EVALUATION OF THE EFFECT OF INSETICIDES ON THE INTESTINAL MICROBIOTA OF *Culex quinquefasciatus*

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José Márcio Gomes Fernandes
UEMG – Universidade do Estado de Minas Gerais
Divinópolis – Minas Gerais.

Adriano Guimarães Parreira
UEMG – Universidade do Estado de Minas Gerais
Divinópolis – Minas Gerais.

Stênio Nunes Alves
UFSJ – Universidade Federal de São João Del Rey
Divinópolis – Minas Gerais.

ABSTRACT: Mosquitoes are insects from the Culicidae family and, mostly, are blood feed. The *Culex* genre has big sanitary importance in Brazil, being vector of filariosis and, recently, related as capable of transmission of Zika Virus. They are in the all country and to fight with them, insecticides are the most common way used. But, an interesting alternative way to combat the diseases transmission from these species is the use of endosymbiont bacteria, in some cases, capable of blocking the virus transmission. For this, the present work intends to identify the effect of insecticides over the microbiota of the intestinal tract of *Culex quinquefasciatus*. Initially, traps were built to obtain larvae samples and, after that, expose to Temephos and Deltamethrin insecticides, in different concentrations and time change. The intestinal tract was cleanly taken with the help of a microscopy. After that, the material was triturated and plaque in agar – MH, to obtain bacterial colonies to identify. It is highly notice that there is influence of the concentration and exposure time and the used insecticide over the living intestinal microbiota in these insects. This represents an important register of the influence from those chemistries under the microorganism's populations.

KEYWORDS: Bacteria; Culicidae; disease; vector.

RESUMO: Mosquitos são insetos da família Culicidae, em sua maioria hematófaga. O gênero *Culex* tem grande importância sanitária no Brasil, sendo transmissor da filariose e, recentemente, relatado como transmissor do Zika Vírus. Disseminada por todo o país, a prática do uso de inseticidas é a mais comumente empregada para o controle da proliferação de mosquitos. Interessante alternativa de controle da transmissão de doenças provocadas por estes seria a utilização de bactérias endosimbiontes, muitas delas capazes de bloquear a transmissão de diversos vírus. Contudo, não se sabe ainda o efeito exercido pelos inseticidas tradicionalmente utilizados sobre a microbiota intestinal daqueles insetos. Deste modo, o presente trabalho buscou avaliar o efeito de inseticidas sobre a microbiota do trato intestinal de espécimes de *Culex quinquefasciatus*. Inicialmente, foram montadas cubas ao ar livre para a obtenção das larvas, em seguida foram expostas aos inseticidas Temefós e Deltametrina, em concentrações e em intervalos de tempo variados. O trato intestinal foi assepticamente retirado e o material obtido triturado e plaqueado em meio Agar MH para posterior identificação das espécies bacterianas. Com base nos resultados encontrados percebe-se que há influência da concentração, tempo de exposição e do inseticida utilizado sobre a microbiota residente no trato intestinal dos insetos, com diversidade de situações observadas. Isso representa importante registro da influência daqueles químicos sobre a população de microrganismos.

PALAVRAS-CHAVE: Bactéria, Culicidae, doenças, vetor.

1 | INTRODUCTION

The presence of microorganism in mosquitoes gut has been of interest for many researches, since they have a beneficial symbiotic relationship to their host. This relationship is associated with dietary supplementation, adaptation to environment variations, homeostasis and immune system control, which may, for example, block the transmission of microorganisms during blood repast (MORAN et al, 2008). The microorganism alto presents themselves with contributions for digesting process, nutrition, growing, reproduction, immunity, behaving and resistance to a variety pathogenic organisms action, mostly against natural enemies that present a threat to the specie (MORAN et al, 2008).

Studies have shown that the presence of a variety of microorganisms into vector insects, in a more specific case the Culicidae family, has inhibited the sporogonic development of some parasites ceasing transmission of the same to the insect (DONG et al, 2009). The gut lumen of mosquitoes has been shown to contain a diverse population of bacteria that varies depending on the sex, developmental stage and ecological factors of the mosquitoes. A recent study showed that the predominant bacterial phylum present in *Aedes*, *Anopheles* and *Culex pipiens* was Proteobacteria, and the most common bacterial class was the *Gammaproteobacteria* (Minard et al., 2013). The presence of bacteria in the midgut could induce immune activation by producing specific compounds that directly interact with pathogens, such as antiviral compounds.

Mosquito lineages obtained in field and in laboratories have a big association from microbiota to the insect’s intestine, consisting mainly in Gram-negatives bacteria, members of the Enterobacteriaceae family (ORFANÓ, 2012).

In studies carried out by Gusmão et al. (2010) the main genres found in intestinal tract of *A. aegypti* were *Serratia*, *Klebsiella*, *Asaia*, *Bacillus*, *Enterococus*, *Kluyvera* and *Pantoea*. Already in studies evaluating intestinal microbiota of *A. aegypti* it was detected the prevalence of Proteobacteria, Actinobacteria and Firmicutes, where during the growing, it was noticed an increase of firmicutes. These divergences in both works confirmed that bacteria composition is different to one mosquito to another for influence of several factors, like the habitat (JARUSEVICIUS, 2013).

In studies evaluating intestinal microbiota diversity of the Anopheles, in the early days of adult life was observed predominance from Proteobacteria, Actinobacteria and Firmicutes with a progressive increase of Bacteroidetes (WANG et al, 2011). Were identifie in *Anopheles gambiae* and *An. funestus* field populations 16 species of bacteria, from 14 genres (ORFANÓ, 2012). In laboratory populations of *An. gambiae* and *An. sthephensi* were identified a several variety of bacteria, mostly of the genres *Asaia*, *Enterobacter*, *Mycrobacterium*, *Sphingomonas*, *Serratia* and *Chryseobacterium* (FAVIA et al, 2007 & DONG et al, 2009).

In the gut of *Culex quinquefasciatus*, Vasanthi and Hoti (1992), Kim et al. (2015) and Chandel et al. (2013) found different genus as *Acinetobacter*, *Aeromonas*, *Bacillus*, *Enterobacter*, *Enterococcus*, *Klebsiella*, *Listeria*, *Pantoea*, *Pseudomonas*, *Serratia*, *Shigella*, *Staphylococcus* and *Stenotrophomonas*. Gao et al. (2018) demonstrated that organophosphate malathion perturbs the gut microbiome development trajectory and quorum sensing, as well as related physiological processes such as motility and pathogenicity.

However, little is known about the relationship of these microorganisms and insecticides used against mosquitoes. In this sense, the present study aimed to evaluate which bacteria genus are present in the intestine of *C. quinquefasciatus* larvae after exposure to different concentrations of temephos and deltamethrin.

2 | METHODOLOGY

2.1 Mosquito

C. quinquefasciatus larvae were obtained from a laboratory culture, as previously described by Gerber (1979).

2.2 Exposure of the larvae to the insecticide

In this study, 4th instars larvae of *C. quinquefasciatus* were exposed for 16 h to 0,01 µg/L, 0,1 µg/L e 1 µg/L of the organophosphate, temephos (Fersol 500CE), and 0,5 µg/L, 5 µg/L e 50 µg/L of the pyrethroid, deltamethrin (Fersol 25CE). For each insecticide tested, the larvae were divided into groups consisting of 20 specimens as well as the control group, which three replications each treatment. The control group larvae were exposed to dechlorinated water. After the exposure time, larvae were washed and transferred to other plastic recipients containing dechlorinated water. The temperature was maintained at 26±1 °C throughout all of the tests.

2.3 Dissection of the gut of mosquitoes, microbiological isolation and identification of the prevalence of bacteria

30 live larvae were selected for dissection and subsequent isolation of gut bacteria.

All the dissections were performed under Zeiss stereomicroscope (Model: Stemi 508). Prior to dissection, all larval samples were disinfected with 75% ethanol for 5 min, followed by washing with phosphate buffered saline (PBS) twice. All dissections were performed under sterile conditions and gut sections were homogenized separately in 100 µl PBS, and stores in Eppendorf tubes with PBS solution under refrigeration, for later use. Then plating and obtaining of bacterial isolates were removed under aseptic. Plating in Agar-Mueller Hinton (Himedia®) media isolated the cultivable microorganisms and pure colonies obtained were sent to identification, by mass spectrometry. The identification of the prevalence of bacteria occurred in the Veterinary School of the Federal University of Minas Gerais.

3 | RESULTS

Table 1 shows the genus identification of gut bacteria of *C. quinquefasciatus* larvae after exposure to different concentrations of temephos and deltamethrin.

Insecticide	Treatment/Bacteria Genus			
	Control	0,01 µg/L	0,1 µg/L	1 µg/L
Temephos	*Lactobacillus* Microbacterium Listeria Bacillus Staphylococcus	*Lactobacillus* Microbacterium Listeria Serratia	*Lactobacillus* Sphingomonas	Bacillus
	Control	0,5 µg/L	5 µg/L	50 µg/L
Deltamethrin	*Lactobacillus* *Microbacterium* *Listeria* Bacillus Staphylococcus	*Staphylococcus* *Bacillus*	*Staphylococcus* *Bacillus*	*Microbacterium*

Table 1. Bacteria genus found in the gut of *C. quinquefasciatus* larvae after exposing to temephos and deltamethrin.

It is observed that the insecticides as well as the dose collaborate directly to diminish the diversity of the intestinal microbiota of the larvae of *C. quinquefasciatus*, favoring the prevalence of certain genera in front of the concentration of insecticide that the larvae were exposed. It is also observed that the insecticide and its concentration favor the proliferation of certain genus.

Figure 1 shows that the genus Lactobacillus is prevalent in the gut of mosquito larvae after exposure to temephos. While the genus *Staphylococcus* was the most prevalent after exposure to deltamethrin (Figure 2).

The two figures show that the prevalence of *Staphylococcus* was reduced to about 10 times when the larvae were exposed to temephos. Indicating that this insecticide impairs the development of this microorganism in the mosquito intestine. However, there is a greater diversity in the prevalence of bacterial genera in the intestine of larvae exposed to temephos.

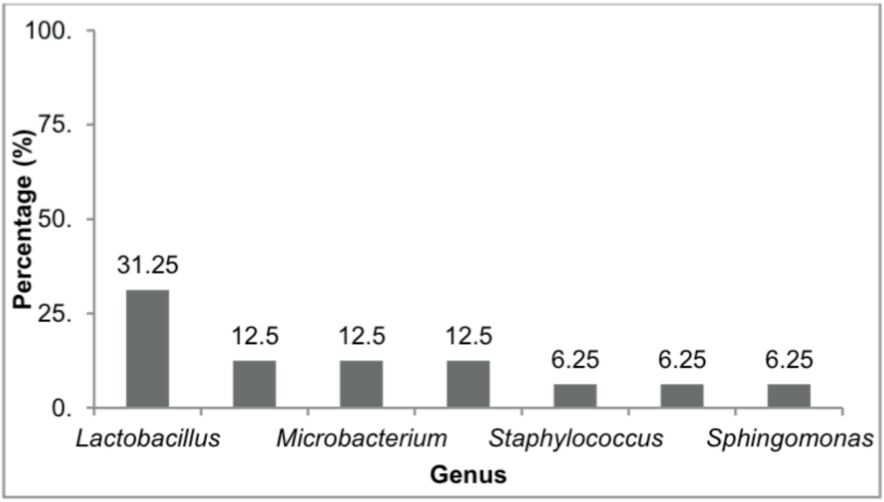

Figure 1. Prevalence of genus found in the gut of *C. quinquefasciatus* larvae after exposure to different concentrations of temephos

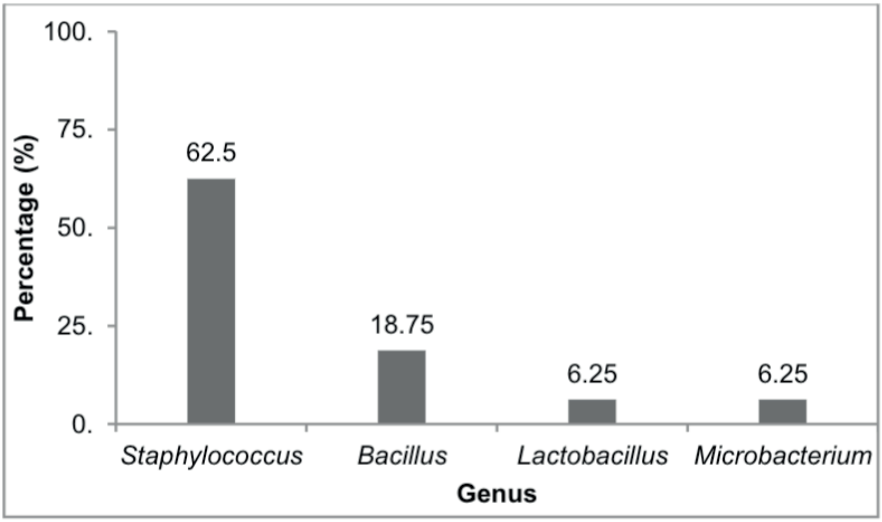

Figure 2. Prevalence of genus found in the gut of *C. quinquefasciatus* larvae after exposure to different concentrations of deltamethrin

4 | DISCUSSION

The results found in this work for control group, are similar to those of Alves et al. (2010), which where related 17 bacterial species of the gut of 13 species of the Culicidae family. Similar genuses to this work were *Bacillus* and *Staphylococcus*. Although there are some disagreement with other species and genus found, factors such as collect place, feeding and exposure to insecticides or cannot explain the observed differences. Others studies, also shown similar genus in the gut of mosquito (Vasanthi and Hoti, 1992; Chandel et al., 2013; Kim et al., 2015). The gut community ecology remains poorly investigated from the standpoint of mosquito interactions within its natural environment and insecticides.

The larvae exposed to different concentrations of insecticides showed a smaller diversity of the microbiota, this could be caused by the change of pH, redox conditions and digestive enzyme present (Dillon & Dillon, 2004; Alves et al., 2010). The insecticides may favor the resistance or susceptibility of mosquitoes to viruses. In this sense, Chadran et al. (2013) have show that bacterial communities may differ between dengue virus-resistant and susceptible *A. aegypti* strains.

Still in this sense, Guedes et al. (2017) observed that *C. quinquefasciatus* were capable of Zika virus transmission when artificially feed by infected blood. This fact shows the importance of study and evaluates ways to control the transmission on that vector, with emphasis on the knowledge of living microbiota and its inter-relations with pathogenic microorganisms, as well as insecticide effects most used under the same. Xi et al. (2008) eliminated gut bacteria using antibiotic treatment and found that the replication of DENV in *A. aegypti* was accelerated, suggesting that the mosquito's endogenous microbiota stimulated a certain level of antiviral gene expression.

In addition, the bacteria may be candidates for paratransgenesis in mosquitoes, since in one study *Bacillus cereus* was genetically modified to produce the larvicidal protein of *Lysinibacillus sphaericus* (Luxananil et al., 2003).

The shifts in the microbiota of larvae exposure to insecticide may to predict the influence of functional and metabolic profile of the gut microbial community, since this was observed in adult females of *A. aegypti* (David et al., 2016).

Moreover, it is known that there is a benign relationship between arbovirus and its vectors, implicated in interactions between microbial communities, parasites and immunity (Dillon & Dillon, 2004). However, the effect of insecticides on the bacterial community and the relation of arbovirus competence of the insect gut have been ignored. It was found only a correlate study that proves the role of midgut symbiotic bacteria in resistance of *Anopheles stephensi* (Diptera: Culicidae) to organophosphate insecticides (Soltani et al., 2017). This becomes relevant because, recent studies has proposed the use of bacteria for the biological control of mosquitoes. However, they did not analyze the effect that the different insecticides used for the chemical control of mosquitoes cause to the microbiota and even the relation of the effects on the mosquitoes.

Although we did not identify any specific genera that were uniquely or consistently associated with insecticide in *C. quinquefasciatus*, results indicate that pesticide exposure could will significantly disrupt populations. Thus, even at lower doses the change in the composition of the microbiota could therefore impact the mosquito biology and ecology. Zouache et al., (2012) demonstrated an increase in *Enterobacter* together with a decrease in *Wolbachia* relating Chikungunya virus (CHIKV) and *A. albopictus*. Zink et al. (2015) relating West Nile virus with *C. pipiens* showed that increased diversity is not associated with an overall increase in microbial load, but rather a change in composition.

It should be noted that the *Serratia* genus was present in larvae exposed in the lowest concentration of temephos, an insecticide recommended by the World Health Organization to combat mosquitoes. However *S. odorifera* have been shown to enhance susceptibility of *A. aegypti* to both CHIKV and Dengue virus with co-feeding experiments (Apte-Deshpande et al., 2012; Apte-Deshpande et al., 2014).

It is important to emphasize that insecticides compromise the biological development of larval and that the gut microbiota can affect vector competence larval growth, adult fitnes and therefore the prevalence of diseases (Strand, 2018).

Thus, further investigation of the intimate relationship between gut bacteria and the mosquito is necessary, because our study shows that in the presence of insecticides there is a modification of the prevalence of certain genera in the gut of *C. quinquefasciatus* larvae. This alteration may also influence the development of larvae and, therefore, the relation of microbiota, arbovirus and the transmission of diseases by mosquitoes.

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