Universidade Federal da Grande Dourados - UFGD
Faculdade de Ciências Biológicas e Ambientais - FCBA
Programa de Pós-Graduação em
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BIOATIVIDADE DO EXTRATO AQUOSO DE *Simarouba versicolor* A. ST-HILL (SIMAROUBACEAE) SOBRE *Plutella xylostella* L. (LEPIDOPTERA: PLUTELLIDAE) E TOXICIDADE EM ORGANISMOS NÃO-ALVO

Silvana Aparecida de Souza

Dourados-MS
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Biografia do Acadêmico

Sou Silvana Aparecida de Souza, nascida em 15 de março de 1997, natural de Dourados-MS, iniciei o ensino fundamental em 2003 na escola municipal Izabel Muzzi Fioravanti e permaneci nesta escola até 2007. Em 2008 comecei o sexto ano do ensino fundamental na escola municipal Maria da Rosa Antunes da Silveira Câmara na qual permaneci até 2011. Me recordo que já em 2007 tinha a absoluta certeza que seria Bióloga. Em 2012 comecei o ensino médio na escola estadual Menodora Fialho de Figueiredo no qual permaneci até 2014.

No último ano do ensino médio, já fazendo a preparação pré-vestibular no CEUD-UFGD, descobri a bolsa de iniciação cientifica para alunos do ensino médio (PIBIC-EM) oferecido pela Universidade Federal da Grande Dourados (UFGD) e fui selecionada para trabalhar na área de Interação Inseto-Planta sob a orientação da Professora Dra. Rosilda Mara Mussury e, foi aí, o início de uma incrível e longa parceria com minha orientadora. Em 2015, ingressei no curso de Ciências Biológicas – Bacharelado na UFGD e me formei em março de 2019.

Durante minha graduação, fui bolsista de iniciação científica durante 3 anos, avaliando o potencial inseticida de plantas nativas do Cerrado sob *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae). Para completar minha formação, fui bolsista de projeto de extensão durante 1 ano, testando o efeito de extratos aquosos de plantas nativas do Cerrado junto a pequenos produtores da região de Dourados-MS. No meu Trabalho de Conclusão de Curso (TCC) avaliei a bioatividade de espécies do gênero *Campomanesia* sob *Plutella xylostella* sob a orientação da Professora Dra. Rosilda Mara Mussury. Ainda, no ano de 2019, ingressei no mestrado do Programa de Pós-graduação em Entomologia e Conservação da Biodiversidade (PPGECB) como bolsista CAPES com a orientação da Professora Dra. Rosilda Mara Mussury.

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Aos meus colegas do Laboratório de Interação Inseto-Planta (LIIP), onde se desenvolveu toda pesquisa com alegria, com muita troca de conhecimento e experiência. Em especial, ao Alberto Domingues, Matheus Moreno, Isabella Padial e Natália Pereira, por toda ajuda e paciência.

Dedicatória

Aos meus pais, José e Sonia.
Ao meu irmão, Roberto.
Ao meu amor, melhor amigo e namorado, André.
Aos meus avós, Palmira (*In memorian*), Dorival (*In memorian*) e, Lourdes

Sumário

RESUMO GERAL

A traça-das-crucíferas (*Plutella xylostella*) (Linnaeus, 1758) (Lepidoptera: Plutellidae) é um dos principais insetos praga que atacam a cultura das Brássicas e causam sérios danos e prejuízos no mundo todo. Devido sua eficácia, rapidez e praticidade, os inseticidas sintéticos ainda são utilizados como método de controle desse microlepidóptero. Entretanto, o uso indiscriminado e incorreto de agrotóxicos vem selecionando, naturalmente, insetos resistentes, além da contaminação do solo e da água e redução na biodiversidade. Sendo assim, os inseticidas a base de plantas surgem como uma alternativa de controle efetiva, seletivos, não acumulativos, pouca ou nenhuma toxicidade ao meio ambiente e aos organismos não-alvos. Diante do exposto, o objetivo do trabalho foi avaliar a ação do extrato aquoso de *Simarouba versicolor* A. St-Hill (Simaroubaceae) em diferentes concentrações na biologia de *P. xylostella* e determinar a concentração de menor toxicidade dessa planta no nematoide de vida livre *Caenorhabditis elegans* (Maupas, 1900) (Rhabditidae). Utilizou-se as concentrações de 0,01; 0,05; 0,10 1,00; 5,00 e 10,00% e o controle composto por água destilada. Foram realizados os seguintes testes: ciclo de vida e toxicidade; preferência de oviposição, embriotoxicidade e preferência alimentar. Para o experimento de ciclo de vida, foram oferecidos discos tratados para lagartas recém eclodidas, sendo avaliadas as características da fase imatura e adulta. O experimento de toxicidade, nematoides *C. elegans* na fase L4 foram incubados com o extrato da planta teste e a testemunha e, posteriormente, foi avaliado a porcentagem de sobrevivência dos nematoides. No experimento de preferência de oviposição com chance de escolha, um casal de *P. xylostella* foram inseridos em gaiolas com 4 discos de couve, sendo 2 imersos na testemunha e 2 no extrato da concentração teste. Diariamente foi contabilizado o número de ovos em cada tratamento. Para o experimento de embriotoxicidade, discos com 10 ovos foram imersos nos tratamentos, extrato da concentração teste e testemunha, e foi contabilizado o número de lagartas eclodidas. No experimento de preferência alimentar, discos de couve tratados com extrato da concentração teste e testemunha foram oferecidos para uma lagarta de terceiro instar, com a possibilidade de escolha. O extrato aquoso de *S. versicolor*, em todas as concentrações, afetou negativamente a traça-das-crucíferas, reduzindo a oviposição, a eclosão das lagartas, a alimentação, afetou a fase imatura e adulta de *P. xylostella* e nas concentrações mais baixas não apresentou toxicidade ao *C. elegans.*

PALAVRAS-CHAVE: Pau-paraíba, antibiose, antixenose, ecotoxicidade, controle alternativo.

ABSTRACT

The diamondback moth (*Plutella xylostella*) (Linnaeus, 1758) (Lepidoptera: Plutellidae) is one of the main insect pests that attack brassica crops and cause serious damage worldwide. Due to their effectiveness, speed, and practicality, synthetic insecticides are still used as a control method for this microlepidoptera. However, the indiscriminate and incorrect use of pesticides has naturally selected resistant insects, in addition to soil and water contamination and a reduction in biodiversity. Thus, plant-based insecticides emerge as an effective, selective, non-accumulative control alternative, with little or no toxicity to the environment and non-target organisms. The objective of this work was to evaluate the action of the aqueous extract of *Simarouba versicolor* A. St-Hill (Simaroubaceae) at different concentrations on the biology of *P. xylostella* and to determine the concentration of less toxicity of this plant on the free-living nematode *Caenorhabditis elegans* (Maupas, 1900) (Rhabditidae). Concentrations of 0.01; 0.05; 0.10 1.00; 5.00 and 10.00% and the control composed of distilled water. The following tests were performed: life cycle and toxicity; oviposition preference, embryotoxicity, and food preference. For the life cycle experiment, treated disks were offered to newly hatched caterpillars, with the characteristics of the immature and adult stages being evaluated. In the toxicity experiment, *C. elegans* nematodes in the L4 phase were incubated with the test plant extract and the control and, subsequently, the percentage of nematode survival was evaluated. In the free-choice oviposition preference experiment, a couple of *P. xylostella* were placed in cages with 4 cabbage discs, 2 immersed in the control and 2 in the test concentration extract. The number of eggs in each treatment was counted daily. For the embryotoxicity experiment, disks with 10 eggs were immersed in the treatments, test concentration extract, and control, and the number of hatched caterpillars was counted. In the experiment of food preference, cabbage discs treated with extract of the test and control concentrations were offered to a third instar caterpillar, with the possibility of choice. The aqueous extract of *S. versicolor*, at all concentrations, negatively affected the diamondback moth, reducing oviposition, hatching of caterpillars, and feeding, affected the immature and adult phase of *P.* xylostella and at lower concentrations did not show toxicity to *C. elegans*.

KEYWORDS: pau-paraíba, antibiosis, antixenosis, ecotoxicity, alternative control.

1. INTRODUÇÃO GERAL

Dentre os problemas fitossanitários que causam perdas significativas nas culturas da família Brassicaceae, a *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), conhecida popularmente como traça-das-crucíferas, é uma das principais pragas que atacam as hortaliças pertencentes a esta família (FURLONG et al., 2013). Este microlepidóptero é uma praga cosmopolita (CHENG et al., 2008; SRINIVASAN et al., 2011) e pode causar sérios danos ao cultivo, podendo causar a perda de 100% da colheita (SHELTON et al., 1993; POONSRI et al., 2015). Os custos com o seu manejo somam em até 5 bilhões de dólares anualmente (ZALUCKI et al., 2012).

Devido a eficácia, rapidez e praticidade, a principal forma de controle da traça-das-crucíferas ainda se dá através da aplicação de inseticidas sintéticos (DE BORTOLI et al., 2013; RIBEIRO et al., 2014; ZAGO et al., 2014). Porém, a sua capacidade de adaptação a grande variação de temperatura (VICKERS et al., 2004), somado a elasticidade genética e o ciclo de vida curto (CAPINERA, 2008), fez com que esse inseto se tornasse resistente a mais de 95 princípios ativos de inseticidas registrados mundialmente (APRD, 2019). Dessa forma, no seu manejo foi necessário incluir uma rotação maior de inseticidas sintéticos (SARFRAZ & KEDDIE, 2005) e, consequentemente, houve um aumento na poluição ambiental e acumulação de resíduos nas hortaliças (LU et al., 2015; AKOTO et al., 2016).

Embora tenha sido considerada por muito tempo insignificante, em 1970, a traça-das-crucíferas começou a se tornar problemática, e está entre os 25 artrópodes mais importantes na China. Sua elevada capacidade de resistir a pesticidas foi observada em 1980, quando os neonicotinóides começaram a falhar (CAPINERA, 2008).

As aplicações indiscriminadas de inseticidas sintéticos além de causar bioacumulação (DENG et al., 2017), contaminação do solo e da água e seleção de insetos resistentes (DE BORTOLI et al., 2013; LU et al., 2015), pode levar a morte de polinizadores, como abelhas e borboletas (KÖHLER & TRIEBSKORN, 2013; BAIDOO & MOCHIAH, 2016), inimigos naturais (AKTAR et al., 2009; MKENDA et al., 2015) e prejudicar a saúde humana (MORAES et al., 2007).

Sendo assim, os inseticidas botânicos surgem como uma alternativa eficaz de controle menos tóxico ao meio ambiente e à organismos não-alvo (DUBEY et al., 2010), podendo contribuir para a redução das aplicações de inseticidas sintéticos. Os inseticidas botânicos possuem vários mecanismos de ação sobre os herbívoros (SOKOVIĆ et al., 2010) tornando-o uma alternativa de controle dentro do Manejo Integrado de Pragas (MIP).

Os inseticidas botânicos possuem mecanismos de ação especifico sobre os insetos, como à antibiose e a antixenose. A antixenose ou a não preferência se dá quando o extrato influencia no comportamento do inseto, afetando a escolha do hospedeiro, fazendo com que ocorra a redução do uso deste hospedeiro para abrigo, alimentação ou deposição dos ovos (PANDA & KHUSH, 1995; PICANÇO, 2010).

Antibiose acontece quando o inseto herbívoro se alimenta da planta com o extrato, e este afeta negativamente a sua biologia (PAINTER, 1951; PICANÇO, 2010), promovendo a redução da biomassa pupal, mortalidade, redução da fecundidade e entre outros efeitos (BALDIN et al., 2019).

Dessa forma, a ideia central do trabalho foi avaliar os efeitos dos extratos aquosos de *Simarouba versicolor* A. St-Hill em diferentes concentrações sobre a biologia de *P. xylostella*, bem como verificar sua toxicidade a organismos não-alvos, como o nematoide *C. elegans*, importante bioindicador da qualidade da água e do solo. Esse trabalho visa fornecer subsídios para preencher as lacunas em relação aos efeitos secundários dos inseticidas botânicos e também servir como base para outros estudos, afim de incentivar o uso de produtos naturais.

2. REVISÃO BIBLIOGRÁFICA

2.1. Importância econômica e aspectos biológicos de *Plutella xylostella*

A *Plutella xylostella* é um microlepidóptero pertencente a superfamília Yponomeutidae e família Plutellidae (CAPINERA, 2008). O ciclo de vida da traça-das-crucíferas, de ovo a adulto, dura em média 27 dias (CAPINERA, 2008) (Figura 1). No entanto, pode variar de 15 a 35 dias dependendo da temperatura (MONNERAT, 1995). Em temperaturas mais baixas, pode chegar a 14 gerações por ano e em temperaturas mais altas, como 27 °C, pode chegar a 30 gerações ao ano (HO, 1965).

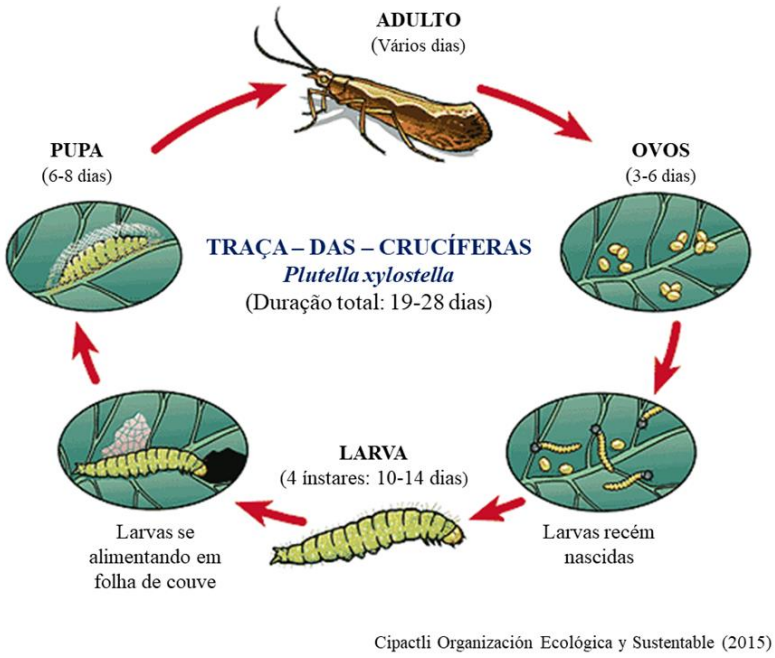

Figura 1. Ciclo de vida de *Plutella xylostella*. Adaptado de Cipactli Organización Ecológica y Sustentable, 2015.

É um inseto holometábolo, com 5 fases de desenvolvimento: Ovo, larva, pré-pupa, pupa e adulto. Os ovos são ovais e achatados, no início são amarelados, medindo cerca de 0,44 mm de comprimento e 0,26 mm de largura. A maioria dos ovos são depositados em grupo, de 2 a 8 ovos. As fêmeas procuram ovipositar em sulcos, depressões ou nas nervuras das folhas, para que as lagartas recém-eclodidas tenham alguma proteção. Quando estão próximos a eclosão, os ovos ficam com coloração escura. O período de incubação dos ovos dura cerca de 3 a 6 dias (MONNERAT, 1995; CAPINERA, 2008; CARDOSO et al., 2010; TRIPLEHORN & JOHNSON, 2015).

A fase larval possui 4 instares, sem grandes alterações na forma ao longo dos instares, mas diferindo no tamanho (TRIPLEHORN & JOHNSON, 2015). As lagartas possuem

aparelho bucal mastigador, se alimentam exclusivamente de folhas de Brássicas e chegam a atingir no quarto instar 10 mm de comprimento (TALEKAR & SHELTON, 1993).

No primeiro instar, as lagartas são incolores com a cabeça escura (MONNERAT, 1995) e possui hábito minador, ou seja, se alimentam do parênquima foliar formando minas, onde permanecem até atingirem o segundo instar. No segundo e terceiro instar, as lagartas se tornam verdes e/ou amareladas e começam a raspar a epiderme das folhas. No quarto instar começam a aparecer perfurações na epiderme foliar. Então, no final do quarto instar, as lagartas entram no estágio de pré-pupa, fase em que as lagartas reduzem a alimentação e começam a produzir o casulo. A fase larval dura de 6 a 11 dias (MEDEIROS et al., 2003; CAPINERA, 2008).

As pupas possuem um casulo de seda (MONNERAT, 1995) e geralmente se fixam na parte inferior das folhas ou no final do caule de Brássicas em busca de proteção, já que neste estágio, as pupas são imóveis e não se alimentam (TRIPLEHORN & JOHNSON, 2015). No início, as pupas possuem coloração esverdeada, ao longo dos dias a coloração vai mudando gradualmente e, quando os adultos estão próximos a emergir, a pupa fica completamente escura. A fase pupal varia de 3 a 8 dias (MEDEIROS et al., 2003; CASTELO BRANCO & FRANÇA, 2001; CAPINERA, 2008; THULER, 2009).

A mariposa é um microlepidóptero de coloração parda com cerca de 10 mm de comprimento, com hábitos noturnos, quando ocorre o acasalamento e deposição dos ovos e se alimentam de orvalho e néctar (TALEKAR & SHELTON, 1993). Os machos e as fêmeas vivem cerca de 12 e 16 dias, respectivamente. Cada fêmea oviposita, em média, 160 ovos, contudo, o número pode variar de 18 a 300 ovos. O período de oviposição dura cerca de 10 dias, no entanto, o pico de oviposição ocorre nos quatro primeiros dias (HARCOURT, 1957; CAPINERA, 2008). Os adultos apresentam características na parte dorsal e ventral dos adultos que permitem diferenciar entre macho e fêmea (VACARI, 2009). As fêmeas possuem coloração mais clara e uniforme e na parte ventral, possui no final do abdômen duas manchas escuras e uma faixa de pelos. Os machos possuem uma faixa mais clara no dorso e coloração mais escura, e na parte ventral, possui uma fenda no final do abdômen.

A escolha do local para oviposição ocorre através de estímulos químicos (olfativo ou gustativo), seja através da liberação de substâncias voláteis do hospedeiro ou de fatores físicos, tátil ou visual, como a presença de tricomas, cera ou a temperatura ambiente (PIVNICK et al., 1990; BUKOVINSZKY et al., 2005).

Dentre os problemas fitossanitários que podem atingir o cultivo das Brássicas, *P. xylostella* é um dos principais fatores limitantes da produção (CASTELO BRANCO &

FRANÇA, 2001), e por ser uma praga cosmopolita (CHEGN et al., 2008), causa sérios prejuízos no mundo todo (JANKOWSKA & WIECH, 2006; PHILIPS et al., 2014).

Durante o primeiro instar, as lagartas se alimentam do parênquima foliar e logo, permanecem protegidas dentro das minas. A partir do segundo instar elas começam a se alimentar de várias partes da planta, como a epiderme foliar, caule e inflorescências (MEDEIROS et al., 2004). Nesse estágio, as lagartas começam a fazer inúmeras perfurações em toda a planta, principalmente na folha, o que acaba comprometendo o processo de fotossíntese, e consequentemente, pode causar a sua morte (ZALUCKI et al., 2012). No terceiro e quarto instar a lagarta é mais voraz em sua alimentação, gerando mais prejuízos do que nos dois primeiros instares (MAU & KESSING, 2007).

Os danos causados pela traça-das-crucíferas reduziram em 60% a produção de repolho (BIO CONTROLE, 2013). Em brócolis e couve-flor, a presença da praga na inflorescência, parte consumida, pode fazer com que essas hortaliças não sejam comercializadas (CAPINERA, 2012). Dependendo da região em que está o plantio de couve e repolho, da temperatura ambiente e da região, os prejuízos chegam a 95% (CASTELO BRANCO, 1999; CZEPAK et al., 2005).

2.2. Inseticidas botânicos no controle de *Plutella xylostella*

Diante de vários problemas oriundos do uso incorreto de inseticidas sintéticos, se fez necessário novas alternativas de controle, que fossem seletivas e menos agressivas ao meio ambiente (KIM et al., 2003; MENEZES, 2005), tais como os inseticidas botânicos.

Os inseticidas botânicos são produtos químicos naturais provenientes de plantas (HIKAL et al., 2017), com ação sinergética dos metabólitos secundários (KIM et al., 2003). Um dos principais métodos de elaboração dos inseticidas botânicos se dá através do processo de maceração. O método consiste em adicionar o pó vegetal em um líquido extrator, como por exemplo a água ou solvente orgânico (SANTOS et al., 2013). A solução permanece em repouso e o solvente não deve ser substituído (OLIVEIRA et al., 2016).

Os metabólitos secundários são substâncias provenientes do metabolismo secundário da planta, não envolvida nos processos vitais do organismo (BERENBAUM & SEIGLER, 1992). Esses aleloquímicos possuem peso molecular baixo e podem ser separados, de acordo com sua composição química, em três grupos diferentes: terpenos, produtos secundários nitrogenados e compostos fenólicos (VIZZOTTO et al., 2010).

Essas substâncias podem ser metabolizadas de forma constitutiva ou induzida. Os metabólitos secundários constitutivos estão presentes durante todo o desenvolvimento da planta e não precisam de nenhum fator de indução. Os metabólitos secundários induzidos são sintetizados em decorrência de algum estresse abiótico ou biótico, como o ataque de herbívoros (GERSHENZON et al., 2000; MCCALL & KARBAN, 2006; BALDIN et al., 2019).

Segundo Whittaker (1970), os aleloquímicos são definidos como compostos não nutritivos com ação intraespecífica, ou seja, são substâncias sintetizadas que vão interagir entre espécies diferentes. Dentro dos aleloquímicos, existem dois grupos de grande interesse agrícola, os cairomônios e os alomônios. Os cairomônios favorecem a espécie que vai receber o estímulo químico, atraindo ou estimulando a alimentação de um herbívoro. Por outro lado, os alomônios atuam de forma contrária aos cairomônios, favorecendo quem produziu o estímulo químico, como por exemplo a planta hospedeira, afastando, inibindo a alimentação ou a oviposição e podem causar a morte do indivíduo (WHITTAKER & FEENY, 1971).

A definição de cairomônios ou alomônios muda de espécie para espécie, por exemplo, os glucosinolatos são compostos produzidos nas hortaliças da família Brassicaceae, e são considerados compostos fagodeterrentes, ou seja, reduzem a alimentação de insetos generalistas (FAHEY et al., 2001). Entretanto, para insetos especialistas, como a *P. xylostella*, estes compostos atuam como estimulantes na alimentação e oviposição (FAHEY et al., 2001; VAN LOON et al., 2002; HERVÉ et al., 2014).

Sendo assim, devido a presença dos aleloquímicos e da ação sinergética entre eles, os inseticidas botânicos podem ser separados em 6 grupos, de acordo com o seu mecanismo de ação, sendo eles: Repelentes, *Antfeedants* ou deterrentes alimentares, tóxicos, retardadores de crescimento e inibidores de desenvolvimento, inibidores de reprodução e atrativos (RAJASHEKAR et al., 2012). Além dos inúmeros mecanismos de ação, os inseticidas botânicos são biodegradáveis, tão eficazes quanto os inseticidas sintéticos, baixa toxicidade e além da disponibilidade vegetal, possui baixo custo para aplicação e manejo (NEERAJ et al., 2017).

2.3. Ecotoxicidade

Os produtos naturais de origem vegetal, principalmente de plantas medicinais, são conhecidos por apresentarem baixa toxicidade ao meio ambiente e ao homem (PENTEADO, 2007; YUET et al., 2013; IBRAHIM et al., 2016). No entanto, a falta de informação em

relação a toxicidade ao meio ambiente e seu efeito em organismos não-alvos, como polinizadores e predadores, dificultam a aplicação dos inseticidas botânicos em áreas de cultivo (ZALLER & BRUHL, 2019).

É de suma importância conhecer os efeitos secundários dos inseticidas botânicos, principalmente, para garantir a segurança dos produtores e evitar efeitos negativos no meio ambiente, como a contaminação ambiental e efeitos sobre organismos não-alvos.

Para avaliar os efeitos de substâncias químicas, são realizados testes de toxicidade, principalmente, em mamíferos. Entretanto, para isso faz-se necessário um número grande de indivíduos, que somado ao seu ciclo de vida longo, torna esses experimentos inviáveis (TEJEDA-BENITEZ & OLIVERO-VERBEL, 2016; HARLOW et al., 2016; HUNT, 2017).

Vários estudos têm utilizado o *Caenorhabditis elegans* (Maupas, 1900) (Rhabditidae) como modelo em testes com produtos naturais, como os extratos de plantas, avaliando a atividade farmacológica, toxicidade ou o mecanismo de ação sob o nematoide (COLLINS et al., 2006; CHENG et al., 2014; DUANGJAN et al., 2019).

C. elegans é um nematoide de vida livre presente no solo (BONGERS & FERRIS, 1999; HOPE, 1999), no entanto, parece ter preferência por habitats úmidos e repletos de vegetação em decomposição (FÉLIX & BRAENDLE, 2010). Em ambientes com pouca intervenção humana é comum encontrar este organismo em flores, frutos, cogumelos e cascas em decomposição (KIONTKE et al. 2011; FÉLIX et al. 2013).

Os ambientes úmidos são repletos de matéria em decomposição, e consequentemente, de micro-organismos, como as bactérias, que são a principal fonte alimentar do nematoide (DIRKSEN et al. 2016; SAMUEL et al. 2016; HUNT, 2017). A disponibilidade de alimento é um dos fatores que pode alterar o ciclo de vida do *C. elegans* (NEAL et al. 2015). Logo, a presença desses organismos em um habitat serve como um indicador de riqueza do solo (YEATES & BONGERS, 1999; YEATES, 2003).

C. elegans é responsável por fazer a decomposição de matéria orgânica, ciclagem de nutrientes, regulação de agentes de fitopatógenos e a degradação de substâncias tóxicas (BONGERS & FERRIS, 1999). Logo, é um organismo sensível a presença de agrotóxicos como herbicidas e inseticidas (PARODI et al., 2015; JACQUES et al., 2019).

Bailey et al. (2018) ao colocar indivíduos de *C. elegans* em contato com um herbicida a base de glifosato (TouchDown®, Syngenta), observaram a inibição da atividade mitocondrial e os nematoides começaram a produzir peróxido de hidrogênio. Neste contexto, estes organismos podem ser utilizados como indicadores ecológicos adequados para identificar estresse oxidativo em ambientes (WANG et al., 2018) e monitorar a qualidade da água (ISO 2010) e do solo (ASTM International 2014).

Este nematoide possui algumas características que o tornam uma excelente opção em testes de toxicidade, tais como, ciclo de vida curto com aproximadamente 21 dias, tamanho pequeno medindo cerca de 1mm de comprimento, facilidade de criação e manutenção, alta taxa de reprodução e possuem a capacidade de autofecundação, chegando a produzir cerca de 300 ovos por indivíduo (KALETTA & HENGARTNER, 2006; HARLOW et al., 2016; RUSZKIEWICZ et al., 2018).

Outra vantagem do modelo é que *C. elegans* foi o primeiro organismo multicelular que teve seu genoma sequenciado (CONSORTIUM, 1998), e foi encontrado uma semelhança de 80% com o genoma humano (KALETTA & HENGARTNER, 2006). Além disso, possui um sistema neuronal estruturalmente e funcionalmente bem semelhante ao dos mamíferos (RUSZKIEWICZ et al., 2018). Sendo assim, testes utilizando esse nematoide tem contribuído para entender diversas doenças humanas, principalmente doenças neurológicas, como a doença de Parkinson (HARRINGTON et al. 2010), Alzheimer (LU et al., 2014), diabetes tipo 2 e depressão (KALETTA & HENGARTNER, 2006).

2.4. *Simarouba versicolor*

A família Simaroubaceae é constituída por 32 gêneros (SIMÃO et al., 1991; SARAIVA et al., 2006), e, no Brasil, encontra-se 6 gêneros, sendo estes: *Quassia* e *Picrolemma*, na Amazônia, *Castela* e *Picrasma*, na região Sul, por fim *Simaba* e *Simarouba*, que se encontram distribuídas por todo o país (ARRIAGA et al., 2002; ALMEIDA et al., 2007).

É uma família de planta extremamente rica em sua composição fitoquímica, com propriedades farmacêuticas e inseticidas (MUHAMMAD et al., 2004). Dentre os compostos presentes nas espécies desta família, encontram-se alcaloides, triterpenos, compostos fenólicos, antraquinonas e os quassinoides. Os quassinoides são considerados marcadores taxonômicos das Simaroubaceae, devido sua síntese em grande quantidade e quase exclusiva (SARAIVA et al., 2006; ALMEIDA et al., 2007; ALVES et al., 2014).

Simarouba versicolor A. St-Hill, conhecida popularmente como mata-cachorro ou pau-paraíba, está presente várias regiões do estado do Mato Grosso do Sul e chega a medir cerca de 3 a 4 metros de altura (CARVALHO et al, 2013). Estudos anteriores verificaram que o extrato de *S. versicolor* afetou negativamente *Atta sexdens rubropilosa* (Forel, 1908) (Hymenoptera: Formicidae) e *Rhodnius milesi* (Carcavallo, Rocha, Galvão & Jurberg, 2001) (Hemiptera: Reduviidae) (PEÑAFLOR et al., 2009; CARVALHO et al., 2013), no entanto, não existem trabalhos com o extrato de *S. versicolor* em *P. xylostella.*

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4. OBJETIVO GERAL

Avaliar os efeitos de extratos aquosos de *Simarouba versicolor* sobre a biologia de *P. xylostella*, bem como determinar a concentração de menor toxicidade ao nematoide de vida livre *Caenorhabditis elegans*.

5. HIPÓTESES

H0 - Os extratos vegetais de *S. versicolor* não interferem na biologia de traça-das-crucíferas e não apresentam toxicidade ao *C. elegans*.

H1 - Os extratos vegetais interferem na biologia de traça-das-crucíferas e apresentam toxicidade ao *C. elegans*.

CAPÍTULO I

Bioactivity of the aqueous extract of *Simarouba versicolor* A. St-Hill: plant with high insecticidal potential on *Plutella xylostella* L. (Lepidoptera: Plutellidae) and ecotoxicological action on the *Caenorhabditis elegans* model

(Formatado seguindo as normas da revista Insects)

Bioactivity of the aqueous extract of *Simarouba versicolor* **A. St-Hill: plant with high insecticidal potential on** *Plutella xylostella* **L. (Lepidoptera: Plutellidae) and ecotoxicological action on the** *Caenorhabditis elegans* **model**

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Abstract

Alternative methods of insect management are an important field of study for agriculture. We hypothesized *Simarouba versicolor* presents a potential insecticide under *Plutella xylostella* with low toxicity to the environment, assessed in *Caenorhabditis elegans*. This work aimed to determine the action of aqueous extracts from S. versicolor (AE-SV) in the biology of *P. xylostella* and to determine the concentration of less toxicity of this plant in the nematode *C. elegans*. AE-SV was chemically investigated by Ultrahigh-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). We evaluated the effect of the botanical extract on the life cycle of *P. xylostella*, from the larval stage to adult stage, at concentrations 10%, 5%, 1%, 0.1%, 0.05%, 0.01% and a control. Subsequently, we analyzed the toxicity of the extract in an in vivo model. EA-SV showed high levels of phenolic and flavonoid compounds. Six compounds were identified based on UHPLC-MS/MS analysis, including flavanone, kaempferol, 4,5-dimethoxycanthin-6-one, 11-acetylamarolide, ailantinone, and glaucarubinone. The median lethal time for P. xylostella was estimated to be 96 h in treatments with EA-SV. Fifty percent of the population died after 96 hours of exposure to all the botanical extract concentrations, and at 120 hours 100% of the individuals were dead. Larvae exposed to EA-SV at concentrations of 0.01%, 0.05% and 0.1% showed a reduction in leaf area consumption, underdevelopment, reduction in movement, and pupa biomass. The lowest concentrations of EA-SV (0.1%, 0.05% and 0.01%) did not cause mortality in nematodes, showing viability of 80% or more, not differing from the control and, therefore, these concentrations did not show toxic action and can be considered selective. Thus, the aqueous extract of *S. versicolor* could be an effective control alternative because it acts mainly in the larval stage, the stage in which the insect causes damage to products.

Keywords: Ecotoxicity, alternative control, botanical insecticides, glaucarubinone.

1. Introduction

For years, chemical products have been sought as attractive alternatives for pest control [1,2]. However, besides their benefits, many consequences should be considered when applying chemical products: environmental problems, human health concerns, pest resistance, mortality of beneficial insects [3-5], toxic residue accumulation, water and soil contamination, toxicity to landholders [6,7], augmentation of secondary pests, pest population explosions, and selection loss of insecticide efficacy [8,9].

Plutella xylostella L. (Lepidoptera: Plutellidae) is one of the most destructive pests worldwide, and its high migratory capacity, biotic potential, and short life cycle, as well as a lack of proper management practices [10], have made the insect resistant to more than 101 active ingredients of pesticides registered worldwide [11]. This requires frequent rotation of synthetic insecticides, generating an estimated cost to control this pest of USD 4–5 billion a year [12]. Consequently, there is greater environmental pollution and accumulation of residues in vegetables [13,14]. Therefore, the elevated cost of agricultural production and the risks to the environment demand novel alternatives to control these insects that are more selective and less harmful to the environment and humans [15,16]. In addition, higher awareness by producers and consumers and policy implementation have resulted in demands for reduced pesticide use during food production and the use of practices that support agroecological intensification [17,18].

Therefore, eco-friendly attitudes and the production of pesticide-free organic products have promoted the search for alternative products that do not harm the environment [19]. Among these alternatives, there are natural botanical insecticide products, derived from the secondary metabolism of plants, that make food repellant, inhibit oviposition and growth, or have larvicidal effects in insect pests of agricultural importance [20-27]. The mode of action of these natural insecticides is based on several compounds; they hinder the evolution of resistance in herbivorous insects [28,29] and, with some exceptions, are less toxic to the soil, water, and non-target organisms, such as natural predators, pollinators, and vertebrates [30]. They also cost less and are easy to obtain, apply, and manage [31]. Thus, botanical insecticides can be important alternative to control insects in laboratory experiments [21-27], agricultural production areas, as in the use of *Azadirachta* [32], or in the plastic tunnel condition [33]. Another advantage is that botanical insecticides do not remain in nature after application, which drastically reduces the chances of environmental contamination [34] and promotes greater environmental conservation [35].

The Simaroubaceae family is composed of 32 genera, constituting approximately 250 species of shrubs and trees, and some *Simarouba* species have been reported to possess insecticidal and repellent activity. For example, *S. amara* Aubl., used as a repellent against larvae of the mosquito *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) [36], and *S. versicolor* showed clear activity against the leaf-cutting ant *Atta sexdens rubropilosa*, Forel, 1908 (Hymenoptera: Formicidae), and antifungal activity against the symbiotic fungi *Leucoagaricus gongylophorus* (Möller) Singer (Agaricales, Agaricaceae) and *Rhodnius milesi* (Hemiptera: Reduviidae) [37-39]. Phytochemical studies on *S. versicolor* A. St-Hill. have mainly concentrated on

stem, branches, fruits, and bark, including quassinoids, triterpenoids, alkaloids, steroids, and coumarins as major compounds [39,40]. Quassinoid compounds are almost exclusive to Simaroubaceae, and are considered taxonomic markers of the group [41]. Although our knowledge of the chemical composition of extract generated from the leaves of this plant is still limited, Simote [38] reported the presence of flavonoids.

Plutella xylostella L. was selected for the present investigation based on its economic importance. We hypothesized that *S. versicolor* would present insecticide activity against *P. xylostella* with low toxicity to the environment, and could be used as an alternative pest control agent.

To test the selective toxicity of plant extracts, many studies were performed using *Caenorhabditis elegans* (Maupas, 1900) as a model of non-target organism [42-45]. Organophosphorus insecticides with eight active ingredients that were tested on *C. elegans* inhibited the enzyme cholinesterase through the accumulation of acetylcholine, a neurotransmitter [45-47]. This finding demonstrates that *C. elegans* is a good model for neurotoxicity testing [48]. *Caenorhabditis elegans* is a free-living nematode present in soil, plant litter [49,50], and rarely in aquatic environments [51]. It is responsible for maintaining soil quality and recycling nutrients [52], is extremely sensitive to the presence of pesticides such as herbicides and insecticides [45-47,53,54], and is considered an important bioindicator of soil pollution [55] and water pollution [56]. Using mammals for toxicity testing is not feasible, as it takes years to evaluate the results and the cost is high due to the long-life cycle [57,58]. The advantage of using *C. elegans* is that because it has a short life cycle, it is possible to observe the results and effects in the short term [59].

In our survey, we found that extract from *S. versicolor* had insecticide activity against *P. xylostella*. In this work, we aimed to determine the action of *S. versicolor* aqueous leaf extract on the biology of *P. xylostella* and the concentration with the least toxicity in *C. elegans*, and to determine its chemical composition by LC-MS/MS, to scientifically support its biological action. The results of this study will increase our understanding of the in vivo toxicity of *S. versicolor* and the possibility of using a botanical extract of this species to minimize damage to the environment. We evaluated the extract from leaves, as they are renewable materials and removing them does not compromise the plant´s development.

2. Materials and Methods

2.1. Botanical material

Fully expanded leaves of *S. versicolor* were collected in the morning during January 2020 at Pousada das Abelhas, in the municipality of Campo Grande, MS (21°13’28” S, 54°11’28” W, 437 m altitude), placed in plastic bags moistened with filter paper, and taken to the laboratory for sorting and identification by an expert, then deposited in the herbarium of the Faculty of Biological and Environmental Sciences of the UFGD under number 6481. The collection of botanical material was authorized by the National Management System. Genetic heritage and associated traditional knowledge (SISGEN) were filled under number AF5E2AA.

2.2. Preparation of aqueous extract

The *Simarouba versicolor* leaves were cleaned in running water and dried in a forced air circulation greenhouse for 72 h at a maximum temperature of 40 °C (±1 °C), then the completely dried leaves were crushed in an industrial mill until they turned into a fine powder. The powder was protected from light and moisture during storage and stored at room temperature (25 ± 2°C). To obtain the aqueous extract (AE-SV) by maceration, 3 g of vegetable matter was added to 30 mL of distilled water. After homogenization, the mixture was kept in a refrigerated environment (10 °C) for 24 hours for the extraction of water-soluble compounds, which was later filtered with filter paper to obtain the AE-SV stock solution at a concentration of 10%. Different concentrations (5, 1, 0.1, 0.05, and 0.01%) were evaluated in assays against *P. xylostella*.

To chemical analysis the aqueous extract (AE-SV) was conducted by process of lyophilization, typically used to water removal, also known as freeze-drying, commonly used samples preservation, resulted in lyophilized AE-SV.

2.3. Chemical composition

The total phenolic content in lyophilized AE-SV (1 mg/mL, dissolved in water) was determined by using Folin–Ciocalteu reagent [60]. 100 μL of AE-SV was mixed with 0.5 mL of Folin-Ciocaleu' s (1:10 v/v), and after 3 min, 1.5 mL of aqueous sodium bicarbonate (2%) was added. The absorbance was measured at 765 nm using a spectrophotometer, after 30 min. We prepared a calibration curve (2.5, 5.0, 10.0, 20.0, 25.0, 50.0, 100.0 and 125.0 μg) using gallic acid prepared in 80% water as the standard. We then used these data to generate a linear regression model, and the line equation was obtained and used for the calculation of the experimental samples. The equation of the gallic acid curve was Y = -0.052 + 7.5x, with a correlation coefficient of R = 0.99727, and the results are expressed in milligrams of gallic acid equivalent (GAE) per gram of extract.

To level of flavonoids, 500 μL of lyophilized AE-SV was mixed with 1.50 mL of ethanol (95%), 0.10 mL of aluminium chloride (10%), 0.10 mL of sodium acetate (1 M) and 2.80 mL of distilled water. After incubation for 40 min, absorbance was measured at 415 nm. The quantification was carried out using a standard curve of quercetin to obtain a line equation (Y = 0.3546 + 12.8030X; R = 0.99972). The results were expressed as quercetin equivalent (QE) in mg per gram of extract [60]. Total flavanol in AE-SV was estimated using a method reported previously [60], absorbance was read at 440 nm, and expressed as quercetin equivalent (QE) in mg per gram of extract using the calibration curve with quercetin.

The condensed tannin content was measured by vanillin−HCl reagent [51]. The lyophilized AE-SV was mixed with 5 mL vanillin-HCl (8% conc. aq. HCl and 4% vanillin). Absorbance was read at 500 nm, after 20 min. Quantification was performed using an calibration curve, using catechin as the standard (Y = 0.00896 + 0.84392X; R = 0.98978). The condensed tannin concentration is expressed as catechin equivalent (CAE) in mg per gram of extract.

All the assays were carried out in triplicate.

2.4. HPLC-MS/MS analysis

The lyophilized AE-SV was solubilized in methanol–acetonitrile (1:1, v: v) at a concentration of 0.5 mg/mL and centrifuged for 5 min, and the supernatant was analyzed, with a UHPLC system (Shimadzu Nexera X2) equipped with a CBM-20A system controller, two LC-30AD pumps, a CTO-30A column oven, and an SIL-30AC autosampler coupled to an HRMS system (QTOF Impact II, Bruker Daltonics Corporation, USA) equipped with an electrospray ionization source, quadrupole time-of-flight (QTOF) analyzer, and multichannel plate (MCP) detector (Impact II, Bruker Daltonics Corporation, USA). The capillary voltage was operated in positive ionization mode and was set to 4500 V with an endplate offset potential of −500 V. The dry gas parameters were set to 8 L/min at 200 °C with a nebulization gas pressure of 4 bar. Data were collected from m/z 50–1300 with an acquisition rate of 5 spectra per second, and the ions of interest were selected by automatic MS/MS scan fragmentation. Chromatographic separation was performed using a C18 column (75 × 2.0 mm i.d.; 1.6 μm Shim-pack XR-ODS III). The gradient mixture of solvents A (H_2O) and B (acetonitrile with 0.1% formic acid; v: v) was as follows: 5% B 0–1 min, 30% B 1–3 min, 95% B 3–12 min, maintained at 95% B 12–16 min, and 5% B 16–17 min. The flow rate was 0.2 mL/ min, the column temperature was 40 °C, and the injection volume was 3 μL. The dried AE-SV was solubilized in methanol–acetonitrile (1:1, v:v) at a concentration of 0.5 mg/mL and centrifuged at 1.200 x g for 5 min, and the supernatant was analyzed. The data were processed by Bruker Compass DataAnalysis 4.3 software. The compounds were proposed based on a bibliographic review of the genus and species, as well as the error value of the mass [62].

2.5. Rearing of P. xylostella

Larvae and pupae of *P. xylostella* were collected from cabbage plantation areas in the city of Dourados (22°13′16″ S and 54°48′20″ W) in the state of Mato Grosso do Sul and reared at the Insect–Plant Interaction Laboratory of the Faculty of Biological and Environmental Sciences at the Federal University of Grande Dourados (UFGD), Mato Grosso do Sul, Brazil. Individuals were maintained under constant temperature (25 ± 2°C) and relative humidity (70 ± 5%) with a photophase of 12 h.

The pupae were deposited in plastic containers (9 × 19 × 19 cm) for the emergence of adults. The adults were fed honey diluted in 10% distilled water using cotton soaked in the solution. Cabbage and filter paper discs moistened with distilled water, both 9 cm in diameter, were added to the same container for egg deposition. This set was changed daily and replaced with new discs.

After eggs were laid, the discs were transferred to transparent plastic containers (30 × 15 × 12 cm), in which the larvae remained from hatched eggs until they reached the pupa stage. They were fed with organic cabbage leaves (*Brassica oleracea* var. *acephala*) previously sanitized with 5% sodium hypochlorite solution.

Cabbage leaves were arranged with the adaxial side of the first leaf facing the plastic container, and the free abaxial side was used to place the larvae. Then, the second leaf was positioned with the abaxial side facing down. Every day, the leaf with the adaxial side facing the plastic container was discarded and replaced by the

second leaf with the abaxial face facing down [63]. Newly formed pupae were removed from the plastic containers and transferred back to the adult cage.

2.6. Bioactivity of aqueous extract on P. xylostella

Organic cabbage leaves (*B. oleracea* var. *acephala*) were cut into discs measuring 4 cm², which were immersed for 15 seconds in AE-SV at concentrations of 10, 5, 1, 0.1, 0.05, and 0.01%; the control consisted of distilled water. Subsequently, the discs were kept at 25 °C for 40 minutes to remove excess moisture and then immediately transferred to Petri dishes. In each Petri dish (12 × 2 cm), a cabbage disc was inserted under a wet filter paper disc and a neonate larva of *P. xylostella* (0-24 h). Cabbage discs were replaced every 24 hours by new discs immersed in the respective treatment, and filter paper discs were changed every 48 h.

Plutella xylostella larvae were monitored daily, and the number of dead individuals, characterized by immobility, was counted. The surviving larvae remained in Petri dishes until they reached the pupal stage. They were weighed 24 h after pupation (pupal biomass) and subsequently isolated in individual test tubes until they emerged as adults. Pupal duration was assessed according to the time (days) that individuals remained in the pupal stage. Pupal survival was calculated according to the percentage of pupae that eclosed.

After the adults emerged in the test tubes and were sexed, two *P. xylostella* (one male and one female) were transferred into a transparent cage with untreated cabbage and moistened filter paper discs as an oviposition substrate. Diluted honey solution was used as feed.

Discs with eggs were removed daily and replaced, and the number of deposited eggs (fecundity) was counted. After counting, the discs were transferred to Petri dishes to count the number of hatched larvae (egg survival). The moths remained in the cage until both died, and during this period the number of days that males and females remained alive (longevity) and that females oviposited (oviposition period) were counted (Figure 1).

Pupal and adult stages data assessments were performed in individuals exposed to AE-SV and survived in the larval stage.

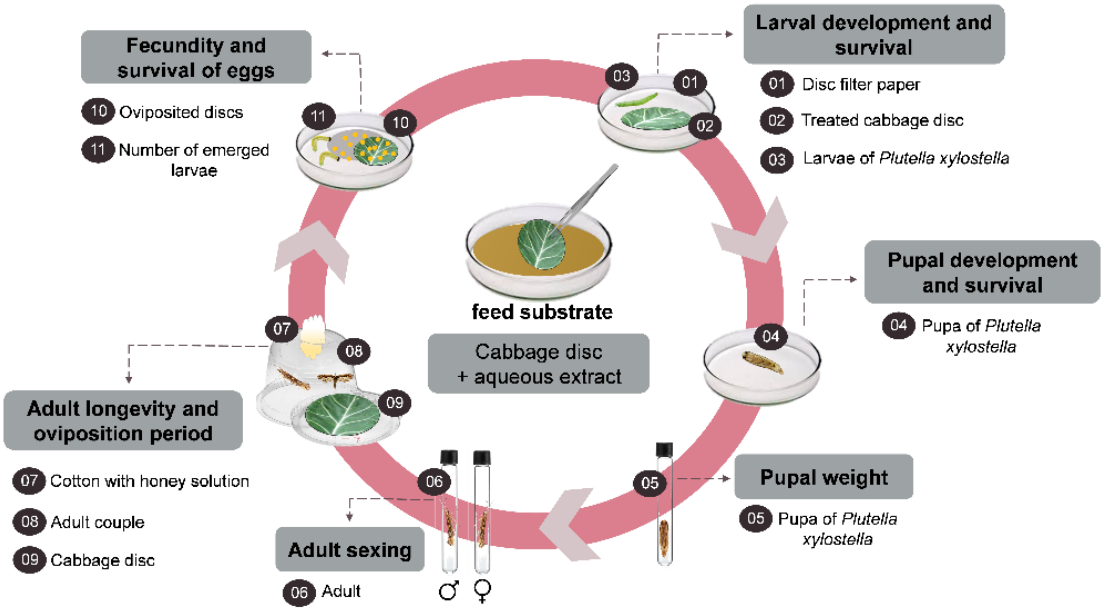

Figure 1. Schematic representation of methodology used to assess potential insecticide.

The experiment for the bioactivity assay of AE-SV on *P. xylostella* was a completely randomized design, with seven treatments (six concentrations and a control) each consisting of 50 larvae. Each individual was considered a replicate. In the pupal and adult stages, the number of replicates was dictated by the number of individuals that survived the larval stage.

All the variables were analyzed with a generalized linear model. The best model for the data of the variables weight and duration of larval and pupal stages was a gamma distribution with the inverse link function. Mortality and rate of development showed binomial and Gaussian distribution, respectively. The goodness of fit of the models was evaluated using half-normal plots with a simulated envelope using R.

As mean development did not show normal distribution, we estimated the rate of development using the following equation: $r(T) = 1.0/e^{\{| \Sigma \ln (di) | n\}}$, [64], where r(T) is development rate, di is individual observations of development time (days), and n is number of observations.

2.7. Rearing of C. elegans

The selective toxicity of different concentrations of AE-SV was evaluated in vivo in the nematode *C. elegans* from wild strain N2. The nematodes were incubated in Petri dishes with nematode growth medium (NGM) agar, at a temperature of 20 °C. The individuals were fed with *Escherichia coli* OP50-1 bacteria added to the Petri dish, and to synchronize nematodes for the bioassay, sodium hypochlorite (2%) and sodium hydroxide (5 M) were used in pregnant hermaphrodites. The eggs were incubated at 20 °C for 48 h to obtain L4 phase nematodes.

2.8. Toxicity assessment

To perform the toxicity experiment, the methodology of Dengg and Meel [65] was used. In a 96-well plate, 10 to 20 nematodes in phase L4 that were incubated with AE-SV at concentrations of 10, 5, 1, 0.1, 0.05, and 0.01% solubilized in M9 minimal medium were added and maintained at a constant temperature of 20 °C (Figure 2). Nematode survival assessment was performed after 24 and 48 hours of incubation, and nematodes were considered dead when they did not show any movement when touched repeatedly with the micro spatula. A Motic SMZ-140 and W10X/23 stereo microscope was used for the survival evaluation.

Two independent experiments were performed in triplicate (10 to 20 nematodes per well). The data were submitted to analysis of variance (ANOVA) and the mean values were compared by the Dunnett test ($P < 0.05$) using the GraphPad Prism 5 program. The data are represented as average ± standard error of the mean (EEM).

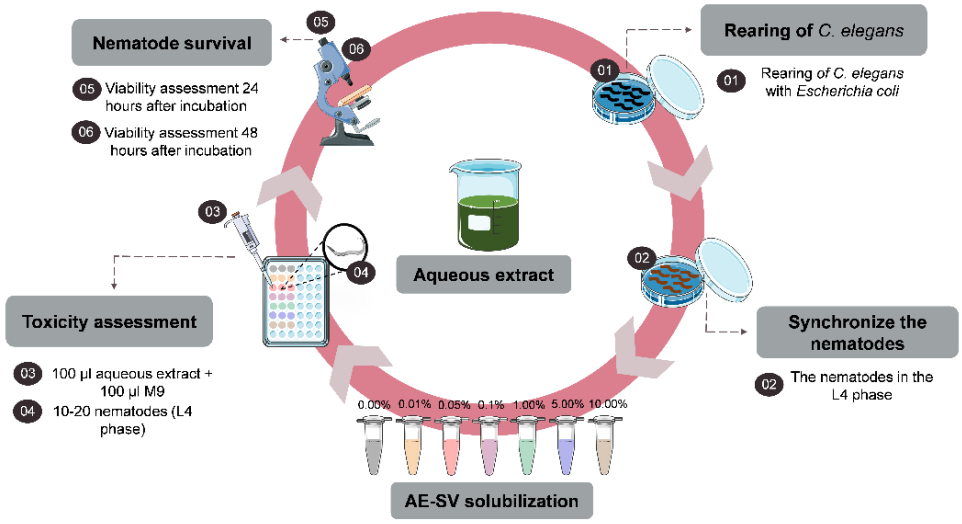

Figure 2. Schematic representation of methodology used to assess toxicity.

3. Results

3.1. Chemical prolife

The chemical analysis of AE-SV showed high phenol concentration (935.08 ± 0.58 254 mg GAE/g extract), followed by flavonoids (623.42 ± 2.44 mg QE/g extract), flavanol (444.56 ± 6.22 mg QE/g extract), and condensed tannins (36.18 ± 10.25 mg CAE/g extract).

HPLC-MS/MS chromatogram reported flavanone (1) (Rt = 6.56 min), kaempferol (2) (Rt = 7.22 min), 4,5-dimethoxycanthin-6-one (3) (Rt = 8.73 min), 11-acetylamarolide (4) (Rt = 9.57 min), ailantinone (5) (Rt = 10.09 min), and glaucarubinone (6) (Rt = 10.58 min) (Figure 3, Table 1).

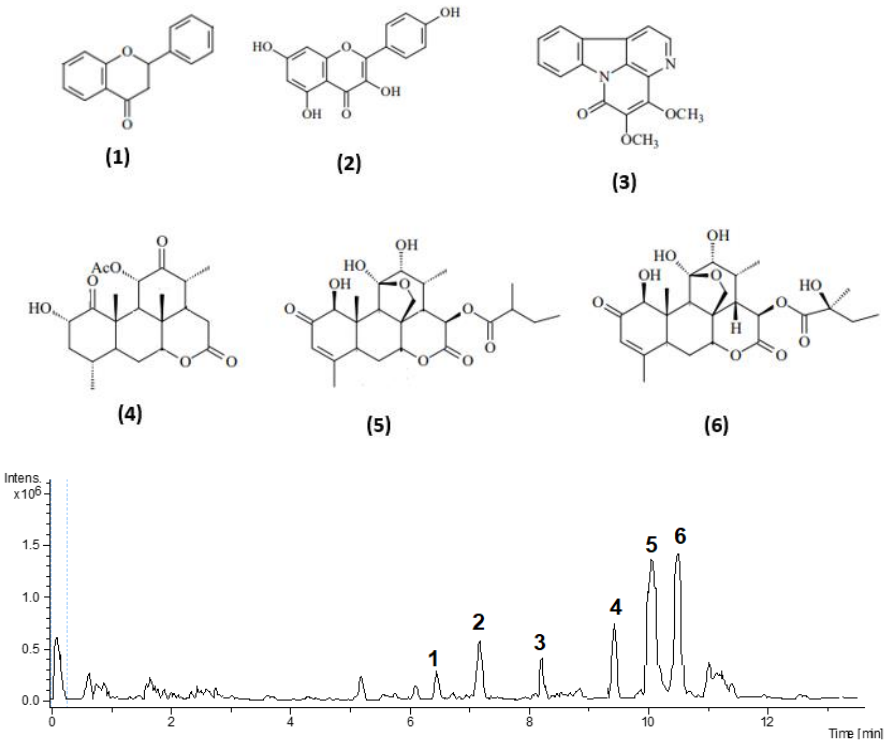

Figure 3. HPLC-MS chromatogram of aqueous extract of *S. versicolor* (AE-SV) leaves obtained in positive mode: flavanone (1), kaempferol (2), 4,5-dimethoxycanthin-6-one (3), 4. 11-acetylamarolide (4), ailantinone (5), glaucarubinone (6).

Table 1: The main compounds observed by HPLC-MS/MS in positive mode of the aqueous extract (AE-SV) leaves.

Peak	Compound	RT (min)	[M+H] *m/z*	Molecular Formula	MS/MS [M+H] Fragments
(1)	Flavanone	6.56	224.0837	$C_{15}H_{12}O_2$	
(2)	Kaempferol	7.22	286.2432	$C_{15}H_{10}O_6$	286, 165, 153, 137, 99
(3)	4,5-Dimethoxycanthin-6-one	8.73	280.0848	$C_{16}H_{12}N_2O_3$	265, 251, 221, 237
(4)	11-Acetylamarolide	9.57	406.1991	$C_{22}H_{30}O_7$	378, 318, 274, 214
(5)	Ailantinone	10.09	495.5009	$C_{25}H_{34}O_9$	
(6)	Glaucarubinone	10.58	494.2151	$C_{25}H_{34}O_{10}$	493, 375, 345, 301, 117

3.2. Bioactivity of EA-SV

All concentrations of *S. versicolor* significantly interfered with the survival rate of *P. xylostella*. The median lethal time for *P. xylostella* was estimated at 96 h under all treatment tested with AE-SV (Figure 4).

The lowest concentrations of the aqueous extract (0.01, 0.05, and 0.1%) caused longer periods in the larval stage than the control, until they died or reached the

pupal stage (Figure 4A-C), with a mortality rate of approximately 80% after 120 h of exposure. After 144 h, the rate of action of the aqueous extract on larvae decreased, causing longer development in the larval stage for some individuals.

The highest percentage of mortality occurred when larvae were fed on cabbage discs treated with the highest concentrations of AE-SV (1, 5, and 10%) (Figure 4D-F). On the first day of the bioassay, larvae took a test bite and continued feeding. In the following days, food consumption was reduced until larval death. In total, >50% of the population died after 96 h of exposure to the aqueous extract, and within 120 h, 100% of individuals were dead. Owing to the 100% larval mortality rate, we could not evaluate the parameters related to pupal development and adult reproduction in larvae treated with 1, 5, and 10% AE-SV. Contrary to the AE-SV groups, for the control group all larvae remained alive after 216 h.

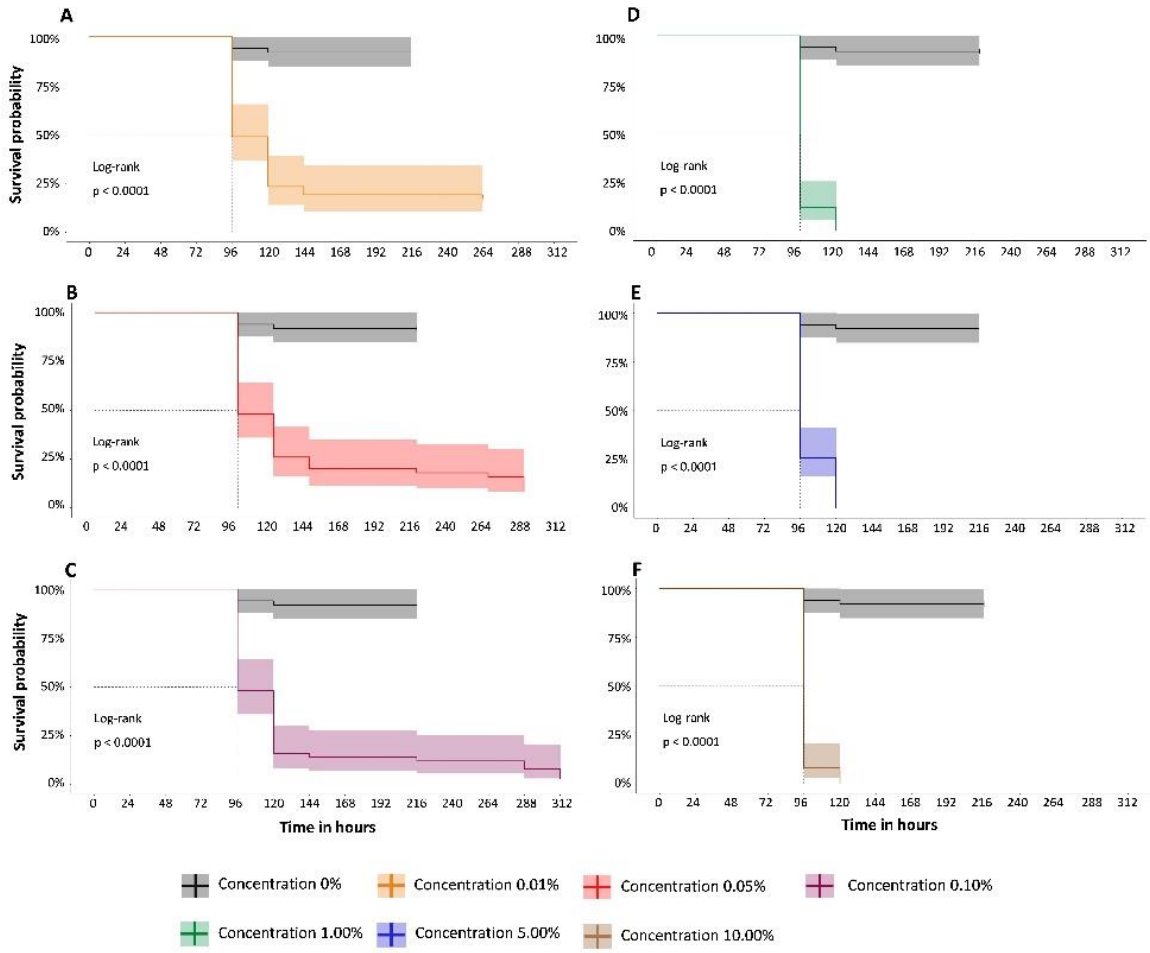

Figure 4. Survival rate of *Plutella xylostella* larvae after exposure to aqueous extract of *Simarouba versicolor* at different concentrations: (A) 0.01%; (B) 0.05%; (C) 0.10%; (D) 1.00%; (E) 5.00%; (F) 10.00%.

For larvae exposed to AE-SV at concentrations of 0.01, 0.05, and 0.1%, there was a significant reduction in the area of leaf consumed, as well as reduced development, change in larval mobility, a shorter period of metamorphosis to the pupal stage, and reduced weight gain. Moreover, the healthy control larvae had a greenish coloration (Figure 5A), whereas those treated with 0.1 and 0.05% AE-SV

had dark spots at the ends of their bodies (Figure 5B, C), and some that were treated with 10% AE-SV presented with a petrified appearance, dark coloration, and an extremely strong odor (Figures 5D). Notably, larvae treated with AE-SV in concentrations of 0.01, 1, and 5% showed the same characteristics as those treated with the other concentrations, except larvae treated with 10% showed a petrified aspect.

Figure 5. Larvae and adult *Plutella xylostella*. (A) Healthy *Plutella xylostella* larvae. (B–D) Larvae treated with 0.05%, 0.1%, and 10% AE-SV, respectively. (E, F) Deformity found in adult *Plutella xylostella* treated with 0.05% AE-SV as larvae.

Regression of the larval development rate increased with a cubic response pattern for all concentrations of *S. versicolor* in comparison with the control (Figure 6). Therefore, there was a reduction in the duration of larval development at intermediate concentrations, and a tendency for it to increase at the highest concentration, 10% (Table 2).

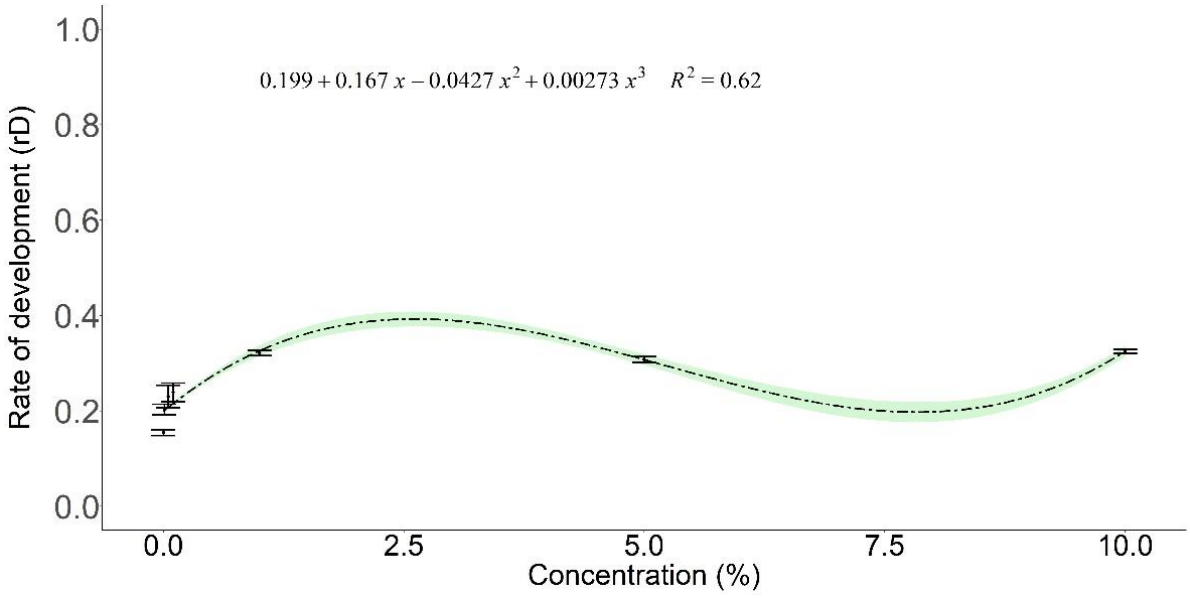

Figure 6. Rate of development (rD) of *Plutella xylostella* larvae exposed to different concentrations of *Simarouba versicolor* aqueous extract. *F-value*: 35.91; *p-value*: < 0.00001.

We observed an average reduction of approximately 33% in pupal biomass developed from larvae fed with cabbage discs treated with 0.01 and 0.05% AE-SV (Table 2). We could not evaluate the adult phase parameters for larvae fed with 0.05% AE-SV due to the emergence of deformed adults (Figure 5E, F).

Although the means were not statistically analyzed due to the low number of repetitions, it is possible to notice that the AE-SV at a concentration of 0.01% negatively affected the adult stage of the diamondback moth (Table 3). Females from larvae fed with 0.01% AE-SV–treated cabbage lived only 6 days, representing a reduction of 67.74% when we observed the control (Table 3). Females oviposited for 3 days, 10 days less than the control, for a reduction of 78.41% in oviposition period. Moreover, comparing the control to the AE-SV at 0.01%, the fecundity (number of eggs) and egg survival were compromised, with reductions of 77.62 and 33.11%, respectively (Table 3).

In table 2, we analyzed the data obtained from surviving individuals from larval stage and reached the pupal stage. The values obtained in the adult phase (Table 3) were not analyzed due to the high mortality in the larval phase at 0.05% concentration, which caused a low number of replicates.

Table 2. Larval and pupal development (days) and biomass (g) of *Plutella xylostella* pupae exposed to aqueous extract of *Simarouba versicolor* at different concentrations (25 ± 2 °C; 70 ± 5 RH; 12h photophase).

Concentration (%)	Larval development (days)	Pupal development (days)	Pupal biomass (g)
0.00	6.54 ± 0.24 a *n*=50	5.95 ± 0.13 a *n*=41	0.0053 ± 0.0001 a *n*=41
0.01	5.06 ± 0.27 b *n*=50	5.50 ± 0.68 a *n*=8	0.0038 ± 0.0001 b *n*=8
0.05	4.92 ± 0.66 b *n*=50	4.66 ± 0.66 * *n*=3	0.0033 ± 0.0004 * *n*=3
0.10	4.48 ± 0.41 b *n*=50	-	-
1.00	3.12 ± 0.06 b *n*=50	-	-
5.00	3.26 ± 0.06 b *n*=50	-	-
10.00	3.08 ± 0.04 b *n*=50	-	-
F and P value	*F* = 19.87; *P* (> *F*) < 0.0001	*F* = 19.87; *P* (> *F*) = 0.0001	*F* = 1. 17; *P* (> *F*) = 0.3324

Means followed by different letters within a column differed from each other at the 5% significance level. n = number of replicates. *Data not considered in the analysis because the low number of replicates.

Table 3. Longevity of females and males (days), fecundity, egg survival and oviposition period of *Plutella xylostella* pupae exposed to aqueous extract of *Simarouba versicolor* at different concentrations (25 ± 2 °C; 70 ± 5 RH; 12h photophase).

Concentration (%)	Longevity of females (days)	Longevity of males (days)	Fecundity (number of eggs)	Egg survival (%)	Oviposition period (days)
0.00	18.60 ± 01.47 *n*=10	19.10 ± 1.81 *n*=10	299.40 ± 13.83 *n*=10	0.72 ± 0.03 *n*=10	13.9 ± 0.82 *n*=10
0.01	06.00 ± 01.52 *n*=3	11.60 ± 3.28 *n*=3	67.00 ± 30.98 *n*=3	0.46 ± 0.02 *n*=3	3.00 ± 1.00 *n*=3
0.05	-	-	-	-	-
0.10	-	-	-		

Means were not compared because the low number of replicates at the concentration 0.01%. n = number of replicates.

3.3. Toxicity of AE-SV in C. elegans

When analyzing the toxicity of AE-SV in *C. elegans*, we observed that lower concentrations (0.1, 0.05, and 0.01%) did not induce mortality, with a viability rate of 80% or more, similar to the control (Figure 7A, B). Thus, these concentrations did not exhibit toxic action and can be considered selective.

However, 1, 5, and 10% concentrations showed toxicity to the non-target biological model; for example, 1 and 5% AE-SV killed approximately 55% within 24 h of incubation and >80% of the population after 48 h. In addition, 10% AE-SV killed 100% of individuals within 48 h of incubation (Figure 7A, B).

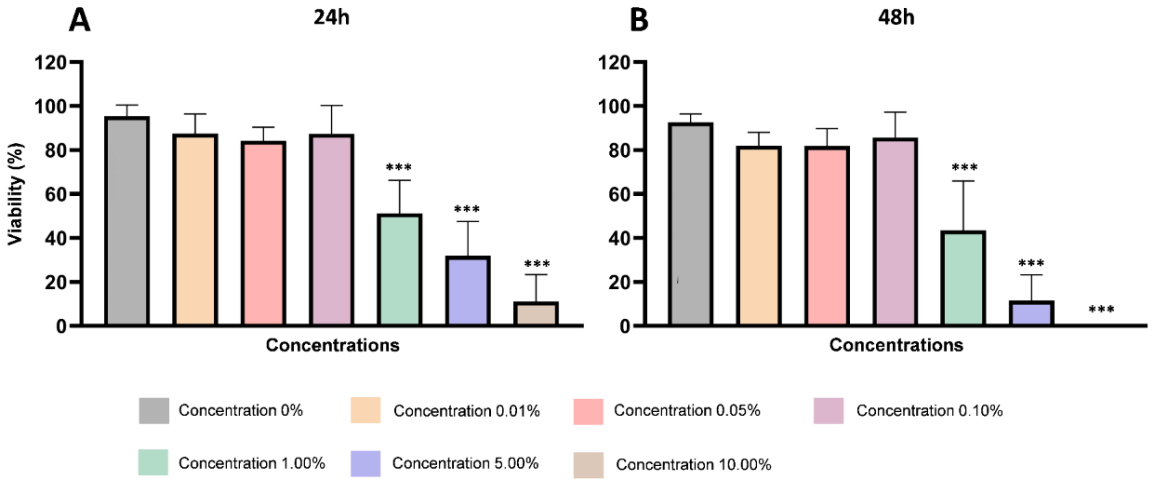

Figure 7. Viability (%) of nematodes (*C. elegans*) treated with different concentrations of *S. versicolor* aqueous extract (AE-SV) during (A) 24 h and (B) 48 h of incubation. *** Statistically significant results ($p < 0.001$) when treated group was compared with control group (concentration 0%).

4. Discussion

The present study is the first to describe the action of AE-SV in the biology of *P. xylostella* and its toxicity against *C. elegans*. We found that only 96 h of incubation with the highest concentrations of *S. versicolor* extract was sufficient to promote 50% mortality of *P. xylostella*. Death was due to hindered feeding and movement, and within 120 h, 100% of the individuals were deceased. Lower concentrations accelerated pupation on those individuals that survived larval stage, reduced weight gain and provoked early metamorphosis into the pupal stage, as well as the promotion of adults with reduced size; this can make copulation difficult, resulting in fewer fertile females [66]. Moreover, due to the need to degrade allelochemicals within the extract, the insect reallocates resources that would otherwise be used to gain weight in the larval stage, and thus there is greater energy expenditure toward degrading toxic compounds and lower conversion rates of ingested nutrients [67], resulting in lower pupal biomass.

Extracts in higher concentrations tended to modify feeding behavior, either by inhibiting feeding or negatively affecting the biology after ingestion. This may be because higher concentrations mean a greater number of secondary compounds will be extracted, consequently increasing their effects on the insect [68]. This result is of great relevance in the field, as the diamondback moth causes a great deal of damage during the larval stage [69]. Therefore, having a pest control method that causes development acceleration and mortality in a significant proportion of the population (>97%) in the larval stage could lead to a reduction in crop losses and a parallel reduction in the cost of pest control [70].

Lower concentrations promoted reductions in pupal biomass, female fecundity, egg survival, and oviposition period. Lower pupal biomass may reflect the difficulty of converting food into biomass [71,72], and thus the quality and quantity of feeding. Additionally, sublethal effects during the developmental stage may cause complications in subsequent life cycle stages [63,73,74], as observed in the results obtained in the pupal and adult phases. Therefore, pupal biomass can be considered an essential factor in adult reproduction that directly affects the quantity of eggs produced by females [68]. Recent studies have shown that reduced pupal biomass is correlated with reduced fecundity [21,26,27,75]. Thus, a reduction in pupal biomass may affect the size of adults, resulting in smaller moths, and this size difference may interfere with mating, consequently reducing the total number of eggs produced per female [27,66].

To determine the environmental impact, the toxicity of the extract was evaluated in a non-target organism, the free-living nematode *C. elegans*. The lowest concentrations of *S. versicolor* extract did not cause mortality, with viability of 80% or more, similar to the control, therefore these concentrations did not exhibit toxic action and can be considered selective.

The *S. versicolor* compounds may have feeding deterrent properties, inhibiting peristalsis in addition to restricting hatching [76], therefore higher concentrations of the extract are not indicated.

Lower concentrations of *S. versicolor* aqueous extract (0.1, 0.01, and 0.05%) negatively affected the development of *P. xylostella*, which means they are potentially effective against this target organism, showing no difference compared

to the control in the selective toxicity test, i.e., they were not toxic to *C. elegans*. However, the highest concentrations showed high mortality rates for both *P. xylostella* and *C. elegans*.

Previous studies described the petrified and dark appearance of lepidopteran larvae when they come in contact with a biotic control agent. For instance, *Oxydia vesulia* larvae (Cramer, 1779; Lepidoptera: Geometridae), when exposed to *Bacillus thuringiensis* var. kurstaki (Bacillaceae), presented symptoms similar to those found in this study, such as reduced movement and feeding as well as dark spots all over the body [77]. In *Helicoverpa zea* larvae (Bodie, 1850; Lepidoptera: Noctuidae), intestinal paralysis and total paralysis were observed after contact with *B. thuringiensis*, with lethal effects 3 days after contamination [78]. In *P. xylostella* larvae treated with aqueous extract of *Alibertia sessilis* (Vell.), K. Schumand (Rubiaceae), and *Alibertia intermedia* (Mart.) (Rubiaceae), mortality occurred during the larval stage, with dark coloration and petrified appearance, in addition to reduced pupal biomass [21].

Owing to the presence of numerous compounds in aqueous extracts of *S. versicolor*, particularly quassinoids and glaucarubinone, which exert direct effects on various biological characteristics of pests (such as those observed in *P. xylostella*), an increasing number of studies have investigated the desirable characteristics of *S. versicolor* for insect pest management. Many quassinoids are responsible for changes in feeding behavior and regulation of insect growth [79-81]. Thus, the changes in development observed in this study are another indication of the insecticidal potential of *S. versicolor*. Changes in feeding behavior and growth regulation have been verified in *Spodoptera litura* (Fabricius, 1775; Lepidoptera, Noctuidae) [80-81], in addition to insecticidal activity against *Tetranychus urticae* (Koch, 1836; Acari, Tetranychidae), *Myzus persicae* (Sulzer, 1776; Hemiptera, Aphididae), and *Meloidogyne incognita* (Kofoid and White, 1919; Nematoda, Heteroderidae) [79].

Reports indicate that quassinoids potently inhibit feeding through the function of bitter taste [82,83], which was observed in this study; individuals remained in the larval stage for an additional 3 to 4 days, and many did not reach the pupal stage due to food reduction/restriction, plant toxicity, and/or food conversion. In other insect species, quassinoids are reported to slow lepidopteran pupation, even when applied at low concentrations, showing that the combination of food restriction and toxicity can delay development [83-84], resulting in growth regulation [81] with overall slowed development. In third instar larvae of *Locusta migratoria migratorioides* (Reiche and Fairmaire; Orthoptera: Acrididae), quassinoids induced antifeeding activity [85], which has also been observed in other insect pests [86].

The analysis of *S. versicolor* showed variations in the contents of phenolic and polyphenolic compounds, highlighting the total phenolics followed by flavonoids and flavonol, being in agreement with LC-MS/MS reported flavanone and kaempferol (Fig.3, Table 1). Phenolic and polyphenolic compounds make up a major group of phytochemicals in plants; flavonoids (polyphenolic substances) present a wide range of biological activities, including insecticide effects (87-89). Thus, we can partially associate the effectiveness of AE-SV in inducing mortality in *P. xylostella* by the presence of these compounds.

Combining quassinoids with other compounds, such as kaempferol, further increases the potential of the plant, since their presence can alter the palatability of plants and reduce their nutritional value; produce free radicals in insects, thus decreasing digestibility; or even function as toxins. Additionally, they can cause rupture of the midgut epithelial membrane or disturbance to its metabolism [90]. Furthermore, they cause increased mortality and sublethal effects in larvae [91]; reduced oviposition [92]; decreased pupal survival, body weight, and fecundity [21,93,94]; and mortality.

Botanical insecticides are considered as alternative pest control because they are safer with respect to biodiversity and the health of humans and the environment, they have no cumulative effect [95,96], and they biodegrade easily [97]. In the trials performed in this study, aqueous extract of *S. versicolor* was shown to be effective for population control of *P. xylostella*, with low toxicity to the non-target organism *C. elegans*. Additionally, it is highly accessible and inexpensive. However, the extract should be used carefully. The data presented here is preliminary, being indispensable to tests on natural enemies and pollinators.

5. Conclusions

Here, we show for the first time that the extract of *S. versicolor* leaves at all concentrations was effective in inducing mortality on *P. xylostella*, and that it contains flavonoids, alkaloids and quassinoids. Among all the tested treatments, the 0.1% concentration stood out and could be explored in future field studies as an alternative control method. The extract with a concentration of 0.1% caused larval mortality, was not toxic to the nematode *C. elegans*, and required less vegetal matter at a low concentration.

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CAPÍTULO II

Simarouba versicolor aqueous extract inhibits feeding, oviposition and affects embryonic development of Diamondback moth

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Simarouba versicolor aqueous extract inhibits feeding, oviposition and affects embryonic development of diamondback moth

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Abstract: *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae) is the main lepidopteran pest of the Brassica crop due to its resistance to numerous insecticides. Multi pesticides resistance in insects of agricultural importance is a global problem, and new forms of effective control that are less harmful to the environment are becoming increasingly necessary. The present study analyzed the effects of aqueous extracts of *Simarouba versicolor* (Simaroubaceae), a plant present in the Brazilian Cerrado, at concentrations of 0.1, 1, 5, and 10% and control (distilled water) on the feeding preference, oviposition and embryonic development of *P. xylostella*. The results showed that the aqueous extract of *S. versicolor* resulted in decreases in oviposition and feeding of *P. xylostella*; a reduction in the hatching of the larvae was also observed, indicating ovicidal properties. However, the concentration of 1% resulted in a greater reduction in oviposition and a reduction in the number of hatched larvae. Dietary concentrations of 10% and 5% caused food intake suppression, and concentrations of 1% and 0.1% reduced dietary intake by 97% and 78%, respectively. This study highlights the efficacy of aqueous extracts of *S. versicolor* in the control of crucifer moths, reducing larval feeding and the number of individuals that reach the larval stage, which is the stage where the insect causes losses to producers.

Keywords: Embryotoxicity; oviposition deterioration; antifeedant; antixenosis.

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1. Introduction

Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae), popularly known as Diamondback moth, is a cosmopolitan insect that feeds exclusively on vegetables belonging to the family Brassicaceae [1,2]. Overall, the costs for managing this pest are estimated at US $4 to $5 billion, and losses in productivity can reach 100% if control measures are not adopted [3,4].

Currently, the main method for controlling *P. xylostella* is through the application of synthetic insecticides [5,6]. However, factors related to extensive and inappropriate applications of broad-spectrum insecticides, in addition to the short life cycle, high reproductive potential, low intraspecific competition, environmental adaptability, and genetic elasticity of the insect [7-9], have favored the evolution of

populations that are resistant to the main classes of insecticides recommended for control [10-12]. In addition to resistance to different types of synthetic insecticides, *P. xylostella* was the first insect pest reported to develop high-level resistance to spraying of *Bacillus thuringiensis* (Bt) toxins in the field [2,13].

In this sense, it is necessary to adopt control methods that are effective and environmentally safe and that reduce losses to producers. A successful alternative is botanical insecticides, which can be used according to the precepts of integrated pest management (IPM) [14,15]. Due to the synergistic action of secondary compounds, botanical insecticides are effective in pest control and have several mechanisms of action on target insects, such as deterring or suppressing feeding and oviposition and having antibiotic effects on eggs, larvae and adults [16-21].

The family Simaroubaceae is widely known for its pharmaceutical, herbicidal and, especially, insecticidal properties [22-26]. Phytochemical studies of *Simarouba versicolor* St. - Hill (Simaroubaceae) found secondary metabolites with insecticidal activity, such as saponins, flavonoids, quassinoids, alkaloids, and terpenes [21,27-31], which can be extracted in different ways, including by the simple maceration of the plant in water and/or extraction using organic solvents by various types of distillation [32].

Some plants with insecticidal properties have already been evaluated for the control of *P. xylostella*, but little is known about the effects of *S. versicolor* extract on the species. Nevertheless, *S. versicolor* can be used in pest management through formulations from crude or partially refined extracts. Due to the multi pesticide resistance of *P. xylostella* to synthetic insecticides, our objective was to evaluate the effects of the aqueous extract of *S. versicolor* at different concentrations on the feeding, oviposition and embryonic development of *P. xylostella*. The results can support the development of subsidies that enable the commercialization and application of this botanical insecticide in Brassicaceae plantations with crucifer moth infestations as well as in other cultivation systems.

2. Materials and Methods

The experiments and rearing were performed in the Laboratory of Insect-Plant Interaction, School of Biological and Environmental Sciences, Federal University of Grande Dourados, Mato Grosso do Sul, Brazil, at a constant temperature (25 ± 2 °C) and relative humidity (70% ± 5%) and a photoperiod of 12 h.

2.1. Insects

The *P. xylostella* stock was established from individuals collected in organic gardens of *Brassica oleracea* var. *acephala* located in the city of Itaporã, Mato Grosso do Sul, Brazil. *P. xylostella* rearing methodology was adapted from Barros et al. [33].

2.2. Obtaining the aqueous extracts of Simarouba versicolor

Leaves of fully expanded *Simarouba versicolor* were collected from a region dominated by Cerrado vegetation at SITES in the municipality of Campo Grande, Mato Grosso do Sul, Brazil (latitude: 21°13'28"S, longitude: 54°11'28"W; altitude: 437 m). The collection of the botanical material was authorized by the National

Council of Brazilian Research (CNPq)/Council for the Management of Genetic Heritage (CGEN/MMA) under permit number AF5E2AA. Exsiccatae were deposited in the herbarium of the School of Biological and Environmental Sciences of UFGD under accession number DDMS 6481.

After collection, the leaves were sanitized in running water, dried in a forced air oven for 72 hours at 45 °C and ground in an industrial knife mill until a fine powder was obtained; the powder was then stored in plastic containers, which provided protection from moisture and light.

To obtain the aqueous extract of *S. versicolor* (EASv), the maceration technique was used. To obtain aqueous extracts with concentrations of 10%, 5%, 1% and 0.1%, 3 g of the powdered material was added to 30 ml of distilled water; 1.5 g to 30 ml; 0.3 g to 30 ml and 0.015 g to 30 ml, respectively. The material was weighed on an analytical balance (Bel Mark Analytical Balance - 0.001 g). The solutions were left to stand for 24 hours in a refrigerated environment and were subsequently filtered using filter paper.

2.3. Effect of EASv on the food preference of P. xylostella with a choice test

Discs of organic cabbage (*Brassica oleracea* var. *acephala*) with a 4 cm Ø were immersed in EASv at concentrations of 0.1, 1, 5 and 10% and left on filter paper for approximately 40 minutes to allow the solution to dry. The control consisted of discs immersed in distilled water.

Subsequently, four cabbage discs were transferred to a Petri dish (9 cm in diameter and 1.5 cm in height). The discs were placed across from each other and equidistant, with two discs immersed in the EASv and the other two discs immersed in water (i.e., the control). Then, third instar stock-reared *P. xylostella* larvae were released in the center of the plate. The larva remained in the Petri dish for 24 hours, with the possibility of choosing between discs treated with EASv or control discs. After 24 hours, the cabbage discs were removed and scanned (Figure 1), the leaf area consumed was measured using ImageJ software, and the Kogan and Goeden [34] food preference index (FPI) was calculated.

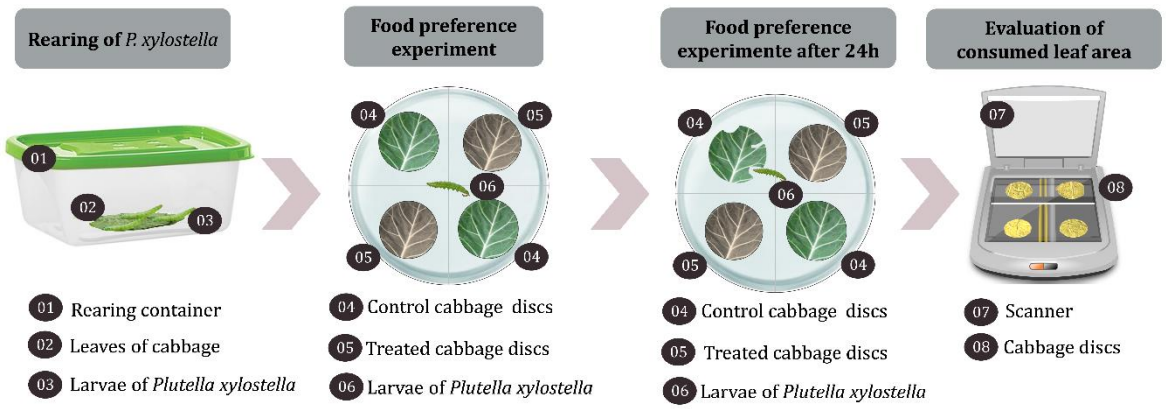

Figure 1. Schematic representation of methodology used to food preference of P. xylostella in a free-choice test.

2.4. Effect of EASv on the oviposition of P. xylostella in a choice test

Stock-reared pupae were kept in individual test tubes until adult emergence. The sex of the moths was determined based on the sexual dimorphism of the adults [35], and couples were formed.

Subsequently, 4 cm Ø cabbage discs were immersed in EASv at concentrations of 0.1, 1, 5 and 10%, and control discs were immersed in distilled water. The discs were placed on sheets of filter paper for approximately 40 minutes to allow the liquid to dry. Then, 1 couple consisting of adults up to 12 hours post-emergence was placed in plastic cages (8 cm in diameter x 6 cm in height), where they remained for 10 days, after which oviposition preferences were evaluated based on the oviposition preference index (IPO) of Kogan and Goeden [34].

A moistened filter paper disc measuring 8 cm Ø and four cabbage discs with 4 cm Ø (two control discs (immersed in water) and two discs immersed in the EASv) were added to each cage as oviposition substrates. The discs were changed every 24 hours, and the number of eggs on the discs was counted. Cotton soaked in honey solution diluted in 10% distilled water were offered as food for the adult insects (Figure 2).

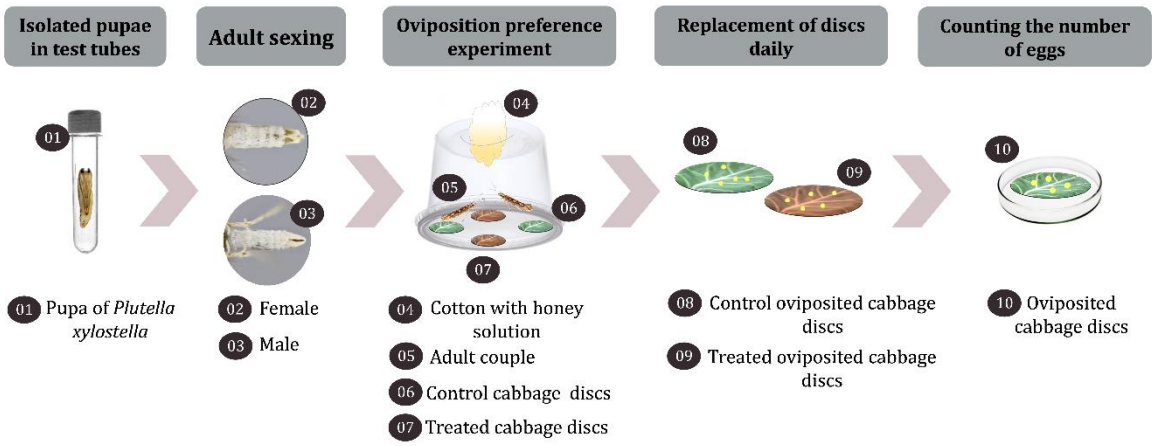

Figure 2. Schematic representation of methodology used to free-choice oviposition preference test of *P. xylostella*.

2.5. Effects of EASv on the embryonic phase of Plutella xylostella

Stock-reared *P. xylostella* pupae were kept in individual test tubes until adult emergence. After the emergence and sexing of adults, 2 couples up to 12 hours old were released into transparent plastic cages (8 cm in diameter x 6 cm in height) containing 3 cabbage discs (4 cm Ø) on filter paper discs (8 cm Ø) used as oviposition substrate.

After 24 hours, the discs on which the insects oviposited were removed, and carefully cut into smaller areas, each containing 10 eggs [36]. Then, these eggs were immersed in EASv at concentrations of 0.1, 1, 5 and 10% for 30 seconds and transferred individually to Petri dishes. The control consisted of distilled water. The

number of hatched larvae was counted daily (Figure 3) and compared with the number of eggs that had the transparent chorion with a stereoscopic magnifying glass.

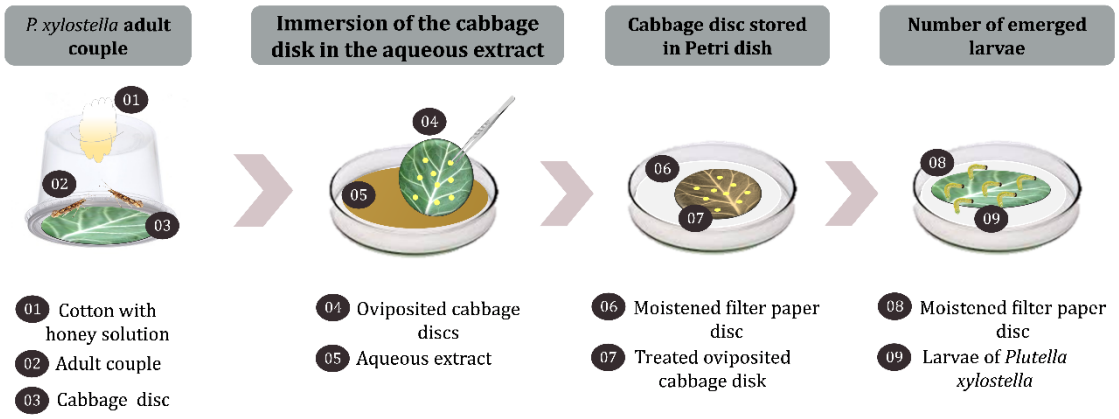

Figure 3. Schematic representation of methodology used to assess effects of EASv on the embryonic phase of *Plutella xylostella*.

2.6. Statistical analysis

2.6.1. Food preference experiment

The experiment was conducted with a completely randomized design with 10 replicates and 3 subsamples; each replicate consisted of a plate with four discs and 1 third instar larva. The data were subjected to analysis of variance (ANOVA), and the means were compared using the T test at a 5% probability. The data is represented as the mean ± standard error of the mean.

2.6.2. Calculation of the food preference index (FPI)

The effect produced by the plant extract was evaluated using the food preference index [34]. The extract was classified as a phagostimulant if the index was greater than 1, neutral if it was equal to 1 and phagodeterrent if it was lower than 1, using the formula PI = 2A/(M+A), where A is the consumed area of the treated discs and M is the consumed area of the untreated discs.

2.6.3. Oviposition choice experiment

The experimental design used to evaluate oviposition during the free-choice test was completely randomized; a total of 5 treatments (4 concentrations and a control) were used, and each treatment consisted of 10 replicates, i.e., 10 cages. The results were subjected to analysis of variance (ANOVA), and the means were compared by T test ($P < 0.05$). Data that did not meet the normality criteria were transformed to $\sqrt{x} + 0.5$. The data are represented as the mean ± standard error.

2.6.4. Calculation of the oviposition preference index (OPI)

The effect of EASv on the oviposition of crucifer moths was evaluated using the oviposition preference index (OPI) described by Kogan and Goeden [34]: ISO = 2A/(M + A), where A is the number of eggs on the leaves immersed in the extract and M is the number of eggs on the leaves immersed in distilled water. The OPI

values range from zero to two, with values greater than 1 being classified as ovistimulants, values equal to 1 classified as neutral and values less than 1 bring classified as ovideterrents.

2.6.5. Effect of EASv on the embryonic phase of Plutella xylostella

The experimental design used was completely randomized with 5 treatments (4 concentrations plus control) and 10 replicates, with 10 eggs in each replicate. The data were subjected to analysis of variance (ANOVA), compared by Tukey's test ($P < 0.05$), and represented as the mean ± standard error. Data that did not meet the normality criteria were transformed to $\sqrt{x} + 0.5$.

2.7. Chemical composition

The extract was lyophilized (Alpha 1–2LD Plus). The process parameters were 0.045 mbr vacuum and -42 °C temperature. The lyophilized extract was solubilized at a concentration of 1 mg/mL.

2.7.1. Content of phenolic compounds by the Folin-Ciocalteu method

To the sample, 0.5 mL of Folin-Ciocalteu reagent (1:10 v/v) and 1 mL of distilled water were added to 0.1 mL of sample, incubating for 1 minute. Subsequently, 1.5 mL of 20% sodium carbonate (w/v) was added and the reaction was waited for 2 hours, keeping the sample in a dark place. Finally, the sample was read in a UV/Vis spectrophotometer (Global Trade Technology) at a wavelength of 760 nm [37].The analysis was performed with three replicates, using distilled water as a blank. For quantification, an analytical curve of gallic acid subjected to the same chemical reaction as the samples was used. The results were expressed in gallic acid equivalent per g of lyophilized extract (mg AGE g-1).

2.7.2. Flavonoid content by the aluminum chloride method

Aluminum chloride 2% (1 mL) in methanol was added to 1 mL of the sample, waiting for the reaction for 15 minutes and reading in a UV/Vis spectrophotometer at a wavelength of 430 nm [37]. The blank used was distilled water and the analyzes were performed in triplicate. An analytical curve using rutin was prepared for quantification and the results were expressed in equivalent rutin per g of lyophilized extract (mg RE g-1).

2.7.3. Determination of Tannins

The tannin content was determined by the Fo-in-Denis spectrophotometric method described by [38] with adaptations in the used volumes of reagents, but maintaining the concentrations. For each 0.5 mL of sample, 0.5 mL of Folin-Denis reagent was added, followed by 0.5 mL of 8% sodium carbonate, allowing it to react for 120 minutes. The reading was performed in a spectrophotometer at a wavelength of 725 nm. To calculate the tannin concentration, an analytical curve was performed using a tannic acid standard. The results were expressed in mg of tannic acid equivalent per g of lyophilized extract (mg ATE g-1).

2.7.4. Antioxidant potential against DPPH

Sample (0.1 mL) and 3 mL of 0.004% DPPH radical and wait 30 minutes in a dark place. After this period, the sample was read at a wavelength of 517 nm. The readings were performed in triplicate using distilled water as a blank. The percentage of DPPH inhibition (%) was calculated as described [39].

3. Results

3.1. Effect of EASv on the feeding preference of Plutella xylostella

The leaf area consumed varied significantly for all the EASv concentrations evaluated. Regarding the mean of the leaf area consumed, there was no consumption of the discs treated with EASv at concentrations of 10% and 5% (Table 1). At the 1% concentration, EASv promoted a reduction in consumption of approximately 97% when compared to the consumption of the control discs. For the 0.1% concentration, the botanical extract promoted a reduction in larval feeding by approximately 78% compared to the control (Table 1).

According to the food preference index, all the EASv concentrations were classified as phagodeterrents, i.e., the EASv-treated discs reduced the intake of food by *P. xylostella* (Table 1).

Table 1: Leaf area consumed (± SE) and feed preference index of *Plutella xylostella* exposed to cabbage discs treated with aqueous extract of *Simarouba versicolor* at different concentrations.

Concentration (%)	Consumed Leaf Area (cm²)		Preference index[1]	Classification [1]
	Extracts	Control		
10.00	0.00 ± 0.00 b n= 30	0.44 ± 0.03 a n= 30	0.00	Fagodeterrent
5.00	0.00 ± 0.00 b n= 30	0.45 ± 0.03 a n= 30	0.00	Fagodeterrent
1.00	0.01 ± 0.00 b n= 30	0.32 ± 0.03 a n= 30	0.01	Fagodeterrent
0.10	0.08 ± 0.002 b n= 30	0.36 ± 0.029 a n= 30	0.11	Fagodeterrent

*Means followed by the same letter in a row do not differ statistically from each other according to a t test at a 5% probability level.

[1] According to [34]

3.2. Effect of EASv on Plutella xylostella oviposition

The results obtained show that all the EASv concentrations had significant effects on the fecundity of *P. xylostella.* Regarding the daily oviposition mean, we observed that the females showed a recognition pattern for the treated discs for up to 3 days, except for the 5% EASv; after this period, there were drastic decreases in oviposition on discs treated with the botanical extracts (Figure 4).

At 10% and 5%concentrations, there was no oviposition on Days 8 and 9, respectively. However, for the 1% and 0.1% concentrations, the absence of oviposition occurred only on the last day of evaluation (Figure 4).

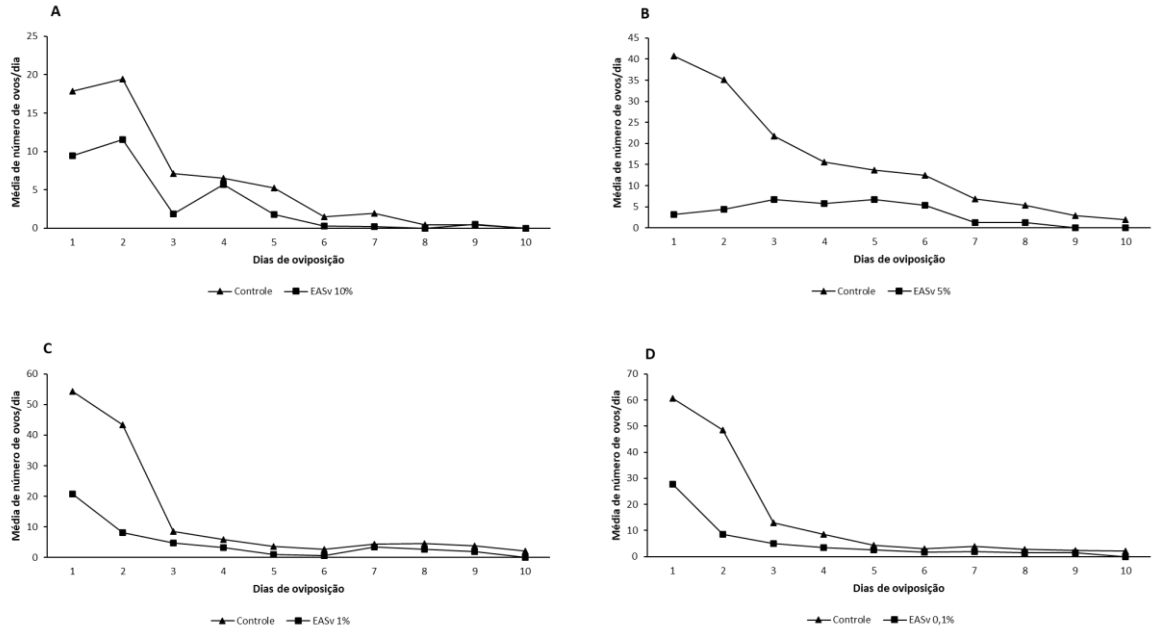

Figure 4. Mean number of *Plutella xylostella* eggs over a 10-day during the oviposition preference experiment using cabbage discs treated with aqueous extracts of *Simarouba versicolor* at different concentrations.

The 10% EASv concentration caused an average reduction of 47.50 eggs/couple, i.e., a reduction of approximately 66% (Table 2). Although the observations were made for 10 days, the females that had contact with the EASv at a concentration of 10% died after 8 days of testing, while the males of the same treatment remained alive throughout the 10 days. The mortality among the females may have occurred because the females came into direct contact with the substrate for oviposition.

Furthermore, at the 5% EASv concentration, a reduction of approximately 62% in the total number of eggs was observed when compared to the control. In addition, the 1% and 0.1% EASv concentrations reduced oviposition by 60% compared to the control, with mean reductions of 66.50 and 80 eggs per couple, respectively (Table 2).

The oviposition preference index (OPI) demonstrated preference of the insect for the untreated substrate (control) and a slight preference for substrates treated with EASv. Thus, the botanical extracts were classified as ovideterrent (Table 2). Note that all the EASv concentrations interfered in the choice of *P. xylostella* females for treated discs and resulted in lower oviposition rates. None of the concentrations suppressed oviposition; however, there was a strong reduction in the number of eggs on the discs. Treatment with EASv resulted in a mean decrease in oviposition by *P. xylostella* females of 63.62% (Table 2).

Table 2: Number of eggs (± SE) and oviposition preference index of *Plutella xylostella* on cabbage discs treated with aqueous extracts of *Simarouba versicolor* at different concentrations.

Concentration (%)	Average Number of Eggs		Preference index[1]	Classification [1]
	Extracts	Control		
10.00	28.30 ± 6.08 b n= 10	60.40 ± 8.57 a n= 10	0.60	Ovideterrent
5.00	55.50 ± 1.27 b n= 10	146.00 ± 7.74 a n= 10	0.55	Ovideterrent
1.00	47.40 ± 9.53 b n= 10	119.80 ± 13.27 a n= 10	0.52	Ovideterrent
0.10	57.00 ± 5.92 b n= 10	133.50 ± 9.37 a n= 10	0.60	Ovideterrent

*Means followed by the same letter in a row do not differ statistically from each other according to a t test at a 5% probability.

[1] According to Kogan & Goeden [34].

3.3. Effects of EASv on the embryonic phase of Plutella xylostella

The aqueous extract of *S. versicolor* affected the embryonic development of *P. xylostella* eggs at all concentrations, reducing the number of hatched larvae ($F = 4.85$; GL = 4; $P = 0.0027$; CV = 8, 93) and showing ovicidal properties (Table 3). The 1% EASv concentration was the treatment that most affected the hatching of the larvae, reducing the number of hatched larvae by 28.42% on average. However, this treatment did not differ from the other concentrations. Thus, the 10% EASv concentration reduced the hatching of crucifer moth eggs by 23.62% on average. In addition, the 5% and 0.1% EASv concentrations reduced the number of hatched larvae by an average of 20.70% and 13.21%, respectively, compared to the control (Table 3).

In general, all the concentrations reduced the number of hatched larvae by 21.48% on average. In addition, there was no significant difference between the concentrations of EASv, which indicates that any of the concentrations used will affect the hatching of *P. xylostella* eggs (Table 3).

Table 3. Mean number (± SE) of hatched *P. xylostella* larvae from eggs exposed to different concentrations of *Simarouba versicolor* aqueous extract.

	Concentrations of *Simarouba versicolor* aqueous extract				
	Control	**EASv 10%**	**EASv 5%**	**EASv 1%**	**EASv 0,1%**
Nº of hatched larvae	9.99 ± 0.00 a	7.63 ± 0.74 b	7.92 ± 0.51 b	7.15 ± 0.41 b	8.67 ± 0.33 ab
F and P value	*F*= 4.85; *P*= 0.0027				

*Means followed by the same letter in the row do not differ statistically from each other according to Tukey's test at a 5% probability level.

3.4. Chemical composition

Tannins, phenolic compounds and flavonoids were found in the aqueous extracts *Simarouba versicolor* leaves (Table 4). Furthermore, antioxidant activity was observed in the botanical extract (Table 4).

Table 4: Antioxidant activity, phenolic compounds, flavonoids, and tannins of *Simarouba versicolor.*

Aqueous Extract	Tannins (mg/g)	Phenolic Compounds (mg/ g)	Flavonoids (mg/g)	Antioxidant Activity (mg/g)
Simarouba versicolor	52.11± 0.43	354.13±2.04	247.11± 5.43	87.31± 2.32

4. Discussion

Our results showed that *S. versicolor* extract can be used as an effective botanical insecticide in the control of crucifer moths. EASv at concentrations of 10% and 5% prevented the onset of feeding by *P. xylostella* larvae, causing them to feed only from untreated discs. Furthermore, the lowest concentrations reduced consumption by 87.5% on average. Phytophagous insects rely on behavioral mechanisms for feeding, including a stage in which host acceptance and adequacy are assessed [40]. However, the presence of secondary metabolites can alter the choice and acceptance of the host [41], making substrates less attractive or unpleasant to palate through smell or bitter taste [42,43].

In particular, *P. xylostella* is a voracious feeder, especially in the third and fourth instars [44]; thus, the larvicidal effect of EASv on third instar larvae and the reduction in feeding by moths on cruciferous plants is an extremely important result, once lower leaf consumption suggests a reduction in damage and, consequently, a reduction in losses to the producers.

During the larval stage, insects must ingest a sufficient amount of nutrients to be converted into growth tissue [45]. Thus, the phagodeterrent effect of EASv at all concentrations compromised larval feeding, and may have caused sublethal effects throughout the life cycle of the insect, such as impaired development and metamorphosis, a reduction in pupal weight, low adult fecundity, and even mortality [17,46-48].

The larvae exposed to the highest concentrations (10% and 5%) consumed the untreated discs more than the larvae exposed to the lower concentrations. This is possibly because at the lower concentrations, the larvae took a test bite and initially fed on the treated discs, affecting the physiology of the insects. We observed that the larvae that fed on the EASv-treated discs showed reduced mobility.

EASv affected the oviposition preference of *P. xylostella* females once the number of eggs deposited on the treated discs reduced by 64% on average. In addition, EASv reduced the number of hatched larvae by 21% on average. Arthropods use chemical, physical or morphological stimuli [49] to choose the substrate on which they oviposit and/or feed. These stimuli are observed through sight, smell, touch and taste [41]. Plant extracts have antixenotic elements, which can confuse females in the process of plant selection, especially in the evaluation and acceptance phase [50], making it less used for oviposition [51]. However, due to their nocturnal habit [9], females may guide themselves by identifying odors.

In particular, *P. xylostella* uses antennas and abdominal segments to grope the substrate and verify whether it is safe for egg deposition [52-54]. Thus, it is assumed that the reduction in the number of eggs on the treated discs was due to the presence of volatile compounds in the EASv, causing repellent action or irritability among females when they came into contact with the treated substrate [36].

A daily fluctuation in mean number of eggs produced by the couples of *P. xylostella* was verified in the different treatments. Moreover, from the third day of the experiment, there was a reduction in the number of eggs for all the treatments, including the control, suggesting the possibility that EASv spread in the cage and interfered with oviposition preference even in the control. Under normal conditions, a female crucifer moth oviposits approximately 160 eggs on average, but a female can oviposit up to 300 eggs [9]. As the test included a choice of substrates, the possibility of the extract interfering in the choice of substrates for oviposition by the females should be considered. Although, this possibility was not evaluated by the authors.

We believe that the EASv penetrated through the egg micropores, causing the death of the embryos and, consequently, the reduction in egg hatching; these micropores are approximately 0.8 mm and allow gas exchange within the egg [36]. In addition, *P. xylostella* eggs have a rough chorion, facilitating the penetration of extract into the eggs [36]. In this sense, the reduction in the number of eggs and egg hatching on treated discs after contact with EASv results in the future reduction of the population and, consequently, decreases the losses and costs associated with the management of *P. xylostella* [55]. Additionally, EASv reduces the number of individuals that reach the larval stage, especially the first instar, the stage in which the larvae have a mining habit, feeding from leaf parenchyma [56]. This behavior prevents the larvae from having contact with insecticides, hindering its control.

When evaluating the effect of *Carum copticum* (Apiaceae) extracts on *Culex pipiens* (Diptera: Culicidae), it was found that the methanolic extract, with a concentration of 0% at 150 μm/mL, had an ovicidal effect on *C. pipiens* egg hatchability [57], and this ovicidal action was directly related to increased concentration. Previously, in the study conducted by Tavares et al. [58], it was found that extracts of citronella (*Cymbogopon nardus*), sassafras (*Ocotea odorifera*) and neem oil (*Azardirachta indica*) had ovicidal effects on *Bemisia* spp.; on average, 58% of the eggs became unviable and did not hatch.

The results of this study may be due to alkaloids, terpenes, steroids, coumarins, anthraquinones, and flavonoids present in *S. versicolor* [27,29,31]. The terpenes found in *Chloroxylon swietenia* DC. (Rutaceae), had a deterrent effect on the

oviposition of *Spodoptera liture* (Fabricius) (Lepidoptera: Noctuidae) [59] and consequently adult mortality [60]. The coumarins present in *Ageratum conyzoides* (Asteraceae) caused high adult mortality in *Rhyzopertha dominica* [61].

Alkaloids are secondary metabolites that act on the nervous system of insects [62]. These compounds have an adulticidal action, i.e., they cause mortality in adults [63]. Conversely, flavonoids can directly affect reproduction, decrease oviposition [64,65] and reduce egg hatching [66]. It was observed a reduction in oviposition when *P. xylostella* females were exposed to aqueous and ethanolic extracts of *Schinus terebinthifolius* Raddi (Anacardiaceae), *Annona coriacea* Mart. (Annonaceae), *Annona crassiflora* Mart. (Annonaceae) and *Serjania marginata* Casar (Sapindaceae) [67]. The authors explain that the ovideterrent effect was due to the presence of tannins, flavonoids, saponins, and tannins.

5. Conclusions

The aqueous extract of *S. versicolor* (EASv) showed a phagodeterrent effect, negatively affected the oviposition and hatching of *P. xylostella* eggs in laboratory experiments, and concomitantly reduced the number of individuals that reached the larval stage, i.e., the stage in which the insect is considered a key pest and causes substantial losses to Brassica crops, reducing productivity and increasing management costs. Therefore, this extract has great potential to be used as a tool in the management of *P. xylostella*, especially on small farms considering the simplicity of its preparation and the availability of the raw material. Our results increase the knowledge about the bioactivity of EASv on *P. xylostella*, contributing to other studies that seek to determine the feasibility of applying this botanical insecticide in organic or ecological food production systems.

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CONSIDERAÇÕES FINAIS

Os ensaios realizados para testar a eficácia de *Simarouba versicolor* como potencial inseticida, mostrou que dependendo da concentração utilizada, sucesso é possível obter sobre qualquer uma das fases do ciclo de vida do inseto, em especial a fase larval, que é a fase destrutiva as culturas de Brássicas, O efeito larvicida, evidenciado no capitulo 1, fagodeterrente ou *antifeedant*, evidenciado no capitulo 2 proporciona a redução da alimentação e concomitantemente, a redução dos danos e prejuízos ao produtor. Além disso, a má alimentação durante a fase larval provoca efeitos ao longo de todo o ciclo do inseto, afetando a fertilidade e a fecundidade dos adultos.

O efeito ovideterrente, evidenciado no capítulo 2, somado a redução da fecundidade devido à má alimentação e ao efeito ovicida, reduzem o número de indivíduos da próxima geração e, consequentemente, afeta a população da traça-das-crucíferas.

Quanto a concentração ideal de utilização, recomendamos concentrações mais baixas pois não apresenta toxicidade ao nematoide de vida livre *C. elegans*, e devido a sua homologia genética aos humanos, pressupõe-se que o extrato aquoso não apresentará toxicidade aos humanos.

Sendo assim, conforme os resultados obtidos, é possível concluir que, o extrato aquoso de *S. versicolor* é promissor no controle de traça-das-crucíferas, e pode ser utilizado como uma alternativa de controle desta praga.