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ÍCARO RODRIGUES SARQUIS

OBTENÇÃO DE FORMULAÇÕES CONTENDO ÓLEO DE *Carapa guianensis* Aublt. ASSOCIADA COM FIBROÍNA DA SEDA ATIVAS EM LARVAS DE *Aedes aegypti*

Macapá
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Orientador: Prof. Dr. Irlon Maciel Ferreira

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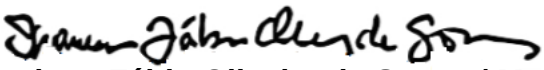

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SUMÁRIO

AchE	Acetilcolina
AO1	Óleo de andiroba extraído no mês de março
AO2	Óleo de andiroba extraída no mês de agosto
CAL-B	Lipase de *Candida antarctica* B
CB1	Recptor canabinóide tipo 1
CB2	Receptor canabinóide tipo 2
CG-EM	Cromatografia gasosa–espectrometria de massa
DL50	Dose letal 50%
DL90	Dose letal 90%
DLS	Dynamic Light Scattering
DmSO	Dimetilsulfóxido
DNA	Ácido Desoxirribonucleico
EHL	Equilíbrio Hidrófilo Lipofílico
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
EtOH	Etanol
FAEE	Ester etílico livre
FAEE1	Ester etílico livre Óleo A1
FAEE2	éster etílico livre Óleo A2
FDA	Food and Drug Administration
FFA	Ácido graxo livre
FFA1	Ácido graxo livre do óleo AO1 associado a fibroína da seda
FFA2	Ácido graxo livre do óleo AO2 associado a fibroína da seda
FFAr	Free Acid Ethyl Ester Óleo A
GABA-A	Canal Iônico Ácido do Gama-aminobutírico
IPEA	Instituto de Pesquisa Econômica Aplicada
IV	Infravermelho
IV-TF	Iree Acid Ethyl Ester Óleo AF
ml	Mililitros
MSR	Metodologia de Superfície de Resposta
nm	Nanômetro
pH	Potencial Hidrogeniônico
PIT	Temperatura de Inversão de Fases
RMN	Espectroscopia por ressonância magnética nuclear
RNA	Ácido Ribonucleico
Rpm	Rotação por minuto
WHO	World Health Organization

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RESUMO

Introdução: O óleo de *Carapa guianensis* Aublt. (Andiroba) é um importante recurso natural utilizado na Amazônia por suas propriedades medicinais, dentre estas, a proteção e repelência contra insetos transmissores de doenças como mosquito *Aedes aegypti*. Devido seu perfil lipílico, o óleo de *C. guianenesis* Aublt. é promissor para aplicações biocatalíticas que podem aprimorar seu uso em sistemas biológicos. **OBJETIVO:** Asssociar o óleo de *C. guianensis* Aublt. em fibroína da seda e avaliar sua aplicação como formulações ativas frente as larvas do mosquito *Aedes aegypti*. **Metodologia:** O óleo de andiroba foi coletados em períodos diferentes do ano (março [AO1] e agosto [AO2]). Suas propriedades fisico-químicas (índice de acidez, indice de peroxido, densidade, índice de refração e ponto de fusão) foram avaliadas. Em seguida realizado as reações de hidrólise, transesterificação e amidação direta utilizando lipase de *Candida antarctica* imobilizada em resina acrílica, como catalizador. Os produtos gerados foram caracterizados por ^{13}C Ressonância Magnética Nuclear (RMN), Infravermelho – Transformada de Fourier (IV-TF) e Cromatografia à Gás – Espectrometria de Massas (CG-EM). A solução de fibroína da seda (2%) foi obtida a partir do casulo picotado de *Bombyx mori* combinado com uma solução pré-aquecida de Na_2CO_3 (2%), solubilizada H_2O: EtOH: $CaCl_2$, dialisada por 3 dias. Por fim, o óleo e seus derivados (ácidos, ésteres e amidas graxas) foram associados com a solução de fibroina-da-seda e seu potencial foi testado frente as larvas de *A.*aegypti nas concentrações de 25, 50, 75 e 100 µg/mL, a mortalidade foi avaliada em 24 e 48 horas. **Resultados e discussões:** O perfil lipídico dos óleos AO1 e AO2 apresentaram diferenças significativas de valores para todas as análises realizadas, vale destacar o índice de acidez de 21,97 para o AO1 enquanto para o AO2 foi de 7.91. Os ácidos graxos identificados nas amostras foram palmitico, linoleico, oleico, vaccenico e estearico. Onde o AO1 composto por 5,4% de ácido linoleico e AO2 por 33,1%, evideciando a infuência da época de coleta na composição lipidica do óleo de andiroba. Entre o óleo e seus derivados, os ácidos graxos livres (FFA) foram que melhor apresentou atividade. O CL50 do ácido graxo livre associado com fibroina da seda (FFA2-SF) foi 16,79 µg/mL, enquanto que, para o FFA1-SF foi de 129.45 µg/mL em 48 h. Isso demonstra que a ação larvicida das formulações foi diretamente relacionada à composição dos ácidos graxos. **Conclusões:** Oos ácidos graxos livre obtido do óleo de *Carapa guianensis* Aublt. por reação de hidrolise enzimática, associados com fibroína da seda se mostrou promissor no controle do *A. aegypti* em concentrações baixas e de fácil preparo e obtenção dos materiais envolvidos.

Palavras-Chave: *Carapa guianensis*; Biocatálise; Formulações; *Aedes aegypti.*

Agradecimentos: CNPq.

OBTAINING FORMULATIONS CONTAINING OIL FROM Carapa guianensis Aublt. ASSOCIATED WITH SILK FIBROIN ACTIVE IN *Aedes aegypti* LARVES

Introduction: *Carapa guianensis Aublt* oil. (Andiroba) is an important natural resource used in the Amazon for its medicinal properties, including protection and repellency against insects that transmit diseases such as the Aedes aegypti mosquito. Due to its lipyl profile, the oil of *C. guianenesis Aublt*. is promising for biocatalytic applications that can improve its use in biological systems. **Objective:** To associate the oil of *C. guianensis Aublt*. in silk fibroin and evaluate its application as active formulations against *Aedes aegypti* mosquito larvae. **Methodology:** Andiroba oil was collected at different times of the year (March [AO1] and August [AO2]). Its physicochemical properties (acidity index, peroxide index, density, refractive index and melting point) were evaluated. Then, the reactions of hydrolysis, transesterification and direct amidation were performed using *Candida antarctica* lipase immobilized in acrylic resin, as a catalyst. The products generated were characterized by 13C Nuclear Magnetic Resonance (NMR), Infrared - Fourier Transform (IV-TF) and Gas Chromatography - Mass Spectrometry (CG-EM). The silk fibroin solution (2%) was obtained from the perforated *Bombyx mori* cocoon combined with a pre-heated solution of Na2CO3 (2%), solubilized H2O: EtOH: CaCl2, dialysed for 3 days. Finally, the oil and its derivatives (acids, esters and fatty amides) were associated with the solution of silk fibroin and its potential was tested against the larvae of *A.aegypti* in concentrations of 25, 50, 75 and 100 µg / mL, mortality was assessed at 24 and 48 hours. **Results and discussions**: The lipid profile of the AO1 and AO2 oils showed significant differences in values for all analyzes performed, it is worth mentioning the acidity index of 21.97 for AO1 while for AO2 it was 7.91. The fatty acids identified in the samples were palmitic, linoleic, oleic, vaccenic and stearic. Where AO1 composed of 5.4% linoleic acid and AO2 by 33.1%, showing the influence of the collection period on the lipid composition of andiroba oil. Among the oil and its derivatives, free fatty acids (FFA) showed the best activity. The LC50 of free fatty acid associated with silk fibroin (FFA2-SF) was 16.79 µg / mL, whereas for FFA1-SF it was 129.45 µg / mL in 48 h. This demonstrates that the larvicidal action of the formulations was directly related to the composition of fatty acids. **Conclusions**: The free fatty acids obtained from *Carapa guianensis Aublt* oil. by enzymatic hydrolysis reaction, associated with silk fibroin was shown to be promising in the control of *A. aegypti* in low concentrations and easy to prepare and obtain the materials involved**.**

Keywords: *Carapa guianensis*; Biocatalysis; Formulations; *Aedes aegypti*.

Acknowledgements: CNPq.

1 INTRODUÇÃO

O mosquito *Aedes aegypti* pertence a ordem Diptera, família Culicidae, área de clima tropical apresenta maiores índices desta espécie, sendo ele o vetor do vírus da Dengue, Zika e febre Chikungunya, consideradas um grave problema de saúde pública no Brasil e no mundo.

Adaptado ao espaço urbano, as fêmeas do mosquito depositam seus ovos em criadouros, que em contato com a água os ovos eclodem e se tornam larvas, pupas e por fim um mosquito adulto, reinciando o ciclo. As principais estratégias para o controle populacional do *A. aegipti* está na utilização de inseticidas sintéticos, no entanto tais inseticidas apresentam sérios problemas como não seletividade, difícil degradação efeitos tóxicos imediatos e crônicos sobre outros seres vivos, como nos humanos.

Uma alternativa para o controle dos mosquitos, frente a resistência aos inseticidas sintéticos, é a bioprospecção de produtos de origem vegetal, como óleos e extratos para tal aplicação. Por este motivo o desenvolvimento de novos bioinseticidas é no controle deste vetor.

Carapa guianensis Aublt. é uma espécie vegetal reconhecida por seu uso na medicina popular, sobretudo na região Norte do Brasil, como anti-inflamatório, analgésica, inseticida e dentre outras, sendo essa versatilidade atribuída aos constituintes do óleo extraído dos seus frutos, obtidos em diferentes épocas do ano. Porém alguns fatores como a alta viscosidade e não solubilidade em água dificultam o aproveitamento desse importante recurso vegetal.

Nesse sentido, o presente trabalho empregou recursos biocatalíticos para impor modificações químicas aos triglicerídeos presentes no óleo de *C. Guianensis* Aublt. na criação de um bioformulação, contendo a fibroína da seda como alternativa aos surfactantes tradicionais, assim viabilizando sua solubilidade em água, para avaliar a capacidade larvicida do óleo e seus derivados.

1.1 Aspectos gerais sobre o *Aedes aegypti*

Os mosquitos são insetos da ordem Diptera, família Culicidae, encontrados em todos os continentes, as áreas de clima tropical possuem maior endemicidade. No Brasil são conhecidos popularmente de carapanã, muriçoca, pernilongos, os adultos são hematófagos e em suas outras fases de desenvolvimento, aquáticos (Simon, Y. G, 2016).

No filo Arthropoda, a família Culicidae apresenta os mais importantes insetos hematófagos de interesse para a saúde pública. O mosquito *Aedes aegypti* foi descrito pela primeira vez em 1762 por Linnaeus, chamado de *Culex aegypti*, *Culex* significa ‘’mosquito’’ e *aegypti* ‘’egípcio’’, assim: mosquito egípcio. Em 1818, o gênero *Aedes* foi descrito, onde

segundo as suas características biológicas e e morfológicas ele foi recolocado no gênero *Aedes* e não no gênero *Culex*, portanto ficou estabelecido *Aedes aegypti* (www.ioc.fiocruz.br)

O mosquito é originário do continente Africano, possivelmente da Etiópia ou região, acompanhou a migração humana pelo globo terrestre, entre os culicídeos, é conhecido como a espécie mais adaptada ao ambiente humano, além de ser o mais importante vetor dos agentes etiológicos da dengue, febre amarela urbana, febre Chikungunya e Zika vírus (Peniche et al., 2019).

A transmissão do vírus da Dengue ocorre principalmente nos centros urbanos, no Brasil é uma das doenças infecciosas mais ocorrente, possui quatro sorotipos conhecidos e apresenta graves complicações podendo levar a morte, do final do ano de 2018 ao início do mês de fevereiro de 2019 foram registrados 54.777 casos prováveis de Dengue (Brasil, 2019)

A febre Chikungunya, também transmitida pelo vetor *A. aegyti,* causa fortes dores nas articulações e em alguns casos pode levar a óbito. Em 2018 ocorreu cerca de 80.940 casos prováveis no Brasil, destaca-se os estados do Rio de Janeiro, São Paulo, Minas Gerais e Pará entre os de maiores casos (Brasil, 2019).

O Zika vírus foi registrado pela primeira vez em 2015 no Brasil e além de causar febre e dor pelo corpo, este vírus pode trazer uma grave complicação, a microcefalia em recém-nascidos, sendo declarado estado de emergência nacional, principalmente por esta grave complicação, em 2016 foram registrados cerca de 10.000 casos e 200 óbitos por conta da microcefalia (IPEA, 2018).

Por estes motivos o mosquito *A. aegypti* representa um grave problema de saúde pública, mesmo com investimentos em medidas de combate ao mosquito, seu controle é complexo e representa uma provocação a comunidade científica e a indústrias que desenvolvem produtos para o controle populacional desse vetor, consequentemente das doenças transmitidas por ele.

1.2 Ciclo de vida do *Aedes aegypti*

O mosquito apresenta um desenvolvimento em ciclo, composto por quatro fases, na seguinte ordem: ovo, larva, pupa e mosquito adulto. Tudo começa no ovo de *A. aegypti*, fase de maior resistência do mosquito, que pode variar de acordo com as condições de temperatura e umidade. A fase larval e de pupa necessitam ocorrer em meio aquoso para chegar a se tornar um mosquito adulto (Simon, Y.G 2016).

As larvas em meio aquoso passam por quatro estágios evolutivos (1° a 4° instar) e todo este período pode levar até 10 dias para ocorrer (figura 1), pois variações ambientais afetam essa fase de maneira a acelerar ou desacelerar o processo. O "corpo" da larva é dividido em cabeça, tórax e abdômen, possuem cerdas espalhadas pelo corpo para auxiliar sua flutuação e movimento na água, apresentam um órgão chamado de sifão respiratório e um trato digestório e aparelho bucal mastigador-rapador (Consoli, et al., 1994).

A fase larval é o período de maior crescimento e alimentação, todas as etapas de digestão dos alimentos: digestão, absorção, formação e eliminação das feses, ocorre no canal alimentar ou intestino. O que significa que o intestino é uma grande interface entre o inseto e o meio onde vive (Terra & Ferreira, 2009).

Os insetos possuem o sistema digestório dividido em três partes: intestino anterior, médio e posterior (Figura 2), a região anterior é responsável pela ingestão,

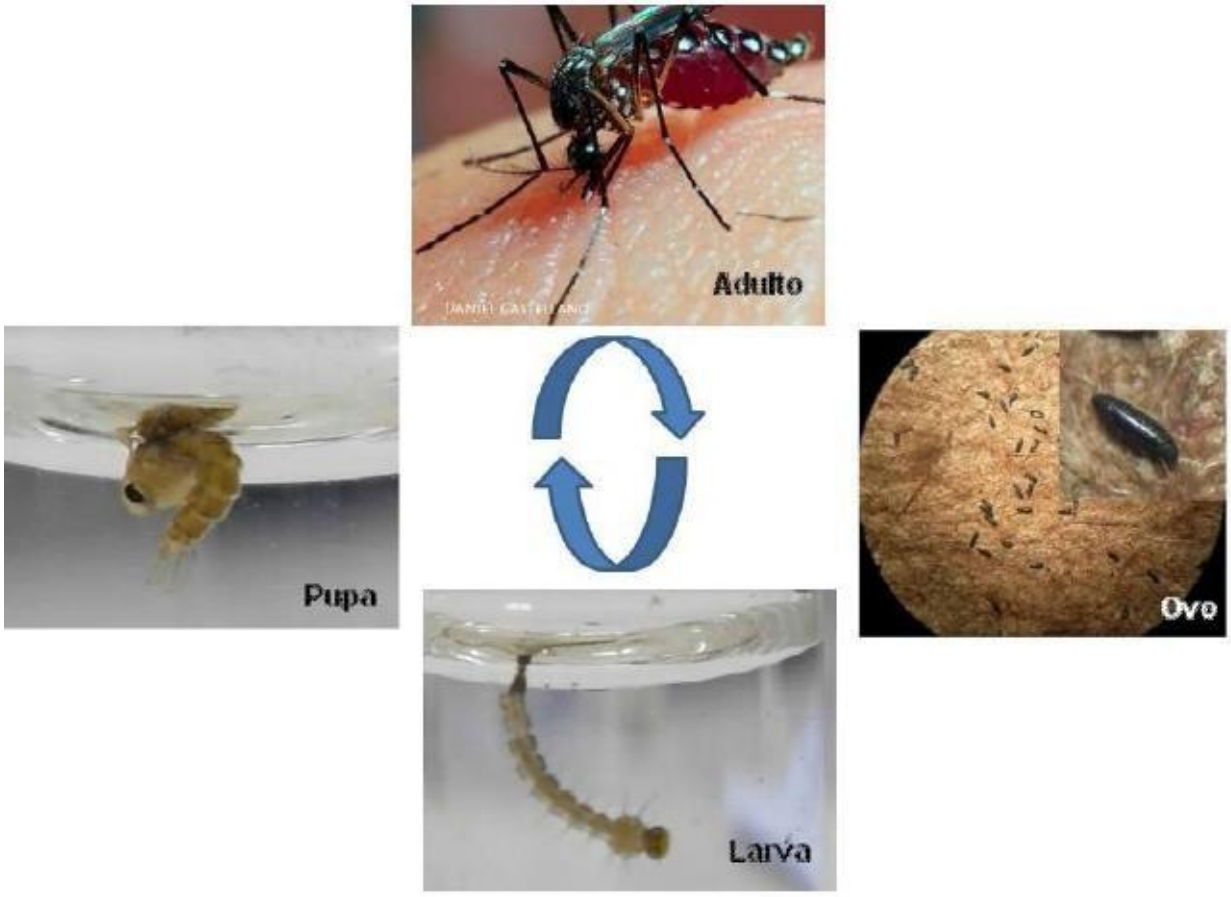
trituração, armazenamento e transporte para região mediana, no intestino médio se encontra ao menos quatro tipos celulares diferentes em uma única camada epitelial. Nesta região ocorre a absorção de produtos da digestão através de enzimas digestivas produzidas e secretadas nesta região. No terço final (posterior) ocorre a absorção de água e sais, antes da eliminação das fezes pelo ânus (Simon, Y.G,2016).

Na maioria dos insetos, os alimentos são envolvidos por uma camada acelular semipermeável composta de quitina e e protéinas, chamada de membrana peritrófica, esta membrana delimita o espaço endoperitrófico, por onde passa o bolo alimentar, e o espaço ectoperitrófico, espaço entre a membrana peritrófica e o epitélio (Terra & Ferreira, 2009; Simon, Y.G, 2016).

A região do intestino médio é responsável por liberar enzimas, que podem ser associadas ao glicocálix ou serem proteínas integrais da membrana das células do intestino. As células do intestino médio são protegidas por uma camada de muco, que a protege contra substâncias muito ácidas, pois o pH natural é descrito como alcalino variando de 6 a 12, para o funcionamento ideal de suas enzimas digestivas, como das lipases dos insetos, resposáveis por adquirir ácidos graxos para o desenvolvimento larval (Terra & Ferreira, 2009). Desta maneira, estudos apontam que os inseticidas sintéticos e naturais afetam principalmente essa região, levando ao colapso celular e consequentemente a morte dos insetos (Alves et al., 2015).

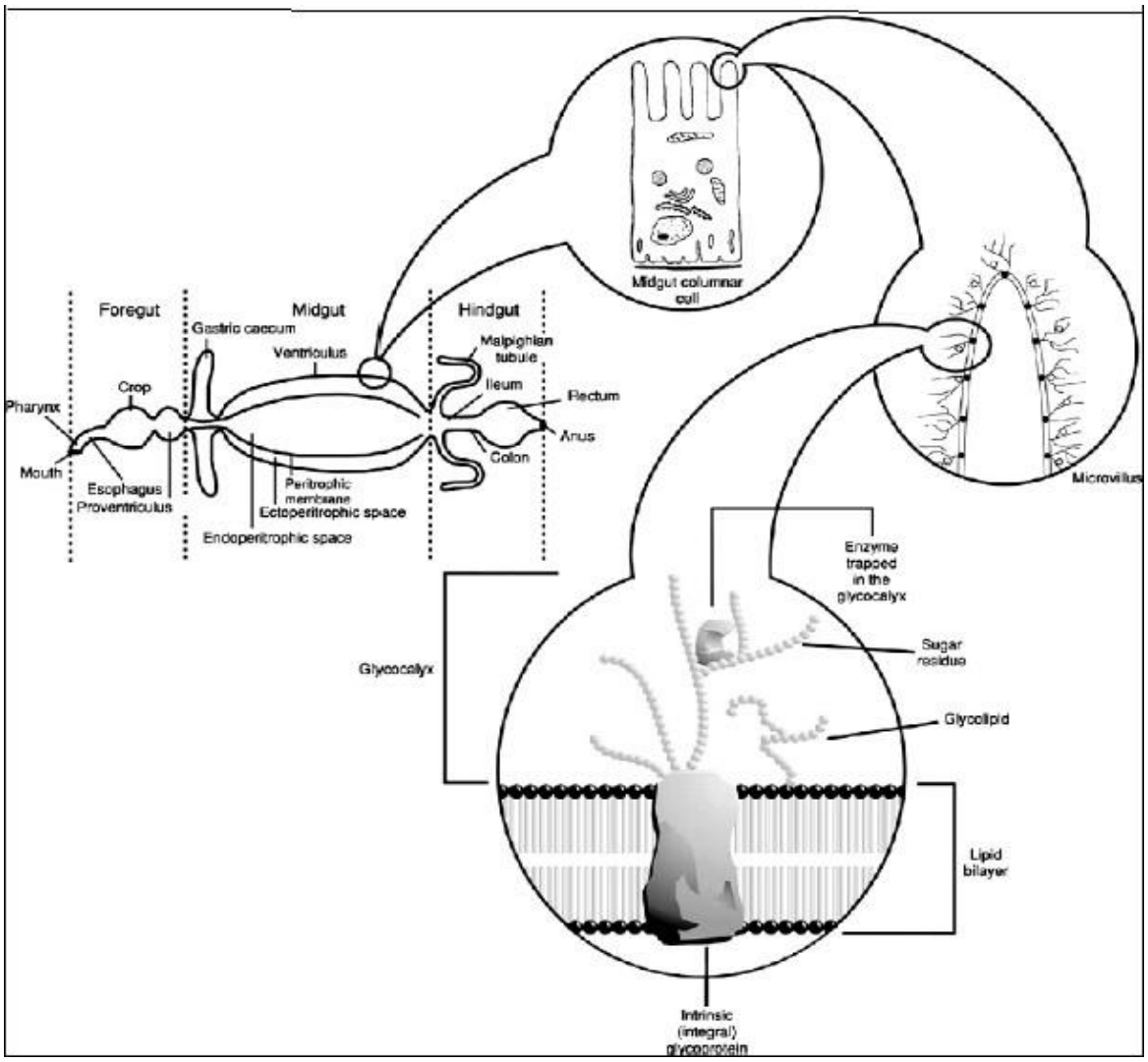
As pulpas não se alimentam e passam por metamorfose para se tornar mosquitos adultos. Possuem aspecto de “vírgula” e geralmente ficam estáticas na água, apenas quando algo as afeta que elas passam a se movimentar, após cerca de dois dias esta fase acaba e inicia a vida adulta. Na fase adulta, os mosquitos apresentam cabeça, tórax e abdômen, asas, patas. As fêmeas após a fecundação necessitam realizar a hematofagia, para maturação de seus ovos, uma vez realizado a maturação ela voa aos criadouros e realiza a ovipozição, reiniciando o ciclo (Camargo, 2008).

Figura 1. Ciclo de vida do mosquito *A. aegypti*



Fonte: SIMON, Y.G 2016

Figura 2. Representação e divisão do intestino do inseto. Representação do glicocálix, proteínas e bicamada lipidica presente nas microvilosidades do intestino médio das larvas.



Fonte: Terra & Ferreira 2012.

1.3 Métodos de controle do *Aedes aegypti*

Diversos métodos biológicos, mecânicos, químicos e até genéticos vêm sendo utilizados para o controle do vetor. No controle ambiental é realizado a diminuição dos focos do mosquito, já o controle biológico pode ser feito através de espécies de peixes

que se alimentam das larvas e pupas ou fungos patógenos para as formas evolutivas e adulto. A genética consiste em produzir mosquitos estéreis prejudicando sua reprodução, essas alternativas são promissoras no sentido de que não se pode adotar uma única medida de

controle, é necessário um conjunto das ações para tornar o processo eficaz (Zara et al., 2016).

As ações químicas são realizadas através de produtos químicos orgânicos e inorgânicos da classe dos organoclorados, organosfosforados, carbamatos e piretróides, que afetam a fase adulta e larval do mosquito (Braga & Vale, 2007).

Com o passar dos anos foi observado que estes inseticidas apresentam alta toxidade ambiental, pois se acumulam na cadeia produtiva e podem levar ao comprometimento genético de espécies animais e vegetais, bem como retornar aos humanos pela via alimentar. Assim como o uso indiscriminado dos inseticidas destas classes, tem realizado uma seleção a populações resistentes dos mosquitos aos produtos, por este motivo o desenvolvimento de novos produtos inseticidas, de menor toxidade ao ambiente e humanos, biodegradáveis e de fontes renováveis, e que apresentem diferentes mecanismos de ação são imprescindíveis para combater a proliferação do mosquito (Braga & Vale, 2007).

Neste contexto a busca por extratos e óleos vegetais ganha importância, por vencer as barreiras da resistência química e serem menos tóxicos ao meio ambiente. Vale citar que óleos fixos e essenciais das espécies *Pterodon emarginatus*, *Rosmarinus officinalis*, *Hyptis suaveolens* e *Carapa guianensis* dentre outras, foram avaliados como métodos alternativos de controle do mosquito (Oliveira et al., 2016; Duarte et al., 2015; Peniche et al.,2018).

1.4 Aspectos gerais de *Carapa guianensis* Aubl.

A Amazônia brasileira é cosiderada uma das áreas mais ricas em biodiversidade do mundo (Milheiras et al., 2020). Em meio a esta riqueza a medicina tradicional vem

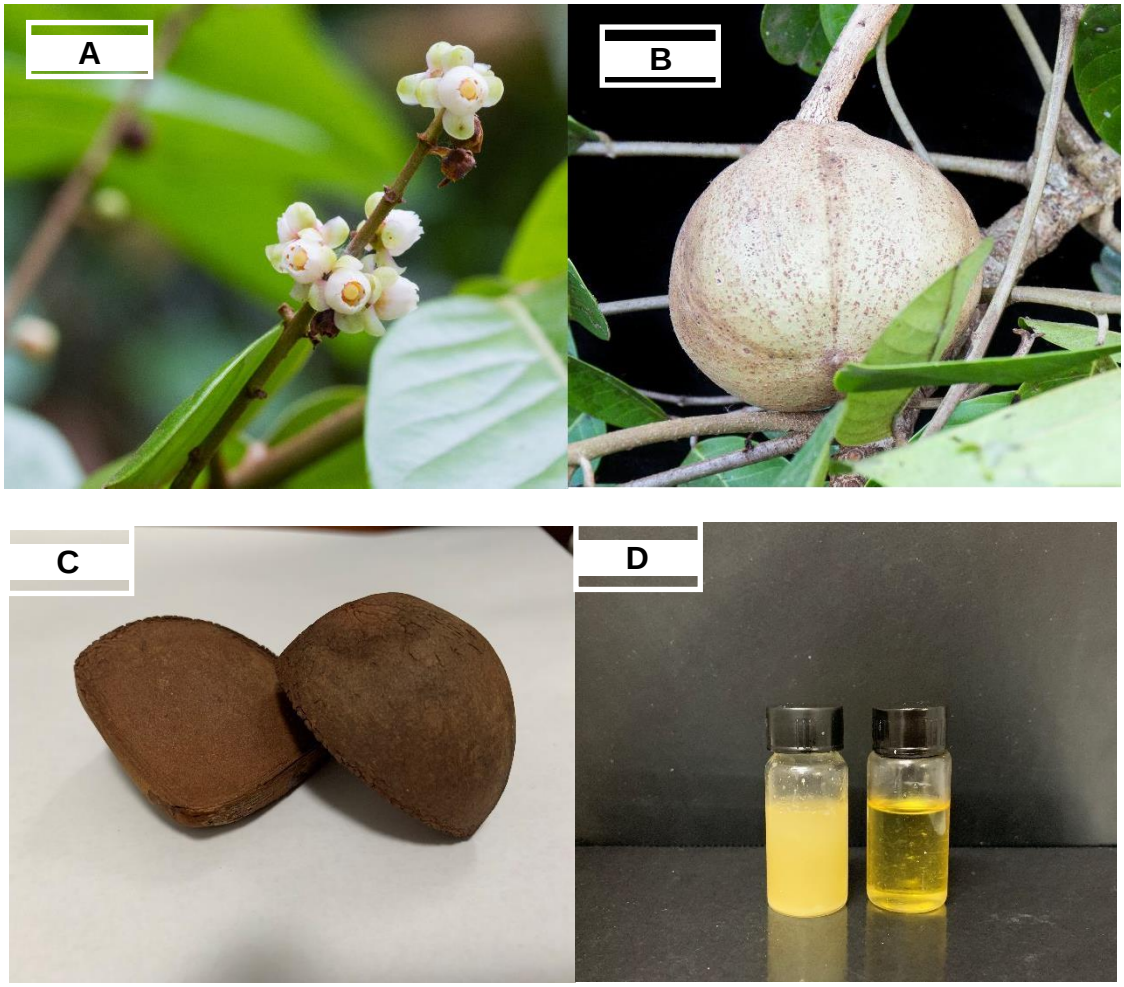
sendo utilizada ao longo dos tempos pela população que vive próximo as áreas florestas. Uma das espécies vegetais mais utilizadas na medicina tradicional é a *Carapa guianensis* Aubl. (Meliaceae), conhecida como Andiroba. A família *Meliaceae* é composta de 192 gêneros, nesta família o gênero *Carapa* possui 38 espécies registradas, sendo uma destas espécies a *Carapa guianensis* Aubl. (www.tropicos.org, 2019).

Esta árvore se localiza geralmente nas várzeas da bacia amazônica, possui flores pequenas, perfumadas e de cor creme, florescendo no período de menor número de chuva, agosto a outubro, logo após gerados os frutos em forma de cápsula que armazena de 4 à 16 sementes com amêndoas em volta que pesam cerca de 21g cada, da qual é extraído o óleo (figura 3). (Lorenzi, H & Matos, F.J.A, 2008).

Os frutos costumam ocorrer nos meses de menor e maior quantidade de chuva, janeiro a fevereiro, março a julho, esta variação é estudada quanto a aspectos do tamanho dos frutos, hidrocória, aspectos reprodutivos, genéticos e quanto ao seu perfil lipídico, ocorrendo algumas diferenças dependendo da época de coleta (Cunha et al.,2017; Cloutier et al., 2007).

Desta maneira o período de coleta ocorre de janeiro a fevereiro e a outra safra de março a julho, fatores ambientais como a pluviosidade, forma de extração, , radição solar, período de coleta, modo de coleta, local de coleta, até mesmo larvas do gênero *Hypspyla* podem influenciar no perfil lipídico, teor de limonóides, aspectos físico-químicos, bem com podem influenciar no seu perfil terapêutico e qualidade do óleo de andiroba. (Silva et al, 2018; Nardi et al, 2017).

Figura 3. A-Inflorescência, B-Fruto, C-Sementes, D-Óleo fixo de *Carapa guianensis* Aubl.



Fonte: A, B (www.tropicos.org), C, D (Próprio autor).

O óleo extraído de seus frutos possui diversas aplicabilidades na medicina tradicional: tratamento de contusões e erupções na pele, inflamações, reumatismo, artrite,

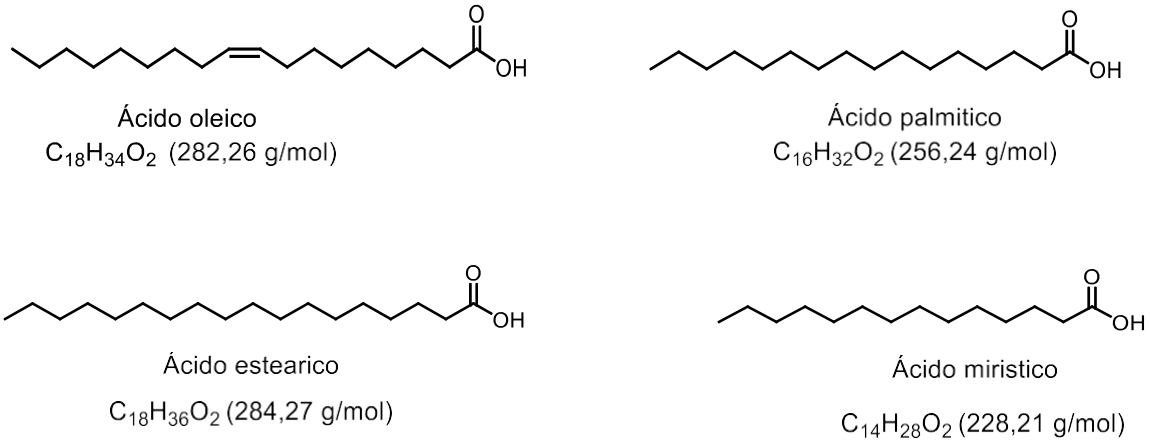
tratamento do diabetes, repelente de mosquitos, analgésico, febre, diarréia antiparasitário, psoríase e anticâncer (Henriques, M.G & Penido, C., 2014; Ambrozin et al., 2006).

As atividades biológicas do óleo de *C. Guianensis* Aublt. são atribuídas aos tetraterpenóides contidos no óleo como 6-α-acetoxigedunina, 7-diacetoxi-7-oxogedunina, andirobina, gedunina entre outros (Henriques, M.G & Penido, C, 2014). Destacando-se pela ação antialérgica e anti-inflamatória, com mecanismos complexos

de inibição de mediadores químicos pró-inflamatórios e bloqueio das células e respostas imunológicas alérgicas (Ferraris et al., 2011).

No entanto, o óleo de andiroba não possui apenas terpenóides, este óleo é rico em ácidos graxos insaturados e saturados, como ácido oleico (57%), linoleico (5%), palmítico (25%), ácido esteárico (10%), ácido mirístico (0,5%) (Figura 4), sendo a maioria destes ácidos de cadeia longa monoinsaturados e de cadeia longa saturados (Stachiw et al., 2016; Iha et al., 2014).

Figura 4. Estrutura química dos ácidos graxos mais frequentes no óleo de andiroba.



Fonte: Próprio autor – ChemBioDraw

Apesar do óleo de *C. guianensis* Aublt. ser amplamente estudado ainda é um desafio a sua produção em larga escala para utilização em produtos padronizados com seus constituintes químicos, no entanto sua composição química o mostra como promissor na obtenção de produtos com ação biológica derivados do óleo (Silva et al., 2010).

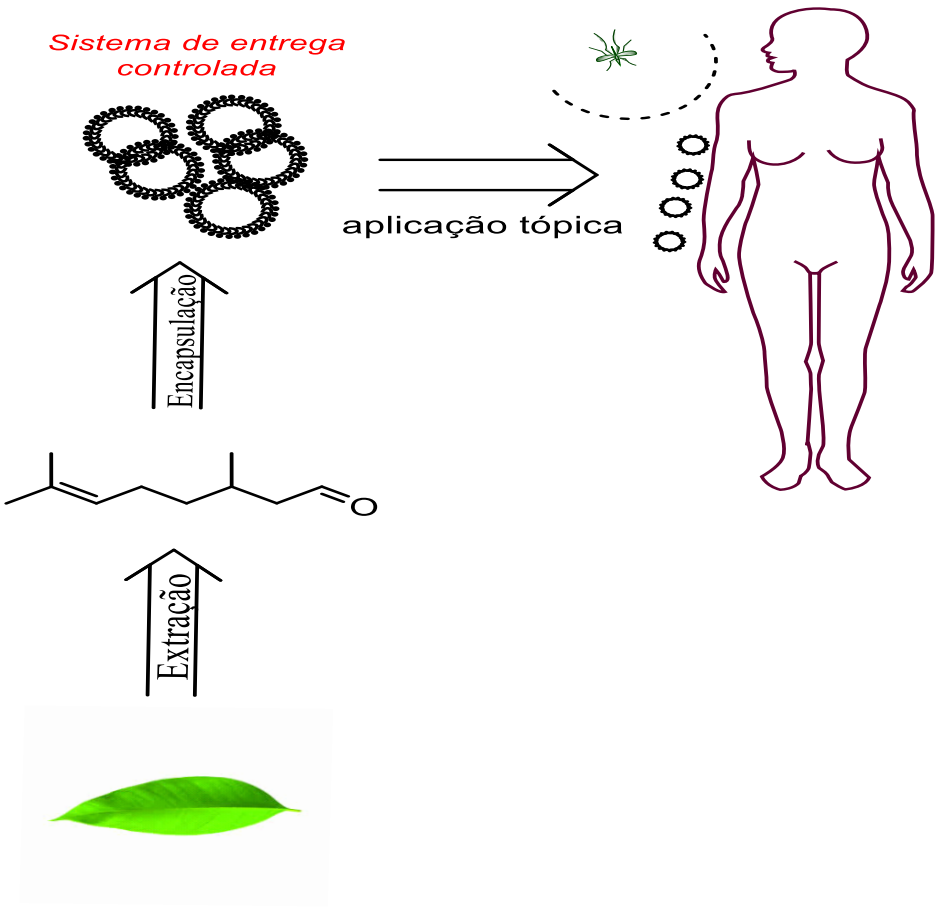
Estes ácidos graxos são importantes para a função energética celular e estão presentes nas membranas plasmáticas dos seres eucariontes, nos mais diversos sistemas: nervoso, muscular, respiratório, circulatório dentre outros. De uma maneira geral estes ácidos graxos juntos com radicais fosfatos e proteínas formam a bicamada lipídica das membranas celulares nos seres vivos, as membranas tem por função proteger as células, permitir trocas com o meio extracelular, garantem a fluidez da

membrana, potencial de ação, respiração celular, assim como alterações na membrana podem gerar a morte celular (Tortora e Hall 2015).

O estudo de materiais e técnicas que carream princípios ativos para atuar em membranas celulares surgem como uma nova tecnologia, neste sentido a encapsulação de óleos vegetais, como o óleo de *C. guianensis* Aublt. em emulsões e nanoemulsões ativas frente a parasitas do gênero *Leishmania*, *Trypanossoma*, assim como ativas frente as larvas de *Aedes aegypti* ou como formulações repelentes tem ganhado cada vez mais interesse da comunidade científica (Baldiserra et al., 2013; Moraes et al., 2018; Jesus et al., 2015).

Segundo a revisão de Silva & Ricci-Junior (2020) o efeito repelente do óleo de andiroba está relacionado com nortriterpenóides e limonóides, por outro lado sobre o efeito larvicida são apontados os limonóides assim como os ácidos graxos insaturados em questão, no entanto técnicas semelhantes de encapsulação são usados para ambas as atividades, na figura 5 está representado um esquema de extração, encapsulação e utilização do produto natural citronelol em formulação repelente.

Figura 5. Esquema de extração do produto natural citronelol, encapsulação e utilização como repelente de mosquitos



Fonte: Adaptado de Silva & Ricci-Junior, 2020.

1.5 Processos biocataliticos por lipases

A biocatálise é um ramo da Biotecnologia com ênfase nas transformações químicas preferencialmente orgânica. A Biotecnologia possui a interdisciplinaridade como principal característica, envolvendo e integrando conhecimentos, como a biologia celular, bioquímica, biofísica e a partir disso desenvolver técnicas e produtos viáveis economicamente e ecologicamente corretas (Da Gonçalves & Marsaioli, 2013).

O termo biocatálise é aplicado em processos enzimáticos, utilizando enzimas oriundas de microrganismos (fungos ou bactérias), plantas ou animais superiores, como catalizadores biológicos, no intuito de acelerar as reações químicas promovendo modificações estruturais, gerando características desejáveis a este novo composto. O uso desses biocatalisadores vem ganhando lugar na Indústria química e farmacêuticas, dentre as vantagens: a alta viabilidade do reuso do catalisador, maior rentabilidade, maior rendimentos, maior enantiosseletividade, maior quimiosseletividade e uso em condições brandas de pH e temperatura e pressão (Omori; Portas; De Souza, De Oliveira, 2012).

Em uma extensa revisão Birolli e colaboradores (2015) apontou que o Brasil é uma fonte de microrganismos biocataliticamente promissores, dentre eles fungos, algas e bactérias encontradas no país, que possuem enzimas promissoras, que podem vir a serem utilizadas em reações biocatalíticas em industrias químicas e farmacêuticas, assim como reações em produtos naturais oriundos da flora brasileira.

Segundo Ferreira (2016) processos enzimáticos aos poucos vêm substituindo processos químicos tradicionais, pois diminuem a poluição ambiental e apresentam rendimentos equivalentes aos métodos tradicionais.

Neste contexto, as lipases são enzimas da classe das hidrolases (hidrolases triacilglicerases; EC 3.1.1.3) , mais estudadas e utilizadas em processos biocatalíticos normalmente nos seres vivos são resposáveis por reações de hidrólise, transesterificação e esterificação de ésteres carboxílicos (Ferreira, 2016), porém em experimentos e na indústria química, farmacêutica e alimentícia ela é utilizada para outros fins, como no desenvolvimento de ésteres aromáticos (Macedo; Pastore, 1997), na síntese de tensoativos naturais, síntese de fármacos ou adjuvantes farmacêuticos enantioméricamente ativos. (Carvalho et al., 2005).

As lipases apresentam versatilidade, pois conseguem se adequar a diferentes meios e com diferentes substratos, condição conhecida como reações de promiscuidades, possuem alta eficiência e seletividade, as reações apresentam um menor consumo de energia sendo ecologicamente aceitáveis (Zanotto et al., 2009) também denominadas triacilglicerol-hidrolases, são conhecidas por hidrolisar

acilgliceróis de óleos em ácidos graxos, sendo também estudada e utilizada para a produção de biocombustíveis a partir de óleos em reações de transesterificação ou esterificação de maneira renovável, biodegradável e ambientalmente aceitável (Tiosso et al., 2014).

1.6 Biocatálise de óleos vegetais

Como já relatado, são muitas as possibilidades de utilização das lipases em distintas reações químicas, por isso serão explorados estudos sobre os três tipos de reações químicas mais interessantes para este trabalho, hidrólise, transesterificação e amidação.

As reações de transesterificação, hidrólise e amidação promovem a síntese de ácidos, ésteres e amidas graxas respectivamente, geralmente na semi-síntese desses grupos são usados catalizadores ácidos ou básicos fortes, gerando subprodutos e resíduos tóxicos ao meio ambiente, além de necessitarem de altas temperaturas e condições drásticas, neste sentido a biocatálise é uma alternativa ao métodos químicos tradicionais, pois a enzima pode atuar como catalisador heterogêneo com alto poder de resuso e em alguns casos mantendo sua capacidade enantioseletiva e quimioseletiva (Ferreira, I.M. 2016).

1.6.1 Reação de transesterificação e hidrólise de óleos vegetais com aplicação na atividade larvicida

Os óleos vegetais são constituídos por triacilglicerídeos, que são moléculas de glicerol ligada a três ésteres de ácidos graxos. Podendo existir os diacilgliceróis e monoacilgliceróis quando dois ou um grupo hidroxila estiver esterificado com o ácido graxo (Carvalho, 2014).

A reação de transesterificação é uma reação relativamente simples, na qual um triéster ligados por uma molécula de glicerol, se transforma em outro éster (mono éster) na presença de um álcool, ficando na sua forma livre na presença de um álcool, nesta

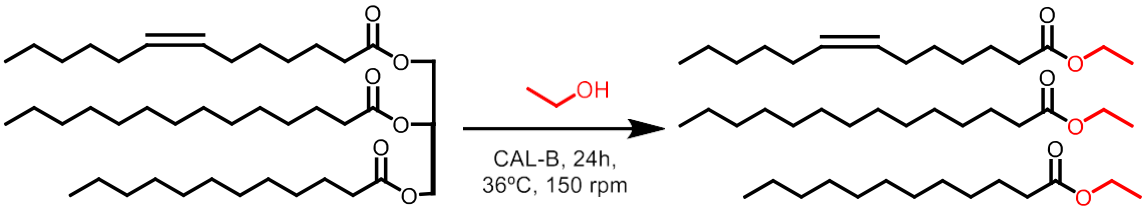
reação propriedades físico-químicas como densidade, viscosidade e fluidez são modificadas (Almeida, 2014).

A reação ocorre em três etapas, primeiro ocorre a conversão de triglicerídeo em diglicerídeo, depois a conversão em monoglicerídeo e este em éster graxo com a liberação de uma molécula de glicerol na presença de um álcool (figura 6). Esta reação sofre influência da temperatura, tipo de catalizador, pressão e agitação. E por ser uma reação

reversível, é possível utiliza-la para isolamento dos ácidos graxos a partir dos ésteres obtidos na reação (Almeida, 2014).

Os álcoois mais utilizados, são álcoois de cadeia curta, como o metanol e o etanol, no entanto o etanol é menos tóxico quando comparado ao metanol e pode ser obtido de maneira mais barata e por fontes renováveis, sendo visto com potencial para aplicação em escala industrial e ecologicamente aceitável.

Figura 6. Exemplo da reação de transesterificação via catálise enzimática (CAL-B)



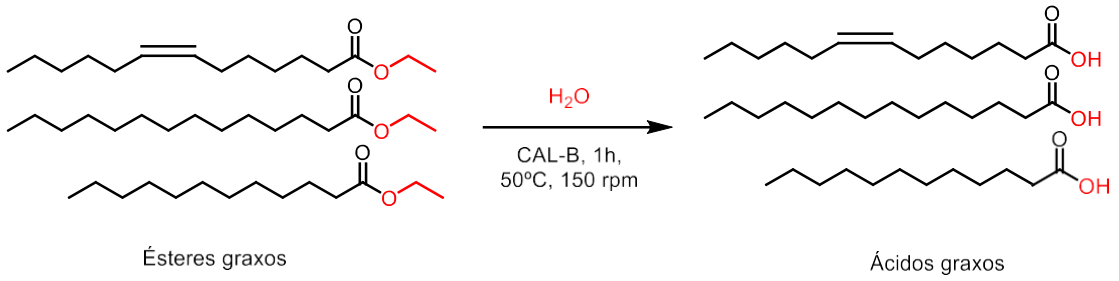
Triglicerideo

Ésteres graxos

Fonte: Próprio autor – ChemBioDraw

Por outro lado, a reação de hidrólise enzimática envolve ésteres ou triglicerídeos de ácidos graxos, água e enzima como catalizador, por fim são obtidos ácidos graxos livres (figura 7) como produto principal e etanol como subproduto, sendo um processo ecologicamente aceito, pois não gera poluentes ao meio ambiente nem resíduo.

Figura 7. Reação de hidrolise dos óleos vegetais via catálise enzimática.



Fonte: Próprio autor – ChemBioDraw

Apesar da grande maioria dos trabalhos citados na literatura relatarem esta reação para produção de biodiesel, a utilização destes ésteres com atividades biológicas como antimicrobiana e larvicida vem ganhando destaque (Araújo et al., 2018; Pant et al., 2016). No entanto, a maioria dos artigos utilizam os metil ésteres para realizar a ação larvicida, Silva e colaboradores (2016) utilizou o óleo de girassol e óleo de soja como fontes de triglicerídeos para reação de transesterificação utilizando o metanol como álcool e teste frente as larvas de *Culex quinquefasciatus*, neste trabalho foram relatadas altereações bioquímicas nas larvas que as levaram a morte.

Pant e colaboradores (2016) utilizaram os ésteres do óleo de Jatropha (*Jatropha curcas*) e Karanja (*Pangamia glabra*) e foi avaliado suas respectivas atividades repelentes e mosquiticida contra adultos de *A. aegypti* no formato de serpentina que já é comercialmente vendida, os ésteres apresentaram eficiência considerada, em uma abordagem ecologicamente correta, demonstrando que é possível utilizar ésteres de óleos vegetais para atividade frente ao mosquito *A.aegypti*.

Assim como os ésteres e ácidos graxos podem ser produzidos através da transesterificação, eles podem ser adquiridos de outras maneiras, desta forma, Silva (2015) isolou ácidos graxos e metil ésteres dos frutos de *Solanum lycocarpum* e testou frente a larvas de *C. quinquefasciatus*, otendo atividade larvicida para ambos.

Os ácidos graxos também são utilizados como fontes de ativos para o desenvolvimento de produtos larvicidas, no entanto a maioria dos estudos conduzidos

com os ácidos graxos são realizados através de ácidos graxos comprados de indústrias químicas ou isolados através de técnicas cromatográficas, no primeiro tem a dificuldade do preço e transporte, no segundo caso há utilização de solventes e fase estacionária, num processo que pode ser lento e custoso, neste sentido a reação de hidrólise enzimática supera estes problemas pois consegue extrair de uma fonte natural esses ativos (Silva et al., 2015).

Neste contexto, Rahuman e colaboradores (2008) foram precursores do uso de ácidos graxos com ação larvicida, ao utilizar extratos de *Citrullus colocynthis (Linn.) Schrad* que apresentou atividade larvicida moderada, depois os componentes majoritários foram isolados, os ácidos oleico e linoleico, em seguida foi realizado o teste com eles isolados e a atividade foi maior, sugerindo estes como os principais responsáveis pela atividade larvicida frente ao *A. aegypti*.

Melo e colaboradores (2018) utilizaram os seguintes ácidos graxos para verificar sua atividade larvicida: ácido oleico, ácido palmítico, ácido esteárico, ácido linoleico, ácido linolenico e seus respectivos metil ésteres: metil oleato, metil palmitato, metil estearato, metil linoleato e metil linolenato, todos estes foram comprados e utilizados frente a larvas de *C.quinquefasciatus*, os ácidos graxos oleico, linoleico e linolênico apresentram as amiores taxas de mortalidade quando comparados aos seus respectivos metil ésteres e ácidos graxos palmíticos e esteárico, sugerindo que estes ácidos por possuírem insaturações podem causar danos letais ao intestino médio das larvas, além disso foram detectadas alterações bioquímicas de glicose, triglicerídeos e proteínas nos grupos tratados com estes ativos.

No mesmo trabalho também fica o questionamento sobre os metis ésteres, que apresentram resultados menores do que os ácidos graxos livres. No entanto Silva (2016) produziu um trabalho utilizando metil ésteres e obteve resultados de 100% de mortalidade na concentração de 100 mg/L, sugerindo que os metis ésteres podem alterar a fuidez da membrana das larvas principalmente no sitema digestivo e cutícula, podendo causar lesões nestes lugares, sendo pormissores como agentes larvicidas.

Perumalsamy e colaboradores (2015) ao estudarem a espécie *Milletia pinnata* obtiveram resutados interessantes para os ácidos graxos livres, explicando um possível mecanismo biológico para os mesmo, ao realizar o estudo estrutura-atividade foi identificado que o grau de insaturação de ácidos graxos e o tamanho da cadeia lateral podem afetar toxicidade frente as larvas *A. aegypti*.

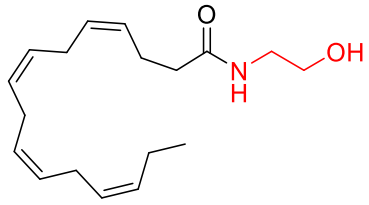
Neste mesmo trabalho, os ácidos elajico e ácido araquídico causaram interferência no sistema octopaminérgico das larvas. Os ácidos linoleico e linolênico foram ativos na inibição da enzima acetilcolinaesterase (AChE) e no sistema octopaminérgico, vale ressaltar que a inibição da AChE é relatada na literatura como chave para atividade larvicida, sendo estas uma das mais estudadas na literatura. Por fim o ácido oleico foi considerado o mais promissor componente ativo pois apresentou a maior taxa de inibição de AChE, consequentemente menor dose letal 50 (DL_{50}), quando comparado a ácidos saturados como o palmítico e etil e metil ésteres do próprio ácido oleico estes apresentaram as menores inibições de AChE consequentemente as menores taxas de mortalidade frente *A.aegypti*.

1.6.2 Reação de amidação e suas aplicações

As amidas graxas, também conhecida como alquilamidas, vêm ganhando importância em estudos científicos devido suas propriedades físicas nas aplicações químicas comerciais como tensoativos na produção de detergentes, lubrificantes, fungicidas, agrotóxicos entre outras e propriedades biológicas (Araújo et al., 2018).

As amidas graxas podem ser incorporadas em matrizes lipídicas, proteicas, lipoproteicas dentre outras, como exemplo o grupo das *N*-aciletanolaminas, neste grupo se destaca a anandamina (figura 8) que possui atividade moduladora do sistema nervoso central, apresentando também propriedades anti-carcinogênicas e anti-inflamatória (Barata et al., 2020) também sendo reconhecida como endocanabinóide por ativar os mesmos receptores de *Cannabis sativa*, apresentando como efeito adverso a queda da pressão e frequência do coração (De Almeida, 2014).

Figura 8. Estrutura química da anandamina.



Fonte: Próprio autor - ChemBioDraw

As amidas graxas possuem efeitos sobre o sistema nervoso principalmente por se ligarem aos receptores CB1 e CB2, podendo apresentar ação sistêmica e semelhantes aos canabinóides produzidos pelo organismo como o palmitoletanolamida e oleoetanolamida (figura 8) (Iannot et al.,2016).

Figura 9. Estrutura química das amidas palmitoletanolamida e oleoetanolamida

Palmitoiletanolamida **Oleoiletanolamida**

Fonte: Próprio autor - ChemBioDraw

Em 1950 foram realizados estudos pioneiros com amidas graxas, descoberto que a partir de frações do óleo de amendoim, gemas de ovos e lecitina de soja, era possível se obter atividades anti-inflamatória, a palmitoetanolamida foi apontada como responsável pela ação (Lopes et al., 2010).

A partir da primeira amida graxa identificadas os pesquisadores passaram a investigar as atividades farmacológicas no organismo como diminuição da contratilidade do coração, proteção dos neurônios frente condições neurológicas crônicas, auxílio na consolidação da memória, regulador do apetite, indutor de sono, demonstrando ter amplo efeito sobre o sistema nervoso, mas apesar destas propriedades farmacológicas promissoras ainda há poucos estudos que relatam a obtenção e melhoramento dos processos, estudos estes que podem tornar as amidas graxas uma realidade, pois podem baratear o processo de obtenção e viabilidade comercial (Lopes et al., 2010; De almeida, 2014).

A síntese das amidas graxas é realizada através da condensação entre ácidos carboxílicos e aminas, porém esta reação não é espontânea e acaba sendo realizada em condições extremas, utilizando diferentes reagentes, muitas etapas envolvidas, tornando este processo custoso na sua obtenção. Sendo assim, uma forma de facilitar esta reação é primeiramente realizar a esterificação dos triglicerídeos, posteriormente realizar a reação de aminólise para obtenção das amidas graxas, porém nesta etapa ainda são descritas condiçoes críticas de reação (D'oca, 2010).

Com o passar do tempo estudos da síntese de amidas graxas a partir de fontes totalmente sintéticas e de óleos naturais seguida de testes frente a organismos vivos vem sendo realizados. Como o realizado por Mata-Santos e colaboradores (2016) que realizaram a síntese de amidas graxas citotóxicas frente as larvas do parasita *Toxocara canis*, utilizando produtos sintéticos. No trabalho de Mäki-Arvela (2017) realizou a amidação de ácidos graxos sintéticos isolados em temperaturas de 180 °C, utilizando a etanolamina, em pressões de 20 bar, para obter amidas graxas com atividades farmacológicas, assim Kothandamapni e colaboradores (2017) utilizaram temperaturas de 120°C para obter amidas graxas.

Por outro lado, estudos que utilizam condições mais brandas e ecologicamente aceitáveis vem sendo realizados. D´Oca (2010) produziu benzilamidas graxas com atividade antituberculose em temperatura de 50°C, 120°C e em temperatura ambiente 25°C a partir de ésteres metílicos previamente obtidos. Liu e colaboradores (2016) conseguiram sintetizar amidas graxas com atividade antibacteriana em 40°C utilizando lipase imobilizada, anilina e triglicerídeos isolados.

Almeida e colaboradores (2013) utilizaram a reação de amidação direta utilizando o óleo de *Passiflora edulis* como fonte de triglicerídeos, etanolamina, apesar de obter sucesso nesta síntese a temepratura de 100°C foi empregada por 8 horas para se obter alto rendimento, mostrando que ainda é um desafio a obtenção de amidas graxas em temperatura ambiente.

No entanto Araújo e colaboradores (2018) realizaram a síntese de amidas graxas com atividade antimicrobiana através da amidação direta usando o óleo de Patawa (*Oenocarpus bataua*), neste estudo foi utilizada a enzima imobilizada CAL-B e

obteve-se um rendimento de 82% de amidas graxas, e rendimento de 98% para o catalizador Al_2O_3 em temperatura ambiente (27°C) por 24 horas, mostrando que é possível realizar a síntese de amidas graxas utilizando óleos naturais em temperatura ambiente e condições reacionais brandas.

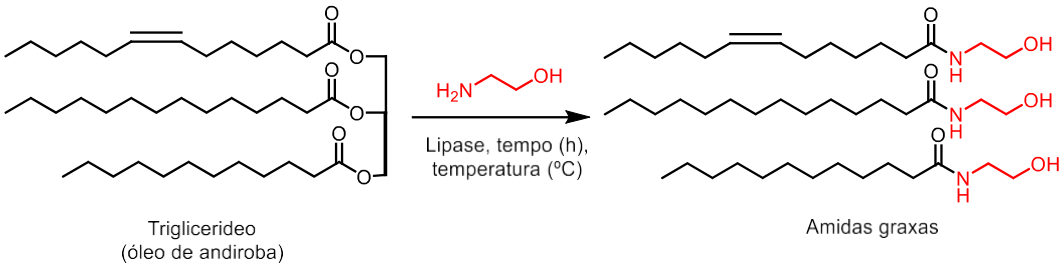
Recentemente Barata e colaboradores (2020) realizaram a síntese quimioenzimática de amidas graxas a partir dos triglicerídeos presentes no óleo de *Bertholetia excelsa* (castanha do Brasil) com atividade anti-imflamtória frente a edema em ratos com atividade semelhante a da endometacina (fármaco anti-inflamatório já comercializado) e baixa toxicidade, utilizando a Amano lipase de *Pseudomonas fluorecens*, demosntrando que a utilização de enzimas para amidação de óleos Amazônicos pode ser um novo caminho para o desenvolvimento de fármacos, oriundos da mais rica flora do Brasil.

Neste sentido, Nascimento e colaboradores (2020) realizaram um trabalho inédito ao utilizar os triglicerídeos do óleo de *Carapa guianensis* (andiroba) para produzir amidas graxas com atividade anticonvulsivante em ratos, com ação moduladora do receptor GABA- A, foi possível inibir convulsões em nos animais, demonstrando que a amidação pode gerar fármacos endocanabinóides com atividade significativa, podendo ser no futuro uma alternativa no tratamento deste agrave, colaborando com o uso dos óleos Amazônicos na obtenção de novos tratamentos para doenças como a epilepsia.

No entanto, poucos trabalhos relatam a obtenção de amidas por aminólise direta a partir de óleos naturais com aplicação frente as larvas de *A. aegypti*, sendo este trabalho com dados inétidos na utilização em utilizar esta reação para este propósito usando o óleo de andiroba.

Portanto, o presente trabalho buscou a síntese direta de amidas graxas a partir do óleo de andiroba, via catálise enzimática, reagindo os ácidos graxos presentes no óleo com a etanolamina a ser realizada em condições brandas e reprodutíveis no laboratório, podendo este estudo contribuir no avanço da biotecnologia para o aproveitamento e melhor custo na obtenção destes produtos farmacologicamente promissores e ativos frente as larvas de *A.aegypti* (figura 10).

Figura 10. Reação de amidação direta de triglicerídeos com etanolamina



Fonte: Próprio autor – ChemBioDraw

1.6.3 Fibroína da seda

Os primeiros relatos do uso da seda iniciam desde os aborígenes australianos utilizando-a para caça e pesca, até a utilização da seda por gregos e romanos para curar ferimentos, porém as seda ainda não era processada, até que na China foram encontrados achados arqueológicos de casulos de *Bombyx mori* para criar tecidos em teares rudimentares, no Paquistão também foram encontradas artefatos, sugerindo que essa prática iniciou em 5000 – 2000 a.C (Holland, et al., 2019).

Por muitos anos, o segredo da obtenção da seda permaneceu na China, porém nas estradas que o valioso tecido percorria, sua fama foi ganhando o mundo, até que a utilização dos casulos de *Bombyx mori* ficaram disponíveis além da Ásia. Atualmente são criados cerca de 980 milhões de larvas, para produzir 400 megatoneladas de seda, hoje em dia são criadas as larvas de maneira controlada, até elas produzirem seu casulo para se tornarem mariposas, neste processo os produtores retiram o casulo para produzir a seda (Holland, et al., 2019). Além disso é considerada um material barato e disponível em grande quantidade no Brasil, pois muitos casulos são descartados por produtores.

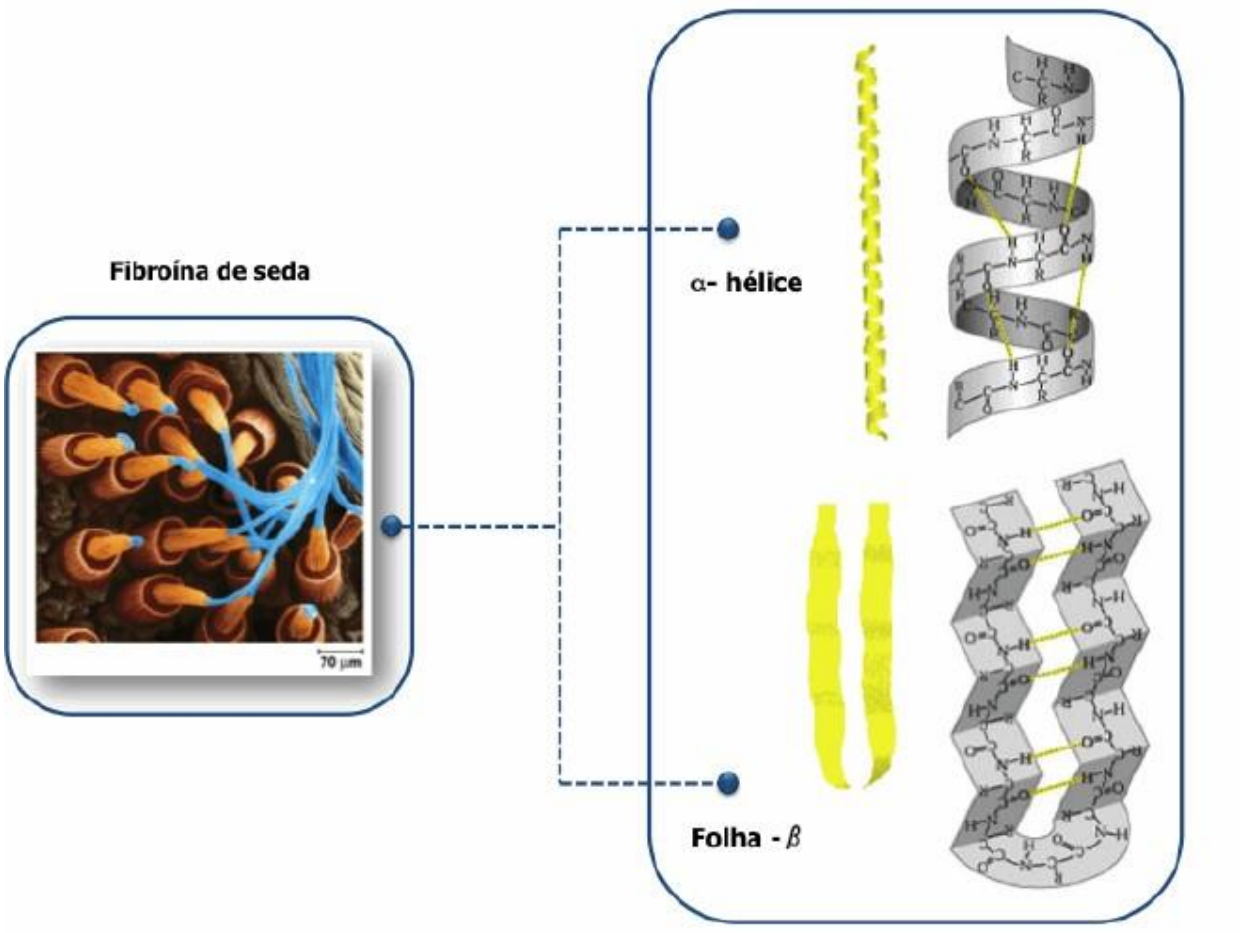
A seda foi documentada pela primeira vez por Claudius Galenus para tratar gladiadores feridos em combates, sobre tudo na pele e tendões, nos últimos anos tem surgido pesquisas que serão citadas no decorrer deste tópico que se referem a fibroína de seda em aplicações de biomédicas.

A seda é um biopolímero proteico, produzidas por glândulas especializadas de alguns artrópodes, principalmente do bicho-da-seda (*Bombyx mori*). O casulo do Bicho-da- seda (Figura 11) é constituído de duas proteínas, a fibroína e a sericina. A fibroína representa cerca de 70 a 80% do peso do polímero, a fibroína é constituída basicamente por 3 aminoácidos: glicina, alanina e serina, e possui caráter hidrofóbico. A sericina é solúvel em água, esta característica, faz com que seja utilizada a técnica de degomagem para separação das proteínas. A sericina se apresenta em folhas α e β, sobretudo na forma α. A fibroína é se apresenta numa forma de folha β com ligações de hidrogênio entre umas e as outras, sendo a conformação mais estável (Ferreira, I.M, 2016; Lopes, J.R, 2017) (Figura 12).

Figura 11. Casulos do Bicho-da Seda

Fonte: Autor 2018

Figura 12. Conformações das proteínas contidas na seda.



Fonte: Lopes, J.R (2017)

Nos últimos anos a fibroína extraída do casulo do bicho-da-seda foi amplamente estudada e utilizada na saúde humana, principalmente no estudo de engenharia de tecidos, sensores elétricos em organismos vivos e recentemente em sistemas de liberação controlada de fármacos (Fan et al., 2019).

No estudo da engenharia de tecidos, a fibroína de seda é aprovada pelo FDA em cirurgias (Food and Drug Administration) com um biomaterial para ser utilizado para substituir tecidos como a pele, nervos, tendões, vasos sanguíneos e ossos. Nesse sentido este biopolímero apresenta compatibilidade com células humanas, demora

para ser eliminado completamente, ao mesmo tempo ele é ‘’degragado’’ e integrado ao corpo humano onde foi inserido, ajudando na recuperação (Fan et al.,2019).

Outra aplicação da fibroína de seda é sua utilização como transportador de medicamentos em formulações para organismos vivos, vale destacar que a fibroína possui blocos hidrofílicos e hidrofóbicos, essa característica faz com que ela seja capaz de se solubilizar na água, bem como estabilizar medicamentos instáveis nesse ambiente (Fan et al., 2019). Neste sentido um dos produtos mais estudados é a utilização de hidrogéis de fibroína para reconstituição de tecidos cartilaginosos, devido sua capacidade física de resistência mecânica e principalmente biocompatibilidade com este tipo de tecido. (Singh et al., 2016). Por outro lado Yang e colaboradores (2019) produziram hidrogéis utilizando fibroína de seda, álcool polivinilico e borax, estes hidrogéis foram capazes de serem condutores de impulsos elétricos, altamente resistentes e totalmente aplicáveis ao desenvolvimento de porteções mecânicas para seres humanos e máquinas como robôs, sendo um novo produto que pode ficar na interface entre equipamentos eletrônicos, medicmanetos e seres humanos.

Outra aplicação da fibroína que vem crescendo ultimamente e sua aplicação biocatalítica, pela grande quantidade de grupos amino (-NH_2) e na sua conformação β, Ferreira e colaboradores (2017) utilizou a fibroína como suporte para a lipase de *P. fluorecens* na síntese enantioseletiva de haloidrinas.

Em outro trabalho Ferreira e colaboradores (2014) utilizaram a fibroína e o alginato (outro biopolímero) para formas esferas que possibilitaram imobilizar uma lipase, estas esferas então foram utilizadas em reações químicas, e o resultado foi que estas apresentaram alta enantioseletividade na obtenção de clorohidrinas, que é um precursor de fármacos importante.

Recentemente tem se utilizado a fibroína no emprego de emulsões e nanoemulsões como carreadora de princípios ativos, no entanto a utilização de fibroína juntamente com o óleo de Andiroba ainda não é relatado na literatura. Mesmo assim, no próximo item será discutido a utilização dos dois para aprimorar a atividade larvicida, bem como consolidar a fibroína como carreador de princípios ativos.

1.6.4 Emulsões e nanoemulsões de *C. guianensis* com atividade larvicida

Segundo a Farmacopéia Brasileira (2019) o conceito de emulsão consiste na forma farmacêutica líquida que envolve uma mistura de dois líquidos imiscíveis, através de um ou mais surfactantes, no qual um líquido está disperso no outro em forma de pequanas gotículas (fase interna da emulsão) e o outro está presente de forma contínua (fase externa da emulsão).

Óleos vegetais geralmente são hidrofóbicos e insolúveis em água, por este motivo é necessário utilizar surfactantes para torna-los úteis em formulações aquosas, emulsões e nanoemulsões constituem formas farmacêuticas que superam este problema e viabilizam óleos em meio aquoso, sendo assim ideias para realizar testes larvicidas, já que as larvas e pulpa de *A.aegypti* se desenvolvem na água (Duarte, et al., 2015).

A diferença entre nanoemulsões e emulsões consiste basicamente no tamanho das gotículas que são formadas no interior da fase interna de uma emulsão, se o tamanho das gotículas estiverem entre 0 e 500 nanômetros (nm) podemos dizer que se trata de uma nanoemulsão, caso estes valores ultrapassem os 500 nanômetros podemos chamar de uma macroemulsão, ou simplesmente emulsão, porém pelo fato das nanoemulsões se apresentarem como partículas em tamanhos menores, várias sãos as atividades especiais a estas formulações descritas na literatura (Ferreira, et al., 2010; Bajerski, et al., 2016).

As nanoemulsões por terem um tamanho diminuto possuem estabilidade cinética, sendo apontadas ideias para uso em diluição ou em meios aquosos, as nanoemulsões são descritas por apresentarem sistema de liberação controlada em relação a formulações convencionais, são apontados menos efeitos adversos por conta da menor dose empregada de ativos, proteção dos componentes ativos frente a degradação química, fotodegradação, dentre outros benefícios (Bajerski et al., 2016).

As nanoemulsões podem ser obtidas por técnicas de alto e baixo aporte de energia (Holkem, et al., 2015).Basicamente, as técnicas de alto aporte de energia utilizam equipamentos robustos e caros para realizar a quebra das gotículas, como agitadores ultrassonicos, microfluidização e agitação em alta pressão. Por outro lado,

técnicas de baixo a porte de energia utilizam a própria energia do sistema para desenvolver nanoemulsões, são utilizadas técnicas de emulsificação espontânea e método da temperatura de inversão de fases (PIT), estas últimas são mais desejáveis por apresentarem menores custos e técnicas mais acessíveis (Holkem et al., 2015).

Neste contexto Ferreira e colaboradores (2010) foram pioneiros na produção de emulsões com o óleo de andiroba, utilizando Span 80 e Tween 20 com surfactantes conseguiram obter emulsões do óleo de *C.guianensis* de 2 µm estáveis em temperatura ambiente, neste estudo foi utilizado a técnica de equilíbrio hidrófilo-lipófilo (EHL), esta técnica consiste em uma escala de vai de 0 a 15 para surfactantes e óleos, quanto mais próximo do 0 mais hidrofílico o surfactante ou óleo, quanto mais próximo de 15 mais hidrofóbico o óleo ou surfactante, noralmente são utilizadas misturas de tensoativos com EHL alto e baixo para obtenção de emulsões e nanoemulsões estáveis.

Prophiro e colaboradores (2012) realizaram o primeiro relato do óleo de *C.guianensis* testado como larvicida frente *A.aegypti*, segundo os autores o óleo foi solubilizado em polisorbato 80 e os testes larvicidas seguiram nesta solução, que pode também ser considerada uma emulsão, com resultados de dose letal 50 (DL50) de 140 mg/L.

Em seguida Prophiro e colaboraores (2012) avaliou em outro trabalho a toxicidade desta emulsão de *C.guianensis* nas larvas de *A.egypti*, sua emulsão teve atividade residual, ou seja, com o passar do tempo a emulsão foi capaz de aumentar gradativamente a mortalidade das larvas, bem como causar toxicidade no estágio de pulpa e no estágio adulto, pois as pulpas foram prejudicadas no processo, afetando os mosquitos adultos, demonstrando que *C.guianensis* afetou nos três estágios o vetor *A.aegypti*, sendo considerada pormissora para este fim.

Jesus e colaboradores (2017) desenvolveram nanoemulsões com o óleo de *C. guianensis*, foi utilizada a técnica de EHL, foram utilizados uma faixa de EHL de 4.3; 5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 15. Foram utilizados diversos tensoativos para chegar estes valores de EHL, neste trabalho foi sugerido que o EHL do óleo de andiroba foi de 11, pois apresentou características macroscópias de emulsões estáveis, não houve

separação de fases, o tamanho de gotícula, índidice de polidispersão se mantiveram estáveis, sendo considerados parâmetro físicos-químicos que garantem estabilidade.

Em seguida foi realizado o teste larvicida residual, apresentando mortalidade superior a 50% após 3 cilcos de 48 horas, revelando que a nanoencapsulação do óleo pormoveu uma liberação controlada dos ativos em decorrer do tempo, apesar de utilizar uma técnica de baixo aporte de energia (Jesus et al., 2017).

Outros trabalhos produziram nanoemulsões independente do valor de EHL utilizando surfactantes convencionais para outras atividades, como Baldissera e colaboradores (2013) usaram Tween 80 e Span 80 para produzir uma nanoemulsão ativa frente parasitas do gênero *Trypanossoma* obtendo resultados promissores e uma alternativa de tratamento a esta parasitose, com técnica de alto aporte de energia.

Em outro experimento, Moraes e colaboradores (2018) utilizou nanoemulsões de *C.guianensis,* com Tween 80 e verificou-se a atividade frente a parasitas do gênero *Leishmania*, in vivo, conseguindo realizar um tratamento preliminar em ratos infectados, podendo ser uma alternativa para esta doença negligenciada, neste trabalho foi utilizado uma técnica de alto aporte de energia para obtenção das nanoemulsões.

Por fim, Milhomem-Paixão (2017) avaliaram a toxicidade de uma nanoemulsão estável de *C.guianensis,* com tamanho de gotícula de 140 nm, e segundo os testes em células humanas esta nanoemulsão não foi considerada citotóxica, genotóxica e hematotóxica para cultura de células, indicando que *C.guianensis* pode ser matéria prima para a produção de nanoemulsões seguras em cosméticos e outros tratamentos.

Segundo Duarte (2015) produtos naturais larvicidas podem ser considerados pormissores levando em consideração a taxa de mortalidade em 48 horas de tratamento, na concentração de 250 ppm (µg/mL): assim com mortalidade >75% é considerado um produto promissor, entre 75% e 50% é considerado parcialmente promissor, entre 50 e 25% é considerado fracamente promissor, abaixo de 25% é considerado inativo.

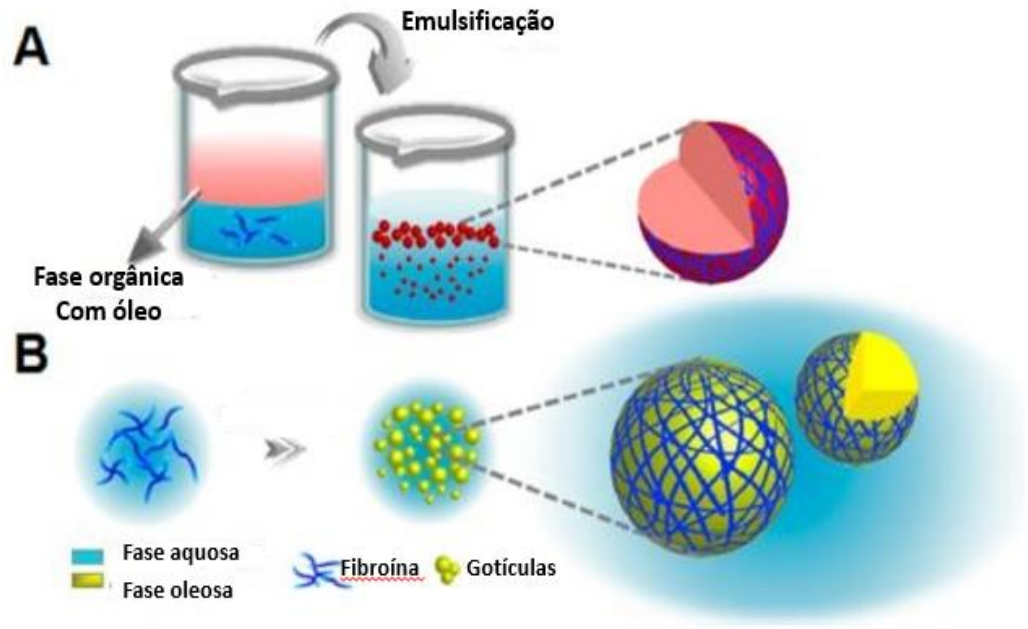
As emulsões e nanoemulsões são possíveis pelo uso de tensoativos, substâncias anfifílicas que permitem a dispersão de gotículas oleosas em meio aquoso,

na maioria dos estudos são utilizados tensoativos sintéticos derivados do petróleo e que são tóxicos ao ser humano e outros seres vivos do meio ambiente, por estes motivos e como a questão ambiental vem ganhando mais destaque, tensoativos naturais são alternativas viáveis para este fim. Por isso a proteína fibroína foi escolhida como tensoativo na produção de emulsões larvicidas neste trabalho.

Vale ressaltar que a fibroína, é uma proteína extraída do casulo do bicho da seda (*Bombix mori*), como o Brasil é o terceiro maior produtor de Seda do mundo, as indústrias de seda acabam por representar uma fonte destes precioso bioproduto. O casulo é formado por duas protéinas, sericina e fibroína, onde a extração da fibroína é realizada através de técnicas simples e economicamente viáveis. Além de apresentar estabilidade térmica, elasticidade, não sofre degradação de microorganismos, se configura como um promissor biopolímero brasileiro (Ferreira, I.M. 2016).

A fibroína possui domínios polares e apolares, o que a caracteriza com potencial de diminuir a tensão interfacial em emulsões, porém poucos trabalhos utilizam a fibroína para este fim, neste contexto Cheng e colaboradores (2017) utilizaram a fibroína como surfactante na produção de emulsões e verificaram que ela possui viabilidade para este fim, obtendo o tamanho de gotícula na faixa de 5 a 20 µm (cerca de 50 a 200 nanômetros), com boa estabilidade, neste trabalho o processo de emulsificação foi descrito na figura 13, sendo demonstrado como simples, também foi verificado que as emulsões com fibroína e o óleo utilizado teve maior capacidade de chegar ao núcleo das células, demonstrando maior biocompatibilidade comparado a outros surfactantes.

Figura 13. Mecanismo de encapsulação da fibroína. A: encapsulação do óleo vermelho; B: gotículas com fibroína ao redor estabilizando a emulsão.



Fonte: Adaptado de Cheng et al., 2017

Neste contexto, poucos trabalhos relatam a utilização da fibroína de seda em nanopartículas. Vale citar o trabalho de Mane e colaboradores (2017) que obteve nanopartículas de ouro e prata ativas frente a larvas e pupas do *A. aegypti*, atribuindo o sucesso da mortalidade do vetor na desativação de proteínase redução do DNA e RNA.

Por estas razões, o primeiro artigo deste trabalho teve o objetivo de produzir emulsões com os ácidos graxos do óleo de *C. guianensis* como componentes ativos da formulação. A inovação se deu pela utilização da fibroína como surfactante para este bioproduto, a fibroína é uma proteína extraída do casulo do bicho da seda, o casulo do bixo da seda é utilizado por industrais têxteis para obtenção da seda, no entanto, muitos destes casulos são descartados, estes descartes foram utilizados para obtenção da fibroina, neste trabalho.

O segundo artigo deste trabalho utilizou as reações de transesterificação enzimática do óleo de andiroba para produzir nanoemulsões com os etil ésteres obtidos

na ausência de tensoativos, uma vez que os etil ésteres possuem extremidades polares e apolares, apresentam comportamento semelhante aos tensoativos, sendo assim, estes foram utilizados diretamente sobre a água, para gerar nanoemulsões, estas nanoemulsões foram testadas nas larvas de *A.aegypti*, de maneira que é a primeira vez que foram obtidas nanoemulsões larvicidas sem tensoativos para o óleo de *C.guianensis.*

Por fim, o terceiro artigo deste trabalho utilizou a reação de amidação direta sobre o óleo de *C.guianensis* para produção de amidas graxas, pelo fato das amidas graxas já serem utilizadas como tensoativos, as amidas graxas obtidas foram utilizadas para nanoencapsular o próprio óleo de andiroba, para posterior avaliação larvicida frente *A. aegypti*, a inovação ocorre por ser um trabalho pioneiro com o óleo de *C.guianensis* e poder criar um tensoativo do próprio óleo, para obter a atividade larvicida.

Neste trabalho também foi proposto a utilização da Metodologia de Superfíce de Resposta (MSR) para obtenção das nanoemulsões com estas amidas graxas, sem a utilização de surfactantes tradicionais e como uma alternativa frente abordagem clássicas como do EHL que necessitam de uma série de tensoativos para obtenção da formulação ideal, no entanto nem sempre há essa disponibilidade de surfactantes, por isso esta técnica aparece como promissora para o futuro no campo das nanoformulações.

Apesar de o óleo de andiroba ter muito aplicabilidade na medicina tradicional e ser promissor, ainda são poucos os estudos que descrevem sua utilização, principalmente em nanoformulações e emulsões, além da sua utilização como produto inseticida/larvicida, neste sentido este trabalho buscou utilizar ferramentas biocatalíticas no óleo de andiroba para este propósito, no objetivo de gerar um produto larvicida inovador e promissor, com tensoativos alternativos e técnicas ambientalmente aceitáveis.

2.1 OBJETIVO GERAL

Utilizar o óleo de *Carapa guianensis* e seu derivados (ácidos, esteres e amidas graxas) para a obtenção de emulsões associadas com fibroína de seda como surfactante natural com atividade larvicida frente à *Aedes aegypti*.

2.2 OBJETIVOS ESPECÍFICOS

a) Comparar quimicamente o óleo de *C.guianensis* do município de Mazagão, obtido em diferentes épocas do ano;

b) Obter ácidos, ésteres e amidas graxas a partir do óleo de *C. guiannesis* via catálise enzimática;

c) Caracterizar as substâncias obtidas por técnicas espectroscópicas, Espectrometria de Massas (CG-EM), Ressonância Magnética Nuclear (RMN) de ^{1}H e ^{13}C e Infravermelho (IV).

d) Determinar a atividade larvicida em *Aedes aegypti* e CL50 após exposição das formulações obtidas.

e) Análisar morfologicamente as larvas tratadas.

Carapa guianensis Aubl. (Meliaceae) oil associated with silk fibroin, as alternative to traditional surfactants, and active against larvae of the vector *Aedes aegypti*

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Abstract

This paper reports on preparation of an oil-in-water new microemulsion of *Carapa guianensis* Abul. (Meliaceae) oil and derivates [fatty acid ethyl ester (FAEE's) and fatty free acid (FFA's)], using silk fibroin (SF), as alternative to traditional surfactants, and its activity against the larvae of the vector *Aedes aegypti.* Among the emulsions that were prepared, FFA2-SF derived from AO2, that mostly contained unsaturated fatty acids, presented the best results against the larvae of *Ae. aegypti* after 24h (LC_{50} = 16.79 $mg.mL^{-1}$), through causing structural alterations to the vector that were

boosted by the fibroin. The lipid profile, enhanced by the fibroin emulsion state, of the oils strongly influences larval mortality.

Keywords: Mini-emulsion; Free fatty acids; Fatty acid ethyl ester; Larvicidal effect; Silkworm cocoon.

INTRODUCTION

Larvicides for controlling mosquitos are generally formulated using synthetic insecticide compounds and are likely to include pyrethroids (Demok et al. 2019, Kushwah et al. 2015). However, most of these synthetics substances have adverse effects: such as bioaccumulation in the environment and toxicity to mammalians (Vontas et al. 2012). Moreover, reports of development of pesticide resistance relating to use of synthetic insecticides on mosquitoes are becoming increasingly common (da Silva-Alves et al. 2013, Carvalho et al. 2018). Bioproducts such as plant extracts (Bouvier, Dogbo and Camara 2003, Araujo et al. 2018), derived compounds (Aher and Roy 2018, Araújo et al. 2020) or microorganisms (Ragavendran, Dubey and Natarajan 2017) have been successfully used as alternative to synthetic larvicides against *Aedes aegypti*;

The Amazon region is an important source of plants that produce different vegetable oils, and these are used by the local population to treat diseases (Ferreira Rodrigues Sarquis et al. 2019). Among these oils, seed oil from *Carapa guianensis* and derivatives has been shown to have anti-inflammatory effects (das Gracas and Penido 2014, Matsui et al. 2014), anticonvulsant action (Oliveira et al. 2020), hepatoprotective and antimicrobial effects (Santos et al. 2012) and larvicide effects against *Aedes aegypti* (de Mendonça et al. 2005). *Carapa guianensis* is a tree belonging to the family Meliaceae and is popularly known as andiroba (Nascimento et al. 2019). It grows preferentially in tropical forests and usually produces 180–200 kg of seeds per year, containing approximately 60% (v/w) oil, by weight (Novello et al. 2015).

Too are reported in the literature the effect of the andiroba oil to be a repellent against *Aedes aegypti* (de Mendonça et al. 2005) (Miot et al. 2004). Prophiro (Prophiro et al. 2012b) found that the

residual larvicidal effect of from *C. guianensis* oil in 72h showed 100% of mortality for *Ae. aegypti*. Silva (Silva et al. 2006) reported on the larvicidal effect of andiroba oil on *Ae. albopictus*, after a 24-hour period. Silva (Silva et al. 2004) also described the larvicidal effect of andiroba oil on *Ae. aegypti*, 8 h after exposure to the oil.

Emulsions can be used in potentialization of the action of the dispersed active, since the essential oils or fixed oils have serious problems of dispersion in aqueous vehicles and that mainly, in the case of contact with insects and other vectors, who have part of their cycle or living environment in contact with water (Echeverría et al. 2019). This helps overcome the low solubility of the oil in water and aids in controlled release (Nuchuchua et al. 2009). Ferreira (Ferreira et al. 2010) reported using a combination of Span 80® and Tween 20® as surfactants to prepare emulsions containing andiroba oil as the oil phase. Nanoemulsions prepared using *C. guianensis* oil via a low-energy solvent-free method have been used in larvicidal tests against *Ae. aegypti* (Jesus et al. 2017).

Silks are protein polymers found in the glands of arthropods such as silkworms, spiders, scorpions, mites and bees. They are spun into fibers during these arthropods' metamorphosis (Kundu et al. 2008, Wang et al. 2004, Zhang 2002). Sequence specificity and subsequent secondary, tertiary and quaternary structures of hydrophobic blocks of amino acids (predominately glycine, alanine, serine and tyrosine), govern the mechanical, physical and chemical properties of silk fibroin (Holland et al. 2019). The mechanical robustness and cell biocompatibility of native silk fiber has been used by humans for biomedical applications both preclinically (Kundu et al. 2010) and as excellent delivery systems for bioactive organic compounds such as drugs, peptides, proteins, etc. (Koh et al. 2015). Silk fibers are processed using a variety of methods to produce forms such as hydrogels (Sah and Pramanik 2011), films (Lu et al. 2009), scaffolds (Kazemimostaghim et al. 2014), microspheres (Ferreira et al. 2016), nanoparticles (Pandiarajan et al. 2018) or enzymatic support (Ferreira et al. 2014).

Natural polymers like silk fibroin have been investigated by researchers working in different fields, particularly as substitutes for synthetic polymers, this paper reports on preparation of an oil-

in-water emulsion of *Carapa guianensis* oil and their derivates (fatty acid ethylic and fatty free acid), using silk fibroin, as alternative to traditional surfactants, and its activity against the larvae of *Aedes aegypti*.

MATERIAL AND METHODS

Chemical materials

Ethanol (99%) and DMSO (99%) were purchased from Syth (Brazil), and *n*-hexane (98.5%) and ethyl acetate (98%) were obtained from Vetec (Brazil). These were used without further purification. Lipase acrylic resin from *Candida antarctica* (CALB ≥ 20,000 U/g) was purchased from Sigma-Aldrich.

Plant material and oil extraction from *Carapa guianensis*

Botanical material was collected in the municipality of Mazagão, State of Amapá, Brazil (00°13′48.3″ S; 51°24′37.4″ W) and identified by a botanist (Dr. Rosângela Sarquis). A voucher specimen was deposited in the herbarium of the Brazilian Agricultural Research Company (EMBRAPA) under the number 194102.

The first collection was performed on March 10 (AO1) and the second on June 10 (AO2), in the same specimen. The oils (AO1 and AO2) were obtained by means of the traditional method of cooking the seeds and leaving them to rest for 72 h. The seeds were then removed from the shells, the fruit mass was prepared and, lastly, the oil was collected (Nardi et al. 2016).

Semi-synthesis of fatty acid ethyl esters (FAEEs) and free fatty acids (FFAs) from lipase B *Candida antarctica* immobilized on immobeads (CAL-B)

The enzymatic transesterification reaction was performed separately for AO1 and AO2, to generate their respective fatty acid ethyl esters (FAEE1 and FAEE2) and fatty acids (FFA1 and FFA2), following the methodology described by Ferreira (Ferreira et al. 2018).

For this reaction, the following reactive were used: andiroba oil OA1 and OA2 separately (3 mL), ethanol (9 mL) and CAL-B (10% w/w of the oil). These were added to a round-bottomed flask (25 mL). The mixtures were incubated at 32 °C on a 130-rpm orbital shaker (Lucadema, Brazil). After 24 h, the reaction was terminated. The reaction mixture was filtered, the organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure. The product (FAEE1 and FAEE2) was purified by means of silica gel column chromatography, with a mixture of *n*-hexane and ethyl acetate (9:1) as the eluent. The product was characterized by means of FT-IR analysis and GC-MS was used to quantify the fatty acids in the AO1 and AO2 oil, via derivation.

The enzymatic hydrolysis reaction was performed on FAEE1 or FAEE2 (1 mL), with water (containing 0.1 mol L^{-1} of phosphate buffer) and CAL-B (10% w/w of the FAEE), which were mixed in the round-bottomed flask (25 mL). The mixtures were incubated at 45 °C in a 130-rpm orbital shaker (Lucadema, Brazil). After 24 h, the reaction was terminated. The reaction mixture was filtered, the organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure. The product (FAEE1 and FAEE2) was purified by silica gel 60 column chromatography, with a mixture of n-hexane and ethyl acetate (9:1) as the eluent, and the product was characterized by of FT-IR and GC-MS.

^{13}C nuclear magnetic resonance (NMR) analysis

^{13}C NMR experiments were performed in a Agilent Technologies 400 MHz Premium Shielded spectrometer. All samples (AO's, FAEE's and FFA's) were prepared dissolving (20 µL, approximately) in 600 µL of deuterated chloroform ($CDCl_3$, Cambridge Isotope Laboratories, Andover, USA) and tetramethylsilane (TMS) as the internal standard. The chemical shifts were expressed in ppm.

Gas chromatography-mass spectrometry (GC-MS)

The analysis on FFA's was conducted using a gas chromatograph (GCMS-QP 2010) equipped with an injection auto-sampler AOC-20i (Shimadzu). Electron impact detection was used (Shimadzu MS2010 Plus detector), with an electronic impact of 70 eV, which enabled detection of fragments from 50 to 500 Da. Separations were performed in a fused silica capillary column (RTX-5MS with internal diameter = 0.25 mm, length = 30 m and film thickness = 0.25 µm) in a helium stream of 1.0 mL/min. The sample was dissolved in ethyl acetate (3 µg/mL), and 1.0 µL of the solution was subjected to the following experimental conditions: injector temperature, 210 °C; detector temperature, 250 °C; carrier gas, helium; flow rate 1.0 mL/min; and split injection with split ratio of 1/15. The column temperature was programmed starting from 130 °C, with an increase of 5 °C/min to 290 °C, ending with a 5 min isothermal period at this temperature. The total duration of the analysis was 39 min. The components present in the FAEE1 and FAEE2 samples were identified through comparison of mass spectra data with those presented in the NIST 5.0 library (Faria e Souza et al. 2017).

Physicochemical analysis on the vegetable oils

The acidity index (mg KOH/g), refractive index, density (mg/mL) and peroxide content (meq/kg) found in the AO1 and AO2 oils were analyzed using the methods described in the analytical standards of the Adolfo Lutz Institute (Lutz 2005).

Preparation of silk fibroin solution

The silk fibroin solution was prepared based on the method developed by Ferreira (Ferreira et al. 2017). A silkworm cocoon (3.0 g) was placed in 500 mL of a 2% Na_2CO_3 solution that had been preheated to 100 ºC, and stirred at this temperature for 30 min. The resultant fiber was separated by means of filtration and washed with distilled water (3 x 500 mL). Lastly, the silk fiber was shredded and placed in an oven to dry (50 ºC) for 24 h.

After this step, 50 mL of a ternary solution of H_2O:EtOH:$CaCl_2$ (8:2:1 molar proportions) were used to dissolve the silk fibroin. This mixture was transferred to a cellulose dialysis tube with an exclusion limit of 16 kDa and dialyzed in water for 3 d at room temperature. The dialysis water was changed every 24 hours. The fibroin solution was stored at 10 °C. It was then centrifuged (6000 rpm for 10 min) to remove impurities and larger particles. The concentration of the silk fibroin solution was adjusted to 2% (w/w) using distilled water. The final concentration was determined by means of the gravimetric method.

Preparation of emulsion

A low-energy emulsification method was used to prepare emulsions by adding water to a mixture of silk fibroin and samples (AO, FAEE or FFA). The final solution of 20 mL had the following constituents: 75% silk fibroin solution (2%); 5% active compounds (AO, FAEE or FFA); and 24% ethanol (99%).

Initially, an organic phase was prepared by adding the active compounds and ethanol to a beaker (50 mL). The mixture was stirred by magnetic agitator (300 rpm) for 30 min at 40 ºC. Next, the aqueous phase (silk fibroin) was added, with a flow of ≈ 0.5 mL/min with continuous agitation for 60 min.

The stability of all of the emulsions was evaluated 1 and 30 days after the preparation. The mean droplet size and polydispersity index of the emulsions were determined by of dynamic light scattering (DLS) (Zetasizer, model ZS, Malvern Instruments). The measurements were performed at 25 °C.

Larvicidal activity

The emulsion samples from OA's, FAEE's and FFA's were prepared at different concentrations (25, 50, 75 and 100 µg/mL) for larvicidal tests on *A. aegypti*. Five replicates were performed, with ten larvae each. The negative controls consisted of silk fibroin solution (2%) and as

positive control was used dichlorvos solution (6.25 ng/mL). that was present in the respective test sample. The larval mortality rate was determined after 24 h of incubation (25 ºC and 75% humidity). Larvae were considered dead when they did not respond to stimuli or did not rise to the solution surface, in contrast with those observed in the control. The bioassay experiments were conducted in accordance with the WHO guidelines of 2005.

Statistical analysis

Lethal concentrations (LC_{50} and LC_{90}) were determined after 24 and 48 h of incubation and were calculated using Probit analysis in the StatGraphics Centurion XV software, version 15.2.11. If the control mortality of the treated groups was between 5 and 20%, the analysis was corrected in accordance with the WHO (2005) formula: mortality (%) = X – Y ⁄ X × 100, where X = percentage survival in the untreated control and Y = percentage survival in the treated sample.

RESULTS AND DISCUSSION

The lipid profiles of the samples (AO1 and AO2) from the seed oil of *Carapa guianensis* are summarized in Table 1. It was possible to identify and indirectly quantify the fatty acids using GC-MS, from their respective FAEEs.

Figure 1 presents the analyses of FAEE1 and FAEE2 samples, using GC-MS. These were described in terms of the retention time (x axis) and the ionic signal intensity of the mass spectrum (y axis). Qualitative analysis demonstrated the presence of the following compounds: palmitic, linoleic, vaccenic and stearic acids. These were present in AO1 (collected during the dry season) and in AO2 (collected during the rainy season), extracted from the seeds of *C. guianensis.* The retention times were well defined and there was no overlapping of peaks, except in relation to vaccenic acid (C18:1, ʊ-7), which was eluted very close to oleic acid.

Figure 1. Here

AO1 (collected during the dry season) and AO2 (collected during the rainy season) presented similar profiles regarding the main fatty acids identified. However, the proportions of these FFAs varied greatly between samples. Parameters such as temperature, solar radiation, precipitation and humidity may have influenced the fatty acid content of the seeds of *C. guianensis*. While AO1 mostly contained saturated fatty acids (41.5%) such as palmitic and stearic acid, AO2 only had a saturated fatty acid proportion of 25.6%. It is noteworthy that the monounsaturated fatty acid oleic acid (C18:1, ʊ -9) was identified as the largest constituent in both samples, accounting for 50.6% of all fatty acids present in AO1 and 40.2% in AO2. In contrast, the polysaturated fatty linoleic acid (C18:2, ʊ-6) only presented a proportion of 5.4% in AO1, but a high proportion of 33.1% in AO2.

One of the advantages of using *C. guianensis* oil is the low toxicity. Some study showed that oral administration of *C. guianensis* seed oil did not induce significant alterations in almost all biochemical, hematological and morphological parameters in Wistar rats. However, indicate a possible hepatic toxicity (Costa-Silva et al. 2008).

Ferreira (Ferreira et al. 2018) identified the fatty acids in the seed oil of andiroba, through derivation from its ethyl esters, and also found that oleic acid (C18:1, ʊ -9) was the largest constituent, with relative abundance of 42.9%, followed by palmitic acid (C16:0) with 32.5%. However, that study cited the presence of myristic and arachidonic acids as part of the range of fatty acids present in the oil of *Carapa* sp. Oleic acid was also found to be highly prevalent in the seed oil of *C. guianensis* Aubl., obtained through different extraction methods by Araújo-Lima (Araujo-Lima et al. 2018).

Table 1. Here

Spectroscopic methods (as ^{13}C NMR) may also be useful for samples which are not amenable to chromatographic methods. In general, it can be noticed from the spectra that carbon signals group

in four main spectral regions: carbonyl atoms region (~175 ppm); carbon atoms involved in -C=C double bonds region (~130 ppm); c) methylene groups region (~20-32 ppm); e) chain ending methyl groups region (~14 ppm) (Sacchi et al. 1993, Sacchi et al. 1994). The spectra (FAEE's and FFA's) show high similarity as they are of saturated and unsaturated alkyl chains derived from the AO's triglyceride (Figure 2A and 2B). For ^{13}C NMR spectrum corresponding to fatty free acid (FFA1 and FFA2), it is possible to notice an absence of the characteristic signals of the glycerol peaks in 68.9–62.1 ppm, present in the spectrum corresponding to the oils (AO1 and AO2). In contrast, the ^{13}C NMR spectrum of FAEE presented the characteristic signal of the ester mixture -CH_2 alkyl carbon; after the transesterification reaction, this signal appears at 60.1 ppm.

Figure 2. Here

Physicochemical properties of AO emulsions and their derivatives containing silk fibroin

The physicochemical parameters of AO1 and AO2 determined in this study are summarized in Table 2.

The acidity content indices for the samples AO1 and AO2 were 21.97 ± 0.14 meq.kg^{-1} and 7.91 ± 0.18 meq.kg^{-1}, respectively. Values close to 7.5 meq.kg^{-1} were found in the study by Ferreira (Ferreira et al. 2018), i.e. closer to the AO2 values.

The peroxide indices for the samples AO1 and AO2 were determined as 0.698 ± 0.199 mg.NaOH.g^{-1} and 1.426 ± 0.11 mg.NaOH.g^{-1}, respectively. These values were lower than those was found by Silva (Silva et al. 2018), which was 3.89 mg.NaOH.g^{-1}. The peroxide index of oils indicates the degree of oxidation presented.

The oils showed different melting points. For AO1, it was 34-35 ºC, but for AO2 the melting point could not be determined. Moreover, different density values were determined: 1.24 mg.mL^{-1} and 0.92 mg.mL^{-1} for AO1 and AO2, respectively. Sousa (Sousa et al. 2018) characterized andiroba

seed oil and found that the density was 1.02 mg.mL^{-1}, i.e. lower than the values for AO1 and AO2 in the present study (Table 2).

The refractive index is specific of each oil, and this is related to the degree of unsaturation of the bonds, the presence of oxidation compounds and the thermal treatment to which it has been subjected. The refractive index for AO1 was 1.45, and this was close to the index for AO2, which was 1.46. It should be noted that no values for andiroba oil are stipulated through legislation.

Table 2. Here

Obtainment of AO emulsions and their derivatives containing silk fibroin

Emulsions are colloidal dispersions of at least two immiscible liquids in which one of the liquids is dispersed in the other. They were stabilized using surfactants with a suitable hydrophile-lipophile balance (HLB value) (Zhu et al. 2019). A conventional emulsion typically has a droplet diameter of between 0.01 and 100 μm. Emulsions are thermodynamically unstable because of the relatively high interfacial tension associated with contact between the oil and water phases (Wang et al. 2015).

In general, proteins are attractive emulsifiers in the food industry because of their good emulsifying properties. Proteins such as β-lactoglobulin, casein, soy protein isolate and pea protein are often used in combination with other emulsifiers to stabilize oil-water emulsions and multiple emulsions (He et al. 2011, Zhu et al. 2019, Xu and Yao 2009).

Silk fibroin solutions were used to obtain oil-water mini-emulsions, as an alternative to using traditional nonionic surfactants such as Tween 80. One of the advantages of replacing traditional surfactants with silk fibroin solutions is in relation to preparation of oil-water mini-emulsions. This enables lower production cost, high availability, good amphiphilic properties, very low cell toxicity, very low environmental toxicity and good biodegradability, given that this is a protein-based biomaterial.

Andiroba oil is easy to obtain and has high availability in the Amazon environment. Its extraction does not compromise the vegetation mass. Moreover, it possesses an arsenal of pharmacological effects (Ambrozin et al. 2006, das Gracas and Penido 2014, Vendramini et al. 2012), including larvicidal activity (de Mendonça et al. 2005). However, the low solubility of andiroba oil in water makes it unviable to apply it directly in an aqueous medium. Therefore, using this oil in emulsified systems has become a viable alternative for its application in aqueous media (Oliveira et al. 2016, Sugumar et al. 2014).

Table x summarizes the values for the polydispersity index (PDI), particle size and zeta potential of the following emulsions, just after their preparation: AO-SF, FAEE-SF and FAA-SF. The PDI indicates the homogeneity of size distribution: the lower the PDI is, the more homogenous the particle diameters are (Ramos et al. 2020). The zeta potential relates to the surface charge on the particles, which is directly affected by the medium in which the particles are placed (Wang et al. 2010).

It should be noted that, only 24 hours after the emulsions prepared from the oils AO1 and AO2, the phases of these emulsions were already visibly separating, thus indicating that they were thermodynamically unstable. On the other hand, the FAEE-SF and FFA-SF emulsions remained visibly stable after this time. This suggests that emulsions containing derivatives of ethyl esters or fatty free acids from either of the oils tended to be more stable than the water-oil-fibroin system.

Because the mini-emulsions containing FFA1-SF and FFA2-SF presented small particle sizes, they were subjected to monitoring of physical stability in a previous experiment, stored at room temperature for 15 days. As shown in Figure 2, the particle sizes of FFA1-SF and FFA2-SF changed through this storage period. In FFA1-SF, the size was of 572 nm on the first day and remained unchanged on the 15th day (Figure 3A). For FFA2-SF, the size changed from 308 nm on the first day to 528.6 nm on the 15th day of storage (Figure 3B). This demonstrated that the hydrodynamic system had lost stability, possibly through precipitation of smaller particles of the silk protein on droplet surfaces, thus increasing the numbers of drop-drop events. The colloidal stability of nanoemulsions

or fibroin nanoparticles could possibly be improved through addition of cationic polymers (Wang et al. 2010).

The zeta potential is directly related to the net charges on the surfaces of the macromolecules and particles. Figure 3 also shows the zeta potential profile of the emulsions that were developed. I both cases, there was a change in charge from the first to the 15th day. For FFA1-SF (Figure 3A), it went from -2.35 mV (1st day) to +1.17 mV (15th day), while for FFA2-SF it went from -0.67 mV (1st day) to +0.65 mV (15th day) (Figure 3B). The low zeta potential values for these two emulsions shows that the long chains of silk proteins on the droplet surfaces restricted the movements of particles when they were subjected to an electrical field. It should be noted that the zeta potential of natural silk fibroin is -20 mV (Wang et al. 2010).

Figure 3. Here

It can be suggested that the difference in physicochemical properties between the emulsions that were prepared from fibroin and the free fatty acids FFA1 and FFA2 (starting from AO1 and AO2) related to variation in the chemical composition, which will have caused changes to the interfacial stability between the phases. It should be noted that one of the important parameters for the stability of emulsification is the efficiency of the oil that is encapsulated in the droplets (Xu and Yao 2009).

Evaluation of the larvicidal activity of AO emulsions and their derivatives containing silk fibroin

To investigate the larvicidal effect of the emulsions on *Ae. aegypti*, they were firstly tested at a concentration of 50 µg/mL, with assessments of 24 and 48 hours after contact with the larvae.

The emulsions with fibroin prepared from the oils AO1 and AO2 did not present any activity against the larvae *Ae. aegypti*, after 24 h or 48 h. On the other hand, the emulsions containing the ethyl esters (FAEE1-SF and FAEE2-SF) showed moderate larvicidal activity. This activity level was

not significant for FAEE1-SF (14% at 24 h). For FAEE2-SF, the activity level was able to cause the death of 32% of the larvae, and the rate was significant at both 24 h and 48 h.

Jesus (Jesus et al. 2017) developed a nanoemulsion using polysorbate 85 as a nonionic tensioactive agent, to obtain a nanoformulation with a particle size of 148.8 nm and PDI of 0.131. They evaluated the residual potential residual in relation to larvae of *Aedes aegypti* that were at a late point within the third instar, in 48-hour cycles. However, they did not do tests using tensioactive solutions as control for the mortality rate.

Among the emulsions tested, FFA1-SF and FFA2-SF were found to be efficient larvicidal over *Ae. aegypti* at 48 h. Both emulsions reached significant larval mortality rates: 68% and 60%, respectively (Figure 4).

Figure 4. Here

In the light of these results, further tests were conducted with different concentrations (10, 25, 50, 75, 100, 125 and 150 µg/mL) using the emulsions derived from fatty acids, to determine the LC_{50} and LC_{90}.

The LC_{50} and LC_{90} results from using the emulsions prepared from FFA1 and FFA2 are summarized in Table 3. Probit analyses were performed with 95% confidence intervals to determine LC_{50} and LC_{90}.

The LC_{50} of FFA2-SF at 24 and 48 hours was 94.455 and 16.796 µg/mL, respectively (Table 3), while for FFA1-SF it was 212.333 µg/mL at 24 h and 129.455 µg/mL at 48 h. This demonstrated that the larvicidal action of the emulsions was directly related to the composition of the fatty acids and that this action was boosted by functional groups, thus: glyceride < ethyl ester < free acid (Table 3).

It is worth highlighting that, to demonstrate the larvicidal effect of the fatty acids in emulsions with silk fibroin, the larvicidal capacity of the FFA's dissolved in DMSO was evaluated. In all the

cases tested, the FFA's in DMSO presented LC_{50} and LC_{90} that were higher than those of the respective emulsions in fibroin (Table 3). This result emphasizes the carrying capacity of the fibroin emulsion and its positive impact on the biological activity.

Our results are corroborated by the findings of Melo (de Melo et al. 2018), who performed larvicidal tests on *Culex quinquefasciatus* using different fatty acids such as oleic acid, palmitic acid, stearic acid, linoleic acid and linolenic acid and their respective methyl esters, diluted in DMSO. The unsaturated acids, like linoleic and oleic acid and their respective methyl esters, showed the highest mortality rates (100%), among the compounds tested. In contract, the mortality rate from saturated fatty acids such as stearic acid and palmitic acid was moderate (30% and 15% respectively).

It might be suggested that seasonal variation in the oil from andiroba seeds is responsible for differences in larvicidal mortality rates against *Ae. aegypti*. AO2 presented a higher level of unsaturated fatty acids such as oleic and linoleic acids, and consequently higher mortality rates than those of emulsions of the fatty derivatives of AO2, because it presented higher levels of saturated acids such as palmitic.

Table 3. Here

It is worth to mention that, this oil has been used efficiently as a repellent against mosquitos and other insects. Prophiro (Prophiro et al. 2012b) were the first to report that the larvae of *Aedes aegypti* were susceptible to the oil of *Carapa guianensis* dissolved in DMSO, with LC_{50} of 136 mg/L and LC_{90} of 551 mg/L. In another study, Prophiro (Prophiro et al. 2012a) investigated the larvistatic action of the oil of *C. guianensis* against *Aedes aegypti* and found that the LC_{50} was 140 mg/L. Despite the proven larvicidal activity of the oil of *C. guianensis*, very few studies have addressed this topic.

Emulsions with smaller droplet sizes can be obtained through high energy input methods using high-cost rotary equipment. Methods with lower energy input use less rotation in cheaper equipment, with techniques involving the temperature. In the present study, a spontaneous emulsification method

was used, with a constant temperature of 25 °C. This method made possible to obtain larvicidal emulsions with the small size of the AO1 and AO2.

Images of the larvae of *Ae. aegypti* after treatment with FFA2-SF (50 µg/mL) showed that fibroin particles precipitated and adhered to the bristles and caused damage to the cuticles (Figure 5). The digestive tract was also seen to be swollen or smoothed, with dark areas. Biomaterial (FFA2-SF) was clearly seen to be adhering to the anal papillae and increasing the size of the respiratory siphon of the larvae, and their digestive and respiratory system no longer functioned well, which gave rise to lethal alterations such as blockage at the anal papillae. This showed that the efficiency of the mini-emulsion of FFA2-SF came from the damage caused to the digestive and respiratory system of the larvae. This morphological damage was similar to what was observed by Araújo (Araújo et al. 2020) using a hexanic extract from the leaves of *Acmella oleracea* dissolved in silk fibroin and by Yu (Yu et al. 2015), who reported the damage caused to the structure of the stigma plate at the apex of the siphon.

Figure 5. Here

Conclusion

In this study, a solution of silk fibroin extracted from *Bombyx mori* was used as an alternative emulsifying agent for preparing emulsions oil/water containing bioactive agents with low solubility (triglycerides, ethyl esters and fatty acids) from the seeds of *Carapa guianensis*, for application larvicides. The oils (AO1 and AO2) presented differences in lipid composition: AO1 mostly contained saturated fatty acids while AO2 mostly contained unsaturated fatty acids. These differences directly influenced the properties of the fibroin emulsion and the larval mortality. Among the emulsions, FFA2-SF derived from AO2 presented LC_{50} = 16.796 $mg.mL^{-1}$ against the larvae of *Ae. aegypti* after 24 h, through causing structural alterations to the vector that were boosted by the fibroin. Because of the biocompatibility and high availability of silk fibroin and the ease of preparation of the

emulsions, these innovative emulsions containing fatty acids derived from the oil of *C. guianensis* showed promise for larvicidal applications.

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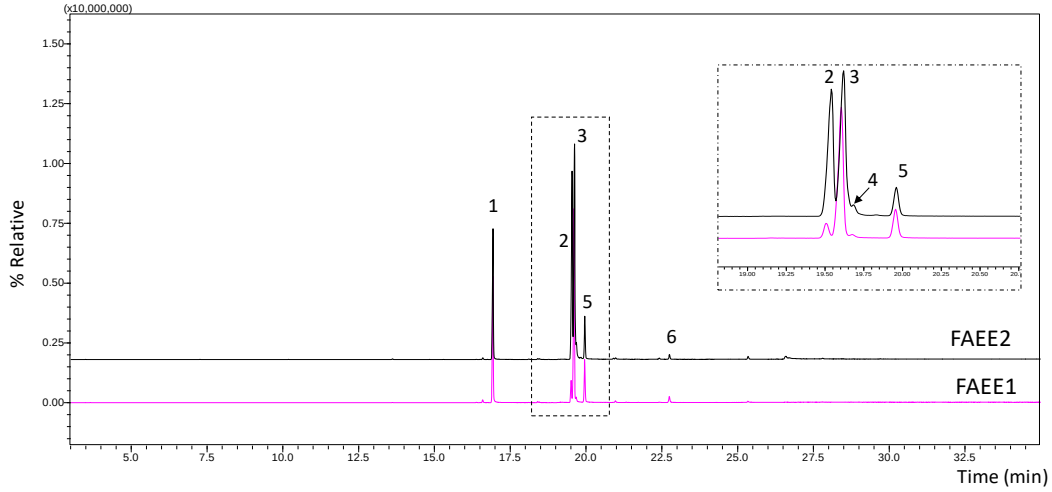

Figure 1. Chromatogram of the samples FAEE1 and FAEE2, from AO1 and AO2 of *C. guianensis.* Peak (retention time): 1 - Palmitic (16.8 min); 2 – Linoleic (19.5 min); 3 – Oleic (19.6 min); 4- Vaccenic (19.7 min); 5 – Stearic (19.9 min); 6 – Not identified (min).

Table 1. FAEE compositions from AO1 and AO2, determined through GC-MS analysis.

Fatty acid[a]	**Peak (Figure 1)**	**FAEE1 Relative concentration**[b] **(%)**	**FAEE2 Relative concentration**[b] **(%)**
Palmitic (C16:0)	1	31.4	19.5
Linoleic (C18:2, ʊ-6)	2	5.4	33.1
Oleic (C18:1, ʊ -9)	3	50.6	40.2
Vaccenic (C18:1, ʊ-7)	4	0.9	0.4
Stearic (C18:0)	5	10.0	6.2
Not identified	6	1.5	0.6
∑ Saturated	-	41.4	25.7
∑Monounsaturated	-	51.5	40.6
∑ Polyunsaturated	-	5.4	33.1

[a]MS database (NIST 5.0). [b] Values selected by the integration of the peaks.

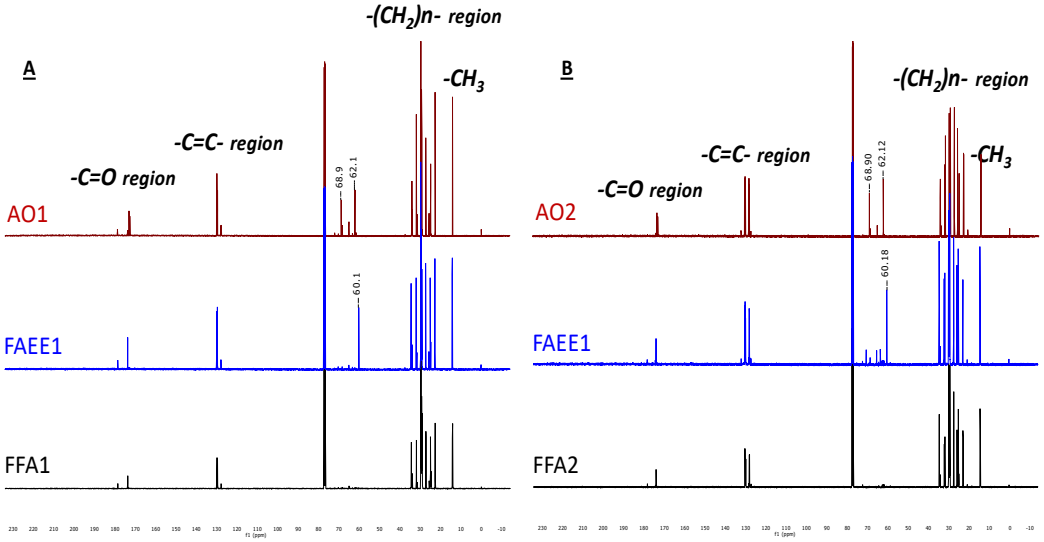

Figure 2. ^{13}C NMR spectrum of the samples AO1, FAEE1 and FFA1 (A); AO2, FAEE2 and FFA2 (B) from of andiroba oil.

Table 2. Comparison of the physicochemical characteristics of the oils.

Physicochemical analysis	AO1 	AO2 
Acidity index (meq.kg^{-1})	21.97 ± 0.14	7.91 ± 0.18
Peroxide index (mg.NaOH.g^{-1})	0.698 ± 0.199	1.426 ± 0.11
Density (mg.mL^{-1})	1.24 ± 0.12	0.92 ± 0.2
Refractive index	1.4594 ± 0.0001	1.4649 ± 0.0005
Melting point (°C)	34-35	nd

Nd. Not determined.

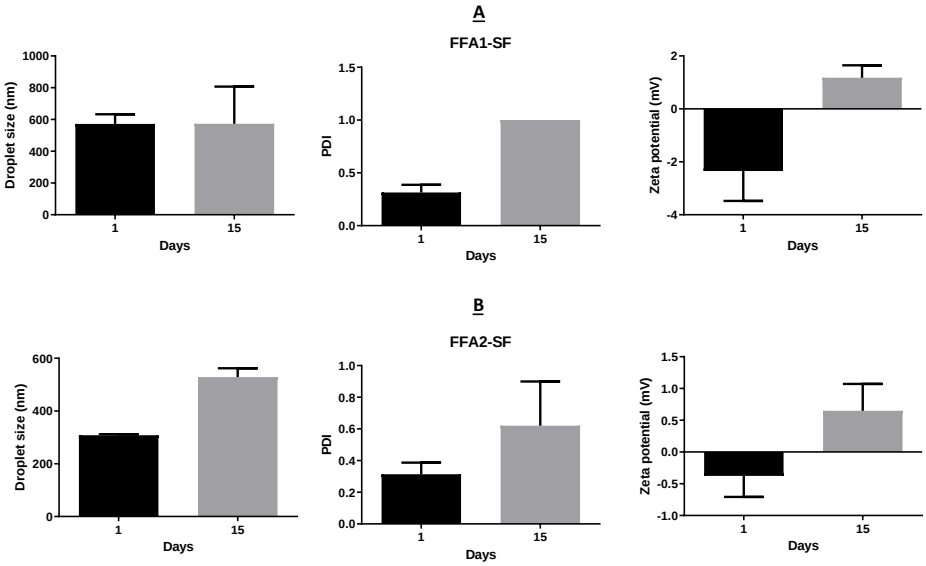

Figure 3. Droplet size, polydispersity index (PDI) and zeta potential in mV of the emulsion from the first to the 15th day of preparation for FFA1-SF (A) and FFA2-SF (B).

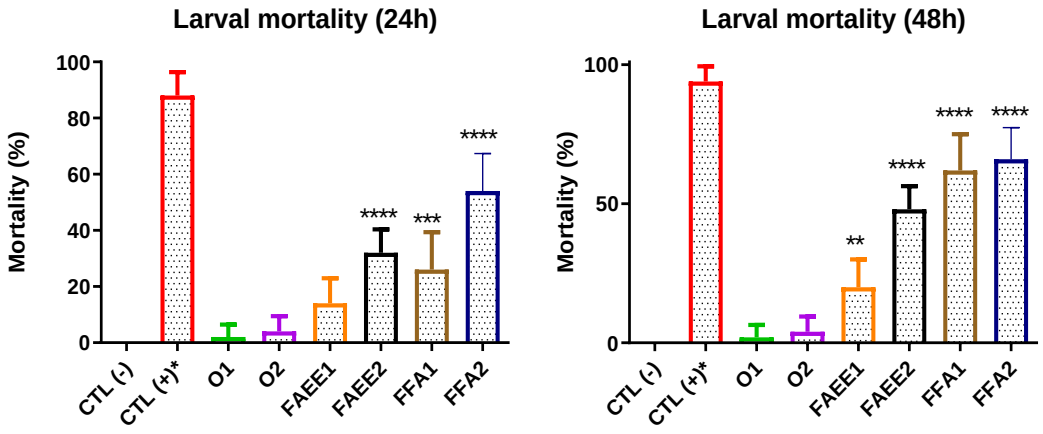

Figure 4. Mortality rates among larvae of *Ae. aegypti* caused by mini-emulsions, assessed 24 and 48 hours after contact with the larvae at a concentration of 50 ppm. CTL(-): silk fibroin (2%); CTL(+): Dichlorvos solution (*6.25 ng/mL).

Table 3. LC_{50} and LC_{90} for mortality among larvae of *Ae. aegypti*, caused by emulsions of FFAs associate silk fibroin (75%) and in DMSO (2%).

Larvicidal active agent	LC_{50} **(µg/mL)**		LC_{90} **(µg/mL)**	
	24 hours	48 hours	24 hours	48 hours
FFA1-SF	212.333	129.455	93.732	94.275
FFA2-SF	94.455	16.796	47.114	38.302
FFA1-DMSO	141.371	114.863	286.759	206.337
FFA2-DMSO	105.752	81.175	239.859	193.601

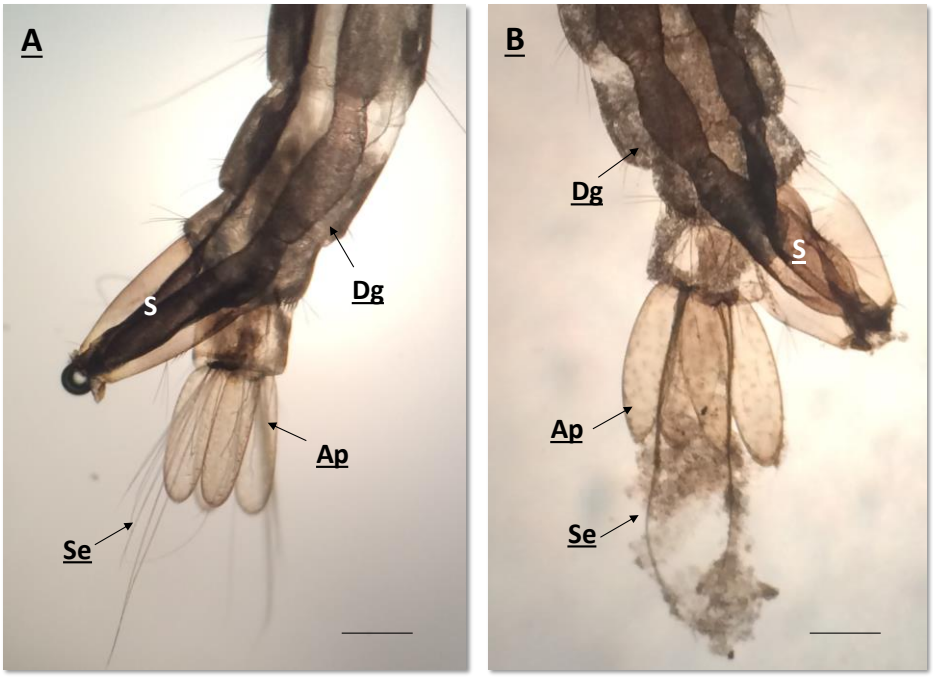

Figure 5. End segment of *Ae. aegypti* larva under stereomicroscope (10×). (A) Control larva with intact anal papillae and siphon. (B) Larva treated with FFA2-SF, showing darkened and deformed anal papillae. Dg: digestive tract; S: siphon; Ap: anal papillae; Se: setae.

Direct aminolysis of *Carapa guianensis* oil for obtaining nanoemulsions active against *Aedes aegypti* larvae

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ABSTRACT

Carapa guianensis (Andiroba) oil is an important natural resource for the inhabitants of the Brazilian Amazon, although it is still little explored. This study aimed to produce fatty amides through the biocatalysis of Andiroba oil (AO) and to use these compounds as a surfactant for AO nanoemulsions using Response Surface Methodology (RSM). A study of the larvicidal activity against Aedes aegypti was conducted to assess the nanoemulsion larvicidal activity. Gas chromatography points to oleic and linoleic fatty acids as constituients. The nanoemulsion obtained by RSM had an acceptable polydispersity index, particle size around 120 nanometers and a zeta potential greater than 60 mV in module. Larval mortality in the third instar was 64 and 74% after 24 and 48 hours respectively. The use of fatty amides from AO can be promissing in the development of nanoformulations against the main vector of the Dengue, Zika and Chikungunya virus.

Key-Words: *Carapa guianensis*; biocatalysis; nanoemulsion; fatty amides; response surface method; *Aedes aegypti*.

1 INTRODUCTION

Carapa guianensis (Andiroba) is an important vegetal specie in the Brazilian Amazonia regarding its medicinal properties, amongst them the anti-inflammatory and insecticide the most popularly used and investigated (Sarquis et al., 2019).

In order to explore its insecticidal potential, the incorporation in aqueous media is necessary, although the hydrophobic characteristic makes it difficult. To overcome this barrier, nanotechnology and biocatalysis are feasible approaches (Speranza et al., 2015; Baldiserra et al., 2013).

The field of nanothecnology has gained great interest in several applications. One of this area is the development of nanoformulations with biocide effects (Bajerski et al., 2016). Biocatalysis is a niche in Green Chemistry that uses the enzymatic apparatus of living beings as a catalyst for chemical reactions, seeking to reduce stages and reduce pollution, the environmental issue is gaining more and more importance, so a process that does not generate pollutants and under mild reaction conditions is desirable.

In recent years, fatty amides have been used as surfactants based on biocatalysis and conventional chemical synthesis, beyo they are part of a family of biologically active lipid compounds, mainly in the central nervous system (Nascimento et al., 2020), anti-inflammatory (Barata et al.,2020), antimicrobial and with physico-chemical properties that allow it to be effective surfactants in the detergent and pesticide industry (Araújo et al., 2018).

However, the potential of biocatalysis applied to *Carapa guianensis* oil is still little explored in obtaining nanoformulations therefore this work shows a type solution for these two problems, using biocatalysis techniques to create surfactants with the same oil used in nanoemulsion and Response Surface Methodology (RSM) with alternative methodology for validation the surfactant.

Then the objective of this article was obtaining nanoemulsions of Andiroba oil using direct aminolisys to produce amphiphilic molecules capable of emulsifying and generating nanoformulation, furthermore the larvicidal activity of nanoemulsion was tested against *Aedes aegypti* larvae to verify the capacity of delivery the active compounds to alive organisms in nano scale.

2 MATERIALS AND METHODS

2.1 Obtaining andiroba oil (AO)

The collection of botanical material was carried out and at the coordinates S 00°13'48.3 " W 51°24'37.4", identified by the botanist Rosângela Sarquis. The exsicatae was deposited in the IAN herbarium of Embrapa in the Eastern Amazon Belém-PA with n° 194102. Andiroba oils were obtained by the traditional method used by local riverside dwellers. The oil collection was carried out on the tenth day of June (AO), by the riverside community of the municipality of Mazagão-AP (Nardi etl al.,2014).

2.2 Chemicals and Reagents

Ethanolamine (99.5%) was purchased from Sigma-Aldrich, Lipase acrylic resin from *Candida antarctica* (CAL-B ≥ 5000 U/g) expressed in *Aspergillus niger* was purchased from Sigma-Aldrich (São Paulo - Brazil). Hexane (98%) and ethanol (>99) was purchased from Synth (SP, Brazil).

2.3 Gas Chromatograph-Mass Spectrometry

The reaction analyses were conducted using a gas chromatograph (GCMS- QP 2010) equipped with an auto-sampler injection AOC-20i (Shimadzu). Electron impact detection was used as detector (Shimadzu MS2010 Plus), electronic impact of 70 eV and fragments detected from 50 to 550 Da were the conditions used. Separations were performed on a fused silica capillary column (RTX-5MS with i.d. = 0.25 mm, length = 30 m and film thickness = 0.25 µm) in a stream of helium 1.03 mL/min. The sample was solubilized in hexane (2 µg/mL) and 1.0 µL of the solution was subjected to the following experimental conditions: injector temperature, 210 °C; detector temperature, 250 °C; carrier gas, Helium; flow rate 3.0 mL/ min; splitinjection with split ratio 1/15. The column temperature was programmed from 90 °C, with an increase of 6 °C/min to 250 °C, ending with a 5 min isothermal at this temperature, the total analysis time was 33.67 min.

2.4 Fourier Transform Infrared Analysis (FTIR)

FTIR spectra were recorded on a Shimadzu IR Affinity spectrometer. Samples Andiroba oil (AO), free acid ethyl ester (FAEE) and fatty amide of Andiroba oil (FAAO) were prepared as thin films on KBr disks. The transmittance was expressed in cm^{-1} in the region between 4000 and 400 cm^{-1} with resolution of 4 cm^{-1} and 64 scans.

2.5 Nuclear Magnetic Resonance(NMR)

^{13}C NMR spectra of AO and FAAO were recorded on an Agilent Technologies 500/54 Premium Shielded spectrometer. The samples were solubilized in deuterated $CDCl_3$ and chemical shifts expressed in ppm relative to internal standard TMS or deuterated solvents. The chemical shifts were given in ppm.

2.6 Aminolysis reaction of Triglycerides from *Carapa guianensis* oil (AO)

The amidation reaction was performed using ethanolamine (3 mL), AO (1 mL), 300 µL of hexane and 10% CAL-B as catalyst on orbital stirring (150 rpm, 28 ± 2 °C) during 48 h. After this period, the enzyme was filtered and washed with hexane (3×1 mL). The filtrate was evaporated under reduced pressure and purified by column chromatography on silica gel using hexane: ethyl acetate (9:1) as eluent.

2.7 Optimization and selection of AO nanoemulsion with FAAO using response surface methodology

In this study, three levels and three variables Box–Behnken factorial design was applied to determine the best combination of factors for smallest droplet size employing the FAAO as surfactant. The formulation (1-5% w/w FAAO concentration), and condition (5-9 pH), and stirring time (1-6 min) that given optimum FAAO nanoemulsion were determined using RSM technique. RSM was performed to evaluate the effects of independent variables: pH (x1), O/A concentration of FAAO (x2) and stirring time (x3) on the responses: droplet size (Y1) and Zeta potential (Y2) of the FAAO nanoemulsions.

The independent variables x1, x2 and x3 and their levels values are shown in table 1. The polynomial equation used for the three variables was described in the Equation 1:

$$Y_i = \beta 0 + \beta 1x1 + \beta 2x2 + \beta 3x3 + \beta 11x1^2 + \beta 22x2^2 + \beta 33x3^2 + \beta 12x1x2 + \beta 13x1x3 + \beta 23x2x3 \text{ [Eq.1]}.$$

Where: Yi is the predicted response (i = 1 or 2); β0 is the constant; β1, β2 and β3 are the linear coefficients; β11, β22 and β33 are the quadratic coefficients; β12, β13 and β23 are the interaction coefficients; and x1, x2 and x3 are the independent variables (Holanda et al., 2019).

Table 1. Three independent variables used in the employed Box-Behnken factorial Design

Factor	Name	Levels		
		-1	0	+1
x1	pH	5	7	9
x2	Amide Concentration % (A/O)	1	3	5
x3	Stirring time (min)	1	3	6

The software STATISTICA® (version 10, Statesoft – Inc., Tulsa, USA trial version, 2011) was used for experimental design and data analysis. For evaluation of independent variables significance, interections and inlfuence, analysis of vairance (ANOVA) was used. The Pareto charts were made to understand which variables most influence the response: particle size and zeta potential.

The RSM with Box–Behnken factorial design of 3 coded levels, 3 independet variables, and 10 experimental runs (Table 2) was used to optimize the nanoemulsification condition including pH (x1), A/O (x2) and Stirring time (x3) of FAAO and destiled water, making possible to determine the best conditions to produce with AO.

Table 2. Box-Behnken design of FAAO

Run	pH	Amide	Time
1	7	3	3
2	9	5	6
3	5	1	1
4	9	3	1
5	5	5	3
6	7	5	1
7	9	1	3
8	7	1	6
9	7	3	3
10	5	3	6

2.8 Preparation of nanoemulsions

Nanoemulsions were prepared according to the low energy method used by Duarte and cols (2015). Using 1, 3 or 5% (w/w) of FAAO, 99, 97 or 95% (w/w) of water, with final mass of 20 g. For the experimental design FAAO was added in one of the concentrations (1, 3 or 5%), with the aqueous phase at a defined pH (5, 7 or 9) in the stirring time (1, 3 or 6 min). After that, the AO was mixed with the best conditions of FAAO stirred at 100 rpm using a Vortex equipment for the optimum time. Nanoemulsion was stored under room temperature (22 ± 2°C) and evaluated after 1 and 30 days of preparation.

2.9 Measurement of droplet size, polydispersity index and zeta potential

Droplet size, Zeta potential and polydispersity index (Pdi) of the nanoemulsions of design experimental and the nanoemulsion involving FAAO and AO was determined by dynamic light scattering with particle size analyzer (Zeta size ZS, Malvern, UK). Nanoemulsion was diluted with water (Duarte, et al., 2015). Measurements were made in triplicate.

2.10 Larvicidal Activity

The larvae of Aedes aegypti were obtained at the Arthropoda Laboratory, kept in a controlled ambient, with a temperature of 25 °C, humidity of 75% and 12h of light: dark cycle. Different concentrations (25, 50, 75 and 100 µg / mL) were tested for the nanoemulsion containing the assets (FAAO with AO) against the Aedes aegypti larvae. All tests were performed in quintuplicate. The vehicle of the emulsions (FAAO with H_2O) was used as a negative control. Mortality was verified after 24 and 48 hours of incubation. The larvae were considered dead when they did not react to stimulus and also compared to the control group, according to protocol of WHO (2005).

3 RESULTS AND DISCUSSION

3.1 Physicochemical characteristics of the oil

The AO extracted by traditional methodology from the seeds of *C. guianensis* (Nardi et al., 2017) demonstrated physico-chemical properties according to other literature reports (Silva, 2018). The volume of NaOH solution (0.1 M) used to neutralize the free fatty acids in the sample corresponds to the acidity index. So, the free acidity value was 7.91%; bulk density 0,8816 g/cm^3; Degree of oxidation was 1.426 meq.kg^{-1}. It is important to know the quality standard of the triglycerides for an efficient reaction.

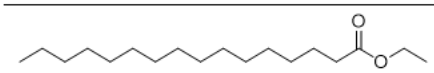
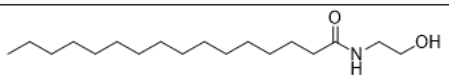
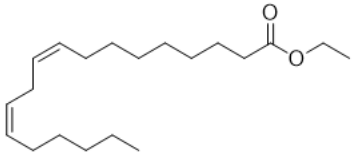
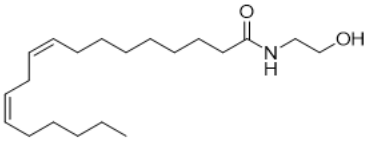
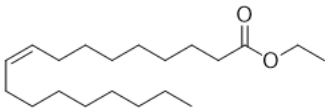
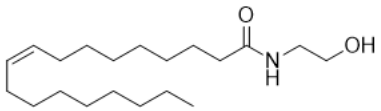
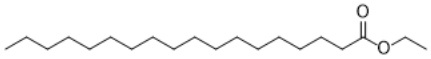
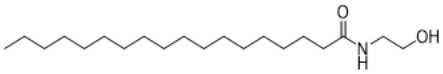
3.2 Synthesis of Fatty Amides

The aminolysis of AO was performed with CAL-B enzyme catalyst at room temperature for 48 hours. The catalyst bets for convention of FA by direct aminolysis from AO was the CAL-B (5%) at 27 ± 2 °C for 48 h, with 44,43% yield. These yields can be considered low compared to Araújo et al., 2018 with direct aminolysis of *Oenocarpus bataua* (Patawa) oil. This work points to 82% yield with CAL-B, however the oil was extracted in Soxhlet with Hexane and the difference in the lipid profile may have contributed to this difference in the yield reaction (Araujo et al., 2018).

AO (expressed in ethyl esters [FAEE]) and FAAO were analyzed by GC- MS, the fatty acid composition of the AO was oleic acid (40.23%), linoleic acid (33.1%), palmitic acid (19.5%), stearic acid (6.17%) and 0.6% without identification. This composition of AO is in accordance

with the reported by Tiosso et al., (2014) that found oleic acid (47.0%) as the most abundant compound, followed palmitic acid (29.0%) and stearic acid (10%). Table 3 show the FAEE and the production of FAAO after aminolysis reaction with ethanolamine.

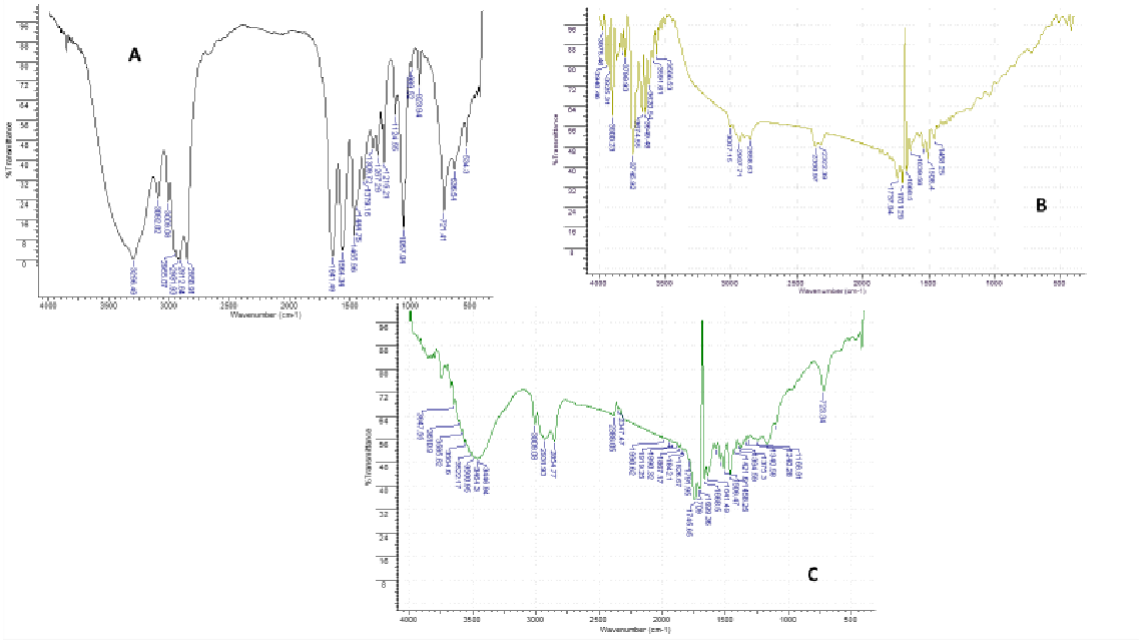
Table.3 FAEE and FAAO identified in the analysis by CG-MS

FAEE	FAAO
 ethyl palmitate (**1**)	 N-isopropylpalmitamide *N*-(2-hydroxyethyl)palmitamide
 ethyl linoleate (**2**)	 (9*Z*,12*Z*)-*N*-isopropyloctadeca-9,12-dienamide
 ethyl oleate (**3**)	 *N*-isopropyloleamide
 ethyl stearate (**4**)	 N-isopropylstearamide (4a)*N*-(2-hydroxyethyl)stearamide

* Retention time (Method described in item 2.3).

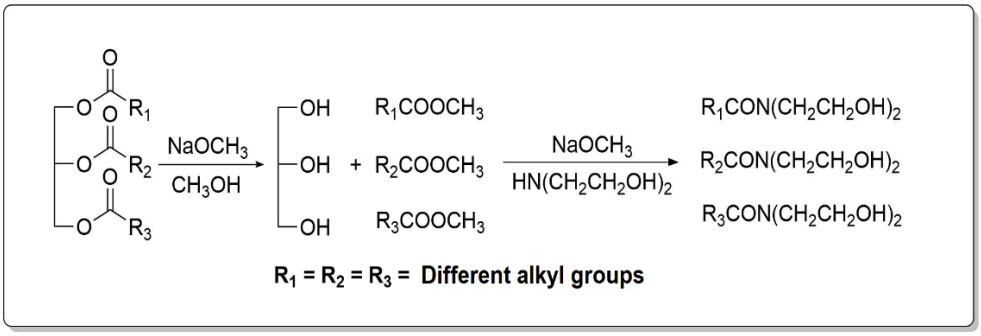
The FT-IR spectra are shown in Figure 1. A-C. FAAO, FAEE and AO respectively. The signals range 3296.49, 3092.02 and 3009.08 cm^{-1} are related to amides by the stretch – NH_2 and the 1641.49 cm_{-1} correspond to bond -N-H. These signals generate some difference to others spectra's, but there is a lot of similarity between them because they do not present major structural differences.

Figure 1. Andiroba oil (A), Free acid ethyl ester (B) Fatty Amide from Andiroba Oil (C).



One of the objectives of this research is to develop an eco-friendly methodology according to the principles of green chemistry, using the least amount of catalyst, lowest energy expenditure and highest atomic yield (Araújo et al.,2018) in this context this is the first time that direct aminolysis of *Carapa guianensis* oil was achieved by CAL-B, an environmentally friendly methodology. Others authors usually obtained in two steps through the amidation of fatty methyl esters in Figure 2 (Almeida et al., 2013).

Figure 2. Conventional amidation reaction



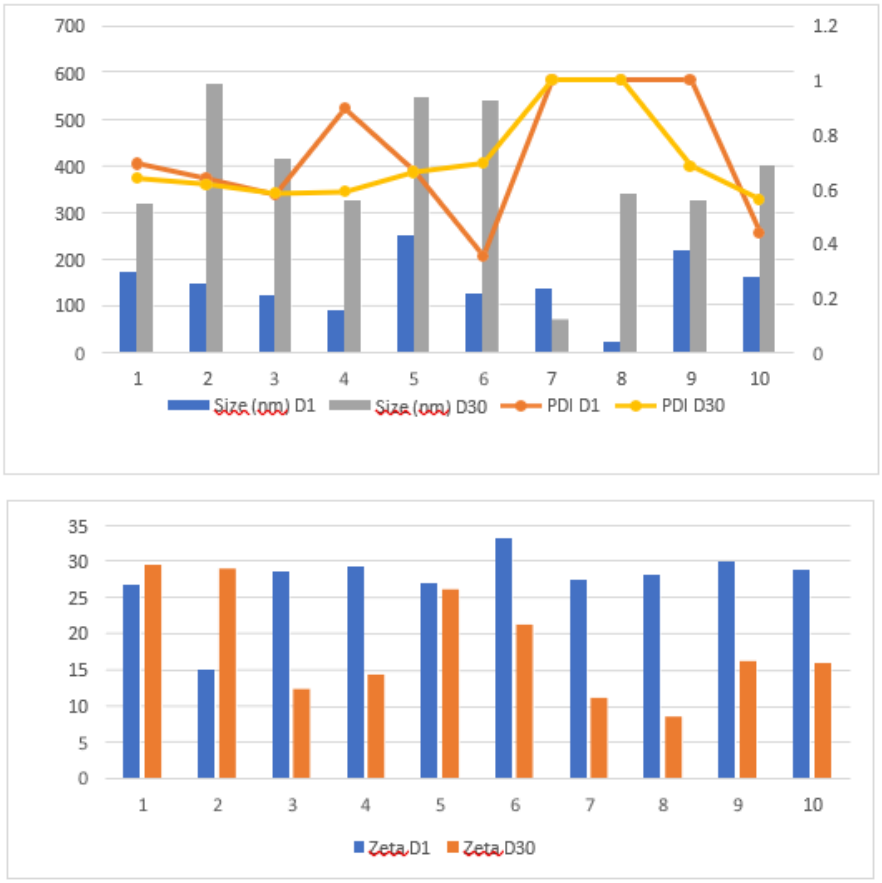
Lopes and co-works (2010) transesterified mamona oil (*Ricinus communis*) as first step, then aminolysis was performed as second step, at high temperatures 75, 90 and 130 C°, in a non-renewable catalyst (CH_3ONa) with yields ranging from 6 to 88%, so this work has advantages such as direct amidation (only one step), this saves time in obtaining fatty

amides, and also proves that amides can be obtained at room temperature and using a renewable catalyzers.

4.1 Response Surface Methodology to validate of FAAO as surfactant

It is the first time that Andiroba oil has been used as a surfactant for the nanoemulsion itself, in the form of fatty amide. For this reason, amide nanoemulsions and distilled water were made with different pH, FAAO concentrations and stirring time following experimental design to determine the most important variable and validate fatty amides from AO as a surfactant. Particle size, polydispersity index and zeta potential were measured on days 1 and 30 (Figure 3).

Figure 3. Results of particle size, polydispersity index and zeta potential on day 1 and day 30 of the nanoemulsions, in the 10 runs of the experimental design.



In general, the values of droplet size of nanoemulsions range between 19.42 and 248 nm in first day, 317.7 and 517.7 nm in thirtieth day. It is important evaluate droplet size because it characterizes nanoformulations, being a sign of stability that must be maintained in storage and in dispersion. So, it is necessary stabilize the size, as the dispersion of nanoemulsion can change the droplet size and alter its bioactivity (Milhomem-Paixão et al., 2017). According to other research with AO and other natural oils, it is common for nanoemulsions with this type of raw material to undergo changes in the droplet size due to the complexity of these oils (Melo et al., 2018; Milhomem-Paixão et al., 2017; Valentim et al., 2018).

The homogeneity of particle populations is represented by the polydispersity index. Interesting values range from 0 (monodispersed) to 0.500 (relatively broad distribution), while values below 0.700 are considered acceptable (Valentim et al., 2017). So, only in 4 compositions the values of polydispersity exceeded 0.700, demonstrating the stability of systems.

The surface charge of the droplets is represented by the Zeta potential, high values positive or negative are attributed to kinetically stable nanoemulsions (Valentim., et al 2018). Previously others authors using *Carapa guianensis* nanoemulsion showed zeta potential of around -27.6 mV; -3.9 mV; -53.39 mV. Then, in ten FAAO formulations, the zeta potential values exceeded the -25-mV mark and this value went up with the inclusion of AO to the mixture (table 4; Figure 6 a-c).

According to research carried out in the field, these parameters are important to guarantee the stability of nanoemulsions in storage and consequently their biological activity, the nanoemulsification process must be able to trap the active component, maintaining the droplet size when stored (Valentim et al., 2018).

Based on these results the FAAO can be useful as a surfactant capable of generating stable AO microemulsions. This result is important because it shows a new methodology compared to the HLB method and other methods that use conventional surfactants, which can be toxic, presenting an alternative in the development of a natural surfactant.

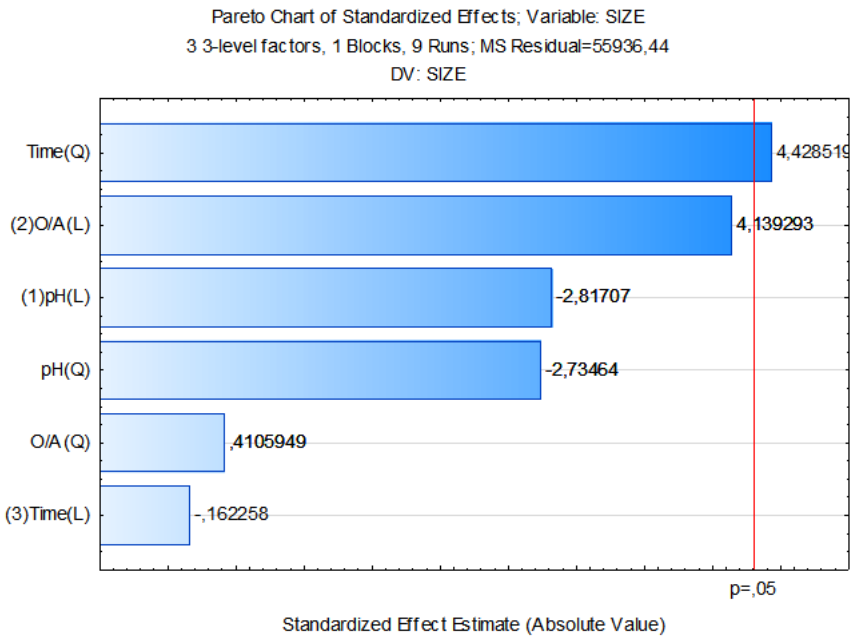
3.4 Response Surface Methodology for select the optimum nanoemulsion conditions

For the non-heating and low-energy method, the determining variable for the generation of smaller nanometer-scale particles was the time, Pareto Chart of standardized effects

presented in Figura 4 showed significant effects in droplet size (nm), time (quadratic), pH and concentration of FAAO (A/O) of the medium variables (quadratic), variables values are showed in table 4. The greater the length of the bar for each parameter, the greater the absolute importance of the estimated effects (Holanda et al., 2019). The vertical red line represents the limit between the significant and less significant effects with a 5% risk of error. Just one effect was significant at 95% confidence level in the studied experimental domain ($P < 0.05$) as shown in Figure 4.

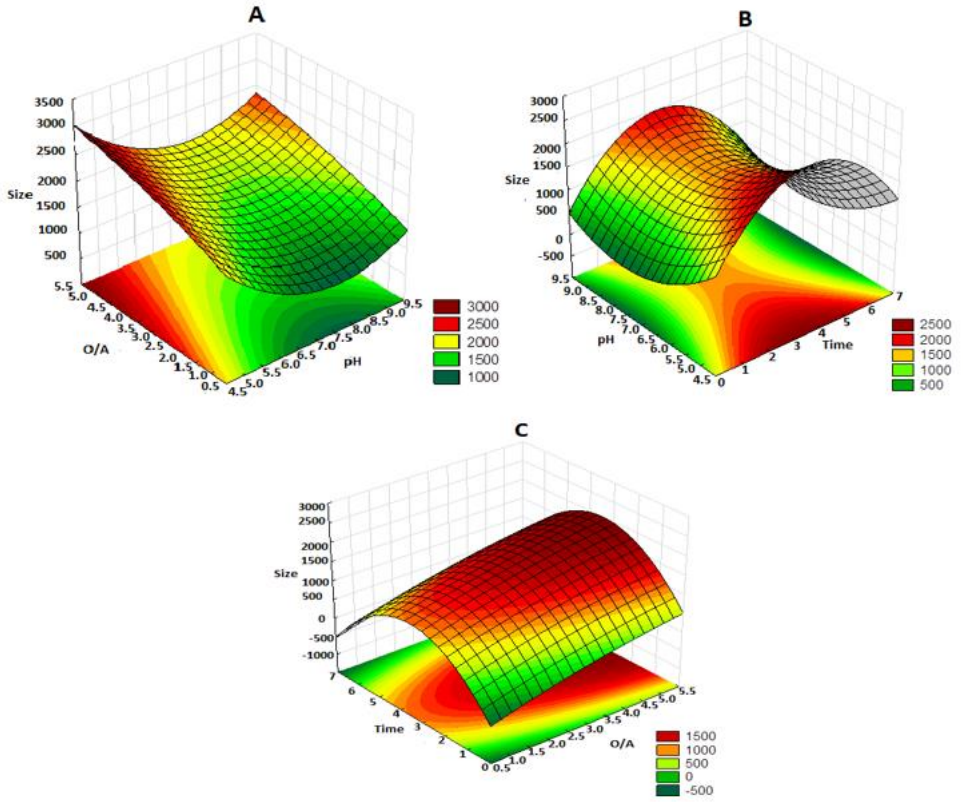
The value of the coefficient of determination (R2) was 0.91. The proficiency of the model is demonstrated if R2 is equal to 0.75 or higher than this value (Haaland,1989). The response surface of the production of AO nanoemulsions using FAAO with surfactant as a function of A/0 (concentration of FAAO) (x2) and pH (x1) is presented in Figure 5 as a 3D surface plot. Analyzing the interactions between these two factors, the idea is clear that the chosen pH range did not influence the responses of the analyzed runs.

Figure 4. Pareto chart illustrating main and interaction effects of the factors affeting the droplet size (nm).



Pareto chart illustrating main and interaction effects of the factors affeting the droplet size (nm) (1) pH (L):linear effect of factor *x1*, (2) O/A Concentration of FAAO (L): linear effect of factor *x2*, (3) Time (L):linear effect of factor *x3*, pH (Q): quadratic effect of factor *x1*, O/A Concentration of FAAO (Q): quadratic effect of factor *x2*, Time (Q): quadratic effect of factor *x3*. The vertical line points the limit between the significant and less significant effects with a 5% risk of error.

Figure 5. Response surface plot and contour plot of the droplet size content as a function of (A) pH (*x1*) and O/A concetration of FAAO (*x2*); (B) Time (*x3*) and pH (*x1*); (C) O/A concentration of FAAO (*x2*) and Time (*x3*).

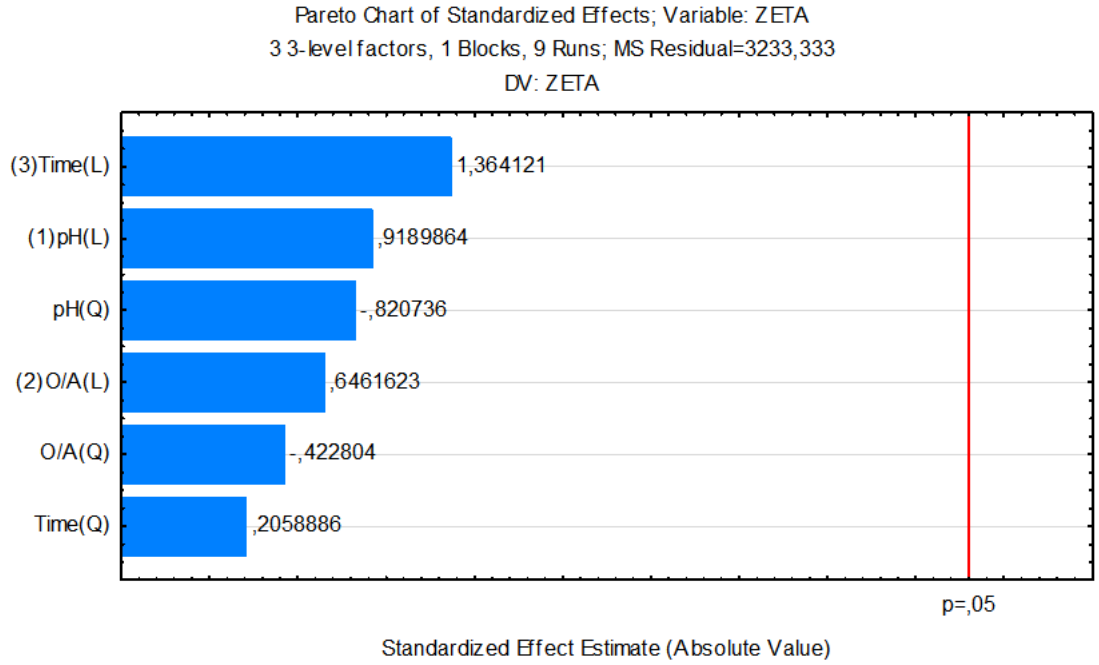


In another way, Figure 4 shows that stirring time (x3) had great influence on the droplet size in relation to the tested pH range and the FAAO concentration, the surface graph shows that there is an ideal time for the smallest particle size, when the time approaches 1 or 7 minutes the smallest particle size is favored, when the time approaches 3 minutes the particle size tends to increase for any concentration of FAAO and pH.

The smallest droplet size (19.42 nm) was obtained for pH 7.0 in the longest time tested (6 min) and with the smallest FAAO concentration (1%), however the PDI was not favorable. For Heydari and co-workers (2019) formulations made with non-ionic surfactants do not undergo significant changes with ionic strength in changing pH, suggesting that FAAO behaves as a non-ionic surfactant.

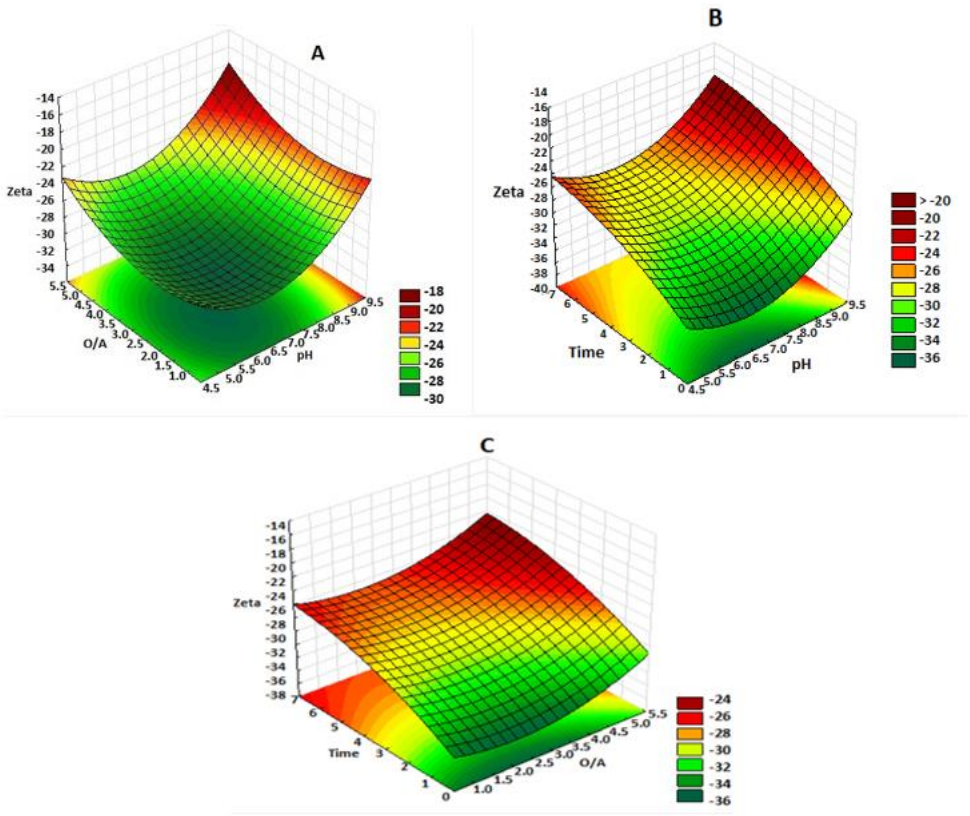
Another important parameter was evaluated by the RSM method, the zeta potential. However, according to Figure 6, the variables evaluated did not statistically influence the results of the Zeta potential.

Figure 6. Pareto chart illustrating main and interaction effects of the factors affeting the zeta potential (mV).



Pareto chart illustrating main and interaction effects of the factors affeting the zeta potential (mV). (1) pH (L):linear effect of factor *x1*, (2) O/A Concentration of FAAO (L): linear effect of factor *x2*, (3) Time (L):linear effect of factor *x3*, pH (Q): quadratic effect of factor *x1*, O/A Concentration of FAAO (Q): quadratic effect of factor *x2*, Time (Q): quadratic effect of factor *x3*. The vertical line points the limit between the significant and less significant effects with a 5% risk of error.

Figure 7. Response surface plot and contour plot of the potencial Zeta content as a function of (A) pH (*x1*) and O/A concetration of FAAO (*x2*); (B) Time (*x3*) and pH (*x1*); (C) O/A concentration of FAAO (*x2*) and Time (*x3*).



But the variable stirring time was the most statistically significant variable, in time close to 1 minute the value of the zeta potential in module was higher, indicating greater stability of these systems, as well as the pH closer to 7 guaranteed higher values also with O/A close to 3% (Figure 7 B-C).

In this case the better results were when the time was close to 1 and 7 minutes, this can be explained as a change in the surfactant by increasing the temperature up to 5 minutes and then stabilizing in 6 and 7 minutes, because this can effect the nanoemulsification process Figure 5-B (Pongsumpun et al.,2020).

Based on the results obtained and in the literature, the parameters that presented the smallest droplet size on day 1 (168 nm) and smallest size variation on day 30 (317.7 nm), lowest PDI on day 1 (0.691) and smallest variation on day 30 (0.639), highest value of the

zeta potential in module of day 1 (26.6 mV) and the value remains high on day 30 (29.5 mV) corresponds to run 1, with pH 7, 3%of Amide concentration and 3 minutes of stirring time.

Stirring time (x3) was statistically the most significant factor to microemulsification, however the concentration of amide (x2) was also important, because in concentrations close to 1% they obtained a smaller particle size. For all the reasons presented above, formulation one was chosen to be repeated with the AO and to produce the nanoemulsion to be tested against the larvae of Aedes aegypti.

Table 4. The design matrix and responses for the independent variables levels availed in droplet size and Zeta potential of nanoemulsions using FAAO

Run	pH	Amide %	Time (min)	Size (nm)	ZP (mV)
1	7	3	3	168.8	26.6
2	9	5	6	144	15.0
3	5	1	1	121.6	28.0
4	9	3	1	87.97	29.2
5	5	5	3	248.7	26.9
6	7	5	1	122.4	33.1
7	9	1	3	134.3	27.3
8	7	1	6	19.42	28.1
9	7	3	3	217.3	29.9
10	5	3	6	159.1	28.8

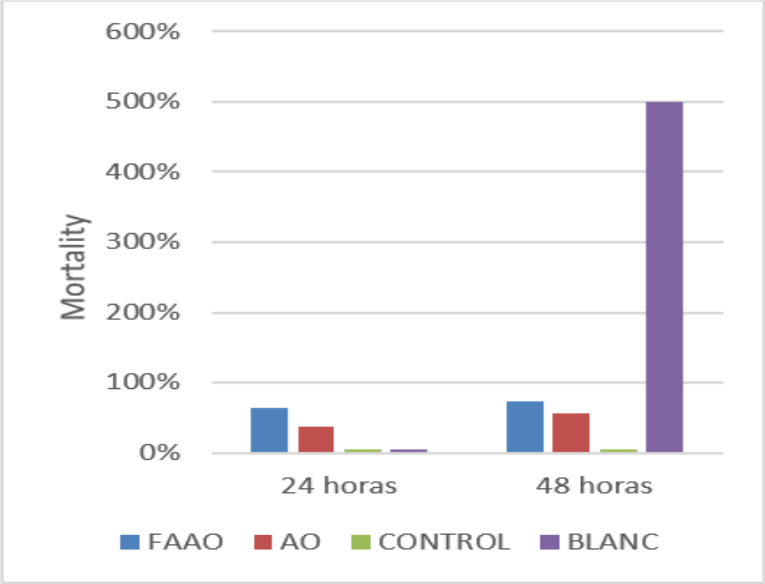
3.4 *Carapa guianensis* larvicidal nanoemulsion with fatty amides as surfactant

For the first time a nanoemulsion with *Carapa guianensis* oil using fatty amides made from Andiroba´s own oil as surfactant, follow the principles of Green Chemistry, with renovable catalyst and solvent-free for the aminolysis of natural triglyceride has been produced. In addition, is the first time the amidation reaction is perfomed with *Carapa guianensis* oil.

The nanoemulsion was carried out in the conditions of formulation 1, as the oil in the concentration of 5% in relation to the total emulsion (20g), 3% of FAAO and supplemented with distilled water (92%), the whole process took 3 minutes.

The result of this approach was a nanoemulsion with 122.2 nm, PDI 0.784 and -67.2 to zeta potential. So, these results indicate that RSM can be used to produce stable nanoemulsions of *Carapa guianensis* oil with nanometric size, using fatty amides from the oil itself as a surfactant. The figure 7 presents larvicidal mortality of AO nanoemulsion with FAAO after 24 and 48 h.

Figure 7. Mortality of nanoemulsion with FAAO and AO, control and blanc nanoemulsion.



In the future, other tests should be carried out to reinforce the idea of controlled release carried out by the FAAO (residual larvicidal method), as the mortality level rises from the cycle from 24 to 48 hours in 10%, this may represent that a controlled release pattern is occurring. As the dose used was low in comparison to other studies (table 6), this nanoemulsion is promising to help on *Ae. Aegypti* control.

One of the objectives of this work is was demonstrating the possibility of obtaining nanoemulsions in the absence of conventional surfactants, and mainly using the oil itself. Despite the great potential of AO, there are still few studies that address its nanotechnological applicability.

A low-energy/solvent-free method of AO nanoemulsification was used effectively against *Aedes aegypti* larvae, however this method used Balance Hydrophile/Lipophile (HLB) approach, therefore synthetic nonionic surfactants polysorbate 80, sorbitol monooleate and mixed of this surfactant to find HLB value and produce the optimal nanoemulsion (Jesus, et

al., 2017). Others authors made a nanoemulsion with AO and surfactant Kolliphor® by phase-inversion temperature (PIT) method (Milhomem-Paixão, et al., 2017). Melo and collaborators (2018) also employed PIT method with different proportions of Tween 80 and Span 80, varying the weight of surfactants ratios (SOR method) for 5 minutes of mix. Baldiserra (2013) used AO to produce nanemulsions and tested against the parasite *Trypanossoma evansi in vitro* by spontaneous nanoemulsification method, achieving success with lipophilic and hidrofilic surfactants (Span 80 and Tween 80 respectively). Organic solvent (acetona) followed by magnetic stirrer and high pressure homogenization. Moraes and co-workers (2018) used AO nanoemulsions with Tween and Span 80 against other parasite *Leishmania infantum* and *Leishmania amazonensis*, this nanoemulsions were able to reduce infection levels in macrophage cultures.

Table 5 is a detailed summary of the articles cited above compared to this one involving nanotechnology and Andiroba oil.

Table 5. Articles involving Carapa guianensis and nanotechnology

Authors	Surfactant	Method	Activity	Particle size	Colety/Acquisition
Sarquis, et al., 2020	FA	SE	Larvicidal	±122.2 nm	Mazagão, Amapá, Brazil
Jesus, et al 2017	PS80, SM	SE	Larvicidal	±151.1 nm	Mazagão, Amapá, Brazil
Milhomem-Paixão, et al., 2017	KO	PIT	Cytotoxity, Genotoxity and Hematotoxicity	±142.5 nm	Uruará, Pará, Brazil
Melo, et al., 2018	TW 80, SP 80	PIT, SOR	Genotoxicity and Antigenotoxicity	±130 nm	Pará State, Brazil
Baldiserra, et al., 2013	TW 80, SP 80	HPH	Trypanocidal	±240.3 nm	Baraca Sabará C.I S/A, SP, Brazil
Moraes, et al., 2018	TW 80, SP 80	MF, SON	Leishmanicidal	±92.20 nm	Jurua Eco-extractivism, Acre, Brazil

Surfactant: FA: Fatty Amides; PS80: Span 80; SM: Sorbitan Monooleate; KO: Kolliphor; TW80: Tween 80; SP 80: Span 80. **Method**: SE: Spontaneus emulsification; PIT: Phase inversion temperature; SOR: Surfactant range; HPH: High preasure homogenization; MF: Microfluidization; SON: Sonication.

Nanoemulsions can be produced by different methods, these methods can be low and high-energy, the first uses PIT, SE and solvent diffusion, the second uses SON, MF and HPH. The stability and safety of the formulation components are characteristics of the ideal nanoemulsion (Milhomem-Paixão., et al., 2017).

In this sense, all of the nanoemulsions exposed in table 5 showed particle size averaging 147.5 nm, but the smaller results was of the high-energy methods in leishmanicidal nanoemulsion ±92,20 nm, while the other nanoemulsions were prepared by high-energy method and resulting in bigger sizes ±240.3 nm (Moraes et al., 2018; Baldiserra et al., 2013).

New procedures to produce nanoemulsions different from high-energy modes are persuaded (Valentim et al.,2017). Therefore, low-methods appears as an alternative, involving simpler equipment, without the use of heat, may even achieve comparable results (Jesus, et al 2017; Milhomem-Paixão, et al., 2017; Melo, et al., 2018; Baldiserra, et al., 2013; Moraes, et al., 2018).

It is important to compare the particle size of formulations to define how nanoemulsion and evaluate its stability, just as this parameter directly affects the bioavailability of the active components of the formulation, variations in size can generate changes in toxicity tests that are not yet understood. However, the small size is desired as it has advantages over conventional size, such as increased stability, controlled release, enhancing the biological activities of the formulation (Milhomem-Paixão et al., 2017).

In recent years, the interest in natural products with inseticidal activity has grown. In this context, some nanoformulations were developed, specially to *Ae. aegypti* (Ricci-Junior et al.,2020). Table 6 shows nanoformulations with larvicidal activities to compare with the nanoformulation of this research.

Table 6. Larvicidal nanoemulsions with essential oils and resin oil

Authors	Oil	Surfactant	Method	Particle size	Zeta potential	PDI	MI/ 24 hrs	MI/ 48 hrs	Dose	Vector
Sarquis et al.,2020	*Carapa guianensis*	FA	SE	±130 nm	-67 mV	±0.784	64%	74%	100 ppm	Aa
Jesus et al., 2017	*Carapa guianensis*	PS80, SM	SE	±151 nm	-27 mV	±0.131	3%	5%	250 ppm	Aa
Oliveira et al., 2017	*Pterodon emerginatus*	PS80, SM	SE	±151 nm	-32 mV	±0.221	26.67%	93.33%	100 mg/L	Cq
Duarte et al., 2018	*Alecrim*	PS20	SE	≥180 nm	NR	±0.550	80.00%	90.00%	250 ppm	Aa
Peniche et al., 2018	*Hyptis suaveoleans*	PS80	SE	±152 nm	NR	±0.523	46.67%	63.33%	250 ppm	Cq
Segumar et al., 2019	*Eucalyptus globulus*	T80	US	±9.4 nm	NR	NR	100.00%	100.00%	250 ppm	Cq
Prophiro et al., 2012	*Carapa guianensis*	PS80	SOL	NN	NR	NR	50.00%		141 mg/L	Aa

Surfactant: FA: Fatty Amides; PS80: Span 80; SM: Sorbitan Monooleate; KO: Kolliphor; TW80: Tween 80; SP 80: Span 80. **Method**: SE: Spontaneus emulsification; US: Ultrasonication; SOL: solubilization; **PDI:** Polydispersity index; **MR:** Mortality rate; **Vector:** Aa: *Aedes aegypti*; Cq: *Culex quinquesfasciatus*.

Compared to other formulations, it's possible to obtain active nanoformulations as new method. Size slightly smaller than the authors Jesus (2017), Oliveira (2017), Duarte (2018), Peniche (2018). However, when compared to Segumar (2019) who used ultrasonication, the particle size was 120 nm larger and compared with Prophiro (2012), which was the first to use AO as a larvicidal agent through solubilization in DMSO.

These differences in particle size are likely to influence larvicidal activity. This is a pioneering work using FAAO as a surfactant to make the AO assets available in aqueous medium. As the particle size was smaller than the works mentioned above, while the low dose used (100 ppm) was successful against the *Ae. aegypti* larvae. Further studies are needed to understand the influence of amides on larvicidal activity, as the amides solely in water.

5. CONCLUSION

The biodiversity is an important source of bioactive compounds. The amazon tree, *Carapa guianensis* is one of the most promising species in the region, but there are still few studies that use this source for product development. In this sense, this work used a natural surfactant for the development of larvicidal nanoformulations, following the principles of green chemistry, obtaining nanofomulations with a droplet size between 100 to 600 nanometers. A nanoformulation was chosen by the Response Surface methodology as more stable, then tested against Aedes aegypti larvae, presenting promising activities of 60 to 70% mortality, presenting results comparable to formulations made with traditional surfactants.

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5 CONSIDERAÇÕES FINAIS E PERSPECTIVAS.

Os processos biocatáliticos com óleo vegetais têm oportunizado pesquisas inovadoras com espécies vegetais de uso tradicional na Amazônia, na tentativa de buscar novas formulações bioativas. Este estudo demonstrou, pela primeira vez, que é possivel através das reações de transesterificação e amidação, sugerir novos usos ao óleo extraído de forma tradicional de *C. guianensis.*

O desenvolvimento de formulações com as amidas, ácidos, esteres da *C. guianensis* demonstraram no teste *in vitro* com as larvas de *A. aegypti* serem ativas, no entanto mais estudos devem ser realizados e outros testes para identificar o mecanismo de ação destas formulações.

A fibroina demostrou ser um transportador de ativos capaz de interagir com a cutícula externa das larvas, esta interação é muito interessante, pois isso demontra que a fibroína pode ser utilizada para o controle de pragas e parasitas que apresentam cutícula (revestimento quitinoso), podendo ser um carreador mais seletivo e específico.

Biosurfactantes do óleo de *C. guianensis* (andiroba) são capazes de produzir nanoemulsões bioativas com o próprio óleo fixo extraído da espécie, sendo que essa capacidade deve ser testada com outros óleos futuramente. As amidas graxas, neste estudo, foram usadas como surfactante para nanoemulsões, deixando claro a versatilidade destas moléculas

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Title: Oil-in-water emulsion of Carapa guianensis using silk fibroin, as an alternative to traditional surfactants, and active against larvae of the vector Aedes aegypti

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Keywords: Mini-emulsion; Free fatty acids; Fatty acid ethyl ester; Larvicidal effect; Silkworm cocoon

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Abstract: This paper reports on preparation of an oil-in-water new microemulsion of Carapa guianensis oil and derivates [fatty acid ethyl ester (FAEE's) and fatty free acid (FFA's)], using silk fibroin, as alternative to traditional surfactants, and its activity against the larvae of the vector Aedes aegypti. Among the emulsions that were prepared, FFA2-SF derived from AO2 presented the best results against larvae of Ae. aegypti after 24h (LC50 = 16.79 mg.mL-1), through causing structural alterations to the vector that were boosted by the fibroin. The lipid profile, enhanced by the fibroin mini-emulsion state, of the oils strongly influences larval mortality, the AO2 mostly contained unsaturated fatty acids.