JENY TATIANA BERNAL ZULUAGA

POTENTIAL OF NOVEL TOOLS FOR THE BIORRATIONAL CONTROL OF *Euschistus heros*

Dissertation submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Eugênio Eduardo de Oliveira

Co-adviser: Elson Santiago de Alvarenga

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“We are like butterflies that fly for a day thinking that they will fly forever.”

Carl Sagan.

Abstract

BERNAL-ZULUAGA, Jeny Tatiana, M.Sc., Universidade Federal de Viçosa, July 2023. **Potential of novel tools for the biorational control of *Euschistus heros***. Adviser: Eugênio Eduardo de Oliveira. Co-Adviser: Elson Santiago Alvarenga.

Brazil is the largest producer of soybeans in the world. This recognition has been achieved through years of research, manipulation, and selection of soybean varieties to be productive in tropical climates. Soybean cultivation is of great global importance, primarily for animal feed and oil production. However, soybeans (*Glycine max*) face limiting factors such as climate, weeds, and pests.one of the most representative pests of soybean crops is *Euschistus heros*, commonly known as the "brown soybean stink bug". This sucking insect attacks pods and seeds, directly affecting production. The main control method for sucking insects in soybean cultivation is the use of neonicotinoid insecticides, which are compounds derived from nicotine. These compounds act on the nervous system of insects, binding to nicotinic acetylcholine receptors. Their effects cause the death of the insect within hours. However, prolonged, and excessive use of the same control method has selected populations of resistant insects, making control difficult. Additionally, the use of chemical insecticides has negative effects on natural and agricultural ecosystems and impacts human health. Therefore, it is important to research alternative control methods that enrich integrated pest management (IPM) programs and diversify agricultural systems. During this study, a prospecting of new synthetic molecules and essential oils were conducted, focusing on the biorational control of *Euschistus heros* and selectivity to pollinators and natural enemies. The toxicity of essential oils from *Ruta graveolens* (rue), *Schinus terebinthifolius* (red pepper), *Cymbopogon citratus* (lemon grass), and *Morinda citrifolia* (noni) was evaluated in concentrations of 25.13 nL/cm^2, 50.26 nL/cm^2 and 100.52 nL/cm^2 on third instar nymphs of *E. heros*. Essential oils of rue, lemon grass and noni showed significant toxicity with a mortality rate ranging from 90% to 100% after 24 hours of exposure. The essential oil of *Morinda citrifolia* had a LC_{50} of 11.06 (9.05 - 13.07) nL/cm^2. The synthetic molecules evaluated belong to the group of tetronamides of mucochloric acid. For this study we call them: BEN, M-AN, 3F, DICL, AN, TO2, 4Cl, 2Cl, 2F, 3F, and 4Br. The DICL molecule was the only one that caused significant mortality in *E. heros*. A dose-response curve was obtained for this molecule, revealing a LC_{50} of 0.019 (0.017 - 0.020) nL/cm^2. Toxicity bioassays were also conducted on pollinators and natural enemies. From the bioassays on pollinators, no significant effect was found on the consumption of DICL-contaminated diet. In the bioassays with the predator, no significant mortality or negative effects on predatory capacity were observed. Our results suggest the potential use of the studied essential oils as insecticidal tools or as a basis to produce new insecticidal products. Additionally, our findings demonstrate the potential use of the DICL molecule as a biorational insecticide, with high efficiency in the control of *E. heros* and minimal to no effect on the pollinators *Apis mellifera* and *Partamona helleri*, as well as the predatory insect *Coleomegilla maculata*. Future studies could evaluate the selectivity potential of the parasitoid *Telenomus podisi*. A biological controller that is currently beingused in some soybean fields in Brazil.

Keywords Agricultural entomology. Biorational pest management. Insecticide selectivity

Resumo

BERNAL-ZULUAGA Jeny Tatiana, M.Sc., Universidade Federal de Viçosa, julho de 2023. **Potencial de novas ferramentas para o controle biorracional do *Euschistus heros*.** Orientador: Eugênio Eduardo de Oliveira. Coorientador: Elson Santiago de Alvarenga.

O Brasil é o maior produtor de soja do mundo. Este reconhecimento foi alcançado a través de anos de pesquisa, manipulação e seleção de variedades de soja para serem produtivas em climas tropicais. O cultivo da soja é de grande importância mundial, principalmente para alimentação animal e produção de óleo. No entanto, a soja (*Glycine max*) enfrenta fatores limitantes, como clima, ervas daninhas e pragas. Umadas pragas mais representativas da cultura da soja é o *Euschistus heros*, popularmente conhecido como "percevejo marrom da soja". Esse inseto sugador ataca vagens e sementes, afetando diretamente a produção. O principal método de controle de insetos sugadores na cultura da soja é o uso de inseticidas neonicotinoides, compostos derivados da nicotina. Esses compostos atuam no sistema nervoso dos insetos, ligando-se aos receptores nicotínicos de acetilcolina. Seus efeitos causama morte do inseto em poucas horas. No entanto, o uso prolongado e excessivo do mesmo método de controle tem selecionado populações de insetos resistentes, dificultando o controle. Além disso, o uso de inseticidas químicos tem efeitos negativos nos ecossistemas naturais e agrícolas e impacta a saúde humana. Portanto, é importante pesquisar métodos alternativos de controle que enriqueçam os programas de manejo integrado de pragas (MIP) e diversifiquem os sistemas agrícolas. Durante este estudo, foi realizada uma prospecção de novas moléculas sintéticas e óleos essenciais, com foco no controle biorracional de *Euschistus heros* e seletividade a polinizadores e inimigos naturais. A toxicidade dos óleos essenciais de *Ruta graveolens* (arruda), *Schinus terebinthifolius* (pimenta roça), *Cymbopogon citratus* (capim limão) e *Morinda citrifolia* (noni) foi avaliada em concentrações de 25.13 nL/cm^2, 50.26 nL/cm^2 e 100.52 nL/cm^2 em ninfas de terceiro instar de *E. heros*. Os óleos essenciais de arruda, capim limão e noni apresentaram toxicidade significativa com taxa de mortalidade variando de 90% a 100% depois de 24 horas de exposição. O óleo essencial de *Morinda citrifolia* apresentou CL_{50} de 11.06 (9.05 - 13.07) nL/cm^2 As moléculas sintéticas avaliadas pertencem ao grupo das tetronamidas do ácido mucoclórico. Para este estudo chamamos elas de: BEN, M-AN, 3F, DICL, AN, TO2, 4Cl, 2Cl, 2F, 3F e 4Br. A molécula DICL foi a única que causou mortalidade significativa em *E. heros*. Uma curva dose-resposta foi obtida para esta molécula, revelando uma LC_{50} de 0.019 (0.017 – 0.020) nL/cm^2. Bioensaios de toxicidade também foram conduzidos em polinizadores e inimigos naturais. A partir dos bioensaios em polinizadores, nenhum efeito significativo foi encontrado no consumode dieta contaminada com DICL. Nos bioensaios com o predador, não foram observadas mortalidade significativa ou efeitos negativos na capacidade predatória. Nossos resultados sugerem o uso potencial dos óleos essenciais estudados como ferramentas inseticidas ou como base para a produção de novos produtos inseticidas. Adicionalmente, nossos achados demonstram o potencial uso da molécula DICL como inseticida biorracional, com alta eficiência no controle de *E. heros* e mínimo ounenhum efeito sobre os polinizadores *Apis mellifera* e *Partamona helleri*, bem como sobre o inseto predador *Coleomegilla maculata*. Estudos futuros, poderiam avaliar o potencial de seletividade do parasitoide *Telenomus podisi*. Um controlador biológico que atualmente está sendo ussado em alguns campos de soja no Brasil.

Palavras-chave: Entomologia agrícola. Manejo biorracional de pragas. Seletividade de inseticidas.

Contents

General Introduction

Brazil is the largest country in South America and the largest producer of soybeans in the world. Brazil managed to cultivate soybeans after years of research, manipulation, and development of resistant and productive varieties in the tropical climate. Soybeans (*Glycine max* (L.) Merrill) became important to the Brazilian economy in the late 1940s when it first appeared in international soybean production statistics. Currently, this crop contributes a significant portion of the countrys income (de Oliveira and de Souza e Silva, 2021), with the state of Mato Grosso being the largest producer, with a 2022-2023 crop of 44,476.4 thousand tons, representing 29 percent of soybean production in the country, followed by Goias, Paraná, and Rio Grande do Sul. The current limiting factors for soybean cultivation are climate, weeds, chemical use, and pests. Among the most important pests are the pod-sucking insects, with a high representation of *Euschistus heros* (Correa-Ferreira and de Azevedo, 2002; Azevedo et al., 2022), known as the "brown stink bug of soybeans." This insect directly attacks the commercially valuable part of the plant, affecting the productivity and quality of pods and seeds. The main control tool is the use of neonicotinoid-based insecticides, which are compounds derived from the nicotine molecule and act on the insect's nervous system, causing death within hours (Bortolotto et al., 2015; Castellanos et al., 2018; Sahani et al., 2022; Somavilla et al., 2020, 2019). Neonicotinoid insecticides are derived compounds of nicotine and were introduced in 1991 for use in agriculture and public health programs. These insecticides have been very successful due to their high effectiveness in insect control and low toxicity to mammals compared to the nicotine molecule that inspired them (Buszewski et al., 2019; Matsuda et al., 2020; Sahani et al., 2022). However, there are different studies on the negative effects of neonicotinoid and pyrethroid insecticides in the natural environment, humans, non-target organisms (Naeem et al., 2022; Rashid et al., 2010), and the imbalance in insect communities that visit or live in agricultural systems (Guedes et al., 2017). The excessive and inadequate use of chemical insecticides is generating populations of resistant insects and causing failure in their control due to the selection of genetic resistance characteristics related to factors such as detoxification enzymes, modification of target sites, and insect behavior (Azevedo et al., 2022; Castellanos et al., 2018; Somavilla et al., 2020, 2019; Tuelher et al., 2017). Additionally, the insecticides used today have low or no selectivity with non-target organisms, resulting in a loss of ecosystem services such as predation and biological control, which are essential for the proper functioning of agricultural systems (van der Sluijs et al., 2013) and natural ecosystems. The discovery of new molecules with insecticidal potential in sucking pests like *E. heros* opensup new possibilities to diversify management strategies, strengthen integrated pest management (IPM) programs, and promote agriculture that is more environmentally friendly (Ademokoya et al., 2022; Nicolopoulou-Stamati et al., 2016; Parra et al., 2023; Parra and Coelho, 2022). In this sense, the present study aims to prospect the insecticidal potential of new synthetic molecules and essential oils from *Ruta graveolens*, *Schinus terebinthifolius*, *Cymbopogon citratus*, and *Morinda citrifolia* for the control of the "brown stink bug of soybeans" *Euschistus heros*, as well as evaluating possible side effects on non-target organisms such as natural enemies and pollinators. This aims to contribute to the understanding of the ecology of agricultural systems and promote alternative pest control methods.

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Chapter 1

Insecticidal activity of essential oils to *Euschistus heros*

BERNAL-ZULUAGA, Jeny Tatiana, M.Sc., Universidade Federal de Viçosa, July, 2023. **Insecticidal activity of essential oils to *Euschistus heros* (Hemiptera: Pentatomidae)** Adviser: Eugênio Eduardo de Oliveira. Co-Adviser: Elson Santiago de Alvarenga.

Abstract

Many plant species produce compounds that are interesting for the control of agricultural pests. Some of these compounds have dissuasive, repellent, and even lethal properties. Research has been conducted for years on the use of essential oils and their constituent compounds to develop products, provide alternative control options, and enhance integrated pest management (IPM) programs. However, competition with chemical methods, legal requirements, and lack of information among rural producers have delayed the availability and use of these alternatives. Currently, the consumer market is demanding more and more chemical-free products and a more environmentally friendly agricultural use of land. Therefore, it is important to integrate synergistic control methods to reduce the negative effectsand excessive use of chemicals. This is the case with soybean cultivation, which faces various pod-sucking pests. One of the most representative pests in this crop is *Euschistus heros*, whose main method of control is the use of chemical insecticides from the neonicotinoid group. For this reason, it is important to develop new alternatives for the control of this pest and to discover new compounds with different active principles, lower impact on ecosystems, and less danger to rural workers. During this study, the toxicity of essential oils from *Ruta graveolens*, *Schinus terebinthifolius*, *Cymbopogon citratus*, and *Morinda citrifolia* on third instar nymphs of *E. heros* was evaluated. Concentration-mortality bioassays were conducted by tarsal contact of theinsects with dried oil residues in glass vials. Based on these bioassays, the biocidal capacity of each oil was evaluated.

Significant toxicity values were found for essential oils of rue, lemon grass and noni, with mortality ranging from 90% to 100% at 48 hours of evaluation in concentrations of 25.13 nL/cm^2, 50.26 nL/cm^2 and 100.52 nL/cm^2. Our results demonstrate the potential use of these essential oils as insecticidal tools for the biorational control of *E. heros* or as a basis for the development of new products.

Keywords Biopeseticides. Integrated pest management. Plants extracts.

1 Introduction

Essential oils from plants have been successfully used in the pharmaceutical and cosmetics industry (Assadpour et al., 2023). However, their use in agriculture has not had the same success, despite the known insecticidal properties of different plant extracts, (REGNAULT-ROGER, 1997). Essential oils or their active ingredients have a low market share compared to synthetic pesticides. The market for biopesticides based on essential oils faces regulatory barriers, strong competition from chemical insecticides, and a lack of information for rural producers (Isman, 2019). Despite this landscape, research on the properties of aromatic plants and their oils related to secondary metabolism and their repellent, deterrent, and toxic effects on different species of agricultural pests is still growing. These compounds produce neurotoxic, cytotoxic, phototoxic, and mutagenic effects on insects (REGNAULT-ROGER, 1997; Assadpour et al., 2023).

Biopesticides based on essential oils or their main active ingredients began to be commercialized over two decades ago in the United States. However, manyyears of inadequate and excessive use of chemical insecticides in agricultural fields have produced negative side effects on humans (Weisenburger, 1993), natural and agricultural ecosystems, and have selected resistant insect populations (REGNAULT-ROGER, 1997). On the other hand, natural products and their active ingredients have low toxicity to humans, soil, and the environment (Corrêa and Salgado, 2011; Assadpour et al., 2023) and produce few collateral effects on non-target organisms such as natural enemies and pollinators (Isman, 2019).

Currently, the biopesticide market is growing, and registration requirements are becoming clearer and more accessible (Giunti et al., 2022)(Giunti et al., 2022). The modern consumer is demanding in terms of chemical residues in food, and the market demands eco-friendly and sustainable agricultural production (Guedes et al., 2017; Isman, 2019; Giunti et al., 2022). Additionally, the excessive use of chemical insecticides has led to legal restrictions and bans in many parts of the world. This panorama makes necessary the ecotoxicological evaluation of this type of biopesticides to understand its environmental impact and open the doors for the inclusion of new molecules based on essential oils from plants as alternatives for pest insect control.

As a result of extensive research on essential oils, the repellent and deterrent properties of ornamental and garden plants such as *Ruta graveolens* and *Cymbopogon citratus* are now known (Ali et al., 2013; Perera et al., 2022; S. and B., 2023; Wadhwa et al., 2023), and the toxic properties on insects of noni seeds *Morinda citrifolia* have been revealed (Guilherme et al., 2023). In this regard, the present study aims to evaluate the toxic properties of different essential oils on *Euschistus heros*, one of the most important pests in soybean cultivation in Brazil, with the objective of increasing alternatives to the use of chemical insecticides and allowing the diversification of agroecosystems using more environmentally friendly control techniques.

2 Material & Methods

2.1 Rearing of insects

The brown soybean bug *E. heros* were obtained from a stock population reared at the laboratory of physiology and neurobiology of insects at the Federal University

of Viçosa – UFV, under controlled conditions (25 ± 2 °C, 10 h of scotophase). For all bioassays with *E. heros* it was used third instar nymph. For the tests with pollinators, Africanized bees *Apis mellifera* and stingless bees *Partamona helleri* were obtained from the UFV apiary. For tests with natural enemies, adult ladybugs of the species *Coleomegilla maculata* were obtained from a stock population reared at the laboratory of physiology and neurobiology of insects at the UFV. For all bioassays with natural enemies and pollinators, it was used adult's insects.

2.2 Mortality bioassays of *Euschistus heros* exposed to essential oils

The insecticidal potential of essential oils from *Ruta graveolens, Schinus terebinthifolius, Cymbopogon citratus* and *Morinda citrifolia* was evaluated. For the mortality bioassays, the third-instar *E. heros* nymphs were exposed, by tarsal contact, to oil residues impregnated in glass vials. A total of 10 replicates were performed, each consisting of 10 individuals, in a total of 100 individuals for each evaluated oil. Mortality assessments were performed at 24 hours of exposure. The control consisted of 10 glass vials impregnated with acetone. A dose-response curve was performed for *Morinda citrifolia* oil.

2.3 Statistical analyses

The toxicity data of essential oils on third instar nymphs did not support the assumption of normality. These data were analyzed through a Kruskal-Wallis test, using the Sigmaplot 14.5 software. Concentration-mortality bioassay data were subjected to a probit analysis (PROC PROBIT, SAS Institute 2010) to estimate the lethal concentrations of essential oils. Graphs were created in sigmaplot 14.5 software (Systat Software, Inc., San Jose, CA, USA).

3 Results

3.1 Mortality bioassays with essential oils

A total of four essential oils were evaluated with respect to their potential for toxicity on third instar nymphs of *E. heros* for 24 hours. The oils of *R. graveolens*, *C. citratus*, and *M. citrifolia* presented the highest mortality values for the 0.5% concentration (figure 1). Mortality of *E. heros* ranged from 90% to 100% (figure 1). However, the oil of *S. terebinthifolius* presented the lowest mortality value, with a percentage lower than 30% for the concentration of 0.5% (figure 1).

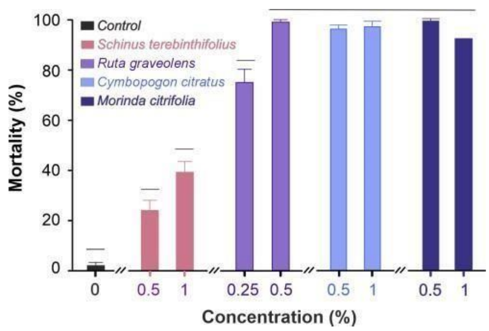

Figure 1.1: Toxicity of the essential oils of *S. terebinthifolius*, *R. graveolens*, *C. citrarus* and *M. citrifolia* on third instar nymphs of *E. heros*.

For the essential oil of *M. citrifolia*, a dose-response curve was carried out with the objective of knowing the lethal concentrations for 50% of the exposed insects. The Probit model satisfactorily described the data of concentration – mortality based on X^2= 2.03 and a P= 0.36, allowing to estimate an LC_{50}= 11.06 (9.05 - 13.07) nL/cm^2 (Figure 2).

3.2 Concentration mortality bioassay with *Morinda citrifolia*

The Probit model satisfactorily described the data of concentration – mortality based on X^2= 2.03 and a P= 0.36, allowing to estimate an LC $_{50}$ 11.06 (9.05 - 13.07) nL/cm^2 (Figure 2). The results obtained from the concentration-mortality bioassay with third instar nymphs of *E. heros*, exposed by tarsal contact to *M. citrifolia* oil are shown in figure 2.

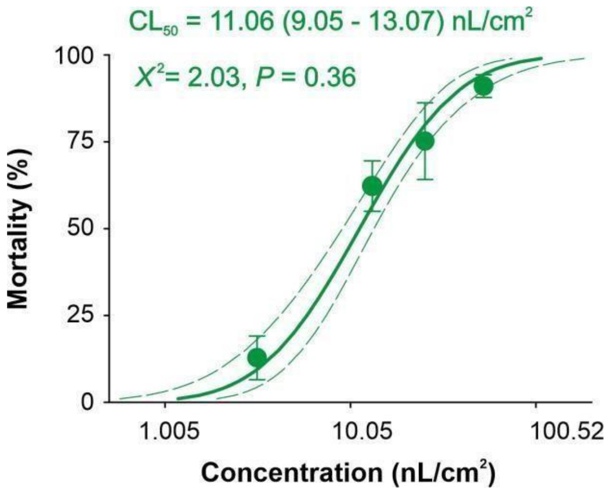

Figure 1.2: Concentration curve - mortality of the essential oil of *M. citrifolia* on third instar nymphs of *E. heros*. Plotted lines indicate 95% confidence intervals from the PROBIT analysis.

4 Discussion

In this chapter, the insecticidal potential of the essential oils from *R. graveolens*, *S. terebinthifolius*, *C. citratus*, and *M. citrifolia* on *E. heros* was evaluated. Third instar nymphs of *E. heros* were used because at this stage, the nymphs start causing damage to the crop by sucking on pods and seeds (Fernandes, 2017). Regarding toxicity potential, the oils from *R. graveolens*, *C. citratus*, and *M. citrifolia* showed significant mortality rates above 90% during the first 24 hours of exposure. The oil from *S. terebinthifolius* did not show any toxicity potential as it did not reach 50% insect mortality within 24 hours of exposure.

Other studies have shown the potential of essential oils from plants of the *Cymbopogon* genus as insect repellents and fungal growth inhibitors, even at concentrations as low as 0.1% (Caballero-Gallardo et al., 2012). Our study demonstrated the insecticidal potential of *C. citratus* *R. graveolens* and *M. citrifolia* resulting in 100% mortality within 24 hours of exposure at a concentration of 0.5%. These results are consistent with those of Moretti et al. (2002), who found toxicity of essential oils on pest insects at concentrations of 0.25%, 0.5%, and 1%.

It is expected that in developing countries, the demand for food by 2025 will increase by 50% to 100%. This poses a challenge for agricultural production, which needs to adopt sustainable and environmentally friendly production strategies (Assadpour et al., 2023). This scenario highlights the importance of exploring the bioactive properties of essential oils as a basis for the development of biopesticides, emphasizing their low toxicity to mammals and their low environmental persistence, which makes them compatible with pollinators and biological control agents.

Due to this low persistence, biopesticides based on essential oils are accepted as compatible with biological control agents and pollinators that may sporadically visit crop fields, such as bees (Assadpour et al., 2023). However, there is little knowledge about the collateral effects that biopesticides based on essential oils may have on non-target organisms (Haddi et al., 2020).

Currently, there has been a worrisome decline in pollinator fauna, which is affecting agriculture globally (Giunti et al., 2022). Therefore, it is recommended for future studies to conduct bioassays to evaluate the selectivity of the oils assessed here on biological control agents and pollinators that frequently visit soybean fields.

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Chapter 2

New synthetic molecules for the biorrational control of *Euschistus heros* (Hemiptera: Pentatomidae) and selectivity to natural enemies and pollinators

BERNAL-ZULUAGA, Jeny Tatiana, M.Sc., Universidade Federal de Viçosa, July 2023. **New synthetic molecules for the biorrational control of *Euschistus heros* (Hemiptera:Pentatomidae) and selectivity to natural enemies and pollinators**. Adviser: Eugênio Eduardo de Oliveira. Co-Adviser: Elson Santiago de Alvarenga.

Abstract

Euschistus heros is one of the most representative pests of the soybean crop in Brazil. This insect sucks content from pods and grains, directly damaging production. Brazil is the second largest producer of soybeans in the world and this crop is of great importance to its economy. The main control method for sucking insects are neonicotinoid insecticides, but their excessive use has generated resistance in insects and negative consequences for the natural environment and for man. This scenario makes it necessary to diversify control alternatives with different modes of action, more friendly to nature and less toxic to humans. For this reason, in this work the toxicity of molecules of synthetic origin was evaluated in third instar nymphs of *E. heros*. The molecules BEN, M-AN, 3F, AN, TO2, 4CL, 2CL and DICL were evaluated.

In addition, the impact of the DICL molecule on the pollinators *Apis mellifera* and *Partamona helleri* and on the predator *Coleomegilla maculata* was evaluated. For the evaluation of mortality of *E. heros* nymphs, concentration-mortality bioassays were performed, through tarsal contact of the insects with dry residues of the molecules in glass flasks. For the evaluation of the impact on pollinators, mortality bioassays were carried out through the consumption of contaminated diet and for the evaluation of the impact on a predator, concentration-mortality bioassays were carried out, through tarsal contact and the impact on the predatory capacity in the days following exposure. Significant values of toxicity of the DICL molecule on *E. heros* were found and no adverse effects on pollinators and natural enemies were found. Our results show the potential for the use of this molecule as an insecticidal tool for the biorational control of *E. heros*.

Keywords Tetronamides. Selectivity. Pollinators. Natural enemies.

1 Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most important crops in the world, itsseed has a high oil and protein content. It is used as animal feed, as a base to produce oil and various foods. In Brazil, soybean is one of the most important crops and contributes to a large extent to the country's economy (de Oliveira and de Souza e Silva, 2021). Soybean is a legume that develops successfully in a temperate climate, however, after many years of research and modification, the legume was successfully cultivated in the tropical climate of Brazil.

Despite its adaptation to the natural conditions of Brazil, soybeans still face limiting factors such as pests, among the most important are pod and grain sucking insects, which directly affect the quality and production of the crop. Among these insects, *Euschistus heros* represents one of the most limiting pests, both nymphs and adults suck the sap from pods and seeds, causing deformation and delay in the development of these organs (Correa-Ferreira and de Azevedo, 2002; Sosa-Gómezet al., 2020).

Neonicotinoid insecticides have become the most widely used control tool worldwide. These insecticides act on the insect's nervous system, binding to the postsynaptic acetylcholine receptors and causing the insect's death in a matter of hours. These insecticides have been successful on the market due to their low toxicity to birds and mammals. However, its excessive and inappropriate use has caused negative effects on non-target organisms, has contaminated both terrestrial and aquatic ecosystems, and has selected resistant insect populations that make their control difficult (Shivanand et al., 2023). In addition, these insecticides are being banned in some countries due to their adverse effects on non-target organisms such as pollinators and natural enemies (Bortolotto et al., 2015; Castellanos et al., 2018;Sahani et al., 2022; Somavilla et al., 2019, 2020).

The main cause of imbalance in ecosystems are the sublethal effects of the products used in agriculture, which leave residues in the long term, causing effects that are not lethal, but modify the structure of communities with small changes (Guedes et al., 2017). A widespread view that insecticides are meant to kill insects, not just repel or control them has also led to an overuse of unnecessary chemicals.

There is a gap in the knowledge of the side and sub-lethal effects of many active ingredients of currently marketed agricultural pesticides, including products of natural origin or with active ingredients of natural origin (Guedes et al., 2017). Modern agriculture needs to produce more food in eroded and scarce soils, for this reason, it is important to enrich integrated pest management (IPM) programs with control alternatives that use different modes of action, that delay the appearance of resistant organisms and that at the same time, they interact in a friendly way with the different factors that constitute the agrosystems (Karuppuchamy and Venugopal,2016).

For all this, it is necessary to discover new molecules with selectivity potential towards non-target organisms and lethal to sucking pests such as *E. heros*. In this work, a prospection of the insecticidal potential of tetronamide molecules derived from mucochloric acid was carried out in mortality bioassays with third instar nymphs of *E. heros* and the selectivity of these molecules in non-target organisms such as the neotropical pollinators *Apis mellifera* and *Partamona helleri* and the natural enemy *Coleomegilla maculata* was evaluated, to offer new biorational control tools for sucking pests in soybean fields.

2 Material & Methods

2.1 *Euschistus heros* mortality bioassays exposed to synthetic molecules

Ten synthetic molecules provided by the chemistry laboratory of the Federal University of Viçosa - UFV were evaluated. For mortality bioassays, third-instar *E. heros* nymphs were exposed, by tarsal contact, to residues of synthetic molecules (BEN, M-AN, 3F, DICL, AN, TO2, 4Cl, 2Cl, 2F, 3F, 4Br) in glass vials. A total of 10 replicates were performed, each consisting of 10 insects, for a total of 100 individuals for each evaluated molecule. Mortality assessments were performed at 24 and 48 hours of exposure. The control consisted of 10 glass vials impregnated with acetone. A dose-response curve was performed with the DICL molecule, which reached a mortality rate of 80%. The CL_{80} of the DICL molecule was used to evaluate the effects on pollinators and natural enemies.

2.2 Pollinator selectivity bioassays

The bee mortality bioassays were carried out at the UFV apiary. Bees from different hives were used to randomize individual and hive-related effects. Adults of *Apis mellifera* and *Partamona helleri* were exposed to a diet contaminated with the DICL molecule that shows potential toxicity on *E. heros* (in the previous bioassay). The bioassays had 5 replicates, each consisting of a pot with 10 bees, for a total of 50 individuals per treatment (contaminated and control). Consumption of the contaminated diet and insect mortality over time (1, 3, 5 and 24 hours) were evaluated. The control consisted of bees exposed to a clean feed mixed with solvent. DMSO was used as solvent.

2.3 Natural enemy selectivity bioassays

Adults of *Coleomegilla maculata* were exposed, by tarsal contact, to the DICL molecule, which shows potential toxicity on *E. heros* in the mortality bioassay. The bioassay consisted of 5 Petri dishes with contaminated filter paper and 5 control Petri dishes with solvent impregnated filter paper. Acetone was used as a solvent. Six individuals were placed in each Petri dish, for a total of 30 individuals per treatment (contaminated and control). Mortality was assessed over time.

2.4 Bioassays of predation ability of biological control agents exposed to insecticide

From the previous bioassay, 40 adults of ladybird *Coleomegilla maculata* were randomly selected for predation bioassay on *Ephestia kuehniella* eggs (Lepidoptera: Pyralidae). 20 ladybugs from the treatment with the DICL molecule and 20 insects from the control treatment was selected. The ladybugs were individualized in glass petri dishes and offered 20 mg of *E. kuehniella* eggs. The initial egg weight and the decrease in weight every two hours during the first 8 hours were recorded, then the 24-hour weight was evaluated and in the next 4 days the weight was evaluated every 12 hours, replacing the initial egg weight at each assessment. The weight loss of the eggs during the experiment was depreciated from the raw data at the time of analysis. The bioassays were carried out in the laboratory of physiology and neurobiology of insects at the UFV in a controlled environment (temperature and photoperiod).

2.5 Statistical analyses

The mortality data of non-target organisms exposed to the DICL molecule were analyzed using the Mann-Whitney rank sum test. Concentration-mortality bioassay data were subjected to a probit analysis (PROC PROBIT, SAS Institute 2010) to estimate the lethal concentrations of DICL molecules. Graphs were created in sigmaplot 14.5 software (Systat Software, Inc., San Jose, CA, USA).

3 Results

3.1 Toxicity bioassays with synthetic molecules

Of the 8 molecules evaluated in the experiment (BEN, M-AN, 3F, DICL, AN, TO2, 4CL and 2CL). Only the DICL molecule presented a mortality higher than 80%, which shows a potential effectiveness for the control of *E. heros*. The other molecules presented a mortality of less than 50% (figure 1).

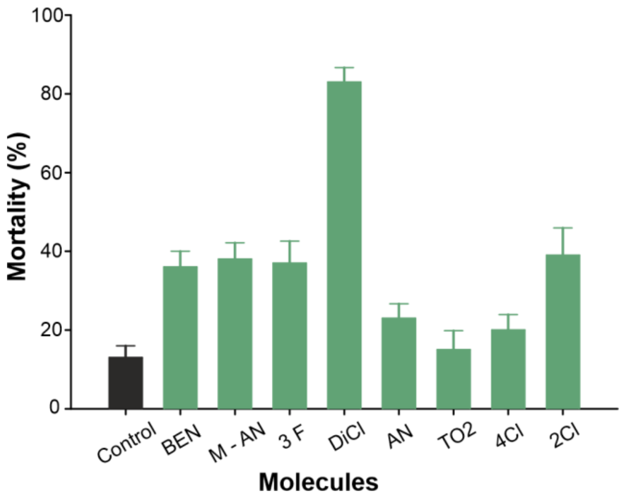

Figure 2.1: Susceptibility of *E. heros* (Nymphs) to molecules of synthetic origin in concentration of 0.033 nL/cm^2 (3000 ppm).

From the toxicity bioassays, the lethal concentrations for the DICL molecule were determined, calculating the dose-response curve. The PROBIT model satisfactorily described the concentration-mortality data with X^2= 0.39 and P= 0.82, allowing estimation of a LC_{50}= 0.019 (0.017 - 0.020) nL/cm^2 and a LC_{80} = 0.030 (0.027 - 0.037) nL/cm^2 (figure 2).

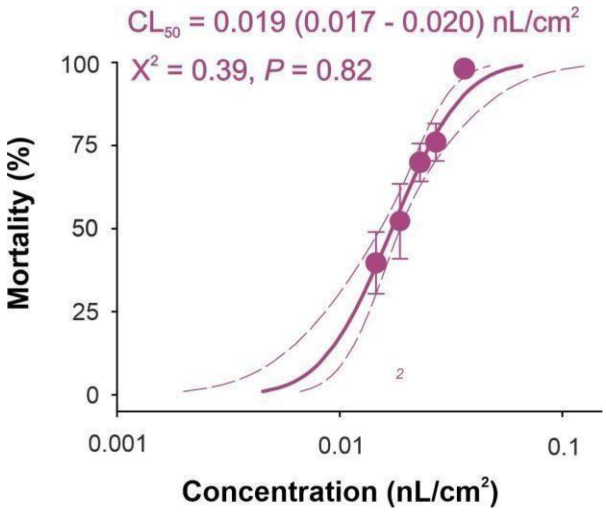

Figure 2.2: Mortality of third instar nymphs of *E. heros* in contact with the DICL molecule at a concentration of 0.033 nL/cm2. The dashed lines indicate the 95% confidence interval of the Probit analysis.

From the dose-response curve of the DICL molecule, the LC_{80} (minimum concentration allowed for selectivity tests in non-target organisms) was chosen to evaluate the selectivity in the pollinators *Apis mellifera* and *Partamona helleri* and in the biological control agent *Coleomegilla maculata* . In this way, pollinators and the biological control agent were subjected to a concentration of 0.027 nL/cm^2.

This latter concentration was used for selectivity bioassays on pollinators commonly found in soybean fields, such as the Africanized bee *A. mellifera* and the stingless bee *P. helleri*, and selectivity and predatory capacity were evaluated with the biological control agent *C. maculata*. From the selectivity bioassays, it was demonstrated that the molecule DICL did not cause significant mortality in *A. mellifera* (P= 1.0), *P. helleri* (P= 0.69), or *C. maculata* (P= 1.0).

From the analysis of repeated measures over time with the pollinators, a significant effect was only found for time (F = 125.59; $P < 0.0001$), time in interaction with species (F = 33.56; $P < 0.0001$), and between species (F = 376.63; $P < 0.0001$). However, no significant effect of the molecule DICL (F = 0.27; P = 0.60) or of the molecule in interaction with species (F = 1.28; P = 0.27), or the molecule in interaction with time and species (F = 0.23; P = 0.87) was found (Table 1).

Table 2.1: Analysis of variance with repeated measures over time for 24 hours, including 5 hours exposure to DICL (2480 ppm) treated sugar syrup, with neotropical pollinator bees *Apis mellifera* and *Partamona helleri*.

Source of variation	*df*	*F*	*P*
Between samples			
DICL (D)	1	0.27	0.6086
Species (S)	1	376.63	<0.0001*
D X S	1	1.28	0.2741
Error	16	–	–

	df_{den} / df_{num}	*Wilks lambda*	*f*	*P*
Within samples				
Time (T)	14/3	0.036	125.59	<0.0001*
T X D	14/3	0.752	1.54	0.2483
T X S	14/3	0.122	33.56	<0.0001*
T X D X S	14/3	0.952	0.23	0.8718

*Significant at $P < 0.05$.

Exposure of Africanized *Apis mellifera* bees to the DICL molecule through dietary intake did not result in significant mortality with respect to the control treatment (Figure 3A). The percentages of mortality both in the control treatment and in the treatment with DICL, were less than 10% (figure 3A). Figure 3B shows that the DICL molecule does not have a significant effect on diet consumption compared to the control treatment during the 24 hours of exposure.

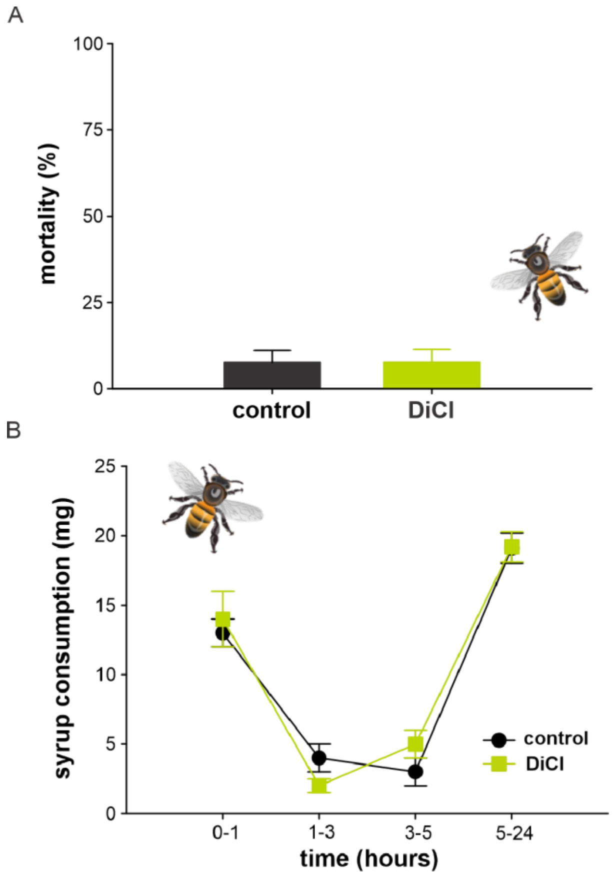

Figure 2.3: Selectivity of the DICL molecule in the Africanized bee *Apis mellifera* during 24 hours of exposure. (A) susceptibility of bees to the DICL molecule and (B) Consumption of diet contaminated with the DICL molecule.

In general, the results suggest that the DICL molecule does not exert a significant effect on mortality or on the diet consumption of the Africanized bee *Apis mellifera*.

Exposure of *Partamona helleri* stingless bees to the DICL molecule through dietary consumption did not result in significant mortality with respect to the control treatment (Table 1). Mortality rates in both DICL treatment and control were less than 5% during the first 5 hours of evaluation (Figure 4A). Figure 4B shows that the DICL molecule does not have a significant effect on diet consumption compared to the control treatment during the 24 hours of exposure (Table 1). In general, the results suggest that the DICL molecule does not exert a significant effect on mortality or on the diet consumption of the stingless bee *Partamona helleri*.

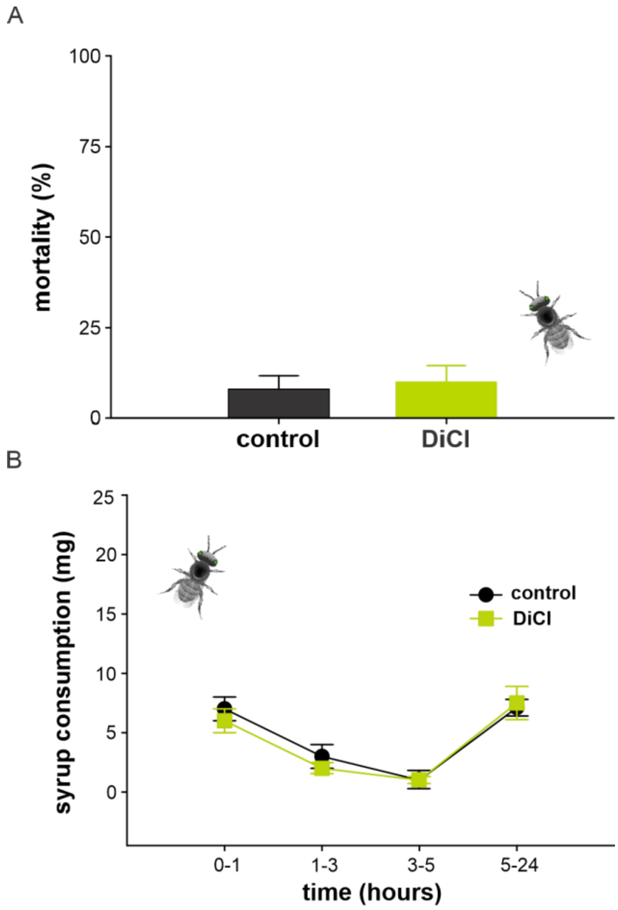

Figure 2.4: Selectivity of the DICL molecule in the stingless bee *Partamona helleri* during 24 hours of exposure. (A) susceptibility of bees to the DICL molecule and (B) Consumption of diet contaminated with the DICL molecule.

The tarsal contact mortality bioassay of the biological control agent *Coleomegilla maculata* with the DICL molecule resulted in 100% survival of the insects (figure 5).

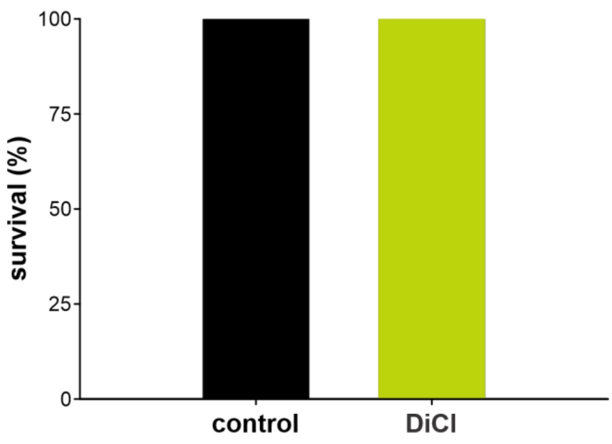

Figure 2.5: Survival of *Coleomegilla maculata* exposed to solid residues of the DICL molecule.

The consumption of *E. kuehniella* eggs by the predator *C. maculata* was not affected by exposure to the DICL molecule (Table 2, Figure 8).

Regarding the analysis of repeated measures over time for the biological control agent *C. maculata* in the first 24 hours, a significant effect was only found for time (F = 14.54; $P < 0.0001$). However, no significant effect of the molecule DICL (F = 3.21; P = 0.08) or of the molecule in interaction with time (F = 0.62; P = 0.65) was found (Table 2).

Table 2.2: Analysis of variance with repeated measures over time for 24 hours, including previous exposure to DICL (2480 ppm, with the predator *Coleomegilla maculata*.

Source of variation	*df*	*F*	*P*
Between samples			
DICL (D)	1	3.21	0.0813
Error	38	–	–

	df_{den} / df_{num}	*Wilks lambda*	*f*	*P*
Within samples				
Time (T)	35/4	0.376	14.54	<0.0001*
T X D	35/4	0.934	0.62	0.6515

*Significant at $P < 0.05$.

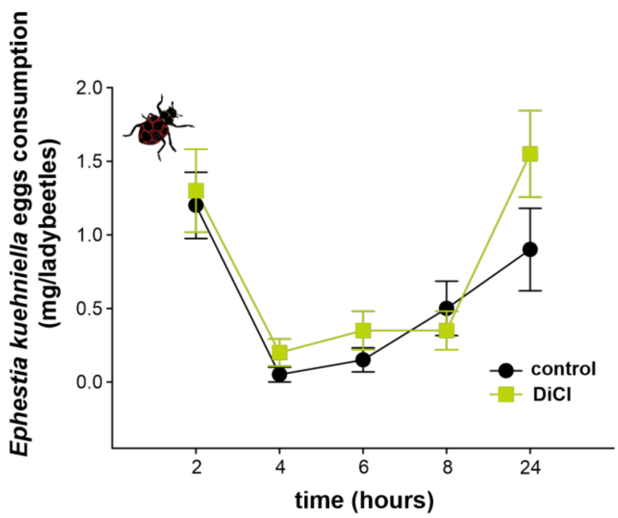

Figure 2.6: Consumption of *E. kuehniella* eggs by *C. maculata* for 24 hours after exposure with the DICL molecule.

The egg predation bioassay continued for 4 days after DICL exposure (Table 3, Figure 9).

During the following 4 days of evaluation, the results were similar to those of the 24-hour assessment. Only a significant effect was found for time (F = 24.61; $P < 0.0001$), and there was no significant effect of the molecule DICL (F = 0.54; P = 0.46)or the interaction of the molecule over time (F = 1.36; P = 0.25) (Table 3).

Table 2.3: Analysis of variance with repeated measures over time for 96 hours after the first 24 hours, including previous exposure to DICL (2480 ppm), with the predator *Coleomegilla maculata*.

Source of variation	*df*	*F*	*P*
Between samples			
DICL (D)	1	0.54	0.4656
Error	38	–	–

	df_{den} / df_{num}	*Wilks lambda*	*f*	*P*
Within samples				
Time (T)	32/7	0.157	24.61	<0.0001*
T X D	32/7	0.770	1.36	0.2537

*Significant at $P < 0.05$.

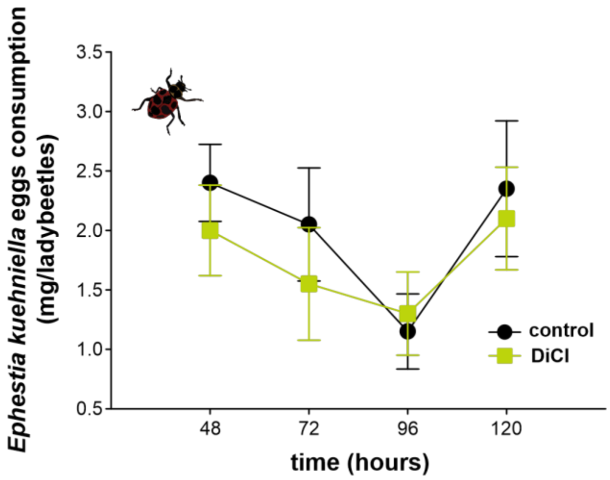

Figure 2.7: Consumption of *E. kuehniella* eggs by *C. maculata* for 4 days after exposure with the DICL molecule.

In general, these results suggest that exposure to the DICL molecule does not cause lethal effects or affect the predatory capacity of *C. maculata*.

4 Discussion

Our results from the 8 molecules evaluated showed the potential for toxicity of the DICL molecule on third instar nymphs of *E. heros*. This molecule showed high toxicity towards *E. heros* with an estimated LC_{50} of 0.019 nL/cm^2 and showed selectivity with the pollinators *Apis mellifera* and *Partamona helleri* and the biological control agent *Coleomegilla maculata* exposed to an LC_{80} = 0.027 nL/cm^2.

From the analysis of repeated measures over time with pollinators, our results showed a mortality rate of less than 10% for *A. mellifera* and less than 5% for *P. helleri*, and no significant differences were found in diet consumption between the treatment with the DICL molecule and the control for the two species of pollinators. Our results are consistent with those of Esteves et al. (2023) who found no significant effect on mortality or diet intake for *A. mellifera* and *P. helleri* exposed to *Persea venosa* essential oil.

Regarding the evaluations of mortality and predatory capacity of *C. maculata*, our results show that the DICL molecule does not have toxic effects on this biological control agent (Figure 7) and does not affect the predatory capacity after its exposure with the molecule (Figures 8 and 9). These results are consistent with those found by Toledo et al. (2020) (2020) who found no significant effect of clove essential oil on mortality and behavior (locomotor and predatory) of *C. maculata*.

Overall, our results demonstrate that the molecule DICL is a tool with biorational potential for the control of *E. heros* due to its high mortality percentage towards this pest and its potential selectivity with pollinators, as it does not affect their diet consumption over time among the evaluated species, nor does it affect the predatory capacity of the biological control agent *C. maculata*. *Apis mellifera* is an important pollination agent in soybean fields, helping with the maintenance of production.In modern agriculture, the selectivity of a pesticide with non-target organisms is a desired characteristic, taking into account the decline of entomofauna in agricultural fields and the loss of ecosystem services such as pollination and natural pest control

(Ndakidemi et al., 2016; Giunti et al., 2022).

Taking into account that currently commonly used chemical insecticides exert negative sublethal effects on natural predators such as ladybugs (He et al., 2019), the current awareness of the consumer market and the constant demand for eco-friendly agriculture (Guedes et al., 2017; Giunti et al., 2022), it is necessary to discover new molecules and compounds with desired characteristics such as acute toxicity for pest organisms and selectivity with non-target organisms, mainly those that provide ecosystem services in agricultural fields, such as pollination and natural pest control (Giunti et al., 2022).

To achieve an eco-friendlier soybean crop, it is necessary to discover new alternatives to the use of neonicotinoid insecticides to control pod and seed sucking insects. Although there are a large number of alternatives, these compounds are generally not highly effective individually, but have shown additive and synergistic effects with other pest control methods Verheggen et al. (2022). For these reasons, it is recommended for future studies, a more holistic view that considers the ecotoxicological parameters for both target and non-target organisms, that considers the interaction between species, and that considers both direct and indirect effects within the ecosystem.

In the same way that Haddi et al. (2020) conclude, our study highlights the importance of ecotoxicological studies not only on potential conventional pesticides but also on potential biorational pesticides, to avoid unwanted effects on the environment, on non-target organisms such as other pests, predators, parasitoids and pollinators and avoid unwanted effects on human and environmental health.

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