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ISOENZYMES OF *Aedes Aegypti* FOUND IN BAIXADA CUIABANA, MATO GROSSO, BRAZIL¹

ISOENZIMAS DE *Aedes Aegypti* ENCONTRADAS NA BAIXADA
CUIABANA, MATO GROSSO, BRASIL

ISOENZIMAS DE *Aedes Aegypti* ENCONTRADAS EN BAIXADA
CUIABANA, MATO GROSSO, BRASI

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ABSTRACT

The infestation of arbovirus vectors results from a combination of human population growth, urbanization in tropical areas and a great expansion of the geographic distribution of *Aedes aegypti* due from climate change, movement of people and the weakness of public policies. The objective of this research was to identify the populations of this vector in urban areas of four municipalities in the Baixada Cuiabana, using ovitraps, captured between October 2015 and November 2016. The electrophoresis technique was used to investigate the expression of esterase in biological samples of the *Ae. aegypti* in different environments. Esterases are involved in the development of resistance to chemical compounds in several insects, including the genus *Aedes*. The staining intensity of the gels was classified as “expressed bands”, with weaker staining; and “overexpressed bands”, with a darker color, indicative of the stronger presence of esterases. Mann-Whitney test analyzes the frequency of bands in the population of *Ae. aegypti* showed an increase in expression, between seasons and locations, whose vector can be more adapted under selection pressures related to the rainfall regime. An important factor in the higher frequency of overexpression was related to the beginning of the rains, in November/2016. Variations in the expression of bands were conclusive for populations considered to be polymorphic, which denotes an intensification of the campaign to combat this vector, focusing on the elimination of mosquito breeding sites that reduce its population dynamics.

KEYWORDS

Culicidae, esterase, expressed bands, vector.

RESUMO

A infestação de vetores de arbovírus resulta de uma combinação de crescimento da população humana, urbanização em áreas tropicais e uma grande expansão da distribuição geográfica do *Aedes aegypti* decorrente das mudanças climáticas, circulação de pessoas e da fragilidade das políticas públicas. O objetivo desta pesquisa foi identificar as populações desse vetor em áreas urbanas de quatro municípios da Baixada Cuiabana, por meio de ovitrampas, capturadas entre outubro de 2015 e novembro de 2016. A técnica de eletroforese foi utilizada para investigar a expressão da esterase em amostras biológicas do mosquito *Ae. aegypti* nos distintos ambientes. Esterases estão envolvidas no desenvolvimento de resistência a compostos químicos em diversos insetos, incluindo o gênero *Aedes*. A intensidade da coloração dos géis foi classificada como “bandas expressas”, com coloração mais fraca; e “bandas superexpressas”, com uma cor mais escura, indicativa da presença mais forte de esterases. As análises pelo teste de Mann-Whitney da frequência das bandas na população de *Ae. aegypti* mostraram um aumento na expressão, entre as estações e locais, cujo vetor pode ser mais adaptado sob pressões de seleção relacionadas ao regime de chuvas. Um fator importante na maior frequência de superexpressão esteve relacionado com o início das chuvas, em novembro/2016. As variações na expressão de bandas foram conclusivas para as populações consideradas como polimórficas, o que denota uma intensificação da campanha de combate desse vetor, com enfoque na eliminação de criadouros do mosquito que reduzam sua dinâmica populacional.

PALAVRAS-CHAVES

Bandas expressas, Culicidae, esterase, vetor.

RESUMEN

La infestación de vectores de arbovirus resulta de una combinación de crecimiento de la población humana, urbanización en áreas tropicales y una gran expansión de la distribución geográfica de *Aedes aegypti* debido al cambio climático, el movimiento de personas y la debilidad de las políticas públicas. El objetivo de esta investigación fue identificar las poblaciones de este vector en áreas urbanas de cuatro municipios de la Baixada Cuiabana, utilizando ovitrampas, capturadas entre octubre de 2015 y noviembre de 2016. La técnica de electroforesis se utilizó para investigar la expresión de esterasa en muestras biológicas de *Ae. aegypti* en diferentes ambientes. Las esterases están involucradas en el desarrollo de resistencia a compuestos químicos en varios insectos, incluido el género *Aedes*. La intensidad de tinción de los geles se clasificó como “bandas expresadas”, con tinción más débil, y “bandas sobreexpresadas”, con un color más oscu-

ro, indicativo de la presencia más fuerte de esterasas. Los análisis de prueba de Mann-Whitney de la frecuencia de bandas en la población de *Ae. aegypti* mostró un aumento en la expresión, entre estaciones y localizaciones, cuyo vector puede adaptarse debajo presiones de selección relacionadas con el régimen de lluvias. Un factor importante en la mayor frecuencia de sobreexpresión estuvo relacionado con el inicio de las lluvias, en noviembre/2016. Las variaciones en la expresión de bandas fueron concluyentes para poblaciones consideradas polimórficas, lo que denota una intensificación de la campaña de combate a este vector, enfocada en la eliminación de criaderos de mosquitos que reducen su dinámica poblacional.

PALABRAS CLAVE

Culicidae, bandas expresadas, esterases, vector

1 INTRODUCTION

The physical and natural conditions resulting from factors related to climate change, air pollution, loss of biodiversity, water, air and soil degradation are the most important indicators that impact the human health populations in the world. The environmental impacts resulting from the lack of infrastructure resulting from urbanization can pose risks to human health and result in the proliferation of different types of insects, including vectors that cause important arboviruses. Ineffective sanitation can lead to waterborne diseases and enable the reproduction of vectors of many diseases (ALMEIDA *et al.*, 2020). An example is the *Aedes aegypti* mosquito (Diptera: Culicidae - Linnaeus, 1762), primary vector for viral diseases, such as dengue, yellow fever, Chikungunya and Zika (CHRISTOFFERSON, 2016), conditioning epidemic mainly due to pollution of the urban environment.

Factors associated with the *Aedes* infestation are the intense flows of people to the urban perimeter, concentration of degraded areas (RODRIGUES; SILVA, 2018), poor basic sanitation, lack of water supply, and insufficient or inadequate garbage collection. These create potential conditions for the development of potential vectors. Its control remains the efficient method to prevent the transmission of the dengue virus and other diseases, such as yellow fever (XAVIER *et al.* 2017).

The dengue burden has expanded for decades and now affects more than 120 countries (PANG *et al.*, 2017). Viruses of this disease have spread rapidly throughout the tropical regions of the world in recent decades and today, its transmission is observed in the Americas, Southeast Asia, Western Pacific, Africa and in non-endemic areas of the USA and Europe (MARQUES-TOLEDO *et al.*, 2019).

Arbovirus vector infestation may result from a combination of human population growth, urbanization in tropical areas and a large expansion of the geographic distribution of *Ae. aegypti*. (MAYER *et al.*, 2017). The elimination of breeding sites to reduce the density of vectors and the consequent reduction in the transmission of the disease also depends on the population's housing and education

conditions. The ideal is to sustainably eliminate the mass reproduction of the mosquito or reduce the potential for transmission of infections of public health concern (OPAS, 2019).

In addition to climatic aspects, such as temperature and rainfall, other social factors (MORIYAMA; NUMATA, 2019) that may also be associated with the increase in the population of insects is the disposal of garbage (SOBRAL; SOBRAL, 2019). Strategies to combat *Ae. aegypti* can be integrated with environmental management programs and their control has been an important challenge in developing countries (ZARA *et al.*, 2016), which takes into account the socio-environmental and socio-economic peculiarities of each region.

The rain regime and duration directly influence the occurrence of cases in the beginning of the warmest and humid period, which is characteristic of tropical climates and the rapid adaptability of the mosquito contribute to the difficult monitoring (KRAEMER *et al.*, 2015). An efficient method of capture, with a quantitative diagnosis of infestation, is the use of ovitraps, which has shown to be a promising strategy in the state of Mato Grosso (MIYAZAKI *et al.*, 2009; BUTAKKA *et al.*, 2019).

Although vaccines are currently being tested, some control measures, such as mosquitoes infected with the wMel strain of *Wolbachia pipientis*, like the newest one, make them less susceptible to dengue virus infection than wild *Ae. aegypti* (UTARINI *et al.*, 2021). Limiting reproduction or reducing mosquito survival is a good strategy (OPAS, 2019). Another strategy for observing mosquito control is to explore genetic variability in vector populations. The ability to adapt is often due to mutations in genes (WULIANDARI *et al.*, 2020), which changes their physiological and behavioral characteristics. Thus, it becomes necessary to investigate genetic variability and the identification of *Ae. aegypti* isoenzyme expression provides a more effective control (TYAGI *et al.*, 2017).

Esterase, such as carboxylesterase and cholinesterase are involved in the development of resistance in several insects, including the genus *Aedes*. This resistance mediated by acetylcholinesterases may involve the occurrence of one or more point mutations in the structure of the gene encoding this isoenzyme, as demonstrated in populations of *Ae. aegypti* (PAIVA *et al.*, 2016).

The molecular bases for the expression of esterase have challenged researchers, since the publication of the *Ae. aegypti* genome and variability in these isoenzymatic patterns allows the identification and characterization of genomic variability (MATTHEWS *et al.*, 2018). Esterase isoenzymes have multifunctional hydrolytic activities and catalyze the hydrolysis of a significant number of esters. In insects, they are associated with several important metabolic functions, such as controlling levels of juvenile hormone and biochemical mechanisms (GIGLIOLLI *et al.*, 2011).

The present research aims to carry out a molecular study of populations to present evidence that there are genetic variations in the vector population and to point to the need for diversified control strategies for a better use of existing methods. In this context, this exploratory descriptive work sought to identify and biochemically characterize the expression of the esterase isoenzyme of this species in different locations, months and seasonal phases.

Butakka and collaborators (2019) found an increase in enzyme expression in rainy periods in two municipalities in the state of Mato Grosso. In other samples from *Ae. aegypti*, we reinforce the hypothesis that in rainy periods there is a higher frequency of expression of esterasic bands of populations of this vector in different municipalities.

2 METODOLOGY

The procedure of this research was the capture of eggs from populations of *Ae. aegypti* mosquito, produced by oviposition in ovitraps in urban areas of four municipalities in the Baixada Cuiabana region. The ovitraps were installed every 15 days with 3 replicas at each sampling point from domiciliary and peridomiliary areas of Cuiabá (CB), Várzea Grande (VG), Chapada dos Guimarães (CG), and Santo Antônio do Leverger (SA), central-southern region of Mato Grosso, Brazil, between October 2015 and November 2016. We selected such regions due to their higher human population movement (CB and VG), distinct geographical position and altitude (SA, at about 100 m, and CP, at about 700 m above sea level).

The geographic co-ordinates of each sampling location, altitude of every municipality, population size and density are shown in Table I, according to information from the Brazilian Institute of Geography and Statistics (IBGE, 2017).

The specimens of *Ae. aegypti* were captured using ovitraps, oviposition traps designed by the classic work of Fay and Eliason (1966) and has been shown to be a more sensitive, inexpensive and fast method for monitoring *Aedes* colonizers in breeding sites (DEPOLI *et al.*, 2016). The ovitrap consists of a 9x12 cm black 580 mL plastic container with a 13.5x2.5 cm Eucatex® reed, whose rough part is facing outward for oviposition. 270 mL of an aqueous solution and 30 mL of straw infusion are added for female attraction and subsequent oviposition. At each point, three traps were placed at a maximum height of 1.5 m, in busy areas with heavy congestion of people, with an ovitrap at each point and 12 samples in the capture. The ovitraps were installed every 15 days and remained for 5 days for oviposition. Subsequently, they were collected and the material sent to the laboratory for egg counting, hatching and larval development.

The work in the laboratory consisted primarily of counting the eggs performed on the straw through a stereoscopic microscope. To certify the presence of *Ae. aegypti*, the breeding was done in small aquariums (500 mL of water), covered by a fillet net as a biosafety to prevent escape at the time of emergence of adults.

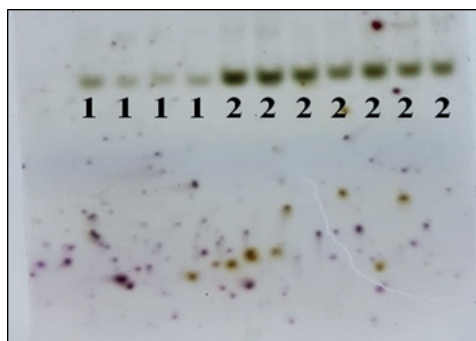
The eggs were treated in the laboratory at a constant temperature of $26\text{ °C} \pm 1.3\text{ °C}$. After the spontaneous hatching of the eggs, the larvae were fed with 100 mg of fish feed was placed as food until the pupae developed. This treatment was only for the production of organisms until the adult stage.

After reaching the adult stage, each individual was sucked into a container, identified and stored separately in a polypropylene tube, labeled and frozen in a freezer at a temperature of approximately -20 °C . The electrophoresis technique was used, which aims to investigate the expression of esterase in biological samples. This technique was used by Lima and collaborators (2021), to assess the total mosquito extract. Mosquitoes were macerated in bromophenol-glycerol blue sample buffer to expose the proteins to an electrical current. The molecules migrate through the gel towards the less anodic pole, separating the isoenzymes according to their molecular weight and/or electrical charge.

The sample “*n*” for the analysis of esterases refers only to the number of mosquitoes in which the molecular analyzes were performed, which corresponds to $81 \pm 12\%$ of the total number of mosquitoes produced. An analysis was performed for each mosquito individually to investigate through molecular analysis whether each organism had expressed bands or overexpressed bands in esterases.

The following molecular analysis method was in accordance with the model by Paiva and collaborators (2016). The polyacrylamide gel preparation consists of a 10% Gel composition associated with 29% acrylamide, 1% bis-acrylamide and tris gel buffer at alkaline pH 8.8. 20 µl TEMED and 10% ammonium persulfate were added to polymerize and give a final volume of 25 mL of gel. The polyacrylamide gel plates were placed in the electrophoresis apparatus, with up to 11 individuals in each electrophoresis plate, with one individual in each locus or band (Figure 1). A run on vertical electrophoresis was performed in 0.1 M Tris-Glycerin Buffer, pH 8.3, refrigerated for three hours, at 200V.

Figure 1 – Electrophoretic gel of *Aedes aegypti* mosquitoes stained with RR-Salt and Alpha isoesterase, with identification of the bands and expression of the esterase enzyme, by means of the intensity (expressed bands (1), lighter tones, overexpressed bands (2) darker shades)



Source: Research Data.

To check the color of the esterase isoenzyme, the samples were stained with alpha naphthyl acetate and RR-Salt, diluted in acetone and phosphate buffer, then placed in an oven at about 37°C, with no light for one hour. The staining intensity of the gels was classified as “expressed bands” (1), with weaker staining; and “overexpressed bands” (2), with a darker color, indicative of the stronger presence of esterases (Figure 1).

The data were tested for normality, using Shapiro-Wilk. The latter had greater robustness, with proof of non-normal distribution. The mean values were taken using the Mann-Whitney non-parametric test, comparing two independent samples repeatedly and to identify the trend and significance of the expression of the bands between populations of the four municipalities. The values of the expression of the esterase bands of mosquitoes in the municipalities were considered in full for statistical analysis using the non-parametric Mann-Whitney test. The results of the 8-month sampling were analyzed to identify differences using the Kruskal-Wallis test and this was also used to verify the significance in the frequency of expression of esterase bands between different months and between seasonal phases. From the number of individuals of *Ae. aegypti* (n) identified during the months and sampling sites (municipalities), the “sites”, “months” or “phases” factor was considered as the independent predictor variable while the esterase factor was the dependent variable.

The calculations were based on the average number of expression of *Ae. aegypti* esterases. specimens (*n*), with the variability of your results analyzed during the months [considering the months and seasonal phases (i.e. Drought- July to October; Flood - November and December; Full - February and March; and Ebb - May and June)] and sampling sites (municipalities), as independent predictor variables, and the esterase as dependent variables.

3 RESULTS

The parcel of *Ae. aegypti* analyzed for differential expression between the predictors was 537 individuals, of which 423 were positive for alpha-esterase and were distributed among the four sampling sites. Of these, 393 (73%) samples had expressed bands and 144 (27%) samples had overexpressed bands. Geographical coordinates, human demographic density and the number of individuals of *Ae. aegypti* registered in each municipality are shown in Table 1. The “n” only refers to the number of molecular analyzes for each individual.

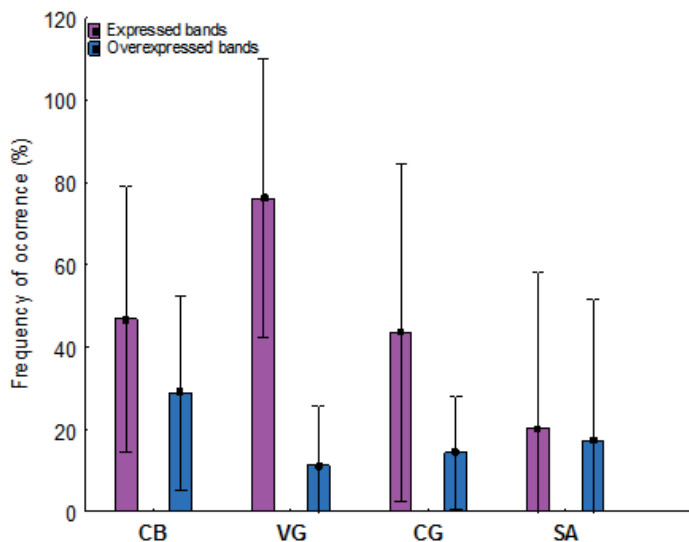
Table 1 - Geographic coordinates description of the biomonitoring sampling sites and n sampling in the four municipalities in the state of Mato Grosso, Brazil

Code	Coordinates	Altitude (m)	Human population	DD	<i>Aedes aegypti</i> (n)
CB-01	15°36'36" S 56°03'76" W				50
CB-02	15°40'18" S 56°02'30" W	176	590.118	157,66	3
CB-03	15°34'48" S 56°03'42" W				114
VG-01	15°42'36" S 56°08'30" W				44
VG-02	15°39'56" S 56°08'06" W	198	274.013	240,98	41
VG-03	15°39'12" S 56°08'01" W				45
CG-01	15°27'40" S 55°44'44" W				22
CG-02	15°28'00" S 55°44'09" W	811	19.049	2,85	48
CG-03	15°28'04" S 55°44'14" W				13
SA-01	15°51'53" S 56°04'38" W				11
SA-02	15°51'51" S 56°04'15" W	141	18.392	1,51	24
SA-03	15°51'43" S 56°04'54" W				8

Legend: N. Number of samples; DD- Demographic density (hab / km²); CB-01; CB-02; CB-03 (Cuiabá); CG-01; CG-02; CG-03 (Chapada dos Guimarães); SA-01; SA-02; SA-03 (Santo Antônio do Leverger); VG-01; VG-02; VG-03 (Várzea Grande). Source: IBGE (2017).

The results of the frequency of occurrence of the expressed and overexpressed bands showed highly significant values for the predictors among the four municipalities (Figure 2) by the Mann-Whitney test (Test $U=2.10$; $p=0.03$; $U=1.83$; $p=0.04$, respectively), with the highest values for the municipality of Várzea Grande identified in the expressed bands.

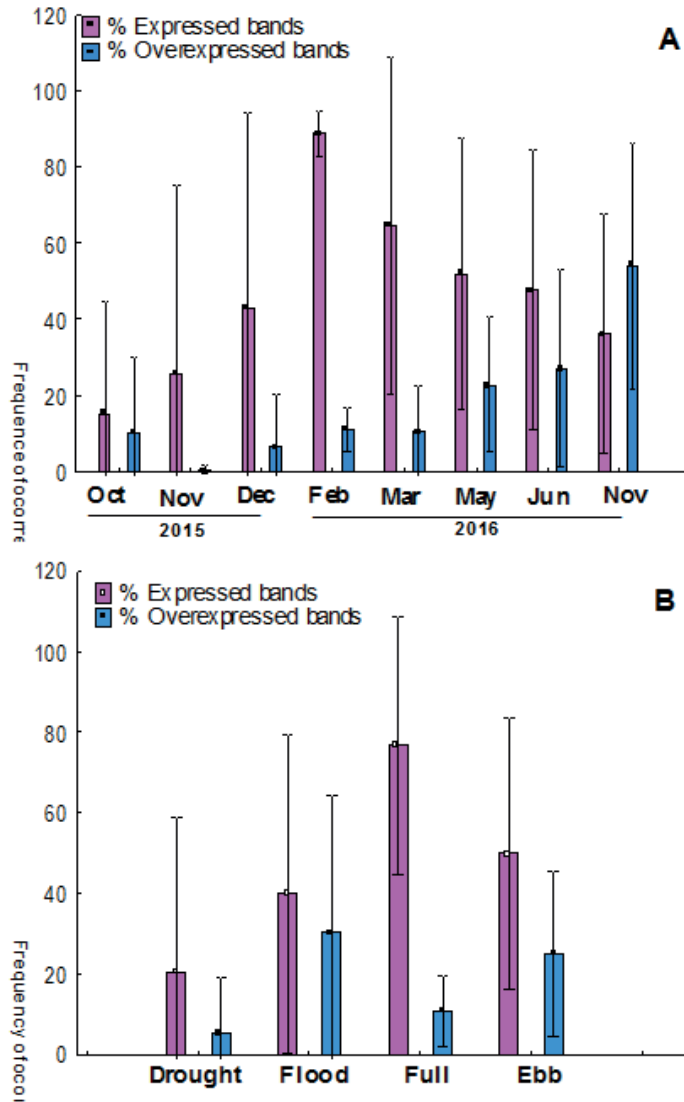
Figure 2 – Mean frequency values (%) expressed bands and overexpressed bands in *Aedes aegypti* between the four sampling sites. Legend: CB-Cuiabá $n=175$; VG-Várzea Grande $n=126$; CG-Chapada dos Guimarães $n=92$; SA-Santo Antônio do Leverger $n=30$



Source: Research Data.

The frequency of expression of esterases may also vary between populations over time. The mean frequency of the bands expressed, increased between October 2015 and February 2016 (FIGURE 3a), with significant differences (Kruskal-Wallis $H_{c,7,32}=13.86$; $p<0.01$) and, consequently, among seasonal phases (FIGURE 3b), especially in the full period ($H_{c,3,32}=6.24$; $p<0.01$). Subsequently, a small reduction was observed until November 2016. In contrast to the bands expressed, the frequency of overexpressed increased from March to November 2016 (FIGURE 3a).

Figure 3 – Mean frequency values (%) “expressed bands” and “overexpressed bands” in *Aedes aegypti* among the months of sampling (A) and seasonal phases (B). Legend: 5b. Drought Express bands n=35, Overexpress bands n=23; Flood Express n=102, Overexpress n=64; Full Express n=153, Overexpress n=17; Ebb Express n=20, Overexpress n=9



Source: Research Data.

4 DISCUSSION

The analyses of the *Ae. aegypti* population in the four municipalities of Mato Grosso showed variability in esterase expression patterns with time, seasons, and sites; which may undergo selection pressures related to the rainfall regime. The increased variability of esterases showed that populations are metabolically active, as this is the most favorable season for vector development.

The egg stage was a precedent in the environment in the surveyed places, whose females were exposed in a period before the capture of eggs in ovitraps. The proliferation of vectors and the recording of overexpressed bands in some organisms were important for detection in rainy periods, and this interaction between organisms in the same population was significant when considering the month of November/2016. The rainfall regime determined the highest frequency of esterase bands in populations of *Ae. aegypti* as registered in the results of this research, reinforcing our hypothesis. In other samples, Butakka and collaborators (2019) also identified a trend of increasing alleles expressed with precipitation in the municipalities of Cuiabá and Várzea Grande.

The dissemination of vectors can be strongly involved with their peculiar characteristics, such as their ecological requirements, their number in each location, their life cycle stage. The drought period limits the production of larvae due to the limited availability of breeding sites. Furthermore, the environmental stress caused by the limitation of food and the amount of water and even the presence of predators with potential to reduce vector population (ZARA *et al.*, 2016) interfere in the metabolism and development of the *Ae. aegypti*.

An important factor that represented a higher frequency of isoenzyme overexpression was the beginning of the rains, in November/2016. The activity of these enzymes may be related to increased expression levels of genes that encode these enzymes, providing vector resistance (BHARATI; SAHA, 2018).

It is relevant to consider the temporal issue in studies involving the recording of overexpression of esterase bands, which increased in this period, as the vector is persistent because it presents a proliferation dynamic in rainy periods that favor its development. The reduction in the frequency of expression of esterase bands in dry months, such as October/2015, would be associated with adverse environmental conditions, less favorable to the development of the mosquito, such as the absence of water puddles in the breeding sites. Climatic factors also interfere in the biology of vectors and the transmission of pathogens associated with them. In a situation of global warming, they can cause an increase in the incidence of dengue (GALATI *et al.*, 2015).

Seasonal climate fluctuations have an effect on insect dynamics, such as the higher incidence of dengue, Chikungunya and Zika in the rainy season (ARAÚJO *et al.*, 2019), together with the planet's climate changes, which have also generated concern about the possible expansion of the current area of incidence of arboviruses (GALATI *et al.*, 2015). Social factors and development and control policies are equally important in the dynamics of vector diseases.

The volume of places with garbage can influence the mosquito population variation, and consequently, the volume of suitable environments for oviposition, related to the sociocultural and biological aspects of each location. The frequency of expression of esterases may depend on the influence of

external factors, such as the process of natural selection and life cycle, even in polluting waters. The variability and intensity of enzyme expression in the population of *Ae. aegypti*, denotes its detoxification by the mosquito organism, which acts through the oxidation, reduction and hydrolysis of harmful compounds (MONTELLA *et al.*, 2012).

The variability of the expression of the bands may have a relationship between the rainy seasons and altitudes in some places. For example, the altitude was thought to be one of the factors that may have contributed to the increase in esterase expression in *Ae. aegypti* populations in the municipality of Chapada dos Guimarães. However, in the municipality of Várzea Grande, this perhaps occurred due to the population density of the people, with the availability of food and energy requirements of the mosquito population. It is important to note that esterase, especially the carboxylesterase and cholinesterase, have been associated with the developmental stages of the insects, that are vectors of the diseases; and their consistent expression is considered primary in developing resistance chemicals. The mechanisms involving carboxylesterases result from the increased activity of this enzyme in resistant insects (DAS; DUTTA, 2014).

The presence or absence of a specific isoenzyme in a certain developmental phase indicates the activity of regulatory mechanism that controls the spatial and temporal expression of the corresponding gene. In the present study, where adult subjects were examined, there were more individuals with “expressed” than with “overexpressed.” Genetic and biochemical understanding is fundamental in the development of management strategies to prevent. This emphasizes that their adaptation to the urban spaces is accompanied by phenotypic and even genotypic changes, generating greater resistance to current control methods.

The results shown allow us to identify characteristics of the *Ae. aegypti* structure and dynamics in different sites, in addition to its importance from the perspective of band expression. This information can significantly help in the establishment of strategies for their biocontrol by ovitraps, which have good efficiency to be used in the surveillance of *Ae. aegypti* (SILVA; LIMONGI, 2018). Biomonitoring by ovitraps in Mato Grosso municipalities is relevant in terms of integrated management, as it has the advantage of minimizing environmental damage resulting from the use of chemical insecticides, and can be adopted to determine population size and/or identify resistant populations in this process. Zara and collaborators (2016) points out that the oviposition trap can be considered an ideal entomological tool for its characteristics and advantages for epidemiological monitoring as part of the surveillance and control of dengue vectors.

A fast and efficient technique allows the identification and characterization of the genomic variability of the studied organism through this isoenzymatic system. It was important to detect quantitative differences between these isoenzymes in different municipalities (higher band expression in Cuiabá and Chapada dos Guimarães) and over time (rainy period).

The results in the expression of esterases bands in populations between municipalities, between months and seasonal phases showed a significant difference in the mean values. The significant increase in the frequency of occurrence in the expression of esterase bands was registered in the months of February and March/2016, contrary to what was observed only for the expression of the bands, with a reduction for these periods.

5 CONCLUSION

The esterase technique made it possible to verify the genes and their enzymatic expression reflects the activity of genes and their differences between these isoenzymes in populations of the species *Ae. aegypti* in the four municipalities studied.

The population's habits and the increase in the movement of people (and consequently viruses), uncontrolled urbanization, climate change and a collapse in public health infrastructure favor the development of the species. At the same time, methods for controlling the size of populations using modern genetic techniques, such as the construction of transgenic mosquitoes with appropriate characteristics, are still under laboratory investigation.

It is necessary to expand the vector control strategies of *Ae. aegypti*, and to include in the program actions aimed at the systematic monitoring of populations. The combination of these actions facilitates a more accurate identification of areas with the greatest risk of viral transmission, which allows for a quick and efficient intervention in order to contain the occurrence of possible proliferation of vectors.

The enzymatic expression, the polymorphism that characterizes the genomic variability of the vector and its genetic knowledge will serve as subsidies for further studies that aim to determine the relationship of this enzymatic system with biological mechanisms, which allows us to monitor and delay the appearance of resistant genotypes and their propagation in populations of *Ae. aegypti*. It is essential to expand the technological innovations used as complementary tools to control programs. The results obtained from this research may contribute to the search for a more efficient form of control for this vector.

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