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BIOMATERIALS AND ESSENTIAL OILS AS PROMISING TOOLS FOR PUBLIC HEALTH PROBLEMS IN BRAZIL: PRELIMINARY STUDY

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ABSTRACT

Biomaterials have been applied for a long time in different areas and, for countless uses and with technological advances, they have helped to solve several problems in the most diverse areas of health, working on the development of different mechanisms, orthoses, prostheses. and, in recent years, in the creation of formulations for the biological control of microorganisms that cause public health problems. Brazil is among the countries that have the most cases of diseases transmitted by arboviruses in the world. There is scientific evidence reporting the use of plants with repellent and bioactive properties for the development of natural, non-toxic and efficient alternative products. Develop a biodegradable polymeric formulation containing encapsulated essential oil, and test the essential oil of *Piper callosum in natura* on larvae of *Aedes aegypti* mosquitoes. The formulation developed and submitted to 35 °C was stable in all evaluations during the 25 days of analysis and the essential oil of *Piper callosum* proved to be a promising natural resource against larvae of the mosquito *Aedes aegypti*.

I. INTRODUCTION

Biomaterials have been applied for a long time in different areas and, for countless uses and with technological advances, they have helped to solve several problems in the most diverse areas of health [1], working on the development of different mechanisms, orthoses, prostheses and, in recent years, in the creation of formulations for the biological control of microorganisms that cause public health problems [2].

The Brazil is among the countries that present the majority of cases of diseases transmitted by arboviruses in the world, mainly the population of the State of Amazonas [3], with high rates of diseases transmitted by the *Aedes aegypti* mosquito. The great proliferation of *Aedes aegypti* in northern Brazil is associated with the tropical and rainy climate that favors its reproduction.

The zika, dengue and *Chikungunya* viruses are responsible for the majority of public health epidemiological problems; however, in recent years, scholars have invested in the development of natural strategies for possible biological and epidemiological controls of arboviruses, aiming at the use of essential oils extracted from plants in the Amazon. There is scientific evidence reporting the use of plants with repellent and bioactive properties for the development of natural, non-toxic and efficient alternative products [4]. The chemical constitution of the essential oils (EO) of certain plants varies according to their species and their biological activities are related to the constituents and functional groups [5]. For this reason, some of them may have specific insecticidal, fungicidal, acaricidal and bactericidal functions. These functions are exploited for the development of new biocidal formulations.

The plants that are part the *Piperaceae* group, in addition to being major producers of essential oils (EO), are rich in bioactive substances. Among these species, *Piper nigrum*, *Piper aduncum*, *Piper hispidinervum* and *Piper marginatum* stand out, which proved insecticidal and larvicidal activities [6,7]. Essential oils from certain plant species have numerous biocidal activities [8,9], antioxidant, antimicrobial and antibacterial functions [10], in addition to fungicidal properties [11].

The Amazon has one of the largest ecosystems in the world, with a wide variety of plants rich in EO (essential oils) with bioactive and medicinal functions [12]. *P. callosum* is the largest genus in the family *Piperaceae* [13] and is widely distributed in the north of the country. This species is a perennial shrub, with twisted stems and branches, alternating permanent leaves on the central face and has small yellow flowers. In addition, it has a predisposition for hot climates and soils rich in organic matter, justifying the fact that it is widely found in the State of Amazonas.

In the northern region of Brazil, *Piper callosum* is known as 'electric oil' because it provides immediate relief from muscle pain (analgesia) and is also used in folk medicine to combat stomach pan and relieve the inconvenience of insect bites [14]. The essential oil of *Piper callosum* is composed of monoterpenes, sesquiterpenes, phenylpropenes and terpenoids. Figure 1. *Piper callosum*.

Figure 1: *Piper callosum*.
Source: Author, (2019).

Biodegradation involves a natural process in which polymers are converted into simpler compounds, because, according to time and/or temperature, structural properties deteriorate. In recent years, nanotechnology has enabled the use of natural materials for the development of biosystems with multidisciplinary applications. Most researches use gelatin as a base material for encapsulating certain assets, such as medicines, oils and products of biological origin. Among the synthetic polymers most found in biotechnological applications, polycaprolactone stands out. Due to the fact that essential oils have volatile substances that are easily lost when exposed to certain temperatures and environments, it is necessary to develop techniques capable of conserving assets so that their properties and functions do not change. For this reason, the essential oil of *Piper callosum* was encapsulated by means of gelatin and polycaprolactone. As gelatin and essential oil are elements that undergo metamorphosis when exposed to certain factors, the biocide stability study was carried out for 25 days at less than 35 °C to verify the stability parameters according to the storage time and temperature. This temperature was chosen based on the climate of the Amazon, which revolves around 32 °C and 36 °C.

To develop a biodegradable polymeric formation on a nanometric scale containing encapsulated essential oil; evaluate the stability of the biosystem as a function of the preservative and temperature considering the parameters of encapsulation efficiency, pH, turbidity, electrical conductivity and organoleptic properties for a determined time, in addition to performing the larvicidal test applying the essential oil of *Piper callosum* in natura to mosquito larvae *Aedes aegypti* to teste the effectiveness of the natural asset.

II. MATERIALS AND METHODS

Two different solutions were prepared for the synthesis of gelatin/polycaprolactone nanoparticles. Solution 1: (1g) od gelatin was solubilized in (100mL) od distilled water and heated to 50 °C with constant stirring. Then, (0.30g) of tween 80 solubilized in 50 mL of distilled water, after solubilization it was poured into the solubilized gelatin. Solution 2: (0.05g) Polycaprolactone, (0.02g) Span 60 and (0.1g) TACC were solubilized in (5mL) dichloromethane. 0.0750g of *Piper callosum* essential oil (EO) supplied by the Brazilian Agricultural Research Corporation (EMBRAPA) was added to Solution II. After the solubilization of the essential oil, solution II was added to solution I using the turrax nanoparticle disperser for 30s. Then, (0.0935g) of the enzyme transglutaminase was added to the final solution, together with the preservative NE. The formulation was stored in the incubator for analysis, the following evaluation parameters were used: encapsulation efficiency, turbidity, pH, electrical conductivity and senses (sight and smell) for the study of organoleptic properties, Figure 2. Formulation development illustration.

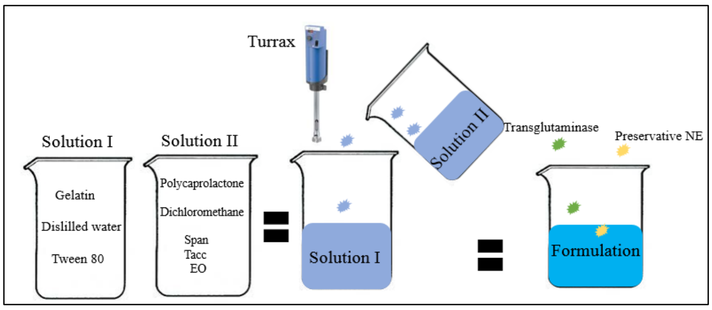

Figure 2: Illustrative image of the development of the formulation.
Source: Author, (2019).

The larvicidal test was performed at the Malaria and Dengue Laboratory/LMD-CSAS (INPA) to test the effectiveness of the essential oil of *Piper callosum in natura*, groups of 10 larvae in the 3rd larval stage were inserted into plastic cups (100 mL) containing initially 1mL of distilled water, 100µL of the larval food and 100µL of the larvicidal concentrations (72 to 48 $mg.mL^{-1}$) (47 to $35mg.mL^{-1}$) of the essential oil solubilized in DMSO. Readings were taken after 24 and 48 hours of exposure; larvae that did not respond to artificial stimuli were considered dead.

III. RESULTS AND DISCUSSION

III.1 STUDY OF FORMULATION STABILITY

After 25 days of studying the behavior of the biocidal formulation submitted to 35 °C and containing the preservative NE, we obtained the following results: Figure 3. Encapsulation efficiency (EE), Figure 4. pH, Figure 5. Electrical conductivity, Figure 6. Organoleptic properties and Figure 7. Turbidity.

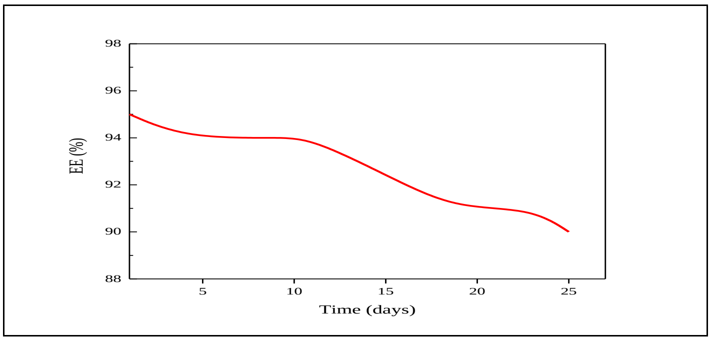

Figure 3: Encapsulation efficiency (EE).
Source: Author, (2019).

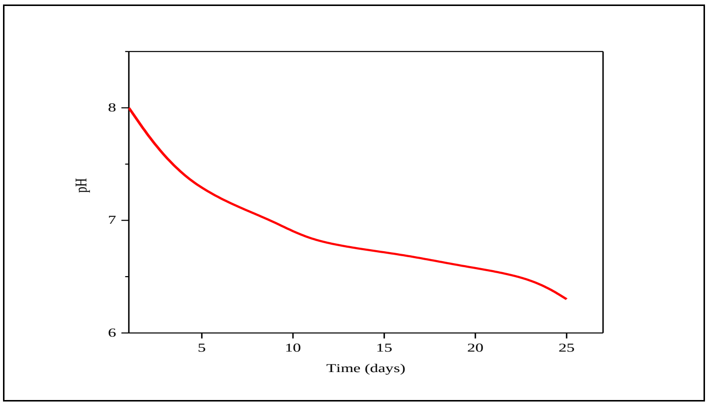

Figure 4: pH.
Source: Author, (2019).

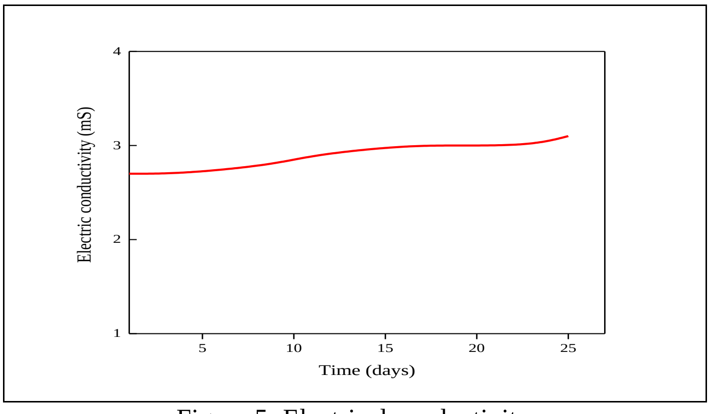

Figure 5: Electrical conductivity.
Source: Author, (2019).

The formulation showed an EE greater than 90%, indicating that the combination of gelatin and polycaprolactone encapsulants was effective in encapsulating the essential oil of *Piper callosum*. The initial value of the encapsulation efficiency (Figure 3) was around 95% and after 30 days of evaluations 90%. Polymeric biocides are considered efficient when their encapsulation efficiency is greater than 70% [15]. If we consider only the analysis of the encapsulation efficiency (EE), the formulation would indicate stability, since the encapsulation efficiency was shown to be high. For this reason, it is important to associate the stability assessment with other analyzes (such as turbidity, organoleptic properties, pH and electrical conductivity). Some studies report that biodegradable polymeric formulations that have two layers are more resistant to extrinsic factors than those that have only one [16,17].

During the 25 days of analysis of the stability of the formulation, the pH values decreased, but remained above 6.3. Based on this result, it can be said that the EO remained protected by the gelatin and polycaprolactone layer during the evaluation period. The pH was adjusted to 8 so that its behavior over time was studied and, after 25 days, it dropped to 6.3. As shown in (Figure 4), the formulation showed a decrease in pH over the 25 days of study, however this value did not compromise the formulation, as the pH of the essential oil of *Piper callosum* is around 5.5. This fact is related to the exposure of the EO to the environment by decreasing the EE, as previously seen, a single isolated evaluation is not enough to analyze the stability of a formulation, therefore, it is necessary to join several evaluation parameters to determine the effectiveness of a product.

The decrease in pH values is not only related to the exposure of the EO to the medium, since the pH of the OE is acidic and, naturally, when exposed to the medium, the formulation will tend to the pH of the essential oil. The electrical conductivity values were in the range between 2.7 and 3.1 mS, as shown in (Figure 5). These values corroborate the EE and turbidity assessments. Because unexpected changes in electrical conductivity are indicative of instabilities [18].

The increase in the electrical conductivity values in systems containing EO encapsulated in biodegradable polymeric nanoparticles is related to the presence of a large amount of free charge (ions) in the solution and, consequently, to the reduction of the encapsulation efficiency and to the destabilization of the formulation. In some cases, these electrical conductivity values can be changed even further, depending on the temperature at which the formulation is packaged. The decrease in pH values can be correlated with the values of electrical conductivity, since this decrease is related to the exposure of greater amounts of EO in the medium and, consequently, to the increase in charges [19]. The electrical conductivity values of the formulation did not compromise its stability.

The organoleptic properties (color and odor) were analyzed in comparison with the initial aspects of the formulation. The purpose of this evaluation was to verify whether the formulation suffered changes in color and odor, in addition to phase separation, excessive turbidity and interruptions. Regarding organoleptic properties, the formulation did not show any significant changes that would compromise pain and appearance. As shown in (Figure 6). Corroborating with the indications found by the evaluation of the organoleptic properties of the formulation, the turbidity was not excessive (Figure 7), pointing out that the materials used for the development of the formulation were not compromised, although we know that biodegradable systems are easily compromised by external factors (chemical, physical and biological) [20].

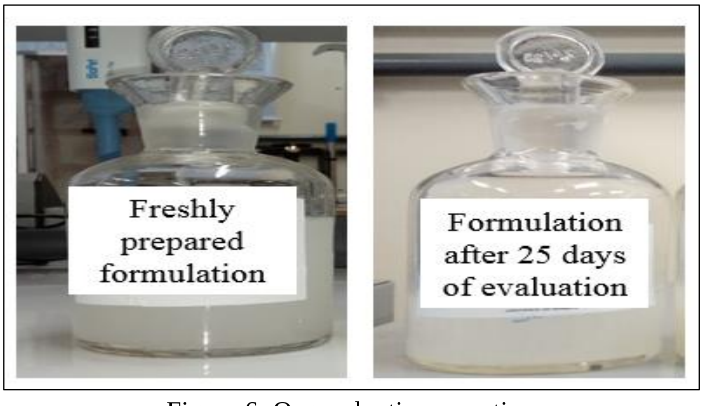

Figure 6: Organoleptic properties.
Source: Author, (2019).

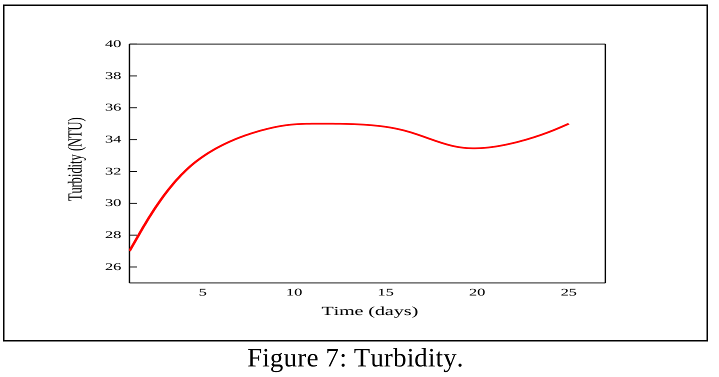

Figure 7: Turbidity.
Source: Author, (2019).

III.2 LARVICIDAL STUDY

The bioassays performed with the essential oil of *Piper callosum* showed greater activity in the first 24h of exposure, with low increases in the mortality percentages after this time interval, as shown in Table 1.

Table 1. Values of LC_{50} (inhibitory concentration) and CL_{90} (lethal concentration) of the essential oil of *Piper callosum in natura* against *Aedes aegypti* larvae.

Species	Time (h)	LC_{50} ± DP (μg. mL^{-1})	(LCI-LCS)	LC_{90} ± DP (μg. mL^{-1})	(LCI-LCS)
Aedes aegypti	24	53.46 ± 1	52.1 -57.9	94.15 ± 1	87.4 – 124
	48	38.13 ± 1	37.4 – 40	55.96 ± 1	53.3 – 67.2

Upper confidence limit (LCS), lower confidence limit (LCI) and standard deviation (SD).
Source: Author, (2019).

Through the applied doses, the values of lethal concentrations CL_{50} and CL_{90} were found through analysis by the POLOPC® program (LeOra Software Berkeley, CA), respecting the confidence interval at the 95% significance level. The larval toxicity study showed that the essential oil has greater larvicidal activity in the first 24 hours. Considerable lethal concentrations were (53.46 ± 1) and (94.15 ± 1) μg.mL^{-1} for LC_{50} and LC_{90}, respectively. However, the activities recorded in 48 h were essential to complement and prove the effectiveness of the essential oil of *Piper callosum* against the larvae of the *Aedes aegypti*. Normally, in the first hours of biological tests, the target organisms have higher mortality, in the following hours the effects are less, but more extensive [21].

P. callosum oil showed larvicidal activity from the concentration of 48 ppm in the first 24h. However, larval mortality was representative only from the concentration of 72ppm. The mortality percentages were quite different between the concentrations used, in the first 24h of the experiment, with a gradual increase in the mortality percentage as the concentration was increased.

IV. CONCLUSIONS

Using the results as a basis, we can say that the essential oils of certain species in the Amazon have biocidal activities, however, we emphasize the essential oil of the *Piper callosum* plant, which has been shown to be very effective against *Aedes aegypti* larvae.

The biocide developed in this study proved to be stable at 35 °C, indicating that it would adapt in the hottest regions of Brazil, mainly in the North, where arbovirus concentrations are higher. This study emphasized that it is possible to develop solutions that are not aggressive to the environment and to man, using natural materials, such as gelatin, and regional ones, such as essential oils.

The small variations found between each evaluation parameter are related to the natural degradation of the polymeric formulations, however they were not able to compromise the stability of the biocide. The effectiveness of the essential oil of the species *Piper callosum in natura* has been demonstrated, the future intention is to test the natural active encapsulated in polymeric nanoparticles against *Aedes aegypti* larvae. Thus, it was possible to develop a biocidal formulation from biodegradable polymers, which behaves well in hot climates in Brazil and has a minimum durability of 25 days, without being compromised by external factors.

V. ACKNOWLEDGMENTS

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