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Effects of pesticide residues in soil substrates on the biology cycle of *Aedes aegypti* from three different setting zones in Benin

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Abstract

In order to evaluate the effects of insecticides residues in soil substrates on the biology cycle of *Aedes aegypti*, we conducted a study in three ecological zones: urban areas (Dandji, southern Benin with few agriculture activities), peri urban areas (Awaya, central of Benin with agriculture practices), and forests (Kaoura, northern Benin with few agriculture activities). These areas were chosen because they provided an ideal environment for the development of *Aedes aegypti*. The purpose of this study is to look for insecticide residues that may have detrimental impacts on the biology cycle of *A. aegypti*. Indirect bioassays were used to investigate the factors affecting mosquito larvae's ability to develop normally at breeding sites, their rate of growth, and their ability to produce an adequate number of larvae. Due to the absence of an HPLC equipment for the direct detection of pesticide residues in samples, *A. aegypti* larvae at breeding sites were reconstituted using water and soil samples obtained at each study site. A comparison of the larval growth in test breeding locations was made using the reference strain SBE as a control. The different bioassays demonstrate the existence of inhibitory elements on test materials. In control samples, larval development was observed to be normal. However, when the breeding sites were established with just a few grams of soil samples from the three study sites, it was found that the *A. aegypti* eggs had a poor hatching rate, along with sluggish larval development and a low production of adult mosquitoes from hatched eggs. The findings of this study indicate that toxic substances that prevent *A. aegypti* eggs from hatching and larvae from growing are most likely leftover pesticides from agricultural operations. In order to quantify the toxic factors likely to affect the biology parameters of *A. aegypti* cited above, these results must be validated using HPLC techniques.

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Introduction

In recent decades, the continued medical significance of vectors like *Aedes aegypti* has been highlighted by global epidemics of viral infections (Lassa, Ebola, Covid-19) and arboviruses (Yellow Fever, Chikungunya, Dengue). The most well-known viruses spread by the mosquito species *A. aegypti* (Linnaeus, 1762) are Dengue (DENV), Yellow Fever (FJV), Chikungunya (CHIKV), and Zika (ZIKV).

DENV is currently the most common arbovirosis and a public health issue in many parts in the world. The prevalence of dengue has been rising at an extraordinary rate over the last decades, with multiple outbreaks increasing the number of cases and impacted areas globally (WHO, 2009). Several outbreaks of dengue fever have been recorded in Sub-Saharan Africa during the past ten years, including those in Côte d'Ivoire (Fofana *et al.*, 2019), Nigeria (Fagbami and Onoja, 2018), and Senegal (Gaye *et al.*, 2021). With approximately 1045 cases and 26 fatalities reported in Burkina Faso in 2019 (Ouédraogo *et al.*, 2019), this outbreak is the most recent.

According to a research conducted by Anges *et al.* (2014) in southern Benin, *A. aegypti* populations are year-round inhabitants of places where numerous used tires are kept and sold (Yadouléton *et al.*, 2014). The only known approach to effectively combat epidemics is vector control based on the use of WHO-approved insecticides given the widespread dispersion of the *A. aegypti* mosquito throughout the world, which has caused numerous cases of mortality and the lack of vaccinations or suitable therapies (Lounibos et Juliano, 2018; Amelia-Yap *et al.*, 2018). In fact, the use of these insecticides has made it feasible to keep this mosquito's population under control in a number of nations, avoiding multiple outbreaks. However, the careless application of these same pesticides without regard for safety regulations would have led to issues with resistance with regard to *A. aegypti* populations, not only in agriculture to control crop pests but also in public health, particularly in the fight against malaria vectors and the impregnation of mosquito nets. The purpose of the current study is therefore to comprehend how the use of pesticides affects the larval development of *A. aegypti*.

Materials and methods

Study Area

The study was carried out in 3 zones in Benin.

Dandji (southern Benin, 6° 24′ 18″N, 2° 22′ 31″ E) located in urban area of Cotonou city with poor urbanisation facilities. Additionally, lots of second-hand vehicle tires from Europe and Asia, which constitute good breeding sites for *A. aegypti*. Awaya (central Benin, 7° 41′ 3.2″N, 2° 18′ 31″E) located in peri-urban area of Dassa city, presence of sparse forest with animals like bats, snakes and other small rodents. Kaoura (North-East of Benin, 1° 7′ 45″N, 2° 61′ 13.6″E) located in forest area of Kandi, presence of animals and birds with limited access to human activities (Fig. 1).

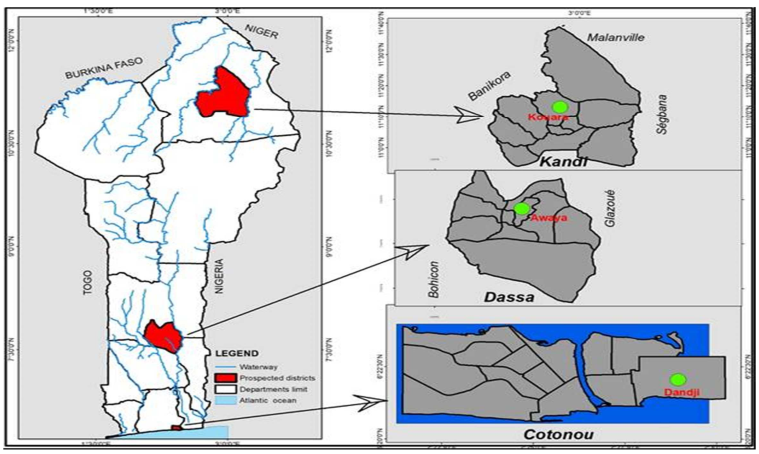

Fig. 1. Map of Benin showing the study sites.

The central and southern Benin are characterized by two rainy seasons (March- July and October-November) and two dry seasons (December-March and August-September). The mean annual rainfall is 1,500 mm in July and the temperature ranging from 23 to 32 °C. The north is characterized by a Sudanian climate with one rainy season (middle of May to October) and one dry season (November-May) with 1,300 mm as mean annual rainfall.

Impact of pesticides on the development of A. aegypti larvae

The approach we took wasn't meant to be a precise assessment of pesticide residues in the breeding locations. Instead, a covert evaluation was conducted to look for any potential problems that would prevent mosquito larvae from developing normally in their nesting locations. Using a technique developped by Yadouleton *et al.* (2011), we investigated egg hatch rate and larval development in *A. aegypti* using breeding facilities built with soil substrates from insecticide-treated regions and tap water. This rearing media's outcomes were contrasted with those of a control medium, which consisted of tap water and CREC (area where there no agriculture activities) soil without any pesticides. The reference strain of *A. aegypti* SBE and wild populations of *A. aegypti* from Dandji, Awaya and Kaoura were used in the rearing.

Samples of the soil substrate were taken at the three sites mentioned above. Using soil samples collected from the research sites, rearing conditions were replicated. Each site included 1000 mL of water and 100 grams of dirt. With a microscope, 200 susceptible SBE strain *Ae. aegypti* eggs were counted and distributed to each location. The *Ae. aegypti* populations at the three research sites underwent the same procedure.

The various sites received and raised more than 4000 *Ae. Aegypti* eggs in total. We were able to observe the fluctuation in the eggs hatching rate and the regularity of adults appearances by daily monitoring of the rearing. The data from the supposedly contaminated environments were compared with those from the control environments to check for the possible existence of a factor inhibiting larval development.

Statistical analysis

Analysis of variance (Anova) at the 5% threshold was used to compare the hatching rates of eggs and the emergence rates of stage 1 larvae according to the origin of the sites. The results of these analyses provided information on the impact of insecticide treatments on larval development.

Results

Impact of agricultural insecticide treatments on A. aegypti egg hatching

Hatching conditions are relatively favourable for larval development in tap water plus control soil (without insecticide). When the control soil is replaced by soil substrates taken from areas where crops are grown in association with insecticide use, hatching rates drop by 20% in the SBE strain and 25%, 15% and 13% respectively in the Dandji, Awaya and Kaoura populations.

In fact, the average hatching rate of eggs from the control locations for the SBE strain is 85% (n=200), whereas it is 82%, 80%, and 81% for the populations of *A. aegypti* in Dandji, Awaya, and Kaoura, respectively. However, these hatching rates decreased to 12% for the SBE strain and to 42%; 18%; and 20%, respectively, for the Dandji; Awaya; and Kaoura populations when a little amount (100 grams) of soil from an insecticide-treated region was introduced.

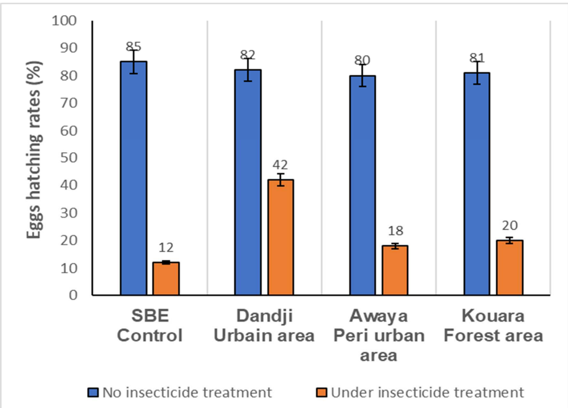

Fig 2. *A. aegypti* egg hatching rate following the different treatments.

Impact of agricultural insecticide treatments on the development of A. aegypti larvae.

Larval development was measured by considering the proportion of eggs that reached the pupal stage after being put in water. This parameter is the best indicator to evaluate the possible existence of a limiting factor that could slow down the development of the larvae in the breeding sites. Indeed, this parameter takes into account the stay of the larvae in the cottages. Contrary to the very limited time taken by the egg to hatch in the water, the duration of this stay is sufficient to allow the effect of the limiting factor to manifest itself by delaying the hatching of the eggs, for example.

An average of 88% (SBE) and 85%; 80% and 83% for Dandji, Awaya, and Kaoura, respectively, of the larvae reached the adult stage in the control sites. The sites containing samples from the treated (insecticide) environments resulted in lower larval development than the control (at least 20% decrease in SBE; 17%; 10% and 12% respectively for the Dandji, Awaya and Kaoura populations. There was a significant difference between the emergence rates of the susceptible strain and the Dandji, Awaya and Kaoura populations when the deposit was prepared from a soil sample from the treated environment ($P<0.05$). Indeed, only 18% emergence was obtained for the SBE strain compared to 48%, 50% and 52% respectively for the Dandji, Awaya and Kaoura populations on average. In essence, the SBE strain only achieved 18% emergence, compared to averages of 48%, 50%, and 52% for the Dandji, Awaya, and Kaoura populations.

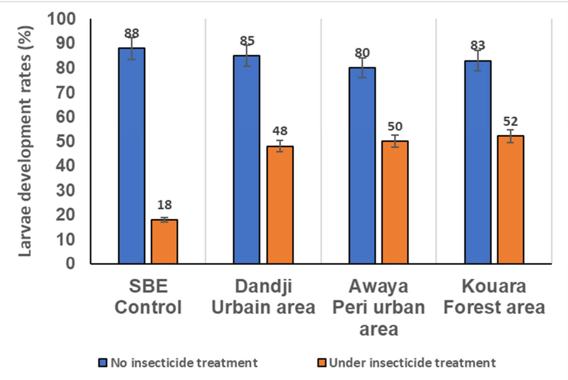

Fig 3. *A. aegypti* larvae development rate following the different treatments.

Discussion

The environment, particularly mosquito breeding grounds, has been contaminated for a number of years due to the extensive use of phytosanitary agents in agriculture. Pesticide molecules are likely to leave the application site after being used, acting as organic micropollutants that contaminate all environmental compartments (Carletto *et al.*, 2010). These compounds pose a concern to the environment because of their ecotoxicity and bioaccumulation capacity (Martín *et al.*, 2010).

The fate of pesticide packaging observed during our investigations is not conducive to safeguarding the environment. Many of them are thrown away in crop fields or in abandoned jars, which constitute real breeding grounds for *A. aegypti* during the rainy season. The pesticide particles in these packages could be leached out and come into contact with the mosquito breeding grounds during the rainy season. Pesticide cans and other spraying materials are often washed and rinsed in water channels and streams, which often serve as mosquito breeding grounds for *Ae. aegypti* in particular (Ouédraogo *et al.*, 2019). This situation explains the emergence of *A. aegypti* resistance observed in crop areas under insecticide treatment such as in Awaya in Dassa-zoumè and Dandji. According to, the presence of insecticide residues in the surface layer of agricultural soils as in Awaya and Kaoura is often sufficient to select for resistance in mosquitoes (Yadouléton *et al.*, 2014; Yadouleton *et al.*, 2011).

This explains the drop in SBE egg hatch rates and *A. aegypti* populations following the addition of a few grams of soil from the study sites. Soil from fields under insecticide treatment for crop pests contains chemical pesticides that are toxic to mosquito larvae. Insecticide treatments, which are applied every fortnight and last for three months, from July to October, when mosquito larvae are developing, project particles of active ingredients, some of which come into direct contact with the breeding sites, and therefore with the mosquito larvae that develop there (Yadouleton *et al.*, 2011).

A. Aegypti larvae develop in field margins or abandoned containers and are directly exposed to these repeated insecticide treatments. This pesticide pressure is intense since no larva can evade it and it affects females and males equally (contrary to the insecticides for anti-malarial use which only target the females, or even the endophagous or endophilic fraction of these females). This circumstance explains the dramatic decline in the *A. aegypti* populations' egg hatch rates from the three research sites, demonstrating that the pesticides employed in the cotton, vegetable, and grain growing areas are really to blame for the mortality.

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