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Study the use of lavivorous fish against larvae of *Culex quinquefasciatus* (Diptera: Culicidae) in laboratory conditions in Dogbo district in South-western Benin, West Africa

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Abstract

The use of chemical insecticides causes important damages to environment and human health and there is a need to search for alternative solutions. This study aims to study the use of larvivorous fish against larvae of *Cx. quinquefasciatus* in laboratory conditions in Dogbo district in south-western Benin, West Africa. Larvae of *Cx. quinquefasciatus* mosquitoes were collected from breeding sites using the dipping method from August to November 2023 during the small rainy season in Dogbo district. Alive *Clarias gariepinus* 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. The results showed that larvivorous fishes ate more larvae of *Cx. quinquefasciatus* 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. Even if it is difficult to use larvivorous fishes against larvae of *Cx. quinquefasciatus* in field conditions, good results were obtained in laboratory conditions in the current study.

Keywords: Larvivorous fish, larvae of *Culex quinquefasciatus,* biological control, laboratory conditions, Benin

Introduction

C*ulex quinquefasciatus* Say, a member of the Culex pipiens group, is a medically important mosquito and major pest species with a worldwide distribution. C*ulex quinquefasciatus* is known to be a major vector of filariasis, St. Louis encephalitis virus (SLEV), West Nile virus (WNV), and Rift Valley Fever virus (RVFV). It is considered to be an opportunistic feeder, and while host choice is regionally variable, it feeds on many species of birds, mammals, and occasionally reptiles and amphibians.

Insecticides are often deliberately introduced into the mosquito habitat in the fight against the many human diseases they transmit (e.g. malaria, dengue fever, yellow fever and filariasis) (Lounibos, 2002). As a consequence mosquito control programs are now threatened by the selection of mosquito populations resistant to these chemical insecticides (Hemingway *et al*., 2002).

The strong level of pyrethroid-resistance in C*ulex quinquefasciatus* in Africa (Chandre *et al*., 1998) represents an obstacle to malaria prevention as people may not perceive the personal protective effect of ITNs if *Culex* fails to be killed.

High frequencies of resistance to permethrin, DDT and carbosulfan were recorded in *Cx. quinquefasciatus* (Corbel *et al*., 2007). In the urban area of Cotonou (Ladji and Asecna), *Culex* mosquitoes exhibited high *kdr* frequency and elevated levels of esterases and GST activity (5- to 7-fold higher than SLAB). The higher mortality rates observed with permethrin compared to DDT may be explained by the presence of an as yet unidentified additional resistance mechanism (e.g. “Leu-Ser” mutation) which might confer higher resistance to DDT than to permethrin (Ranson *et al*., 2000; Martinez-Torres *et al*., 1999). Since no cross-resistance has been detected between OPs and carbamates (very low frequency of *Ace.1* gene), the high survival rates observed with carbosulfan in all populations may be explained by the presence of detoxifying enzymes (Corbel *et al*., 2007). Indeed, elevated oxidases and esterases are responsible for carbamate resistance in many insect species (Grafton-Cardwell *et al*., 2004). No resistance to malathion was found in *Culex*, suggesting the absence of malathion-specific carboxyesterases in Benin (Corbel *et al*., 2007). Dieldrin resistance in *Cx. quinquefasciatus* shows the classical semi-dominance (Davidson, 1964). With dieldrin resistance at this high frequency, new insecticides that show cross-resistance to dieldrin (e.g. fiproles) are unlikely to find use in vector control (Corbel *et al*., 2007).

Current researches have to explore several alternative avenues of controlling the bites of adult *Cx. quinquefasciatus*, 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 (Gerberich and Laird, 1968). Certain exotic fishes such as *Poecilia reticulata, Gambusia affinis* and *Oreochromis mossambicus* have been used in various ecological conditions in India for mosquito control (Sitaraman *et al*., 1976; Chand and Yadav, 1994; Prasad *et al.,* 1994). Use of these exotic fish has raised environmental concerns in view of their suspected adverse effects on local aquatic fauna (Hulbert *et al*., 1972). 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 *Cx. quinquefasciatus* 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 *Cx. quinquefasciatus* 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.

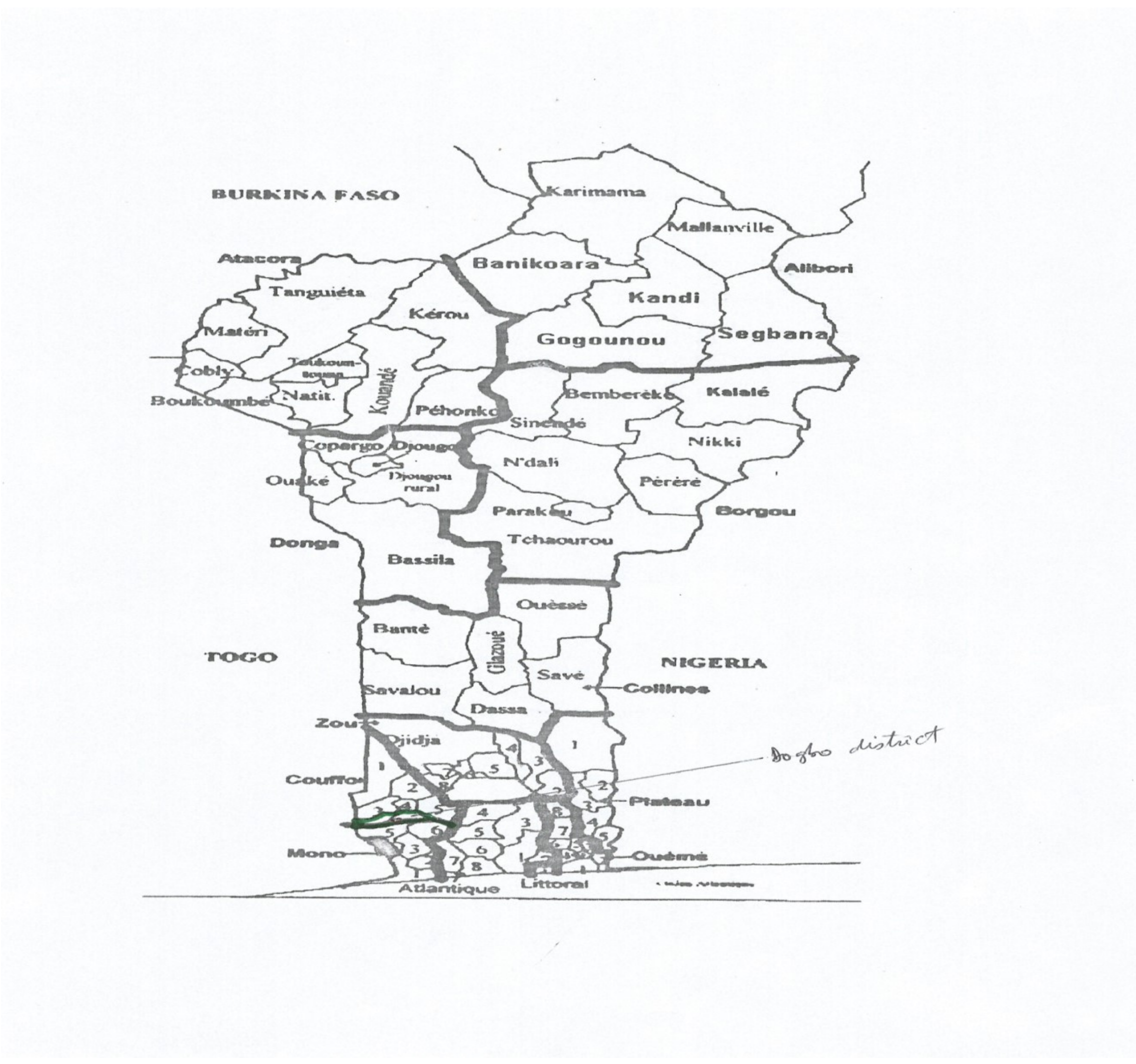

Figure 1: Map of Republic of Benin showing Dogbo District

Mosquito sampling

Cx. quinquefasciatus 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. 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 in Normal High School of Technical Teaching (ENSET) of Lokossa.

Figure 2: A breeding site of *Culex quinquefasciatus* larvae surveyed in Dogbo district

Clarias gariepinus fish collection

Alive *Clarias gariepinus* 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, *Clarias gariepinus* fishes bought were put in some jars contained water and carried by car from Ganvié location to the Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET).

Laboratory evaluation for larvivorous efficacy

The sample of fishes (*Clarias gariepinus*), 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 of Pluridisciplinary Researches of Technical Teaching (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 *Clarias gariepinus* to prey upon mosquito larvae, laboratory evaluation was conducted on larvae of the vector mosquito specie, *Cx. quinquefasciatus* (Diptera: Culicidae). Two fishes of the same specie of *Clarias gariepinus* 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 *Clarias gariepinus* 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.

Data 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 *Cx. quinquefasciatus*) before tests comparatively to when they were unfed.

Results and Discussion

Larva-eating capacity of fed *Clarias gariepinus* fish in the laboratory conditions

The eating capacity of fed larvivorous fish against larvae of *Cx. quinquefasciatus* 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 *Cx. quinquefasciatus* was reduced. The maximum reduction was 82% whereas the minimum reduction was 49% and the mean was 65.5%.

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

Number of glass jars	Number of larvae tested: Before larvivorous fish introduction	Number of larvae tested: After larvivorous fish introduction	%Reduction
Control	0	0	0
1	100	37	63
2	100	28	72
3	100	51	49
4	100	44	56
5	100	18	82

Larva-eating capacity of unfed *Clarias gariepinus* fish in the laboratory conditions

The eating capacity of unfed larvivorous fish against larvae of *Cx. quinquefasciatus.* 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 *Cx. quinquefasciatus* was dramatically reduced. The maximum reduction was 84 % whereas the minimum reduction was 58% and the mean was 71%.

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

Number of glass jars	Number of larvae tested		%Reduction
	Before larvivorous fish introduction	After larvivorous fish introduction	
Control	0	0	0
1	100	32	68
2	100	21	79
3	100	42	58
4	100	36	64
5	100	16	84

The analysis of Table 3 shows that there are many advantages in the use of lavivorous fish (*Clarias gariepinus* 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 *Clarias gariepinus* fish also have larva-eating capacity Local lavivorous fishes can be reared easily	Larvae of *Culex quinquefasciatus* breeed in dirty or polluted water (sump water, gutter water…) where lavivorous fishes cannot live easily
Exotic lavivorous fishes also can be used if their larva-eating capacity is higher than that of local lavivorous fishes	

Figure 3: *Clarias gariepinus* fish

Mosquitoes are responsible for the transmission of many pathogens such as malaria, yellow fever, dengue fever, and so on. The control of mosquitoes using chemical insecticides is not always a sensible approach, so, 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 *Cx. quinquefasciatus* was reduced. In addition, after the introduction of unfed larvivorous fishes in each of the five glass jars, the number of larvae of *Cx. quinquefasciatus* was dramatically reduced. These results showed that larvivorous fishes ate more larvae of *Cx. quinquefasciatus* 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 *Cx. quinquefasciatus*. 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 (*Clarias gariepinus* 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 *Clarias gariepinus* 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, larvae of *Culex quinquefasciatus* breed in dirty or polluted water (sump water, gutter water…) where lavivorous fishes cannot live easily.

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 *Cx. quinquefasciatus* 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 (Kaneko *et al.*, 2000). 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 (Fletcher *et al*., 1993; Louis and Albert, 1988). 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 (Homski *et al.,* 1994). 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 (Yildirim and Karacuha, 2007).

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. It is worth mentioning that larvae of *Cx. quinquefasciatus* live in polluted waters as their breeding sites.

Conclusion

The use of larvivorous fishes against larvae of *Cx. quinquefasciatus* has given good results in laboratory conditions in the current study. However, researches must also be carried out in field conditions in a context where it is useful to search for alternative solutions to damages cause by chemical insecticides to environment and human health.

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