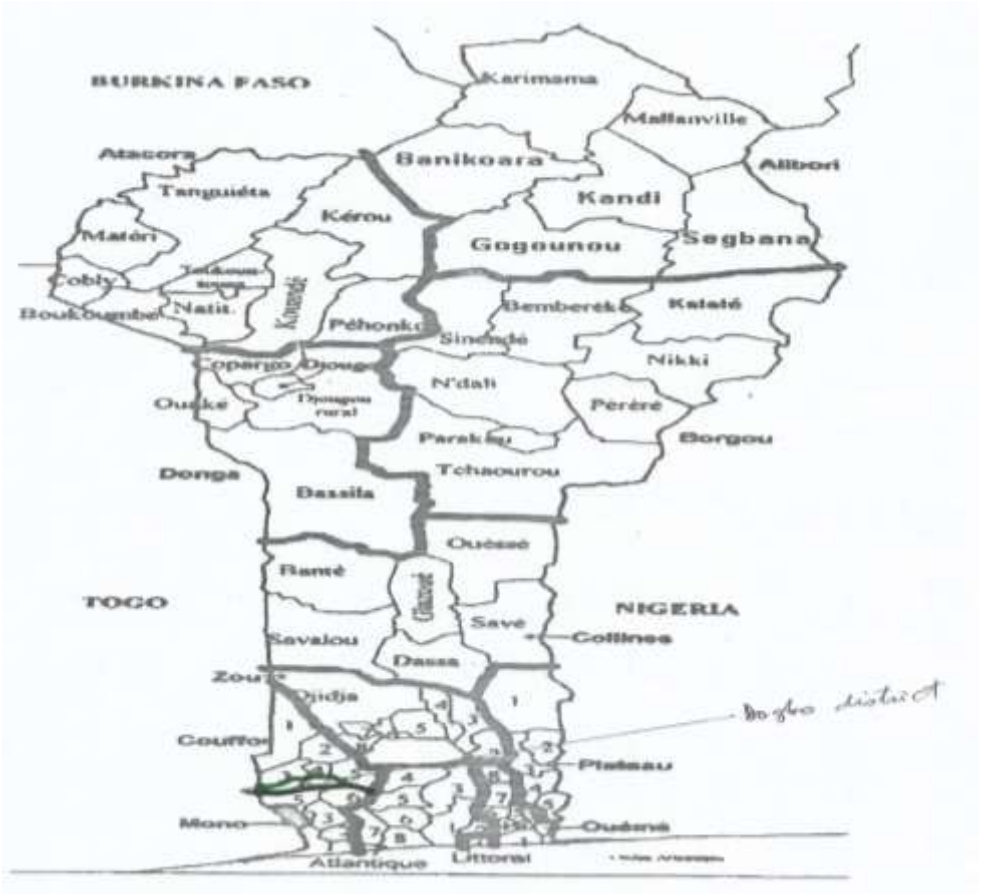

Fig. 1: Map of Republic of Benin showing Dogbo District.

Mosquito sampling

Aedes aegypti mosquitoes were collected from August to November 2023 during the small rainy season in Dogbo district. Larvae and pupae were collected from breeding sites using the dipping method and kept in labeled bottles. Adult *Aedes aegypti* from these breeding sites were previously identified. The samples were then carried out to the Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) of the Department of Sciences and Agricultural Techniques located in Dogbo district.

Orechromis niloticus fish collection

Alive *Orechromis niloticus* fishes were bought immediately once catched by fishers of Ganvié location in So-Ava district which southern border is Cotonou district. The habitations of Ganvié location are built on water. Fishing is a main activity of people. Then, *Orechromis niloticus* fishes bought were put in some jars contained water and carried by car from Ganvié location to the Laboratory (LaRPET) of the Department of Sciences and Agricultural Techniques located in Dogbo district in southern Benin.

Laboratory evaluation for larvivorous efficacy

The sample of fishes (*Orechromis niloticus*), indigenous larvivorous fishes were brought from their natural habitats from Ganvié location in So-Ava district in Atlantic department in southern Benin to the Laboratory (LaRPET) of the Department of Sciences and Agricultural Techniques located in Dogbo district in south-western Benin. To determine the natural propensity of the sample of *Orechromis niloticus* to prey upon mosquito larvae, laboratory evaluation was conducted on larvae of the vector mosquito specie, *Aedes aegypti* (Diptera: Culicidae). Two fishes of the same specie of *Orechromis niloticus* were released in five glass jars of same dimensions contained each 1 litre of water. A batch of 100 larvae of four instars reared in the insectary of the Laboratory (LaRPET) was added in each glass jar for the two fishes in the morning and larval consumption was observed every two hours. Total larval consumption was recorded at the end of 24 hours when all remainder larvae removed. A glass jar (without larvae) containing only two fishes of same specie of *Orechromis niloticus* were used as control for biological tests. The tests were repeated for three consecutive days to establish the maximum devouring capacity of the fishes when they were fed with fish food (without larvae) before tests comparatively to when they were unfed before tests.

Statistical analysis

Analysis using Fisher's exact test was performed to compare the maximum devouring capacity of the fishes when they were fed with fish food (without larvae of *Aedes aegypti*) before tests comparatively to when they were unfed.

RESULTS

Larva-eating capacity of fed Orechromis niloticus fish in the laboratory conditions

The eating capacity of fed larvivorous fish against larvae of *Aedes aegypti* in the laboratory conditions was showed in table 1. The analysis of this table showed that after the introduction of fed larvivorous fishes in each of the five glass jars, the number of larvae of *Aedes aegypti* was reduced. The maximum reduction was 79% whereas the minimum reduction was 43% and the mean was 61%.

Table 1: Reduction in the number of larvae in the glass jars after the introduction of fed larvivorous fish against larvae of *Aedes aegypti.* in the laboratory conditions.

	Number of larvae tested		
Number of glass jars	**Before larvivorous fish introduction**	**After larvivorous fish introduction**	**%Reduction**
Control	0	0	0
1	100	21	79
2	100	46	54
3	100	57	43
4	100	29	71
5	100	36	64

Larva-eating capacity of unfed Orechromis niloticus fish in the laboratory conditions

The eating capacity of unfed larvivorous fish against larvae of *Aedes aegypti.* in the laboratory conditions was showed in Table 2. The analysis of this table showed that after the introduction of unfed larvivorous fishes in each of the five glass jars, the number of larvae of *Aedes aegypti* was dramatically reduced. The maximum reduction was 81% whereas the minimum reduction was 51% and the mean was 66%.

Table 2: Reduction in the number of larvae in the glass jars after the introduction of unfed larvivorous fish against larvae of *Aedes aegypti* in the laboratory conditions.

	Number of larvae tested		
Number of glass jars	**Before larvivorous fish introduction**	**After larvivorous fish introduction**	**%Reduction**
Control	0	0	0

1	100	19	81
2	100	34	66
3	100	49	51
4	100	26	74
5	100	31	69

The analysis of Table 3 shows that there are many advantages in the use of lavivorous fish (*Orechromis niloticus* fish) to control mosquito larvae.

Table 3: Advantages and disadvantages of the use lavivorous fish.

Advantages	Disadvantages
Respects human health (by contrast to chemical control) Respects or preserves the environment (by contrast to chemical control) Local fishes such as *Orechromis niloticus* fish also have larva-eating capacity Local lavivorous fishes can be reared easily Exotic lavivorous fishes also can be used if their larva-eating capacity is higher than that of local lavivorous fishes	Difficulty to reach breeding sites in the field

Fig. 2: *Orechromis niloticus* fish.

DISCUSSION

Alternative biological control methods, especially the use of larvivorous fishes, can play a significant role in controlling of mosquito larvae. Some of larvivorous fishes are important predators of mosquito larvae. In fact, in the current study, after the introduction of fed larvivorous fishes in each of the five glass jars, the number of larvae of *Aedes aegypti* was reduced. In addition, after the introduction of unfed larvivorous fishes in each of the five glass jars, the number of larvae of *Aedes aegypti* was dramatically reduced. These results showed that larvivorous fishes ate more larvae of *Aedes aegypti*. when they were unfed than when they were fed. The presence of fish food in glass jars may play a role by limiting the eating capacity of the larvivorous fishes. In fact, because of the presence of fish food in glass jars, larvivorous fishes first tried to eat this food before eating larvae of *Aedes aegypti*. The results obtained after the introduction of unfed larvivorous fishes in glass jars showed that the larva-eating capacity of these fishes was high.

The use of lavivorous fish (*Orechromis niloticus* fish) to control mosquito larvae has many advantages. In fact, it respects the human heath (by contrast to chemical control). It also respects or preserves the environment (by contrast to chemical control). Local fishes such as *Orechromis niloticus* fish also have larva-eating capacity. Local lavivorous fishes can be reared easily. Exotic lavivorous fishes also can be used if their larva-eating capacity is higher than that of local lavivorous fishes. However, *Aedes aegypti* larvae breeding sites are difficult to be reached in the field.

In Dogbo district as in many other districts of Benin, there is the presence of breeding sites and there is no doubt that introduction of this larvivorous fish in these water sources will dramatically reduce the mosquito

population and hence malaria infection. Biological control using larvivorous fishes against larvae of *Aedes aegypti* has given good results in the current study and may be a very efficient method of tackling malaria. However, the field conditions are not the same as laboratory conditions. Even if very few studies were published in the country on the use of larvivorous fishes for malaria control, there are many reports elsewhere or in other countries. For example, larvivorous fish were part of an integrated control programme that succeeded in eradicating malaria from the Southwest Pacific.[12] Based on the results of similar studies on the indigenous larvivorous fish *Aphanius dispar* in Ethiopia and Djibouti, fish have been introduced on an operational scale for the control of malaria transmission on those countries.[13-14] It also was reported that under the laboratory conditions *A. dispar* was more successful than *G. affinis* in preying upon the III and IV instars and pupae, and that the two species could complement each other as mosquito control agents in different habitat conditions.[15] The results of the field trial showed that *A. dispar* is capable of controlling mosquito breeding in confined water bodies effectively within a fortnight of its application. An experimental study in Turkey showed high mosquito larval consumption by *A. chantrei* and recommended its use in biological control instead of *Gambusia* spp.[16]

In the current study, during the laboratory evaluation for larvivorous efficacy, otherwise when the biological tests were in progress in laboratory, all larvae which had pupated and all pupae which had emerged or become adult were taken into account in result recording. The results obtained in the current study in laboratory conditions are encouraging and must permit to envisage the implement of such a strategy to the whole of the territory of the Republic of Benin in order to measure its impact.

CONCLUSION

Less effort has been made using strategy to control larvae and pupae in breeding sites such as the use of larvivorous fishes in Benin. Biological control against larvae of *Aedes aegypti* using larvivorous fish has given good results in laboratory conditions in the current study. Biological control also preserves or respects environment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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