UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA E FISIOLOGIA
TESE DE DOUTORADO

INGRIDD AYSLANE TORRES DE ARAÚJO RIBEIRO

ÓLEOS ESSENCIAIS DE *Croton rudolphianus* e *Algrizea macrochlamys* NO COMBATE À DOENÇAS TROPICAIS NEGLIGENCIADAS:

Esquistossomose e dengue

Recife
2020

INGRIDD AYSLANE TORRES DE ARAÚJO RIBEIRO

ÓLEOS ESSENCIAIS DE *Croton rudolphianus* e *Algrizea macrochlamys* NO COMBATE À DOENÇAS TROPICAIS NEGLIGENCIADAS:

Esquistossomose e dengue

Tese apresentada ao Programa de Pós-graduação em Bioquímica e Fisiologia da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Doutor em Bioquímica e Fisiologia.

Área de concentração: Bioquímica e Fisiologia

Orientadorora: Profª. Drª. Maria Tereza dos Santos Correia

Co-orientadora: Profª. Drª. Daniela Maria do A. Ferraz Navarro

Recife

2020

Catalogação na fonte
Elaine C Barroso
(CRB4 1728)

Ribeiro, Ingridd Ayslane Torres de Araújo
Óleos essenciais de *Croton rudollphianus* e *Algrizea macrochlamys* no combate à doenças negligenciadas: esquistossomose e dengue / Ingridd Ayslane Torres de Araújo Ribeiro – 2020.

195 f.: il., fig., tab.

Orientadora: Maria Tereza dos Santos Correia
Coorientadora: Daniela Maria do A. Ferraz Navarro
Tese (doutorado) – Universidade Federal de Pernambuco. Centro de Biociências. Programa de Pós-Graduação em Bioquímica e Fisiologia, 2020.
Inclui referências, apêndice e anexos.

1. Óleos vegetais 2. Doenças negligenciadas 3. Metabólitos secundários I. Correia, Maria Tereza dos Santos (orient.) II. Navarro, Daniela Maria do A.

665.3 CDD (22.ed.) UFPE/CB – 2020-169

INGRIDD AYSLANE TORRES DE ARAÚJO RIBEIRO

ÓLEOS ESSENCIAIS DE *Croton rudolphianus* e *Algrizea macrochlamys* NO COMBATE À DOENÇAS TROPICAIS NEGLIGENCIADAS:

Esquistossomose e dengue

Tese apresentada ao Programa de Pós-graduação em Bioquímica e Fisiologia da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Doutor em Bioquímica e Fisiologia.

Aprovada em: 25 / 08 / 2020.

BANCA EXAMINADORA

_____

Profª. Drª. Maria Tereza dos Santos Correia (Orientador)
Universidade Federal de Pernambuco

_____

Profª. Drª. Ana Maria Mendonça de Albuquerque Melo (Examinador Externo)
Universidade Federal de Pernambuco

_____

Drª. Shyrlane Torres Soares Veras (Examinador Externo)
Universidade Federal de Pernambuco

_____

Profª. Drª. Patrícia Maria Guedes Paiva (Examinador Interno)
Universidade Federal de Pernambuco

_____

Profª. Drª. Luana Cassandra Breitenbach Barroso Coelho (Examinador Interno)
Universidade Federal de Pernambuco

Dedico este trabalho a
minha amada família

AGRADECIMENTOS

Agradeço primeiramente a Deus pela dádiva da vida, pelas pessoas que colocou em meu caminho, por ter me dado força e saúde.

À minha família, em especial, a mãe Fabiana Torres, minha avó Socorro Torres, tia Fátima Torres e tio Fabiano Veras por todo amor, cuidado, dedicação, apoio, ensinamentos, ajuda e compreensão em todos os momentos da minha vida.

Ao meu noivo João Henrique pelo amor, ajuda, paciência, compreensão e por sempre estar comigo nos momentos em que mais preciso.

Ás minhas amigas, Aline Maria, Micherle Silva, Amanda Silva e Rosimere Silva pelos momentos de descontração e companheirismo.

Aos meus amigos do Laboratório de Radiobiologia, Hianna Silva, Douglas Araújo, Dewson Pereira, Maíra Vasconcelos, José Luís, Katarine Mizan e Vinícius pela força, ajuda, disponibilidade e companheirismo.

Aos meus amigos do Laboratório de Ecologia Química, Jéssica Nascimento e Júlio Cesar, pela disponibilidade, ajuda e momentos de descontração.

Ao Dr. Marcelo Moreno pela disponibilidade, ajuda e incentivo.

À Profª. Drª. Ana Maria Mendonça de Albuquerque, do Departamento de Biofísica e Radiobiologia da UFPE, por ter me acolhido de forma muito carinhosa e pelos ensinamentos, disponibilidade e colaboração.

À minha orientadora, Profª. Drª. Maria Tereza dos Santos Correia, pela oportunidade, acolhimento, paciência, confiança e ensinamentos em todas as etapas da minha pesquisa.

À minha co-orientadora, Profª. Drª. Daniela Maria do Amaral Ferraz Navarro, pelas sabias contribuições, compreensão, apoio, disposição e acolhimento.

À Profª. Drª. Márcia Vanusa da Silva, do Departamento de Bioquímica da UFPE, pela colaboração, incentivo e ajuda.

Ao Prof. Dr. Fábio Marcel do Departamento de Parasitologia da UFPB pelo acolhimento, disponibilidade e ajuda.

Ao Dr. Alexandre Gomes da Silva (*in memoriam*), do Instituto Nacional do Semiárido, pelos ensinamentos, conselhos e coletas realizadas.

Aos Professores da Pós-Graduação do Departamento de Bioquímica da UFPE.

Aos funcionários do Departamento de Bioquímica, em especial a João Antônio Virgínio, pela disposição em nos ajudar, ensinar e resolver alguns problemas.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa.

À Universidade Federal de Pernambuco, aos Departamentos de Bioquímica, Biofísica e Química fundamental, e ao Programa de Pós-Graduação em Bioquímica e Fisiologia pela oportunidade de estudo.

E enfim, a todos aqueles que de uma forma direta ou indiretamente contribuíram para que esse momento se tornasse uma realidade na minha vida.

“Para realizar grandes conquistas, devemos não apenas agir, mas também sonhar; não apenas planejar, mas também acreditar.”

Anatole France

RESUMO

Doenças tropicais negligenciadas (DTNs) são patologias que ocorrem predominantemente nos países em desenvolvimento. A esquistossomose é uma doença parasitária que no Brasil é causada por *Schistosoma mansoni*, enquanto a dengue é uma flavovirose transmitida, principalmente, por *Aedes aegypti*. Devido ao caráter endêmico dessas doenças, torna-se importante pesquisas por estratégias para combatê-las. O estudo de substâncias de origem vegetal, como os óleos essenciais (OEs), tem sido alvo para combater diferentes patologias. Este trabalho teve como objetivo avaliar o potencial tóxico dos OEs das folhas de *Croton rudolphianus* e *Algrizea macrochlamys* frente *Biomphalaria glabrata*, hospedeiro intermediário da esquistossomose, cercárias de *S. mansoni* e do inseto *A. aegypti*. A composição química dos OEs foi determinada por análise de CG-EM. Os testes de toxicidade sobre *B. glabrata* foram realizados com embriões e adultos. Os ensaios cercaricidas foram realizados com cercárias de *S. mansoni*. Os bioensaios com *A. aegypti* foram realizados usando ovo, larva e pulpa. O ensaio ecotoxicológico foi realizado com *Artemia salina*. O óleo de *C. rudolphianus* mostrou ser tóxico para todas as fases embrionárias de *B. glabrata* testadas (CL_{50} = 126,54, 133,51, 143,53 e 161,95 µg/mL para as fases de blástula, gástrula, trocófora e véliger, respectivamente). Para os caramujos adultos e cercárias tratados com óleo de *C. rudolphianus* a CL_{50} foi de 47,88 e 14,81 µg/mL, respectivamente. Esse óleo também apresentou efeito genotóxico para hemócitos de *B. glabrata*. O óleo de *A. macrochlamys* apresentou ação tóxica para embriões de *B. glabrata* (CL_{50} = 55,70, 56,83, 52,85 e 49,85 µg/mL para as fases de blástula, gástrula, trocófora e véliger, respectivamente). Esse óleo também apresentou ação tóxica para adultos de *B. glabrata* (CL_{50} = 46,15 µg/mL) e cercárias de *S. mansoni* (CL_{50} = 11,36 µg/mL). O (*E*)-cariofileno, composto majoritário de ambos OEs, apresentou ação tóxica para embriões de *B. glabratra* (CL_{50} = 10,08, 10,27, 11,43 e 12,5 µg/mL para as fases de blástula, gástrula, trocófora e véliger, respectivamente) e cercárias de *S. mansoni* (CL_{50} = 3,32 µg/mL e CL_{90} = 5,45 µg/mL). O óleo de *C. rudolphianus* também causou mortalidade em larvas quarto instar de *A.aegypti* (CL_{50} = 21.86 µg/mL) e diminuiu a taxa de eclosão de ovos de *A. aegypti*. Entretanto, este óleo não apresentou efeito tóxico para pupas de *A. aegypti*. O OE de *A. macrochlamys* não apresentou nenhuma toxicidade para larvas e pupas de *A. aegypti*, mas causou uma diminuição na taxa de eclosão dos ovos de *A. aegypti*. O ensaio com organismo não alvo demonstrou que o óleo de *A. macrochlamys* não apresentou toxicidade. Por sua vez, o OE de *C. rudolphianus* foi mais tóxico para adultos de *B. glabrata*, cercárias de *S. mansoni* e larvas de *A. aegypti* do que para organismo não alvo testado (*A. salina*). Os resultados

demonstraram que os óleos de *A. macrochlamys* e *C. rudolphianus* são potencial ferramenta para o controle da esquistossomose e dengue, uma vez que ambos apresentaram efeitos deletérios contra o hospedeiro intermediário da esquistossomose, cercárias de *S. mansoni* e pelo menos uma fase do ciclo de *A. aegypti*.

Palavras-chave: *Croton*. Dengue. Esquistossomose. Inseticida. Metabólitos secundários. Moluscicida.

ABSTRACT

Neglected tropical diseases (NTDs) include a set of diseases that occur predominantly in developing countries. Schistosomiasis is a parasitic disease that in Brazil is caused by *Schistosoma mansoni*, while dengue is a flavovirose transmitted mainly by *Aedes aegypti*. Due to the endemic character of these diseases, the search for strategies to combat them becomes important. The study of substances from plants, such as essential oils (EOs), has been targeted to combat different pathologies. This work aimed to evaluate the toxic potential of EOs from leaves of *Croton rudolphianus* and *Algrizea macrochlamys* against *Biomphalaria glabrata*, intermediate host of schistosomiasis, cercariae of *S. mansoni* and the insect *A. aegypti*. The chemical composition of EOs was performed by GC-MS analysis. The toxicity tests on *B. glabrata* were carried out with embryos and adults. The cercaricidal assays were performed with cercariae of *S. mansoni*. The bioassays with *A. aegypti* were performed using egg, larvae and pulpae. The ecotoxicological test was performed with *Artemia salina*. *C. rudolphianus* oil showed to be toxic for all embryonic phases of *B. glabrata* tested (LC_{50} = 126.54, 133.51, 143.53 and 161.95 µg/mL for blastulae, gastrulae, trochophore and veliger phases, respectively). For adult snails and cercariae treated with *C. rudolphianus* oil the LC_{50} was 47.88 and 14.81 µg/mL, respectively. This oil also had a genotoxic effect on *B. glabrata* hemocytes *A. macrochlamys* oil showed toxic action for embryos of *B. glabrata* (LC_{50} = 55.70, 56.83, 52.85 and 49.85 µg/mL for the blastulae, gastrulae, trochophore and veliger phases, respectively). This oil also showed toxic action for adults of *B. glabrata* (LC_{50} = 46.15 µg/mL) and cercariae of *S. mansoni* (LC_{50} = 11.36 µg/mL). The (*E*)-caryophyllene, major compound of both EOs, showed toxic action to embryos of *B. glabratra* (LC_{50} = 10.08, 10.27, 11.43 and 12.5 µg/mL for blastulae, gastrulae, trochophore and veliger phases, respectively), and *S. mansoni* cercariae (LC_{50} = 3.32 µg/mL). *C. rudolphianus* oil also caused mortality in fourth instar larvae of *A. aegypti* (LC_{50} = 21.86 µg/mL) and decreased the hatching rate of *A. aegypti* eggs. However, this oil did not show toxic effect on pupae of *A. aegypti*. The EO of *A. macrochlamys* did not present any toxicity to fourth instar larvae and pupae of *A. aegypti*, but caused a decrease in the hatching rate of *A. aegypti* eggs. The non-target organism assay showed that *A. macrochlamys* oil was not toxic. In turn, *C. rudolphianus* EO was more toxic to adults of *B. glabrata*, cercariae of *S. mansoni*, and larvae of *A. aegypti* than to the non-target organism tested (*A. salina*).The results showed that the oils of *A. macrochlamys* and *C. rudolphianus* are a potential tool for the control of schistosomiasis and dengue, since both had deleterious effects

against the intermediate host of schistosomiasis, *S. mansoni* cercariae and at least one phase of the life cycle of *A. aegypti*.

Keywords: *Croton.* Dengue. Insecticide. Molluscicide. Secondary metabolites. Schistosomiasis.

LISTA DE FIGURAS

Artigo 1

Artigo 2

Artigo 3

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

ABTS	2,2’-azino-bis-(3-etilbenzotiazolina-6- acido sulfônico)
ANVISA	Agência Nacional de Vigilância Sanitária
CG-EM	Cromatografia gasosa acoplada à espectrometria de massa (do inglês: GC-MS)
CL_{50}	Concentração letal para 50% da população (do inglês: LC_{50})
CL_{90}	Concentração letal para 90% da população (do inglês: LC_{90})
$CL_{99,9}$	Concentração letal para 99,9% da população (do inglês: $LC_{99,9}$)
CL_{100}	Concentração letal para 100% da população (do inglês: LC_{100})
CLAE	Cromatografia líquida de alta eficiência (do inglês: HPLC)
CMI	Concentração mínima inibitória (do inglês: MIC)
DPPH	1,1-difenil-2-picrilhidrazil
DTN	Doença Topical Negligenciada (no plural: DTNs)
IC_{50}	Inibição de crescimento para 50% da população
IDH	Índice de Desenvolvimento Humano
MVA	Mevalonato ou ácido mevalônico (em inglês: mevalonate).
MEP	Metileritritol 4-fosfato (em inglês: methylerythritol 4-phosphate)
OE	Óleo essencial (no plural: OEs)
OMS	Organização Mundial da Saúde (no inglês: WHO)
PCE	Programa de Controle da Esquistossomose
PZQ	Praziquantel
SEVS	Secretaria Executiva de Vigilância em Saúde
SUS	Sistema Único de Saúde

SUMÁRIO

1 INTRODUÇÃO

As doenças tropicais negligenciadas (DTNs) incluem um conjunto de doenças que ocorrem majoritariamente nos países em desenvolvimento. Elas são responsáveis por elevada morbilidade e mortalidade (BRASIL, 2020a). Normalmente, as DNTs estão relacionadas com a falta de saneamento adequado, água potável, habitação adequada, e serviços de saúde. Elas ainda causam grande impacto econômico, social e político, pois afetam negativamente a aprendizagem, a produtividade e a renda da população (ROSÁRIO *et al*., 2017).

A esquistossomose é uma DTN causada por vermes trematóides do gênero *Schistosoma* (WHO, 2018a). Ela é endêmica em 78 países distribuídos entre as Américas, África, Ásia e Europa (WHO, 2018b). No Brasil, a esquistossomose é causada pela espécie *Schistosoma mansoni*, que utiliza caramujos do gênero *Biomphalaria* como hospedeiro intermediário (NEVES, 2016). Devido a sua extensa distribuição geográfica, altos índices de infecção e eficiência na transmissão da esquistossomose a espécie *Biomphalaria glabrata* é considerada o hospedeiro intermediário de *S. mansoni* mais importante nas Américas (BRAGA *et al.*, 2012; BRASIL, 2014).

Uma das principais formas de combate a esta doença ocorre por meio do controle dos caramujos vetores (BRASIL, 2017), atualmente, o único moluscicida recomendado pela Organização Mundial de Saúde é a niclosamida (Bayluscide®, Bayer) (COLLEY *et al*., 2014). Substância sintética com comprovada eficiência contra os moluscos, porém apresenta alto custo em sua aplicação, se decompõe sob luz solar e apresenta elevado nível de toxicidade para organismos não-alvo, como peixes, anfíbios e plantas (FARIA *et al*., 2018)

A dengue, outra DTN, é uma doença viral que causa sérios problemas econômicos e a saúde da população que residem nas regiões tropicais e subtropicais (CHURAKOV *et al*., 2019). Ela é transmitida por mosquitos, do gênero *Aedes*, principalmente da espécie *Aedes aegypti*. Esse mosquito também transmite os vírus da chikungunya, zika e febre amarela (WHO, 2017; WHO, 2019a). A forma mais eficiente de prevenção da dengue é através do controle dos mosquitos (AGUIAR *et al.*, 2016), que atualmente é realizada através da utilização de inseticidas sintéticos (BRASIL, 2020b).

No entanto, o uso de moluscicidas e inseticidas sintéticos trazem várias desvantagens, como eliminação de espécies não alvo, desenvolvimento de populações resistentes, além do alto custo (MARTINS *et al*., 2014; ZARA, 2016). Por esse motivo, é importante desenvolver pesquisas científicas voltadas para a descoberta de novas substâncias com estas ações e que

sejam menos tóxicas ao ambiente. Os compostos derivados do metabolismo secundário dos vegetais, como os óleos essenciais (OEs), seriam uma possível fonte dessas substâncias.

Os OEs são uma mistura complexa de substâncias voláteis e lipofílicas (PAVELA; BENELLI, 2016). Esses óleos possuem diversas funções para plantas, como por exemplo, atrair polinizadores e dispersores de sementes, proteger contra patógenos, além de proteger o aparelho fotossintético contra altas temperaturas (PAVELA; BENELLI, 2016; SHARIFI-RAD *et al*., 2017). Alguns deles possuem diversas atividades biológicas, como inseticida (RIBEIRO *et al*., 2020), antimicrobiana (ARAÚJO *et al*., 2017) e antinociceptiva (XIMENES *et al*., 2013; NOGUEIRA *et al*., 2015).

Esses óleos podem ser encontrados em diversas famílias vegetais, como Anacardiaceae, Annonaceae, Euphorbiaceae, Fabaceae, Lamiaceae e Myrtaceae. Na família Euphorbiaceae, o gênero mais diverso é o *Croton*, que possui cerca de 1300 espécies (BRITO *et al*., 2018). Algumas espécies desse gênero são empregadas na medicina popular para tratar diversas enfermidades (BARRERA *et al*., 2016), como diabetes, constipação intestinal, diarreia e outros problemas digestivos, feridas externas, febre, colesterol alto, hipertensão, inflamação, dor, úlceras, obesidade, dentre outras (ALVES, 2017). *Croton rudolphianus* é uma espécie endêmica do Brasil, e há relatos na literatura que seu OE possui atividade antimicrobiana e inseticida contra *Sitophilus zeamais* (RIBEIRO 2016a; RIBEIRO *et al*., 2020)

Por sua vez, o gênero *Algrizea* pertence à família Myrtaceae e possui duas espécies endêmicas no Brasil distribuídas na Bahia e Pernambuco (FLORA DO BRASIL, 2020a). Há relatos que o OE de *Algrizea minor* possui efeito antioxidante, antinociceptivo e antimicrobiano (VERAS *et al*., 2019). No entanto, não há relatos para atividades biológicas com o OE de *Algrizea macrochlamys*.

Diante do exposto, esse trabalho teve como objetivo avaliar o efeito dos OEs obtidos a partir das folhas de *C. rudolphianus* e *A. macrochlamys* frente ao caramujo hospedeiro intermediário da esquistossomose (*B. glabrata*), cercárias de *S. mansoni*, ovos, larvas e pupas de *A. aegypti*, bem como, testar a ecotoxicidade desses OEs utilizando o organismo não-alvo *A. salina*.

1.1 OBJETIVOS

1.1.1 Objetivo geral

Avaliar o potencial tóxico dos OEs extraídos das folhas de C. *rudolphianus* e *A. macrochlamys* sobre o hospedeiro intermediário da esquistossomose (*B. glabrata*), cercárias de *S. mansoni* e *A. aegypti* (ovos, larvas e pulpas), bem como, testar a ecotoxicidade dos OEs utilizando a *A. salina* (organismo não-alvo).

1.1.2 Objetivos específicos

- Obter e caracterizar quimicamente por meio da cromatografia gasosa acoplada a espectrometria de massas (CG-EM) os OEs das folhas de *C. rudolphianus* e *A. macrochlamys*;

- Avaliar a toxicidade do OE de *C. rudolphianus* contra o molusco *B. glabrata* em diferentes estágios embrionários (blástula, gástrula, trocófora e veliger) e fase adulta, cercárias de *S. mansoni* e *A. salina* (organismo não-alvo);

- Investigar o efeito citotóxico do OE de *C. rudolphianus* nos hemócitos de *B. glabrata*;

- Avaliar a toxicidade do OE de *A. macrochlamys* e do seu composto majoritario ((E)-cariofileno), também contra embriões e adultos de *B. glabrata*, cercárias de *S. mansoni* e *A. salina* (organismo não-alvo);

- Analisar o efeito dos OEs de *C. rudolphianus* e *A. macrochlamys* sobre pupas e larvas no quarto instar do principal vetor da dengue, bem como, a interferência destes óleos na eclosão de ovos de *A. aegypti*.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 DOENÇAS TROPICAIS NEGLIGENCIADAS

As doenças tropicais negligenciadas (DTNs), causadas por agentes infecciosos ou parasitas, incluem um conjunto de doenças que ocorrem predominantemente nos países em desenvolvimento e são responsáveis por elevada morbidade e mortalidade. Estas enfermidades incapacitam ou matam milhões de pessoas e representam uma necessidade médica importante até os dias atuais (BRASIL, 2020a).

As áreas onde se concentram as DTNs têm como principal característica a marca da pobreza e do subdesenvolvimento, e compreendem principalmente as regiões da África Subsaariana, Ásia e América Latina. A maioria dessas doenças é determinada pelo acesso insuficiente à água potável, ao saneamento, à habitação adequada, à educação e aos serviços de saúde. Além disso, elas podem causar complicações crônicas que afetam negativamente a aprendizagem, a produtividade e a renda (ROSÁRIO *et al*., 2017).

As DTNs causam grande impacto econômico, social e político, e podem ser classificadas em três categorias baseado na emergência, controle e disponibilidade de medicamentos (Tabela 1) (LINDOSO; LINDOSO, 2009).

Tabela 1 - Distribuição das DTNs de acordo com categorias baseadas na Organização Mundial de Saúde (OMS).

Categoria 1	Categoria 2	Categoria 3
Doença descontrolada e emergindo (ex.: dengue, tripanossomíase humana africana e leishmaniose).	Doença possui estratégia de controle disponível, porém a mesma ainda persiste (ex.: malária e esquistossomose).	Doença com estratégia de controle eficaz, na qual a carga de doença está caindo e há um plano para sua eliminação (ex.: lepra, doença de Chagas, filariose linfática e oncocercose).

Fonte: Lindoso e Lindoso (2009).

Várias DTNs ocorrem no Brasil, principalmente nas regiões Norte e Nordeste, onde há áreas com menor Índice de Desenvolvimento Humano (IDH). A malária, esquistossomose, dengue, doença de Chagas, leishmaniose, hanseníase, oncocercose e filariose linfática são as DTNs com maior prevalência no Brasil (ROSÁRIO *et al*., 2017).

2.2 ESQUISTOSSOMOSE

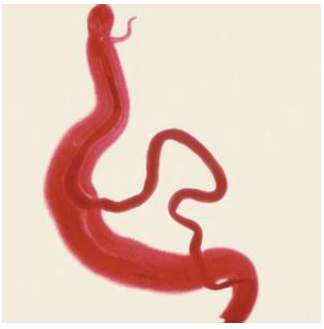
2.2.1 Aspectos gerais da esquistossomose

A esquistossome é uma doença parasitária de veiculação hídrica, de caráter agudo ou crônico, causada por vermes trematódeos do gênero *Schistosoma* (WHO, 2018a). Ela encontra-se distribuída entre a Ásia, África, América e Oriente Médio (MARTINS-MELO *et al*., 2014; SCHOLTE *et al*., 2014, WHO, 2018a). Além disso, é considerada um importante problema de saúde pública, sendo prevalente em comunidades pobres sem acesso à água potável e saneamento adequado (SCHOLTE *et al*., 2014, WHO, 2018a).

Estima-se que mais de 240 milhões de pessoas no mundo estão infectadas por esses trematódeos e cerca 700 milhões vivem em áreas consideradas de risco (WHO, 2018a). Adicionalmente, de acordo com a Organização Mundial da Saúde (WHO, 2018a), pelo menos 66,5 milhões de pessoas foram infectadas e 218 milhões necessitaram de tratamento preventivo para esquistossomose em 2015.

Em relação aos parasitas que causam essa doença, eles pertencem ao Filo Platelminto, Classe Trematodea e Família Schistosomatidae. São representados por vermes que parasitam mamíferos, crocodilianos e aves, tendo como habitat o sistema venoso desses animais (NEVES, 2016). Algumas características diferenciam o gênero *Schistosoma* dos demais trematódeos, são digenéticos; apresentam uma fase de desenvolvimento infectante para o hospedeiro definitivo (cercárias); e na fase adulta apresentam sexos distintos (dióicos) e dimorfismo sexual (SILVA *et al.*, 2008). As fêmeas são mais longas (7-17 mm de comprimento), finas e possuem tegumento liso. Por sua vez, os machos são robustos, medem 6-12 mm de comprimento, possuem tegumento coberto por tubérculos e espinhas, e um canal ginecóforo para abrigar à fêmea e fecundá-la (CDC, 2017), como mostra a Figura 1.

Figura 1 - Adultos de *S. mansoni*.

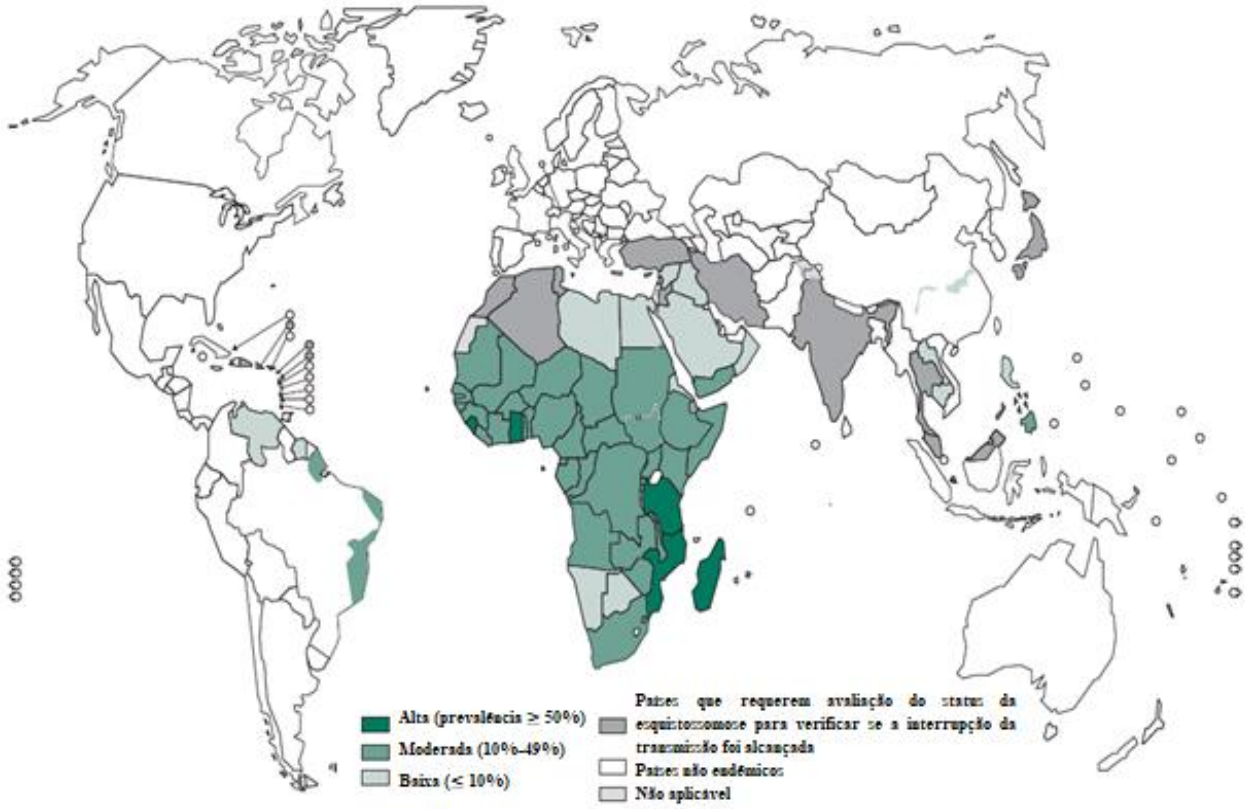


Nota: A fêmea é a mais fina e reside no canal ginecóforo do macho, que é o mais espesso.
Fonte: CDC (2017).

2.2.2 Aspectos epidemiológicos da esquistossomose

A esquistossomose é classifica como DTN e é endemica em áreas tropicais e subtropicais (Figura 2). Sua distribuição é focal, uma vez que, a transmissão depende de hospedeiros intermediários específicos (caramujos do gênero *Biomphalaria, Bulinus e Oncomelania*e) e atividades humanas infecciosas (WHO, 2018a).

Figura 2 - Distribuição da esquistossomose no mundo em 2012.



Fonte: WHO (2015).

Conforme a Organização Mundial de Saúde (WHO, 2018a), há duas principais formas de esquistossomose, intestinal e urogenital, causada por seis espécies de *Schistosoma*: *S. mansoni*, *S. japonicum*, *S. haematobium*, *S. mekongi*, *S. guineenses* e *S. intercalatum*, sendo as três primeiras as mais relevantes. Além disso, as espécies desse gênero utilizam caramujos gastrópodes aquáticos como hospedeiros intermediários do seu ciclo de vida (WHO, 2018a). A Tabela 2 mostra à distribuição geográfica das espécies a cima, e seus respectivos hospedeiros intermediários.

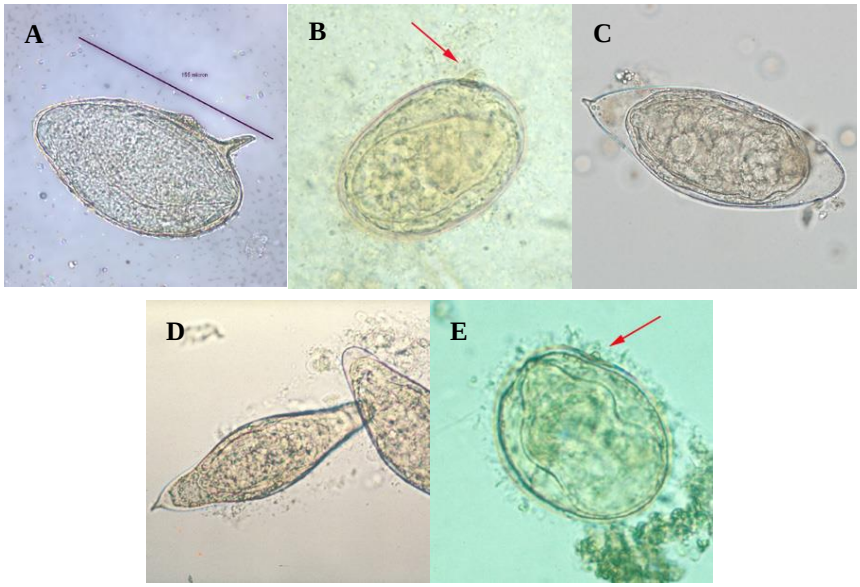
Tabela 2 - Distribuição geográfica e hospedeiros intermediários das espécies parasitas do gênero *Schistosoma*.

Espécie	Distribuição geográfica	Hospedeiro intermediário
S. mansoni	África, Oriente Médio, Caribe, Brasil, Venezuela e Suriname	*Biomphalaria* spp.
S. japonicum	China, Indonésia e Filipinas	*Oncomelania* spp.
S. mekongi	Vários distritos do Camboja e da República Democrática Popular do Lau	*Neotricula aperta*
S. guineenses e *S. intercalatum*	Áreas da floresta tropical da África Central	*Bulinus* spp.
S. haematobium	África, Oriente Médio e Córsega (França)	*Bulinus* spp.

Fonte: WHO (2018a).

Geralmente, as espécies que infectam o homem são facilmente identificadas através do tamanho e morfologia do ovo (Figura 3), origem geográfica do isolado e a especificidade pelo hospedeiro intermediário (CDC, 2017). Dessas espécies de *Schistosoma* citadas acima, a *S. mansoni* possui maior distribuição global e a única espécie causadora da esquistossomose no Brasil (BERGQUIST, 2002).

Figura 3 - Ovos das diferentes espécies de *Schistosoma.*



Nota: O tamanho e a morfologia do ovo variam de acordo com cada espécie. **A.** *S. mansoni* (espinho lateral proeminente perto da extremidade posterior). **B.** *S. japonicum* (ovos maiores e mais redondos que as outras especies, e espinho menor). **C.** *S. haematobium* (são eliminados na urina e possuem espinho terminal conspícuo). **D.** *S. intercalatum* (semelhante aos ovos de *S. haematobium*, porém mais longos e eliminados nas fezes). **E.** *S. mekongi* (ovos semelhante aos de *S. japonicum*, porém menores).
Fonte: CDC (2017).

A esquistossomose é endêmica em 78 países distribuídos entre as Américas, África, Ásia e Europa (WHO, 2018a; WHO, 2018b). Além disso, 92% das pessoas que necessitam de

terapia preventiva contra esquistossomose vivem no continente Africano (WHO, 2018b). Os principais países da África afetados por esta doença, são: Nigéria, Etiópia, República Democrática do Congo, Moçambique, Quênia, República Unida da Tanzânia, Camarões, Uganda, Malawi e Gana (WHO, 2015).

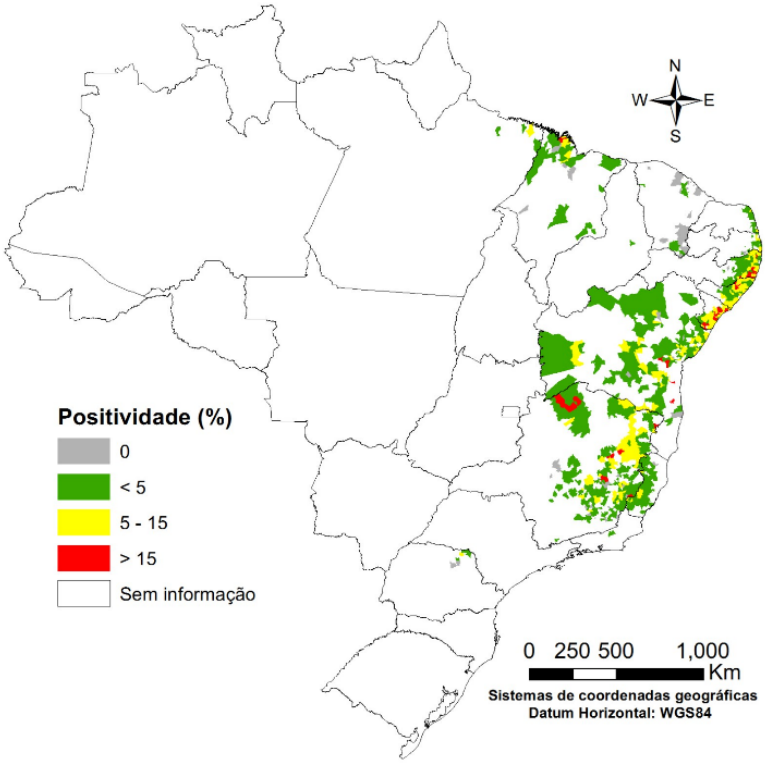
A esquistossomose foi erradicada com sucesso na Tunísia e Japão, e controlada na China. No entanto, continua predominante na Indonésia e Filipinas. A morbidade associada a *S. mekongi* foi controlada em Camboja, mas focos endêmicos persistem na República Democrática Popular do Lau (WHO, 2015).

Em 2014, foi relatado a presença de focos de transmissão de esquistossomose urogenital na Europa. A doença foi descrita, atingindo a França, Alemanha e Itália, acreditando-se que a infecção tenha se iniciado em Córsega (França), em um rio ao norte de Porto-Vecchio, um destino turístico popular. Possivelmente, a chegada da esquistossomose nesses países está relacionada com migração de pessoas infectadas de regiões endêmicas da África, para a Europa, uma vez que o parasita (*S. haematobium*) tem distribuição exclusiva em países africanos (BOISSIER *et al*., 2015).

O Brasil responde por aproximadamente 96% dos casos de esquistossomose de toda América Latina e Caribe (MARTINS-MELO *et al*., 2015). Outros focos de transmissão no continente americano são: Colômbia, Venezuela, Porto Rico, República Dominicana, Santa Lúcia, Guadalupe, Martinica, São Cristóvão e Nevis, Monserrate, Haiti, San Martin e Suriname. Muitos pesquisadores acreditam que a esquistossomose chegou ao Brasil no período colônial com a vinda de escravos africanos infectados pelo *S. mansoni*, e se expandiu devido à migração de pessoas infectadas para áreas com novas atividades econômicas e produção industrial (MORAES *et al*., 2014). No Brasil, estima-se que aproximadamente 1,5 milhões de pessoas vivem em áreas sob risco de contrair essa doença, onde, a maioria delas estão localizadas nos estados do Nordeste (BRASIL, 2018a).

A esquistossomose tem uma área de transmissão extensa no Brasil, com áreas de endemias e focais abrangendo 19 dos 27 estados, concentrando-se nas regiões Nordeste e Sudeste (Figura 4) (MARTINS-MELO *et al.*, 2014). Os principais estados atingidos são: Alagoas, Sergipe, Bahia, Minas Gerais, Espírito Santo, Paraíba, Rio Grande do Norte e Pernambuco. Os estados do Pará, Maranhão, Piauí, Ceará, Rio de Janeiro, São Paulo, Santa Catarina, Paraná, Rio Grande de Sul são considerados áreas focais com menor número de prevalência da doença (BRASIL, 2018a; VITORINO *et al*., 2012).

Figura 4 - Distribuição da esquistossome no Brasil entre 2010 a 2015.



Fonte: Brasil (2018b).

O estado de Pernambuco ocupa o terceiro lugar em prevalência da doença na Região Nordeste, dos 186 municípios do Estado, 102 são endêmicos para esquistossomose. Na região endêmica da zona da mata ocorre em 46 municípios. Além disso, a expansão da esquistossomose para o litoral do Estado vem sendo registrada desde 1992, com a detecção de casos agudos da doença em indivíduos de classe média/alta e focos do hospedeiro intermediário da esquistossomose, caramujos do gênero *Biomphalaria* (BARBOSA *et al*., 2014; SILVA; DOMINGUES, 2011).

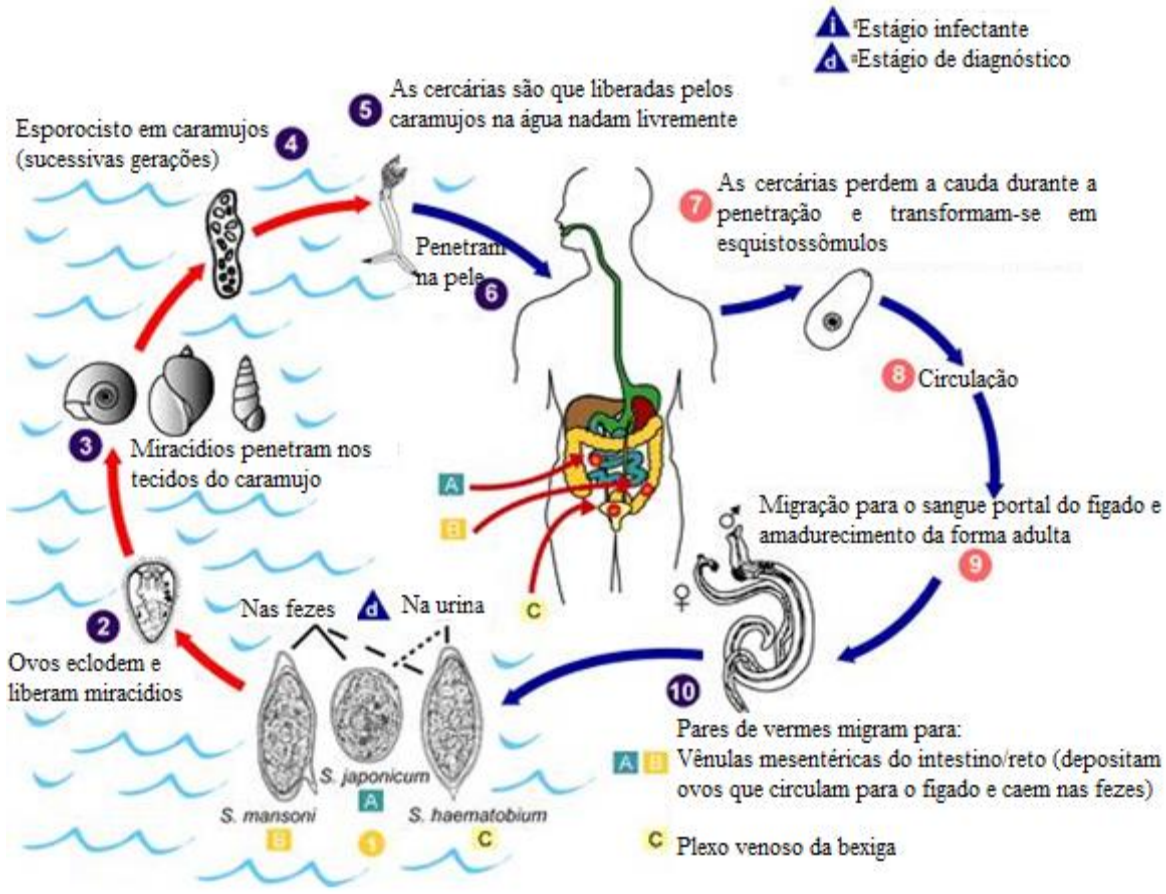
2.2.3 Ciclo biológico de *S. mansoni*

S. mansoni apresenta um complexo ciclo biológico (Figura 5), demonstrando uma excelente capacidade adaptativa entre o parasito, seus hospedeiros intermediário e definitivo, e meio ambiente (NEVES, 2016). O seu ciclo de vida é composto por duas fases: uma fase assexuada e outra sexuada (BARSOUM *et al*., 2013).

A fase assexuada do ciclo de vida de *S. mansoni* inicia-se quando as fezes de indivíduos contaminados, contendo ovos do parasito, entram em contato com a água doce. Os ovos (Figura 3), em contato com a água e estimulado por alguns fatores (temperaturas mais altas, luz intensa

e oxigenação da água), eclodem, liberando miracídios (Figura 6A), que são a forma infectante dos caramujos (hospedeiros intermediários). A capacidade do miracídio em penetrar o caramujo se limita a 8 horas após a sua eclosão e é notavelmente influenciada pela temperatura. Nesse período, a ação em conjunto dos intensos movimentos miracidianos e enzimas proteolíticas liberadas por sua glândula de penetração, permitem ao miracídio ultrapassar os tecidos do caramujo do gênero *Biomphalaria*. Em seguida, após 48 horas da infecção do hospedeiro intermediário, o parasita sofre replicações transformando-se em esporocisto, que dará origem a forma infectante de vertebrados, as cercárias. Essa fase do ciclo de vida no caramujo requer de 4 a 6 semanas antes que as cercárias sejam liberadas. Além disso, um único miracídio gera em torno de 100 a 300 mil cercárias (NEVES, 2016; BARSOUM *et al*., 2013).

Figura 5 - Ciclo de vida de *S. mansoni*.

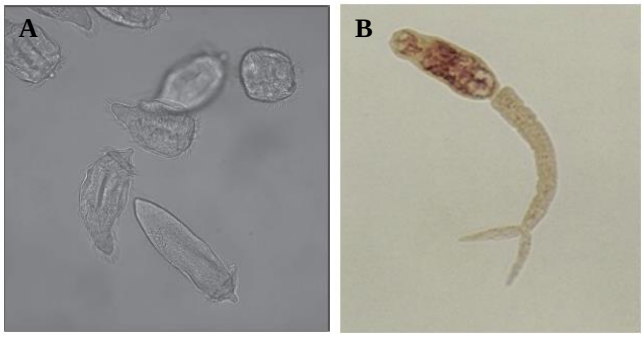


Nota: O ciclo biológico de *S. mansoni* apresenta uma alternância de gerações entre o hospedeiro intermediário, moluscos do gênero *Biomphalaria* spp., e os hospedeiros definitivos vertebrados, dentre eles o homem.
Fonte: CDC (2017).

As cercárias (Figura 6B) liberadas na água nadam a procura do seu hospedeiro definitivo permanecendo ativas por até 48 horas em condições favoráveis, no entanto, o período de maior

infectividade ocorre nas primeiras 8 horas após a liberação delas no meio aquático. As cercárias se fixam na pele ou mucosas do hospedeiro, preferencialmente entre os folículos pilosos, com o auxílio de suas duas ventosas e de uma substância mucoprotéica secretada por suas glândulas acetabulares. Ao atingir os capilares subcutâneos, o parasito perde a cauda bífida, se transformando em esquistossômulo, esse processo dura de 5 a 15 minutos (NEVES, 2016).

Figura 6 - Miracídio e cercária de *S. mansoni*. **A.** Miracídio. **B.** Cercária.



Fonte: Brasil (2014).

Os esquistossômulos, por sua vez, migram para os pulmões cerca de 7 dias após a penetração e posteriormente, para o sistema intra-hepático, podendo usar duas vias para realizar essa migração. (i) via sanguínea, tradicionalmente aceita, os esquistossômulos saem das arteríolas pulmonares e dos capilares alveolares, ganhando as veias pulmonares (pequena circulação) chegando ao lado esquerdo do coração, acompanhando o fluxo sanguíneo, disseminados pela aorta (grande circulação) até o sistema intra-hepático onde se fixam. Pela (ii) via transtissular, os esquistossômulos saem dos alvéolos pulmonares através da penetração do parênquima pulmonar, pleura e diafragma, alcançando a cavidade peritoneal e perfurando a cápsula e parênquima hepático, chegando ao sistema porta intra-hepático (NEVES, 2016).

Uma vez no sistema porta intra-hepático, os esquistossômulos se alimentam e se desenvolvem transformando-se em machos e fêmeas 25 a 28 dias após a penetração. Posteriormente, migram, acasalados (fase sexuada), para a veia mesentérica inferior, onde farão oviposição. As fêmeas realizam a postura dos ovos na veia mesentérica inferior e, aproximadamente, 400 ovos são postos por dia, demorando cerca de 8 dias para se tornarem maduros (formação completa do miracídio). Cerca de metade dos ovos vão para o exterior junto com o bolo fecal, a outra metade fica no intestino ou fígado. Os ovos podem ser observados 42 dias após a infecção, nas fezes do hospedeiro (BARBOZA *et al*., 2012; BARSOUM *et al*., 2013; COLLEY *et al.*, 2014; NEVES, 2016; VITORINO *et al*., 2012).

A morbidade causada pela esquistossomose é induzida não pela presença do próprio verme no organismo, mas pelos ovos postos por ele. Já que, muitos deles não são eliminados nas fezes, permanecendo por um longo tempo no intestino e fígado, causando uma resposta imunológica no hospedeiro (BURKE *et al*., 2009; COLLEY *et al*., 2014).

2.2.4 Aspectos clínicos da esquistossomose

A infecção por *S. mansoni* pode ocasionar manifestações clinicas distintas, dependente da idade do paciente, estado nutricional, localização do parasito, intensidade do parasitismo, resposta imune à infecção e reação do organismo do paciente a presença do ovo de *Schistosoma* (CASTRO, 2009; CARVALHO *et al*., 2008).

A esquistossomose pode ser classificada em duas fases: aguda e crônica. A fase aguda se divide em duas fases distintas: fase pré-postural e a fase pós-postural. A fase pré-postural possui sintomatologia variada e ocorre cerca de 10 a 35 dias após a infecção. Nesse período alguns pacientes são assintomáticos, e outros apresentam sintomas, como: dermatite cercariana, mal-estar, com ou sem febre, tosse, dores musculares, desconforto abdominal e hepatite aguda (BRASIL, 2014; NEVES, 2016).

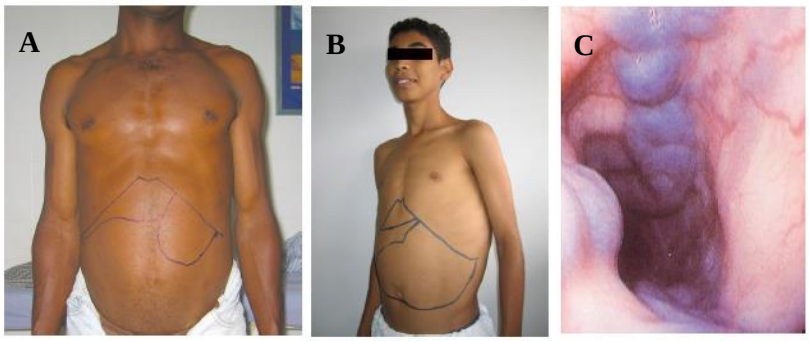
A fase pós-postural aparece em torno de 50 a 120 dias após a infecção, nessa fase começa a postura de ovos, principalmente, no intestino e fígado, e até mesmo no pulmão. Esses ovos provocam uma resposta imunológica no hospedeiro, resultando na formação de granulomas (COLLEY *et al.*, 2014). Os sintomas dessa fase incluem febre alta, sudorese, calafrios, emagrecimento, diarréia, cólicas, tosse, hepatoesplenomegalia discreta, linfademia, leucocitose com eosinofilia. A maioria dos casos evolui para esquistossomose crônica (BRASIL, 2014; CASTRO, 2009; NEVES, 2016).

O início da fase crônica dá-se por volta do sexto mês após infecção. Podem surgir sinais de progressão da doença em diversos órgãos, chegando a causar hipertensão pulmonar e portal, ascite, hepatoesplenomegalia, ruptura de varizes do esôfago (Figura 7). Essa fase apresenta-se nas seguintes formas: hepatointestinal, hepática e hepatoesplênica (BRASIL, 2017).

A forma hepatointestinal é caracterizada pela presença de surtos diarréicos, intercalados com constipação intestinal crônica. Na forma hepática há fibrose no fígado sem esplenomegalia, e pode ser assintomática ou sintomática, exibindo os mesmos sintomas da forma hepointestinal (BRASIL, 2014; BRASIL, 2017; NEVES, 2016). Por sua vez, na esquistossomose hepatoesplênica os sintomas característicos são: hipertensão portal, levando à hepatoesplenomegalia e ao aparecimento de varizes no esôfago (Figura 7); dores abdominais;

alterações das funções intestinais; diminuição acentuada da função do fígado e ascite; encefalopatia hepática, que pode evoluir para o coma hepático e a morte (BRASIL, 2014; BRASIL, 2017).

Figura 7 - Pacientes com esquistossomose hepatoesplênica.



Nota: **A.** Adulto com aumento do fígado e baço. **B**. Adolescente com aumento do fígado e baço. **C.** Varizes no esôfago causada pela hipertensão portal.
Fonte: Brasil (2014).

2.2.5 Diagnóstico e tratamento da esquistossomose

O diagnóstico da esquistossome é realizado através de exames laboratoriais, além disso, o histórico do paciente e o fato de ser originário ou ter morado em áreas endêmicas orientam o médico no diagnóstico desta doença. Os métodos de diagnóstico podem ser categorizados em diretos e indiretos (BRASIL, 2017).

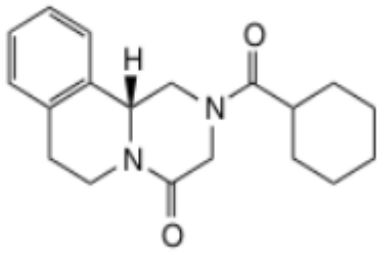
Os diretos consistem na visualização ou demonstração da presença de ovos de *S. mansoni* nas fezes ou tecidos. Os indiretos são baseados em mecanismos imunológicos envolvendo reações de antígeno-anticorpo. Adicionalmente, o diagnóstico também pode ser realizado por exames de imagem, como: ultrassonagrafia de abdômen, ressonância magnética, radiografia do tórax, endoscopia digestiva alta, entre outros (BRASIL, 2017).

O tratamento dessa enfermidade tem como finalidade sua cura, redução da carga parasitária do hospedeiro, e minimização da produção e eliminação dos ovos de *Schistosoma*. Visando impedir a evolução para as formas crônicas da doença, além de prevenir a transmissão da esquistossomose (GOMES *et al.*, 2016a; VITORINO *et al.*, 2012). O tratamento pode ser direcionado individualmente ou em nível populacional (tratamento coletivo) (GOMES *et al.*, 2016a).

Atualmente, o único medicamento utilizado no tratamento da esquistossomose é o praziquantel (PZQ) (Figura 8), um derivado da isoquinolina-pirazina. Ele atua em vermes

adultos, no entanto, possui pouca eficácia contra os outros estágios de *Schistosoma* (COLLEY *et al.*, 2014). Sua ação esquistossomicida ocorre dentro de 15 minutos após sua administração (VITORINO *et al.*, 2012). A dose padrão utilizada no tratamento de *S mansoni* é 50 mg/kg para adultos e de 60 mg/kg para crianças (BRASIL, 2017). Os efeitos colaterais mais comuns do PZQ são: dor abdominal, cefaleia, tonturas, sonolência (COLLEY *et al.*, 2014; NEVES, 2016), palpitação, vômito, prurido, distúrbios visuais e tremores. Os índices de cura da esquistossomose mansônica com uso do praziquantel variam de 60% a 90%, associada à substancial redução da carga parasitária e de produção de ovos pelo *S. mansoni* (VITORINO *et al.*, 2012).

Figura 8 - Estrutura química do Praziquantel (PZQ).



Praziquantel

Fonte: Rios (2015).

Diante do exposto, é necessário implementar estratégias para controlar a disseminação dessa doença que atinge milhões de pessoas por ano.

2.2.6 Estratégias de controle e prevenção da esquistossomose

Os principais métodos de controle da esquistossomose envolvem o tratamento de pessoas infectadas pelo uso de quimioterapia por meio do PZQ; interrupção do ciclo de vida do hospedeiro intermediário através do controle químico; e medidas complementares como: educação em saúde, saneamento básico e higiene (BRASIL, 2017; TLAMÇANI; ER- RAMI, 2014).

O único agente moluscicida utilizado em campanhas de controle dessa doença é a niclosamida (COLLEY *et al*., 2014). No entanto, há várias controvérsias sobre o seu uso. Esse assunto será abordado de forma mais ampla no tópico 2.3.3.

Alguns países, como o Brasil e China, criaram programas específicos para o controle da esquistossome. Esses programas, geralmente, dão mais atenção ao uso de quimioterapia em massa, utilizando o PZQ para reduzir o número de pessoas infectadas nas regiões endêmicas (GURARIE *et al*., 2015).

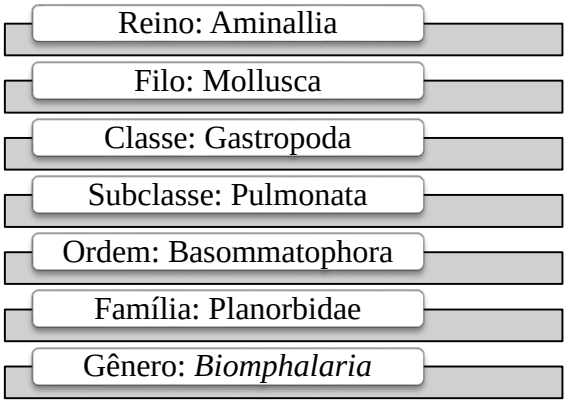
No Brasil, há o "Programa de Controle da Esquistossomose" (PCE), que atualmente é responsável por capacitar e dar suporte aos municípios nas ações que envolvem o diagnóstico e tratamento da infecção. Adicionalmente, ele identifica os focos de moluscos vetores (caramujos do gênero *Biomphalaria*), incentiva as ações de controle, a mobilização social, a educação em saúde e a inserção dos dados gerados no sistema de informação correspondente (QUITES *et al*., 2016)

Em Pernambuco, a Secretaria Executiva de Vigilância em Saúde (SEVS) vem desenvolvendo desde 2011 o Programa Sanar. Essse programa tem como objetivo reduzir ou eliminar as DNTs, como a esquistossomose (GOVERNO DO ESTADO DE PERNAMBUCO, 2018).

2.3 GÊNERO *Biomphalaria*

No Brasil, a esquistossomose é causada pela espécie *S. mansoni*, que usa caramujos do gênero *Biomphalaria* como hospedeiro intermediário (NEVES, 2016). A classificação taxonômica desses caramujos pode ser observada na Figura 9.

Figura 9 - Classificação taxonômica dos caramujos hospedeiros intermediários de *S. mansoni.*



Fonte: Neves (2016).

Mesmo sendo hermafroditas, ou seja, podem se autofecundar, eles têm preferência pela reprodução cruzada. A maturidade sexual dos caramujos é atingida a partir de 30 dias de idade.

As posturas de ovos são realizadas quase que diariamente, geralmente a noite, e as desovas são depositadas em qualquer estrutura sólida submersa. Os ovos são contidos em massas gelatinosas, que podem conter até mais de 100 ovos. Os embriões contidos nos ovos passam por alguns estágios de desenvolvimento, o quais estão discutidos no próximo tópico, até seu completo desenvolvimento e eclosão. Esse processo dura de 7 a 9 dias após a postura dos ovos (NEVES, 2016).

Foram identificadas onze espécies pertencentes a esse gênero no Brasil. No entanto, apenas três delas foram encontradas eliminando cercárias na natureza sendo, portanto, hospedeiras de *S. mansoni* nas Americas, são elas: *Biomphalaria glabrata* (Say, 1818), *Biomphalaria straminea* (Dunker, 1848) e *Biomphalaria tenagophila* (D'orbigny, 1835) (NEVES, 2016). Alem disso, infecções experimentais foram documentadas nas espécies *Biomphalaria peregrina* (D'orbigny, 1835), *Biomphalaria amazonica* (Paraense, 1966) e *Biomphalaria cousini* (Paraense, 1966), mas nunca foram encontradas com infecção natural (BRASIL, 2014; NEVES, 2016). *B. glabrata* é uma espécie que merece destaque, pois é um dos hospedeiros intermediários de *S. mansoni* mais importante nas Américas (NEVES, 2016), por isso essa espécie será abordada no tópico 2.3.2.

2.3.1 Características embrionárias dos moluscos do gênero *Biomphalaria*

O estudo da embriologia de caramujos do gênero *Biomphalaria* é importante tanto para conhecer as etapas de desenvolvimento das espécies desse gênero quanto para controle da esquistossomose, uma vez que, os embriões que não forem erradicados com uso de substâncias moluscicidas logo que eclodirem poderão habitar novamente as áreas tratadas e continuar o ciclo da esquistossomose.

Normalmente, os caramujos do gênero *Biomphalaria* fazem a oviposição próximo a superfície da água, e seus embriões passam por sete estágios de desenvolvimento até a eclosão, são eles: blástula, gástrula, trocófora jovem, trocófora, véliger jovem, véliger e hippo stage (KAWANO *et al*., 1992; KAWANO *et al*., 2008).

O ovo inicialmente passa por diversas clivagens mitóticas até atingir o primeiro estágio de desenvolvimento embrionário, blástula, cerca de 15 horas após a primeira clivagem (Figura 10A). O estádio seguinte de gástrula tem início após 24 horas da primeira clivagem (Figura 10B). Na gastrulação o embrião contém três camadas celulares: a ectoderme, mesoderme e endoderme (KAWANO, 1995). Ao final da gastrulação, cerca de 39 horas após a primeira clivagem, há o aparecimento da boca. O terceiro estágio, trocófora (Figura 10C), ocorre entre

48 a 87 horas da primeira clivagem e é caracterizado pela formação do prototroco que separa o corpo em duas partes (região pré-trocal e pós-trocal). A região pré-trocal dará origem a região cefálica, placa apical, futura região dos olhos e tentáculos e da vesícula cerebral. Enquanto na região pós-trocal encontra-se a boca, situada abaixo da placa apical e na região oposta encontra-se a glândula da concha. O estágio de véliger (Figura 10D), 96 a 120 horas após a primeira clivagem, é caracterizado pela formação da concha e do velum, este originado a partir do prototroco e responsável pela intensa movimentação do embrião. No hippostage, 144 horas após a primeira ciclagem, há o aparecimento de tentáculos bem desenvolvidos e dos olhos, que, a princípio, se evidenciam como um halo pigmentado na região das placas cefálicas. Neste estádio há a formação quase completa de um caramujo jovem. A partir do sétimo dia após a primeira clivagem, a temperatura de 25 °C os caramujos podem eclodir das desovas (KAWANO *et al*., 1992; KAWANO *et al*., 2008).

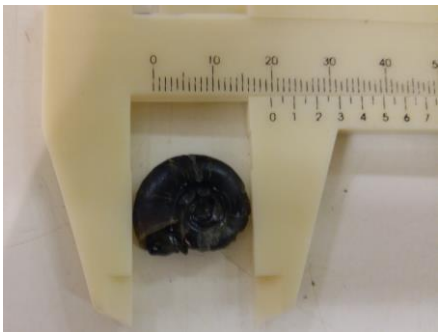
Figura 10 - Embriões da *B. glabrata* em diferentes estádios de desenvolvimento embrionário.

Nota: **A.** blástula, **B.** gástrula, **C**. trocófora e **D.** véliger.
Fonte: Araújo (2016).

2.3.2 A espécie *B. glabrata*

B. glabrata (Figura 11), maior molusco da família Planorbidae, é um dos hospedeiros intermediários de *S. mansoni* mais importante nas Américas, em decorrência de sua extensa distribuição geográfica, altos índices de infecção e eficiência na transmissão da esquistossomose – altamente suceptível ao trematódeo (BRAGA *et al.* 2012; BRASIL, 2014; NEVES, 2016). Adicionalmente, de acordo com Neves (2016) pesquisas relatam que mais de 80% das infecções por *S. mansoni* ocorrem devido à transmissão por *B. glabrata*, além disso, há relatos que um único caramujo dessa espécie possa eliminar até 18 mil cercarias por dia.

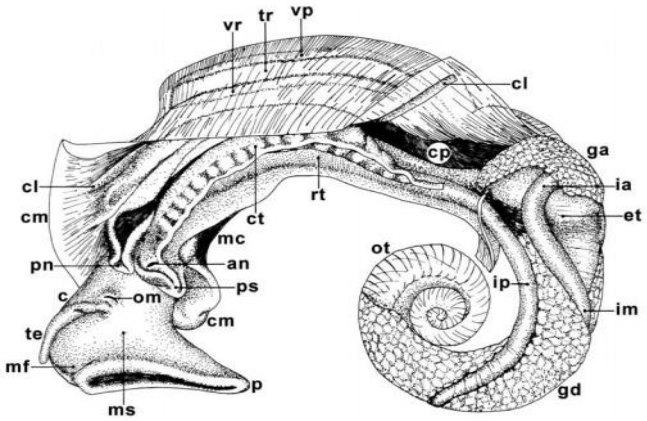
Figura 11 - *B. glabrata* (Say, 1818).



Fonte: A autora (2020).

Essa espécie apresenta como característica concha lisa de coloração escura que possui a função de proteção e abrigo do corpo do molusco, envolvendo as vísceras (Figura 12), com seis a sete giros arredondados, aumentando gradativamente, podendo atingir até 40 mm de diâmetro e 11 mm de largura (BRASIL, 2014; NEVES, 2016). A principal característica da sua anatomia interna é a presença de uma crista renal pigmentada, que confere característica exclusiva dessa espécie (NEVES, 2016; REY, 2001).

Figura 12 - Esquema de um animal do gênero *Biomphalaria* retirado da concha, com a indicação de seus órgãos internos.



Nota: Massa cefalopodal (ms), cavidade pulmonar (cp), mufla (mf), tentáculo (te), colo (c), abertura genital masculina (om), colar ou borda do manto (cm), pseudobrânquia (ps), pneumóstoma (pn), abertura anal (an), músculo columelar (mc), crista lateral (cl), crista retal (ct), veia renal (vr), veia pulmonar (vp), tubo renal (tr), reto (rt), glândula do albúmen (ga), intestino anterior (ia), intestino médio (im), intestino posterior (ip), estômago (et), glândula digestiva (gd), pé (p), ovoteste (ot).
Fonte: Paraense (1975).

B. glabrata possui uma ampla distribuição geográfica, sendo sua presença notificada em 16 estados brasileiros (Alagoas, Bahia, Espírito Santo, Goiás, Maranhão, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Piauí, Rio Grande do Norte, Rio Grande do Sul, Rio de Janeiro,

São Paulo, Sergipe), além do Distrito Federal, abrangendo cerca de 806 municípios (Figura 13) (CARVALHO *et al.*, 2008; BRASIL, 2014; NEVES, 2016).

Figura 13 - Distribuição espacial da espécie *B. glabrata* no Brasil.

Fonte: Carvalho *et al*. (2008).

De acordo com Carvalho *et al*. (2008), a área central de sua distribuição corresponde, principalmente, aos estados da Bahia, Minas Gerais e Espírito Santo. Adicionalmente, *B. glabrata* ocorre de forma quase contínua, para o norte, numa faixa costeira que compreende os estados de Sergipe, Alagoas, Pernambuco, Paraíba e Rio Grande do Norte. Está ausente no estado do Ceará e apenas uma população é reportada no estado do Piauí. No estado do Maranhão são encontradas várias populações distribuídas pelo interior e litoral (CARVALHO *et al.*, 2008).

Na região Sul, *B. glabrata* ocorre no estado do Paraná, com um aglomerado na divisa com o estado de São Paulo. Ocorre em uma única população no estado do Rio Grande do Sul. Além do mais, a espécie foi encontrada na região metropolitana de Porto Alegre, que se localiza cerca de 500 km do município mais ao sul do Brasil, onde era conhecida a presença de *B. glabrata*. Essas observações são relevantes, uma vez que, *B. glabrata* pode alcançar outras regiões do sul do Brasil ou mesmo países vizinhos como Argentina, Paraguai e Uruguai, até o momento livres da doença (CARVALHO *et al.*, 2008).

Uma das estratégias de controle e prevenção da esquistossomose seria a interrupção do ciclo de vida dos moluscos hospedeiros intermediários dessa doença, através do uso de substâncias chamadas moluscicidas.

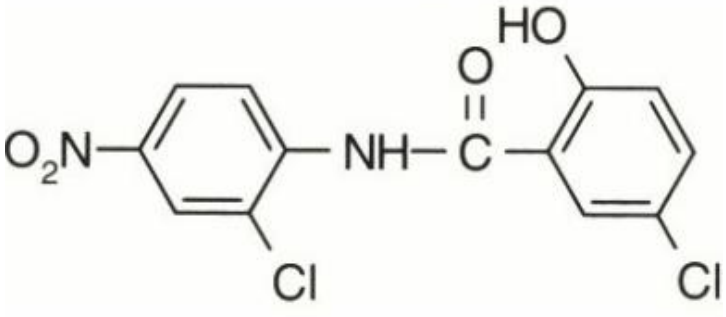
2.3.3 Moluscicida utilizado no controle dos hospedeiros intermediários da esquistossomose

As substâncias denominadas moluscicidas são utilizadas para o controlar moluscos que causam prejuízo as culturas ou são vetores de patologias. Esses moluscicidas podem ser classificados em sintéticos e naturais (CANTANHEDE *et al.*, 2010).

Os sintéticos são largamente empregados em programas de controle da esquistossomose com o objetivo de combater os caramujos vetores da doença. Estima-se que aproximadamente 7.000 produtos químicos, tais como sulfato de cobre, hidróxido de cálcio, e niclosamida, foram testados com este objetivo. No entanto, poucos mereceram destaque (CANTANHEDE *et al.*, 2010; NEVES, 2016; REY *et al*., 2001).

Como mencionado anteriormente, a niclosamida (Figura 14) é o único agente moluscicida utilizado em campanhas de controle da esquistossomose (COLLEY *et al*., 2014). É produzida comercialmente pela Bayer e recomendada pela Agência Nacional de Vigilância Sanitária (ANVISA) e a OMS. Essa substância possui elevada toxidade frente a caramujos, 1 µg/mL causa 100% de mortalidade em 8 horas de exposição para os gêneros *Biomphalaria* e *Bulinus* (REY *et al*., 2001). No entanto, é tóxica contra espécies não-alvo, como peixes, anfíbios e outros organismos aquáticos, produzindo um impacto ambiental negativo ao ecossistema aquático (COLLEY *et al*., 2014). Ainda, possui custo elevado, é fotodegradável e não preveni a recolonização de áreas previamente tratadas (RAPADO, 2012; OLIVEIRA-FILHO; PAUMGARTTEN, 2000; ABREU *et al.*, 2002).

Figura 14 - Estrutura química da niclosamida.



Fonte: Brasil (2018c).

Nesse contexto, pesquisas científicas direcionadas à descoberta de substâncias de origem vegetal com ação moluscicida vem sendo cada vez mais estimuladas (CANTANHEDE *et al.*, 2010; MARTINS *et al*., 2014; MIYASATO *et al.*, 2012; ROCHA-FILHO *et al*., 2015).

No Brasil, as primeiras pesquisas com moluscicidas naturais de origem vegetal demonstraram atividade de extratos aquosos do caule de *Sejania* sp. (cipó-timbó) e *Sapindus saponaria* L. (saboneteira) contra *B. glabrata* (CANTANHEDE *et al.*, 2010).

Atualmente, há pesquisas com látex (OLIVEIRA-FILHO *et al.*, 2010), extratos (FARIA *et al*., 2018; MEDINA *et al*., 2009; ROCHA-FILHO *et al*., 2015), e óleos essenciais (DIAS *et al*., 2013; FONTES-JÚNIOR *et al.*, 2012; TELES *et al*., 2010) de plantas de diversas famílias frente aos hospedeiros intermediários da esquistossomose.

O Látex de *Euphorbia milli* foi tóxico para embriões de *B. glabrata* (OLIVEIRA-FILHO *et al.*, 2010). Além disso, o extrato hidroalcoólico e a fração acetetato de etila de *Manilkara subsericea* mostrou serem tóxicos para *B. glabrata* com um tempo de exposição de 96 horas (CL_{50} = 118,70 e 23,41 µg/mL, respectivamente). Três substâncias isoladas desse mesmo vegetal, quercetina, miricetina e ácido ursólico, também mostraram ser tóxicas para a mesma espécie de caramujo mencionado anteriormente (CL_{50} = 1,57, 0,05 e 0,08 µg/mL, respectivamente) (FARIA *et al*., 2018). Os extratos de hexânico e etanólico das folhas, metanólico do caule, e o ácido caurenóico de *Croton floribundus* também apresentaram ação moluscicida contra *B. glabrata*, com valores de CL_{50} de 37,4, 14,8, 4,2 e 1,16 µg/mL, respectivamente (MEDINA *et al*., 2009). Além disso, extrato aquoso de *Moringa oleifera* apresentou efeito tóxico para *B. glabrata* com um tempo de exposição de 24 horas (CL_{50} = 2,37 mg/mL) (ROCHA-FILHO *et al*., 2015). Por sua vez, a ação moluscicida de OEs será abordada no tópico 2.7.3.

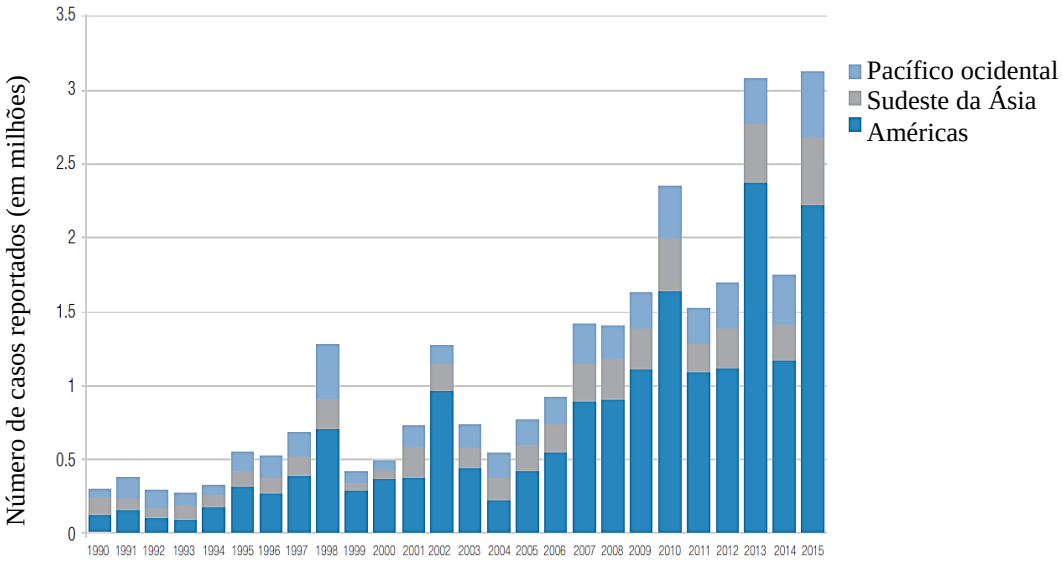
2.4 DENGUE

2.4.1 Aspectos gerais e epidemiológicos da dengue

A dengue é uma arbovirose que causa sérios problemas de saúde e econômicos nas regiões tropicais e subtropicais (CHURAKOV *et al*., 2019). O seu vírus é transmitido por mosquitos, principalmente da espécie *Aedes aegypti* e, em menor extensão, *Aedes albopictus* (WHO, 2017; WHO, 2019a).

A incidência dessa doença cresceu drasticamente em todo o mundo nas últimas décadas, como mostra a Figura 15. Além disso, acredita-se que o número real de casos dessa doença seja subnotificado, pois a maioria deles são assintomáticos ou classificados erroneamente (WHO, 2019a).

Figura 15 - Casos de dengue notificados à OMS, por região, 1990–2015.



Fonte: Adaptado da WHO (2017).

A cada ano, cerca de 390 milhões de casos de dengue são notificadas, dos quais 96 milhões se manifestam clinicamente com qualquer gravidade da doença. Ao mesmo tempo, estima-se que 3,9 bilhões de pessoas, em 128 países, estão em risco de infecção pelo vírus da dengue (WHO, 2019a).

Atualmente, ela é endêmica em mais de 100 países nas regiões da África, Américas, Mediterrâneo Oriental, Sudeste Asiático e Pacífico Ocidental, e se tornou uma das principais causas de hospitalização e morte entre crianças e adultos nessas regiões (WHO, 2019a).

Acredita-se que a dengue seja endêmica em muitas partes do continente Africano por causa da presença da doença e a alta prevalência de anticorpos contra o vírus da dengue em investigações sorológicas. Atualmente, 22 países da África, como Angola, República da Maurícia, Moçambique, República Unida da Tanzânia e Burkina Faso, notificaram surtos de dengue (WHO, 2017).

Estima-se que mais de 1,8 bilhões de pessoas estejam em risco de contrair a doença nos países da Ásia-Pacífico. Em 2015, mais de 450 mil casos de dengue foram noticifados nessa região. Na Europa, a transmissão local do vírus foi relatada pela primeira vez na Croácia e na França em 2010. Foi relatado um surto na ilha da Madeira (Portugal) em 2012 resultou em mais de dois mil casos e importação de casos em 17 outros países europeus. A transmissão autóctone da dengue foi relatada duas vezes no sul da França em 2015 (WHO, 2017).

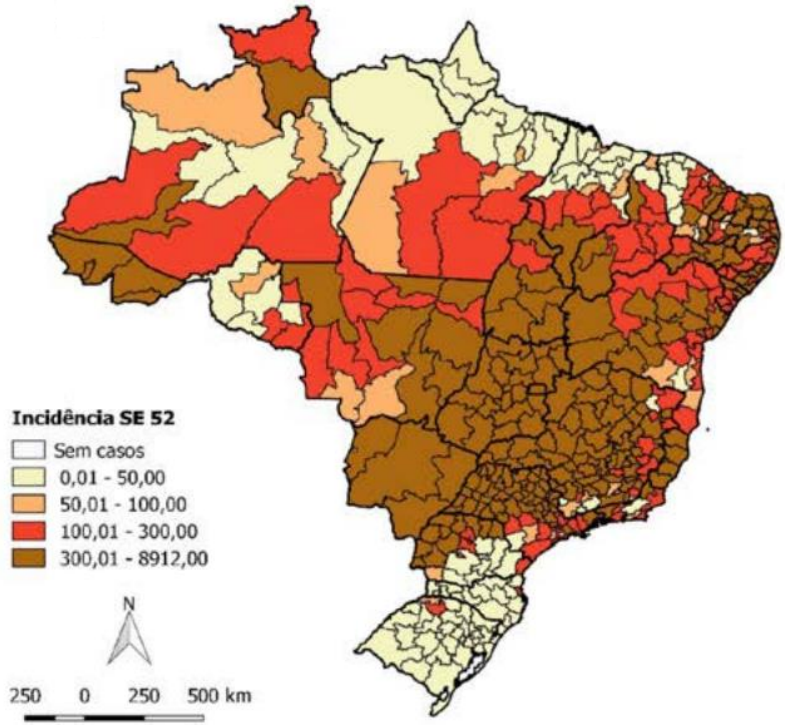
Por sua vez, na região do Pacífico Ocidental, em 2015, mais de 44 mil casos de dengue foram relatados, com uma taxa de letalidade de 0,22%. A maior incidência desses casos nessa região foi nas Filipinas, no Camboja e na Malásia. No Mediterrâneo Oriental há relatado de

dengue nas duas décadas. Essa doença está emergindo como um grande problema de saúde pública no Paquistão, na Arábia Saudita e no Iêmen, com repetidos surtos em centros urbanos (WHO, 2017).

Entre os anos 1960 de 1970 a transmissão dos vírus da dengue foi interrompida em grande parte da Região das Américas, após campanhas para erradicar *A. aegypti*. No entanto, como a vigilância de vetores não foi mantida, os mosquitos prosperaram e surtos de dengue ocorreram no Caribe e nas Américas Central e do Sul (WHO, 2017). Entre os anos de 2001 a 2009, cerca de 30 países das Américas notificaram mais de 6 milhões casos de dengue, nos quais quase 181 mil deles foram de dengue hemorrágica com aproximadamente 2500 mortes. A Venezuela, Brasil, Costa Rica, Colômbia, Honduras e México foram responsáveis por mais de 75% de todos os casos na região (WHO, 2010). Atualmente, essa região vem relatando o maior número de casos de dengue, porém, apresentando menor taxa de letalidade de todas as regiões da OMS (WHO, 2017). Em 2016, foram notificados mais de 2,3 milhões casos dessa doença na região das Américas, onde o Brasil contribuiu com pouco menos de 1,5 milhões de casos (WHO, 2019a).

O Brasil possui relatos de dengue em todos os 27 estados, concentrando-se nas regiões Sudeste, Centro-oeste, Nordeste e Norte (Figura 16) (BRASIL, 2020c).

Figura 16 - Situação epidemiológica da dengue no Brasil em 2019.



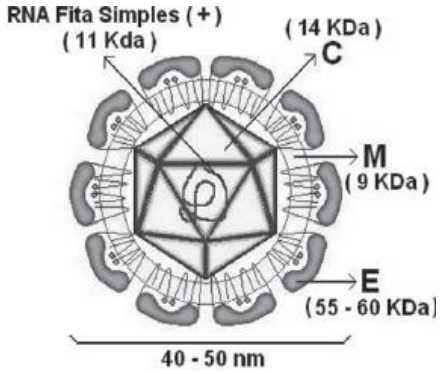
Fonte: Brasil (2020c).

2.4.2 O vírus da dengue e sua transmissão

A dengue é causada por um arbovírus da família Flaviviridae e do gênero *Flavivirus* (BIASSOTI *et al*., 2017). Esse vírus possui RNA de polaridade positiva como material genético e contém cerca 11.000 nucleotídeos que codificam três tipos diferentes de moléculas de proteínas (C, M e E) que formam a partícula viral. O vírus da dengue (DENV) tem formato esférico e diâmetro de 40 a 60 nanômetros (nm) (Figura 17) (DETTOGNI; LOURO, 2017).

No momento foram identificados quatro sorotipos para o vírus causador da dengue (DENV-1, DENV-2, DENV-3 E DENV-4). Quando uma pessoa é infectada por um dos quatro sorotipos, torna-se imune a todos os tipos de vírus durante alguns meses e em seguida mantém-se imune, pelo resto da vida, ao tipo pelo qual foi infectado. Além disso, infecções subsequentes (infecção secundária) por outros sorotipos aumentam o risco de desenvolvimento de dengue grave, também conhecida como dengue hemorrágica (WHO, 2019a).

Figura 17 - Morfologia do vírion de um *Flavivirus.*



Nota: Kda: quilodaltons; C: proteína do capsídeo; M: proteína da membrana; E: proteína do envelope; nm: nanômetros.
Fonte: Dettogni e Louro (2017).

A transmissão do DENV ocorre através da picada de mosquitos fêmeas do gênero *Aedes*, que adquire o vírus enquanto se alimenta do sangue de uma pessoa infectada (BIASSOTI *et al*., 2017). Dentro do mosquito, o vírus infecta intestino e depois se espalha para as glândulas salivares durante um período de 8 a 12 dias. Após esse período, o vírus pode ser transmitido para seres humanos durante a alimentação (WHO, 2019b). Por isso, os indivíduos sintomáticos ou assintomáticos infectados são os principais multiplicadores do vírus, servindo como fonte do vírus para os mosquitos não infectados. Também há registro de transmissão por transfusão sanguínea. Na gravidez, a infecção pelo vírus da dengue não passa da grávida para feto, porém

pode ocasionar aborto ou parto prematuro. Além do mais, as gestantes têm maior chance de desenvolver o quadro grave dessa doença (BRASIL, 2019a).

2.4.3 Aspectos clínicos, diagnóstico e tratamento da dengue

Os sintomas, formas de diagnóstico e tratamento da dengue estão descritos na tabela abaixo (Tabela 3).

Tabela 3 - Sintomas, diagnóstico e tratamento da dengue.

Sintomas	Diagnóstico	Tratamento
Febre alta (39 a 40 °C), dor de cabeça, dores no corpo e articulações, prostração, fraqueza, vômito, dor atrás dos olhos, erupção, coceira na pele, manifestações hemorrágicas e colapso circulatório (nas formas grave da doença).	Exames laboratoriais de sorologia, biologia molecular e isolamento viral.	Analgésicos e antitérmicos, hidratação (oral ou venosa), e internação hospitalar (para paciente com a forma grave da doença).

Fonte: WHO (2019a) e Brasil (2019ab).

A infecção por dengue pode ser assintomática, leve ou grave, e raramente causa a morte. No entanto, idosos e pessoas com doença crônica - como diabetes e hipertensão - têm maior risco de desenvolver dengue grave e outras complicações que podem levar à morte (WHO, 2019a; BRASIL, 2019b).

2.4.4 Estratégias de controle e prevenção da dengue

Atualmente, a forma mais eficiente de prevenção da dengue é a erradicação dos mosquitos vetores, uma vez que, a vacina disponível para a dengue possui várias contraindicações (AGUIAR *et al.*, 2016) e ainda não está disponível no sistema único de saúde (SUS) (DOMINGUES; GARCIA, 2020). No momento, outra vacina contra dengue esta sendo desenvolvida pelo Instituto Butantan. Essa vacina que encontra-se em fase de teste, e até o presente momento é considerada potencialmente eficaz (INSTITUTO BUTANTAN, 2020).

No Brasil, o controle do mosquito *A. aegypti* é feito por forma mecânica, biológica e química (BRASIL, 2019b). O controle mecânico do vetor da dengue compreende ações preventivas simples, eficazes e integradas, como a eliminação de água armazenada em

possíveis criadouros (ex.: em vasos de plantas, pneus, garrafas pláticas, piscinas sem uso e sem manutenção, tampas de garrafas, entre outros) (BRASIL, 2019b) e ações que diminuam o contato do mosquito com o homem, através de telas em portas e janelas (ZARA, 2016), e uso de mosquiteiros (BRASIL, 2019b). O controle biológico utiliza parasitas, patógenos ou predadores naturais para o controle de populações do vetor, como o peixes que comem as larvas do mosquito *A. aegypti*. Por sua vez, o controle químico é realizado através do uso de inseticidas sintéticos das classes dos carbamatos, piretróides e organofosforados para controlar as diferentes fases dos insetos, como as larvas e mosquistos adultos (BRASIL, 2020b). No entanto, o uso de inseticidas sintéticos traz várias desvantagens, como eliminação de espécies não alvo, desenvolvimento de populações resistentes, além do alto custo (ZARA, 2016).

Algumas pesquisas relatam que materiais vegetais apresentam atividades inseticidas frente ao *A. aegypti*. Como por exemplo, os extratos de hexano, acetato de etila, metanol e etanol de *Crotalaria pallida* mostraram ser tóxicos para larvas de *A. aegypti* com um tempo de exposição de 24 horas. As CL_{50} foram de 366,16, 346,27, 262,02, 245,79 mg/mL, respectivamente. Além disso, os extratos metanólico e etanólico inibiram a eclosão de ovos de *A. aegypti* (TAKAGI *et al*., 2020). O OE de *Aniba rosaeodora* também apresentou efeito tóxico para larvas de *A. aegypti* com um tempo de exposição de 24 horas, a CL_{50} foi de 41,07 µg/mL (FERREIRA *et al*., 2020)

Nos tópicos anteriores foi mostrado os aspectos gerais, epidemilógicos, sintomas, dignóstico, tratamento e estratégias de controle e prenção da dengue. Por sua vez, o próximo tópico abordará o principal vetor da dengue: a espécie *A. aegypti*

2.5 A ESPÉCIE *A. aegypti*

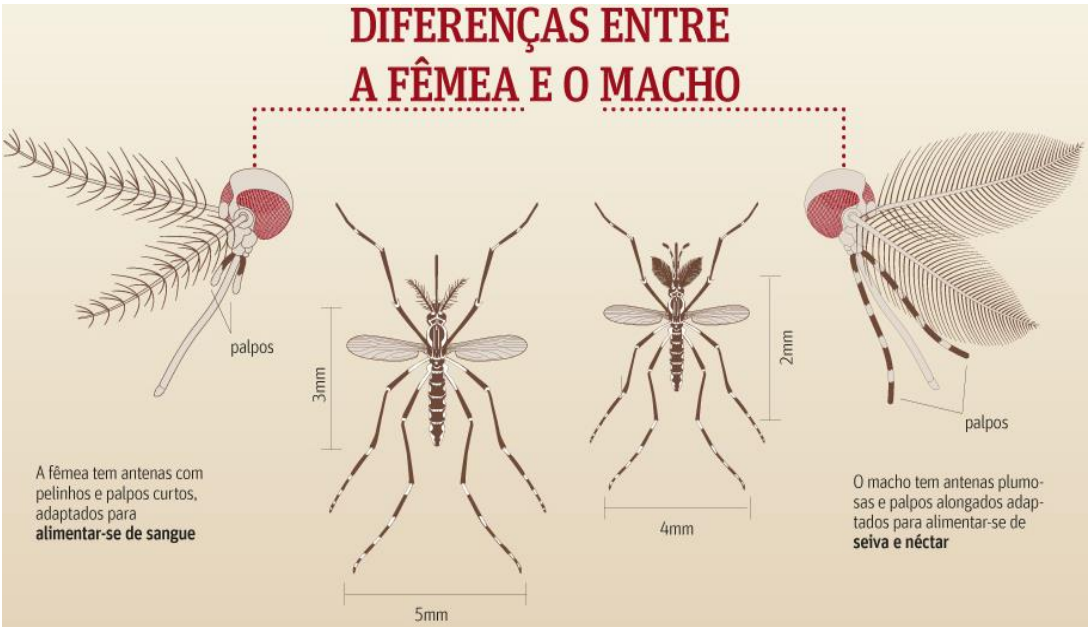
A. aegypti (Figura 18) é um inseto que pertence a família Culicidae e que se originou no continente africano e, provavelmente, dispersou-se para América no período colonial. Esses mosquitos encontram-se distribuídos nas regiões tropicais e substropicais, e possuem uma grande capacidade de dispersão e adaptação ao meio, além disso, eles podem ser encontrados em áreas urbanas, suburbanas e rurais (SILVA *et al*., 2018; TOLLE, 2009).

Na fase adulta de *A. aegypti* ocorre dimorfismo sexual, onde há diferença no tamanho dos mosquitos, nas antenas e no aparelho bucal. As fêmeas são maiores que os machos; possuem antenas pilosas, enquanto os machos possuem antenas plumosas; e o aparelho bucal das fêmeas é modificado para se alimentar de sangue, já a aprelho bucal dos machos é modificado para alimentação de néctar (Figura 19) (ZETTEL; KAUFMAN, 2020).

Figura 18 - Mosquitos de *A. aegypti* (Linnaeus, 1762).

Fonte: CDC (2020).

Figura 19 - Dimorfismo sexual em mosquito *A. aegypti* adulto.



Fonte: Marques (2020).

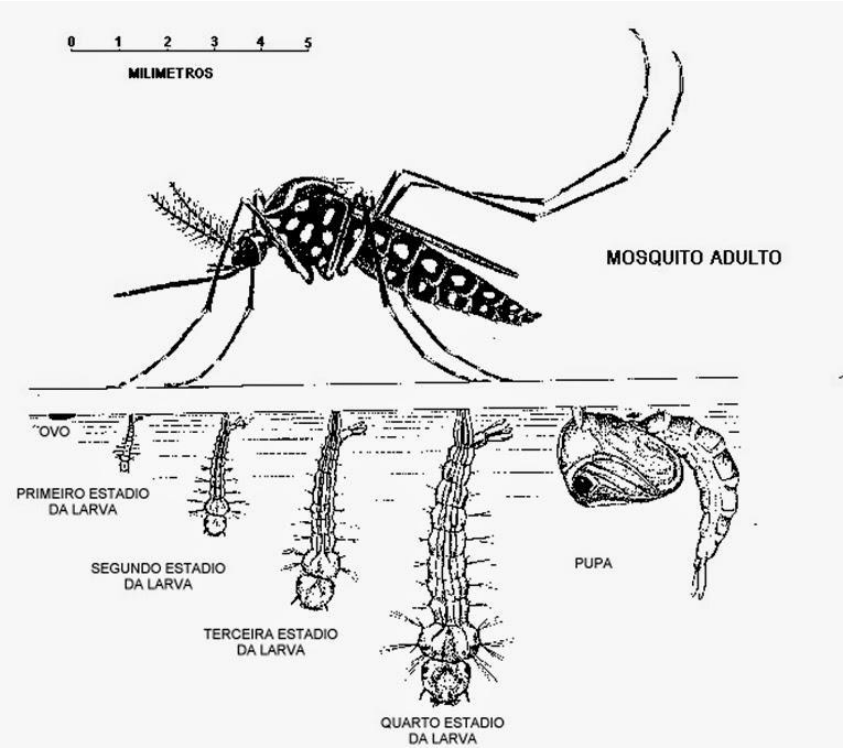
2.5.1 Ciclo de vida do *A. aegypti*

O ciclo de vida completo desse inseto é composto por quatro fases, ovo, larva, pupa e mosquito adulto (Figura 20) (SILVA *et al*., 2018), na qual as fases de larva e pupa requerem um ambiente com água parada para seu desenvolvimento (BRASIL, 2019a). Esse ciclo varia de acordo com a temperatura, disponibilidade de alimentos e quantidade de larvas existentes no mesmo criadouro. Em condições ambientais favoráveis, leva um período de sete a dez dias da eclosão do ovo até o desenvolvimento do mosquito adulto (BRASIL, 2019c).

Após a cópula, as fêmeas de *A. aegypti* precisam realizar a hematofagia para o desenvolvimento e maturação completa dos ovos (Figura 21A). Geralmente, elas fazem oviposição três dias após a ingestão de sangue, e os ovos são postos em superfícies próximas a locais com água parada. Eles medem cerca de 1 mm de comprimento e possuem contorno

alongado e fusiforme. Mesmo sendo necessário água para a eclosão dos ovos, parte deles podem ficar viáveis por mais de 450 dias sem ter contato com água (BRASIL, 2020d).

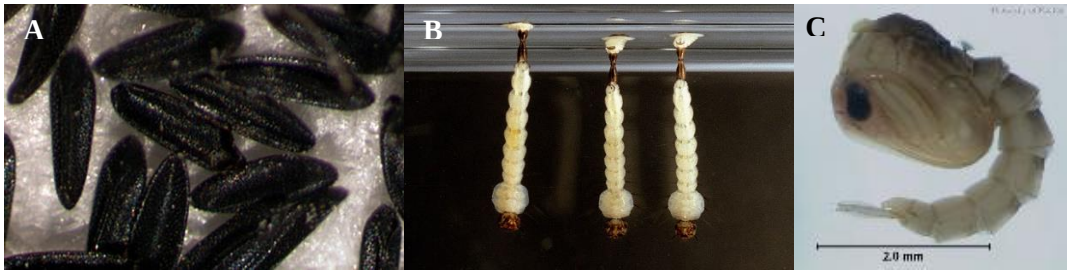
Figura 20 - Ciclo de vida do *A. aegypti*.



Fonte: Santos (2016).

Após a cópula, as fêmeas de *A. aegypti* precisam realizar a hematofagia para o desenvolvimento e maturação completa dos ovos (Figura 21A). Geralmente, elas fazem oviposição três dias após a ingestão de sangue, e os ovos são postos em superfícies próximas a locais com água parada. Eles medem cerca de 1 mm de comprimento e possuem contorno alongado e fusiforme. Mesmo sendo necessário água para a eclosão dos ovos, parte deles podem ficar viáveis por mais de 450 dias sem ter contato com água (BRASIL, 2020d).

Figura 21 - Ovos, larvas e pupa de *A. aegypti*.



Fonte: Brasil (2020d), Zettel e Kaufma (2020).

Após a eclosão dos ovos, as larvas (Figura 21B) se alimentam de partículas orgânicas presentes na água, e elas passam por quatro instares (L1, L2, L3 e L4). O primeiro ínstar (L1) surge após eclosão do ovo. Os ínstares L2 e L3 caracterizam-se por constante alimentação. Por sua vez, no final do quarto e último ínstar (L4), a larva cessa sua alimentação para a transformação em pupa (BRASIL, 2020d).

A fase de pupa (Figura 21C) caracteriza-se por ser uma etapa de transição entre o ambiente aquático e o terrestre (FORATTINI, 2002). Normalmente, as pupas transformam-se em adultos após cerca de 48h. Os mosquitos adultos apresentam coloração escura e o tórax revestido por escamas escuras e branco-prateadas, o abdomem escurecido com manchas anelares branco-prateadas e as pernas traseiras possuem faixas brancas semelhantes a listras (Figura 18) (CLEMONS *et al*., 2010).

2.5.2 Outras doenças transmitidas pelo *A. aegypti*

A febre amarela, chikungunya e zika são doenças causadas por vírus que, assim como a dengue, são transimitidas pelo *A. aegypti*. Elas causam sintomas similares em humanos, como febre alta de 4 a 12 dias e dor nas articulações. No entanto, cada um tem sua patologia única, com altas taxas de mortalidade para febre amarela, mas raramente por chikungunya e zika (SOUZA-NETO *et al*., 2019).

A febre amarela é uma doença infecciosa, não contagiosa, de curta duração (no máximo de 12 dias) e de gravidade variável (CAVALCANTE; TAUIL, 2017). Em áreas urbanas a disseminação dessa doença ocorre através da picada de mosquitos *A. aegypti* infectados pelo vírus da febre amarela. Os sintomas mais frequentes incluem febre, calafrios, dor de cabeça, dor no corpo, náuseas, vômitos, fraqueza e fadiga (BRASIL, 2020e). As formas graves dessa doença é caracterizada por insuficiência hepática e renal, e hemorragia, podendo levar a morte (CAVALCANTE; TAUIL, 2017). A principal ferramenta de prevenção e controle da febre amarela é a vacina, que esta disponível no sistema único de saúde (BRASIL, 2020e)

A Chikungunya se caracteriza por febre, dor articular intensa e debilitante, cefaleia e mialgia. Essa doença possui alguns sintomas semelhantes aos da dengue, mas se diferencia da mesma pela poliartrite/artralgia simétrica (principalmente nos punhos, tornozelos e cotovelos), que normalmente melhora após 10 dias, no entanto, pode durar meses (DONALISIO; FREITAS, 2015). O nome Chikungunya significa "aquele que se curva", no idioma africano Makonde, em a razão posição antálgica que os pacientes adquirem durante o período de doença (HONÓRIO *et al*., 2015). Além disso, algumas pessoas podem desenvolver quadros mais

graves com manifestações neurológicas, como encefalite, meningoencefalite, mielite e síndrome Guillain-Barré, cutâneas bolhosas e miocardite (DONALISIO; FREITAS, 2015).

O vírus da zika, além de ser transmitidos por *A. aegypti* e *A. albopictus*, também pode ser transmitido por transfusão sanguínea, via sexual e pela grávida ao feto (LUZ *et al*. 2015). Os sintomas mais comuns dessa doença são manchas vermelhas na pele, dor articular, conjuntivite, dor de cabeça e febre, que persistem por aproximadamente 7 dias (BRASIL, 2019c). Algumas complicações podem manifestar com a infecção por zika vírus, como a síndrome de Guillain-Barré. A infecção por zika vírus em mulheres grávidas também pode levar a morte fetal, insuficiência placentária, restrição de crescimento fetal e microcefalia congênita (WHO, 2020). Entre março de 2015 e abril de 2016, mais de 5000 casos de microcefalia foram relatados entre recém-nascidos no Brasil, em decorrência ao surto de zika no país (SCHRAM, 2016).

Diante do que foi mostrado, o uso de materiais vegetais vem recebendo grandes incentivos para o combate tanto aos moluscos causadores da esquistossomose quanto para o combate ao mosquito *A. aegypti*. Diversas plantas vem sendo testadas como possíveis agentes moluscicidas e inseticidas (FARIAS et al., 2018; ROCHA-FILHO *et al*., 2015; DIAS *et al*., 2013), porém pouco se sabe sobre seus metabolitos ativos.

2.6 METABÓLITOS SECUNDÁRIOS DOS VEGETAIS

O Reino Vegetal possui uma grande diversidade de espécies que podem ser empregadas como alternativas na síntese de produtos químicos utilizados para diversos fins (WINK, 2015), como no controle de insetos pragas (RIBEIRO *et al*., 2020; SANTOS *et al*., 2017), vetores de doenças (PAVELA; BENELLI, 2016), bem como no tratamento de diversas enfermidades (BRUSOTTI *et al.*, 2014). Dessa maneira, estudos sobre essas potenciais fontes de compostos biologicamente ativos são necessárias (RIBEIRO, 2016a).

Os vegetais possuem dois metabolismos responsáveis pela produção de metabólitos com diferentes funções, são eles: (i) metabólitos primários e (ii) metabólitos secundários. Os primários são susbtâncias imprescindíveis ao desenvolvimento e sobrevivência dos organismos, e desempenham funções essenciais no vegetal, como, fotossíntese, respiração e transporte de soluto. Além do mais, os compostos envolvidos no metabolismo primário possuem uma distribuição universal nas plantas (ANULIKA *et al*., 2016; VERMA; SHUKLA, 2015), por exemplo, açúcares, proteínas, ácidos nucléicos, lipídios.

Por outro lado, os metabólitos secundários podem estar presentes ou não nos vegetais dependendo das variáveis ecológicas (ANULIKA *et al*., 2016; VERMA; SHUKLA, 2015). Eles desempenham um papel importante nas interações entre as plantas e o ambiente, como na defesa contra herbívoros, patógenos, e estresses ambientais, bem como, na atração de polinizadores e microrganismos simbiontes (ZAYNAB *et al*., 2018; VERMA; SHUKLA, 2015). Esses compostos podem ser armazenados em vacúolos ou em algumas estruturas secretoras, como por exemplo, tricomas e ductos de resina (PAVELA; BENELLI, 2016). Além disso, esses metabólitos secundários possuem uma contribuição significante nas indústrias farmacêutica, nutricional e cosmética (VERMA; SHUKLA, 2015), e alguns deles são específicos de gêneros e espécies podendo ser utilizados como caracteres taxonômicos na classificação das plantas (AHARONI; GALILI, 2011).

A síntese de metabólitos secundários está intimamente associada a vias de metabolismo primário, como a glicólise, a via do chiquimato, ciclo do ácido tricarboxílico e produção de aminoácidos aromáticos e alifáticos (AHARONI; GALILI, 2011). No entanto, a extração, purificação e caracterização dos metabólitos secundários ainda é um grande desafio no processo de descoberta de novos compostos bioativos, uma vez que, esses metabólitos estão presentes em pequenas quantidades no material vegetal (BRUSOTTI *et al.*, 2014).

Dentre as substâncias sintetizadas pelos vegetais como metabólitos secundários, encontram-se os componentes dos OEs (BAKKALI *et al*., 2008), o qual será abordado mais a frente.

2.6.1 Biossíntese de metabólitos secundários em vegetais

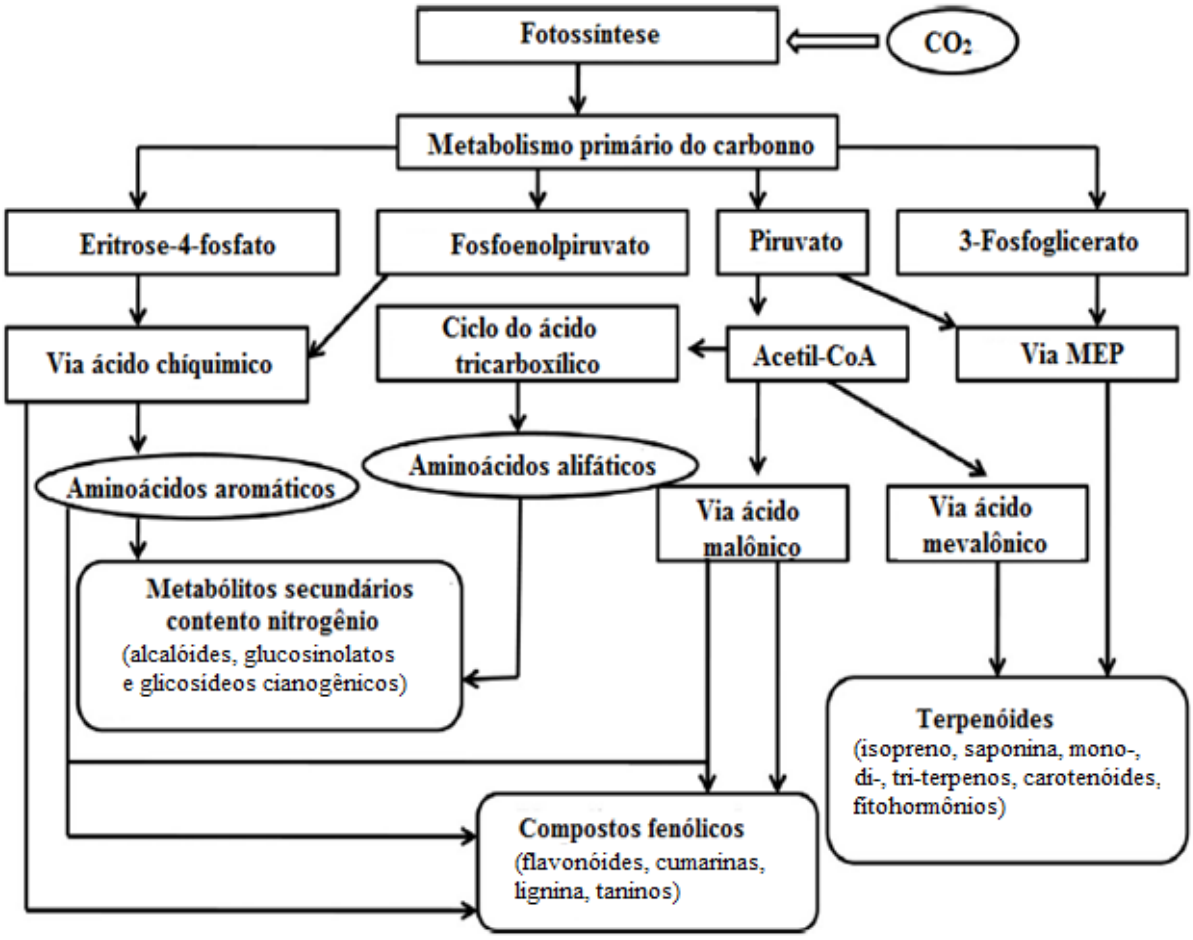
Os metabólitos secundários dos vegetais podem ser divididos em três grupos quimicamente distintos, são eles: terpenos, compostos fenólicos (fenilpropanóides e flavonóides) e compostos nitrogenados (alcalóides, glicosídeos cianogênicos e glucosinolatos) (ANULIKA *et al*., 2016; VERMA; SHUKLA, 2015). A Figura 22 mostra o esboço geral das vias de biossíntese dos metabólitos secundários em vegetais.

2.6.1.1 Terpenos

Os terpenos ou terpenóides são considerados o maior grupo de metabólitos secundários dos vegetais, e normalmente são insolúveis em água. Eles são formados pela fusão de unidades de cinco carbonos (unidade de isopreno) (Figura 23), e classificados de acordo com número

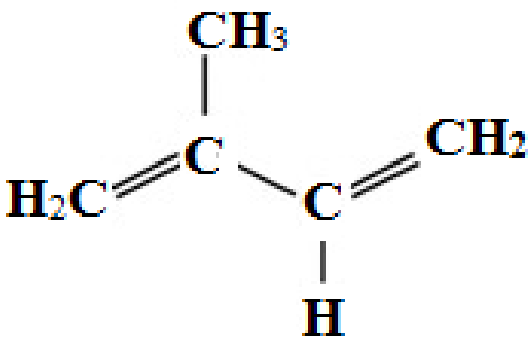
dessas unidades que se ligam entre si, como mostra a Tabela 4 (ANULIKA *et al*., 2016; VERMA; SHUKLA, 2015; WINK, 2015).

Figura 22 - Esboço geral das vias de biossíntese dos metabólitos secundários em vegetais.



Fonte: Verma e Shukla (2015).

Figura 23 - Estrutura química do isopreno (C_5H_8).



Fonte: Ribeiro (2016a).

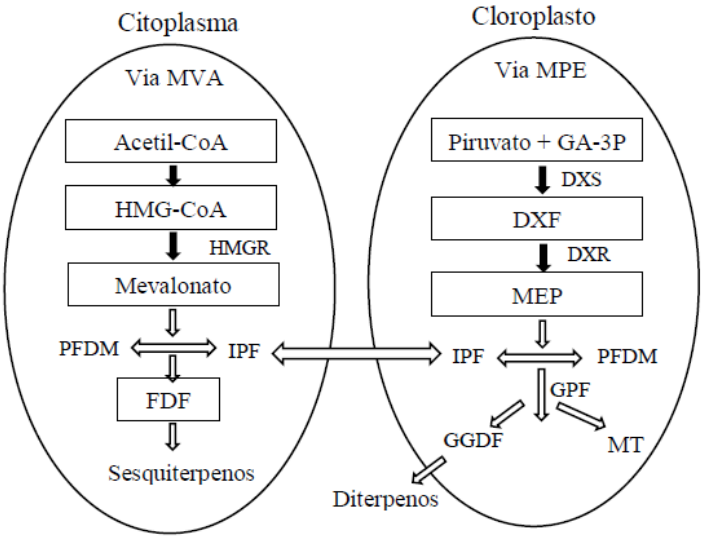
Tabela 4 - Classificação dos terpenos encontrados nos vegetais.

Número de unidades de isopreno	Número de átomos de carbono	Classes	Exemplos
1	5	Isopreno	Cadeia lateral das citocininas
2	10	Monoterpenos	Óleos essenciais
3	15	Sesquiternos	Ácido abscísico
4	20	Diterpenos	Giberelina e paclitaxel
6	30	Triterpenos	Brassinoesteroide e saponinas
8	40	Tetraterpenos	Carotenoides e xantofilas
N	N	Politerpenos	Borracha

Fonte: adaptado de Anulika *et al*. (2016), Verma e Shukla (2015), e Wink (2015).

Os terpenos são sintetizados a partir de duas vias metabólicas distintas. A primeira via ocorre no citoplasma através do mevalonato ou ácido mevalônico (em inglês: MVA, mevalonate). A segunda nos cloroplastos pelo metileritritol 4-fosfato (em inglês: MEP, methylerythritol 4-phosphate) derivado piruvato ou 3-fosfoglicerato (Figura 24) (PAVELA; BENELLI, 2016; VERMA; SHUKLA, 2015; WINK, 2015).

Figura 24 - Vias do Mevalonato (MVA) e metileritritol 4-fosfato (MPE).



Nota: HMG-CoA: 3-hidroxi-3-metilglutaril coenzima A; HMGR: 3-hidroxi-3-metilglutaril redutase; PFDM: pirofosfato de dimetilalilo; IPF: isopentenil pirofosfato; FDP: farnesil difosfato; GA-3P: gliceraldeído-3-fosfato; DXS: 1-desoxi-D-xilulose 5-fosfato sintase; DXF: 1 desoxi-D-xilulose 5-fosfato; DXR: 1-desoxi-D-xilulose 5-fosfato reductoisomerase; GPF: geranil pirofosfato; GGDF geranilgeranil difosfato; MT: monoterpenos.

Fonte: Adaptado de Pavela e Benelli (2016).

Os terpenos desempenham um importante papel defensivo nas plantas, pois muitos deles são tóxicos e possuem efeito deterrente na alimentação de insetos e mamíferos herbívoros (ANULIKA *et al*., 2016). Alguns deles ainda desempenham um papel importante no crescimento das plantas, como por exemplo, giberelinas (diterpeno), esteróis (triterpenos), carotenóides (tetraterpenos) e ácido abscísico (sesquiterpenos) (VERMA; SHUKLA, 2015). Além disso, estudos mostraram que eles interagem com as membranas celulares, aumentando a fluidez e a permeabilidade dessas membranas, podendo ocasionar o fluxo descontrolado de íons e metabólitos, e até mesmo o vazamento das células resultando em morte celular por necrose ou apoptose (WINK, 2015).

2.6.1.2 Compostos fenólicos

Considerados o segundo principal grupo de metabólitos secundários de plantas, os compostos fenólicos são formados por pelo menos um anel aromático com no mínimo um grupamento hidroxila (OH-). Esses compostos podem ser sintetizados por duas rotas metabólicas são elas: a do ácido malônico e a do ácido chiquímico (Figura 22). A rota do ácido malônico é altamente significativa em bactérias e fungos, mas é menos comum em plantas superiores. Por sua vez, a via ácido chíquimico é mais comum nos vegetais, sendo ela responsável pela biossíntese da maioria dos compostos fenólicos nesses organismos (VERMA; SHUKLA, 2015; RIBEIRO, 2016a).

Esses compostos exercem funções importantes nos vegetais, incluindo crescimento, defesa contra diferentes estresses bióticos ou abióticos, e reprodução. Eles também podem ser utilizados como um indicador de estresse, uma vez que, há um aumento na síntese de compostos fenólicos em vegetais expostos a produtos químicos e condições de estresse (RIBEIRO, 2016a; VERMA; SHUKLA, 2015).

2.6.1.3 Compostos nitrogenados

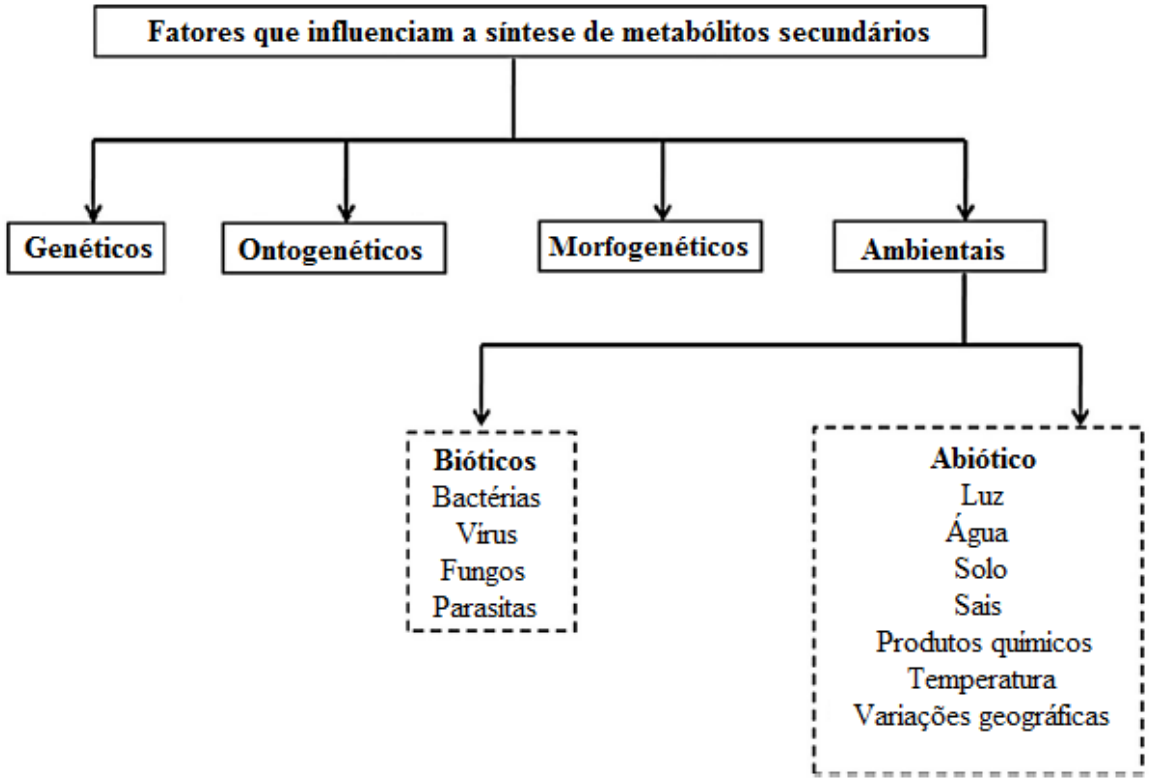
Os compostos contendo nitrogênio representam a terceira classe de metabólitos secundários. Glicosídeos cianogênicos, glucosinolatos e alcaloides são exemplos desses compostos. Eles são sintetizados tanto a partir de alguns aminoácidos aromáticos, como tirosina e triptofano (ambos derivados do ácido chíquimico) quanto aminoácidos alifáticos como a ornitina e lisina (VERMA; SHUKLA, 2015). Alguns compostos nitrogenados encontrados nos

vegetais, como glicosídeos cianogênicos e glucosinolatos, fazem parte do sistema de defesa desses organismos (ANULIKA *et al*., 2016).

2.6.2 Fatores que influenciam a biossíntese de metabólitos secundários

Como mostra a Figura 25, a síntese dos metabólitos secundários é influenciada por quatro principais fatores. Dentre eles, os genéticos, ontogenéticos, morfogenéticos e ambientais, como detalhados a seguir.

Figura 25 - Fatores que afetam a síntese dos metabólitos secundários em vegetais.



Fonte: Verma e Shukla (2015).

2.6.2.1 Fatores genéticos

A síntese das enzimas utilizadas nas rotas metabólicas dos produtos naturais está diretamente relacionada com o código genético (processos de transcrição e tradução). Então, se houver alguma alteração nos genes do vegetal isso pode acarretar a síntese de diferentes enzimas que vão catalizar diferentes reações metabólicas e gerar um metabótito secundário distinto (RIBEIRO, 2016a; VERMA; SHUKLA, 2015).

2.6.2.2 Fatores ontogenéticos

A ontogenia é toda a sequência de eventos envolvidos no desenvolvimento de um organismo. Os vegetais passam por diversos estágios de desenvolvimento, que começam a partir da semente, seguido por estágio de plântulas, juvenil, de maturação e termina no estágio de senescência (VERMA; SHUKLA, 2015). Durante essas diferentes fases, os vegetais possuem necessidades distintas, como proteção, atração de polinizadores e dispersores de sementes, que influencia diretamente a síntese e a acumulação de metabólitos secundários.

2.6.2.3 Fatores morfogenéticos

Os vegetais possuem tecidos com funções especializadas, como armazenamento, secreção, suporte e transporte. De acordo com Verma e Shukla (2015), diferentes tecidos possuem vias metabólicas distintas, e consequentemente metabólitos secundários diferentes. Sendo assim, a biossíntese de determinado metabólito pode esta restrita a um tecido específico (RIBEIRO, 2016a).

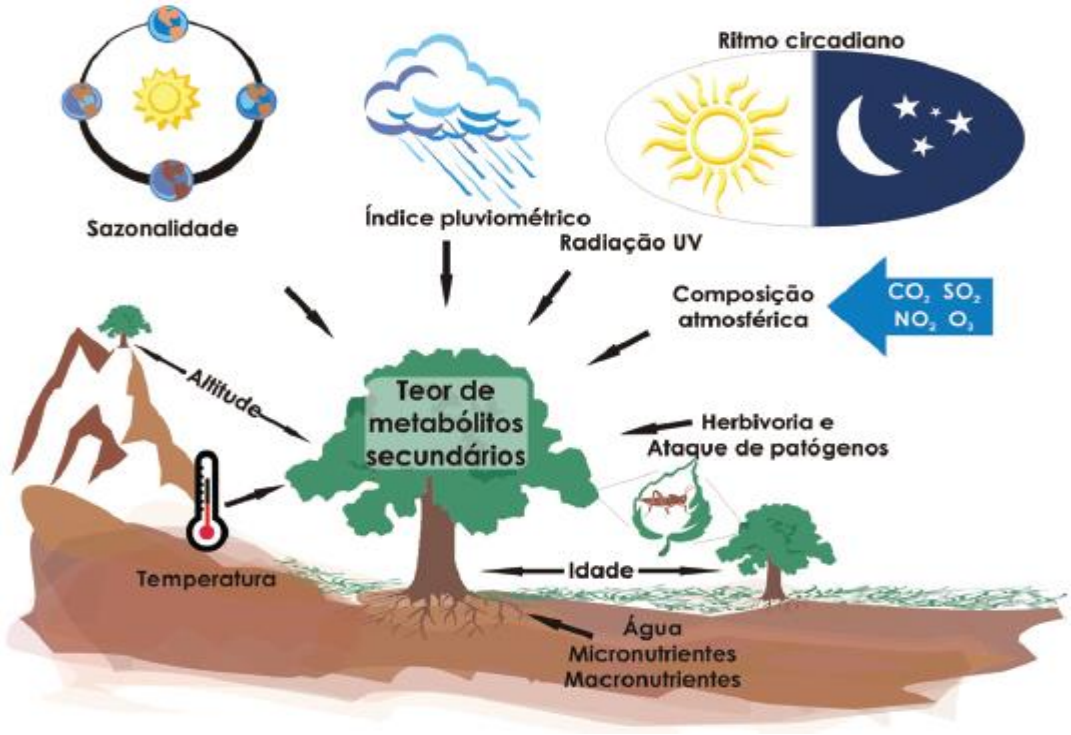
2.6.2.4 Fatores ambientais

As plantas interagem com o ambiente para sua sobrevivência e são influenciadas por vários fatores ambientais, incluindo estímulos abióticos e bióticos que regulam a síntese de metabólicos secundários (VERMA; SHUKLA, 2015).

- Fatores abióticos: os vegetais interagem com o ambiente e entram em contato com diversos fatores abióticos, como mostra a Figura 26, que influenciam o desenvolvimento e a sobrevivência dos mesmos. Um desequilíbrio entre esses componentes pode causar estresses e consequentemente alterar a produção e acúmulo de metabólitos secundários (VERMA; SHUKLA, 2015).

- Fatores bióticos: os vegetais são fisicamente atacados por muitos agentes biológicos, como bactérias, fungos, nematoides, vírus, entre outros, que causam estresse nas plantas. Para se protegerem desses ataques as plantas utilizam seus alguns dos seus metabólitos secundários que possuem atividades antimicrobianas e inseticidas (VERMA; SHUKLA, 2015).

Figura 26 - Principais fatores ambientais que influenciam a síntese de metabólitos secundários nos vegetais.



Fonte: Gobbo-Neto e Lopes (2007).

2.7 ÓLEOS ESSENCIAIS

2.7.1 Definição, características e importância dos OEs

OEs são misturas lipofílica complexa de metabólitos secundários, constituídas principalmente por monoterpenos e sesquiterpenos. Esses óleos são produzidos e secretados por tricomas glandulares, cavidades e canais secretores que estão difundidos na superfície dos órgãos vegetais, particularmente nas folhas e flores (PAVELA; BENELLI, 2016; SHARIFI-RAD *et al*., 2017). Eles caracterizam-se por serem líquidos a temperatura ambiente, altamente voláteis, possuírem baixo peso molecular e densidade geralmente mais baixa que a água, na maioria das vezes são límpidos, apresentam forte odor, e normalmente são obtidos por destilação a vapor ou hidrodestilação (BAKKALI *et al*., 2008; TUREK; STINTZING, 2013).

Os OEs, geralmente, desempenham papéis importantes na defesa dos vegetais contra herbívoros e patógenos (PAVELA; BENELLI, 2016; SHARIFI-RAD *et al*., 2017). Devido a essas propriedades de defesa, esses óleos são alternativas para uso como pesticidas de origem vegetal (KAMANULA *et al.*, 2017; RIBEIRO *et al*., 2020), com a finalidade de minimizar os danos ecológicos. Adicionalmente, os OEs são importantes na reprodução das plantas, uma vez

que, o aroma e sabor dos mesmos podem atrair agentes polinizadores e dispersores de sementes (PAVELA; BENELLI, 2016), atuando assim no ciclo de vida do vegetal. Eles também desempenham papel importante na termotolerância dos vegetais, protegendo o aparelho fotossintético e ajudando a manter a atividade fotossintética sob estresse de alta temperatura (PAVELA; BENELLI, 2016).

Além da importância ecológica, os OEs também são importantes na economia, em virtude das suas aplicações nas indústrias de alimentos, cosméticos, farmacêuticas, entre outras. Devido a suas propriedades antioxidante, antimicrobiana, anti-inflamatórias, hidratantes e cicatrizantes os OEs e seus compostos são utilizados na fabricação de perfumes (NOUR, 2018), maquiagens, aditivos alimentares, produtos sanitários e agrícolas (BAKKALI *et al*., 2008).

Adicionalmente, alguns óleos são empregados na aromaterapia, como os óleos de lavanda e ylang-ylang utilizados no tratamento de estresse, ansiedade e depressão (ALI *et al*., 2015). Em virtude de alguns OEs possuírem propriedades repelentes, outro importante uso desses óleos é na fabrição de repelentes aplicados sobre a pele (BAKKALI *et al*., 2008).

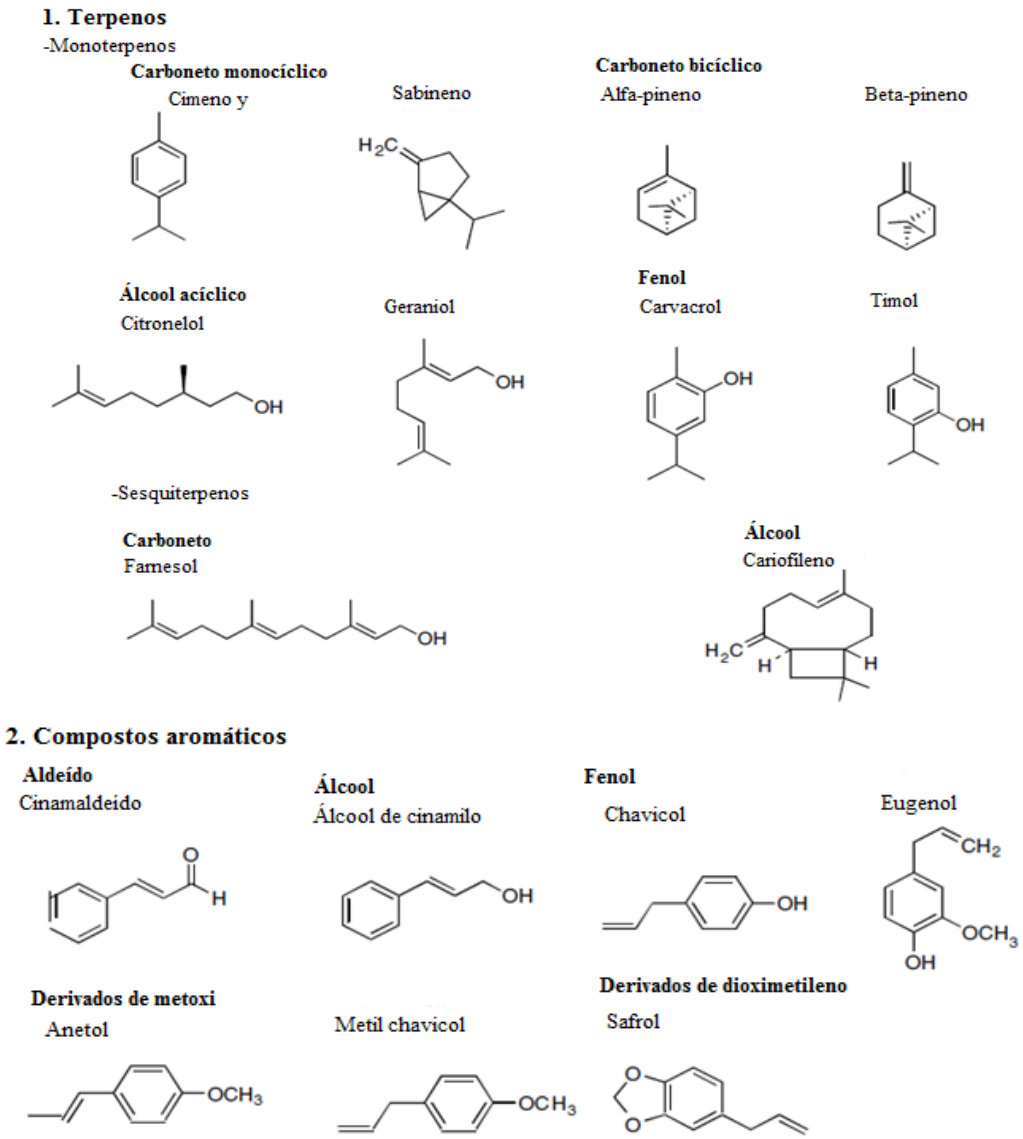
2.7.2 Composição química e métodos de extração dos OEs

Os componentes dos OEs podem ser divididos em dois grupos de origens biosintéticas distintas: (i) terpenos, e (ii) constituintes aromáticos, todos caracterizados por baixo peso molecular (Figura 27) (BAKKALI *et al*., 2008; DHIFI *et al*., 2016).

Vale ressaltar que a composição química dos OEs é determinada geneticamente, porém, ela pode ser modificada por vários fatores, como parte do vegetal (morfogenéticos), fase de denvolvimento do vegetal (oncogenéticos), fatores ambientais (abióticos e bióticos) (VERMA; SHUKLA, 2015), quimiotipo, época de colheita, forma de secagem do material, entre outros (BAKKALI *et al.*, 2008).

De acordo com Bakkali *et al*. (2008), os OEs podem conter cerca de 20 a 60 compostos em concentrações bastante distintas. Além disso, eles são caracterizados, geralmente, por dois ou três compostos majoritários em concentrações razoavelmente altas (20-70%) em comparação com outros componentes presentes em menores quantidades. Esses compostos majoritários podem estar relacionados com as atividades biológicas presentes nos óleos (BAKKALI *et al*., 2008). No entanto, outros autores atribuem as atividades biológicas a ação sinérgica entre os compostos (YOU *et al*., 2014), ou até mesmo a um composto específico (RIBEIRO *et al*., 2020). Também foi sugerido que a atividade dos compostos majoritários pode ser modulada por outros compostos em menores concentrações (BAKKALI *et al*., 2008).

Figura 27 - Estrutura química dos componentes dos OEs.



Fonte: Bakkali *et al.* (2008).

Em relação ao método para identificação química dos componentes dos OEs, a Cromatografia Gasosa acoplada à Espectrometria de Massas (CG-EM) vem sendo utilizada habitualmente em diversos trabalhos (KADHIM *et al*., 2016; RIBEIRO *et al*., 2020; SANTOS *et al*., 2017). Além desse método, a Cromatografia Líquida de Alta Eficiência (CLAE) também pode ser utilizada com a mesma finalidade (RIBEIRO, 2016a).

Os OEs podem ser obtidos por diferentes métodos de extração e é importante ressaltar que as propriedades desses óleos dependem do método de extração utilizado (RIBEIRO, 2016a). Esses métodos podem ser classificados em duas categorias: convencionais/métodos clássicos e inovadores/métodos avançados (ASBAHANI *et al.*, 2015; ROOHINEJAD *et al*., 2018).

Os métodos convencionais são normalmente baseados na destilação por aquecimento, sendo exemplos de métodos convencionais a hidrodestilação, arraste a vapor e prensagem a frio (ASBAHANI *et al.*, 2015). Por sua vez, os métodos inovadores objetivam reduzir o tempo de extração, uso de solvente, consumo de energia, emissões de CO_2, e ter a maior probabilidade de conservar a composição química dos OEs. Extração por fluido supercrítico, extração assistida por ultrassom e por micro-ondas, são exemplos de alguns métodos inovadores (ASBAHANI *et al.*, 2015).

A hidrodestilação é o método convencional menos complexo utilizado na extração de OEs. Nesse método o material vegetal é aquecido junto com água destilada dentro de um balão de vidro, no qual o vapor, proveniente do aquecimento do material e da água, passa por um condensador e a mistura de água e óleo, previamente condensada, vai para um decantador a fim de ser separada por densidade. Por sua vez, o arraste a vapor é um método clássico baseado no mesmo princípio da hidrodestilação, mas, nesse método não há contato direto entre o material vegetal e água (ASBAHANI *et al.*, 2015).

A prensagem a frio é um método de extração mecânica utilizado na extração de OEs de frutas cítricas. Durante o processo de extração as frutas são prensadas e delas é extraído tanto o OE, que anteriormente estava nas glândulas de armazenamento, quanto o suco. Em seguida, é feita uma centrifugação com a finalidade de separar o OE puro, que pode ser utilizado nas indústrias alimentícias e farmacêuticas, e como aromatizante (ASBAHANI *et al.*, 2015).

A extração por fluido supercrítico baseia-se no uso solventes em estado supercrítico, o que significa que eles estão sujeitos a temperaturas e pressões acima de seu ponto crítico (REYES-JURADO *et al*., 2015). O dioxido de carbono é um solvente bastante usado na extração por fluido supercrítico de compostos de plantas, pois ele não é tóxico, atinge o estado supercrítico em pressões relativamente baixas e perto da temperatura ambiente, além de não ser inflamável (ASBAHANI *et al.*, 2015). O processo de extração utilizando esse método consiste em duas etapas: (i) extração do componentes solúveis em um solvente supercrítico e (ii) separação dos solutos extraídos do solvente. Essa separação entre os compostos solúveis do solvente supercrítico pode ser realizada modificando as propriedades termodinâmicas do solvente supercrítico, alterando a temperatura ou pressão do sistema, por exemplo (REYES-JURADO *et al*., 2015).

Na metodologia de ultrassom o material vegetal é imerso em água ou em algum solvente orgânico e, concomitantemente é submetido à ação de ultrassons. Esssas ondas de ultrassom induzem a vibração mecânica das paredes e membranas dos vegetais e isso ocasiona a rápida

liberação dos OEs, com uma relativa dimuniação na temperatura empregada durante o processo de extração (ASBAHANI *et al.*, 2015; RIBEIRO, 2016a).

Na extração assistida por micro-ondas o aquecimento é instantâneo e ocorre no interior da amostra, levando a extrações muito rápidas. Nesse método a extração ocorre devido a alterações na estrutura celular causadas por ondas eletromagnéticas (REYES-JURADO *et al*., 2015). A aplicação da extração de OEs usando micro-ondas reduz drasticamente o tempo de extração e o volume de solvente necessário (ASBAHANI *et al.*, 2015).

2.7.3 Uso de OEs no controle dos hospedeiros intermediários da esquistossomose e de *A. aegypti*

Há uma diversidade de vegetais que possuem efeitos tóxicos contra moluscos e insetos, especialmente, entre as espécies da família Euphorbiaceae (PEREIRA *et al.*, 2017). Essas substâncias de origem vegetal, geralmente, são uma alternativa segura, biodegradável e de baixo custo quando comparado com a niclosamida.

Os OEs podem ser uma alternativa para o controle dos hospedeiros intermediários da esquistossomose e dos vetores da dengue, uma vez que, constituem uma fonte rica de compostos bioativos que são, geralmente, biodegradáveis e não-tóxicos, potencialmente adequados para uso em programas de controle (FONTES-JÚNIOR *et al.*, 2012). Vários óleos demostram efeito tóxico contra os caramujos do gênero *Biomphalaria*, hospedeiros intermediários da esquistossomose, e frente ao *A. aegypti*, como os citados na Tabela 5.

2.8 FAMÍLIA EUPHORBIACEAE

A família Euphorbiaceae é uma das mais complexas, maiores e mais diversas famílias de angiosperma. Ela é composta por cerca de 334 gêneros e mais de 8000 espécies, que estão distribuídas nas regiões tropical e sub-tropical, especialmente na África e América. Essa família é considerada uma importante fonte de compostos bioativos, como terpenos, taninos, alcaloides e esteroides (ISLAM *et al*., 2019; RAMALHO *et al*., 2018).

Tabela 5 - OEs com efeito tóxico para os hospedeiros intermediários da esquistossomose e *A. aegypti*

OEs	Parte vegetal/ Família	Efeito tóxico	Referências
Critus limon	Casca do fruta/ Rutaceae	Moluscicida frente a *B. glabrata* (CL_{50} = 13,18 µg/mL).	Jorge (2017)
Croton rhamnifolioides	Folhas/ Euphorbiaceae	Inseticida frente a larvas de *A. aegypti* no quarto instar (CL_{50} = 122,35 e 89,03 µg/mL, para os OEs fresco e armazenado, respectivamente) e efeito deterrente de oviposição para *A. aegypti*.	Santos *et al*. (2014)
Cymbopogon winterianus	Folhas/ Poaceae	Moluscicida frente a *B. tenagophila* (CL_{50} e CL_{100} = 60 e 80 µg/mL, respectivamente).	Costa *et al.* (2015)
Eugenia punicifolia	Partes aéreas/ Myrtaceae	Moluscicida frente a *B. glabrata* (CL_{50} e CL_{90} = 98,91 e 170,13 µg/mL, respectivamente).	Ribeiro *et al*. (2016b)
Hyptis dilatata	Partes aéreas/ Lamiaceae	Moluscicida frente a *B. glabrata* (CL_{50} e CL_{90} = 112,64 e 182,33 µg/mL, respectivamente).	Ribeiro *et al*. (2016b)
Lippia acutidens	Partes aéreas/ Verbenaceae	Moluscicida frente a *B. glabrata* (CL_{50} e CL_{90} = 76,08 e 98,5 µg/mL, respectivamente).	Ribeiro *et al*. (2016b)
Lippia gracilis	Folhas e partes aéreas/ Verbenaceae	Moluscicida frente a *B. glabrata* (CL_{50} = 62,2 µg/mL; (CL_{50} e CL_{90} = 19,09 e 27,41 µg/mL, respectivamente).	Teles *et al*. (2010), Ribeiro *et al*. (2016b)
Schinus terebinthifolius	Folhas/ Anacardiaceae	Inseticida frente larvas e pupas de *A. aegypti* (CL_{50} = 0,370 e 0,552 mg/mL e $CL_{99,9}$ = 2,30 e 2,361 mg/mL, respectivamente).	Bortolucci *et al*. (2019)
Syagrus coronata	Semente/ Arecaceae	Inseticida para larvas de *A. aegypti* no quarto instar (CL_{50} = 21,07 µg/mL) e efeito deterrente de oviposição para *A. aegypti*.	Santos *et al*. (2017)
Syzygium cumini	Folhas/ Myrtaceae	Moluscicida frente a *B. glabrata* (CL_{50} = 90 µg/mL).	Dias *et al*. (2013)
Porophyllum ruderale	Folhas/ Asteraceae	Moluscicida frente a *B. glabrata* (CL_{50} = 774,82 µg/mL).	Fontes-Júnior *et al.* (2012)
Zingiber officinale	Rizomas/ Zingiberaceae	Inseticida para larvas de *A. aegypti* no terceiro instar (CL_{50} = 76,07 µg/mL).	Gomes *et al*. (2016b)

Nota: CL_{50}: Concentração letal para 50% da população; CL_{90}: Concentração letal para 90% da população.

Ela compreende gêneros de grande importância econômica e com grande potencial de pesquisa, como *Hevea, Manihot*, *Ricinus* e *Croton*. São exemplos de espécies dos genêros citados acima: *Hevea brasiliensis*, conhecida popularmente como seringueira, *Manihot esculenta*, conhecida como macaxeira, aipim, mandioca e cassava, e *Ricinus communis*, conhecida como mamona (RIBEIRO, 2016a). Adicionalmente, o gênero *Croton* é um dos mais estudado devido a seus compostos biologicamente ativos (RAMALHO *et al*., 2018).

2.8.1 Gênero *Croton*

Croton é o mais diverso gênero da família Euphorbiaceae e possui cerca de 1300 espécies distribuídas, principalmente, na região tropical (BRITO *et al*., 2018). Cerca 350 espécies de *Croton* foram descritas no Brasil, e elas têm sido reportadas na Caatinga, Campos Rupestres, Campos Sulinos, Cerrado, Floresta Amazônica, Mata Atlântica, Campos de Altitude e Restingas (SECCO *et al*., 2012). Por sua vez, 35 espécies desse gênero ocorrem no estado de Pernambuco e a maior parte delas tem distribuição exclusiva da Caatinga (SILVA *et al.*, 2010).

Diversas espécies desse gênero são empregadas na medicina popular para tratar diversas enfermidades, como infecções, resfriados, febre, ansiedade, anorexia, distúrbios gastrointestinais, inflamações, úlceras, cólicas, câncer (BARRERA *et al*., 2016). Por exemplo, a espécie *Croton linearis* é utilizada para tratar febre associada a infecções, reduzir dor, tratar resfriados e possui efeito sedativo (DÍAZ *et al*., 2019). *Croton zehntneri*, conhecido popularmente como "canela da cunha", é empregado no tratamento de ansiedade, anorexia, disturbios gastrointestinais, possui efeito sedativo e estimula apetite (ALMEIDA *et al*., 2018). *Croton cajucara*, conhecido popularmente como "sacaca", é usada no tratamento de diabetes, colesterol alto e problemas gastroinstestinais (BARRERA *et al*., 2016).

Esses efeitos farmacológicos relatados em várias espécies do gênero *Croton* foram atribuídos a diferentes tipos de compostos, mas principalmente a terpenos, alcaloides, flavonóides e outros compostos fenólicos (MARCELINO *et al*., 2012). Adicionalmente, algumas delas são aromáticas, indicando a presença de OEs (ALMEIDA *et al*., 2018).

2.8.1.1 Composição química dos OEs do gênero *Croton*

A composição química dos OEs obtidos de espécies do gênero *Croton* é bem diversa. De acordo com Salatino e colaboradores (2007), esses óleos são constituídos usualmente por terpenos (mono e sesquiterpenos) e fenilpropanóides. Na Tabela 6 é possivel observar os

componentes majoritários dos OEs das folhas de algumas espécies do gênero *Croton*. O (*E*)-cariofileno e o germacreno D são os compostos mais representativos da classe dos sesquiterpenos nesses OEs.

Tabela 6 - Composição química dos OEs obtidos das folhas de algumas espécies do gênero *Croton*.

Espécies	Compostos majoritários	Referência
C. adamantinus	Metil-eugenol (14,81%), 1,8-cineol (13,74%) e (*E*)-cariofileno (5,80%).	Ximenes *et al.* (2013)
C. argyrophyllus	Espatulenol (22,80%), (*E*)-cariofileno (15,41%), α-pineno (14,07%), e biciclogermacreno (10,43%).	Cruz *et al*. (2017)
C. blanchetianus	Eucaliptol (16,9%), (*E*)-cariofileno (15,9%) e germacreno D (14,5%).	Rodrigues *et al*. (2019)
C. campestres	(*E*)-cariofileno (15,91%), 1,8-cineol (16,98%) e germacreno D (14,51%).	Oliveira-Tintino *et al*. (2018)
C. ceanothifolius	Biciclogermacreno (26,3%), germacreno D (14,7%) e (*E*)-cariofileno (11,7%).	Araújo *et al*. (2020)
C. cordiifolius	1,8-Cineol (25,09%) e α-felandreno (15,43%).	Nogueira *et al.*(2015)
C. conduplicatus (*C. heliotropiifolius*)	1,8-cineol (16,76 - 18,99%), α-felandreno (8,73 - 13,47%), biciclogermacreno (5,51 - 13,76%), (*E*)-cariofileno (6,95 - 9,75%) e espatulenol (3,98 - 7,80%).	Almeida *et al.* (2014)
C. grewioides	α-pinene (47,43%).	Medeiros *et al*. (2017)
C. linearis	Guaiol (7,93%), eudesma-4(15),7-dien-1β-ol (4,94%) e guaia-3,10(14)-dien-11-ol (4,52%).	Díaz *et al*. (2018)
C. rhamnifolioides	Espatulenol (22,46%) e1,8-cineol (18,32%).	Vidal *et al*. (2016)
C. rudolphianus	Composto desconhecido (40,9%), Metil chavicol (22.96%), (*E*)-cariofileno (4,22%), eugenol (4,03%), bicicloelemeno (3,96%), biclogermacreno (3,81%) e espatulenol (2,79%).	Ribeiro *et al*. (2020)
C. piauhiensis	(*E*)-cariofileno (21,01-34,69%), D-limoneno (13,47-16,35%), γ- terpineno (8-10,08%), germacreno D (8,71-10,42%).	Silva *et al*. (2019)
C. tetradenius	Cânfora (25,49%), γ-Terpineol (15,06%), α-terpinene (6,48%).	Carvalho *et al*. (2016)
C. zehntneri	Estragole (90,1 %).	Fonseca *et al.* (2019)

2.8.1.2 Atividades biológicas dos OEs do gênero *Croton*

Os OEs das espécies do gênero *Croton* possuem diversas atividades biológicas como podem ser observadas na tabela abaixo (Tabela 7).

Tabela 7 - Atividades biológicas dos OEs de espécies do gênero *Croton*.

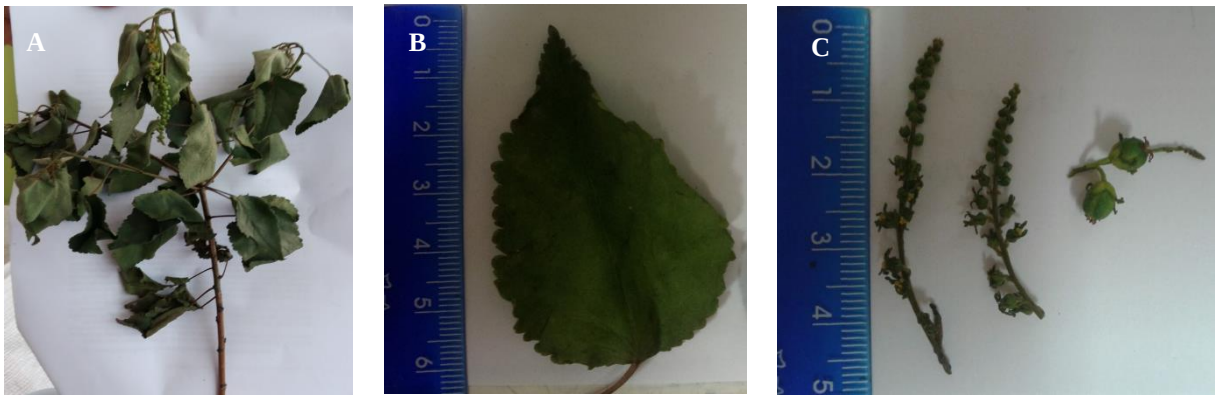
Espécies	Atividades biológicas	Referências
C. adamantinus (folhas)	Atividade antinociceptiva, cicatrizante e antimicrobiana.	Ximenes *et al.* (2013)
C. argyrophylloides (folhas)	Efeito inseticida contra ovos e larvas de *A. aegypti* (CL_{50} = 116,2 e 94,6 μg/mL, respectivamente). Deterrente de ovipiposição para *A. aegypti* (CL_{50} = 458 μg/mL).	Lima *et al.* (2013)
C. argyrophyllus (folhas e partes aéreas)	Efeito inseticida contra larvas terceiro e quarto instar de *A. aegypti* (CL_{50} e CL_{90} = 0,31 e 0,70 mg/mL; 5,92 e 8,94 mg/mL, respectivamente). Atividade antibacteriana e efeito antioxidante (DPPH e ABTS).	Cruz *et al*. (2017) e Brito *et al*. (2018)
C. campestris (folha)	Efeito antiproliferativo para as linhagens de câncer MCF-7 (mama), OVCAR-3 (ovário), 786-0 (rim), HT29 (cólon) e K-562 (leucemia) (IC_{50} = 8,61, 10,34, 30,85, 9,94 e 22,35 μg/mL, respectivamente)	Monteiro *et al*. (2017)
C. cordiifolius (folhas)	Atividade antinociceptiva	Nogueira *et al.* (2015)
C. grewioides (*C. zehntneri*) (folhas)	Efeito inseticida contra ovos, larvas terceiro instar e pulpas de *A. aegypti* (CL_{50} = 45,7, 26,2 e 456,6 μg/mL, respectivamente). Deterrente de ovipiposição para *A. aegypti* (CL_{50} = 45,3 μg/mL).	Lima *et al.* (2013)
C. heliotropiifolius (folhas e parte áerea)	Atividade inseticida frente *Tribolium castaneum*. Atividade antibacteriana contra *B. subtilis* e *S. aureus* (CMI = 62,5 e 500 μg/mL, respectivamente).	Magalhães *et al.* (2015), Araújo *et al*. (2017)
C. nepetaefolius (folhas)	Efeito inseticida contra ovos e larvas terceiro instar de *A. aegypti* (CL_{50} = 141,3 e 66,4 μg/mL, respectivamente).	Lima *et al.* (2013)
C. rhamnifolioides (folhas)	Ação moduladora de gentamicina e oxacilina para as espécies *E. coli* e *S. aureus*, amoxilina para *P. aeruginosa*. Atividade inseticida para larvas de *A. aegypti* (CL_{50} = 89.03 - 122.35 μg/mL). Efeito deterrente de oviposição para mosquistos de *A. aegypti* nas concentrações de 50 a 100 μg/mL.	Vidal *et al*. (2016), Santos et al. (2014)
C. sonderianus (folhas)	Efeito inseticida contra ovos, larvas e pulpas de *A. aegypti* (CL_{50} = 143,2, 54,5 e 494,9 μg/mL, respectivamente).	Lima *et al.* (2013)
C. piauhiensis (folhas)	Efeito inseticida contra larvas terceiro instar de *A. aegypti* (CL_{50} = 252,5 - 336,8 μg/mL).	Silva *et al*. (2019)
C. pulegiodorus (folhas)	Efeito inseticida frente *T. castaneum*.	Magalhães *et al.* (2015)
C. tetradenius (folhas)	Efeito inseticida contra larvas e mosquitos de *A. aegypti* (CL_{50} 0,152 e 1,842 mg/mL mg/mL, respectivamente).	Carvalho *et al*. (2016)
C. zambesicus (caule)	Atividade antibacteriana. Efeito antiproliferativo para as linhagens de câncer de cólon (HT29 e HCT116) e mama (MCF7 e MDA-MB23) (IC_{50} = 23,81, 25,40, 41,37 e 53,77 μg/mL, respectivamente).	Yagi *et al*. (2016)

Nota: DPPH: 1,1-difenil-2-picrilhidrazil, ABTS: 2,2'-azino-bis-(3-etilbenzotiazolina-6- acido sulfônico), CL_{50}: Concentração letal para 50% da população, CL_{90}: Concentração letal para 90% da população, CMI: Concentração mínima inibitória, IC_{50}: Inibição de crescimento para 50% da população.

2.8.2 A espécie *C. rudolphianus*

C. rudolphianus (Figura 28), popularmente conhecido como velame-branco, é uma espécie endêmica do Brasil pertencente à família Euphorbiaceae e à ordem Malpighiales (RIBEIRO *et al*., 2020). Ela é encontrada nos estados de Alagoas, Bahia, Ceará, Minas Gerais, Paraíba, Pernambuco, Piauí e Sergipe (FLORA DO BRASIL, 2020b), crescendo em vegetação de Caatinga, Campos Rupestres, Mata Atlântica e sobre solo argiloso com afloramento rochosos (SILVA *et al.*, 2010).

Figura 28 - *Croton rudolphianus* Müller Argoviensis.



Nota: **A**. ramos, **B**. folha, **C**.inflorências e fruto.
Fonte: Ribeiro (2016a).

Seus exemplares podem variar de 80 a 150 cm de altura, possuem látex translúscido, folhas alternas, frequentemente dispostas no ápice dos ramos, inflorescência solitária e racemiforme, flores estaminadas (3-4 mm) e pistiladas (3-5 mm), e possuem cápsulas orbicular e amarronzada. As sementes são elipsóides, rugosas e castanhas (SILVA *et al*., 2009).

O OE isolado das folhas frescas de *C. rudolphianus*, coletadas no Parque Nacional do Vale do Catimbau (Caatinga), apresentou atividade inseticida contra o gorgulho do milho, *Sitopilus zeamais*, usando diferentes metodologias, como toxicidade por ingestão (CL_{50}= 102.66 µL/g), contato (CL_{50}= 70.64 µL/mL) e fumigação (43.75% de mortalidade na contração de 64 µL/L). Esse óleo também apresentou atividade atrativa para *S. zeamais*, o que pode utilizado para montar armadilhas visando proteger as mercadorias armazenadas do gorgulho do milho (RIBEIRO *et al*., 2020). Além disso, o OE de *C. rudolphianus* apresentou atividade contra algumas bactérias fitopatogênicas, como *Pectobacterium carotovorum* subsp. carotovorum, *Ralstonia solanacearum*, *Xanthomonas campestris* pv. campestres, *Xanthomonas campestris*

pv. malvacearum, *Xanthomonas campestris* pv. viticola (RIBEIRO, 2016a). Os compostos majoritários desse OE estão descritos na Tabela 6

Outra família vegetal que possuem inúmeras espécies produtoras de OEs é a Myrtaceae. Os OEs das espécies dessa família, geralmente, apresentam atividades biológicas (YOKOMIZO; NOKAOKA-SAKITA, 2014).

2.9 FAMÍLIA MYRTACEAE

Myrtaceae é uma família de angiospermas que possui cerca de 140 gêneros e aproximadamente 6000 espécies distribuídas por toda região tropical (FRAUCHES *et al*., 2016; LUCAS *et al*., 2019). No Brasil, a família Myrtaceae é representada por 23 gêneros e mais 1020 espécies, e encontra-se distribuída em todas as regiões do país (FLORA DO BRASIL, 2020c).

Os membros dessa família são árvores ou arbustos com grande importância ecológica, uma vez que, suas flores e frutos carnudos são fonte de alimentos para diversos animais, como mamíferos, pássaros e insetos (STAGGEMEIER *et al*., 2017). Além disso, a família Myrtaceae possui grande valor econômico, pois várias espécies dessa família são usadas como alimento, que são tanto consumidas frescas, quanto usadas na confecção de sobremesas, geleias, vinhos, licores e vinagre (FRAUCHES *et al*., 2016).

Em adição, muitas espécies dessa família são utilizadas na medicina popular no tratamento de algumas doenças. O pó das sementes da azeitona-roxa (*Syzygium cumini*), por exemplo, é usado na medicina popular indiana no tratamento da diabetes de mellitus, inflamações, infecções e diarréia (FRAUCHES *et al*., 2016).

Os gêneros mais representativos dessa família são: *Eucalyptus* (300 espécies), *Malaleuca* (100 espécies), *Eugenia* (600 espécies), *Myrcia* (300 espécies), *Psidium* (100 espécies) e *Syzygium* (200 espécies) (FRAUCHES *et al*., 2016).

Existe um gênero da família Myrtaceae que é endêmico do Brasil e só ocorre em dois estados do Nordeste (Bahia e Pernambuco): *Algrizea* (FLORA DO BRASIL, 2020a). Esse gênero foi descrito em 2006 (PROENÇA *et al*., 2006) e possui duas espécies que ocorrem em regiões de altitudes, e podem crescer em terrenos rochosos.

Veras *et al.* (2019) caracterizaram o OE das folhas da espécie *Algrizea minor* e descreveram suas atividades biológicas. Esse óleo possuiu efeito antioxidante, antinociceptivo e antimicrobiano (VERAS *et al.*, 2019). No entanto, não há relatos para atividade biológicas com o OE de *Algrizea macrochlamys*.

2.9.1 A espécie *A. macrochlamys*

A. macrochlamys (Figura 29) é uma espécie endêmica do Nordeste do Brasil pertencente à família Myrtaceae e à ordem Myrtales. Ela é encontrada na Bahia e Pernambuco, crescendo em vegetação de Caatinga e Campos Rupestres (FLORA DO BRASIL, 2020d).

Seus exemplares são arbustos que variam de 100 a 250 centímetros de altura, possui folhas oposta e inflorescência tipo dicásio terminal ou sub terminal. As flores apresentam 2,5 a 7 mm de altura com cerca de 70 a 85 estames e ovulo bilocular com 3 a 6 óvulos por lóculo. Os frutos apresentam coloração vinho quando maduros com cerca de 2 sementes por fruto (PROENÇA *et al.*, 2006).

Figura 29 - *A. macrochlamys* (DC.) Proença & NicLugh.

Fonte: A autora (2020).

3 RESULTADOS

Os resultados dessa pesquisa estão apresentados na forma de artigos.

3.1 ARTIGO 1 - TOXIC EFFECT OF *Croton rudolphianus* ESSENTIAL OIL FROM LEAVES AGAINST *Biomphalaria glabrata* AND *Schistosoma mansoni* CERCARIAE

ARTIGO A SER SUBMETIDO AO PERIÓDICO ACTA TROPICA

Ingridd Ayslane Torres de Araújo Ribeiro[a], José Luiz Ferreira de Sá[a], Maíra de Vasconcelos Lima[b], Shyrlane Torres Soares Veras[c], Júlio César Ribeiro de Oliveira Farias de Aguiar[d], André L. Aires[e,f], Mônica C. P. A. Albuquerque[e,f], Márcia Vanusa da Silva[a], Ana Maria Mendonça de Albuquerque Melo[g], Daniela Maria do Amaral Ferraz Navarro[d], and Maria Tereza dos Santos Correia[a]

[a] *Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[b] *Departamento de Energia Nuclear, Centro de Tecnologia e Geociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[c] *Departamento de Engenharia Civil e Ambiental, Centro de Tecnologia e Geociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[d] *Departamento de Química Fundamental, Centro de Ciências Exatas e da Natureza, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[e] *Departamento de Medicina Tropical, Centro de Ciência da Saúde, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[f] *Laboratório de Imunologia Keizo Asami - LIKA, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[g] *Departamento de Biofísica e Radiobiologia, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

*Corresponding author. Tel: +558121268540, fax: +558121268576.

E-mail address: ingridd.torres@hotmail.com

Highlights

- *C. rudolphianus* EO from leaves possess lethal effects on adults and embryos of *B. glabrata*.
- For *B. glabrata* adults, LC_{50} and LC_{90} were 47.88 and 78.86 µg/mL, respectively.
- The oil presented genotoxic effect to *B. glabrata* hemocytes.
- Mortality to *S. mansoni* cercariae at all concentration tested was observed.
- Less toxicity for *A. salina* than for *B. glabrata* and *S. mansoni* was observed.

Abstract

This research investigated the effect of the *Croton rudolphianus* leaves essential oils (EO) on *Biomphalaria glabrata* embryos (on different development stages) and adults, *Schistosoma mansoni* cercariae. It was possible to identify 31 compounds in the *C. rudolphianus* EO through GC-MS analysis. The major compounds from this oil were (*E*)-caryophyllene (17.33%), unknown compound (16.87%), bicyclogermacrene (7.1%), δ-cadinene (6.62%) and germacrene D (5.38%). After incubation for 24 h, the EO of *C. rudolphianus* induced the occurrence of non-viable embryos (dead and malformed), with a LC_{50} value of 126.54, 133.51, 143.53 and 161.95 µg/mL and LC_{90} value of 202.61, 216.48, 232.98 and 271.16 µg/mL to blastulae, gastrulae, trochophore and veliger embryonic stages, respectively. The EO was more effective to *B. glabrata* adults (LC_{50} and LC_{90} = 47.89 and 78.86 µg/mL, respectively), and *S. mansoni* cercariae (LC_{50} and LC_{90} = 14.81 and 22.15 after 120 minutes of exposure, respectively) than *B. glabrata* embryos. Concerning to micronucleus assay, the mean frequency of apoptosis, binucleation and micronucleus were 45.33 ± 3.51, 19.33 ± 1.53 and 0.67 ± 0.58 per 1000 cells at 25 µg/mL, which is the highest concentration tested. The oil killed *A. salina* with a LC_{50} and LC_{90} values (68.33 and 111.5 µg/mL, respectively) higher than that determined for adult snails and *S. mansoni* cercariae. In conclusion, *C. rudolphianus* EO had toxic effect on *B. glabrata* adults and embryos, and *S. mansoni* cercariae. Besides, this oil showed to be genotoxic to hemocytes of *B. glabrata*. Concerning to non-target organism assay, *C. rudolphianus* EO was less toxic to *A. salina* then to adult snails and *S.mansoni* cercariae. Due to this, the EO from *C. rudolphianus* leaves is a potential alternative for schistosomiasis control. It would be remarkable to perform other tests with non-target species.

Keywords: embriotoxicity; lethal concentration; natural product; neglected diseases; schistosomiasis; molluscicidal agents.

1. Introduction

Schistosomiasis (or bilharziasis) is a parasitic illness caused by worms of the genus *Schistosoma* (Colley et al., 2014). This sickness is mostly found in tropical and sub-tropical regions, underprivileged populations without suitable sanitary conditions and drinkable water (WHO, 2019). According to World Health Organization (WHO, 2019), the schistosomiasis affects about 240 million people in the world, and around 700 million people live in risk zones for schistosomiasis.

Schistosoma mansoni is the main specie of the genus *Schistosoma* that infects humans in Africa, the Arabian Peninsula, and South America, which use *Biomphalaria* snails as intermediate host and causes hepatic and intestinal schistosomiasis (Araújo et al., 2018a; Rey, 2014; Scholte et al., 2014). In Brazil, the specie *Biomphalaria glabrata* is a vector of *S. mansoni* (Araújo et al 2018b) and its distributed in the northeast, north, southeast and south regions of this country (Scholte et al., 2012).

According to World Health Organization (WHO, 2019) the control of this disease involve chemotherapy, basic sanitary services, hygiene education and snails control. Nowadays, the niclosamide is the only compound recommended by WHO for use as a molluscicidal. Nevertheless, the utilization of this compound might lead to many disadvantages, such as, toxicity to non-target species (WHO, 2017), sunlight sensibility, elevated cost (Martins et al., 2014) and development of resistant population (WHO, 2017). In this sense, the search for natural and low cost molluscicidal agents is crucial.

Natural plants products, such as extracts, lectins and essential oils (EOs), are regarded valuable sources of new bioactive compounds (Nicoletti et al., 2016). Currently, some of this products have been tested about their molluscicidal activities (Gomes et al., 2019a; Gomes et al., 2019b; Rocha-Filho et al., 2015; Sá et al., 2016) and many of them possess toxic effect against snails, being a promising alternative to control of this animals.

EOs are complex compounds that are formed by the secondary metabolites from plants. These compounds are involved in the mechanism of defense against several natural enemies (Bakkali et al., 2008). Some EOs possess a range of biological activities, such as antimicrobial (Khalil et al., 2018), antioxidant (Avanço et al., 2017), insecticidal (Santos et al., 2017; Ribeiro et al., 2020). In fact, numerous researchers have described the effect of plants EOs against *B.*

glabrata, main intermediate host for transmission of schistosomiasis (Gomes et al., 2019a; Gomes et al., 2019b, Araújo et al., 2019).

Croton genus belongs to Euphorbiaceae family and presents approximately 1,300 species largely found in tropical regions (Brito et al., 2018). *Croton rudolphianus* Müll. Arg, is an endemic specie of Brazil and found from the Northeast to Southeast areas (Silva et al., 2010; Ribeiro et al., 2020). Thus, this study aimed to investigate the effect of the EO from *C. rudolphianus* leaves on adults and embryos of *B. glabrata* and cercariae *S. mansoni*, besides, its toxicity in non-target organisms (*Artemia salina* Leach).

2. Materials and Methods

2.1 Plant collection and extraction of the EO

The collection of *C. rudolphianus* leaves (Sisbio 26743-3) was performed in September 2016 at Parque Nacional do Catimbau (8°34'19.8"S 37°14'12.3"W), located in Pernambuco state, Brazil; under authorization of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). A sample (voucher number IPA 91.091) was placed at the Herbarium *Dárdano de Andrade Lima* (IPA, Recife, PE, Brazil).

The procedure to EO extraction was adapted from Ribeiro et al. (2020). A blender was used to triturate fresh leaves (300 g) with distilled water (3 L), and thereafter, this mixture was subject to hydrodistillation technique during 4 h. The EO was stowed in amber glass recipients at - 4°C until the chemical characterization and biological tests. The EO yield was determined as a percentage based on the plant weight (% w/w).

2.2 Chemical analysis of the EO

The chemical analysis from EO of *C. rudophianaus* leaves was executed using a gas chromatography (model 7890A; Agilent Technologies; Palo Alto, CA, USA) fitted out with an Agilent J&W non-polar HP-5ms™ column (30 m x 0.25 mm.; 0.25 μm film thickness) and connected to a selective mass detector (model 5975C; Agilent Technologies; Palo Alto, CA, USA). The oven temperature was programmed for 40 °C and increased by 4 °C /min until 230 °C where it was maintained for 5 min. It was used helium as a carrier gas at a flow rate of 1 mL/min, sustained at a constant pressure of 7.0 psi. The interface with MS was set at 230 °C;

quadrupole temperature was set at 150 °C; mass spectra were recorded at 70 eV in Electron impact ionization mode and scanned in the range *m/z* 35-350 at a speed of 1.0 s/scan.

An EO solution was prepared in n-hexane at 2 mg/mL, then 1 μL of this solution was injected in split mode (1:50). The identification of individual components was performed comparing previously reported retention index values gotten from co-injection of a standard solution of linear C9-C30 hydrocarbons (Sigma-Aldrich) obtained in second the equation of Van den Dool and Kratz (1963). Afterward, the MS results gotten for each component were compared with library of mass spectral from GC-MS system (MassFinder 4, NIST08 and Wiley Registry™ 9th Edition) and with spectra from Adams, 2009.

An aliquot of 1 μl of the sample solution was injected on a Trace GC Ultra gas chromatography (Thermo Scientific, Milan, Italy) equipped with a flame ionization detector (FID) (Thermo Scientific, Milan, Italy), a split/splitless injector and a VB-5 fused silica capillary column (ValcoBond 30m x 0.25 mm; film thickness 0.25 mm) (Valco Instruments Company Inc., Houston, TX, EUA), to quantify the relative amount of each compound in relation to the total amount of compounds in the oil. The temperature conditions of the oven was the same as reported above. It was employed nitrogen as carrier gas at a flow rate of 1 L/min and inlet pressure 30 psi. The detector and injector temperature were set to 250 °C. This process was conducted in splitless mode and repeated thrice.

2.3 Molluscicidal assay

2.3.1 Snails

B. glabrata adults, free from *Schistosoma mansoni* infection, were reared at the *Laboratório de Radiobiologia* of the *Centro de Biociências* of the *Universidade Federal de Pernambuco* (Recife, Brazil) in plastics aquarium (50 x 23 x 17 cm) with filtered water pH 7.0 and at temperature of 25 ± 2 ºC. Daily, the animals were fed with organic lettuce leaves, and the aquariums water were changed once a week. Polyethylene sheets (10 x 10 cm) were placed on aquarium where the mollusks were cultured to allow the snails oviposition.

2.3.2 Bioassays using embryos of *B. glabrata*

This assay was carried out according to Araújo et al. (2018a, 2018b). Briefly, intact egg masses containing 100 embryos at blastulae, gastrulae, trochophore and veliger (0-15, 24-39,

48-87 and 96-111 hours after spawning, respectively) (Kawano et al., 1992) stage were chosen in a stereomicroscope (Wild M3B, Heerbrugg, Switzerland) based on following criteria: intact eggs masses and one embryo per egg. These embryos were placed in Petri dishes (60 mm x 15 mm). Then, the eggs masses were exposed for 24h to a solution of *C. rudolphianus* oil prepared with 0.3% tween 80 at 50, 100, 150, 200, 250 and 300 µg/mL, filtered water (C-, negative control), 0.3% of tween 80 (CSC, co-solvent control) and 1 µg/mL niclosamide (Bayluscide®, Bayer) (NCL, positive control). After this period, the eggs were washed with filtrated water and putted in Petri dishes with filtered water at 25 ± 2 °C. During 7 consecutive days, the embryos were observed with a stereomicroscope to determine the number of viable and non-viable (dead or malformed) embryos. This bioassay had two independents experiments and it was replicated thrice (totalizing 600 embryos per treatment).

2.3.3 Bioassays using adults of *B. glabrata*

Molluscicidal bioassays of *C. rudolphianus* EO were carried out based on Silva et al. (2018). Groups of ten *B. glabrata* (*S. mansoni* negative) with 13 ± 1 mm shell diameter were putted in plastic container (60 mL) and exposed to EO solution (0.3% tween 80) at 12.5, 25, 50, 75 and 100 µg/mL for 48h at 25 ± 2 °C. In the control groups, the snails were treated to filtered water (C-, negative control), 0.3% of tween 80 (CSC, co-solvent control) and 1 µg/mL niclosamide (Bayluscide®, Bayer) (NCL, positive control). After that, the animals were washed with filtrated water, placed in a recipient full of filtrated water, fed with lettuce and observed daily for 7 days at 25 ± 2 °C to record the mortality rate. The death was confirmed when the snails show inactivity, discolored color in the body and shell, and heart beats absence. The test was carried out in triplicate for each treatment totalizing 30 snails per treatment.

2.3.4 Citotoxicity: micronucleus assay using hemocytes of *B. glabrata*

The micronucleus assay was conducted to assess the genotoxicity caused in hemocytes of *B. glabrata* adult snails that were exposed to sublethal concentrations of EO from *C. rudolphianus* leaves. This assay was carried out according to Silva et al. (2019), and it was composed by 5 treatments, filtrated water (C-, negative control), 0.3% of tween 80 (CSC, co-solvent control) and 15, 20 and 25 µg/mL of *C. rudolphianus* oil. Each treatment contained 5 snails, which were exposed for 48 hours. After this period, the mollusks were washed under filtrated water and dried with absorbent tissue paper. Then, the hemolymph collection was

realized by a puncture in the cephalopodal region with a pipette. Aliquots of 100 µL of hemolymph were deposited on microscopy slides with 100µL ethylenediaminetetraacetic acid (EDTA) in Ringer's solution 10mM. Subsequently, the slides were putted in humidified chamber at 25 ± 2 °C for 30 minutes. Afterwards, the hemocytes were fixed with 200 µL of glutaraldehyde in Ringer's solution at 1% for 10 minutes and then washed with Ringer's solution. After dried, the slides were stained with Giemsa (5% v/v) for 2 minutes, washed again with distilled water, and dried at 25 ± 2 °C. The slides were analyzed in optical microscope (Kyowa Medilux-12, Japan) with 100 x magnification. The morphological changes observed in the cells were classified: as apoptosis, binucleation and micronucleus, as described elsewhere (Silva et al., 2019). For each treatment was analyzed 1000 hemocytes. This test was performed in thrice totalizing 3000 cells per treatment.

2.4 Cercaridal activity

The cercaricide bioassays of the *C. rudolphianus* oil were carried out according to Silva et al. (2019). *B. glabrata* snails infected with *S. mansoni* (Belo Horizonte strain) were exposed to artificial light (60 W) during 2h for liberation of cercariae. An oil solution of *C. rudolphianus* was prepared using 0.3% tween 80 to achieve the final concentrations of 5, 10, 15, 20 and 30 µg/mL. A suspension of 100 cercariae was kept in a watch glass and exposed to those concentrations of oil. In the control groups, the cercariae were exposed to filtered water (pH 7.0) (C-, negative control), 0.3% of tween 80 (CSC, co-solvent control) and 1.0 µg/mL niclosamide (Bayluscide®, Bayer) (NCL, positive control). The assays were kept at 27 ± 2 ºC. The mortality of cercariae was observed with a stereomicroscope (Wild M3B, Heerbrugg, Switzerland) at 15, 30, 60 and 120 minutes after exposure to *C. rudolphianus* EO. The bioassay was performed in triplicate and repeated twice (totalizing 600 cercariae per treatment). The mortality rate and cercariae behaviour (motility, atypical rotations and vibrations) were registered. The LC_{50} and LC_{90} were calculated in the end of the assay after 120 minutes.

2.5 Bioassay using larvae of *A. salina*

The brine shrimp (*A. salina*) was used as non-target organism to evaluate the *C. rudolphianus* oil environmental toxicity. This test was executed in accordance with Araújo et al. (2018b). Briefly, 20 mg of eggs from *A. salina* were hatched in a plastic container with 400 mL of natural seawater at 25 ± 2 ºC, pH 8.0, under constant aeration for 48h. After this period,

ten larvae were treated with an oil solution of *C. rudolphianus* (0.3% tween 80) at concentrations 12.5, 25, 50, 75, 100 and 120 µg/mL diluted in natural sea water (5 ml). In the control groups, the larvae were treated to sea water (C-, negative control), 0.3% of tween 80 diluted in seawater (CSC, co-solvent control) and 1 µg/mL niclosamide (NCL, positive control). After 24 h, the mortality rate were recorded. Two independent assays were peformed in quadruplicate for each treatment totalizing 80 larvae per treatment.

2.6 Data analysis

The results were expressed by mean ± Standard Deviation (SD). The One-way fixed-effects ANOVA and Tukey's test (significance at $p < 0.05$) were obtained using the Graph prism 5.0 software for Windows (GraphPad Software, San Diego, California, USA). The lethal concentrations required to kill 50% and 90% of individuals (LC_{50} and LC_{90}) were obtained using the probit analysis with a reliability interval of 95% in StatPlus version 5.98 for Windows.

3. Results

3.1 Yield and chemical analysis of the EO

The EO from *C. rudolphianus* leaves had a yield of 0.96% (w/w; 2.88 g). It was possible to identified 31 compounds (Table 1) by GC-MS analysis. The majors compounds found in the *C. rudolphianus* oil were (*E*)-caryophyllene (17.33%), unknown compound (16.87%), bicyclogermacrene (7.1%), δ-cadinene (6.62%) and germacrene D (5.38%).

3.2 Toxicity to embryos and adults of *B. glabrata*

EO of *C. rudolphianus* promoted an increase in a dose dependent manner of the occurrence of non-viable embryos (dead and malformed) in all embryonic stages of *B. glabrata*. The total of non-viable embryos ranged from 5% to 100%. All embryonic stages showed statistic differences between the treatments. Blastulae and gastrulae stages were the most sensitive to *C. rudolphianus* oil, showing 100% of non-viable embryos at 250 and 300 µg/mL. On the other hand, embryos at veliger stage were less sensitive to this oil. Besides that, trochophore stage only present 100% of non-viable embryos at 300 µg/mL. Mortality reached 100% during NCL treatment at 1 µg/mL in all embryonic stages. On the other hand, C- and

CSC treatments showed a total of viable embryos higher than 98%. The LC_{50} and LC_{90} to blastulae, gastrulae, trochophore, and veliger are shown in (Table 2).

This EO was also toxic to *B. glabrata* adults in a dose dependent manner (Figure 1). The NCL and the treatment at 100 µg/mL of the *C. rudolphianus* oil resulted in 100% mortality of *B. glabrata* adults in 48h, while in C- and CSC was observed no mortality. At 12.5, 25, 50 and 75 µg/mL were observed a mortality rate of 10 ± 0, 16.67 ± 5.77, 53.33 ± 5.77 and 83.33 ± 5.77%, respectively. Statistical analysis revealed significant differences in mortality between the treatments ($F_{7,16} = 468.5$; $p < 0.0001$) and LC_{50} and LC_{90} are shown in (Table 3).

3.3 Micronucleus assay

The *C. rudolphianus* oil promoted an increase in a dose dependent manner of the occurrence of morphological changes, such as apoptosis and binucleation, in hemocytes of *B. glabrata.* The control groups only present apoptosis with a mean frequency of 1.67 ± 0.58 and 1.33 ± 0.58 per 1000 cells to C- and CSC, respectively (Figure 2a). The most abundant morphological alterations observed in the hemocytes treated with *C. rudolphianus* EO was apoptosis. The treatments at 15, 20 and 25 µg/mL showed a frequency of apoptotic cells of 8.33 ± 1.15, 21.67 ± 3.06 and 45.33 ± 3.51 per 1000 cells, which are statistically different to the control (Figure 2a) ($F_{4,10} = 217.3$; $p < 0.0001$). On the other hand, micronucleus was the less abundant morphological changes observed in this study. The mean frequency of micronucleus for all treatment with *C. rudolphianus* oil were the same (0.67 ± 0.58 per 1000 cells) (Figure 2b). For this morphological changes no difference between the treatments were observed ($F_{4,10} = 2$; $p = 0.17$). The mean frequency of binucleation found at 15, 20 and 25 µg/mL were 6 ± 1, 17.33 ± 2.08 and 19.33 ± 1.53 per 1000 cells, respectively, and this results were statistically similar in the last two concentrations (Figure 2c) ($F_{4,10} = 169.3$; $p < 0.0001$).

3.4 Toxicity to *S. mansoni* cercariae

The mortality of *S. mansoni* cercariae exposed to *C. rudolphianus* oil increased with the concentration and exposure time. These results are shown in the Table 4. After 15 minutes of exposure, the highest concentration (30 µg/mL) present 15.83% of mortality. In the end of this assay, after 120 minutes, the same concentration showed 100% mortality. Oppositely, the lowest concentration (5 µg/mL) only present significative mortality (8.17%) after 120 minutes. The death of cercariae was followed by structural separation of the cercariae in the tail and

body. Additionally, since the first minute of exposure it was observed changes in cercariae motility at all concentrations, such as, decrease in the motility and rotation in the own axis. The control groups (C- and CSC) showed absence of mortality, preservation of the body and tail and normal movements of swimming. However, the NCL group present 100% mortality after just one minute of exposure. After 120 minutes of exposure time all treatments showed significant differences when compared to the control ($F_{7,40} = 1497$; $p < 0.0001$). The LC_{50} and LC_{90} value calculated after 120 minutes of exposure time are shown in Table 3.

3.5 Toxicity to non-target organisms

The bioassay with non-target organisms revealed that *C. rudolphianus* EO was not toxic to *A. salina* at 12.5 µg/mL. However, above 25 µg/mL were found significant results. The NCL (1 µg/mL) and the treatment at 120 µg/mL of the *C. rudolphianus* oil resulted in 100% mortality of *A. salina*, while in C- and CSC had absence of mortality. At 12.5, 25, 50, 75 and 100 µg/mL were observed a mortality of 2.5 ± 4.63, 10 ± 7.56, 27.78 ± 6.77, 61.25 ± 8.35 and 81.25 ± 6.41%, respectively. Statistical analysis revealed significant differences in *A. salina* mortality between doses ($F_{8,63} = 572.2$; $p < 0.0001$). The LC_{50} and LC_{90} are shown in (Table 3).

4. Discussion

The EO from *C. rudolphianus* (1.14 %) obtained by Ribeiro et al. (2020) presents similar yield than the oil of *C. rudolphianus* from this study (0.96%). Several EOs from leaves of *Croton* species also possess similar yield than the obtained EO from *C. rudolphianus* leaves. For example, *Croton cajucara* (0.97%) (Chaves et al., 2006), *Croton cordiifolius* (0.81%) (Nogueira et al., 2015) and *Croton rhamnifolioides* (accepted name: *Croton heliotropiifolius*) (0.80%) (Santos et al., 2014).

The majoritarian compounds of *C. rudolphianus* oil obtained in this study were: (*E*)-caryophyllene, bicyclogermacrene, δ-cadinene, and germacrene D. Similar results were obtained by Ribeiro et al. (2020), where *C. rduolphianus* oil presented (*E*)-caryophyllene (4.22%) and bicyclogermacrene (3.81%) as some of its majoritarian compounds. Several EO's from *Croton* species possess the same majoritarian compounds than the obtained in this research, for example, (*E*)-caryophyllene was found in *Croton eriocladus* (24.1%), *Croton campestris* (23%), *Croton glandulosus* (8.9%), *Croton chaetocalyx* (7.1%) (Turiel et al., 2016) and *C. rhamnifolioides* (6.33%) (Santos et al., 2014). The *C. chaetocalyx, C. glandulosus, C.*

eriocladus and *C. campestris* showed bicyclogermacrene in the respectively percentage 13.9, 9.6, 5.2 and 4.7 % (Turiel et al., 2016). The δ-cadinene represents 8 and 4.8% of the oils from *C. chaetocalyx* (Turiel et al., 2016) and *C. cajucara* (Azevedo et al., 2014). The germacrene D was found in *C. campestris* (13.7%), *C. chaetocalyx* (9.3%), and *C. eriocladus* (17.9%) (Turiel et al., 2016).

The EO from *C. rudolphianus* leaves caused mortality in all stage of development of *B. glabrata* embryos. However, the initial embryonic stages (blastulae and gastrulae) are more susceptive to this oil than final stages (trochophore and veliger), as shown in the Table 2. The usnic acid from *Cladonia substellata* (lichen) (Araújo et al., 2018b), piplartine amide isolated from *Piper tuberculatum* (Rapado et al., 2013) and dichloromethane fraction from *Liagora farinose* (algae) extract (Miyasato et al., 2012) showed toxic effects to *B. glabrata* embryos. In the present study, the results were similar. The initial embryonic stages were more sensible to those substances and extract than the final stages. According to Rapado et al. (2011; 2013) and Miyasato et al. (2012), the higher sensibility might be associated to intensive cell proliferation activity at the beginning of embryos development. The potassium usnate from *C. substellata* (Araújo et al., 2018a) and ether extract of *Ramalina aspera* (lichen) (Silva et al., 2019) also present toxic effect in all development stage of embryos from *B. glabrata*. However, the initial embryonic stages were more resistant to those natural products than the final stages, contrary to the results obtained with *C. rudolphianus* oil.

The EO of *C. rudolphianus* also presented toxicity against *B. glabrata* adults with a LC_{50} and LC_{90} = 47.88 and 78.86 µg/mL, respectively, being below than the recommended by WHO (1983) ($LC_{90} \leq 100$ µg/mL), which is a very good result pointing that the *C. rudolphianus* oil might be used as a molluscicidal agent. Besides, the adult snails were more sensitive to *C. rudolphianus* oil then embryos. Others EO from leaves also present molluscicidal effect. For example, the EO from *Lippia gracilis* (Verbenaceae), which have (*E*)-caryophyllene as one of its major constituents same that *C. rudolphianus* oil, exhibited molluscicidal activity against *B. glabrata* adults with LC_{50} and LC_{90} of 62.2 and 82.8 µg/mL, respectively (Teles et al., 2010). The EOs from leaves of *Pimenta dioica* (Myrtaceae) and rhizome of *Zingiber officinale* (Zingiberaceae) also presented lethal effect against *B. glabrata* (LC_{50} = 18.62 and 56.23 µg/mL, respectively; Gomes et al., 2019a; Gomes et al., 2019b).

Many species from Euphorbiaceae family showed molluscicidal activity (WHO, 1983), for example, the extracts from seeds and leaves of *Croton macrostachyus* using different solvents (water, ethanol, petroleum ether and chloroform) were toxic to *Biomphalari*a sp. and *Bulinus* sp. (Garoy et al., 2017). The crude methanolic, n-hexane and ethyl acetate extracts from

aerial parts of *Euphorbia laurifolia* present toxic effect to *B. glabrata* (LC_{50}= 8.89, 9.43 and 5.57 µg/mL; LC_{90}= 11.60, 12.32 and 7.28 µg/mL, respectively) (Mogollón-Morales et al., 2016). The latex of *Euphorbia umbellata* presented lethal effects to *B. glabrata* (LC_{50} value of 1.36 µg/mL and LC_{90} value of 3.69 µg/mL) (Pereira et al., 2017). The latex from *Euphorbia milii* showed toxic effect against *B. glabrata* adults and in embryos (LC_{50}= 0.27 and 34.03 µg/mL, respectively; Oliveira-Filho et al., 2010; Oliveira-Filho; Paumgartten, 2000). Similar to results found in this research, the *B. glabrata* adults were more sensitive to *E. milii* latex than embryos. This fact might be related with egg gelatinous capsule and egg membrane, which provide some protection for embryo, since to achieve the embryos the substances should be capable to get inside in the egg gelatinous capsule and cross the egg membrane (Miyasato et al., 2012).

The micronucleus assay has been used as an index for genotoxic damage in various organisms, such as fish, amphibians and mollusks, exposed to several substances (Rocha; Rocha, 2016). The results have shown that *C. rudolphianus* oil present genotoxic effect to hemocytes of *B. glabrata* at all concentrations tested, due to the presence of cells with morphological changes, such as apoptosis and binucleation. None study was found with hemocytes of *B. glabrata* treated with EOs, for this reason the results of micronucleus assay realized in this reasearch with hemocytes of *B. glabrata* were compared with other substances.

The apoptosis process occurs in both pathological and physiological circumstances (Oral et al., 2016). This point can explain the fact of apoptosis had been the only morphological alteration found in the control groups, probably as physiological process. However, it also was noticed that the number of such apoptotic cells increased with *C. rudolphianus* oil concentrations, which might be related with a pathological process. The apoptosis observed in the hemocytes of *B. glabrata* might be linked to the presence of terpenes, such as (*E*)-caryophyllene, bicyclogermacrene, germacrene D, once those compounds increase the permeability and fluidity of the cells membranes, leading to uncontrolled efflux of metabolites and ions and even to cell leakage, resulting in cell death by apoptosis (Wink, 2015). Similar results were found using ether extract of *R. aspera* in hemocytes of *B. glabrata*, the number of apoptotic cells increased with the concentration (Silva et al., 2019). This extract at 6.5, 7.5 and 8.5 µg/mL showed a frequency of apoptotic cells of 32.66, 81.66 and 364.33 per 1000 cells (Silva et al., 2019). The oxyfluorfen (herbicide) also showed the presence of apoptotic cells in *B. glabrata* hemocytes, at 0.5 µg/mL showed a frequency of apoptotic cells of 37.75 per 1000 cells (Lima et al., 2019).

Micronucleus occurs when a chromosome or chromosome fragment is not incorporated into one of the nuclei during the process of cell division (Minhas et al., 2016). The cells that possess micronucleus have suffered unrepaired DNA damage, and they are considered unviable (Hintzsche et al., 2012). In this study, it was not possible to observe a significant difference between the micronucleus frequency of the controls and the treatments with EO. Similar results were obtained in erythrocytes of *Tilapia rendalli* exposed to 5 and 15 µg/mL of Roundup® (herbicide) during 5 and 10 days (Pires et al., 2018). The oxyfluorfen and ether extract of *R. aspera* promoted the presence of micronucleus in hemocytes of *B. glabrata* (Lima et al., 2019; Silva et al., 2019). At 0.5 µg/mL of oxyfluorfen was possible to observe a micronucleus frequency of 1.8 per 1000 cells (Lima et al., 2019). The extract of *R. aspera* at 6.5, 7.5 and 8.5 µg/mL showed a micronucleus frequency of 2.33, 5.66 and 3.66 per 1000 cells (Silva et al., 2019).

Binucleation, which is the presence of two nuclei in a cell, occurs when the nucleus divides without cytoplasmic division. This occurs due to some damage in the cell membrane, such as peroxidation (Minhas et al., 2016). In this study, the frequency of binucleation increase with the *C. rudolphianus* EO concentration with the highest mean frequency of binucleated cells was 19.33 per 1000 cells at 25 µg/mL. The oxyfluorfen also presented similar results, at 0.25 and 0.5 µg/mL showed a binucleation frequency of 4 and 11.4 per 1000 cells, respectively in hemocytes of *B. glabrata* (Lima et al., 2019). On the other hand, the frequency of binucleated cells in *B. glabrata* hemocytes decrease with the concentration of the ether extract of *R. aspera* increased (Silva et al., 2019).

In addition to its molluscicidal and genotoxic effects to *B. glabrata*, the EO from *C. rudolphianus* leaves also showed lethal effect against *S. mansoni* cercariae (LC_{50} and LC_{90} = 14.81 and 22.15 µg/mL, respectively), which is the infectious form for humans. (Colley et al., 2014). EO from *C. rudolphianus* leaves also caused changes in cercariae motility, decrease in the motility and rotation in the own axis. According to Araújo et al. (2018a), these characteristics are not compatible with the cercariae infectious ability. Therefore, *C. rudolphianus* oil can be considered a significant tool for of the schistosomiasis. Other natural products showed effect to *S. mansoni* cercariae as potassium usnate (Araújo et al., 2018b) and divaricatic acid (Silva et al., 2018), with LC_{50} calculated after 120 minutes of expouse of 0.71 and 0.89 µg/mL, respectively, which is a better result than the one found in this study. The curcumin, a compound isolated from *Curcuma longa*, showed toxic effect to *S. mansoni* cercariae with LC_{50} and LC_{90} of 6.36 and 23.80 µg/mL after 60 minutes (Matos et al., 2020). The kaurenoic acid isolated from *C. floribundus* extract also present toxic effect to *S. mansoni*

cercariae, at 10 µg/mL during 30 minutes of exposure was observed 99.5% of mortality (Medina et al., 2009).

C. rudolphianus oil was more toxic to *B. glabrata* adults (LC_{50} and LC_{90} of 47.88 and 78.86 µg/mL, respectively) and *S. mansoni* cercariae (LC_{50} and LC_{90} of 14.81 and 22.15 µg/mL, respectively) than *A. salina* (LC_{50} and LC_{90} of 68.33 and 111.5 µg/mL, respectively). Hence, it might be possible use of *C. rudolphianus* EO as a molluscicide and cercaricide agent. Similar results were observed in the EOs from *Syzygium cumini* (Dias et al., 2013) and *Cymbopogon winterianus* (Rodrigues et al., 2013), which present molluscicidal effects to *B. glabrata* snails (LC_{50} = 90 and 54 µg/mL, respectively) and showed a lower toxicity to *A. salina* with LC_{50} of 175 and 181 µg/mL, respectively.

5. Conclusion

C. rudolphianus EO presented toxicity to adults and embryos in all development stage of *B. glabrata*. As well as, it also caused mortality in *S. mansoni* cercariae, which is the phase that infects human. Furthermore, it was less toxic to non-target organism (*A. salina*) than to *B. glabrata* adults and *S. mansoni* cercariae. Due to it, this EO might be a promising alternative to control schistosomiasis.

Competing interest

The authors declare no conflict of interest.

Acknowledgments

We thank the CNPq, CAPES, and FACEPE for financial support; CNPq and Capes provided PhD and Master fellowships for the students involved in this work. We also thank the parasitology laboratory from UFPE for assisting with cercariae bioassay.

References

Adams, R.P. 2009. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publishing Co. 804 p.

Araújo, F.P.; Albuquerque, R.D.D.G.; Rangel, L.S.; Calda, G.R.; Tietbohl, L.A.C.; Santos, M.G.; Ricci-Júnior, E.; Thiengo, S.; Fernandez, M.A; Santos, J.A.A.; Faria, R.X.; Rocha, L. 2019. Nanoemulsion containing essential oil from *Xylopia ochrantha* Mart. produces molluscicidal effects against different species of *Biomphalaria* (Schistosoma hosts). Memorias do Instituto Oswaldo Cruz, 114, 1-8.

Araújo, H.D.A.; Melo, A.M.M.A.; Siqueira, W.N.; Martins, M.C.B.; Aires, A.L.; Albuquerque, M.C.P.A.; Silva, N.H.; Lima, V.L.M. 2018a. Potassium usnate toxicity against embryonic stages of the snail *Biomphalaria glabrata* and *Schistosoma mansoni* cercariae. Acta Tropica, 188, 132-137.

Araújo, H.D.A.; Silva, L.R.S.; Siqueira, W.N.; Fonseca, C.S.M.; Silva, N. H.; Melo, A.M.M.A.; Martins, M.C.B.; Lima, V. L.M. 2018b. Toxicity of Usnic Acid from *Cladonia substellata* (Lichen) to embryos and adults of *Biomphalaria glabrata*. Acta Tropica, 179, 39-43.

Avanço, G. B.; Ferreira, F.D.; Bomfiim, N.S.; Santos, P.A.S.R; Peralta, R.M.; Brugnari, T.; Mallmann, C.A.; Filho Abreu, B. A.; Mikcha, J.M.; Machinski Jr.; M. 2017. *Curcuma longa* L. essential oil composition, antioxidant effect, and effect on *Fusarium verticillioides* and fumonisin production. Food Control, 73, 806-813.

Azevedo, M.M.B.; Almeida, C.A.; Chaves, F.C.M.; Campos-Takaki, G.M.; Rozental, S.; Bizzo, H.R.; Alviano, C.S.; Alviano, D.S. 2014. Effects of 7-hydroxycalamenene isolated from *Croton cajucara* essential oil on growth, lipid content and ultrastructural aspects of *Rhizopus oryzae*. Planta Medica, 80, 550-556.

Bakkali, F., Averbeck, S., Averbeck, D.; Idaomar, M. 2008. Biological effects of essential oils - A review. Food and Chemical Toxicology, 46, 446-475.

Brito, S.S.S.; Silva, F.; Malhheiro, R.; Baptista, P.; Pereira, J.A. 2018. *Croton argyrophyllus* Kunth and *Croton heliotropiifolius* Kunth: Phytochemical characterization and bioactive properties. Industrial Crops and Products, 113, 308-315.

Chaves, F.C.M., Bizzo, H.R., Angelo, P.C.S., Xavier, J.J.B.N., Sá Sobrinho, A.F. 2006. Rendimento e composição química do óleo essencial de folhas de dois morfotipos de sacaca (*Croton cajucara* Benth.). Revista Brasileira de Plantas Medicinais, 8, 117-119.

Colley, D. G.; Bustinduy, A. L.; Secor, W. E.; King, C. H. 2014. Human schistosomiasis. Lancet, 383, 2253–2264.

Dias, C.N.; Rodrigues, K.A.F.; Carvalho, F.A.A.; Carneiro, S.M.P.; Maia, J.G.S.; Andrade, E.H.A.; Moraes, D.F.C. 2013. Molluscicidal and Leishmanicidal Activity of the leaf Essential Oil of *Syzygium cumini* (L.) Skeels from Brazil. Chemistry & Biodiversity, 10, 1133-1141.

Garoy, E.Y.; Goje, T.; Mebrahtu, D.; Kaushik, A.; Asfaha, R.; Andemariam, A.; Brhane, D.; Efream, E.; Teweldebrhan, K.; Araya, Z. 2017. Evaluation of Molluscicidal Activity of *Croton microstachyus* and *Cissus quadrangularis* against *Biomphalaria* and *Bulinus* species of snails. European Journal of Medicinal Plants, 14, 1-8.

Gomes, P.R.B.; Reis, J. B.; Fernandes, R.P.; Mouchrek Filho, V.E.; Souza, A.G.; Fontenele, M.A.; Silva, J.C. 2019a. Toxicity and molluscicidal activity of the essential oil *Pimenta dioica* against the snail *Biomphalaria glabrata*. Revista Peruana de Biologia, 26, 101-108.

Gomes, P.R.B.; Fernandes, R.P.; Reis, J.B.; Fontenele, M.A.; Freitas, A.C.; Silva, J.C.; Oliveira, R.W.S.; Lyra, W.S.; Paula, M.L. Louzeiro, H.C.; Mouchrek Filho, V. 2019b. Molluscicidal Activity of the Essential Oil of *Zingiber officinale* Roscoe Rhizomes. Journal of Essential Oil Bearing Plants, 22, 526-534.

Hintzsche, H.; Polat, B.; Schewe, V.; Djuzenova, C. S.; Pfreundner, L.; Flentke, M.; Stopper, H. 2012. Micronucleus formation kinetics in buccal mucosa cells of head and neck cancer patients undergoing radiotherapy. Toxicology Letters, 212, 33-37.

Lima, M.V.; Siqueira, W.N.; Silva, H.A.M.F.; Lima Filho, J.M.; França, E.J.; Melo, A.M.M.A. 2019. Cytotoxic and genotoxic effect of oxyfluorfen on hemocytes of *Biomphalaria glabrata*. Environmental Science and Pollution Research, 26, 3350-3356.

Kawano, T., Okazaki, K., Ré, L. 1992. Embryonic development of *Biomphalaria glabrata* (Say, 1818) (Mollusca, Gastropoda, Planorbidae): A practical guide to the main stages. Malacologia, 34, 25-32.

Khalil, N.; Ashour, M. Fikry, S.; Singab, A.N.; Salama, O. 2018. Chemical composition and antimicrobial activity of the essential oils of selected Apiaceous fruits. Future Journal of Pharmaceutical Sciences, 4, 88-92.

Martins, M.C.B.; Silva, M.C.; Silva, L.R.S.; Lima, V.L.M.; Pereira, E.C.; Falcão, E.P.S.; Melo, A.M.M.A.; Silva, N.H. 2014. Usnic Acid Potassium Salt: An Alternative for the Control of *Biomphalaria glabrata* (Say, 1818). Plos one, 9, 1-6.

Matos, J.L.; Silva, K.R.; Lima, P.L.A.; Cunha, W.R.; Ramos, S.B.; Rodrigues, V.; Cabral, F.J.; Magalhães, L.G. 2020. Molluscicidal and cercaricidal activities of curcumin on *Biomphalaria glabrata* and *Schistosoma mansoni* cercariae. Pest Management Science, 76, 1228-1234.

Medina, J. M.; Peixoto, J. L. B.; Silva, A. A.; Haraguchi, S. K.; Falavigna, D. L. M.; Zamuner, M. L. M.; Sarragiotto, M. H.; Vidotti, G. J. 2009. Evaluation of the molluscicidal and *Schistosoma mansoni* cercariae activity of *Croton floribundus* extracts and kaurenoic acid. Revista Brasileira de Farmacognosia, 19, 207-211.

Minhas, S.; Kashif, M.; Nagi, A. H. 2016. Evaluation of Various Nuclear Cytological Changes in Normal Buccal Mucosa and Peritumoral Area in Patients with Oral Squamous Cell Carcinoma Receiving Concomitant Chemoradiotherapy. Pathology Research International, 2016, 1-8.

Miyasato, P. A.; Kawano, T.; Freitas, J. C.; Berlinck, R. G. S.; Nakano, E.; Tallarico, L. F. 2012. Molluscicidal activity of some marine substances against the snail *Biomphalaria glabrata* (Mollusca, Planorbidae). Parasitology Research, 110, 1873-1879.

Mogollón-Morales, J.A.; Nieves, E.; Rondón, M.; Rondón-Rivas, M.E. 2016. Molluscicidal property of *Euphorbia laurifolia* A. Juss (Euphorbiaceae) against *Biomphalaria glabrata* Say intermediate host of Schistosoma mansoni. Avances en Biomedicina, 5, 83-89.

Nicoletti, M.; Murugan, K.; Canale, A.; Benelli, G. 2016. Neem-borne molecules as eco-friendly control tools against mosquito vectors of economic importance. Current Organic Chemistry, 20, 2681 - 2689.

Nogueira, L.M., Silva, M.R., Santos, S.M., Alburquerque, J.F.C., Ferraz, I.C., Albuquerque, T.T., Motta, C.R.F.C., Araújo, R.M., Viana, G.S.B., Martins, R.D., Havt, A., Ximenes, R.M. 2015. Antinociceptive effect of the essential oil obtained from the leaves of *Croton cordiifolius* Baill. (Euphorbiaceae) in mice. Evidence-Based Complementary and Alternative, 2015, 1-7.

Oral, O.; Akkoc, Y.; Bayraktar, O.; Gozuacik, D. 2016. Physiological and pathological significance of the molecular cross-talk between autophagy and apoptosis. Histology and histopathology: cellular and molecular biology, 31, 479-498.

Oliveira-Filho, E.C.; Paumgartten, F.J.R. 2000. Toxicity of *Euphorbia milii* Latex and Niclosamide to Snails and Nontarget Aquatic Species. Ecotoxicology and Environmental Safety, 46, 342-350.

Oliveira-Filho, E.C; Geraldino, B.R.; Coelho, D.R.; Carvalho, R.R.; Paumgartten, F.J. R. 2010. Comparative toxicity of *Euphorbia milii* latex and synthetic molluscicides to *Biomphalaria glabrata* embryos. Chemosphere, 81, 218-227.

Pereira, L.P.L.A.; Dias, C.N.; Miranda, M.V.; Firmo, W.C.A.; Rosa, C.S.; Santod, P.F.; Brito, M.C.A.; Araruna, F.O.S.; Araruna, F.B.; Silva-Souza, N.; Coutinho, D.F. 2017. Molluscicidal effect of *Euphorbia umbellata* (Pax) Bruyns latex on *Biomphalaria glabrata*, *Schistosoma mansoni* host snail. Journal of the São Paulo Institute of Tropical Medicine, 59, 1-5.

Pires, A. A; Manelli-Oliveira, R.; Boschini-Filho, J. 2018. Evaluation of micronuclei frequencies and other nuclear alterations in erythrocytes of *Tilapia rendalli* (Perciformes: Cichlidae) induced by roundup herbicide. Journal of FisheriesSciences.com, 12, 29-32.

Rapado, L.N; Nakano, E.; Ohlwiler, F.P.; Kato, M.J.; Yamaguchi, L.F.; Pereira, C.A.; Kawano, T. 2011. Molluscicidal and ovicidal activities of plant extracts of the Piperaceae on *Biomphalaria glabrata* (Say, 1818). Journal of Helminthology, 85, 66-72.

Rapado, L. N.; Pinheiro, A. S.; Lopes, P. O. M. V.; Fokoue, H. H.; Scotti, M. T.; Marques, J. V.; Ohlweiler, F. P.; Borrelly, S. I.; Pereira, C. A. B.; Kato, M. J.; Nakano, E.; Yamaguchi, F. 2013. Schistosomiasis control using Piplartine against *Biomphalaria glabrata* at different developmental stages. Plos neglected tropical diseases, 7, 1-8.

Rey, L., 2014. Parasitologia e Doenças Parasitárias do Homem nos Trópicos Ocidentais. 4ª Ed. Guanabara Koogan. Rio de Janeiro.

Ribeiro, I.A.T.A.; Silva, R.; Silva, A.G.; Milet-Pinheiro, P.; Paiva, P.M.; Navarro, D.M.A.F.; Silva, M.V.; Napoleão, T.H.; Correia, M.T.S. 2020. Chemical characterization and insecticidal effect against *Sitophilus zeamais* (maize weevil) of essential oil from *Croton rudolphianus* leaves. Crop Protection, 129, 1-7.

Rocha-Filho, C.A.A.; Albuquerque, L.P.; Silva, L.R.S.; Silva, P.C.B.; Coelho, L.C.B.B.; Navarro, D.M.A.F.; Albuquerque, M.C.P.A.; Melo, A.M.M.A; Napoleão, T.H.; Pontual, E.V.; Paiva, P.M.G. 2015. Assessment of toxicity of *Moringa oleifera* flower extract to *Biomphalaria glabrata*, *Schistosoma mansoni* and *Artemia salina*. Chemosphere, 132, 188-192.

Rocha, S.M.; Rocha, C.A.M. 2016. Micronucleus test in bivalve mollusks as an important tool for xenobiotic exposure risk assessment. Acta of Fisheries and Aquatic Resources, 4, 70-79.

Rodrigues, K.A.F.; Dias, C.N.; Amaral, F.M.M.; Moraes, D.F.C.; Mouchrek-Filho, V.E.; Andrade, E.H.A.; Maia, J.G. 2013. Molluscicidal and larvicidal activities and essential oil composition of *Cymbopogon winterianus*. Pharmaceutical Biology, 51, 1293-1297.

Sá, J.L.F.; Siqueira, W.N.; Silva, H.A.M.F.; Santos, M.L.O.; Santos, F.T.J.; Silva, L.R.S.; Cabral, D.L.V ; Bezerra, I.C.F.; Soares, L.A.L.; Melo, A.M.M.A 2016. Evaluation of molluscicidal activity of *Anadenanthera colubrina* extracts on adult mollusc and embryos of the species *Biomphalaria glabrata* (Say, 1818). Scientia Plena, 12, 1-9.

Santos, G.K.N., Dutra, K.A., Lira, C.S., Lima, B.N., Napoleão, T.H., Paiva, P.M.G., Maranhão, C.A., Brandão, S.S.F., Navarro, D.M.A.F. 2014. Effects of *Croton rhamnifolioides* essential oil on *Aedes aegypti* Oviposition, larval toxicity and trypsin activity. Molecules. 19, 16573-16587.

Santos, L. M. M.; Nascimento, J. S.; Santos, M. A.G.; Marriel, N. B.; Bezerra-Silva, P. C.; Rocha, S. K. L.; Silva, A.G.; Correia, M.T.S.; Paiva, P. M. G.; Martins, G. F.; Navarro, D. M. A. F.; Silva, M. V.; Napoleão, T. H. 2017. Fatty acid-rich volatile oil from *Syagrus coronata* seeds has larvicidal and ovipositiondeterrent activities against *Aedes aegypti*. Physiological and Molecular Plant Pathology, 100, 35-40.

Scholte, R.G.C., Carvalho, O.S., Malone, J.B., Utzinger, J., Vounatsou, P., 2012. Spatial distribution of *Biomphalaria* spp., the intermediate host snails of *Schistosoma mansoni* in Brazil. Geospatial Health, 6, 95-101.

Scholte, R.G.C., Gosoniu, L., Malone, J.B., Chammartin, F., Utzinger, J., Vounatsou, P., 2014. Predictive risk mapping of schistosomiasis in Brazil using Bayesian geostatistical models. Acta Tropica, 132, 57-63.

Silva, H.A.M.F.; Sá, J.L.F.; Siqueira, W.N.; Lima, M.V.; Martins, M.C.B.; Aires, A.L.; Albuquerque, M.C.P.A.; Falcão, E.P.S.; Buril, M.L.L; Pereira, E.C.; Melo, A.M.M.A.; Silva, N.H. 2019. Toxicological effects of *Ramalina aspera* (lichen) on *Biomphalaria glabrata* snails and *Schistosoma mansoni* cercariae. Acta Tropica, 196, 172-179.

Silva, H.A.M.F.; Siqueira, W.N.; Sá, J.L.F.; Martins, M.C.B.; Aires, A.L.; Amâncio, F.F.; Pereira, E.C.; Albuquerque, M.C.P.A.; Melo, A.M.M.A.; Silva, N.H. 2018. Laboratory assessment of divaricatic acid against *Biomphalaria glabrata* and *Schistosoma mansoni* cercariae. Acta Tropica, 178, 97-102.

Silva, J.S., Sales, M.F., Gomes, A. P. S., Carneiro-Torres, D.S. 2010. Sinopse das espécies de *Croton* L. (Euphorbiaceae) no estado de Pernambuco, Brasil. Acta Botonica Brasilica. 24, 441-153.

Teles, T.V.; Bomfim, R.R.; Alves, P.B.; Blank, A.F.; Jesus, H.C.R.; Quintans-Júnior, L.J.; Serafini, M.R.; Bonjardim, L.R.; Araújo, A.A.S. 2010. Composition and evaluation of the lethality of *Lippia gracilis* essential oil to adults of *Biomphalaria glabrata* and larvae of *Artemia salina*. African Journal of Biotechnology, 9, 8800-8804.

Turiel, N.A.; Ribeiro, A.F.; Carvalho, N.C.C.; Monteiro, O.S.; Lucas, F.C.A.; Carreira, L.M.M.; Andrade, E.H.A.; Maia, J.G.S. 2016. Variability in Essential Oil Composition of *Croton* Species with Occurrence in the Eastern Brazilian Amazon. Records of Natural Products, 10, 380-384.

Van Den Dool, H., Kratz, P.D. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography A, 11, 463-471.

Wink, M. 2015. Modes of action of herbal medicines and plant secondary metabolites. Medicines, 2, 251-286.

World Health Organization, 1983. The control of schistosomiasis: Second report of the. WHO Expert Committee. World Health Organization, Geneva.

World Health Organization, 2017. Field use of molluscicides in schistosomiasis control programmes: an operational manual for programme managers. Geneva: WHO/HTM /NTD/PCT/2017.02, Licence: CC BY-NC-SA 3.0 IGO.

World Health Organization. Schistosomiasis. Available in: < https://www.who.int/en/news-room/fact-sheets/detail/schistosomiasis/> (Accessed 16 October 2019).

Figures legends

Figure 1 - Mortality of *B. glabrata* adults treated with the essential oil *C. rudolphianus* leaves for 48h. The Tukey's tests was used to evaluate significant differences between treatments, which is represented by different letters ($p < 0.05$). The data are expressed as average ± SD (n=30). C- (negative control): filtered and dechlorinated water; CSC (co-solvent control): 0.3% of tween 80; and NCL (niclosamide): 1 μg/mL positive control.

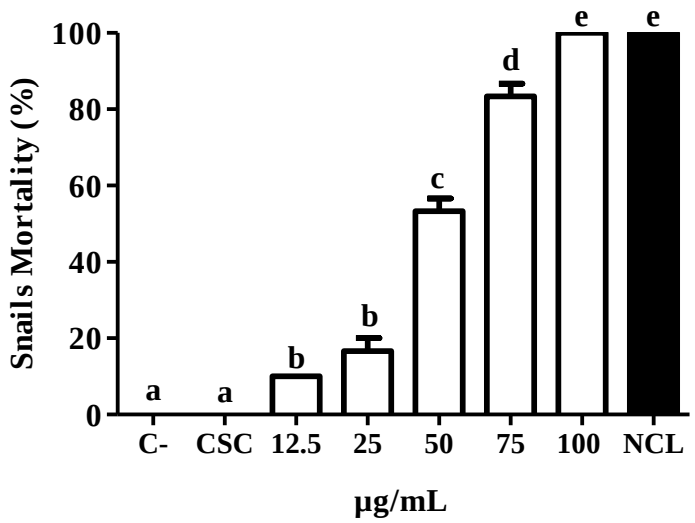
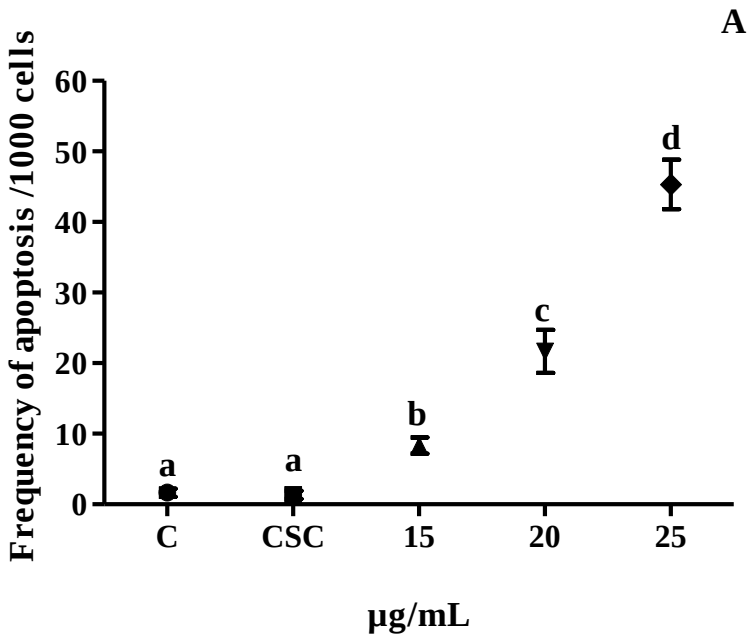
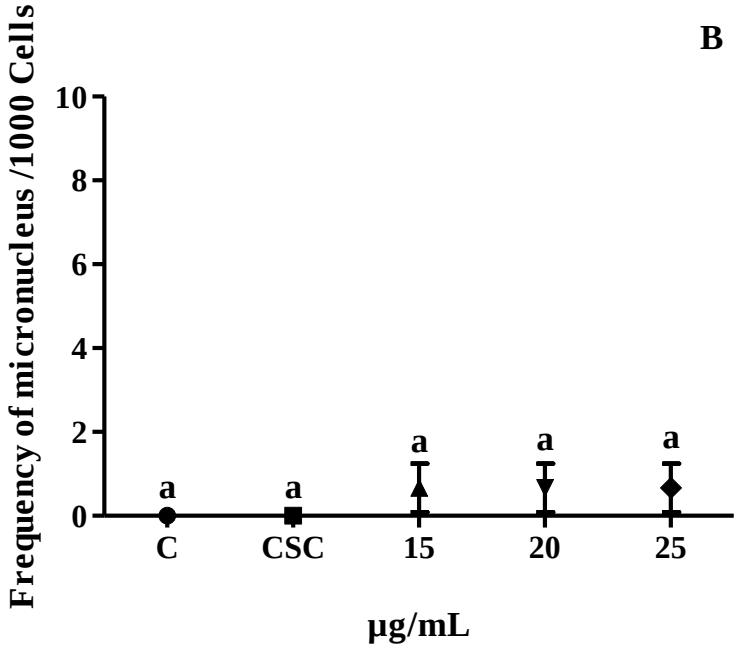
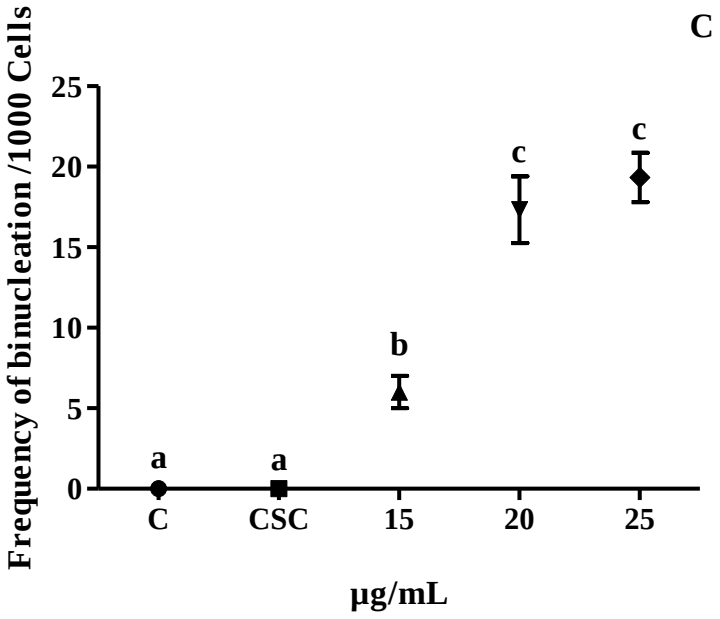


Figure 2 - Average of number of alterations per 1000 cells found in hemocytes of *B. glabrata* adults treated with the essential oil *C. rudolphianus* leaves for 48h at 15, 20 and 25 µg/mL. The Tukey's tests was used to evaluate significant differences between treatments. These differences are represented by different letters ($p < 0.05$). The data are expressed as average ± SD (n=3000). C- (negative control): filtered and dechlorinated water; CSC (co-solvent control): 0.3% of tween 80. **(A)** Number of apoptosis per 1000 cells analyzed. **(B)** Number of micronucleus per 1000 cells analyzed. **(C)** Number of binucleation per 1000 cells analyzed.







Tables

Table 1 - Organic compounds contained in the essential oil from *C. rudolphianus* leaves.

Compound	RI calc[1]	RI lit[2]	Relative area (%) ± SD
α-Pinene	931	932	4.22 ± 0.23
Sabinene	972	988	0.25 ± 0.02
β-Myrcene	991	1002	0.30 ± 0.02
α-Phellandrene	1003	1014	0.01 ± 0.00
α-Terpinene	1015	1021	0.01 ± 0.00

Limonene	1027	1022	3.78 ± 0.19
(E)-β-Ocimene	1048	1026	0.23 ± 0.02
γ-Terpinene	1058	1054	0.17 ± 0.00
Terpinolene	1087	1065	0.01 ± 0.00
Bicycloelemene	1338	1336	1.05 ± 0.12
α-Cubebene	1351	1348	1.00 ± 0.06
Cyclosativene	1366	1369	0.01 ± 0.00
α-Copaene	1377	1374	1.47 ± 0.05
β-Bourbonene	1386	1387	0.01 ± 0.00
β-Cubebene	1391	1387	4.25 ± 0.15
α-Gurjunene	1411	1409	0.01 ± 0.00
(*E*)-Caryophyllene	1421	1419	17.33 ± 0.23
β-Copaene	1430	1430	0.78 ± 0.04
epi-Cedrane	1445	1447	0.10 ± 0.01
α-Humulene	1455	1452	1.48 ± 0.06
allo-Aromadendrene	1462	1458	0.41 ± 0.02
trans-Cadina-1(6),4-diene	1475	1475	0.86 ± 0.02
Germacrene D	1483	1480	5.38 ± 0.17
Bicyclogermacrene	1498	1500	7.10 ± 0.12
Germacrene A	1507	1508	0.10 ± 0.01
Cubebol	1517	1514	2.30 ± 0.43
δ-Cadinene	1525	1522	6.62 ± 0.08
trans-Cadina-1,4-diene	1534	1533	0.29 ± 0.01
Germacrene B	1559	1559	0.13 ± 0.04
Spathulenol	1580	1577	0.26 ± 0.07
Unknown	1680	-	16.87 ± 2.60

[1]Retention index calculated from retention times in relation to those of a series of *n*-alkanes separated on a non-polar DB-5 capillary column. [2]Retention index obtained from literature (Adams, 2007).

Table 2 - Effect of *C. rudolphianus* essential oil on *B. glabrata* embryonic stages.

Embryonic stages	Concentrations* µg/mL	N° of dead embryos (%)	N° of malformed embryos (%)	Total of non-viable embyos (%)	Mean of non-viable embyos ± SD	LC_{50} (confidence limits) µg/mL	LC_{90} (confidence limits) µg/mL
Blastulae	C-	4 (0.67)	3 (0.5)	7 (1.17)	1.17 ± 0.75 a	126.54 (124.83 – 128.25)	202.61 (200.9 – 204.32)
	50	28 (4.67)	26 (4.33)	54 (9)	9 ± 1.90 b		
	100	237 (39.50)	19 (3.17)	256 (42.67)	42.67 ± 1.86 c		
	150	252 (42)	98 (16.33)	350 (58.33)	58.33 ± 1.97 d		
	200	426 (71)	54 (9)	480 (80)	80 ± 3.41 e		
	250	588 (98)	12 (2)	600 (100)	100 ± 0 f		
	300	600 (100)	0 (0)	600 (100)	100 ± 0 f		
Gastrulae	C-	4 (0.67)	6 (1)	10 (1.67)	1.67 ± 0.82 a	133.51 (131.64 – 135.38)	216.48 (214.61 – 218.85)
	50	36 (6)	16 (2.67)	52 (8.67)	8.67± 1.21 b		
	100	213 (35.5)	36 (6)	249 (41.5)	41.5± 5.89 c		
	150	184 (30.67)	136 (22.67)	320 (53.33)	53.33± 3.08 d		
	200	206 (34.33)	224 (37.33)	430 (71.67)	71.67 ± 7.5 e		
	250	320 (53.33)	280 (46.67)	600 (100)	100 ± 0 f		
	300	597 (99.5)	3 (0.5)	600 (100)	100 ± 0 f		
Trochophore	C-	3 (0.50)	3 (0.5)	6 (1)	1 ± 0.63 a	143.53 (141.52 – 145.54)	232.98 (230.97 – 234.99)
	50	36 (6)	4 (0.67)	40 (6.67)	6.67 ± 1.37 a		
	100	104 (17.33)	126 (21)	230 (38.33)	38.33 ± 5.72 b		
	150	265 (44.17)	45 (7.5)	310 (51.67)	51.67 ± 1.51 c		
	200	358 (59.67)	42 (7)	400 (66.67)	66.67 ± 7.87 d		
	250	448 (74.67)	102 (17)	550 (91.67)	91. 67 ± 3.78 e		
	300	345 (57.5)	255 (42.5)	600 (100)	100 ± 0 f		
Veliger	C-	5 (0.83)	3 (0.5)	8 (1.33)	1.33 ± 1.03 a	161.95 (159.49 – 164.41)	271.16 (268.7 – 273.62)
	50	27 (4.5)	3 (0.5)	30 (5)	5 ± 0.89 a		
	100	194 (32.33)	6 (1)	200 (33.33)	33.33± 3.98 b		
	150	266 (44.33)	3 (0.5)	269 (44.83)	44.83 ± 3.49 c		
	200	343 (57.17)	7 (1.17)	350 (58.33)	58.33± 2.25 d		
	250	452 (75.33)	38 (6.33)	490 (81.67)	81.67± 2.25 e		
	300	516 (86)	79 (13.17)	595 (99.17)	99.17 ± 3.43 f		

*Each concentration was performed in triplicate and repeated twice (n = 600). C-: filtered water. The Tukey's tests was used to evaluate significant differences between treatments, which is represented by different letters ($p < 0.05$)

Table 3 - Lethal concentrations to *B. glabrata* adults, *S. mansoni* cercariae and *A. salina* treated with *C. rudolphianus* essential oil.

Bioassay	LC_{50} (confidence limits) µg/mL	LC_{90} (confidence limits) µg/mL
B. glabrata adults	47.88 (44.3 – 51.5)	78.86 (75.26 – 82.46)
S. mansoni cercariae*	14.81 (14.65 – 14.97)	22.15 (21.99 – 22.31)
A. salina	68.33 (65.67 – 70.99)	111.5 (108.84 – 114.16)

* After 120 minutes of exposure time

Table 4 - Mortality rate to *S. mansoni* cercariae treated with *C. rudolphianus* essential oil in relation to exposure time. The data are expressed as average ± SD (n=600). The Tukey's tests was used to evaluate significant differences between treatments, which is represented by different letters ($p < 0.05$).

Experimental groups	**Exposure time (minutes)**			
C. rudolphianus EO (µg/mL)	**15**	**30**	**60**	**120**
5	0 a	0 a	1 ± 1.26 a	8.17 ± 1.94 a
10	0 a	0 a	7.67 ± 1.37 b	21 ± 3.46 b
15	0 a	7.67 ± 2.25 b	16.5 ± 2.95 c	40 ± 4.15 c
20	4 ± 3.69 b	20 ± 3.35 c	37.33 ± 3.88 d	80.33 ± 5.29 d
30	15.83 ± 4.5 C	23.5 ±4.93 c	49.33 ± 4.27 e	100 e
Control groups				
C-	0 a	0 a	0 a	0 f
CSC	0 a	0 a	0 a	0 f
NCL	100 d	100 d	100 f	100 e

3.2 ARTIGO 2 - EFFECT OF THE ESSENTIAL OIL FROM *Algrizea macrochlamys* LEAVES AND ITS MAJORITARIAN COMPOUND ((*E*)-CARYOPHYLLENE) ON *Biomphalaria glabrata* ADULTS AND EMBRYOS, AND *Schistosoma mansoni* CERCARIAE

ARTIGO A SER SUBMETIDO AO PERIÓDICO ACTA TROPICA

Ingridd Ayslane Torres de Araújo Ribeiro[a], Rosimere da Silva[a], José Luiz Ferreira de Sá[a], Shyrlane Torres Soares Veras[b], André L. Aires[c,d], Mônica C. P. A. Albuquerque[c,d], Márcia Vanusa da Silva[a], Ana Maria Mendonça de Albuquerque Melo[e], Daniela Maria do Amaral Ferraz Navarro[f], and Maria Tereza dos Santos Correia[a]

[a] *Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[b] *Departamento de Engenharia Civil e Ambiental, Centro de Tecnologia e Geociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[c] *Departamento de Medicina Tropical, Centro de Ciência da Saúde, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[d] *Laboratório de Imunologia Keizo Asami - LIKA, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[e] *Departamento de Biofísica e Radiobiologia, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[f] *Departamento de Química Fundamental, Centro de Ciências Exatas e da Natureza, Universidade Federal de Pernambuco, 50740-540 Recife, Pernambuco, Brazil.*

Highlights

- Oil lethal effects on *B. glabrata* embryos and adults was proven.
- (*E*)-caryophyllene showed toxic action to *B. glabrata* embryos.
- *A. macrochlamys* oil had LC_{50} and LC_{90} = 46.15 and 61.99 µg/mL, respectively to *B. glabrata* adults.
- Mortality to *S. mansoni* cercariae at all concentration tested of *A. macrochlamys* oil and (*E*)-caryophyllene was observed.

- This oil and (*E*)-caryophyllene did not show significative mortality to *A. salina*.

Abstract

This research aimed to assess the chemical composition and toxicity against the intermediate host of schistosomiasis (*Biomphalaria glabrata*) and non-target organism (*Artemia salina*) from the essential oil (EO) of *Algrizea macrochlamys* leaves and its majoritarian compound ((*E*)-caryophyllene). As well as evaluated the toxicity of this oil and compound against *Schistosoma mansoni* cercariae. The GC/MS analysis revealed 74 compounds, which 56 were identified. The majoritarian constituents of *A. macrochlamys* oil were (*E*)-caryophyllene (15.10%), followed by γ-eudesmol (13.43%), β-eudesmol (7.76%), α-eudesmol (7.36%), guaiol (5.62%) and germacrene D (5.18%). The EO of *A. macrochlamys* and (*E*)-caryophyllene showed toxic effect in a dose dependent manner on *B. glabrata* at all embryonic stage assayed in this study. In addition, the oil was toxic to *B. glabrata* adults in a dose dependent manner after 24h exposure (LC_{50} = 46.15 µg/mL and LC_{90} = 61.99 µg/mL). However, the (*E*)-caryophyllene at all concentrations assayed did not show any mortality rate in adults. Moreover, *A. macrochlamys* oil and (*E*)-caryophyllene cause mortality of *S. mansoni* cercariae (LC_{50} = 11.36 and 3.32 µg/mL and LC_{90} = 22.47 and 5.45 µg/mL, respectively). The oil and its major compound also decreased the motility of the cercariae, which decrease the ability of these organisms to infect humans. Both oil and (*E*)-caryophyllene did not present toxicity to the non-target organism used in this study.

Keywords: Cercariae; lethal concentration; molluscicidal agents; neglected disease; schistosomiasis.

1. Introduction

Schistosomiasis is a parasitic disease caused by trematode worms of the genus *Schistosoma*. This disease has been reported in 78 countries, and is prevalent in tropical and subtropical areas, particularly in poor communities without access to safe drinking water and adequate sanitation (WHO, 2020). In Brazil, schistosomiasis is caused by the species *Schistosoma mansoni*, which uses snails of the genus *Biomphalaria* as an intermediate host (Neves, 2016). Due to its high rates of infection, extensive geographical distribution and

efficiency in the transmission of schistosomiasis, the species *Biomphalaria glabrata* is considered the most important intermediate host of *S. mansoni* in the Americas (Brasil, 2014).

In accordance with the World Health Organization (WHO, 2020), the strategies control of this disease involves chemotherapy with praziquantel, sanitation, health education, and control of intermediate hosts using molluscicidal agents. Nowadays, the niclosamide (Bayluscide®, Bayer) is the only molluscicide recommended by the World Health Organization Pesticide Evaluation Scheme (WHOPES) (WHO, 2017). However, its use can cause some damage to the environment, because of its toxicity to organism such as amphibians, fishes, and aquatic plants (Martins et al., 2014; WHO, 2017).

Essential oils (EOs) are a mixture of substances originated from plants of secondary metabolism. Usually, the EOs are liquid, soluble in organic solvents, volatile and less dense than water (Oliveira et al., 2019). The EOs play an important role in plants such as the protection against herbivores and microorganisms, plant-plant interaction and pollination (Maffei et al., 2011). Some of them had a wide spectrum of biological activities, such as insecticidal (Santos et al., 2017; Ribeiro et al., 2020), antimicrobial, antioxidant (Veras et al., 2019), and molluscicidal (Gomes et al., 2019).

The Myrtaceae family comprises about 150 genera and 4,630 species of woody shrubs to tall trees. This family is mostly distributed in tropical and subtropical regions, presents large dispersion in Americas and Australia, although it is found all over the world (Dluzniewski et al., 2018). Several species of this family have been used in folk medicine, mainly as an antimicrobial, antioxidant, anti-inflammatory agent and to reduce the blood cholesterol level (Ebadollahi, 2013). Besides that, various studies have reported that EOs from Myrtaceae family showed a good potential to control the intermediate host of *S. mansoni* (Dias et al., 2013; Gomes et al 2019; Pinheiro et al., 2017).

The *Algrizea* genus has two species, *Algrizea minor* and *Algrizea macrochlamys* (Sobral et al., 2010). The EO from the first specie has chemical composition, antimicrobial and antioxidant activities described (Veras et al., 2019). However, for the EO from *A. macrochlamys* there is no records of biological activities studies. In this sense, this work aimed to investigate the toxic effect from the EO of *A. macrochlamys* leaves and its majoritarian compound, (*E*)-caryophyllene, in embryos at all development stage and adults of *B. glabrata*, and *S. mansoni* cercariae, as well as, test their environment toxicity in non-target organism.

2. Materials and Methods

2.1 Collection of plant material and EO extraction

Leaves of *A. macrochlamys* were collected in March 2016 from shrubs in the *Parque Nacional do Catimbau*, Pernambuco, Brazil. Voucher specimens was deposited at the Herbarium of the *Instituto Agronômico de Pernambuco* (IPA, Recife, PE, Brazil), with number IPA 96257.

The extraction of EO was carried out according to Ribeiro et al. (2020), where the fresh leaves (200 g) were triturated with distilled water (2 L) and subjected to the process of hydrodistillation using a Clevenger apparatus for 6 hours. The oil was stored at 4 ºC until chemical analysis and biological assays, and its yield was calculated as a percentage based on plant weight (% w/w).

2.2 Chemical characterization of EO

The chemical analysis of the EO from *A. macrochlamys* leaves was carried out using a gas chromatograph (model 7890A; Agilent Technologies; Palo Alto, CA, USA) equipped with an Agilent J &W non-polar HP-5ms™ column (30 m x 0.25 mm id.; 0.25 μm film thickness) and coupled to a selective mass detector (model 5975C; Agilent Technologies; Palo Alto, CA, USA).

An EO solution was prepared in *n*-hexane (2 mg/mL), then 1 μL of this solution was injected in split mode (1:50) with the injector temperature set to 250 °C. The oven temperature was programmed for 40 °C minutes and increased by 4 °C /min until 230 °C where it was maintained at this temperature for 5 min. It was used helium as a carrier gas at a flow rate of 1 mL/min, sustained at a constant pressure of 7.0 psi. The interface with MS was set at 230 °C; quadrupole temperature was set at 150 °C; mass spectra were recorded at 70 eV in Electron impact ionization mode and scanned in the range *m/z* 35-350 at a speed of 1.0 s/scan.

The individual components of the EO were identified by comparison of retention index (RI), obtained by co-injection of each oil sample with C_9–C_{30} linear hydrocarbons (Sigma-Aldrich, St. Louis, MO, USA) and calculated according to the Van den Dool and Kratz equation (1963), with those reported in the literature. The MS data acquired for each component were compared with those stored in the mass spectral library of the GC-MS system (MassFinder 4, NIST08 and Wiley Registry™ 9th Edition) and with published spectra (Adams, 2009) in order

to confirm identity. Whenever possible, identity of compounds was confirmed using retention times and mass spectra of authentic standards available in the *Laboratório de Ecologia Química* (UFPE). The peak areas on the chromatograms were integrated using the software Agilent MSD Productivity ChemStation (Agilent Technologies, Palo Alto, USA) to obtain the total ion current signal, which was used to determine the relative percentages of each oil component.

2.3 Molluscicidal assay

2.3.1 Maintenance of snails

B. glabrata adults, not infected by *S. mansoni*, were raised in the *Departamento de Biofísica e Radiobiologia, Centro de Biociências, Universidade Federal de Pernambuco, Brazil*. They were maintained in plastic aquarium (50 x 23 x 17 cm) with filtered and dechlorinated water at a temperature of 25 ± 2 ºC. The snails were feed daily with organic lettuce, and water of the aquarium were change once a week. Polyethylene sheets (10 x 10 cm) were placed in the aquarium to allow the snail's oviposition.

2.3.2 Bioassays using *B. glabrata* embryos and adults

The bioassay using embryos was performed according to Araújo et al. (2018ab). Briefly, solutions of *A. macrochlamys* oil (30, 40, 50, 60, 70 and 80 µg/mL) and, its majoritarian compound, (*E*)-caryophyllene (Sigma-Aldrich: purity ≥80%) (5, 10, 15, 20 and 25 µg/mL) were prepared with 0.3% of tween 80. After that, 100 embryos were selected using a stereomicroscope (Wild M3B, Heerbrugg, Switzerland) based on following criteria: intact eggs masses and one embryo per egg. After that, they were placed in the Petri dishes (60 x 15 mm). Then, the embryos were exposured to the *A. macrochlamys* oil and (*E*)-caryophyllene for 24h at 25 ± 2 °C. In the control groups, the embryos were exposured for 24h to dechlorinated water (negative control, C-), 0.3% of tween 80 (co-solvent control, CSC) and 1 µg/mL niclosamide (positive control, NCL). After this period, the egg masses were washed under filtered water and placed in Petri dishes containing filtered and dechlorinated water at 25 ± 2 °C. The number of viable and non-viable (dead and malformed) embryos were determined by observation during 7 consecutive days using a stereomicroscope. The embryonic stage used in this assay were blastulae (0-15 h after spawning), gastrula (24 - 39 h), trochophore (48 - 87 h) and veliger (96 - 111 h) (Kawano et al., 1992). This assay was performed in triplicate.

The bioassay with *B. glabrata* adults was carrying out in accordance with World Health Organization (WHO, 1965). Briefly, groups of 10 snails with 12-14 mm of shell diameter were put in plastics recipients (80 mL) containing solutions of EO from *A. macrochlamys* at 30, 40, 50, 60 and 70 µg/mL and (*E*)-caryophyllene (5, 10, 15, 20 and 25 µg/mL) for 24h at 25 ± 2 °C. In the control groups, the adult snails were exposured for 24h to dechlorinated water (negative control, C-), 0.3% of tween 80 (co-solvent control, CSC) and 1 µg/mL niclosamide (positive control, NCL). Subsequently, the snails were washed under filtrated water, fed with lettuce and observed daily for 7 days at 25 ± 2 °C to record the mortality. The death was confirmed by snail's inactivity; discolored color on the body and shell, and heart beats absence. This bioassay was performed in triplicate.

2.4 Bioassay with *S. mansoni* cercariae

The cercaricide assays were performed according to Silva et al. (2019). *B. glabrata* snails infected with *S. mansoni* (Belo Horizonte strain) were exposed to artificial light (60 W) during 2h for liberation of cercariae. The solutions of *A. macrochlamys* oil and (*E*)-caryophyllene were prepared using 0.3% tween 80. The concentrations used in the cercaricidal bioassay with *A. macrochlamys* oil were 1, 5, 10, 15, 20 and 25 µg/mL. To (*E*)-caryophyllene the concentrations used in this assay were 1, 2, 3, 4, 5 and 6 µg/mL. A suspension of 100 cercariae was kept in a watch glass and exposed to those concentrations. In the control groups, the cercariae were exposed to filtered water (pH 7.0) (negative control, C-), 0.3% of tween 80 (co-solvent control, CSC) and 1.0 µg/mL niclosamide (Bayluscide, Bayer) (positive control, NCL). The assays were maintained at 28 ± 1 ºC. The mortality of cercariae was observed with a stereomicroscope (Wild M3B, Heerbrugg, Switzerland) after 15, 30, 60 and 120 minutes after exposure to the EO or (*E*)-caryophyllene. The bioassays were performed in triplicate. The mortality rate and cercariae behaviour, such as motility, atypical rotations and vibrations, were registered. The LC_{50} and LC_{90} were calculated in the end of the assay after 120 minutes.

2.5 Assessment of environmental toxicity in non-target organisms (*Artemia salina*)

The *A. macrochlamys* oil and (*E*)-caryophyllene toxicity evaluation on non-target organisms was performed according to Araújo et al (2018b) using *A. salina*. Briefly, 20 mg of encysted eggs of *A. salina* were hatched in a plastic container with 400 mL of natural seawater at 25 ± 2 ºC, pH 8.0, under constant aeration for 48h. Afterwards, 10 newly hatched larvae were

separated and exposed to *A. macrochlamys* oil in 0.3% tween 80 at 30, 40, 50, 60, 70 and 80 µg/mL and (*E*)-caryophyllene in 0.3% tween 80 at 5, 10, 15, 20 and 25 µg/mL diluted in natural sea water (5 mL) for 24h. In the controls groups, the larvae were exposed to sea water (negative control, C-), 0.3% of tween 80 diluted in seawater (co-solvent control, CSC) and 1 µg/mL niclosamide (positive control, NCL). After the exposure time, the mortality rate were recorded. This assay was performed in quadruplicate.

2.6 *Statistical analysis*

The results were expressed by average ± standard deviation (SD). The Graph prism 5.0 software for Windows (GraphPad Software, San Diego, California, USA) was used to perform the One-way fixed-effects ANOVA and Tukey's test (significance at $p < 0.05$). The LC_{50} and LC_{90} (lethal concentrations required to kill 50% and 90% of individuals) were performed using probit analysis with a reliability interval of 95% in StatPlus version 5.98 for Windows.

3. Results

3.1. Acquisition and chemical constitution of the oil

The yield of EO obtained by hydrodistillation of fresh leaves from *A. macrochlamys* was 3.4 ± 0.7% (w/w). This oil was composed of 74 constituents, which 56 were identified. The sum of the identified components represented more than 88% of the EO. Sesqui- and monoterpenes accounted for 84.50% and 4.03% of the oil, respectively. (*E*)-caryophyllene (15.10%), γ-eudesmol (13.43%), β-eudesmol (7.76%), α-eudesmol (7.36%), guaiol (5.62%) and germacrene D (5.18%) were the major compounds (Table 1).

3.2 Toxicity to embryos and adults of *B. glabrata*

The EO from *A. macrochlamys* leaves and (*E*)-caryophyllene, which is the major compound of *A. macrochlamys* oil, have toxic effect in a dependent dose manner on *B. glabrata* embryos at all embryonic stage assayed in this study. The trochophore and veliger stages were more sensitive to *A. macrochlamys* oil than the other phases. On the other hand, the blastulae and gastrulae stages were more sensitive to (*E*)-caryophyllene than trochophore and veliger. The control groups showed more than 98% viability. However, NCL at 1 µg/mL presented

100% of mortality in all embryonic stage. The LC_{50} and LC_{90} of all embryonic stage are shown in the Tables 2 and 3 to the *A. macrochlamys* oil and (*E*)-caryophyllene, respectively.

This oil also showed toxic effects against *B. glabrata* adults in a dependent dose manner after 24h exposure. Nevertheless, the (*E*)-caryophyllene at all concentration tested (5-25 µg/mL) did not show any mortality rate. The controls groups C- and CSC present absence of mortality. On the other hand, the NCL at 1 µg/mL presented 100% of mortality. The treatments with the EO from *A. macrochlamys* at 30, 40, 50, 60 and 70 µg/mL present 6.67, 36.67, 56.67, 80 and 100% of mortality after 7 days, respectively (Figure 1). These treatments showed significant differences when compared to the control ($F_{7,16}$=185.8; p <0.0001). The LC_{50} for *B. glabrata* adults treated with *A. macrochlamys* oil was 46.15 (44.29- 47.97) µg/mL and LC_{90} was 61.99 (60.15 – 63.83) µg/mL.

3.3 Toxicity to *S. mansoni* cercariae

The toxicity from *A. macrochlamys* oil to cercariae of *S. mansoni* increased with the concentration and exposure time. These results are shown in the Tables 4 and 5. The highest concentration of *A. macrochlamys* EO (25 µg/mL) and (*E*)-caryophyllene (6 µg/mL) at 15 minutes showed a mortality rate of 38.67 ± 3.5 % and 29 ± 7.07 %, in the end of the experiment, after 120 minutes, the mortality rate were 100%. In addition to death, the oil and (*E*)-caryophyllene caused changes in the motility of the cercariae, such as, decrease in the motility and rotation in the own axis. The control groups (C- and CSC) did not present any mortality and the cercariae showed normal movements of swimming, and preservation of the body and tail. On the other hand, the NCL group present 100% mortality in the first minute of exposure. According to the statistical analysis, there were significant differences among the concentrations assayed after 120 minutes of exposure time to *A. macrochlamys* oil and (*E*)-caryophyllene ($F_{6,14}$ = 193.4; p < 0.0001; $F_{5,12}$ = 109.4; p < 0.0001). The LC_{50} were 11.36 (11.05 – 11.67) and 3.32 (3.25 – 3.39) µg/mL and LC_{90} were 22.47 (22.16 – 22.78) and 5.45 (5.38 – 5.52) µg/mL to *A. macrochlamys* EO and (*E*)-caryophyllene, respectively. Both values calculated after 120 minutes.

3.6 Toxicity to non-target organisms

In relation to the non-target organism toxicity bioassay, *A. macrochlamys* oil (30- 80 µg/mL) and (*E*)-caryophyllene (1-25 µg/mL) in all treatment assayed did not show any statistics

relevant toxicity to *A. salina*. The controls groups C- and CSC presented no mortality. However, the NCL at 1 µg/mL presented 100% of mortality.

4. Discussion

The yield of the EO from leaves of *A. macrochlamys* are 3.4 % (w/w), which is better than other species from Myrtaceae family, such as, *Campomanesia aurea* (0.17%), *Campomanesia xantocarpha* (0.02%), *Myrciaria delicatula* (0.19%), *Calyptranthes clusiifolia* (0.15%), *Calyptranthes concinna* (0.16%), *Myrcia splendens* (0.01%), *Eugenia osoriana* (0.19%), *Myrciaria tenella* (0.08%), *Myrceugenia reitzii* (1.59%) (Amaral et al., 2018), *Myrcia eximia* (0.01 - 0.36%) (Ferreira et al., 2020), *Eugenia hiemalis* (0.04 - 0.12%) (Zatelli et al., 2016).

The *A. macrochlamys* EO majoritarian compounds, (*E*)-caryophyllene, γ-eudesmol, β-eudesmol, α-eudesmol, guaiol and germacrene D, can also be found in EOs of species of the same family. For example, the (*E*)-caryophyllene was found in *M. eximia* (20.3-15%), *M. tenella* (17.2%), *C. clusiifolia* (6.7%), *M. splendens* (5.6%), *C. aurea* (5.1%), *E. osoriana* (4.1%) (Ferreira et al., 2020) and *A. minor* (3.76%) (Veras et al., 2019). The β-eudesmol and guaiol were found in *C. xantocarpha* (6.0 and 2.3%, respectively) and *M. delicatula* (3.1 and 2%, respectively) (Ferreira et al., 2020). The *A. minor* and *M. eximia* showed germacrene D (4.67 and 0.08 - 2.93%, respectively) (Veras et al., 2019; Ferreira et al., 2020). These data point similarity among the chemical composition of the EOs from *A. macrochlamys* leaves and other species of Myrtaceae.

The EO from leaves of *A. macrochlamys* and (*E*)-caryophyllene induced mortality in all stage of development of *B. glabrata* embryos. To *A. macrochlamys* oil, the final stages (trochophore and veliger) were more susceptive than the initial embryonic stages (blastulae and gastrulae) (Table 2). Similar results were found in the *B. glabrata* embryos treated with potassium usnate at 1-5 µg/mL (Araújo et al., 2018a) and ether extract of *Ramalina aspera* (lichen) at 10-30 µg/mL (Silva et al., 2019). According to Araújo et al. (2018a), this happen because in the final stages of development, the embryos are completely formed and the substance, in this case *A. macrochlamys* oil, has a greater area of contact with the mollusk, thus enabling a great action of the oil. The opposite happened with (*E*)-caryophyllene, the blastulae and gastrulae stages were more sensitive to this compound than trochophore and veliger (Table 3). Similar results have been observed in *B. glabrata* embryos treated with usnic acid from *Cladonia substellata* (lichen) (Araújo et al., 2018b), piplartine amide isolated of *Piper*

tuberculatum (Rapado et al., 2013) and dichloromethane fraction of the extract from *Liagora farinose* (algae) (Miyasato et al., 2012).

According to the World Health Organization (WHO, 1983), crude preparation of the plant material, such as EOs, should be active at 100 µg/mL or less and kill 90% of snails exposed for 24h. In turn, pure molecules, such as (*E*)-caryophyllene, should cause 90% mortality in concentrations equal to or less than 20 µg/mL. (*E*)-caryophyllene did not cause any mortality to *B. glabratra* adults in all concentrations assayed (5-25 µg/mL). In contrast, the EO of *A. macrochlamys* leaves showed toxic effect on adults of *B. glabrata,* with LC_{50} and LC_{90} values of 46.15 and 61.99 µg/mL, respectively, being below that recommended by WHO (1983). Other EOs from leaves of the same family, Myrtaceae, also show molluscicidal activity. For example, the EO of *Pimenta dioica* showed toxic effect to *B. glabrata* (LC_{50} = 18.62 µg/mL; Gomes et al 2019), which is a better result than the one found in this study. *Syzygium cumini* EO from leaves showed molluscicidal effect against *B. glabrata* after 24h of exposure, with LC_{50} and LC_{90} of 107 and 240 µg/mL (Dias et al., 2013). The EO from *Eugenia uniflora* had deleterious effect on *Biomphalaria tenagophila* (LD_{100} = 60 µg/mL) and *Lymnaea columella*, an intermediate host of the parasite *Fasciola hepatica*, (LD_{100} = 100 µg/mL) after 24h of exposure (Pinheiro et al., 2017).

Extracts from Myrtaceae species also present molluscicidal effect, such as the methanolic extract from fruits, barks and leaves of *Callistemon viminali* cause death on *Biomphalaria alexandrina* (LC_{50}= 6.2, 32 and 40 µg/mL, respectively) after 24h of exposure (Gohar et al., 2014). The methanolic extracts from leaves of *Callistemon citrinus*, *Eucalyptus globulus*, and *Melaleuca styphelioides* were toxic to *B. alexandrina* after 24h of exposure, with a LC_{90} values of 37.8, 245.5 and 783.4 µg/mL, respectively (Mohamed et al., 2018; Al-Sayed et al., 2014).

A. macrochlamys oil and (*E*)-caryophyllene had toxic effect to *S. mansoni* cercariae at all concentration assayed, which is the infectious form for humans (Colley et al., 2014), with LC_{50} = 11.36 and 3.32 µg/mL and LC_{90} = 22.47 and 5.45 µg/mL, respectively, calculated after 120 minutes. In addition to cause the death of *S. mansoni* cercariae, this oil and its major compound decreased the motility of these organisms. According to Araújo et al. (2018a), these characteristics are not compatible with the cercariae infectious ability. Similarly, to the results found in this study, other natural products also showed toxic effect to *S. mansoni* cercariae, such as, potassium usnate Araújo et al. (2018a) and divaricatic acid (Silva et al., 2018). The potassium usnate possess LC_{50} and LC_{90} values calculated after 120 minutes of 0.71 and 2.41 µg/mL, respectively (Araújo et al., 2018a), and the divaricatic acid present a LC_{50} of 0.89 µg/mL (Silva et al., 2018), which is a better result than the one found in this study.

The oil from *A. macrochlamys* and (*E*)-caryophyllene did not showed any significant toxicity to *A. salina* at all concentration assayed, which is a very good point because the oil and its majoritarian compound are toxic to embryos and adults of *B. glabrata*, cercariae of *S. mansoni*, but is not toxic to non-target organisms, in this case *A. salina*. Notwithstanding, the niclosamide, the only moluscicide recommend by WHOPES (WHO, 2017), at 1 µg/mL showed a mortality rate of 100% to *B. glabrata* (embryos an adults), cercariae of *S. mansoni* and to the non-target organism (*A. salina*). Hence, it might be possible use *A. macrochlamys* oil and (*E*)-caryophyllene as molluscicide and cercaricide agent. Nevertheless, futures research about this EO toxicity in others non-target species are necessary. Dias et al. (2013) evaluated the toxicity to *Syzygium cumini* EO to *A. salina*, in addition to molluscicide tests with *B. glabrata* (LC_{50} and LC_{90} of 107 and 240 µg/mL). The LC_{50} of *S. cumini* EO to *A. salina* was 175 µg/mL, which is worse result than the one found in this study. The EO from *P. dioica* was more toxic to *A. salina* (LC_{50} = 14.13 µg/mL) than to *B. glabrata* (LC_{50} = 18.62 µg/mL; Gomes et al 2019).

In conclusion, the EO from *A. macrochlamys* leaves show a high potential to control the *S. mansoni* intermediate host (*B. glabrata*) in its embryonic and adult stages. This oil also had toxic effect to *S. mansoni* cercariae and is not toxic to non-target organism (*A. salina*). The (*E*)-caryophyllene, the majoritarian compound of *A. macrochlamys* oil, also might be a candidate to control schistosomiasis, because its showed toxic effect to embryos of *B. glabrata* and *S. mansoni* cercariae, besides it did not show any toxicity to *A. salina*.

Competing interest

The authors declare no conflict of interest.

Acknowledgments

We thank the CNPq, CAPES, and FACEPE for financial support. Capes provided PhD and Master fellowships for the students involved in this work. The authors also thank the parasitology laboratory from UFPE for assisting with cercariae bioassay.

References

Adams, R.P. 2009. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publishing Co. 804 p.

Al-Sayed, E.; Hamid, H.A.; El-Einin, H.M.A. 2014. Molluscicidal and antischistosomal activities of methanol extracts and isolated compounds from *Eucalyptus globulus* and *Melaleuca styphelioides*. Pharmaceutical Biology, 52, 698-705.

Amaral, W.; Deschamps, C.; Silva, L.E.; Bizzo, H.R.; Pinto, M.A.S.; Biasi, L.A. 2018. Essential oil yield and composition of native species of the Myrtaceae family from "Campos Gerais" of the Atlantic Forest in Parana State. Ciência e Natura, 40, 1-9.

Araújo, H.D.A.; Melo, A.M.M.A.; Siqueira, W.N.; Martins, M.C.B.; Aires, A.L.; Albuquerque, M.C.P.A.; Silva, N.H.; Lima, V.L.M. 2018a. Potassium usnate toxicity against embryonic stages of the snail *Biomphalaria glabrata* and *Schistosoma mansoni* cercariae. Acta Tropica, 188, 132-137.

Araújo, H.D.A.; Silva, L.R.S.; Siqueira, W.N.; Fonseca, C.S.M.; Silva, N. H.; Melo, A.M.M.A.; Martins, M.C.B.; Lima, V. L.M. 2018b. Toxicity of Usnic Acid from *Cladonia substellata* (Lichen) to embryos and adults of *Biomphalaria glabrata*. Acta Tropica, 179, 39-43.

Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Vigilância da Esquistossomose Mansoni - diretrizes técnicas: 4. ed. Brasília: Ministério da Saúde, 2014. 144 p.

Colley, D. G.; Bustinduy, A. L.; Secor, W. E.; King, C. H. 2014. Human schistosomiasis. Lancet, 383, 2253–2264.

Dias, C.N; Rodrigues, K.A.F; Carvalho, F.A.A; Carneiro, S.M.P; Maia, J.G.S.; Andrade, E.H.A.; Moraes, D.F.C. 2013. Molluscicidal and Leishmanicidal Activity of the Leaf Essential Oil of *Syzygium cumini* (L.) Skeels from Brazil. Chemistry & Biodiversity, 10, 1133-1141.

Dluzniewski, F.S.; Vettorato, J.G.; Müller, N.T.G. 2018. Ethnobotanical approach of Myrtaceae in the municipality of Sete de Setembro, Rio Grande do Sul, Brazil. Revista Interdisciplinar em Ciências da Saúde e Biológicas, 2, 21-31.

Ebadollahi, A. 2013. Essential Oils Isolated from Myrtaceae Family as Natural Insecticides. Annual Review & Research in Biology, 3, 148-175.

Ferreira, O.O.; Cruz, J.N.; Franco, C.J.P.; Silva, S.G.; Costa, W.A.; Oliveira, M.S.; Andrade, E.H. 2020. A first report on yield and chemical composition of essential oil extracted from *Myrcia eximia* DC (Myrtaceae) from the Brazilian Amazon. Molecules, 25, 783-794.

Gohar, A.A.; Maatooq, G.T.; Gadara, S.R.; Aboelmaaty, W.S.; El-Shazly, A.M. 2014. Molluscicidal Activity of the Methanol Extract of *Callistemon viminalis* (Sol. ex Gaertner) G.Don ex Loudon Fruits, Bark and Leaves against *Biomphalaria alexandrina* Snails. Iranian Journal of Pharmaceutical Research, 13, 505-514.

Gomes, P.R.B.; Reis, J.B.; Fernandes, R.P; Mouchrek Filho, V.E; Souza, A.G.; Fontelene, M.A.; Silva, J.C. 2019. Toxicity and molluscicidal activity of the essential oil *Pimenta dioica* against the snail *Biomphalaria glabrata*. Revista peruana de biología, 26, 101-108.

Kawano, T., Okazaki, K., Ré, L. 1992. Embryonic development of Biomphalaria glabrata (Say, 1818) (Mollusca, Gastropoda, Planorbidae): A practical guide to the main stages. Malacologia, 34, 25-32.

Martins, M.C.B.; Silva, M.C.; Silva, L.R.S.; Lima, V.L.M.; Pereira, E.C.; Falcão, E.P.S.; Melo, A.M.M.A.; Silva, N.H. 2014. Usnic Acid Potassium Salt: An Alternative for the Control of Biomphalaria glabrata (Say, 1818). Plos one, 9, 1-6.

Maffei, M.E.; Gertschb, J.; Appendino, G. 2011. Plant volatiles: Production, function and pharmacology. Natural Product Reports, 28, 1359-1380.

Miyasato, P.A.; Kawano, T.; Freitas, J.C.; Berlinck, R.G.S.; Nakano, E.; Tallarico, L.F. 2012. Molluscicidal activity of some marine substances against the snail *Biomphalaria glabrata* (Mollusca, Planorbidae). Parasitology Research, 110, 1873-1879.

Mohamed, A.H.; Osman, G.Y.; El-Emam, M.A.; Abdel-Hamid, H.; Ali, R.E.M. 2018. Deteriorations in biological and biochemical aspects of *Biomphalaria alexandrina* snails

exposed to methanol extract of *Callistemon citrinus* leaves. Journal of the Egyptian Society of Parasitology, 48, 343-355.

Neves, D.P. 2016. Parasitologia Humana. 13ª ed. São Paulo: Editora Atheneu.

Oliveira, J.A.C.; Lima, R.K.; Marques, E.A.; Gavilanes, M.L. 2019. Phytochemical aspects and biological activities of essential oil of species of the family Canellaceae: A review. Plant Science Today, 6, 315-320.

Pinheiro, P.F; Gonçalves, L.V.; Criscco, K.B.; Pinheiro, C.A,; Tuler, A.; Cunha, J.B.; Costa, A.V; Pereira Júnior, O.S; Ignacchiti, M.D.C. 2017. Chemical Characterization and Molluscicidal Activity of Essential Oil from Leaves of *Eugenia uniflora* L. on *Lymnaea columella* (Say, 1817) and *Biomphalaria tenagophila* (D'Orbigny, 1835). Journal of Essential Oil Bearing Plants, 20, 1482-1491.

Rapado, L.N.; Pinheiro, A.S.; Lopes, P.O.M.V.; Fokoue, H.H.; Scotti, M.T.; Marques, J.V.; Ohlweiler, F.P.; Borrelly, S.I.; Pereira, C.A.B.; Kato, M.J.; Nakano, E.; Yamaguchi, F. 2013. Schistosomiasis control using Piplartine against Biomphalaria glabrata at different developmental stages. Plos neglected tropical diseases, 7, 1-8.

Ribeiro, I.A.T.A.; Silva, R.; Silva, A.G.; Milet-Pinheiro, P.; Paiva, P.M.; Navarro, D.M.A.F.; Silva, M.V.; Napoleão, T.H.; Correia, M.T.S. Chemical characterization and insecticidal effect against *Sitophilus zeamais* (maize weevil) of essential oil from *Croton rudolphianus* leaves. Crop Protection, 129, 105043, 2020.

Santos, L.M.M.; Nascimento, J.S.; Santos, M.A.G.; Marriel, N.B.; Bezerra-Silva, P.C.; Rocha, S.K.L.; Silva, A.G.; Correia, M.T.S.; Paiva, P.M.G.; Martins, G.F.; Navarro, D.M.A.F.; Silva, M.V.; Napoleão, T.H. 2017. Fatty acid-rich volatile oil from *Syagrus coronata* seeds has larvicidal and ovipositiondeterrent activities against *Aedes aegypti*. Physiological and Molecular Plant Pathology, 100, 35-45.

Silva, H.A.M.F.; Sá, J.L.F.; Siqueira, W.N.; Lima, M.V.; Martins, M.C.B.; Aires, A.L.; Albuquerque, M.C.P.A.; Falcão, E.P.S.; Buril, M.L.L; Pereira, E.C.; Melo, A.M.M.A.; Silva,

N.H. 2019. Toxicological effects of *Ramalina aspera* (lichen) on *Biomphalaria glabrata* snails and *Schistosoma mansoni* cercariae. Acta Tropica, 196, 172-179.

Silva, H.A.M.F.; Siqueira, W.N.; Sá, J.L.F.; Martins, M.C.B.; Aires, A.L.; Amâncio, F.F.; Pereira, E.C.; Albuquerque, M.C.P.A.; Melo, A.M.M.A.; Silva, N.H. 2018. Laboratory assessment of divaricatic acid against *Biomphalaria glabrata* and *Schistosoma mansoni* cercariae. Acta Tropica, 178, 97-102.

Sobral, M.; Faria Júnior, J.E.Q.; Proença, C.E.B. 2010. A new species of *Algrizea* (Myrteae, Myrtaceae) from Bahia, Brazil. Neodiversity, 5, 1-6.

Van Den Dool, H., Kratz, P.D. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography A, 11, 463-471.

Veras, B.O.; Santos, Y.Q.; Oliveira, F.G.S.; Almeida, J.R.G.S.; Silva, A.G.; Correia, M.T.S.; Diniz, K.M.; Oliveira, J.R.S.; Lima, V.L.M.; Navarro, D.M.A.F.; Aguiar, J.C.O.F.; Oliveira, M.B.M.; Lopes, A.C.S.; Silva, M.V. 2019. *Algrizea Minor* Sobral, Faria & Proença (Myrteae, Myrtaceae): chemical composition, antinociceptive, antimicrobial and antioxidant activity of essential oil. Natural Product Research, 4, 1-5.

World Health Organization (WHO). Integrating neglected tropical diseases into global health and development: Fourth WHO report on neglected tropical diseases. Geneva, 2017.

World Health Organization (WHO). Schistosomiasis. Available in: < https://www.who.int/en/news-room/fact-sheets/detail/schistosomiasis/> (Accessed 02 February 2020).

World Health Organization. 1965. Molluscicide screening and evaluation. Bull World Health Organ 33, 567-581.

World Health Organization. 1983. Report of the Scientific working Group on Plant Molluscicide & Guidelines for evaluation of plant molluscicides. TDR/SCH-SWG(4)/83.3. 11 p.

Zatelli, G. A.; Zimath, P.; Tenfen, A.; Cordova, C. M.; Scharf, D. R.; Simionatto, E. L.; Alberton, M. D. Falkenberg, M. 2016. Antimycoplasmic activity and seasonal variation of essential oil of *Eugenia hiemalis* Cambess. (Myrtaceae). Natural Product Research, 30, 1961-1964.

Figures legends

Figure 1 - Mortality of *B. glabrata* adults exposed to the essential oil from *A. macrochlamys* leaves for 24h. The Tukey's tests was used to evaluate significant differences between treatments, which is represented by different letters ($p < 0.05$). The data are expressed as average ± SD (n=30). C- (negative control): filtered and dechlorinated water; CSC (co-solvent control): 0.3% of tween 80; and NCL (niclosamide): 1 µg/mL positive control.

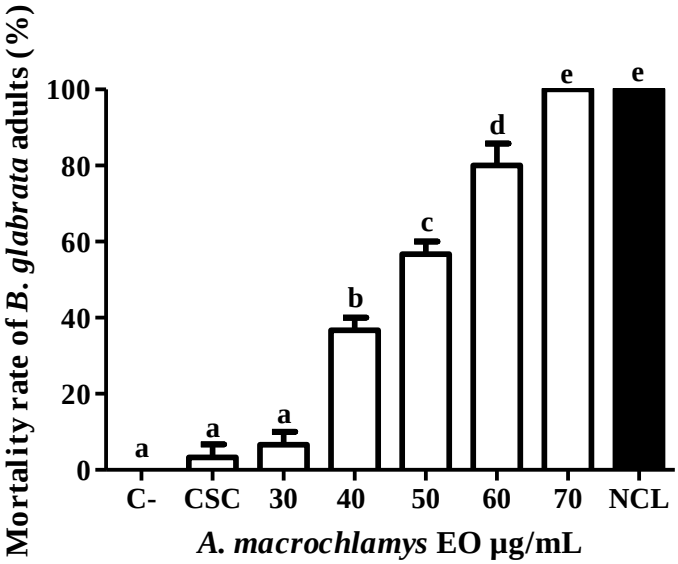


Table 1 - Organic compounds contained in the essential oil from *A. macrochlamys* leaves.

Compounds	RI cal[1]	RI lit[2]	Relative area % ± SD
α-Thujene	925	924	0.02 ± 0.03
α-Pinene	932	932	0.33 ± 0.27
Sabinene	972	969	0.01 ± 0.01
β-Pinene	974	979	0.27 ± 0.20
Myrcene	992	988	0.02 ± 0.02
α-Phellandrene	1003	1002	0.01 ± 0.02
α-Terpinene	1016	1014	0.02 ± 0.04
p-Cymene	1024	1022	0.04 ± 0.04
Limonene	1028	1024	0.17 ± 0.10

1,8-Cineole	1030	1026	2.02 ± 0.71
γ-Terpinene	1059	1054	0.09 ±0.13
(*Z*)-linalool oxide (furanoid)	1073	1067	0.02 ± 0.03
(Z)-linalool oxide (furanoid)	1088	1084	0.02 ± 0.03
Terpinolene	1088	1086	0.02 ± 0.04
Linalool	1100	1095	0.26 ± 0.18
Terpinene-4-ol	1178	1074	0.09 ± 0.05
α-Terpineol	1191	1186	0.64 ± 0.22
N.I.	1329		0.01 ± 0.02
δ-Elemene	1339	1335	0.39 ± 0.19
α-Cubenene	1351	1345	0.61 ± 0.13
α-Ylangene	1373	1373	0.04 ± 0.02
α-Copaene	1378	1374	1.44 ± 0.22
β-Bourbonene	1387	1387	0.27 ± 0.09
β-Cubebene	1392	1387	0.23 ± 0.06
β-Elemene	1394	1389	0.98 ± 0.17
α-Gurjunene	1412	1409	0.57 ± 0.15
(*E*)-Caryophyllene	1422	1417	15.10 ± 2.23
β-Copaene	1431	1430	0.17 ± 0.04
γ-Elemene	1436	1434	0.06 ± 0.02
α-Guaiene	1441	1437	0.18 ± 0.05
Guaia-6,9-diene	1446	1442	0.08 ± 0.03
(*E*)-3,5-Muuroladiene	1453	1451	1.31 ± 0.32
Humulene	1456	1452	2.98 ± 0.62
9-epi-Caryophyllene	1463	1464	0.60 ± 0.17
(*E*)-Cadina-1(6),4-diene	1476	1475	1.42 ± 0.42
γ-Muurolene	1480	1478	0.23 ± 0.04
Germacrene D	1483	1484	5.18 ± 0.91
β-Selinene	1489	1489	0.18 ± 0.03
N.I.	1491		0.07 ± 0.04
(*E*)-Muurola-4(14),5-diene	1494	1493	0.93 ± 0.46
Viridiflorene	1497	1496	0.89 ± 0.41
Bicyclogermacrene	1500	1500	2.49 ± 1.41
α-Muurolene	1503	1500	0.57 ± 0.11
N.I.	1510		0.11 ± 0.03
N.I.	1512		0.16 ± 0.05
γ-Cadinene	1517	1513	0.32 ± 0.11
δ-Cadinene	1526	1522	4.02 ± 1.17
(Z)-Calamenene	1527	1528	2.02 ± 0.58
Zonarene	1529	1528	0.89 ± 0.18
(*E*)-Cadina-1,4-diene	1536	1533	1.08 ± 0.20
N.I.	1541		0.15 ± 0.01
α-Calacorene	1545	1544	0.12 ± 0.04
N.I.	1551		2.75 ± 0.78
N.I.	1556		0.13 ± 0.06
Germacrene B	1561	1559	0.09 ± 0.05
N.I.	1566		0.04 ± 0.01
N.I.	1570		0.26 ± 0.15

Spathulenol	1580	1577	0.65 ± 0.45
N.I.	1587		1.65 ± 0.90
N.I.	1595		0.39 ± 0.19
N.I.	1597		0.22 ± 0.08
Guaiol	1601	1600	5.62 ± 4.48
N.I.	1606		0.48 ± 0.21
5-epi-7-epi-α-Eudesmol	1610	1607	0.51 ± 0.18
N.I.	1613		0.34 ± 0.11
N.I.	1617		0.27 ± 0.04
10-epi-gama-Eudesmol	1623	1622	1.34 ± 0.32
N.I.	1632		1.70 ± 0.80
γ-Eudesmol	1636	1630	13.43 ± 3.65
N.I.	1644		0.43 ± 0.05
N.I.	1647		2.19 ± 0.66
β-Eudesmol	1656	1649	7.76 ± 0.73
α-Eudesmol	1660	1652	7.36 ± 1.46
Bulnesol	1672	1670	2.48 ± 2.18

[1]Retention index calculated from retention times in relation to those of a series of *n*-alkanes separated on a non-polar DB-5 capillary column. [2]Retention index obtained from literature (Adams, 2007). N.I.: not identified.

Table 2 - Effect of *A. macrochlamys* essential oil on *B. glabrata* embryonic stages.

Embryonic stages	Concentrations* µg/mL	Nº Dead embryos (%)	N° of Malformed embryos (%)	Total of non-viable embyos (%)	Mean of non- viable embyos ± SD	LC_{50} (confidence limits) µg/mL	LC_{90} (confidence limits) µg/mL
Blastulae	C-	0	1 (0.33)	1 (0.33)	0.33 ± 0.58 a	55.70 (55.20 - 56.20)	71.36 (70.86 - 71.86)
	30	8 (6.27)	3 (1)	11 (3.67)	3.67 ± 3.06 a		
	40	20 (6.67)	16 (5.33)	36 (12)	12 ± 1.73 ab		
	50	53 (17.67)	31 (10.33)	84 (28)	28 ± 2 b		
	60	148 (49.33)	29 (9.67)	177 (59)	59 ± 4.58 c		
	70	234 (78)	14 (4.67)	248 (82.67)	82.67 ± 15.14 d		
	80	297 (99)	3 (1)	300 (100)	100 ± 0 e		
Gastrulae	C-	5 (1.67)	0	5 (1.67)	1.67 ± 1.15 a	56.83 (56.32 - 57.34)	72.94 (72.43 - 73.45)
	30	12 (4)	3 (1)	15 (5)	5 ± 2 a		
	40	12 (4)	11 (3.67)	23 (7.67)	7.67 ± 1.53 a		
	50	61 (20.33)	21 (7)	82 (27.33)	27.33 ± 4.04 b		
	60	141 (47)	16 (5.33)	157 (52.33)	52.33 ± 3.51 c		
	70	222 (74)	19 (6.33)	241 (80.33)	80.33 ± 3.51 d		
	80	300 (100)	0	300 (100)	100 ± 0 e		
Trochophore	C-	2 (0.67)	0	2 (0.67)	0.67 ± 0.58 a	52.85 (52.29 - 53.41)	70.56 (70 - 71.12)
	30	15 (5)	1 (0.33)	16 (5.33)	5.33 ± 1.53 a		
	40	49 (16.33)	20 (6.67)	69 (23)	23 ± 5.29 b		
	50	103 (34.33)	20 (6.67)	123 (41)	41 ± 1.73 c		
	60	149 (49.67)	34 (11.33)	183 (61)	61 ± 1.73 d		
	70	228 (76)	25 (8.33)	253 (84.33)	84.33 ± 4.62 e		
	80	300 (100)	0	300 (100)	100 ± 0 f		
Veliger	C-	0	1 (0.33)	1 (0.33)	0.33 ± 0.58 a	49.85 (49.31 - 50.39)	69.95 (69.41 – 70.49)
	30	9 (3)	13 (4.33)	22 (7.33)	7.33 ± 1.53 a		
	40	40 (13.33)	58 (19.34)	98 (32.67)	32.67 ± 5.51 b		
	50	64 (21.33)	92 (30.67)	156 (52)	52 ± 13.75 bc		
	60	148 (49.33)	52 (17.34)	200 (66.67)	66.67 ± 7.77 c		
	70	201 (67)	57 (19)	258 (86)	86 ± 9.64 cd		
	80	300 (100)	0	300 (100)	100 ± 0 d		

*Each concentration was performed in triplicate (n = 300). C-: filtered water. The Tukey's tests was used to evaluate significant differences between treatments, which is represented by different letters ($p < 0.05$).

Table 3 - Effect of (*E*)-caryophyllene, major compound of *A. macrochlamys* essential oil, on *B. glabrata* embryonic stages.

Embryonic stages	Concentrations* µg/mL	Nº of dead embryos (%)	Nº of malformed embryos (%)	Total of non-viable embyos (%)	Mean of non- viable embyos ± SD	LC_{50} (confidence limits) µg/mL	LC_{90} (confidence limits) µg/mL
Blastulae	C-	3 (1)	2 (0.67)	5 (1.67)	1.67 ± 0.58 a	10.08 (9.83 – 10.33)	19.05 (18.8 – 19.3)
	1	11 (3.67)	25 (8.33)	36 (12)	12 ± 3.61 a		
	5	11 (3.67)	72 (24)	83 (27.67)	27.67 ± 3.06 b		
	10	10 (3.33)	127 (42.34)	137 (45.67)	45.67 ± 8.5 c		
	15	13 (4.33)	183 (61)	196 (65.33)	65.33 ± 5.86 d		
	20	19 (6.33)	255 (85)	274 (91.33)	91.33 ± 1.15 e		
	25	300 (100)	0	300 (100)	100 e		
Gastrulae	C-	2 (0.67)	1 (0.33)	3 (1)	1 ± 0 a	10.27 (10.01 – 10.53)	19.25 (18.99 – 19.51)
	1	16 (5.33)	20 (6.67)	36 (12)	12 ± 1 b		
	5	24 (8)	49 (16.33)	73 (24.33)	24.33 ± 5.13 c		
	10	21 (7)	128 (42.67)	149 (49.67)	49.67 ± 4.16 d		
	15	39 (13)	149 (49.67)	188 (62.67)	62.67 ± 2.52 e		
	20	66 (22)	205 (68.33)	271 (90.33)	90.33 ± 1.15 f		
	25	300 (100)	0	300 (100)	100 e		
Trochophore	C-	2 (0.67)	3 (1)	5 (1.67)	1.67 ± 0.58 a	11.43 (11.16 – 11.7)	20.9 (20.63 – 21.17)
	1	17 (5.67)	11 (3.67)	28 (9.33)	9.33 ± 1.53 a		
	5	24 (8)	44 (14.67)	68 (22.67)	22.67 ± 1.15 b		
	10	46 (15.33)	85 (28.34)	131 (43.67)	43.67 ± 6.66 c		
	15	116 (38.67)	58 (19.34)	174 (58)	58 ± 8.72 d		
	20	170 (56.67)	77 (25.66)	247 (82.33)	82.33 ± 3.21 e		
	25	300 (100)	0	300 (100)	100 f		
Veliger	C-	1 (0.33)	3 (1)	4 (1.33)	1.33 ± 1.15 a	12.5 (12.21 – 12.79)	22.77 (22.48 – 23.06)
	1	14 (4.67)	12 (4)	26 (8.67)	8.67 ± 0.58 a		
	5	48 (16)	14 (4.67)	62 (20.67)	20.67 ± 2.52 ab		
	10	66 (22)	60 (20)	126 (42)	42 ± 17.78 bc		
	15	89 (29.67)	66 (22)	155 (51.67)	51.67 ± 11.72 cd		
	20	116 (38.67)	106 (35.33)	222 (74)	74 ± 2.65 d		
	25	300 (100)	0	300 (100)	100 ± 0 e		

*Each concentration was performed in triplicate (n = 300). C-: filtered water. The Tukey's tests was used to evaluate significant differences between treatments, which is represented by different letters ($p < 0.05$).

Table 4 - Mortality rate to *S. mansoni* cercariae treated with *A. macrochlamys* essential oil in relation to exposure time. The data are expressed as average ± SD (n = 300). The Tukey's tests was used to evaluate significant differences between treatments, which is represented by different letters ($p < 0.05$).

Experimental groups	**Exposure time (minutes)**			
	15	**30**	**60**	**120**
	A. macrochlamys EO (µg/mL)			
1	0 a	4.67 ± 1.53 a	7.67 ± 2.08 ab	11.33 ± 3.21 a
5	0 a	8.33 ± 2.08 a	16.67 ± 3.51 bc	28.67 ± 3.51 b
10	0 a	10.33 ± 2.52 a	24.33 ± 5.51 c	39.67 ± 9.02 b
15	3.67 ± 1.53 a	28.33 ± 5.03 b	40 ± 4.58 d	57.67 ± 2.52 c
20	28.67 ± 6.03 b	39 ± 4 b	69 ± 4 e	88 ± 6.56 d
25	38.67 ± 3.51 c	64.67 ± 6.66 c	100 f	100 d
	Control groups			
C-	0 a	0 a	0 a	0 a
CSC	0 a	0 a	0 a	0 a
NCL	100 d	100 d	100 f	100 d

Table 5 - Mortality rate to *S. mansoni* cercariae treated with (*E*)-caryophyllene in relation to exposure time. The data are expressed as average ± SD (n = 300). The Tukey's tests was used to evaluate significant differences between treatments, which is represented by different letters ($p < 0.05$).

Experimental groups	**Exposure time (minutes)**			
	15	**30**	**60**	**120**
	(*E*)-Caryophyllene (µg/mL)			
1	0 a	5 ± 3 ab	13 ± 4.36 b	18.67 ± 3.51 b
2	0.33 ± 0.58 a	7.33 ± 2.52 ab	20.67 ± 1.53 b	30.67 ± 2.08 bc
3	6 ± 2 a	19 ± 3.61 bc	25.33 ± 2.52 b	39 ± 4 c
4	7.67 ± 3.21 ab	29.67 ± 5.69 c	40.33 ± 5.86 c	54.33 ± 5.03 d
5	15 ± 5 b	28 ± 10.15 c	41.67 ± 3.06 c	78 ± 9.85 e

6	24 ± 7.07 c	44.5 ± 4.95 d	77 ± 11.31 d	100 f
		Control groups		
C-	0 a	0 a	0 a	0 a
CSC	0 a	0 a	0 a	0 a
NCL	100 d	100 e	100 f	100 f

3.3 ARTIGO 3 - TOXIC EFFECTS OF *Algrizea macrochlamy*s AND *Croton rudolphianus* ESSENTIAL OILS FROM LEAVES ON *Aedes aegypti*

ARTIGO A SER SUBMETIDO AO PERIÓDICO PARASITOLOGY RESEARCH

Ingridd Ayslane Torres de Araújo Ribeiro[a], Rosimere da Silva[a], Júlio César Ribeiro de Oliveira Farias de Aguiar[b], Jéssica Silva Nascimento[b], Shyrlane Torres Soares Veras[c], Márcia Vanusa da Silva[a], Daniela Maria do Amaral Ferraz Navarro[b] and Maria Tereza dos Santos Correia[a]

[a] *Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[b] *Departamento de Química Fundamental, Centro de Ciências Exatas e da Natureza, Universidade Federal de Pernambuco, 50740-540 Recife, Pernambuco, Brazil.*

[c] *Departamento de Engenharia Civil e Ambiental, Centro de Tecnologia e Geociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

Abstract

This study aimed to evaluate the toxic potential of the essential oils (EOs) from *Algrizea macrochlamys* and *Croton rudolphianus* leaves against eggs, fourth instar larvae, and pupae from the main vector of dengue, *Aedes aegypti*. These oils were obtained by hidrodestilation. The chemical characterization of the EOs were performed by GC-MS analysis. The majoritarian compounds from *A. macrochlamys* oil were (*E*)-caryophyllene (15.10%), followed by γ-eudesmol (13.43%), β-eudesmol (7.76%), α-eudesmol (7.36%), guaiol (5.62%) and germacrene D (5.18%). On the other hand, (*E*)-caryophyllene (17.33%), unknown compound (16.87%), bicyclogermacrene (7.1%), δ-cadinene (6.62%) and germacrene D (5.38%) were the majoritarian compounds from *C. rudolphianus* oil. The *A. macrochlamys* oil did not showed any toxic action to fourth instar larvae of *A. aegypti*. On the other hand, *C. rudolphianus* oil cause mortality on larvae of *A.aegypti*, with a LC_{50} value of 21.86 µg/mL. The EOs from *A. macrochlamys* and *C. rudolphianus* did not showed any mortality to pupae from *A. aegypti*. Both oils present ovicidal effect to eggs of *A. aegypti*. The highest concentration of EOs from *A. macrochlamys* (300 µg/mL) and *C. rudolphianus* (250 µg/mL) assayed reduce hatching rate of eggs from *A. aegypti* in 55.34% in relation to control. Thus, *A. macrochlamys* and *C. rudolphianus* oils represent promising sources of bioactive compounds against *A. aegypti*.

Keywords: Dengue fever; essential oils; mosquitoes; natural insecticide; secondary metabolites.

1. Introduction

The dengue is a mosquito-borne viral infection caused by a virus from Flaviviridae family. The symptoms of this illness include severe headache, muscle and joint pains, pain behind the eyes, nausea, vomiting and rash. (WHO, 2020a). According to World Health Organization (2020a), in recent decades the worldwide incidence of dengue has increased dramatically, besides that, about half of the global population is now at risk to get this disease.

The dengue is transmitted by mosquitoes mainly of the specie *Aedes aegypti*, which are found in tropical and sub-tropical regions (Silva et al., 2018). Those mosquitoes also transmit chikungunya virus (CHIKV), Zika virus (ZIKV) and yellow fever virus (Kraemer et al., 2019; WHO, 2020a).

Chikungunya is characterized by fever, severe and debilitating joint pain, headache and myalgia. Some people may develop more severe conditions from this disease, with neurological manifestations, such as encephalitis, meningoencephalitis, myelitis and Guillain-Barre syndrome, bullous skin and myocarditis (Donalisio; Freitas, 2015). The most common symptoms of Zika virus are red patches on the skin, joint pain, conjunctivitis, headache and fever (Brasil, 2019). Zika virus infection in pregnant women can also lead to fetal death, placental failure, fetal growth restriction and congenital microcephaly (WHO, 2020b). The most frequent symptoms of yellow fever include fever, chills, headache, body pain, nausea, vomiting, weakness and fatigue (Brasil, 2020). The severe forms of this disease are characterized by liver and kidney failure, and hemorrhage, leading to death (Cavalcante; Tauil, 2017).

Currently, the most efficient way to prevent those disease transmitted by *A. aegypti* is to eradicate vector mosquitoes, normally using chemical insecticides such as organophosphates (Shaw; Catteruccia, 2019). However, the use of this synthetic substances can cause several damages to the human health and environment (Benelli, 2015), besides, the development of resistant populations of mosquitoes (Bravo et al., 2011).

Many products derived from plants have been assayed about their insecticidal effect against insects, such as lectins (Oliveira et al., 2016), extracts (Gebreziher et al., 2018) and essential oils (EOs) (Ribeiro et al., 2020; Santos et al., 2017a).

EOs are liquid mixtures of volatile compounds derived from plant secondary metabolic (Pavela; Benelli, 2016; Sharifi-Rad et al., 2017). Those oils are normally obtained by hydro or steam distillation, and characterized by a strong odor (Bakkali et al., 2008; Kar et al., 2018). They play an important role in the protection of plants against herbivores and microorganisms, pollination and plant-plant interactions (Maffei et al., 2011). Due to this, many studies have been reported showing biological activities from essential oils, such as antimicrobial (Araújo et al., 2017; Brito et al., 2018), insecticide (Ribeiro et al., 2020; Santos et al., 2017a), molluscicidal (Costa et al., 2015). Several EOs also have been described as potent larvicidal, pupicidal and for had oviposition-deterrent effect against *A. aegypti* (Bortolucci et al., 2019; Gomes et al., 2016; Santos et al., 2017a; Santos *et al*., 2014).

The *Croton* genus belonging to Euphorbiaceae family and possess about 1300 species broadly found in tropical regions (Brito et al., 2018). Some EOs from species of *Croton* genus have revealed insecticide effects, such as *Croton linearis* (Amado et al., 2020), *Croton rhamnifolioides* (Santos et al., 2014) *Croton nepetaefolius* (Santos et al., 2017b) to *Aedes aegypti*, and *Croton pulegiodorus* (Silva et al., 2019a) to *Sitophilus zeamais*. *Croton rudolphianus* Müll. Arg., popularly known as “velame-branco”, is a species endemic in Brazil, distributed through the Northeast to Southeast regions (Silva et al., 2010). The EO from this specie showed insecticidal and attractive effects to *Sitophilus zeamais*, the major compounds of this oil were a not known compound (40.90%) and methyl chavicol (22.96%) (Ribeiro et al., 2020).

Algrizea is a genus of the Myrtaceae family endemic to two states in the Northeast (Bahia and Pernambuco). Currently, this genus has two species: *Algrizea minor* and *Algrizea macrochlamys* (Proença et al., 2006; Sobral et al., 2010). Veras et al. (2019) characterized the EO of *A. minor* leaves and described their biological activities. However, there is no information about the chemical composition and biological activities of the EO of *Algrizea macrochlamys*.

Due to this, this paper aimed to evaluate the ovicidal, larvicidal, and pupicidal effects to the EOs obtained from *A. macrochlamys* and *C. rudolphianus* leaves against *A. aegypti*.

2. Materials and Methods

2.1 Plant material and essential oil extraction

Leaves of *A. macrochlamys* and *C. rudolphianus* were collected in March and September 2016, respectively, at *Parque Nacional do Vale do Catimbau* (PARNA do Catimbau, Pernambuco, Brazil). Voucher specimens of *A. macrochlamys* and *C. rudolphianus* were deposited at the Herbarium of the *Instituto Agronômico de Pernambuco* (IPA, Recife, PE, Brazil), with numbers IPA 96.257 and IPA 91.091, respectively. The EOs of *A. macrochlamys* and *C. rudolphianus* leaves were obtained by hidrodestilation (100g of fresh leaves and 1L of distilled water) in a Clevenger-type apparatus for 4 - 6h, according Ribeiro et al. (2020).

2.2 GC-MS analysis

The chemical characterization from these EOs were executed using GC-MS analysis. The majoritarian compounds from *A. macrochlamys* oil were (*E*)-caryophyllene (15.10%), followed by γ-eudesmol (13.43%), β-eudesmol (7.76%), α-eudesmol (7.36%), guaiol (5.62%) and germacrene D (5.18%). On the other hand, (*E*)-caryophyllene (17.33%), unknown compound (16.87%), bicyclogermacrene (7.1%), δ-cadinene (6.62%) and germacrene D (5.38%) were the majoritarian compounds from *C. rudolphianus* oil.

2.4 Bioassay with *A. aegypti*

2.4.1 Insects

The insects colony used in this study belongs to Rockefeller strain and it was maintained in the laboratory at 70% ± 5% relative humidity and 28 ± 1 °C, under a 12:12 light-dark photoperiod. *A. aegypti* adults were maintained in cages (33 × 33 × 33 cm) and fed with on 10% sucrose solution (males) and bird blood (females) once a week. To eggs collection, a recipient with distillated water and a piece of filter paper (support for oviposition) were placing in the cage three days after the blood meal. The eggs were hatched by submersion in distilled water, and larvae were maintained in plastic basins and fed on a diet of commercial cat food.

2.4.2 Larvicidal bioassay

This bioassay was carrying out using the method recommended by the World Health Organization (2005), with modifications. A stock solution at 200 and 100 µg/mL of *A.*

macrochlamys and *C. rudolphianus* oils, respectively, were prepared with 0.1% tween 80 and distillated water. Early fourth instar larvae of *A. aegypti* were transferred to disposable cups (20 larvae per cup) containing EOs at different concentrations prepared by dilution of the stock solution with distilled water. To *A. macrochlamys* oil was used concentrations at 10, 50, 100, 150 and 200 µg/mL, and the *C. rudolphianus* oil was used at 10, 15, 20, 25, 30 and 35 µg/mL. The concentrations were defined through pilot tests carried out before the experiments. The control (C) was a solution at 0.1% tween 80. To determine LC_{50} values, the number of dead larvae was determined after 48 h of incubation at 70% ± 5% relative humidity and 28 ± 1 °C, under a 12:12 light-dark photoperiod. Larvae were considered dead when did not respond to mechanical stimulus or were unable to reach the surface solution (WHO, 2005). The experiments were performed in triplicate.

2.3 Pupicidal bioassays

The same procedure was carried out to the pupicidal bioassay using 24h pupae of *A. aegypti*. Different concentrations (50, 100, 150, 200, 300, 500 and 700 µg/mL) were prepared in disposable cups by dilution of the stock solutions (700 µg/mL) of *A. macrochlamys* and *C. rudolphianus* oils with distilled water. Then, twenty pupae (10 males and 10 females) were transferred these cups. The control (C) was a solution at 0.1% tween 80. After 48h of incubation at 28 ± 1 °C, under a 12:12 light-dark photoperiod, the number of dead pupae was determined. Three independent experiments were performed.

2.4 Ovicidal bioassay

This assay was performed in accordance with the methodology described by Santos et al. (2017a). The eggs (50 per test) adhered on filter paper were selected by considering their integrity and counted using a stereomicroscope (Leica M80). Subsequently, the eggs were placed into 50 mL backers containing different concentrations of *A. macrochlamys* (150, 200, 250 and 300µg/mL) and *C. rudolphianus* oils (100, 150, 200 and 250 µg/mL). Percent egg viability was calculated by dividing the number of larvae that emerged from the eggs after 72 hours treatment by the number of eggs.

2.5 Statistic analysis

The results were expressed by mean ± Standard Deviation (SD). The One-way fixed-effects ANOVA and Tukey's test (significance at $p < 0.05$) were performed in Graph prism 5.0 software for Windows (GraphPad Software, San Diego, California, USA). In addition, the StatPlus version 5.98 for Windows was used to perform LC_{50} (lethal concentrations required to kill 50% of individuals).

3. Results and Discussion

Numerous EOs have been demonstrated insecticidal effects against a range spectrum of insects (Lira et al., 2015; Ribeiro et al., 2020; Santos et al., 2017a). Besides that, they are, generally, low cost and cause less damage to the environment and people when compared to synthetic insecticides (Blank et al., 2019). In search for alternative ways to the control of the dengue fever vector, the oils from leaves of *A. macrochlamys* and *C. rudolphianus* were tested against *A. aegypti*. However, the EO from *A. macrochlamys* did not possess any larvicidal effect to fourth instar of *A. aegypti* larvae after 48 hours at all concentration tested (10-200 µg/mL). On the other hand, *C. rudolphianus* oil was effective in promoting the death of this larvae after 48 hours (Figure 1), with a LC_{50} value of 21.86 [20.51 – 23.25] µg/mL. No mortality was observed in the control. Statistical analysis revealed significant differences in mortality between doses ($F_{6,14} = 43.46$; $p < 0.0001$).

Other EOs also exhibited larvicidal effect against *A. aegypti*, such as the oils from *Syagrus coronata* (Santos et al., 2017a), *Piper marginatum*, *Piper aduncum* and *Piper arboretum* (Santana et al., 2015). The EO from *Syagrus coronata* (Arecaceae) seeds showed toxic effects to fourth instar from *A. aegypti*, with a LC_{50} value of 21.07 µg/mL (Santos et al., 2017a), which is quite similar to the results found in *C. rudolphianus* oil ($LC_{50} = 21.86$ µg/mL). The oils from *P. marginatum*, *P. aduncum* and *P. arboreum* (Piperaceae) leaves were less active against *A. aegypti* larvae than *C. rudolphianus* oil since they showed LC_{50} values of 34, 46 and 55 µg/mL, respectively) (Santana et al., 2015).

Some EOs from Euphorbiaceae family possess effect against larvae of *A. aegypti*. As example, the EOs from leaves, stalks and inflorescences of *Croton jacobinenesis* and the EO from *Croton piauhiensis* and *Croton rhamnifolioides* leaves showed toxic effects to third instar larvae of *A. aegypti*. The LC_{50} values were 79.3, 117.2 and 65.8 µg/mL (Pinto et al., 2016), 336.8 µg/mL (Silva et al., 2019b) and 89.03 µg/mL (Santos et al., 2014), which are less active to *A. aegypti* than *C. rudolphianus* oil ($LC_{50} = 21.86$ µg/mL). The oil from leaves of *Croton*

argyrophyllus also had a toxic effect against larvae in the third and fourth instars of *A. aegypti* (LC_{50} and LC_{90} of 0.31 and 0.70 mg/mL; 5.92 and 8.94 mg/mL, respectively) (Cruz et al., 2017). The oil from *Croton tetradenius* leaves present toxic effect against *A. aegypti* larvae in the third or fourth instar, the LC_{50} and LC_{90} after 24 h of exposure were 152 and 297 μg/mL (Carvalho et al., 2016), which are worse results than the ones found in this study.

The (*E*)-caryophyllene, the major compound from both EOs, showed a weak deleterious effect to larvae of *A. aegypti* with LC_{50} values in the range of 1038-1202 μg/mL (Silva et al., 2008; Dória et al., 2010). Due to it, this compound may not be the main responsible for the larvicidal effect observed in the *C. rudolphianus* oil. Germacrene D, other main compound from the oils, possess better larvidal effect to *A. aegypti* (LC_{50} = 63.3 μg/mL) (Kiran et al., 2006) when compared to (*E*)-caryophyllene. According to some authors (You et al., 2014; Bakkali et al., 2008), the biological activities from EOs, in this case deleterious effect to *A. aegypti*, may be related to synergistic action among the compounds, a particular compound, or the main components can be modulated by other minor molecules.

In relation to pupicidal bioassay, the EOs of *A. macrochlamys* and *C. rudolphianus* did not show any mortality for pupae at all concentrations assayed (50 - 700 μg/mL) in 48 hours. The control group also did not present mortality. Unlike the results found in this study, the EOs from *Croton sonderianus* and *Croton grewioides* possess toxic effects to pupae of *A. aegypti*, with LC_{50} value of 494.9 and 456.6 μg/mL, respectively (Lima et al., 2013). The *Eucalyptus globulus* oil possess pupicidal effect to pupae of *A. aegypti*, with LC_{50} and LC_{90} values of 144.5 and 741.3 μg/mL, respectively (Kaura et al., 2019).

The EOs from *A. macrochlamys* and *C. rudolphianus* showed ovicidal proprieties, because these oils decreased the eggs hatching rate of *A. aegypti* in all concentration assayed, when compared with control, as showed in Table 1. The highest concentration of EOs from *A. macrochlamys* (300 μg/mL) and *C. rudolphianus* (250 μg/mL) assayed reduced the eggs hatching rate of *A. aegypti* in 55.34% in relation to the control. Statistical analysis revealed significant differences in mortality between doses from *A. macrochlamys* and *C. rudolphianus* EOs ($F_{4,10}$ = 31.85; $p < 0.0001$/ $F_{4,10}$ = 66.54; $p < 0.0001$, respectively). Similar results were found by Lima et al (2013), the EOs from aerial parts of *Croton argyrophylloides*, *Croton nepetaefolius*, *Croton sonderianus* and *Croton grewioides* decreased the eggs hatching rate of *A. aegypti* (LC_{50}= 116.2, 141.2, 143.2 and 45.7 μg/mL, respectively). These oils also possess toxic effects to third instar of *A. aegypti* larvae, with a LC_{50} value of 94.6, 66.4, 54.5 and 26.2 μg/mL, respectively (Lima et al., 2013). The oil from *Azadirachta indica* (neem), *Eucalyptus sp.*, *Syzygium aromaticum* (clove) reduced the eggs hatching rate of *A. aegypti* (Mirza; Zehra,

2018), similar to *A. macrochlamys* and *C. rudolphianus* oils. However, *S. coronata* EO from seeds did not possess ovicidal proprieties (Santos et al., 2017a).

4. Conclusion

A. macrochlamys oil from leaves did not present any toxic effect to larvae and pupae of the main dengue vector. However, this oil possess ovicidal effect to eggs from *A. aegypti*, and it could be a tool to control the hatching rate from eggs of the main dengue vector. On the other hand, the EO from *C. rudolphianus* leaves possess toxic effect to eggs and larvae in the fourth instar of *A. aegypti*. Thus, *A. macrochlamys* and *C. rudolphianus* oils represent promising sources of bioactive compounds against *A. aegypti*.

References

Amado, J. R. R.; Prada, A. L.; Diaz, J. G.; Souto, R. N. P.; Arranz, J. C. E.; Souza, T. P. 2020. Development, larvicide activity, and toxicity in nontarget species of the *Croton linearis* Jacq essential oil nanoemulsion. Environmental Science and Pollution Research, 20, 9410-9423.

Araújo, F. M.; Dantas, M. C. S. M.; Silva, L. S.; Aona, L. Y. S.; Tavares, I. F.; Souza-Neta, L. C. 2017. Antibacterial activity and chemical composition of the essential oil of *Croton heliotropiifolius* Kunth from Amargosa, Bahia, Brazil. Industrial Crops and Products, 105, 203-206.

Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. 2008.Biological effects of essential oils - A review. Food and Chemical Toxicology, 46, 446-475.

Blank, A.F; Arrigoni-Blank, M.F.; Bacci, L.; Costa Junior, L. M.; Nizio, D. A. C. 2019. Chemical Diversity and Insecticidal and Anti-tick Properties of Essential Oils of Plants from Northeast Brazil. In: Malik, S. (Ed.). Essential Oil Research: Trends in Biosynthesis, Analytics, Industrial Applications and Biotechnological Production. Springer International Publishing. pp. 235-258.

Benelli, G. 2015. Research in mosquito control: current challenges for a brighter future. Parasitology Research, 114, 801-805.

Bortolucci, W. C.; Oliveira, H. L. M.; Silva, E. S.; Campo, C. F. A. A.; Gonçalves, J. E.; Piau Junior, R.; Colauto, N. B.; Linde, G. A.; Gazi, Z. C. 2019. *Schinus terebinthifolius* essential oil and fractionsin the control of *Aedes aegypti*. Bioscience Journal, 35, 1575-1587.

Brasil. Fundação Oswaldo Cruz: Uma Instituição a Serviço da Vida. Vírus zika. Available in:<https://portal.fiocruz.br/pergunta/como-e-o-ciclo-de-vida-do-mosquito-aedes-aegypti> (Accessed 23 May 2019).

Brasil. Ministério da Saúde. Febre amarela: sintomas, tratamento, diagnóstico e prevenção. Available in: <http://saude.gov.br/saude-de-a-z/febre-amarela-sintomas-transmissao-e-prevencao> (Accessed in 24 January 2020).

Bravo, A., Likitvivatanavong, S., Gill, S.S., Soberón, M. 2011. *Bacillus thuringiensis*: a story of a successful bioinsecticide. Insect biochemistry and molecular biology, 41, 423–431.

Brito, S. S. S.; Silva, F.; Malheiro, R.; Baptista, P. Pereira, J. A. 2018. *Croton argyrophyllus* Kunth and *Croton heliotropiifolius* Kunth: Phytochemical characterization and bioactive properties. Industrial Crops & Products, 113, 308-315.

Carvalho, K. S.; Silva, S. L. C.; Souza, I. A.; Gualberto, S. A.; Cruz, R. C. D.; Santos, F. R. S.; Carvalho, M.G. 2016. Toxicological evaluation of essential oil from the leaves of *Croton tetradenius* (Euphorbiaceae) on *Aedes aegypti* and *Mus musculus*. Parasitology Research, 115, 3441-3448.

Cavalcante, K. R. L. J.; Tauil, P. L. 2017. Risk of re-emergence of urban yellow fever in Brazil. Revista Epidemiologia e Serviços de Saúde, 26, 617-620.

Costa, A. V.; Almeida, B. R; Gonçalves, L. V.; Crico, K. B.; Ignacchiti, M. D. C.; Pereira Junior, O. S.; Pinheiro, P. F.; Queiroz, V. T. 2015. Efeito moluscicida do óleo essencial de *Cymbopogon winterianus* Jowitt (Poaceae) sobre *Lymnaea columella* (Say, 1817) e *Biomphalaria tenagophila* (D'Orbigny, 1835). Revista Brasileira de Plantas Medicinais, 17, 707-712.

Cruz, R. C. D.; Silva, S. L. C. E.; Souza, I. A.; Gualberto, S. A.; Carvalho, K. S.; Santos, F. R.; Carvalho, M. G. 2017. Toxicological evaluation of essential oil from the leaves of *Croton argyrophyllus* (Euphorbiaceae) on *Aedes aegypti* (Diptera: Culicidae) and *Mus musculus* (Rodentia: Muridae). Journal of Medical Entomology, 54, 985-993.

Donalisio, M. R.; Freitas, A. R. R. 2015. Chikungunya in Brazil: an emerging challenge. Revista Brasileira de Epidemiologia, 18, 283-285.

Dória, G.A.A; Silva, W. J.; Carvalho, G.A.; Alves, P. B.; Cavalcanti, S.C.H. 2010. A study of the larvicidal activity of two *Croton* species from northeastern Brazil against *Aedes aegypti*. Pharmaceutical Biology, 48, 615-620.

Gebreziher, H. G.; Hailu, Z.; Abrha, E. 2018. Effect of botanical extracts from indigenous plant, Kotsili Mariam, on mortality of carmine cochineal insect (*Dactylopius coccus* Costa). Journal of Entomology and Zoology Studies, 6, 1107-1116.

Gomes, P. R. B.; Silva, A. L. S.; Pinheiro, L. L.; Lima, H. S.; Silva, E. F.; Silva, R. P.; Louzeiro, C. H.; Oliveira, M. B.; Filho, V. E. M. 2016. Evaluation of the larvicidal effectof the essential oil of *Zingiber officinale* Roscoe (ginger) against the mosquito *Aedes aegypti*. Revista Brasileira de Plantas Medicinais, 18, 597-604.

Kar, S.; Gupta, P.; Gupta, J. 2018. Essential Oils: Biological Activity Beyond Aromatherapy. Natural Product Sciences, 24, 139-147.

Kaura, T.; Mewara, A.; Zaman, K.; Sharma, A.; Agrawai, S. K.; Thakur, V.; Garg, A.; Sehgal, R. 2019. Utilizing larvicidal and pupicidal efficacy of *Eucalyptus* and neem oil against *Aedes* mosquito: An approach for mosquito control. Tropical Parasitology, 9, 12-17.

Kiran, S. R.; Bhavani, K; Devi, P. S.; Rao, B. R. R.; Reddy, K. J.2006. Composition and larvicidal activity of leaves and stem essential oils of *Chloroxylon swietenia* DC against *Aedes aegypti* and *Anopheles stephensi*. Bioresource technology, 97, 2481-2484.

Kraemer, M. U. G; Reiner Junior, R. C.; Brady, O. J.; Messina, J. P.; Gilbert, M.; Pigott, D. M.; Yi, D.; Johnson, K.; Earl, L.; Marczak, L. B.; Shirude, S.; Weaver, N. D.; Bisanzio, D.

Alexperkins, T.; Lai, S.; Lu, X.; Jones, P.; Coelho, G. E.; Carvalho, R. G.; Bortel, W. V.; Marsboom, C.; Hendrickx. G.; Schaffner, F.; Moore, C. G.; Nax, H. H.; Bengtsson, L.; Wetter, E.; Tatem, A. J.; Brownstein, J. S.; Smith, D. L.; Lambrechts, L.; Cauchemez, S.; Linard, C.; Faria, N. R.; Pybus, O. G.; Scott, T. W.; Liu, Q.; Yu, H. Wint, G. R. W.; Hay, S. I.; Golding, N. 2019. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. Nature Microbiology, 4, 854-863.

Lima, G. P. G.; Souza, T. M.; Freire, G. P.; Farias, D. F.; Cunha, A. P.; Ricardo, N. M. P. S.; Morais, S. M. 2013. Further insecticidal activities of essential oils from *Lippia sidoides* and *Croton* species against *Aedes aegypti* L. Parasitology Research, 112, 1953-1958.

Lira, C. S.; Pontual, E. V.; Albuquerque, L. P.; Paiva, L. M. P.; Paiva, P. M. G.; Oliveira, J. V.; Napoleão, T. H.; Navarro, D. M. A. F. 2015. Evaluation of the toxicity of essential oil from *Alpinia purpurata* inflorescences to *Sitophilus zeamais* (maize weevil). Crop Protection, 71, 95-100.

Maffei, M. E.; Gertsch, J.; Appendino, G. 2011. Plant volatiles: Production, function and pharmacology. Natural Product Reports, 28, 1359-1380.

Mirza, A.; Zehra, A. 2018. Bioefficacy of plant essential oils for the ovicidal, larvicidal and pupicidal activities against the Dengue vector *Ae. aegypti*. Journal of Entomology and Zoology Studies, 6, 1819-1823.

Oliveira, A. E. M. F. M.; Duarte, J. L.; Amado, J. R. R.; Cruz, R. A. S.; Rocha, C.F.; Souto, R. N. P.; Ferreira, R. M. A.; Santos, K., Conceição, E. C.; Oliveira, L. A. R.; Kelecom, A.; Fernandes, C. P.; Carvalho, J. C. T. 2016. Development of a larvicidal nanoemulsion with *Pterodon emarginatus* Vogel oil, 7, 1-19.

Pavela, R.; Benelli, G. 2016. Essential Oils as Ecofriendly Biopesticides? Challenges and Constraints. Trends in Plant Science, 12, 1000-1007.

Pinto, C. C. C.; Menezes, J. E. S. A.; Siqueira, S. M. C.; Melo, D. S.; Feitosa, C. R. S.; Santos, H. S. 2016. Chemical Composition and larvicidal activity against *Aedes aegypti* of essential

oils from *Croton jacobinenesis* Baill. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 15, 122-127.

Proença, C. E. B.; Lughadha, E. M. N.; Lucas, E. J.; Woodgyer, E. M. 2016. *Algrizea* (Myrteae, Myrtaceae): A New Genus from the Highlands of Brazil. Systematic Botany, 31, 320-326.

Ribeiro, I. A. T. A.; Silva, R.; Silva, A. G.; Milet-Pinheiro, P.; Paiva, P. M.; Navarro, D. M. A. F.; Silva, M. V.; Napoleão, T. H.; Correia, M. T. S. 2020. Chemical characterization and insecticidal effect against *Sitophilus zeamais* (maize weevil) of essential oil from *Croton rudolphianus* leaves. Crop Protection, 129, 1-7.

Santana, H. T.; Trindae, F. T. T.; Stabeli, R. G.; Silva, A. A. E.; Militão, J. S. L. T.; Facundo, V. A. 2015. Essential oils of leaves of *Piper* species display larvicidal activity against the dengue vector, *Aedes aegypti* (Diptera: Culicidae). Revista Brasileira de Plantas Medicinais, 17, 105-111.

Santos, G. K. N.; Dutra, K. A.; Lira, C. S.; Lima, B. N.; Napoleão, T. H.; Paiva, P. M. G.; Maranhão, C. A.; Brandão, S. S. F.; Navarro, D. M. A. F. 2014. Effects of *Croton rhamnifolioides* Essential Oil on *Aedes aegypti* Oviposition, Larval Toxicity and Trypsin Activity. Molecules, 19, 16573-16587.

Santos, H. S.; Bandeira, P. N.; Lemos, T. L. G.; Santiago, M. P. 2017b. Chemical composition and larvicidal activity against *Aedes aegypti* L. (Diptera: Culicidae) of essential oils from leaves, stalks and roots of the *Croton nepetaefolius* Baill (Euphorbiaceae). International Journal of Mosquito Research, 4, 19-22.

Santos, L. M. M.; Nascimento, J. S.; Santos, M. A. G.; Marriel, N. B.; Bezerra-Silva, P. C.; Rocha, S. K. L.; Silva, A. G.; Correia, M. T.; Paiva, P. M. G.; Martins, G. F.; Navarro, D. M. A. F.; Silva, M. V.; Napoleão, T. H. 2017a. Fatty acid-rich volatile oil from *Syagrus coronata* seeds has larvicidal and oviposition-deterrent activities against *Aedes aegypti*. Physiological and Molecular Plant Pathology, 100, 35-40.

Sharifi-Rad, J.; Sureda, A.; Tenore, G. C.; Daglia, M.; Sharifi-Rad, M.; Valussi, M.; Tundis, R.; Sharifi-Rad, M.; Loizzo, M. R.; Ademiluuyi, A. O.; Sharifi-Rad, R.; Ayatollahi, S. A.; Iriti, M. 2017. Biological activities of essential oils: from plant chemoecology to traditional healing systems. Molecules, 22, 1-55.

Shaw, W.R.; Catteruccia, F. 2019. Vector biology meets disease control: using basic research to fight vector-borne diseases. Nature Microbiology, 4, 20-34.

Silva, J. S.; Sales, M. F.; Gomes, A. P. S.; Carneiro-Torres, D. S. 2010. Sinopse das espécies de *Croton* L. (Euphorbiaceae) no estado de Pernambuco, Brasil. Acta Botânica Brasilica, 24, 441-153.

Silva, L. S. B.; Cardoso, R. T. N.; Fernandes, J. L. A.; Silva, C. A.; Eiras, A. E. 2018. Modelo Entomológico Determinístico sob Efeito da Pluviosidade para o *Aedes aegypti* e o *Aedes albopictus*. Tendências em Matemática Aplicada e Computacional, 19, 289-303.

Silva, P. T.; Silva, H. C.; Vale, J. P. C.; Frota, V. M.; Rodrigues, T. H. S.; Souza, E. B.; Santiago, G. M. P.; Bandeira, P. N.; Teixeira, A. M. R.; Santos, H. S. 2019b. Circadian rhythm and larvicidal activity against *Aedes aegypti* of essential oils from *Croton piauhiensis*. Revista Virtual de Química, 11, 1682-1692.

Silva, T. L.; Oliveira, C. R. F.; Matos, C. H. C.; Badji, C. A.; Morato, R. P. 2019a. Leaf essential oil from Croton pulegiodorus baill shows insecticidal activity against Sitophilus zeamais motschulsky. Revista Caatinga, 32, 354-363.

Silva, W. J.; Dória, G. A. A.; Maia, R. T.; Nunes, R. S.; Carvalho, G. A.; Blank, A. F.; Alves, P. B.; Marçal, R. M; Cavalcanti, S. C. H. 2008. Effects of essential oils on *Aedes aegypti* larvae: Alternatives to environmentally safe insecticides. Bioresource Technology, 99, 3251-3255.

Sobral, M.; Faria Júnior, J. E. Q.; Proença, C. E. B. 2010. A new species of Algrizea (Myrteae, Myrtaceae) from Bahia, Brazil. Neodiversity, 5, 1-6.

Veras, B. O.; Santos, Y. Q.; Oliveira, F. G. S.; Almeida, J. R. G. S.; Silva, A. G.; Correia, M. T. S.; Diniz, K. M.; Oliveira, J. R. S.; Lima, V. L. M.; Navarro, D. M. A. F.; Aguiar, J. C. R.

O. F.; Oliveira, M. B. M.; Lopes, A. C. S.; Silva, M. V. 2019. *Algrizea minor* Sobral, Faria & Proença (Myrteae, Myrtaceae): Chemical composition, antinociceptive, antimicrobial and antioxidant activity of essential oil. Natural Product Research, 4, 1-5.

World Health Organization (WHO). Dengue and severe dengue. Fact sheet. Available in: <https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue> (Accessed 10 February 2020a).

World Health Organization (WHO). Zika virus. Available in: <http://www.who.int/gho/neglected_diseases/schistosomiasis/en/> (Accessed 23 January 2020b.

World Health Organization. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO, Geneva, 2005.

You, C.X., Yang, K., Wu, Y., Zhang, W.J., Wang, Y., Geng, Z.F., Chen, H.P., Jiang, H.Y., Du, S.S., Deng, Z.W., 2014. Chemical composition and insecticidal activities of the essential oil of *Perilla frutescens* (L.) Britt aerial parts against two stored product insects. European Food Research and Technology, 239, 481–490.

Figures legends

Figure 1 - Larvicidal effect of the essential oil from *C. rudolphianus* leaves against *A. aegypti*. The data are expressed as average ± SD (n=60). The Tukey's tests was used to evaluate significant differences between treatments, which is represented by different letters ($p < 0.05$). C: negative control (0.1%, v/v, Tween 80).

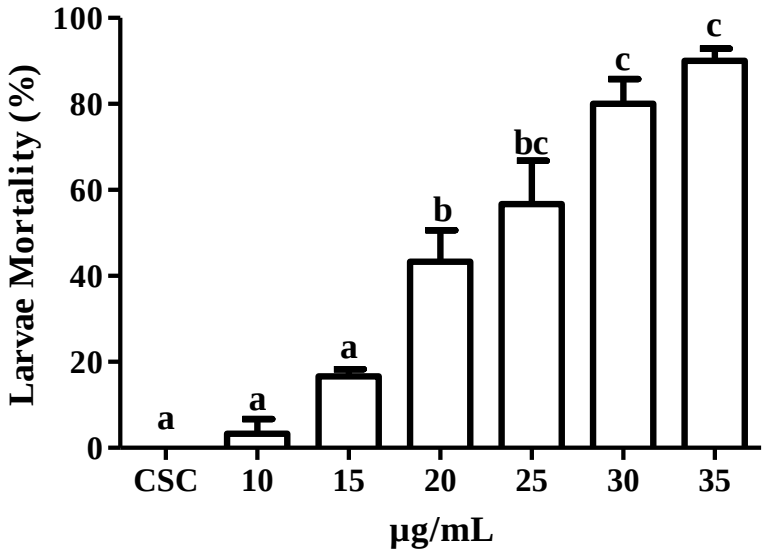


Table 1 - Effect of *A. macrochlamys* and *C. rudolphianus* essential oils on hatching of *A. aegypti* eggs. The data are expressed as average ± SD (n=150). The Tukey's tests was used to evaluate significant differences between treatments, which is represented by different letters ($p < 0.05$). C: negative control (0.1%, v/v, Tween 80).

Treatment	Hatching rate (%) ± SD
Control	78.67 ± 3.06 a
EO from *A. macrochlamys* (µg/mL)	
150	48 ± 7.21 bc
200	31.33 ± 6.11 c
250	28 ± 9.17 c
300	23.33 ± 7.57 c
EO from *C. rudolphianus* (µg/mL)	
100	48.67 ± 4.16 b
150	42.00 ± 7.21 b
200	29.33 ± 3.06 c
250	23.33 ± 4.16 c

4 CONCLUSÕES

O OE obtido a partir das folhas de *C. rudolphianus* apresentou efeito moluscicida contra adultos e todas as fases embrionárias testadas (blástula, gástrula, trocófora e veliger) de *B. glabrata*, e cercárias de *S. mansoni*. Adicionalmente, esse óleo possuiu efeito citotóxico para hemócitos de *B. glabrata* e apresentou uma baixa toxidade para *A. salina* (organismo não alvo).

Por sua vez, o OE das folhas de *A. macrochlamys* mostrou ser tóxico contra adultos e embriões (nas fases de blástula, gástrula, trocófora e veliger) de *B. glabrata*, e cercárias de *S. mansoni*. Além disso, esse óleo não apresentou nenhum efeito tóxico para o organismo não alvo testado (*A. salina*) nas concentrações utilizadas nesse estudo.

O (*E*)-cariofileno, composto majoritário dos dois OEs, apresentou efeito deletério contra todas as fases embrionárias testadas (blástula, gástrula, trocófora e veliger) de *B. glabrata* e cercárias de *S. mansoni*, além disso, não foi tóxico para o organimo não alvo testado (*A. salina*).

Os óleos de *A. macrochlamys* e *C. rudolphianus* reduziram a taxa de eclosão dos ovos de *A. aegypti*. Além disso, o OE de *C. rudolphianus* apresentou ação tóxica para larvas no quarto instar de *A. aegypti*

Devido ao exposto, esses dois OEs e o composto (*E*)-cariofileno poderiam ser utilizados no futuro como ferramentas para o controle de duas doenças tropicais negligenciadas: esquistossomose e dengue.

REFERÊNCIAS

ABREU, F. C.; GOULART, M. O. F.; OLIVEIRA BRETT, A. M. Detection of the damage caused to DNA by niclosamide using an electrochemical DNA-biosensor. **Biosensors and Bioelectronics**, v. 17, p. 913-919, 2002.

AGUIAR, M.; STOLLENWERK, N.; HALSTEAD, S. B. The risks behind Dengvaxia recommendation. **The Lancet Infectious Diseases**, v. 16, p. 882-883, 2016.

AHARONI, A.; GALILI, G. Metabolic engineering of the plant primary–secondary metabolism interface. **Current Opinin in Biotechology**, v. 22, p. 239-244, 2011.

ALI, B.; AL-WABEL, N.; SHAMS, S.; AHAMAD, A.; KHAN, S. A.; ANWAR, F. Essential oils used in aromatherapy: A systemic review. **Asian Pacific Journal of Tropical Biomedicine**, v. 5, p. 601-611, 2015.

ALMEIDA, J. R. G. S.; RAMALHO, A. C. M.; SILVEIRA, F. G. *Croton zehntneri* Pax & K. Hoffm (Euphorbiaceae). **Medicinal and Aromatic Plants of South America**, v. 5, p. 173-182, 2018.

ALMEIDA, J.; SOUZA, A. V.; OLIVEIRA, A. P.; SANTOS, U.; SOUZA, M.; BISPO, L.; TURATTI, Z. C.; LOPES, N. Chemical composition of essential oils from *Croton conduplicatus* (Euphorbiaceae) in Two Different Seasons. **Journal of Essential Oil Bearing Plants**. v. 17, p. 1137-1145, 2014.

ALVES, I. A. B. S. Estudo farmacognóstico e etnofarmacológico DE *Croton cordiifolius* BAIL. (EUPHORBIACEAE). Tese de doutrado. Recife, PE: Universidade Federal de Pernambuco, Recife, 2017, 126 p.

ANULIKA, N. P.; IGNATIUS, E. O.; RAYMOND, E. S.; OSASERE, O. I.; ABIOLA, A. H. The chemistry of natural product: plant secondary metabolites. **International Journal of Technology Enhancements and Emerging Engineering Research**, v. 4, p. 1-8, 2016.

ARAÚJO, A. C. J.; FREITAS, P. R.; BARBOSA, C. R. S.; MUNIZ, D. F.; ROCHA, J. E.; ARAÚJO NETO, J. B.; SILVA, M. M. C.; MOURA, T. F.; PEREIRA, R. L. S.; RIBEIRO-FILHO, J.; SILVA, L. E.; AMARAL, W.; DESCHAMPS, C.; TINTINO, S. R.; IRITI, M.; VITALINI, S. COUTINHO, H. D. M. Essential Oil of *Croton ceanothifolius* Baill. Potentiates the effect of antibiotics against multiresistant bacteria. **Antibiotics**, v. 9, p.1-8, 2020.

ARAÚJO, F. M.; DANTAS, M. C.S.M.; SILVA, L.S.; AONA, L. Y. S.; TAVARES, I. F.; SOUZA-NETA, L. C. Antibacterial activity and chemical composition of the essential oil of *Croton heliotropiifolius* Kunth from Amargosa, Bahia, Brazil**. Industrial Crops and Products**, v. 105, p. 203-206, 2017.

ARAÚJO, H. D. A. **Atividade moluscicida do ácido úsnico e do usnato de potássio sobre a *Biomphalaria glabrata***. Dissertação de mestrado. Recife, PE: Universidade Federal de Pernambuco, Recife, 2016, 115 p.

ASBAHANI, A.; MILADI, K.; BADRI, W.; SALA, M.; ADDI, E. H. A.; CASABIANCA, H.; MOUSADIK, A.; HARTMANN, D.; JILALE, A.; RENAUD, F. N. R.; ELAISSARI, A. Essential oils: From extraction to encapsulation. **International Journal of Pharmaceutics**, v. 483, p. 220-243, 2015.

BAKKALI, F.; AVERBECK, S.; AVERBECK, D.; IDAOMAR, M. Biological effects of essential oils - A review. **Food and Chemical Toxicology**, v. 46, p. 446-475, 2008.

BARBOSA, C. S.; SANTOS, R. S.; GOMES, E. S.; ARAUO, K.; ALBUQUERQUE, J.; MELO, F.; SEVILHA, M. A.; BRASILEIRO, D.; BARRETO, M. I.; LEAL-NETO, O. B.; BARBOSA, V. CORREIA, W. C.; GUIMARÃES, R. J. P. S. Epidemiologia da esquistossomose no litoral de Pernambuco. **Revista de Patologia Tropical**, v, 43, p. 436-445, 2014.

BARBOZA, D. M.; ZHANG, C.; SANTOS, N. C.; SILVA, M. M. B. L.; ROLLEMBERG, C. V. V.; AMORIM, F. J. R.; UETA, M. T.; MELO, C. M. M.; ALMEIDA, J. A. P.; JERALDO, V. L. S.; JESUS, A. R. *Biomphalaria* species distribution and its effect on human *Schistosoma mansoni* infection in an irrigated area used for rice cultivation in northeast Brazil. **Geospatial health**, v.6, n. 3, p. 103-109, 2012.

BARRERA, C. A. C.; GÓMES, D.; CASTIBLANCO, F. A. Importancia medicinal del género *Croton* (euphorbiaceae). **Revista Cubana de Plantas Medicinales**, v. 21, p. 234-247, 2016.

BARSOUM, R. S.; ESMAT, G.; EL-BAZ, T. Human Schistosomiasis: Clinical Perspective: Review. **Journal of Advanced Research**, v. 4, p. 433-444, 2013.

BERGQUIST, N. R. Schistosomiasis: from risk assessment to control. **Trends in Parasitology**, v.18, p. 309-314, 2002.

BIASSOTI, A. V.; ORTIZ, M. A. L. Laboratorial diagnostic of dengue. **Revista Uningá Review**, v. 29, p. 122-126, 2017.

BOISSIER, J.; MONÉ, H.; MITTA, G.; BARGUES, M. D.; MOLYNEUX, D.; MAS-COMA, S. Schistosomiasis reaches Europe. **The Lancet**, v. 15, p. 757-758, 2015.

BORTOLUCCI, W. C.; OLIVEIRA, H. L. M.; SILVA, E. S.; CAMPO, C. F. A. A.; GONÇALVES, J. E.; PIAU JUNIOR, R.; COLAUTO, N. B.; LINDE, G. A.; GAZI, Z. C. *Schinus terebinthifolius* essential oil and fractionsin the control of *Aedes aegypti*. **Bioscience Journal**, v. 35, p. 1575-1587, 2019.

BRAGA, L. B. ***Biomphalaria tenagophila guaibensis* (Mollusca: Planorbidae): avaliação da suscetibilidade a *Schistosoma mansoni* e do status de subespécie**. Dissertação de Mestrado. Belo Horizonte, MG: Fundação Oswaldo Cruz, Belo Horizonte, 2012, 70 p.

BRASIL. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. **Niclosamida**. Disponível em: <http://portal.anvisa.gov.br/documents/111215/117782/n07.pdf/180560e6-ee19-4f95-9c1c-d8a52448b7bb> Acesso em: 23 Mar. 2018c.

BRASIL. FUNDAÇÃO OSWALDO CRUZ. Agência FIOCRUZ de notícias: **Doenças negligenciadas**. Disponível em <https://agencia.fiocruz.br/doen%C3%A7as-negligenciadas> Acesso em: 15 Jan. 2020a.

BRASIL. FUNDAÇÃO OSWALDO CRUZ: UMA INSTITUIÇÃO A SERVIÇO DA VIDA. **Vírus zika**. Disponível em: <https://portal.fiocruz.br/pergunta/como-e-o-ciclo-de-vida-do-mosquito-aedes-aegypti> Acesso em: 23 Maio 2019c.

BRASIL. INSTITUTO OSWALDO CRUZ. **Dengue: vírus e vetor**. Disponível em <http://www.ioc.fiocruz.br/dengue/textos/oportunista.html>. Acesso em: 27 Jan. 2020d.

BRASIL. INSTITUTO RENÉ RACHOU. FIOCRUZ MINAS. **Dengue**. Disponível em: <http://www.cpqrr.fiocruz.br/pg/dengue/> Acesso em: 10 de Maio de 2019a.

BRASIL. MINISTÉRIO DA SAÚDE. **Dengue causas, sintomas, tratamento e prevenção**. Disponível em: <http://portalms.saude.gov.br/saude-de-a-z/dengue> Acesso em: 10 Maio 2019b.

BRASIL. MINISTÉRIO DA SAÚDE. **Controle de Vetores**. Disponível em: <http://www.saude.gov.br/saude-de-a-z/controle-de-vetores-inseticidas-elarvicidas/controle-de-vetores> Acesso em: 27 Jan. 2020b.

BRASIL. MINISTÉRIO DA SÁUDE. **Distribuição da esquistossomose**. Disponível em: <http://portalarquivos2.saude.gov.br/images/png/2017/setembro/14/mapadistribuicaoesquitossomose-2010-1015.png> Acesso em: 16 Fev. 2018b.

BRASIL. MINISTÉRIO DA SÁUDE. **Esquistossomose**. Disponível em: <http://portalms.saude.gov.br/saude-de-a-z/esquistossomose> Acesso em: 16 Fev. 2018a.

BRASIL. MINISTÉRIO DA SAÚDE. **Febre amarela: sintomas, tratamento, diagnóstico e prevençã**o. Disponível em <http://saude.gov.br/saude-de-a-z/febre-amarela-sintomas-transmissao-e-prevencao> Acesso em: 24 Jan. 2020e.

BRASIL. MINISTÉRIO DA SAÚDE. Secretaria de Vigilância em Saúde. **Boletim epidemiológico 02**. Disponível em <http://portalarquivos2.saude.gov.br/images/pdf/2020/janeiro/20/Boletim-epidemiologico-SVS-02-1-.pdf> Acesso 30 Jan 2020c.

BRASIL. MINISTÉRIO DA SAÚDE. Secretaria de Vigilância em Saúde. Coordenação-Geral de Desenvolvimento da Epidemiologia em Serviços. **Guia de Vigilância em Saúde**: volume 3, 1. ed. Brasília: Ministério da Saúde, 2017. 751p.

BRASIL. MINISTÉRIO DA SAÚDE. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. **Vigilância da Esquistossomose Mansoni - diretrizes técnicas**: 4. ed. Brasília: Ministério da Saúde, 2014. 144 p.

BRITO, S. S. S.; SILVA, F.; MALHEIRO, R.; BAPTISTA, P. PEREIRA, J. A. *Croton argyrophyllus* Kunth and *Croton heliotropiifolius* Kunth: Phytochemical characterization and bioactive properties. **Industrial Crops & Products**, v. 113, p. 308-315, 2018.

BRUSOTTI, G.; CESARI, I.; DENTAMARO, A.; CACCIALANZA, G.; MASSOLINI, G. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. **Journal of Pharmaceutical and Biomedical Analysis**, v.87, p. 218-228, 2014.

BURKE, M. L.; JONES, M. K.; GOBERT, G. N.; LI, Y. S.; ELLIS, M. K.; McMANUS, D. P. Immunopathogenesis of human schistosomiasis. **Parasite Immunology**, v. 31, p. 163-176, 2009.

CANTANHEDE, S. P. D.; MARQUES, A. M.; SILVA-SOUZA, N.; VALVERDE, A. L. Atividade moluscicida de plantas: uma alternativa profilática. **Revista Brasileira de Farmacognosia**, v. 20, p. 282-288, 2010.

CARVALHO, K. S.; SILVA, S. L. C.; SOUZA, I. A.; GUALBERTO, S. A.; CRUZ, R. C. D.; SANTOS, F. R. S.; CARVALHO, M.G. Toxicological evaluation of essential oil from the leaves of *Croton tetradenius* (Euphorbiaceae) on *Aedes aegypti* and *Mus musculus*. **Parasitology Research**, v. 115, p. 3441-3448, 2016.

CARVALHO, O. S.; AMARAL, R. S.; DUTRA, L. V.; SCHOLTE, R. G. C.; GUERRA, M. A. M. **Distribuição Espacial de *Biomphalaria glabrata, B. straminea* e *B. tenagophila,* Hospedeiros Intermediários do *Schistosoma mansoni* no Brasil**. In: CARVALHO, O. S.; COELHO, P. M. Z.; LENZI, H. L. *Schistosoma mansoni* e Esquistossomose: Uma visão multidisciplinar. 1ª ed. Rio de Janeiro: Editora Fiocruz, 2008, p. 393-418,

CASTRO, A. K. S. **Avaliação clínica-epidemiológica da esquistossomose mansoni em Comercinho, MG (1981-2005)**. Dissertação de Mestrado. Belo Horizonte, MG: Santa Casa de Belo Horizonte, 2009, 138 p.

CAVALCANTE, K. R. L. J.; TAUIL, P. L. Risk of re-emergence of urban yellow fever in Brazil. **Revista Epidemiologia e Serviços de Saúde**, v. 26, p. 617-620, 2017.

CENTER FOR DISEASE CONTROL AND PREVENTION (CDC). Laboratory Identification of Parasitic Diseases of Public Health Concern. **About Dengue: What You Need to Know**. Disponível em: <https://www.cdc.gov/dengue/about/index.html> Acesso em: 01 Fev. 2020.

CENTER FOR DISEASE CONTROL AND PREVENTION (CDC). Laboratory Identification of Parasitic Diseases of Public Health Concern. **Schistosomiasis infection**. Disponível em: <https://www.cdc.gov/dpdx/schistosomiasis/index.html> Acesso em: 07 Nov. 2017.

CHURAKOV, M.; VILLABONA-ARENAS, C.; KRAEMER, M. U. G.; SALJE, H.; CAUCHEMEZ, S. Spatio-temporal dynamics of dengue in Brazil: Seasonal travelling waves and determinants of regional synchrony. **PLOS Neglected Tropical Diseases**, v. 13, p. 1-13, 2019.

CLEMONS, A.; HAUGEN, M.; FLANNERY, E.; TOMCHANEY, M.; KAST, K.; JACOWSKI, C.; LE, C.; MORI, A.; HOLLAND, W. S.; SARRO, J.; SEVERSON, D. W.; SCHEEL, M. D. *Aedes aegypti*: an emerging model for vector mosquito development. **Cold Spring Harbor protocols**, v. 2010, p. 1-17, 2010.

COLLEY, D. G.; BUSTINDUY, A. L.; SECOR, W. E.; KING, C. H. Human schistosomiasis. **The Lancet**. v. 383, p. 2253-2264, 2014.

COSTA, A. V.; ALMEIDA, B. R; GONÇALVES, L. V.; CRICO, K. B.; IGNACCHITI, M. D. C.; PEREIRA JUNIOR, O. S.; PINHEIRO, P. F.; QUEIROZ, V. T. Efeito moluscicida do óleo essencial de *Cymbopogon winterianus* Jowitt (Poaceae) sobre *Lymnaea columella* (Say, 1817) e *Biomphalaria tenagophila* (D'Orbigny, 1835). **Revista Brasileira de Plantas Medicinais**, v. 17, p. 707-712, 2015.

CRUZ, R. C. D.; SILVA, S. L. C. E.; SOUZA, I. A.; GUALBERTO, S. A.; CARVALHO, K. S.; SANTOS, F. R.; CARVALHO, M. G. Toxicological evaluation of essential oil from the leaves of *Croton argyrophyllus* (Euphorbiaceae) on *Aedes aegypti* (Diptera: Culicidae) and *Mus musculus* (Rodentia: Muridae). **Journal of Medical Entomology**, v. 54, p. 985-993, 2017.

DETTOGNO, R. S.; LOURO, I. D. Desafios da extração do RNA do vírus da dengue (2017). In: RESENDE, R. R. (org.) Biotecnologia Aplicada à Agro&Indústria. 1. ed. São Paulo: Blucher, 2017. pp. 937-966.

DHIFI, W.; BELLILI, S.; JAZI, S.; BAHLOUL, N. MNIF, W. Essential oils' chemical characterization and investigation of some biological activities: a critical review. **Medicines**, v. 3, p. 1-16, 2016.

DIAS, C. N.; RODRIGUES, K. A. F.; CARVALHO, F. A. A.; CARNEIRO, S. M. P.; MAIA, J. G. S.; ANDRADE, E. H. A.; MORAES, D. F. C. Molluscicidal and Leishmanicidal Activity of the Leaf Essential Oil of *Syzygium cumini* (L.) Skeels from Brazil. **Chemistry & Biodiversity**, v. 10, p. 1133-1141, 2013.

DÍAZ, J. G.; ARRANNZ, J. C. E.; BATISTA, D. G. J.; FIDALGO, L. M.; ACOSTA, J. V. MACEDO, M. B.; COS, P. Antileishmanial Potentialities of *Croton linearis* Leaf Essential Oil. **Natural Product Communications**, v. 13, p. 629-634, 2018.

DÍAZ, J. G.; TUENTER, E.; ARRANZ, J. C. E.; MAURY, G. L.; COS, P.; PIETERS, L. Antimicrobial activity of leaf extracts and isolated constituents of *Croton linearis*. **Journal of Ethnopharmacology**, v. 236, p. 250-257, 2019.

DOMINGUES, C. M. A. S.; GARCIA, M. H. O. **Dengvaxia®: Ministério da Saúde emite nota informativa em resposta à Abrasco.** Disponível em: <https://www.abrasco.org.br/site/outras-noticias/saude-da-populacao/dengvaxia-respostaministerio-da-saude-dengue/32911/> Acesso em: 27 Jan 2020.

DONALISIO, M. R.; FREITAS, A. R. R. Chikungunya in Brazil: an emerging challenge**.** **Revista Brasileira de Epidemiologia**, v. 18, p. 283-285, 2015.

FARIA, R. X.; ROCHA, L. M.; SOUZA, E. P. B. S. S.; ALMEIDA, F. B.; FERNANDES, C. P.; SANTOS, J. A. A. Molluscicidal activity of *Manilkara subsericea* (Mart.) dubard on *Biomphalaria glabrata* (Say, 1818). **Acta Tropica**, v. 178, p. 163-168, 2018.

FERREIRA, A. M.; MOUCHREK-FILHO, V. E.; MAFRA, N. S. C.; SALES, E. H.; SANTOS-JÚNIOR, P. S.; EVERTON, G. O. Chemical constituents, toxicity, antioxidant potential and larvicidal activity against *Aedes aegypti* larvae of *Aniba rosaeodora* Ducke essential oil. **Research, Society and Development**, v. 9, p. 1-23, 2020.

FLORA DO BRASIL 2020. Jardim Botânico do Rio de Janeiro. ***Algrizea***. Dispovível em: <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB36819> Acesso em: 21 Jan. 2020a.

FLORA DO BRASIL 2020. Jardim Botânico do Rio de Janeiro. ***Algrizea macrochlamys***. Disponível em: <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB36820> Acesso em: 23 Jan. 2020d.

FLORA DO BRASIL 2020. Jardim Botânico do Rio de Janeiro. ***Croton***. Dispovível em <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB17536> Acesso em: 17 Jan. 2020b.

FLORA DO BRASIL 2020. Jardim Botânico do Rio de Janeiro. ***Myrtaceae***. Dispovível em <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB171> Acesso em: 21 Jan. 2020c.

FONSECA, L.; ROCHA, M. S.; BRITO, L. C.F.; SOUSA, E. S.; REINALDO, F. S.; PEREIRA, F. M.M.; SANTOS, F. E.P; LIMA, S. G. Characterization of Inclusion Complex of *Croton zehntneri* Essential Oil and β-Cyclodextrin Prepared by Spray Drying and Freeze Drying. **Revista Virtual de Química**, v. 11, p. 529-542, 2019.

FONTES-JÚNIOR, U. R.; RAMOS, C. S.; SERAFINI, M. R.; CAVALCANTI, S. C. H.; ALVES, P. B.; LIMA, G. M.; ANDRADE, P. H. S.; BONJARDIM, L. R.; QUINTANS-JÚNIOR, L. J.; ARAÚJO, A. A. S. Evaluation of the lethality of *Porophyllum ruderale* essential oil against *Biomphalaria glabrata*, *Aedes aegypti* and *Artemia salina*. **African Journal of Biotechnology**, v.11, p. 3169-3172, 2012.

FORATTINI, O. P. **Culicidologia Médica: Identificação, Biologia, Epidemiologia**. 1ª Edição.São Paulo: Edusp; v. 2, 2002.

FRAUCHES, N. S.; AMARAL, T. O. LARGUEZA, C. B.; TEODORO, A. J. Brazilian Myrtaceae fruits: a review of anticancer proprieties. **British Journal of Pharmaceutical Research**, v. 12, p. 2231-2919, 2016.

GOBBO-NETO, L.; LOPES, N. P. Medicinal plants: factors of influence on the content of secondary metabolites. **Química Nova**, v. 30, p. 374-381, 2007.

GOMES, A. C. L.; GALINDO, J. M.; LIMA, N. N.; SILVA, E. V. G. Prevalência e carga parasitária da esquistossomose mansônica antes e depois do tratamento coletivo em Jaboatão dos Guararapes, Pernambuco. **Epidemiologia e Serviços de Saúde**. v. 25, p. 243-250. 2016a.

GOMES, P. R. B.; SILVA, A. L. S.; PINHEIRO, L. L.; LIMA, H. S.; SILVA, E. F.; SILVA, R. P.; LOUZEIRO, C. H.; OLIVEIRA, M. B.; FILHO, V. E. M. Evaluation of the larvicidal effectof the essential oil of *Zingiber officinale* Roscoe (ginger) against the mosquito *Aedes aegypti*. **Revista Brasileira de Plantas Medicinais**, v. 18, p. 597-604, 2016b.

GOVERNO DO ESTADO DE PERNAMBUCO. SECRETARIA ESTADUAL DE SAÚDE. **Programa sanar – Doenças negligenciadas**. Disponível em: < http://portal.saude.mpe.gov.br /programa/secretaria-executiva-de-vigilancia-em-saude/programa-sanar-doencas-negligenciadas> Acesso em: 05 Abr. 2018.

GURARIE, D.; YOON, N.; LI, E.; NDEFFO-MBAH, M.; DURHAM, D.; PHILLIPS, A. E.; AURELIO, H. O.; FERRO, J.; GALVANI, A. P.; KING, C. H. Modelling control of *Schistosoma haematobium* infection: predictions of the long-term impact of mass drug administration in Africa. **Parasites & vectors**, v. 8, p. 1-14, 2015.

HONÓRIO, N. A.; CÂMARA, D. C. P.; CALVET, G. A.; BRASIL, P. Chikungunya: an arbovirus infection in the process of establishment and expansion in Brazil. **Cadernos de Saúde Pública**, v. 31, p. 906-908, 2015.

INSTITUTO BUTANTAN. **Nota de esclarecimento vacina contra dengue**. Disponível em: <http://www.butantan.gov.br/noticias/nota-de-esclarecimento--vacina-contra-dengue> Acesso em: 27 Ago. 2020.

ISLAM, M. S.; ARA, H. AHMAD, K. I.; UDDIN, M. M. A review on medicinal uses of different plants of Euphorbiaceae family. **Universal Journal of Pharmaceutical Research**, v. 4, p. 47-51, 2019.

JORGE, N. A. **Estudo químico e avaliação da atividade moluscicida do óleo essencial de *Citrus limon* (limão) frente ao caramujo transmissor da esquistossomose (*Biomphalaria glabrata*)**. Monografia. São Luís, MA: Universidade Federal do Maranhão, São Luís, 2017, 68 p.

KADHIM, M. J; SOSA, A. A.; HAMMED, I. H. Evaluation of anti-bacterial activity and bioactive chemical analysis of *Ocimum basilicum* using Fourier transform infrared (FT-IR) and gas chromatographymass spectrometry (GC-MS) techniques. **Journal of Pharmacognosy and Phytotherapy**, v. 8, p. 127-146, 2016.

KAMANULA. J. F.; BELMAIN, S. R.; HALL, D. R.; FARMAN, D. I.; GOYDER, D. J.; MVUMI, B. M.; MASUMBU, F. F.; STEVENSON, P. C. Chemical variation and insecticidal activity of *Lippia javanica* (Burm. f.) Spreng essential oil against *Sitophilus zeamais* Motschulsky. **Industrial Crops and Products**, v. 110, p.75-82, 2017.

KAWANO, T. Embriologia. In: BARBOSA, F. **Tópicos em Malacologia Médica**. Editora Fiocruz, Rio de Janeiro, p. 157-200. 1995.

KAWANO, T., OKAZAKI, K., RÉ, L. Embryonic development of *Biomphalaria glabrata* (Say, 1818) (Mollusca, Gastropoda, Planorbidae): A practical guide to the main stages. Malacologia, v. 34, p. 25-32, 1992.

KAWANO, T.; NAKANO, E.; WATANABE, L. C. Estudo do desenvolvimento embrionário de *Biomphalaria glabrata* (Mollusca, Planorbidae) e suas aplicações. In: CARVALHO, O. S.; COELHO, P. M. Z.; LENZI, H. L. *Schistosoma mansoni* e Esquistossomose uma Visão Multidisciplinar. **Ed**. **FIOCRUZ**. Rio de Janeiro, 347-391, 2008.

LIMA, G. P. G.; SOUZA, T. M.; FREIRE, G. P.; FARIAS, D. F.; CUNHA, A. P.; RICARDO, N. M. P. S.; MORAIS, S. M. Further insecticidal activities of essential oils from *Lippia sidoides* and *Croton* species against *Aedes aegypti* L. **Parasitology Research**, v. 112, p. 1953-1958, 2013.

LINDOSO, J. A. L.; LINDOSO, A. A. B. P. Neglected tropical diseases in Brazil. **Revista do Instituto de Medicina Tropical de São Paulo**, v.51, p. 247-253, 2009.

LUCAS, E.J.; HOLST, B.; SOBRAL, M.; MAZINE, F. F.; LUGHADHA, E. M. N.; PROENÇA, C. E. B.; COSTA, I. R.; VASCONCELOS, T. N. C. A New Subtribal Classification of Tribe Myrteae (Myrtaceae). **Systematic Botany**, v. 44, p. 560-569, 2019.

LUZ, K. G.; SANTOS, G. I. V.; VIEIRA, R. M. Zika Virus Fever. **Epidemiologia e Serviços de Saúde**, v. 24, p. 785-788, 2015.

MAGALHÃES, C. R. I.; OLIVEIRA, C. R. F.; MATOS, C. H. C.; BRITO, S. S. S.; MAGALHÃES, T. A.; FERRAZ, M. S. S. Potencial inseticida de óleos essenciais sobre *Tribolium castaneum* em milho armazenado **Revista Brasileira de Plantas Medicinais**, v. 17, n. 4, p. 1150-1158, 2015.

MARCELINO, V.; FECHINE, J.; GUEDES, J. R.; TOSCANO, V.; FILGUEIRAS, P.; LEITAO, E. V.; BARBOSA- FILHO, J. M.; SOBRAL, M. Phytochemistry of the genus *Croton*. **Natural Products Research Reviews**, v. 1, p. 221-370, 2012.

MARQUES, L. **Amplie os cuidados contra o mosquito que transmite a dengue**. Disponível em<http://luizamarques2015.blogspot.com/2015/09/amplie-os-cuidados-contra-o-mosquito.ht ml> Acesso em: 28 de Jan. 2020.

MARTINS, M. C.; SILVA, M. C.; SILVA, L. R.; LIMA, V. L.; PEREIRA, E. C.; FALCÃO, E. P.; MELO, A. M.; DA SILVA, N. H. Usnic acid potassium salt: na alternative for the control of *Biomphalaria glabrata* (Say, 1818). **Plos One**, v. 9, p. 1-6, 2014.

MARTINS-MELO, F. R.; PINHEIRO, M. C. C.; RAMOS JR, A. N.; ALENCAR, C. H.; BEZERRA, F. S. M.; HEUKELBACH, J. Trends in schistosomiasis-related mortality in Brazil, 2000–2011. **International Journal for Parasitology**, v. 44, p. 1055-1062, 2014.

MARTINS-MELO, F. R.; PINHEIRO, M. C. C.; RAMOS JR, A. N.; ALENCAR, C. H.; BEZERRA, F. S. M.; HEUKELBACH, J. Spatiotemporal Patterns of Schistosomiasis-Related Deaths, Brazil, 2000-2011. **Emerging Infectious Diseases**, v. 21, p. 1820-1823, 2015.

MEDEIROS, V. M.; NASCIMENTO, Y. M.; SOUTO, A. L.; MADEIRO, S. A. L.; COSTA, V. C. O.; SILVA, S. M. P. M.; SILVA, V. S. F.; AGRA, M. F.; SIQUEIRA-JÚNIOR, J. P.; TAVARES, J. F. Chemical composition and modulation of bacterial drug resistance of the essential oil from leaves of *Croton grewioides*. **Microbial Pathogenesis**, v. 111, p. 468-471, 2017.

MEDINA, J. M.; PEIXOTO, J. L. B.; SILVA, A. A.; HARAGUCHI, S. K.; FALAVIGNA, D. L. M.; ZAMUNER, M. L. M.; SARRAGIOTTO, M. H.; VIDOTTI, G. J. Evaluation of the molluscicidal and *Schistosoma mansoni* cercariae activity of *Croton floribundus* extracts and kaurenoic acid. **Revista Brasileira de Farmacognosia**, v. 19, p. 207-211, 2009.

MIYASATO, P. A.; KAWANO, T.; FREITAS, J. C.; BERLINCK, R. G. S.; NAKANO, E.; TALLARICO, L. F. Molluscicidal activity of some marine substances against the snail *Biomphalaria glabrata* (Mollusca, Planorbidae). **Parasitology Research**, v. 110, p. 1873-1879, 2012.

MONTEIRO, P. A.; ZELIOLI, I. A. M; SOUSA, I. M. O.; RUIZ, A. L. T. G.; VENDRAMINI-COSTA, D. B.; FOGLIO, M. A.; CARVALHO, J. E. Chemical composition and antiproliferative activity of *Croton campestris* A.St.-Hil. essential oil. **Natural Product Research**, v. 33, p. 580-583, 2017.

MORAES, C. N; BICHARA, C. N. C.; PONTES, A. N.; PINTO, S. C. A.; GASÁRETTO, D. Correlação de criadouros de *Biomphalaria* sp., hospedeiro do *Schistosoma mansoni*, em área de baixa infraestrutura sanitária no Distrito de Mosqueiro, Belém, Pará. **Hygeia**, v. 10, p. 216-233, 2014.

NEVES, D. P. **Parasitologia Humana**. 13ª ed. São Paulo: Editora Atheneu, 2016.

NOGUEIRA, L. M.; SILVA, M. R.; SANTOS, S. M.; ALBURQUERQUE, J. F. C.; FERRAZ, I. C.; ALBUQUERQUE, T. T.; MOTTA, C. R. F. C.; ARAÚJO, R. M.; VIANA, G. S. B.; MARTINS, R. D.; HAVT, A.; XIMENES, R. M. Antinociceptive effect of the

essential oil obtained from the leaves of *Croton cordiifolius* Baill. (Euphorbiaceae) in mice. **Evidence-Based Complementary and Alternative Medicine**, v. 2015, p. 1-7, 2015.

NOUR, A. H. Formulation of solid/liquid perfumes of essential oils from different medicinal plants. **Journal of Faculty of Sciences**, v. 5, p. 95-117, 2018.

OLIVEIRA-FILHO, E. C.; GERALDINO, B. R.; COELHO, D. R.; DE-CARVALHO, R. R.; PAUMGARTTEN, F. J. R. Comparative toxicity of *Euphorbia milii* latex and synthetic molluscicides to *Biomphalaria glabrata* embryos. **Chemosphere**, v. 81, p. 218-227, 2010.

OLIVEIRA-FILHO, E. C.; PAUMGARTTEN, F. J. R. Toxicity of *Euphorbia milii* latex and niclosamide to snails and nontarget aquatic species. **Ecotoxicology and Environmental Safety**, v. 46, p. 342-350, 2000.

OLIVEIRA-TINTINO, C. D. M.; PESSOA, R. T.; FERNANDES, M. N. M.; ALCÂNTRA, I. S.; SILVA, B. A. F.; OLIVEIRA, M. R. C.; MARTINS, A. O. B. P. B.; SILVA, M. S.; TINTINO, S. R.; RODRIGUES, F. F. G.; COSTA, J. G. M.; LIMA, S. G.; KERNTOP, M. R.; SILVA, T. G.; MENEZES, I. R. A. Anti-inflammatory and anti-edematogenic action of the *Croton campestris* A. St.-Hil (Euphorbiaceae) essential oil and the compound β-caryophyllene in *in vivo* models. **Phytomedicine**, v. 41, p. 82-95, 2018.

PARAENSE, W. L. Estado atual da sistemática dos planorbídeos brasileiros. **Arquivos do Museu Nacional**, v. 55, p. 105-128, 1975.

PAVELA, R.; BENELLI, G. Essential Oils as Ecofriendly Biopesticides? Challenges and Constraints. **Trends in Plant Science**, v. 12, p. 1000-1007, 2016.

PEREIRA, L. P. L. A.; DIAS, C. N.; MIRANDA, M. V.; FIRMO, W. C. A.; ROSA, C. S.; SANTOS, P. F.; BRITP, M. C. A.; ARARUNA, F. O. S.; ARARUNA, F. B.; SILVA-SOUZA, N.; COUTINHO, D. F. Molluscicidal effect of *Euphorbia umbellata* (Pax) Bruyns latex on *Biomphalaria glabrata*, *Schistosoma mansoni* host snail. **Revista do Instituto de Medicina Tropical de São Paulo**, v. 59, p. 1-5, 2017.

PROENÇA, C. E. B.; LUGHADHA, E. M. N.; LUCAS, E. J.; WOODGYER, E. M. *Algrizea* (Myrteae, Myrtaceae): A New Genus from the Highlands of Brazil. **Systematic Botany**, v. 31, p. 320-326, 2006.

QUITES, H. F. O.; ABREU, M. N. S.; MATOSO, L. F.; GAZZINELI, A. Avaliação das ações de controle da esquistossomose na Estratégia de Saúde da Família em municípios do Vale do Jequitinhonha em Minas Gerais. **Revista brasileira de epdidemiologia**, v. 19, p. 375-389, 2016.

RAMALHO, S. D.; PINTO, M. E. F.; FERREIRA, D.; BOLZANI, V. S. Biologically active orbitides from the Euphorbiaceae family. **Planta medica**, v. 84, p. 558-567, 2018.

RAPADO, L. N. **Obtenção e avaliação da atividade de compostos isolados de *Piper* em modelos biológicos para o controle da esquistossomose mansônica**. Tese de Doutorado. São Paulo, SP: Universidade de São Paulo/Instituto Butantan, São Paulo, 2012, 142 p.

REY, L. **Parasitologia**. 3ª ed. Rio de Janeiro: Guanabara Koogan, 2001.

REYES-JURADO, F.; FRANCO-VEGA, A.; RAMÍREZ-CORONA, N.; PALOU, E.; LÓPEZ-MALO, A. Essential oils: antimicrobial activities, extraction methods, and their modeling. **Food Engineering Reviews**, v. 7, p. 275-297, 2015.

RIBEIRO, E. C. G. **Atividade moluscicida de óleos essenciais de plantas aromáticas da região Amazônica maranhense.** Dissertação de Mestrado.São Luís, MA: Universidade Federal do Maranhão, 2016b, 91 p.

RIBEIRO, I. A. T. A. **Caracterização química e atividades biológicas do óleo essencial de *Croton rudolphianus* Müll. Arg. (Euphorbiaceae)**. Dissertação de mestrado. Recife, PE: Universidade Federal de Pernambuco, 2016a, 119 p.

RIBEIRO, I. A. T. A.; SILVA, R.; SILVA, A. G.; MILET-PINHEIRO, P.; PAIVA, P. M.; NAVARRO, D. M. A. F.; SILVA, M. V.; NAPOLEÃO, T. H.; CORREIA, M. T. S. Chemical characterization and insecticidal effect against *Sitophilus zeamais* (maize weevil) of essential oil from *Croton rudolphianus* leaves. **Crop Protection**, v. 129, p. 1-7, 2020.

RIOS, J. S. H. **Identificação de variantes em alvos farmacológicos do praziquantel e potenciais alvos para novas drogas esquistossomicidas**. Dissertação de Mestrado. Belo Horizonte, MG: Faculdade Infórium de Tecnologia, Belo horizonte, 2015, 69 p.

ROCHA-FILHO, C. A. A.; ALBUQUERQUE, L. P.; SILVA, L. R. S.; SILVA, P. C. B.; COELHO, L. C. B. B.; NAVARRO, D. M. A. F.; ALBUQUERQUE, M. C. P. A.; MELO, A. M. M. A.; NAPOLEÃO, T. H.; PONTUAL, E. V.; PAIVA, P. M. G. Assessment of toxicity of *Moringa oleifera* flower extract to *Biomphalaria glabrata*, *Schistosoma mansoni* and *Artemia salina*. **Chemosphere**, v. 132, p. 188-192, 2015.

RODRIGUES, O. G.; FALCÃO, B. R. M.; BARBOSA, B. C.; PEREIRA, A. V.; AQUINO, V. V. F. *In vitro* biological activity of the *Croton blanchetianus* (Baill) essential oil against *Rhipicephalus* (Boophilus) *microplus* (Acari: Ixodidae). **Journal of Applied Biology & Biotechnology**, v. 7, p. 55-58, 2019.

ROOHINEJAD, S.; KOUBAA, M.; BARBA, F. J.; LEONG, S. Y.; KHELFA, A.; GREINER, R.; CHEMAT, F. **Extraction methods of essential oils from herbs and spices**. In: HASHEMI, S. M. B.; KHANEGHAH, A. M. SANTÁNA, A. S. Essential oils in food processing: Chemistry, safety and applications. India: wiley, 2018, p. 21-56.

ROSÁRIO, M. S.; OLIVEIRA, M. L.; VIEIRA, M. A.; CARNEIRO, J. A.; COSTA, F. M. Neglected tropical diseases: characteristics of the affected individuals and their spatial distribution. **Brazilian Journal of Health Research**, v. 19, p. 118-127, 2017.

SALATINO, A.; SALATINO, F. S.; NEGRI, G. Traditional uses, Chemistry and Pharmacology of *Croton* species (Euphorbiaceae). **Journal of the Brazilian Chemical Society**, v. 18, p. 11-33, 2007.

SANTOS, G. K. N.; DUTRA, K. A.; LIRA, C. S.; LIMA, B. N.; NAPOLEÃO, T. H.; PAIVA, P. M. G.; MARANHÃO, C. A.; BRANDÃO, S. S. F.; NAVARRO, D. M. A. F. Effects of *Croton rhamnifolioides* Essential Oil on *Aedes aegypti* Oviposition, Larval Toxicity and Trypsin Activity. **Molecules**, v. 19, p. 16573-16587, 2014.

SANTOS, L. M. M. **Avaliação do potencial do óleo essencial de sementes de *Syagrus coronata* (martius) beccari (arecaceae: arecoideae) para controle do *Aedes aegypti***. Dissertação de Mestrado. Recife, PE: Universidade Federal de Pernambuco, Recife, 2016, 70 p.

SANTOS, L. M. M.; NASCIMENTO, J. S.; SANTOS, M. A. G.; MARRIEL, N. B.; BEZERRA-SILVA, P. C.; ROCHA, S. K. L.; SILVA, A. G.; CORREIA, M. T.; PAIVA, P. M. G.; MARTINS, G. F.; NAVARRO, D. M. A. F.; SILVA, M. V.; NAPOLEÃO, T. H. Fatty acid-rich volatile oil from *Syagrus coronata* seeds has larvicidal and oviposition-deterrent activities against *Aedes aegypti*. **Physiological and Molecular Plant Pathology**, v. 100, p. 35-40, 2017.

SCHOLTE, R. G. C.; GOSONIU, L.; MALONE, J. B.; CHAMMARTIN, F.; UTZINGER, J.; VOUNATSOU, P. Predictive risk mapping of schistosomiasis in Brazil using Bayesian geostatistical models. **Acta tropica**, v. 132, p. 57-63, 2014.

SCHRAM, P. C. F. Zika virus and public health. **Journal of Human Growth and Development**, v. 26, p 7-8, 2016.

SECCO, R. S.; CORDEIRO, I.; SENNA-VALE, L.; SALES, M. F.; LIMA, L. R.; MEDEIROS, D.; HAIAD, B. S.; OLIVEIRA, A. S.; CARUZO, M. B. R.; CANEIRO-TORRES, D.; BIGIO, N. C. An overview of recent taxonomic studies on Euphorbiaceae s.l. in Brazil. **Rodriguésia**, v. 63, p. 227-242, 2012.

SHARIFI-RAD, J.; SUREDA, A.; TENORE, G. C.; DAGLIA, M.; SHARIFI-RAD, M.; VALUSSI, M.; TUNDIS, R.; SHARIFI-RAD, M.; LOIZZO, M. R.; ADEMILUUYI, A. O.; SHARIFI-RAD, R.; AYATOLLAHI, S. A.; IRITI, M. Biological activities of essential oils: from plant chemoecology to traditional healing systems. **Molecules**, v. 22, p. 1-55, 2017.

SILVA, J. R. M.; NEVES, R. H.; GOMES, D. C. Filogenia, co-evolução, aspectos morfológicos e biológicos nas diferentes fases de desenvolvimento do *Schistosoma mansoni*. In: CARVALHO, O. S.; COELHO, P. M. Z.; LENZI, H. L. ***Schistosoma mansoni* e esquistossomose: uma visão multidisciplinar**. 1ª ed. Rio de Janeiro: Editora Fiocruz, 2008, p. 43-84.

SILVA, J. S.; SALES, M. F.; GOMES, A. P. S.; CARNEIRO-TORRES, D. S. Sinopse das espécies de *Croton* L. (Euphorbiaceae) no estado de Pernambuco, Brasil. **Acta Botânica Brasilica**, v. 24, p. 441-453, 2010.

SILVA, J. S.; SALES, M. F.; CARNEIRO-TORRES, D. S. The genus *Croton* (Euphorbiaceae) from the microregion of Ipanema Valley, Pernambuco, Brazil. **Rodriguésia**, v. 60, p. 879-901, 2009.

SILVA, L. S. B.; CARDOSO, R. T. N.; FERNANDES, J. L. A.; SILVA, C. A.; EIRAS, A. E. Modelo Entomológico Determinístico sob Efeito da Pluviosidade para o *Aedes aegypti* e o *Aedes albopictus*. **Tendências em Matemática Aplicada e Computacional**, v. 19, p. 289-303, 2018.

SILVA, P. C. V.; DOMINGUES, A. L. C. Aspectos epidemiológicos da esquistossomose hepatoesplênica no Estado de Pernambuco, Brasil. **Epidemiologia e Serviços de Saúde**, v. 20, p. 327-336, 2011.

SILVA, P. T.; SILVA, H. C.; VALE, J. P. C.; FROTA, V. M.; RODRIGUES, T. H. S.; SOUZA, E. B.; SANTIAGO, G. M. P.; BANDEIRA, P. N.; TEIXEIRA, A. M. R.; SANTOS, H. S. Circadian rhythm and larvicidal activity against *Aedes aegypti* of essential oils from *Croton piauhiensis*. **Revista Virtual de Química**, v. 11, p. 1682-1692, 2019.

SOUZA-NETO, J. A.; POWELL, J. R.; BONIZZONI, M. *Aedes aegypti* vector competence studies: A review. **Infection, Genetics and Evolution**, v. 67, p. 191-209, 2019.

STAGGEMEIER, V. G.; CAZETTA, E.; MORELLATO, L. P. Hyperdominance in fruit production in the Brazilian Atlantic rain forest: The functional role of plants in sustaining frugivores. **Biotropica**, v. 49, p. 71-82, 2017.

TAKAGI, B. A.; SOUZA, T. G. B.; OLIVEIRA, M. D.; BERNARDES, L. G.; ODA, J. Y.; MACHADO, A. R. S. R.; MACHADO, A.M. Larvicidal and ovocidal effects of *Crotalaria pallida* extracts on the vector *Aedes aegypti*. **Brazilian Journal of Development**, v. 6, p. 23060-23074, 2020.

TELES, T. V.; BONFIM, R. R.; ALVES, P. B.; BLANK, A. F.; JESUS, H. C. R.; QUINTANS-JÚNIOR, L. J.; SERAFINI, M. R.; BONJARDIM, L. R.; ARAÚJO, A. A. S. Composition and evaluation of the lethality of *Lippia gracilis essential* oil to adults of *Biomphalaria glabrata* and larvae of *Artemia salina*. **African Journal of Biotechnology**, v. 9, p. 8800-8804, 2010.

TLAMÇANI, Z.; ER-RAMI, M. Schistosomiasis control: moroccan experience compared to other endemic countries. **Asian Pacific Journal of Tropical Disease**, v. 4, p. 329-332, 2014.

TOLLE, M. A. Mosquito-borne diseases. Current Problems in Pediatric and Adolescent Health Care, v. 39, p. 97-140, 2009.

TUREK, C.; STINTZING, F. C. Stability of Essential Oils: A Review. **Comprehensive Reviews in Food Science and Food Safety**, v. 12, p. 40-53, 2013.

VERAS, B. O.; SANTOS, Y. Q.; OLIVEIRA, F. G. S.; ALMEIDA, J. R. G. S.; SILVA, A. G.; CORREIA, M. T. S.; DINIZ, K. M.; OLIVEIRA, J. R. S.; LIMA, V. L. M.; NAVARRO, D. M. A. F.; AGUIAR, J. C. R. O. F.; OLIVEIRA, M. B. M.; LOPES, A. C. S.; SILVA, M. V. *Algrizea minor* Sobral, Faria & Proença (Myrteae, Myrtaceae): chemical composition, antinociceptive, antimicrobial and antioxidant activity of essential oil. **Natural Product Research**, v. 4, p. 1-5, 2019.

VERMA, N.; SHUKLA, S. Impact of various factors responsible for fluctuation in plant secondary metabolites. **Journal of Applied Research on Medicinal and Aromatic Plants**, v. 2, p. 105-113, 2015.

VIDAL, C. S.; TINTINO-OLIVEIRA, C. D. M.; TINTINO, S. R.; GALVÃO, H. B. F.; COSTA, J. G. M.; COUTINHO, H. D. M.; MENEZES, I. R. A. Chemical composition, antibacterial and modulatory action of the essential oil of *Croton rhamnifolioides* leaves pax and hoffman. **Bioscience Journal**, v. 32, p. 1632-1643, 2016.

VITORINO, R. R.; SOUZA, F. P. C.; COSTA, A. P.; FARIA-JÚNIOR, F; C.; SANTANA, L. A.; GOMES, A. P. *Schistosomiasis mansoni*: diagnosis, treatment, epidemiology, prophylaxis and control. **Revista da Sociedade Brasileira de Clínica Médica**, v. 10, p. 39-45, 2012.

WINK, M. Modes of Action of Herbal Medicines and Plant Secondary Metabolites. **Medicines**, v. 2, p. 251-286, 2015.

WORLD HEALTH ORGANIZATION (WHO). **Dengue and severe dengue**. Fact sheet. Disponível em: <https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue> Acesso 9 Maio 2019a.

WORLD HEALTH ORGANIZATION (WHO). **Dengue control**. The mosquito. Disponível em: <https://www.who.int/denguecontrol/mosquito/en/> Acesso 21 Maio 2019b.

WORLD HEALTH ORGANIZATION (WHO). **Global Health Observatory (Schistosomiasis)**. Disponível em: <http://www.who.int/gho/neglected_ diseases/schistosomiasis/en/> Acesso em: 17 Fev. 2018b.

WORLD HEALTH ORGANIZATION (WHO). **Integrating neglected tropical diseases into global health and development: Fourth WHO report on neglected tropical diseases**. Geneva, 2017.

WORLD HEALTH ORGANIZATION (WHO). **Investing to overcome the global impact of neglected tropical diseases: Third WHO report on neglected tropical diseases**. Geneva, 2015.

WORLD HEALTH ORGANIZATION (WHO). **Working to overcome the global impact of neglected tropical diseases: First WHO report on neglected tropical diseases**. Geneva, 2010

WORLD HEALTH ORGANIZATION (WHO). **Zika virus**. Disponível em: <http://www.who.int/gho/neglected_diseases/schistosomiasis/en/> Acesso em: 23 jan. 2020.

WORLD HEALTH ORGANIZATION. **Schistosomiasis**. Disponível em: <http://www.who.int/mediacentre /factsheets/fs115/en/> Acesso em: 16 Fev. 2018a.

XIMENES, R. M.; NOGUEIRA, L. M.; CASSUNDÉ, N. M. R.; JORGE, R. J. B.; SANTOS, S. M.; MAGALHÃES, L. P. M.; SENA, K. X. F. R.; ALBUQUERQUE, J. F. C.; MARTINS, R.D. Antinociceptive and wound healing activities of *Croton adamantinus* Müll. Arg. essential oil. **Journal of Natural Medicines**, v. 67, p. 758-764, 2013.

YAGI, S.; BABIKER, R.; TZANOVA, T.; SCHOHN, H. Chemical composition, antiproliferative, antioxidant and antibacterial activities of essential oils from aromatic plants growing in Sudan. **Asian Pacific Journal of Tropical Medicine**, v. 9, p. 763-770, 2016.

YOKOMIZO, N. K. S.; NOKAOKA-SAKITA, M. Antimicrobial activity and essential oils yield of *Pimenta pseudocaryophyllus* var. pseudocaryophyllus (Gomes) Landrum, Myrtaceae. **Revista Brasileira de Plantas Medicinais**, v. 16, p. 513-520, 2014.

YOU, C. X.; YANG, K.; WU, Y.; ZHANG, W. J.; WANG, Y.; GENG, Z. F.; CHEN, H. P.;JIANG, H. Y.; DU, S. S.; DENG, Z. W. Chemical composition and insecticidal activities of the essential oil of *Perilla frutescens* (L.) Britt aerial parts against two stored product insects. **European Food Research and Technology**, v. 239, p. 481-490, 2014.

ZARA, A. L. S. A.; SANTOS, S. M.; FERNANDES-OLIVEIRA, E. S.; CARVALHO, R. G.; COELHO, G. E. *Aedes aegypti* control strategies: a review. **Revista Epidemiologia e Serviços de Saúde**, v. 25, p. 391-404, 2016.

ZAYNAB, M.; FATIMA, M.; ABBAS, S.; SHARIF, Y.; UMAIS, M.; ZAFAR, M. H.; BAHADAR, K. Role of secondary metabolites in plant defense against pathogens. **Microbial Pathogenesis**, v. 124, p. 198-202, 2018.

ZETTEL, C.; KAUFMAN, P. **Yellow fever mosquito Aedes aegypti (Linnaeus) (Insecta: Diptera: Culicidae)**. Disponivel em <https://edis.ifas.ufl.edu/in792> Acesso em: 28 Jan. 2020.

APÊNDICE A – ARTIGO PUBLICADO NO PERIÓDICO CROP PROTECTION

Crop Protection 129 (2020) 105043

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Chemical characterization and insecticidal effect against *Sitophilus zeamais* (maize weevil) of essential oil from *Croton rudolphianus* leaves

Ingridd Ayslane Torres de Araújo Ribeiro [a], Rosimere da Silva [a], Alexandre Gomes da Silva [a,b], Paulo Milet-Pinheiro [c,d], Patrícia Maria Guedes Paiva [a], Daniela Maria do Amaral Ferraz Navarro [c], Márcia Vanusa da Silva [a,b], Thiago Henrique Napoleão [a,*], Maria Tereza dos Santos Correia [a,b]

[a] Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil
[b] Núcleo de Bioprospecção e Conservação da Caatinga, Instituto Nacional do Semiárido, Campina Grande, Paraíba, Brazil
[c] Departamento de Química Fundamental, Centro de Ciências Exatas e da Natureza, Universidade Federal de Pernambuco, 50740-540, Recife, Pernambuco, Brazil
[d] Departamento de Botânica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil

ARTICLE INFO

Keywords:
Botanical insecticide
Croton rudolphianus
Essential oil
Maize weevil

ABSTRACT

The control of insect pests (such as *Sitophilus zeamais*) depends mostly on the utilization of synthetic pesticides. Nevertheless, the application of these pesticides leads to many problems; environmental contamination, development of insect resistance, and adverse effects on human health are some of them. This study investigated the chemical constitution, insecticidal activity, and feeding deterrent and repellent effects on *S. zeamais* of essential oil (EO) obtained from *Croton rudolphianus* leaves. Fifty-four compounds were revealed in the *C. rudolphianus* oil. The major compounds of the oil consisted of an unknown compound (40.90%), methyl chavicol (22.96%), (*E*)-caryophyllene (4.22%), eugenol (4.03%), bicycloelemene (3.96%), bicyclogermacrene (3.81%), and spathulenol (2.79%). The EO was toxic to *S. zeamais* when ingested (LC_{50} 102.66 μL/g) and caused changes in the nutritional parameters (relative consumption rate, relative biomass gain, and efficiency of food conversion). The oil was also toxic by contact (LC_{50} 70.64 μL/mL) and fumigation [64 μL/L in the air caused the highest mortality (43.75%)]. However, no repellent property was detected. The results of this study showed that EO from *C. rudolphianus* leaves is toxic in different ways to *S. zeamais* adults, pointing to its potential use for grain protection.

1. Introduction

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae), also known as the maize weevil, is a cosmopolitan insect and considered the primary pest of maize; it also attacks wheat, rice, sorghum, and processed food (Tripathi, 2018). The maize weevil can infect healthy grains during cultivation and storage (Suleiman et al., 2015). According to Ojo and Omoloye (2012), *S. zeamais* and other storage insect pests (such as *Sitophilus oryzae*, *Sitotroga cerealella*, and *Prostephanus truncatus*) are responsible for about 14–50% and 1–2% of maize loss during each season in developing and developed countries, respectively. Maize weevil infestations affect food security, nutritional value, seed viability, and market value (Goñi et al., 2017). Additionally, the infestation increases the temperature and moisture content of the stored grains, which may favor the growth of fungi like *Aspergillus flavus* (Chu et al., 2013). It is estimated that the Brazilian production of maize in 2019/2020 will be around 98.4 million tons (Companhia Nacional de Abastecimento, 2019); it is, however, reported that 20% of this amount will be lost due to the attack of pests on crops, mainly *S. zeamais* (Silva et al., 2017). In this scenario, the control of this insect is extremely important.

The control of maize weevils depends mostly on the use of chemical insecticides (e.g. bifenthrin, fenitrothion, and pirimiphos-methyl). Nevertheless, the uncontrolled application of these substances leads to many problems such as environmental contamination, development of resistant insects, and adverse effects on human health (Bravo et al., 2011). This fact has highlighted the need for more environmentally friendly pesticides, which are safe for humans (Dutta, 2015; Ebadollahi, 2011). In this respect, primary and secondary metabolites from plants, such as lectins, organic and aqueous extracts, and essential oils (EOs), have been reported as insecticidal agents against insect pests (Benelli

* Corresponding author.
E-mail address: thiagohn86@yahoo.com.br (T.H. Napoleão).

https://doi.org/10.1016/j.cropro.2019.105043
Received 23 August 2019; Received in revised form 29 November 2019; Accepted 2 December 2019
Available online 3 December 2019

et al., 2017; Raliya et al., 2018).

EOs are volatile compounds derived from secondary plant metabolites. They are characterized by a strong odor and are usually involved in defense mechanisms against a large spectrum of natural enemies, such as insects and microorganisms (Arnason et al., 2004; Bakkali et al., 2008). For this reason, many studies have investigated the insecticidal effects of EOs. For example, EO from *Alpinia purpurata* inflorescences was described as an insecticidal agent against *S. zeamais* (Lira et al., 2015), EOs from *Croton heliotropiifolius*, *Croton pulegiodorus*, *Myracrodruon urundeuva*, and *Ocimum basilicum* were active against *Tribolium castaneum* (Magalhães et al., 2015), and EO from *Croton rhamnifolioides* caused mortality in *Aedes aegypti* (Santos et al., 2014).

The *Croton* genus (Euphorbiaceae family) includes about 1300 species broadly found in tropical regions (Brito et al., 2018). Some EOs from species of that genus have demonstrated insecticide effects (Magalhães et al., 2015; Santos et al., 2014; Lima et al., 2013). *Croton rudolphianus* Müll. Arg., commonly known as "velame-branco", is a species endemic to Brazil, distributed throughout the Northeast to Southeast regions (Silva et al., 2010). The chemical characterization and insecticidal potential of its EO have never been investigated. The aims of this study were to determine the chemical composition of EO extracted from *C. rudolphianus* leaves, to evaluate the survival and nutritional parameters of *S. zeamais* that ingested an artificial diet containing the oil, and to assess the oil's toxicity by contact and fumigation.

2. Materials and methods

2.1. Plant material and EO extraction

Fresh leaves of *C. rudolphianus* were collected from the *Parque Nacional do Catimbau*, Pernambuco (8° 34′ 01.7″ S 37° 14′ 31.6″ W), Brazil, in December 2014 and March and April 2015. A voucher specimen (number 91,091) was deposited in the Herbarium of the *Instituto Agronômico de Pernambuco* (IPA, Recife, Brazil). The *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio) authorized the collection of plant material (SISBIO 26743). The accession number in the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* (SisGen) is AC7C705.

EO extraction was performed by using a hydrodistillation technique in a Clevenger apparatus for 6 h; fresh leaves (200 g) were triturated with distilled water (2 L) by using a blender. Subsequently, the EO was transferred to amber-glass vials. This procedure was carried out according to Santos et al. (2014), with modifications, and repeated thrice using material from different collections. The extraction yield was expressed in % ± SD (w/w). The three oil samples were then pooled and stored in amber-glass at 4 °C until chemical analysis and biological assays were performed.

2.2. Chemical composition analysis of the EO

The chemical constitution analysis of the *C. rudolphianus* EO was carried out using a gas chromatograph (model 7890A; Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent J&W non-polar HP-5ms™ column (30 m × 0.25 mm; 0.25 μm film thickness) and coupled to a selective mass detector (model 5975C; Agilent Technologies; Palo Alto, CA, USA).

The oven temperature was programmed at 40 °C for 5 min, increased by 2 °C/min until a temperature of 230 °C was reached, and remained constant at this level for 5 min. Helium carrier gas flow was maintained at 100 kPa. The interface with mass spectrometry (MS) was set at 230 °C. The quadrupole temperature was set at 150 °C. Mass spectra were recorded at 70 eV in electron impact ionization mode and scanned in the range *m/z* 35–350 at a speed of 0.5 s/scan.

An oil solution was prepared with pooled EO and hexane (1:50, v/v, with hexane) and a 1 μL aliquot of this solution was injected in split mode (1:50) in a mass spectrometer. To identify the components of the EO, we compared the Retention Indices (RI) reported in the literature and those we acquired by co-injecting a standard solution of linear C9–C30 hydrocarbons (Sigma-Aldrich, St. Louis, MO, USA) obtained in accordance with the equation of Van den Dool and Kratz (1963). To confirm the identification of the compounds, the mass spectrum results obtained for each component were matched to the mass spectral library of the GC-MS system (MassFinder 4, NIST08, and Wiley Registry™ 9th Edition) and to published spectra (Adams, 2009).

The same oil solution was analyzed on a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a flame ionization detector (FID), a split/splitless injector, and a Hamilton Bonaduz (Bonaduz Switzerland) HB-5 fused silica capillary column (30 m × 0.25 mm; film thickness 0.25 μm) to quantify the relative amount of each compound in relation to the total amount of compounds in the oil. The oven temperature conditions were the same as described above for the GC-MS analysis. The detector and injector temperatures were set to 250 °C. A 1 μL aliquot of the sample was injected three times in splitless mode.

2.3. Insecticidal assay

2.3.1. Insects

A *S. zeamais* colony was reared at the *Laboratório de Bioquímica de Proteínas*, *Departamento de Bioquímica*, *Universidade Federal de Pernambuco*, Recife, Brazil, in glass containers (1 L capacity) covered with non-woven fabric and kept in a BOD chamber at a 12:12 L:D photoperiod, 28 ± 2 °C, and 70% relative humidity. Their diet consisted of non-transgenic organic maize grains (100 g per container). Male and female insects (30–60 days old) were used in the assays. The authorization number of the *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio) for rearing *S. zeamais* was 36301.

2.3.2. Assessment of the ingestion toxicity of the EO

For this bioassay, an artificial diet was prepared for the insects in accordance with Lira et al. (2015) by using autoclaved wheat flour (2.0 g; Dona Benta®, Bunge Alimentos S.A., Benevides, PA, Brazil) in a 5 mL oil solution (in 1% Tween 80). The concentrations used in this test were 31.25, 62.5, and 125 μL/g (μL of neat oil/g of wheat flour). In the control treatment, the diet was prepared with 5 mL of a solution consisting of 1% Tween 80 and autoclaved wheat flour. Subsequently, five aliquots (200 μL) of the suspension or control treatments, which were measured with a micropipette fitted with a disposable tip cut at the narrow end (2 mm of internal diameter) were added in Petri dishes with known weight (90 mm × 100 mm). The dishes were left to dry in an oven for 48 h at 30 °C. Then, twenty *S. zeamais* adults were transferred to each dish. After 7 days in darkness at 28 ± 2 °C the weights of the broken flour disks and the weights and mortality rate of the insects were recorded. These tests were conducted five times.

The deterrent and nutritional indices were determined by using the toxicity results obtained by ingestion assays. The feeding deterrence index (FDI) was calculated by the formula FDI (%) = 100 × (C-T)/C, according to Isman et al. (1990), where C and T represent the diet consumption in the control and treatment groups, respectively. Based on these values, the diets including oil were classified in accordance with Liu et al. (2007) as follows: no feeding deterrence (FDI < 20%), weak (50% > FDI ≥ 20%), moderate (70% > FDI ≥ 50%), or strong (FDI ≥ 70%) feeding deterrence.

The relative consumption rate was calculated as (RCR) = (mg of biomass ingested)/(mg of initial insect biomass × days). The relative biomass gain rate was calculated as (RBG) = (mg of biomass gained)/(mg of initial insect biomass × day). Then, the efficiency of food conversion was calculated as (ECI) (%) = 100 × (biomass gained)/(food ingested). All these nutritional parameters were calculated in accordance with Xie et al. (1996).

2.3.3. Assessment of the contact toxicity of the EO

To evaluate the contact toxicity of *C. rudolphianus* oil, we used a method described by Lira et al. (2015). Briefly, solutions of *C. rudolphianus* oil were prepared in 1% (v/v) Tween 80. The concentrations used in this bioassay were 0.75, 1.88, 7.5, 37.5, and 75 μL/mL. Then, 0.5 μL of these solutions was topically applied on the back of the insects' thoracic region by using a micropipette. The insects in the negative control group were treated with 0.5 μL of 1% (v/v) Tween 80. Twenty insects were used in each assay and maintained in plastic recipients (4.0 cm wide and 6.0 cm long). The plastic recipients were kept for 7 days in darkness at 28 ± 2 °C. The mortality rate was recorded after 7 days. The tests were conducted five times.

2.3.4. Assessment of the fumigant toxicity of the EO

This bioassay was carried out in accordance with the method proposed by Chu et al. (2010). Briefly, the lid of the plastic recipient (4.0 cm wide, 6.0 cm long, and 80 mL in volume) was covered with a filter paper that had previously been impregnated with 1.28, 2.56, or 5.12 μL of neat EO from *C. rudolphianus* to achieve final concentrations of 16, 32, and 64 μL/L in the air, respectively. In the control group, we used paper that did not receive any treatment. Polytetrafluoroethylene (Sigma-Aldrich, St. Louis, MO, USA) was applied around the paper in order to prevent the insects from coming into contact with it. Twenty *S. zeamais* adults were put in each recipient. After the evaporation time (30 s) had passed, the lids were tightly closed in order to form a sealed chamber. The insects stayed in these closed containers for 24 h at 28 ± 2 °C. After that, the insects were placed in clean recipients and incubated for 7 days in darkness at 28 ± 2 °C. After the incubation period, the mortality rate was recorded. The tests were conducted five times.

2.3.5. Assessment of the repellent effect of the EO

The repellent assay was performed by using arenas analogous to those described by Lira et al. (2015). Each arena was formed by three plastic recipients (4.0 cm wide and 6.0 cm long), where the central recipient was connected symmetrically with the two other recipients through silicone tubes (11.5 mm diameter and 7.0 cm long). Filter papers were used to cover the lids of the recipients on the left and right sides. The papers on the left side were impregnated with 20 μL of the EO solution at 18.5, 37.5, and 75 μL/mL in 1% Tween 80. The filter papers on the right side were impregnated with 20 μL of 1% Tween 80 (control). After that, ten insects were placed in the central recipient. The arenas were kept in darkness, at 28 ± 2 °C, for 7 days. To calculate the repellency index (REI), the number of *S. zeamais* present in each container was registered once after the incubation period. The REI was obtained according to Mazzonetto and Vendramim (2003) as $2T/(T + C)$, where T is the percentage of *S. zeamais* in the oil recipient (treatment) and C is the percentage of *S. zeamais* in the control recipient. Standard deviations (SD) were calculated and the results were considered as attractive effect (REI ± SD > 1) or repellent effect (REI ± SD < 1). Each assay had four replicates in different arenas.

2.4. Statistical analysis

Data were expressed as mean ± SD. The GraphPad Prism 5.0 software for Windows (GraphPad Software, San Diego, California, USA) was used to perform one-way fixed effects ANOVA and Tukey's tests (significance at $p < 0.05$) for the assays of toxicity by ingestion, contact, and fumigation and for the repellent test. The lethal concentration required to kill 50% of insects (LC_{50}) was calculated by StatPlus version 5.98 (AnalystSoft, Canada) for Windows by probit analysis with a reliability interval of 95% by using the mortality rates calculated by Abbott's correction for the assays of toxicity by ingestion and contact.

3. Results

3.1. Acquisition and chemical constitution of the oil

The EO yield obtained by the hydrodistillation of *C. rudolphianus* leaves was 1.138 ± 0.25% (w/w) and a total of 2.28 g was extracted. The GC-MS analysis revealed 54 volatile compounds (Table 1). The sum of the identified compounds corresponded to more than 50% of the total components detected in the EO. Among the identified compounds, sesquiterpenes were the dominant compound class (24.41%) followed by phenylpropanoids (22.96%) and monoterpenes (5.15%). We could not

Table 1
Relative amount of chemical constituents of the essential oil of *C. rudolphianus* leaves.

Compounds	RI calc.[a]	RI lit.[b]	Relative amount (%)	SD
α-Pinene	932	932	0.08	0.01
Myrcene	990	988	0.18	0.01
Limonene	1027	1024	0.72	0.00
(*E*)-β-Ocimene	1048	1044	0.09	0.00
Linalool	1099	1095	0.05	0.00
Methyl chavicol	1198	1195	22.96	0.02
NI	1331	–	0.29	0.02
Bicycloelemene	1338	–	3.96	0.46
NI	1353	–	0.12	0.01
Eugenol	1358	1356	4.03	0.08
α-Copaene	1377	1374	0.21	0.00
β-Cubenene	1387	1387	0.13	0.00
β-Elemene	1393	1389	0.50	0.01
(*E*)-Caryophyllene	1421	1417	4.22	0.04
β-Copaene	1431	1430	0.24	0.00
α-(*E*)-Bergamotene	1438	1432	0.21	0.00
Aromandrene	1440	1439	0.26	0.00
NI	1447	–	0.14	0.01
(*E*)-3,5-Muuroladiene	1452	1451	0.12	0.00
Humulene	1455	1452	0.64	0.01
9-epi-Caryophyllene	1464	1464	0.36	0.01
(*E*)-Cadina-1(6),4-diene	1476	1475	0.15	0.00
γ-Muurolene	1479	1478	0.23	0.01
Germacrene D	1482	1480	1.24	0.01
Bicyclogermacrene	1499	1499	3.81	0.06
Germacrene A	1509	1508	0.16	0.00
γ-Cadinene	1515	1513	0.43	0.00
δ-Cadinene	1525	1522	1.14	0.02
(*E*)-Cadina-1,4-diene	1535	1533	0.10	0.00
NI	1543	–	0.10	0.00
NI	1564	–	0.06	0.00
NI	1570	–	0.57	0.00
Spathulenol	1581	1577	2.79	0.03
NI	1589	–	0.91	0.01
NI	1597	–	0.32	0.01
NI	1601	–	0.20	0.01
NI	1608	–	0.32	0.01
NI	1615	–	0.13	0.00
NI	1628	–	0.15	0.01
1-epi-Cubenol	1631	1627	0.71	0.00
NI	1642	–	0.36	0.01
NI	1647	–	0.54	0.01
NI	1659	–	0.48	0.02
NI	1671	–	1.14	0.02
NI	1683	–	40.90	0.92
NI	1698	–	0.12	0.04
(2*E*,6*Z*)-Farnesal	1715	1713	0.22	0.06
(2*Z*, 6*E*)-Farnesol	1722	1722	0.53	0.05
(2*E*,6*E*)-Farnesal	1743	1740	0.26	0.01
NI	1749	–	0.07	0.00
NI	1766	–	0.42	0.06
NI	1775	–	0.07	0.01
(2*E*,6*E*)-Methyl farnesoate	1785	1783	1.79	0.01
NI	1792	–	0.05	0.00

[a] Linear Retention Indices (RI) calculated from retention times in regard to a standard mixture of hydrocarbons separated on a non-polar DB-5 capillary column.

[b] LRI literature from Adams (2007), NI: not identified compound, SD: standard deviation.

elucidate the major constituent of the oil, which alone accounted for about 40.90% of the composition. Further major constituents were phenylpropanoid methyl chavicol (22.96%), the sesquiterpene (*E*)-caryophyllene (4.22%), the monoterpene eugenol (4.03%), and the sesquiterpenes bicycloelemene (3.96%), bicyclogermacrene (3.81%), and spathulenol (2.79%).

3.2. Assessment of the ingestion toxicity

The increase of the *C. rudolphianus* oil concentration in the diet led to an increase in the mortality of *S. zeamais* adults (Fig. 1). The mean mortality rates of *S. zeamais* after 7 days were 1.4 ± 0.9, 3.4 ± 0.5, 5.4 ± 1.1, and 13.6 ± 1.0 insects for the control, 31.25, 62.5, and 125 μL/g treatments, respectively. Statistical analysis revealed significant differences in mortality between doses ($F_{3,14} = 195.1$; $p < 0.0001$) and the LC_{50} was 102.66 (93.12–112.21) μL/g. Furthermore, the presence of *C. rudolphianus* oil in the diet interfered with all the parameters of insect nutrition. After 7 days of treatment, three nutritional parameters were analyzed: relative consumption rate, relative biomass gain rate, and efficiency in the conversion of ingested food. Relative consumption rates differed significantly among treatments ($F_{3,12} = 285.8$; $p < 0.0001$) and showed that insects ingested significantly more food in the diet with EO included at a 125 μL/g rate than in the control diet (Fig. 2A).

The relative biomass gain rates (Fig. 2B) were equal to the control in the 31.25 and 62.5 μL/g treatments and lower in the 125 μL/g concentration ($F_{3,12} = 30.98$; $p < 0.0001$). In respect to the efficiency in the conversion of ingested food, there was also a significant difference among treatments ($F_{3,10} = 55.95$; $p < 0.0001$). In the treatments with oil at the 62.5 and 125 μL/g concentrations, the efficiency was lower than in the control treatment (Fig. 2C). In relation to the feeding-deterrence index, no deterrence was observed at any of the concentrations tested.

3.3. Assessment of toxicity by contact

The EO of *C. rudolphianus* leaves caused the death of *S. zeamais* when applied topically on the insects (Fig. 3). Mortality increased with increasing oil concentrations applied on the insects ($F_{5,23} = 55.27$; $p < 0.0001$). The LC_{50} determined was 70.64 (62.96–78.31) μL/mL.

3.4. Assessment of fumigant toxicity and repellent effect

The *C. rudolphianus* EO had a fumigant toxicity effect on *S. zeamais* adults. An increase in oil concentration was directly related to an increase in insect mortality (Fig. 4). The highest mortality rate (43.75 ± 4.79%) was observed in the 64 μL/L treatment in the air. According to the statistical analysis, there were significant differences among the concentrations assayed ($F_{2,8} = 7.90$; $p = 0.0127$).

The *C. rudolphianus* oil did not have a repellent effect at any of the tested concentrations. On the contrary, REI values ranged from 1.55 to 1.36, without significant differences among treatments ($F_{2,8} = 0.8459$; $p = 0.4642$), indicating an attractive action.

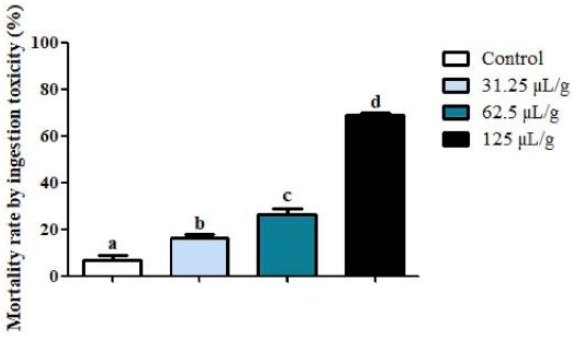


Fig. 1. Mortality rate of *S. zeamais* that were 7 days on an artificial diet containing *C. rudolphianus* essential oil. The control was 1% (v/v) Tween 80. The Tukey's test was used to evaluate significant differences between treatments [represented by different letters ($p < 0.05$)]. Each bar corresponds to the mean ± SD of five replicates.

4. Discussion

The EO yield of *C. rudolphianus* leaves (1.138 ± 0.25%) can be compared to that of oils extracted from leaves of other *Croton* species found in Brazil, such as the red variation of *Croton cajucara* (0.97%) (Chaves et al., 2006), *Croton cordiifolius* (0.81%) (Nogueira et al., 2015), *Croton rhamnifolioides* (accepted name: *Croton heliotropiifolius*) (0.80%), and *Croton regelianus* (0.80%) (Santos et al., 2014; Bezerra et al., 2009).

In general, some EOs from *Croton* species' leaves contain the major constituents of *C. rudolphianus* detected in this study. For instance, *Croton zehntneri* (accepted name: *Croton grewioides*) has methyl chavicol (93.6%) (Donati et al., 2015); *Croton campestris*, *Croton conduplicatus* (accepted name: *C. heliotropiifolius*), *Croton argyrophyllus*, *C. rhamnifolioides*, and *Croton adamantinus* contain (*E*)-caryophyllene (17.0%, 2.96–9.14%, 6.79%, 4.37–6.33%, and 5.80%, respectively) (Almeida et al., 2013, 2014; Ramos et al., 2013; Santos et al., 2014; Ximenes et al., 2013); *C. campestris*, *C. argyrophyllus*, *C. conduplicatus*, and *Croton heterocalyx* oils contain bicyclogermacrene (16.2%, 14.6%, 1.06–13.76%, and 11.2%, respectively) (Almeida et al., 2013, 2014; Ramos et al., 2013; Moreno et al., 2009); *C. conduplicatus* and *C. heterocalyx* oils contain spathulenol (3.98–13.38% and 6.9%, respectively) (Almeida et al., 2014; Moreno et al., 2009).

When ingested, the oil from the *C. rudolphianus* leaves caused mortality in *S. zeamais*; it did not, however, have any feeding deterrent effect. Furthermore, the insects fed more on the artificial diet with the 125 μL/g EO concentration than they did on the control and other diets, while showing reduced efficiency in the conversion of the ingested food. Although they ate more food than insects in the other treatment groups, the insects treated with the 125 μL/g EO concentration had a lower weight gain, indicating a difficulty in *S. zeamais* to incorporate the nutrients from their diet. These results indicate an interesting potential of *C. rudolphianus* oil for the control of *S. zeamais*, since the insects preferred to feed on diets containing this oil, which may be toxic for them. It could be suggested that the anti-nutritional properties and the mortality rates observed in this study are possibly related to post intake effects caused by the oil compounds.

Some natural products, such as EO and lectins, have been evaluated for toxicity by ingestion in *S. zeamais*. The ingestion of the *Alpinia purpurata* inflorescences EO did not kill the insects in 7 days; it did, however, decrease the biomass gain rate and the efficiency in the conversion of ingested food (Lira et al., 2015) and had an anti-nutritional effect similar to that caused by the *C. rudolphianus* oil. The lectins from *Myracrodruon urundeuva* and *Schinus terebinthifolius* leaves and *Opuntia ficus-indica* cladodes also had anti-nutritional effects when ingested by *S. zeamais* (Napoleão et al., 2013; Camaroti et al., 2018; Souza et al., 2018).

The *C. rudolphianus* EO also had a toxicity by contact effect on *S. zeamais* adults ($LC_{50} = 70.64$ μL/mL). Previous studies showed that the major compounds of *C. rudolphianus* EO, such as methyl chavicol and eugenol [($LC_{50} = 76.1$ and 186.2 μg/cm^2, respectively; Zaio et al., 2018)] have insecticidal effects by contact against *S. zeamais* and other stored-grain pests, corroborating our results. The latter compound also had a contact toxicity effect on *S. oryzae* (Chaubey, 2016), *Sitophilus granarius* (Plata-Rueda et al., 2018), and the early fourth-instar of *Spodoptera litura* (Hummelbrunner and Isman, 2001). Moreover, methyl chavicol and (*E*)-caryophyllene have also shown insecticidal effects by contact against *T. castaneum* ($LD_{50} = 20.41$ and 41.72 μg/adult, respectively) and *Liposcelis bostrychophila* ($LD_{50} = 30.22$ and 74.11 μg/adult, respectively) (Guo et al., 2015).

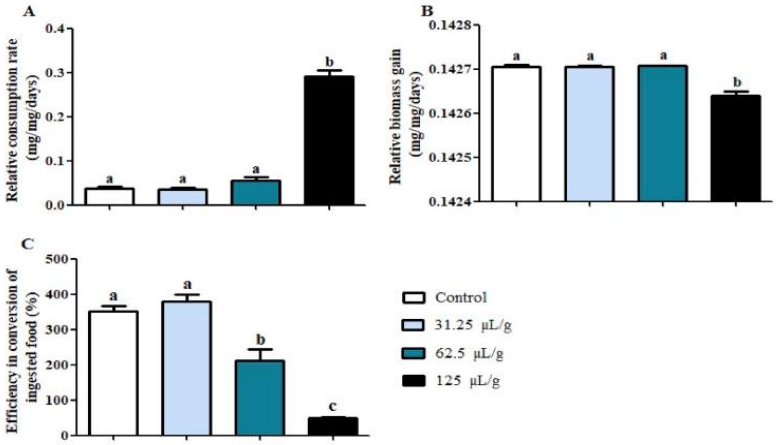


Fig. 2. Nutritional parameters of *S. zeamais* adults maintained on artificial diets containing solutions of *C. rudolphianus* essential oil at 31.25, 62.5, 125 µL/g, or the control (1% Tween 80). (A) Relative consumption rate: quantity of food ingested (mg) per mg of body. (B) Relative biomass gain rate: biomass (mg) obtained every day per mg of initial body weight. (C) Efficiency in conversion of ingested food (%): quantity of ingested food turned into biomass by insects. The Tukey's test was used to evaluate significant differences between treatments [represented by different letters ($p < 0.05$)]. Each bar corresponds to the mean ± SD of five replicates.

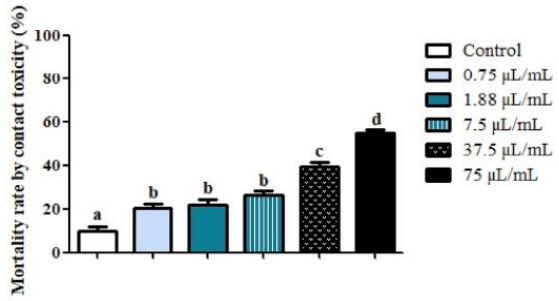


Fig. 3. Mortality rate in *S. zeamais* by contact toxicity of *C. rudolphianus* essential oil. The control was 1% (v/v) Tween 80. The Tukey's test was used to evaluate significant differences between treatments [represented by different letters ($p < 0.05$)]. Each bar corresponds to the mean ± SD of five replicates.

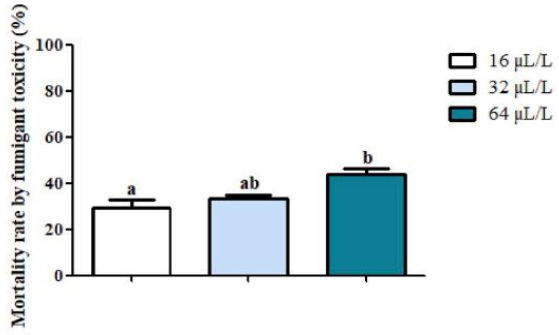


Fig. 4. Mortality rate in *S. zeamais* by fumigant toxicity of *C. rudolphianus* essential oil. No mortality was observed in the control group. The Tukey's test was used to evaluate significant differences between treatments [represented by different letters ($p < 0.05$)]. Each bar corresponds to the mean ± SD of five replicates.

Many EOs (and their major constituents) from members of the Euphorbiaceae family have been found to possess fumigant toxicity effects on *S. zeamais* and other grain pests. For example, the EOs from *C. heliotropiifolius* and *C. pulegiodorus* leaves were found to be toxic by fumigation against *T. castaneum* (Magalhães et al., 2015). The oil from *C. pulegiodorus* leaves also caused the death of *Rhyzopertha dominica* adults ($LC_{50} = 48.66$ µL/L in air) (Souza et al., 2016), which is a better result than the one found in this study. The EOs from *C. grewioides* leaves and stems exhibited toxicity against the Mexican bean weevil (*Zabrotes subfasciatus*) ($LC_{50} = 4.0$ and 13.7 48.66 µL/L in air, respectively) (Silva et al., 2008). The *Mallotus apelta* EO was toxic by fumigation on *S. zeamais* ($LC_{50} = 48.42$ mg/L in air; Liu et al., 2014). Furthermore, the compound methyl chavicol had a fumigant toxicity effect on *S. zeamais* adults ($LC_{50} = 14.10$ mg/L in air; Zhou et al., 2012). The compounds (*E*)-caryophyllene and eugenol were toxic by fumigation to *S. oryzae* ($LC_{50} = 1.98$ µL/cm^3; $LC_{50} = 0.167$ and 0.152 µL/cm^3 for 24 and 48 h, respectively) (Chaubey, 2012, 2016). Methyl chavicol, (*E*)-caryophyllene, and eugenol constituted around 31% of the *C. rudolphianus* leaves' EO, therefore these compounds are possibly related to the fumigant effect observed in this paper.

The mechanism of the *C. rudolphianus* EO toxicity on *S. zeamais* may be related to an acetylcholinesterase (AChE) inhibition. Evidence for this comes from studies by López and Pascual-Villalobos (2014) and Chaubey (2012, 2016), who found that some of the major constituents of the *C. rudolphianus* EO, i.e., methyl chavicol, (E)-caryophyllene, and eugenol, exert a strong inhibition of AChE on congeneric *S. oryzae*. AChE is an enzyme responsible for the catalysis of the acetylcholine hydrolysis (neurotransmitter), which acts by transmitting the message from one neuron to another in the nervous system in several organisms. When an AChE inhibition occurs, there is an acetylcholine accumulation at the cholinergic synapses, disturbing nerve impulse transmission (López and Pascual-Villalobos, 2014).

The potential repellent effects of EOs and their chemical constituents against insects have been evaluated for many plant species (Mossa, 2016). In this paper, the EO of *C. rudolphianus* exerted an attractive effect on *S. zeamais* adults (REI >1), which could be useful in traps aiming to protect stored commodities from the maize weevil (Bayisa et al., 2017). However, methyl chavicol (Bedini et al., 2016), (E)-caryophyllene (Benelli et al., 2012), and eugenol (Chaubey, 2016), from the three primary components of *C. rudolphianus* EO, were found to have repellent activity against *S. zeamais*, *Sitophilus granarius*, and *S. oryzae*, respectively. In addition, methyl chavicol and (E)-caryophyllene also have repellent effects against *R. dominica* and *Tribolium confusum* (Bedini et al., 2016; Bougherra et al., 2015). A previous paper indicates that the

attractive effect of EOs is commonly attributed to a synergistic action among the compounds (You et al., 2014). However, in some cases the EO effect is related to a particular compound. It has also been suggested that the activity of the main components can be modulated by other minor molecules (Bakkali et al., 2008). In our study, it is possible that the major component of the *C. rudolphianus* EO, which was not identified, is involved in this attractive effect.

In summary, the EO from *C. rudolphianus* leaves represents a promising alternative for maize weevil management, due to its many different modes of action and its attractive effects that could be useful in creating lures in maize weevil traps.

Author contributions

Ingridd A.T.A. Ribeiro: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. Writing – review & editing. **Rosimere da Silva:** Investigation. **Alexandre G. Silva**: Data curation; Formal analysis; Resources. **Paulo Milet-Pinheiro:** Data curation; Formal analysis; Investigation; Writing - review. **Patrícia M.G. Paiva:** Funding acquisition; Methodology; Resources. **Daniela M.A.F. Navarro**: Data curation; Formal analysis; Funding acquisition; Methodology; Resources; Supervision; Validation; Writing - review. **Márcia V. Silva:** Conceptualization; Funding acquisition; Project administration; Resources; Supervision. **Thiago H. Napoleão**: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Supervision; Validation; Writing - original draft; Writing - review & editing. **Maria T.S. Correia:** Conceptualization; Formal analysis; Funding acquisition; Project administration; Resources; Supervision; Writing - review.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the *Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco* (FACEPE; APQ-0108-2.08/14) for financial incentive, the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq; 446902/2014-4) for research grants and fellowship (PMGP, THN, DMAFN and MTSC).

References

Adams, R.P., 2009. Identification of Essential Oil Components by Gas Chromatography/ mass Spectrometry. Allured Publishing Co, p. 804.

Almeida, J., Souza, A.V., Oliveira, A.P., Santos, U., Souza, M., Bispo, L., Turatti, Z.C., Lopes, N., 2014. Chemical composition of essential oils from *Croton conduplicatus* (Euphorbiaceae) in two different seasons. J. Essent. Oil Bearing Plants 17, 1137–1145.

Almeida, T.S., Rocha, J.B.T., Rodrigues, F.F.G., Campos, A.R.C., Costa, J.G.M., 2013. Chemical composition, antibacterial and antibiotic modulatory effect of *Croton campestris* essential oils. Ind. Crops Prod. 44, 630–633.

Arnason, J.T., Guillet, G., Durst, T., 2004. Phytochemical diversity of insect defenses in tropical and temperate plant families. In: Cardé, R.T., Millar, J.G. (Eds.), Advances in Insect Chemical Ecology. Cambridge University Press, Cambridge, pp. 1–10.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils - a review. Food Chem. Toxicol. 46, 446–475.

Bayisa, N.G., Hundesa, N., Tefera, B.N., 2017. Essential oils applied on sticky traps increase trapping of sucking pests under greenhouse condition. Int. J. Res. Stud. Agric. Sci. 3, 1–5.

Bedini, S.B., Bougherra, H.H., Flamini, G., Cosci, F., Brlhamel, K., Ascrizzi, R., Conti, B., 2016. Repellency of anethole- and estragole-type fennel essential oils against stored grain pests: the different twins. Bull. Insectol. 69, 149–157.

Benelli, G., Canale, A., Toniolo, C., Higuchi, A., Murugan, K., Pavela, R., Nicoletti, M., 2017. Neem (*Azadirachta indica*): towards the ideal insecticide? Nat. Prod. Res. 31, 369–386.

Benelli, G., Flamini, G., Canale, A., Molfetta, I., Cioni, P.L., Conti, B., 2012. Repellence of *Hyptis suaveolens* whole essential oil and major constituents against adults of the granary weevil *Sitophilus granarius*. Bull. Insectol. 65, 177–183.

Bezerra, D.P., Marinho Filho, J.D.B., Alves, A.P.N.N., Pessoa, C., Moraes, M.O., Pessoa, O. D.L., Torres, M.C.M.L., Silveira, E.R., Viana, F.A., Casta-Lotufo, L.V., 2009. Antitumor activity of the essential oil from the leaves of *Croton regelianus* and its component ascaridole. Chem. Biodivers. 6, 1224–1231.

Bougherra, H.H., Bedini, S., Flamini, G., Cosci, F., Belhamel, K., Conti, B., 2015. *Pistacia lentiscus* essential oil has repellent effect against three major insect pests of pasta. Ind. Crops Prod. 63, 249–255.

Bravo, A., Likitvivatanavong, S., Gill, S.S., Soberón, M., 2011. *Bacillus thuringiensis:* a story of a successful bioinsecticide. Insect Biochem. Mol. Biol. 41, 423–431.

Brito, S.S.S., Silva, F., Malhheiro, R., Baptista, P., Pereira, J.A., 2018. *Croton argyrophyllus* Kunth and *Croton heliotropiifolius* Kunth: phytochemical characterization and bioactive properties. Ind. Crops Prod. 113, 308–315.

Camaroti, J.R.S.L., Almeida, W.A., Belmonte, B.R., Oliveira, A.P.S., Lima, T.A., Ferreira, M.R.A., Paiva, P.M.G., Soares, L.A., Pontual, E.V., Napoleão, T.H., 2018. *Sitophilus zeamais* adults have survival and nutrition affected by *Schinus terebinthifolius* leaf extract and its lectin (SteLL). Ind. Crops Prod. 116, 81–89.

Chaubey, M.K., 2012. Fumigant toxicity of essential oils and pure compounds against *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Biol. Agric. Hortic. 28, 111–119.

Chaubey, M.K., 2016. Insecticidal activities of *Cinnamomum tamala* (Lauraceae) essential oil against *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Int. J. Entomol. Res. 04, 91–98.

Chaves, F.C.M., Bizzo, H.R., Angelo, P.C.S., Xavier, J.J.B.N., Sá Sobrinho, A.F., 2006. Rendimento e composição química do óleo essencial de folhas de dois morfotipos de sacaca (*Croton cajucara* Benth.). Ver. Bras. Plantas Med. 8, 117–119.

Chu, S.S., Du, S.S., Liu, Z.L., 2013. Fumigant compounds from the essential oil of Chinese *Blumea balsamifera* leaves against the maize weevils (*Sitophilus zeamais*). J. Chem. 1e7.

Chu, S.S., Liu, Q.R., Liu, Z.L., 2010. Insecticidal activity and chemical composition of the essential oil of *Artemisia vestita* from China against *Sitophilus zeamais*. Biochem. Syst. Ecol. 38, 489–492.

Companhia Nacional de Abastecimento, OUTUBRO 2019. Acompanhamento da safra brasileira de grãos V. 7 - SAFRA 2019/20 - N. 1 - primeiro levantamento. Brasília: CONAB. Available in. https://www.conab.gov.br/info-agro/safras/graos?view=default. (Accessed 16 October 2019).

Donati, M., Mondin, A., Chen, Z., Miranda, F.M., Nascimento, B.B.J., Schirato, G., Pastore, P., Froldi, G., 2015. Radical scavenging and antimicrobial activities of *Croton zehntneri, Pterodon emarginatus* and *Schinopsis brasiliensis* essential oils and their major constituents: estragole, trans-anethole, β-caryophyllene and myrcene. Nat. Prod. Res. 29, 939–946.

Dutta, S., 2015. Biopesticides: an ecofriendly approach for pest control. World J. Pharm. Pharm. Sci. 4, 250–265.

Ebadollahi, A., 2011. Susceptibility of two *Sitophilus* species (Coleoptera: Curculionidae) to essential oils from *Foeniculum vulgare* and *Satureja hortensis*. Ecol. Balk. 3, 1–8.

Goñi, M.L., Gaña, N.A., Herrera, J.M., Strumia, M.C., Andreatta, A.E., Martini, R.E., 2017. Supercritical CO_2 iof LDPE films with terpene ketones as biopesticides against corn weevil (*Sitophilus zeamais*). J. Supercrit. Fluids 122, 18–26.

Guo, S.S., You, C.X., Liang, J.Y., Zhang, W.J., Geng, Z.F., Wang, C.F., Du, S.S., Lei, N., 2015. Chemical composition and bioactivities of the essential oil from *Etlingera yunnanensis* against two stored product insects. Molecules 20, 15735–15747.

Hummelbrunner, L.A., Isman, M.B., 2001. Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). J. Agric. Food Chem. 49, 715–720.

Isman, M.B., Koul, O., Luczynski, A., Kaminski, J., 1990. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J. Agric. Food Chem. 38, 1406–1411.

Lima, G.P.G., Souza, T.M., Freire, G.P., Farias, D.F., Cunha, A.P., Ricardo, N.M.P.S., Morais, S.M., 2013. Further insecticidal activities of essential oils from *Lippia sidoides* and *Croton* species against *Aedes aegypti* L. Parasitol. Res. 112, 1953–1958.

Lira, C.S., Pontual, E.V., Albuquerque, L.P., Paiva, L.M.P., Paiva, P.M.G., Oliveira, J.V., Napoleão, T.H., Navarro, D.M.A.F., 2015. Evaluation of the toxicity of essential oil from *Alpinia purpurata* inflorescences to *Sitophilus zeamais* (maize weevil). Crop Protect. 71, 95–100.

Liu, X.C., Chen, X.B., Liu, Z.L., 2014. Gas chromatography-mass spectrometric analysis and insecticidal activity of essential oil of aerial parts of *Mallotus apelta* (Lour.) Muell.-Arg. (Euphorbiaceae). Trop. J. Pharm. Res. 13, 1515–1520.

Liu, Z.L., Goh, S.H., Ho, S.H., 2007. Screening of Chinese medicinal herbs for bioactivity against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). J. Stored Prod. Res. 43, 290–296.

López, M.D., Pascual-Villalobos, M.J., 2014. Are monoterpenoids and phenylpropanoids efficient inhibitors of acetylcholinesterase from stored product insect strains? Flavour Fragrance J. 30, 108–112.

Magalhães, C.R.I., Oliveira, C.R.F., Matos, C.H.C., Brito, S.S.S., Magalhães, T.A., Ferraz, M.S.S., 2015. Potential inseticida de óleos essenciais sobre *Tribolium castaneum* em milho armazenado. Rev. Bras. Plantas Med. 17, 1150–1158.

Mazzonetto, F., Vendramim, J.D., 2003. Efeito de pós de origem vegetal sobre *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) em feijão armazenado. Neotrop. Entomol. 32, 145–149.

Moreno, P.R.H., Lima, M.E.L., Caruzo, M.B.R., Torres, D.S.C., Cordeiro, I., Young, M.C. M., 2009. Chemical composition and antimicrobial activity of the essential oil from *Croton heterocalyx* Baill. (Euphorbiaceae *s.s.*) leaves. J. Essent. Oil Res. 21, 190–192.

Mossa, A.T.H., 2016. Green pesticides: essential oils as biopesticides in insect-pest management. J. Environ. Sci. Technol. 9, 354–378.

Napoleão, T.H., Belmonte, B.R., Pontual, E.V., Albuquerque, L.P., Sá, R.A., Paiva, L.M., Coelho, L.C.B.B., Paiva, P.M.G., 2013. Deleterious effects of *Myracrodruon urundeuva* leaf extract and lectin on the maize weevil, *Sitophilus zeamais* (Coleoptera, Curculionidae). J. Stored Prod. Res. 54, 26–33.

Nogueira, L.M., Silva, M.R., Santos, S.M., Alburquerque, J.F.C., Ferraz, I.C., Albuquerque, T.T., Motta, C.R.F.C., Araújo, R.M., Viana, G.S.B., Martins, R.D., Havt, A., Ximenes, R.M., 2015. Antinociceptive effect of the essential oil obtained from the leaves of *Croton cordiifolius* Baill. (Euphorbiaceae) in mice. Evid. Based Complement Altern. Med. 1–7, 2015.

Ojo, J.A., Omoloye, A.A., 2012. Rearing the maize weevil, *Sitophilus zeamais*, on an artificial maize-cassava diet. J. Insect Sci. 12, 1–9.

Plata-Rueda, A., Campos, J.M., Rolim, G.S., Martínez, L.C., Santos, M.H., Fernandes, F.L., Serrão, J.E., Zanuncio, J.C., 2018. Terpenoid constituents of cinnamon and clove essential oils cause toxic effects and behavior repellency response on granary weevil, *Sitophilus granarius*. Ecotoxicol. Environ. Saf. 156, 263–270.

Raliya, R., Saharan, V., Dimkpa, C., Biswas, P., 2018. Nanofertilizer for precision and sustainable agriculture: current state and future perspectives. J. Agric. Food Chem. 66, 6487–6503.

Ramos, J.M.O., Santos, C.A., Santana, D.G., Santos, D.A., Alves, P.B., Thomazzi, S.M., 2013. Chemical constituents and potential anti-inflammatory activity of the essential oil from the leaves of *Croton argyrophyllus*. Braz. J. Pharmacogn. 23, 644–650.

Santos, G.K.N., Dutra, K.A., Lira, C.S., Lima, B.N., Napoleão, T.H., Paiva, P.M.G., Maranhão, C.A., Brandão, S.S.F., Navarro, D.M.A.F., 2014. Effects of *Croton rhamnifolioides* essential oil on *Aedes aegypti* Oviposition, larval toxicity and trypsin activity. Molecules 19, 16573–16587.

Silva, C.G.V., Zago, H.B., Júnior, H.J.G.S., Camara, C.A.G., Oliveira, J.V., Barros, R., Schwartz, M.O.E., Lucena, M.F.A., 2008. Composition and insecticidal activity of the essential oil of *Croton grewioides* Baill. against Mexican bean weevil (*Zabrotes subfasciatus* Boheman). J. Essent. Oil Res. 20, 179–182.

Silva, E.C., Vieira, D.D., Leonel, L.V., 2017. Comparação da atividade inseticida de *Chenopodium ambrosoides* E *Azadirachta indica* no controle de *Sitophilus zeamais*. Cult. Agron. 26, 554–559.

Silva, J.S., Sales, M.F., Gomes, A.P.S., Carneiro-Torres, D.S., 2010. Sinopse das espécies de *Croton* L. (Euphorbiaceae) no estado de Pernambuco, Brasil. Acta Bot. Bras. 24, 441-153.

Souza, C.S., Procópio, T.F., Belmonte, B.R., Paiva, P.M.G., Albuquerque, L.P., Pontual, E. V., Napoleão, T.H., 2018. Effects of *Opuntia ficus-indica* lectin on feeding, survival, and gut enzymes of maize weevil, *Sitophilus zeamais*. Appl. Biol. Chem. 61, 337–343.

Souza, V.N., Oliveira, C.R.F., Matos, C.H.C., Almeida, D.K.F., 2016. Fumigation toxicity of essential oils against *Rhysopertha dominica* (f.) in stored maize grain. Rev. Caatinga. 29, 435–440.

Suleiman, R., Rosentrater, K.A., Bern, C.J., 2015. Evaluation of maize weevils *Sitophilus zeamais* Motschulsky infestation on seven varieties of maize. J. Stored Prod. Res. 64, 97–102.

Tripathi, A.K., 2018. Pests of stored grains. 311-359. In: Omkar (Ed.), Pests and Their Management. Springer, Singapore.

Van den Dool, H., Kratz, P.D., 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. A 11, 463–471.

Xie, Y.S., Bodnaryk, R.P., Fields, P.G., 1996. A rapid and simple flour-disk bioassay for testing substances active against stored-product insects. Can. Entomol. 128, 865–875.

Ximenes, R.M., Nogueira, L.M., Cassundé, N.M.R., Jorge, R.J.B., Santos, S.M., Magalhães, L.P.M., Sena, K.X.F.R., Albuquerque, J.F.C., Martins, R.D., 2013. Antinociceptive and wound healing activities of *Croton adamantinus* Müll. Arg. essential oil. J. Nat. Med. 67, 758–764.

You, C.X., Yang, K., Wu, Y., Zhang, W.J., Wang, Y., Geng, Z.F., Chen, H.P., Jiang, H.Y., Du, S.S., Deng, Z.W., 2014. Chemical composition and insecticidal activities of the essential oil of *Perilla frutescens* (L.) Britt aerial parts against two stored product insects. Eur. Food Res. Technol. 239, 481–490.

Zaio, Y.P., Gatti, G., Ponce, A.A., Larraide, N.A.S., Martinez, M.J., Zunino, M.P., Zygadlo, J.A., 2018. Cinnamaldehyde and related phenylpropanoids, natural repellents and insecticides against *Sitophilus zeamais* (Motsch.). A chemical structure bioactivity relationship. J. Sci. Food Agric. 98, 5822–5831.

Zhou, H.Y., Zhao, N.N., Du, S.S., Yang, K., Wang, C.F., Liu, Z.L., Qiao, Y.J., 2012. Insecticidal activity of the essential oil of *Lonicera japonica* flower buds and its main constituent compounds against two grain storage insects. J. Med. Plants Res. 6, 912–917.

ANEXO A - ACTA TROPICA (GUIDE FOR AUTHORS)

ACTA TROPICA

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0001-706X

DESCRIPTION

Acta Tropica, is an international journal on infectious diseases that covers public health sciences and biomedical research with particular emphasis on topics relevant to human and animal health in the tropics and the subtropics.

Its scope includes the biology of pathogens and vectors, host-parasite relationships, mechanisms of pathogenicity, clinical disease and treatment, and we welcome contributions in basic or applied research in disciplines such as epidemiology, disease ecology, diagnostics, interventions and control, mathematical modeling, public health and social sciences, climate change, parasite and vector taxonomy, host and parasite genomics, biochemistry and immunology and vaccine testing.

Contributions may be in the form of original research papers, review articles, short communications, opinion articles, or letters to the Editors.

Only manuscripts of high scientific significance and innovation will be considered for publication. Manuscripts of minimal international relevance, case reports, and control strategies at very early inconclusive laboratory stages of development will not be considered for publication.

Important Guidelines for Acceptance
Editors and the Editorial Board of *Acta Tropica* provide the following guidelines to help authors prepare manuscripts of high quality that can be considered for publication. Maximize your chances of acceptance by making sure your manuscript: Matches the scientific scope of the journal, Presents results that significantly advance science including innovative new approaches, Meets quality standards of presentation and literature citation, Demonstrates potential health or biomedical impact.

The above points are critical for publication of original papers. Be aware Editors carefully evaluate initial manuscript submissions and only those meeting the above criteria will be forwarded to review. If reviewed favorably and the authors seriously address all concerns, than chances of acceptance are increased. Review papers, in addition, are expected to carefully synthesize the literarture and make recommendations to advance respective scientific fields.

Benefits to authors
We also provide many author benefits, such as free PDFs, a liberal copyright policy, special discounts on Elsevier publications and much more. Please click here for more information on our author services .

Please see our Guide for Authors for information on article submission. If you require any further information or help, please visit our Support Center

AUDIENCE

All clinicians and researchers dealing with tropical diseases, including parasitologists, microbiologists, immunologists and epidemiologists

IMPACT FACTOR

2019: 2.555 © Clarivate Analytics Journal Citation Reports 2020

ABSTRACTING AND INDEXING

BIOSIS Citation Index
Chemical Abstracts
Current Contents
Embase
PubMed/Medline
Science Citation Index
Abstracts on Hygiene and Communicable Diseases
Helminthological Abstracts
Tropical Diseases Bulletin
Veterinary Bulletin
Ecological Abstracts
CAB International
Scopus

EDITORIAL BOARD

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.
To find out more, please visit the Preparation section below.

INTRODUCTION

Acta Tropica publishes original research papers, short communications, review articles and letter to the editor. Original papers **should normally not exceed 10 printed pages** including tables and figures. Short communications should not exceed 4 printed pages including tables and figures. Manuscripts must be accompanied by a letter signed by all the authors. Submission of a paper to *Acta Tropica* is understood to imply that it has not previously been published (except in an abstract form), and that it is not being considered for publication elsewhere. The act of submitting a manuscript to *Acta Tropica* carries with it the right to publish the paper. Responsibility for the accuracy of the material in the manuscript, including bibliographic citations, lies entirely with the authors. Letters to the Editor is considered for publication provided it does not contain material that has been submitted or published elsewhere. The text, not including references, must not exceed 1000 words. The letter can have one figure or small table. When a letter refers to an article recently published in *Acta Tropica*, the opportunity for reply will be given to the authors of the original article. Such a reply will be published along with the letter. Start the letter with "Dear Editor".

Types of Paper
Acta Tropica publishes the following types of papers:

1. Original research articles
2. Short Communications
3. Review articles
4. Opinion articles
5. Letters to the Editor

Original research articles should report highly significant innovative results not previously published elsewhere. Original articles are limited to 7,000 words per article (all text excluding tables and figure legends).

Short Communications should consist of original results or new methods within the scope of the journal. Short Communications need not be formally structured as full papers, but should give sufficient methods and data necessary for their comprehension. Short Communications are limited to 3,000 words per article (all text excluding tables and figure legends).

Review articles should cover subjects falling within the scope of the journal which are of active current interest. They may be submitted or invited by the Editors in Chief. Review articles should include insightful recommendations for future directions needed for achieving public health impacts. Review articles are limited to 15,000 words per article (all text excluding tables and figure legends).

Opinion articles should include scientifically backed points of view regarding currently relevant, controversial or future-oriented topics pertinent to the scope of Acta Tropica. Note that only outlining recent advances in a given field is not acceptable for an Opinion article for Acta Tropica. Besides stimulating scientific discussion or future research, opinion articles should provide a novel conceptual framework for an old or timely issue. The authors should outline which research directions should be prioritized and highlight specific points explaining why they should be prioritized in the forthcoming years to achieve public health impact. Opinion articles are limited to 6,000 words per article (all text excluding tables and figure legends).

Letters to the Editor offering comment or useful critique on material published in the journal are welcome. Letters on "hot topics" about parasite and vector biology, ecology, as well as epidemiology, control of infections, and public health are also welcome. Note that Letters to the Editors will also be externally reviewed but the decision to publish submitted letters rests with the Editors in Chief. A goal is to publish constructive letters that will permit an exchange of views which will be of benefit to both the journal and its readers. Letters to the Editor are limited to 2,000 words per article (all text excluding tables and figure legends).

Special Issues

Special issues represent the state of the art and recent advances in the knowledge of selected topics fitting within the scope of Acta Tropica. The addressed topics cover basic or applied research on epidemiology, intervention and control of parasitic infections, global health, pathogenicity, innovative technology in diagnostics, biology of pathogens and vectors as well as on advances in the understanding of host-parasite relationships. The original research, review and opinion articles in Special Issues are composed by invited leading scientists in their respective fields and are curated by Guest Editors in cooperation with the Editors in Chief. Inquiries regarding ideas for new Special issues should be addressed to any of the Editors in Chief.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A competing interests statement is provided, even if the authors have no competing interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/ registrations, and grants or other funding. Authors should complete the declaration of interest statement using this template and upload to the submission system at the Attach/Upload Files step. **Note: Please do not convert the .docx template to another file type. Author signatures are not required.** If there are no interests to declare, please choose: 'Declarations of interest: none' in the template. This statement will be published within the article if accepted. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Preprints

Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Author contributions

For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service
This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. Please note that your article will be reviewed again by the new journal. More information.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights
As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Open access

Please visit our Open Access page for more information.

Elsevier Researcher Academy
Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's Author Services.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Referees
Authors must send the names, addresses and email addresses of at least 3 potential reviewers for this manuscript meeting the following criteria: They must be experts or active workers in the field; They must not be from the same university; They must not be current or prior mentors or collaborators;

PREPARATION

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.
As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

Please submit the manuscript with double line spacing and with continuous line numbering.

References
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements
There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.
If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.
Divide the article into clearly defined sections.

Figures and tables embedded in text
Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

REVISED SUBMISSIONS

Use of word processing software
Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Please submit the manuscript with double line spacing and with continuous line numbering.

Article structure

Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Theory/calculation
A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

Please provide, when submitting your article, a graphical abstract. This comprises the title, authors and affiliations, identical to the article itself, a summary of about 25 words, and a pictogram: one figure representative of the work described. Maximum image size: 400 × 600 pixels (h × w, recommended size 200 × 500 pixels). Preferred file types: TIFF, EPS, PDF or MS Office files. See https://www.elsevier.com/graphicalabstracts for examples.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

Artwork

Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Indicate per figure if it is a single, 1.5 or 2-column fitting image.
• For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
• Please note that individual figure files larger than 10 MB must be provided in separate source files.

A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
• Supply files that are too low in resolution.
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF) or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) in addition to color reproduction in print. Further information on the preparation of electronic artwork.

Illustration services
Elsevier's Author Services offers Illustration Services to authors preparing to submit a manuscript but concerned about the quality of the images accompanying their article. Elsevier's expert illustrators can produce scientific, technical and medical-style images, as well as a full range of charts, tables and graphs. Image 'polishing' is also available, where our illustrators take your image(s) and improve them to a professional standard. Please visit the website to find out more.

Figure captions
Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:
http://open.mendeley.com/use-citation-style/acta-tropica
When preparing your manuscript, you will then be able to select this style using the Mendeley plug-ins for Microsoft Word or LibreOffice.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: All citations in the text should refer to:
1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.
Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown …'

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.
Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary material
Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data
This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking
If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Mendeley Data
This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to *Mendeley Data*. The datasets will be listed and directly accessible to readers next to your published article online.

For more information, visit the Mendeley Data for journals page.

Data in Brief
You have the option of converting any or all parts of your supplementary or additional raw data into one or multiple data articles, a new kind of article that houses and describes your data. Data articles ensure that your data is actively reviewed, curated, formatted, indexed, given a DOI and publicly available to all upon publication. You are encouraged to submit your article for *Data in Brief* as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to *Data in Brief* where it will be editorially reviewed and published in the open access data journal, *Data in Brief*. Please note an open access fee of 600 USD is payable for publication in *Data in Brief*. Full details can be found on the Data in Brief website. Please use this template to write your Data in Brief.

Data statement
To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

AFTER ACCEPTANCE

Online proof correction
To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Author Services. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.
You can also check the status of your submitted article or find out when your accepted article will be published.

ANEXO B – PARASITOLOGY RESEARCH (GUIDE FOR AUTHORS)

Submission guidelines

Contents

Instructions for Authors

Before you submit: Editorial checklist

To give your manuscript the best chance of publication, follow these policies and formatting guidelines.

- Make sure your manuscript is accurate and readable – Language editing services
- Make sure your submission is complete, including correct author names and correct affiliations – Title page
- General formatting rules for all article types, including page and line numbers in the manuscript – Text, Scientific style and References
- Figures should be submitted as separate files – Artwork and illustrations guidelines
- Make sure you include ethics statements – please carefully check Compliance with Ethical Standards, Research involving humans and animals, and Informed consent
- Make sure you acknowledge all the sources and that overlap with other publications is kept to a minimum (the journal may use software to screen for plagiarism) – Ethical responsibilities of Authors
- Ensure that DNA/RNA sequencing data is submitted to a suitable repository (e.g. GenBank accession numbers) – Research Data Policy

Authorship Policy

Authorship should incorporate and should be restricted to those who have contributed substantially to the work in one or more of the following categories:

• Conceived of or designed study

• Performed research

• Analyzed data

• Contributed new methods or models

• Wrote the paper

Important Notes:

Manuscripts that were previously rejected by this journal cannot be resubmitted.

Changes of authorship or in the order of authors are not accepted after acceptance of a manuscript. Authors are strongly advised to ensure the correct author group when submitting the manuscript.

Types of Papers

All manuscripts should be clear, straightforward and concise.

Original Papers

Original Papers should report data from original research and have no strict limitations in length; however, they should not exceed 10 printed pages (one printed page corresponds to approximately 850 words of text, or 3 illustrations with their legends).

Reviews

Reviews should be comprehensive, fully referenced expositions of subjects of general interest, including background information and detailed critical analyses of current work in the field and its significance. Review articles have no limitation in length. The editors reserve the right to ask for abbreviation of the text if too excessive.

Short Communications

Short Communications should deal with a single point. They should not exceed 3 printed pages (one printed page corresponds to approximately 850 words of text, or 3 illustrations with their legends). “Results” and “Discussions” should be combined. Up to 20 references are recommended. The editors reserve the right to decide what constitutes a Short Communication.

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the

work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit online" on the right and upload all of your manuscript files following the instructions given on the screen.

Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

Submission of revisions

Please submit your revised manuscript as a marked-up text (Word) file in which all changes can be tracked. Additionally, please upload a line-by-line response to all reviewers' remarks when you submit the revised manuscript.

Preprint Policy

Springer accepts posting of preprints of primary research manuscripts on preprint servers, authors' or institutional websites. Details of our policy on posting, licensing, citation of preprints and communications with the media about preprints of primary research manuscripts may be found here.

Authors should disclose details of preprint posting, including DOI and licensing terms, upon submission of the manuscript or at any other point during consideration at a Springer journal. Once the preprint is published, it is the author's responsibility to ensure that the preprint record is updated with a publication reference, including the DOI and a URL link to the published version of the article on the journal website.

Preprints may be cited in the reference list of articles under consideration at Springer journals as shown below:

Babichev, S. A., Ries, J. & Lvovsky, A. I. Quantum scissors: teleportation of single-mode optical states by means of a nonlocal single photon. Preprint at http://arxiv.org/abs/quant-ph/0208066 (2002). .

Costs of Colour Illustrations
Online publication of color illustrations is always free of charge.

Title page

Title Page
Please use this template title page for providing the following information.

The title page should include:

The name(s) of the author(s)
A concise and informative title
The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
A clear indication and an active e-mail address of the corresponding author
If available, the 16-digit ORCID of the author(s)
If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

For life science journals only (when applicable)

Trial registration number and date of registration

Trial registration number, date of registration followed by “retrospectively registered”

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations'.

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

To be used for non-life science journals

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)

Availability of data and material (data transparency)

Code availability (software application or custom code)

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

To be used for life science journals + articles with biological applications

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)

Ethics approval (include appropriate approvals or waivers)

Consent to participate (include appropriate statements)

Consent for publication (include appropriate statements)

Availability of data and material (data transparency)

Code availability (software application or custom code)

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

Text

Text Formatting

Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10-point Times Roman) for text.
Use italics for emphasis.
Use the automatic page numbering function to number the pages.
Do not use field functions.
Use tab stops or other commands for indents, not the space bar.
Use the table function, not spreadsheets, to make tables.
Use the equation editor or MathType for equations.
Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).
Manuscripts with mathematical content can also be submitted in LaTeX.

LaTeX macro package (Download zip, 188 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Important note:
Authors are requested to use automatic continuous line numbering throughout the manuscript and in double space.

Scientific style

Please always use internationally accepted signs and symbols for units (SI units).

Nomenclature

The International Code of Zoological Nomenclature (ICZN) must be observed. Genus and species names should be in italics. Authors of scientific names of the genus and species group should not be italicized. At first mention, a specific name should be cited with nomenclatural author and year, e.g. Catenula lemnae (in italics) Dugès, 1832. When three or more joint authors have been responsible for a name, then the citation of the name of the authors may be expressed by use of the term "et al." following the name of the first author, provided that all authors of the name are cited in full elsewhere in the same work, either in the text or in a bibliographic reference. Authors unfamiliar with the taxonomy of the group to which a species belongs should consult an expert to ensure that it is properly identified and that the correct name is used.

References

Citation

Cite references in the text by name and year in parentheses. Some examples:

Negotiation research spans many disciplines (Thompson 1990).
This result was later contradicted by Becker and Seligman (1996).
This effect has been widely studied (Abbott 1991; Barakat et al. 1995a, b; Kelso and Smith 1998; Medvec et al. 1999, 2000).
Reference list
The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work. Order multi-author publications of the same first author alphabetically with respect to second, third, etc. author. Publications of exactly the same author(s) must be ordered chronologically.

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

ISSN LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

EndNote style (Download zip, 3 kB)

Tables

All tables are to be numbered using Arabic numerals.
Tables should always be cited in text in consecutive numerical order.
For each table, please supply a table caption (title) explaining the components of the table.
Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission
Supply all figures electronically.
Indicate what graphics program was used to create the artwork.
For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
Vector graphics containing fonts must have the fonts embedded in the files.
Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.
Line Art

Definition: Black and white graphic with no shading.
Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
All lines should be at least 0.1 mm (0.3 pt) wide.
Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
Vector graphics containing fonts must have the fonts embedded in the files.
Halftone Art

Definition: Photographs, drawings, or paintings with fine shading, etc.
If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
Halftones should have a minimum resolution of 300 dpi.
Combination Art

Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
Combination artwork should have a minimum resolution of 600 dpi.
Color Art
Color art is free of charge for online publication.
If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
If the figures will be printed in black and white, do not refer to color in the captions.
Color illustrations should be submitted as RGB (8 bits per channel).
Figure Lettering
To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
Avoid effects such as shading, outline letters, etc.
Do not include titles or captions within your illustrations.
Figure Numbering
All figures are to be numbered using Arabic numerals.
Figures should always be cited in text in consecutive numerical order.
Figure parts should be denoted by lowercase letters (a, b, c, etc.).

If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.
Figure Captions
Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.
Figure Placement and Size
Figures should be submitted separately from the text, if possible.
When preparing your figures, size figures to fit in the column width.
For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.
For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
Any figure lettering has a contrast ratio of at least 4.5:1

Electronic Supplementary Material

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as electronic supplementary material, authors should read the journal’s Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

Supply all supplementary material in standard file formats.

Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.
Audio, Video, and Animations
Aspect ratio: 16:9 or 4:3
Maximum file size: 25 GB
Minimum video duration: 1 sec
Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
A collection of figures may also be combined in a PDF file.
Spreadsheets
Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).
Specialized Formats
Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

It is possible to collect multiple files in a .zip or .gz file.
Numbering
If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.
Accessibility
In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

The manuscript contains a descriptive caption for each supplementary material
Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

Ethical Responsibilities of Authors

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation is helped by following the rules of good scientific practice, which include*:

The manuscript should not be submitted to more than one journal for simultaneous consideration.
The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. (Please provide transparency on the re-use of material to avoid the concerns about text-recycling ('self-plagiarism').
A single study should not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (i.e. 'salami-slicing/publishing').
Concurrent or secondary publication is sometimes justifiable, provided certain conditions are met. Examples include: translations or a manuscript that is intended for a different group of readers.

Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors should adhere to discipline-specific rules for acquiring, selecting and processing data.
No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are used for verbatim copying of material, and permissions secured for material that is copyrighted.
Important note: the journal may use software to screen for plagiarism.

Authors should make sure they have permissions for the use of software, questionnaires/(web) surveys and scales in their studies (if appropriate).
Research articles and non-research articles (e.g. Opinion, Review, and Commentary articles) must cite appropriate and relevant literature in support of the claims made. Excessive and inappropriate self-citation or coordinated efforts among several authors to collectively self-cite is strongly discouraged.
Authors should avoid untrue statements about an entity (who can be an individual person or a company) or descriptions of their behavior or actions that could potentially be seen as personal attacks or allegations about that person.
Research that may be misapplied to pose a threat to public health or national security should be clearly identified in the manuscript (e.g. dual use of research). Examples include creation of harmful consequences of biological agents or toxins, disruption of immunity of vaccines, unusual hazards in the use of chemicals, weaponization of research/technology (amongst others).
Authors are strongly advised to ensure the author group, the Corresponding Author, and the order of authors are all correct at submission. Adding and/or deleting authors during the revision stages is generally not permitted, but in some cases may be warranted. Reasons for changes in authorship should be explained in detail. Please note that changes to authorship cannot be made after acceptance of a manuscript.
*All of the above are guidelines and authors need to make sure to respect third parties rights such as copyright and/or moral rights.

Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results presented. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential or proprietary data is excluded.

If there is suspicion of misbehavior or alleged fraud the Journal and/or Publisher will carry out an investigation following COPE guidelines. If, after investigation, there are valid concerns, the author(s) concerned will be contacted under their given e-mail address and given an opportunity to address the issue. Depending on the situation, this may result in the Journal's and/or Publisher's implementation of the following measures, including, but not limited to:

If the manuscript is still under consideration, it may be rejected and returned to the author.
If the article has already been published online, depending on the nature and severity of the infraction:
- an erratum/correction may be placed with the article

- an expression of concern may be placed with the article

- or in severe cases retraction of the article may occur.

The reason will be given in the published erratum/correction, expression of concern or retraction note. Please note that retraction means that the article is maintained on the platform, watermarked "retracted" and the explanation for the retraction is provided in a note linked to the watermarked article.

The author's institution may be informed

A notice of suspected transgression of ethical standards in the peer review system may be included as part of the author's and article's bibliographic record.
Fundamental errors
Authors have an obligation to correct mistakes once they discover a significant error or inaccuracy in their published article. The author(s) is/are requested to contact the journal and explain in what sense the error is impacting the article. A decision on how to correct the literature will depend on the nature of the error. This may be a correction or retraction. The retraction note should provide transparency which parts of the article are impacted by the error.

Suggesting / excluding reviewers

Authors are welcome to suggest suitable reviewers and/or request the exclusion of certain individuals when they submit their manuscripts. When suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way. It is strongly recommended to suggest a mix of reviewers from different countries and different institutions. When suggesting reviewers, the Corresponding Author must provide an institutional email address for each suggested reviewer, or, if this is not possible to include other means of verifying the identity such as a link to a personal homepage, a link to the publication record or a researcher or author ID in the submission letter. Please note that the Journal may not use the suggestions, but suggestions are appreciated and may help facilitate the peer review process.

Authorship principles

These guidelines describe authorship principles and good authorship practices to which prospective authors should adhere to.

Authorship clarified

The Journal and Publisher assume all authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out, before the work is submitted.

The Publisher does not prescribe the kinds of contributions that warrant authorship. It is recommended that authors adhere to the guidelines for authorship that are applicable in their specific research field. In absence of specific guidelines it is recommended to adhere to the following guidelines*:

All authors whose names appear on the submission

1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work;

2) drafted the work or revised it critically for important intellectual content;

3) approved the version to be published; and

4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

* Based on/adapted from:

ICMJE, Defining the Role of Authors and Contributors,

Transparency in authors' contributions and responsibilities to promote integrity in scientific publication, McNutt at all, PNAS February 27, 2018

Disclosures and declarations

All authors are requested to include information regarding sources of funding, financial or non-financial interests, study-specific approval by the appropriate ethics committee for research involving humans and/or animals, informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals (as appropriate).

The decision whether such information should be included is not only dependent on the scope of the journal, but also the scope of the article. Work submitted for publication may have implications for public health or general welfare and in those cases it is the responsibility of all authors to include the appropriate disclosures and declarations.

Data transparency

All authors are requested to make sure that all data and materials as well as software application or custom code support their published claims and comply with field standards. Please note that journals may have individual policies on (sharing) research data in concordance with disciplinary norms and expectations.

Role of the Corresponding Author
One author is assigned as Corresponding Author and acts on behalf of all co-authors and ensures that questions related to the accuracy or integrity of any part of the work are appropriately addressed.

The Corresponding Author is responsible for the following requirements:

ensuring that all listed authors have approved the manuscript before submission, including the names and order of authors;

managing all communication between the Journal and all co-authors, before and after publication;*
providing transparency on re-use of material and mention any unpublished material (for example manuscripts in press) included in the manuscript in a cover letter to the Editor;
making sure disclosures, declarations and transparency on data statements from all authors are included in the manuscript as appropriate (see above).
* The requirement of managing all communication between the journal and all co-authors during submission and proofing may be delegated to a Contact or Submitting Author. In this case please make sure the Corresponding Author is clearly indicated in the manuscript.

Author contributions

In absence of specific instructions and in research fields where it is possible to describe discrete efforts, the Publisher recommends authors to include contribution statements in the work that specifies the contribution of every author in order to promote transparency. These contributions should be listed at the separate title page.

Examples of such statement(s) are shown below:

• Free text:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [full name], [full name] and [full name]. The first draft of the manuscript was written by [full name] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Example: CRediT taxonomy:

• Conceptualization: [full name], …; Methodology: [full name], …; Formal analysis and investigation: [full name], …; Writing - original draft preparation: [full name, …]; Writing - review and editing: [full name], …; Funding acquisition: [full name], …; Resources: [full name], …; Supervision: [full name],….

For review articles where discrete statements are less applicable a statement should be included who had the idea for the article, who performed the literature search and data analysis, and who drafted and/or critically revised the work.

For articles that are based primarily on the student's dissertation or thesis, it is recommended that the student is usually listed as principal author:

A Graduate Student's Guide to Determining Authorship Credit and Authorship Order, APA Science Student Council 2006

Affiliation
The primary affiliation for each author should be the institution where the majority of their work was done. If an author has subsequently moved, the current address may additionally be stated. Addresses will not be updated or changed after publication of the article.

Changes to authorship

Authors are strongly advised to ensure the correct author group, the Corresponding Author, and the order of authors at submission. Changes of authorship by adding or deleting authors, and/or changes in

Corresponding Author, and/or changes in the sequence of authors are not accepted after acceptance of a manuscript.

Please note that author names will be published exactly as they appear on the accepted submission! Please make sure that the names of all authors are present and correctly spelled, and that addresses and affiliations are current.

Adding and/or deleting authors at revision stage are generally not permitted, but in some cases it may be warranted. Reasons for these changes in authorship should be explained. Approval of the change during revision is at the discretion of the Editor-in-Chief. Please note that journals may have individual policies on adding and/or deleting authors during revision stage.

Author identification

Authors are recommended to use their ORCID ID when submitting an article for consideration or acquire an ORCID ID via the submission process.

Deceased or incapacitated authors
For cases in which a co-author dies or is incapacitated during the writing, submission, or peer-review process, and the co-authors feel it is appropriate to include the author, co-authors should obtain approval from a (legal) representative which could be a direct relative.

Authorship issues or disputes

In the case of an authorship dispute during peer review or after acceptance and publication, the Journal will not be in a position to investigate or adjudicate. Authors will be asked to resolve the dispute themselves. If they are unable the Journal reserves the right to withdraw a manuscript from the editorial process or in case of a published paper raise the issue with the authors' institution(s) and abide by its guidelines.

Confidentiality

Authors should treat all communication with the Journal as confidential which includes correspondence with direct representatives from the Journal such as Editors-in-Chief and/or Handling Editors and reviewers' reports unless explicit consent has been received to share information.

Compliance with Ethical Standards
To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" when submitting a paper:

Disclosure of potential conflicts of interest
Research involving Human Participants and/or Animals
Informed consent
Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline. Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

Conflicts of Interest / Competing Interests

Authors are requested to disclose interests that are directly or indirectly related to the work submitted for publication. Interests within the last 3 years of beginning the work (conducting the research and preparing the work for submission) should be reported. Interests outside the 3-year time frame must be disclosed if they could reasonably be perceived as influencing the submitted work. Disclosure of interests provides a complete and transparent process and helps readers form their own judgments of potential bias. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is inappropriate.

Interests that should be considered and disclosed but are not limited to the following:

Funding: Research grants from funding agencies (please give the research funder and the grant number) and/or research support (including salaries, equipment, supplies, reimbursement for attending symposia, and other expenses) by organizations that may gain or lose financially through publication of this manuscript.

Employment: Recent (while engaged in the research project), present or anticipated employment by any organization that may gain or lose financially through publication of this manuscript. This includes multiple affiliations (if applicable).

Financial interests: Stocks or shares in companies (including holdings of spouse and/or children) that may gain or lose financially through publication of this manuscript; consultation fees or other forms of remuneration from organizations that may gain or lose financially; patents or patent applications whose value may be affected by publication of this manuscript.

It is difficult to specify a threshold at which a financial interest becomes significant, any such figure is necessarily arbitrary, so one possible practical guideline is the following: "Any undeclared financial interest that could embarrass the author were it to become publicly known after the work was published."

Non-financial interests: In addition, authors are requested to disclose interests that go beyond financial interests that could impart bias on the work submitted for publication such as professional interests, personal relationships or personal beliefs (amongst others). Examples include, but are not limited to: position on editorial board, advisory board or board of directors or other type of management relationships; writing and/or consulting for educational purposes; expert witness; mentoring relations; and so forth.

Primary research articles require a disclosure statement. Review articles present an expert synthesis of evidence and may be treated as an authoritative work on a subject. Review articles therefore require a disclosure statement. Other article types such as editorials, book reviews, comments (amongst others) may, dependent on their content, require a disclosure statement. If you are unclear whether your article type requires a disclosure statement, please contact the Editor-in-Chief.

Please note that, in addition to the above requirements, funding information (given that funding is a potential conflict of interest (as mentioned above)) needs to be disclosed upon submission of the manuscript in the peer review system. This information will automatically be added to the Record of CrossMark, however it is not added to the manuscript itself. Under ‘summary of requirements’ (see below) funding information should be included in the ‘Declarations’ section.

Summary of requirements

The above should be summarized in a statement and placed in a ‘Declarations’ section before the reference list under a heading of ‘Funding’ and/or ‘Conflicts of interests’/’Competing interests’. Other declarations include Ethics approval, Consent, Data, Material and/or Code availability and Authors’ contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

When all authors have the same (or no) conflicts and/or funding it is sufficient to use one blanket statement.

Examples of statements to be used when funding has been received:

Partial financial support was received from [...]
The research leading to these results received funding from […] under Grant Agreement No[…].
This study was funded by […]
This work was supported by […] (Grant numbers […] and […]
Examples of statements to be used when there is no funding:

The authors did not receive support from any organization for the submitted work.
No funding was received to assist with the preparation of this manuscript.
No funding was received for conducting this study.
No funds, grants, or other support was received.
Examples of statements to be used when there are interests to declare:

Financial interests: Author A has received research support from Company A. Author B has received a speaker honorarium from Company Wand owns stock in Company X. Author C is consultant to company Y.
Non-financial interests: Author C is an unpaid member of committee Z.

Financial interests: The authors declare they have no financial interests.
Non-financial interests: Author A is on the board of directors of Y and receives no compensation as member of the board of directors.

Financial interests: Author A received a speaking fee from Y for Z. Author B receives a salary from association X. X where s/he is the Executive Director.
Non-financial interests: none.

Financial interests: Author A and B declare they have no financial interests. Author C has received speaker and consultant honoraria from Company M and Company N. Dr. C has received speaker honorarium and research funding from Company M and Company O. Author D has received travel support from Company O.

Non-financial interests: Author D has served on advisory boards for Company M, Company N and Company O.

Examples of statements to be used when authors have nothing to declare:

The authors have no relevant financial or non-financial interests to disclose.
The authors have no conflicts of interest to declare that are relevant to the content of this article.
All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.
The authors have no financial or proprietary interests in any material discussed in this article.
Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Research involving human participants, their data or biological material

Ethics approval

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

Retrospective ethics approval

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

Ethics approval for retrospective studies

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

Ethics approval for case studies

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the individual (or parent or guardian if the participant is a minor or incapable) See also section on Informed Consent.

Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the NCBI database for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

Further information is available from the International Cell Line Authentication Committee (ICLAC).

Authors should include a statement that confirms that an institutional or independent ethics committee (including the name of the ethics committee) approved the study and that informed consent was obtained from the donor or next of kin.

Research Resource Identifiers (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively similar to a DOI) for research resources. This journal encourages authors to adopt RRIDs when reporting key biological resources (antibodies, cell lines, model organisms and tools) in their manuscripts.

Examples:

Organism: Filip1tm1a(KOMP)Wtsi RRID:MMRRC_055641-UCD

Cell Line: RST307 cell line RRID:CVCL_C321

Antibody: Luciferase antibody DSHB Cat# LUC-3, RRID:AB_2722109

Plasmid: mRuby3 plasmid RRID:Addgene_104005

Software: ImageJ Version 1.2.4 RRID:SCR_003070

RRIDs are provided by the Resource Identification Portal. Many commonly used research resources already have designated RRIDs. The portal also provides authors links so that they can quickly register a new resource and obtain an RRID.

Clinical Trial Registration

The World Health Organization (WHO) definition of a clinical trial is "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes". The WHO defines health interventions as “A health intervention is an act performed for, with or on behalf of a person or population whose purpose is to assess, improve, maintain, promote or modify health, functioning or health conditions” and a health-related outcome is generally defined as a change in the health of a person or population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register prospective clinical trials (phase II to IV trials) in suitable publicly available repositories. For example www.clinicaltrials.gov or any of the primary registries that participate in the WHO International Clinical Trials Registry Platform.

The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered' should be included as the last line of the manuscript abstract.

Purely observational trials will not require registration.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the EQUATOR Network when preparing their manuscript.

Exact requirements may vary depending on the journal; please refer to the journal's Instructions for Authors.

Checklists are available for a number of study designs, including:

Randomised trials (CONSORT) and Study protocols (SPIRIT)

Observational studies (STROBE)

Systematic reviews and meta-analyses (PRISMA) and protocols (Prisma-P)

Diagnostic/prognostic studies (STARD) and (TRIPOD)

Case reports (CARE)

Clinical practice guidelines (AGREE) and (RIGHT)

Qualitative research (SRQR) and (COREQ)

Animal pre-clinical studies (ARRIVE)

Quality improvement studies (SQUIRE)

Economic evaluations (CHEERS)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Examples of statements to be used when ethics approval has been obtained:

• All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. ...).

• This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).

• Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

• The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: ...).

Examples of statements to be used for a retrospective study:

• Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

• This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

• This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

• This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.

• The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images

in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

• Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.

• Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered “informed”. However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

Consent to Participate
For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues

were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

Consent to Publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be foundhere. (Download docx, 36 kB)

Summary of requirements

The above should be summarized in a statement and placed in a ‘Declarations’ section before the reference list under a heading of ‘Consent to participate’ and/or ‘Consent to publish’. Other declarations include Funding, Conflicts of interest/competing interests, Ethics approval, Consent, Data and/or Code availability and Authors’ contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for "Consent to participate":

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

Sample statements for “Consent to publish”:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

Research Data Policy

A submission to the journal implies that materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

The journal strongly encourages that all datasets on which the conclusions of the paper rely should be available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible. Please see Springer Nature's information on recommended repositories.

List of Repositories

Research Data Policy

General repositories - for all types of research data - such as figshare and Dryad may be used where appropriate.

Datasets that are assigned digital object identifiers (DOIs) by a data repository may be cited in the reference list. Data citations should include the minimum information recommended by DataCite: authors, title, publisher (repository name), identifier.

DataCite

Where a widely established research community expectation for data archiving in public repositories exists, submission to a community-endorsed, public repository is mandatory. Persistent identifiers (such as DOIs and accession numbers) for relevant datasets must be provided in the paper

For the following types of data set, submission to a community-endorsed, public repository is mandatory:

Mandatory deposition Suitable repositories
Protein sequences Uniprot
DNA and RNA sequences Genbank
DNA DataBank of Japan (DDBJ)

EMBL Nucleotide Sequence Database (ENA)

DNA and RNA sequencing dataNCBI Trace Archive
NCBI Sequence Read Archive (SRA)

Genetic polymorphisms dbSNP dbVar

European Variation Archive (EVA)

Linked genotype and phenotype data dbGAP
The European Genome-phenome Archive (EGA)

Macromolecular structure Worldwide Protein Data Bank (wwPDB)

Biological Magnetic Resonance Data Bank (BMRB)

Electron Microscopy Data Bank (EMDB)

Microarray data (must be MIAME compliant) Gene Expression Omnibus (GEO)
ArrayExpress

Crystallographic data for small molecules Cambridge Structural Database
For more information:

Research Data Policy Frequently Asked Questions

Data availability

The journal encourages authors to provide a statement of Data availability in their article. Data availability statements should include information on where data supporting the results reported in the article can be found, including, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. Data availability statements can also indicate whether data are available on request from the authors and where no data are available, if appropriate.

Data Availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

1. The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]
2. The datasets generated during and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.
3. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.
4. Data sharing not applicable to this article as no datasets were generated or analysed during the current study.
5. All data generated or analysed during this study are included in this published article [and its supplementary information files].
More examples of template data availability statements, which include examples of openly available and restricted access datasets, are available:

Data availability statements

Springer Nature provides a research data policy support service for authors and editors, which can be contacted at researchdata@springernature.com.

This service provides advice on research data policy compliance and on finding research data repositories. It is independent of journal, book and conference proceedings editorial offices and does not advise on specific manuscripts.

Helpdesk

Back to top
After acceptance

Upon acceptance of your article you will receive a link to the special Author Query Application at Springer's web page where you can sign the Copyright Transfer Statement online and indicate whether you wish to order OpenChoice, offprints, or printing of figures in color.

Once the Author Query Application has been completed, your article will be processed and you will receive the proofs.

Copyright transfer

Authors will be asked to transfer copyright of the article to the Publisher (or grant the Publisher exclusive publication and dissemination rights). This will ensure the widest possible protection and dissemination of information under copyright laws.

Offprints

Offprints can be ordered by the corresponding author.

Color illustrations

Online publication of color illustrations is free of charge. For color in the print version, authors will be expected to make a contribution towards the extra costs.

Proof reading

The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor.

After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

Open Choice

Open Choice allows you to publish open access in more than 1850 Springer Nature journals, making your research more visible and accessible immediately on publication.

Article processing charges (APCs) vary by journal – view the full list

Benefits:

Increased researcher engagement: Open Choice enables access by anyone with an internet connection, immediately on publication.
Higher visibility and impact: In Springer hybrid journals, OA articles are accessed 4 times more often on average, and cited 1.7 more times on average*.

Easy compliance with funder and institutional mandates: Many funders require open access publishing, and some take compliance into account when assessing future grant applications.
It is easy to find funding to support open access – please see our funding and support pages for more information.

* Within the first three years of publication. Springer Nature hybrid journal OA impact analysis, 2018.

Open Choice

Funding and Support pages

Copyright and license term – CC BY
Open Choice articles do not require transfer of copyright as the copyright remains with the author. In opting for open access, the author(s) agree to publish the article under the Creative Commons Attribution License.

Find more about the license agreement

English Language Editing

For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood. If you need help with writing in English you should consider:

Asking a colleague who is a native English speaker to review your manuscript for clarity.
Visiting the English language tutorial which covers the common mistakes when writing in English.
Using a professional language editing service where editors will improve the English to ensure that your meaning is clear and identify problems that require your review. Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts. Springer authors are entitled to a 10% discount on their first submission to either of these services, simply follow the links below.
English language tutorial

Nature Research Editing Service

American Journal Experts

Please note that the use of a language editing service is not a requirement for publication in this journal and does not imply or guarantee that the article will be selected for peer review or accepted.

If your manuscript is accepted it will be checked by our copyeditors for spelling and formal style before publication.