University of São Paulo
College of Agriculture “Luiz de Queiroz”

Bioactive insectistatic compounds from Solanaceae against *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae)

Gabriel Luiz Padoan Gonçalves

Dissertation presented to obtain the degree of Master in Science. Area: Entomology

Piracicaba
2018

Gabriel Luiz Padoan Gonçalves
Agricultural Engineer

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **JOSÉ DJAIR VENDRAMIM**

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2018

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Gonçalves, Gabriel Luiz Padoan

Bioactive insectistatic compounds from Solanaceae against *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae: Bruchinae) / Gabriel Luiz Padoan Gonçalves. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011 - - Piracicaba, 2018.

121 p.

Dissertação (Mestrado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Bioprospecção 2. Caruncho-do-feijão 3. Inseticidas botânicos 4. Metabolitos secundários 5. Cromatografia 6. Ácidos graxos. I. Título

RESUMO

Compostos insetistáticos de Solanaceae bioativos contra *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae: Bruchinae)

As plantas produzem um ampla gama de compostos químicos insetistáticos para sua própria proteção contra os danos causados por insetos fitófagos. Tais compostos podem prejudicar os insetos nas fases de ovo, ninfa, larva e adulto promovendo mortalidade, repelência, fagodeterrência, deformações morfológicas, prolongamento do desenvolvimento, redução da absorção de nutrientes etc. Compostos bioativos de plantas têm sido usados para o controle de insetos-praga na agricultura desde a antiguidade, mas durante a Revolução Verde muitos deles foram substituídos por inseticidas sintéticos como os organoclorados, organofosforados, carbamatos e piretroides. No entanto, o uso excessivo e irracional dos inseticidas sintéticos na agricultura gerou problemas ambientais, de saúde e a seleção de populações resistentes de insetos. Como uma resposta a esses aspectos negativos dos inseticidas sintéticos, foram elaboradas regulamentações legais mais rigorosas para o registro de defensivos agrícolas nas agencias regulatórias governamentais resultando em custos mais elevados para o desenvolvimento de novos inseticidas sintéticos comerciais. Inevitavelmente, isso incentivou pesquisas científicas para a bioprospecção de compostos insetistáticos com o intuito de se descobrirem inseticidas mais seguros com novos modos de ação. Portanto, no presente estudo, foi realizada uma triagem com 25 extratos etanólicos provenientes de 17 espécies de Solanaceae para avaliar a bioatividade deles sobre o caruncho-do-feijão, *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae: Bruchinae). Os extratos etanólicos das folhas de *Solanum lycocarpum* A. St.-Hil e de flores de *Brugmansia suaveolens* (Willd.) Bercht. & J.Presl foram os melhores dentre todos; dessa forma eles foram selecionados para uma série de fracionamentos químicos (Extração em Fase Sólida) com base em bioensaios toxicológicos para identificar compostos bioativos. As frações químicas de *S. lycocarpum* mataram os adultos de *Z. subfasciatus*, reduziram o número de ovos (devido à deterrência de oviposição e redução na fecundidade), redução na progênie F_1 e nos danos nos grãos de feijão, e também reduziu a fecundidade da progênie F_1. A bioatividade promovida pelas frações de *S. lycocarpum* se deve, possivelmente, à presença de vitanolídeos e alcaloides. Por outro lado, as frações bioativas de *B. suaveolens* promoveram um efeito ovicida inibindo o desenvolvimento embrionário nos ovos de *Z. subfasciatus*. Consequentemente, a progênie F_1 e os danos nos grãos também foram completamente inibidos. Além disso, tais frações também promoveram mortalidade dos adultos de *Z. subfasciatus*, que demostraram sinais de hiperexcitação, um sintoma característico de inseticidas neurotóxicos. Na fração bioativa BSHidAcF1-1-C foram identificados derivados de ácidos graxos por meio de Cromatografia Gasosa com Espectrometria de Massas. Os resultados adquiridos no presente estudo demonstram o potencial de prospectar compostos insetistáticos de espécies da família Solanaceae para proteger grãos de feijão armazenado contra os danos promovidos por *Z. subfasciatus* em armazéns.

Palavras-chave: Bioprospecção; Caruncho-do-feijão; Pesticidas botânicos; Metabolitos secundários; Cromatografia.

ABSTRACT

Bioactive insectistatic compounds from Solanaceae against *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae: Bruchinae)

Plants produce a wide range of insectistatic phytochemicals for protection against the damages caused by phytophagous insects. They can harm insect eggs, nymphs, larvae and adults promoting mortality, repellency, phagodeterrence, morphological deformations, prolongation of development, reduce nutrient-intake etc. Plant bioactive compounds have been used to control insect pests in agriculture since ancient times, but during Green Revolution many of them were replaced by synthetic compounds such as organochlorines, organophosphates, carbamates and pyrethroids. Nonetheless, the excessive and irrational use of synthetic insecticides in agriculture led to environmental and health issues and the selection of resistant insect pest populations. As a response to these negative aspects of synhtetic insecticides, it was developed stringent regulatory laws and requirements for pesticide registration at Governmental Regulatory Agencies resulting in higher costs to develop new commercial synthetic insecticides. Inevitably, scientific studies with bioprospection of insectistatic compounds from plants have grown in order to discover safer insecticides with new modes of action. Therefore, in the present study, it was performed a screening with 25 ethanolic extracts from 17 Solanaceae species in order to evaluate their bioacticity against the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae: Bruchinae). Ethanolic extracts from leaves of *Solanum lycocarpum* A. St.-Hil and flowers of *Brugmansia suaveolens* (Willd.) Bercht. & J.Presl were the best ones; thereby they were selected for bioguided fractionations (Solid Phase Extraction) based on toxicological bioassays in order to identify their bioactive compounds. The chemical fractions from *S. lycocarpum* killed adults of *Z. subfasciatus*, reduced the number of eggs per sample (due to oviposition deterrence and the decrease of fecundity), and reduce F_1 progeny and damages on bean grains, and also reduced the fecundity of F_1 progeny. The observed bioacticity promoted by fractions of *S. lycocarpum* is possibly due to the presence of whitanolides and alkaloids. On the other hand, bioactive fractions from *B. suaveolens* promoted an intense reduction on egg-adult viability preventing the embryonic development in *Z. subfasciatus* eggs. Consequently, F_1 progeny and damages on bean grains were completely inhibited as well. Moreover, they also killed adults of *Z. subfasciatus*, which demonstrated signs of hyperexcitation, a symptom related to neurotoxic insecticides. It was identified fatty acid derivatives in the bioactive fraction BSHidAcF1-1-C through a Gas Chromatography Mass Spectrometry analysis. The results acquired in the present study demonstrate the potential of prospecting insectistatic compounds in Solanaceae species in order to protect bean grains against the damages promoted by *Z. subfasciatus* in warehouses.

Keywords: Bioprospection; Mexican bean weevil; Botanical pesticides; Secondary metabolites; Chromatography.

1. INTRODUCTION

A constant concern not only for agriculture, forestry, livestock and food scientists but also for all humankind is the future situation of land-use for food production. Humanity has to sustainably produce enough food for 9 billion people in 2050 under a scenario of climate changes that will reshape agriculture, forest, pasture and urban landscape worldwide (Cohen 2003, Ewert et al. 2005, Godfray et al. 2010, Schmitz et al. 2014). Thereby, designing sustainable farm systems that not excessively rely on non-renewable resources and maintain the functionality of agroecosystems for future generations is a priority goal reaching all spheres related to food production, considering its technical, social, economic and political aspects (Reganold and Wachter 2016). Concerning technical aspects, Integrated Pest Management (IPM) will play an important role in this journey for producing food for billions of human beings because the attack of insect-pests result in tremendous losses of food every year (Oerke 2006, Akoijam et al. 2014).

The problems related to quantitative and qualitative damages on stored grains promoted by insects date back to antiquity. Consequently, the elaboration of controlling methods for these pests has emerged more than 3,000 years ago with the use of ash and soil dust (dehydration and mechanical damage), plant material (resins, powders etc.) and sulfur dioxide for fumigation and repellency of different stored grain pests (Levinson and Levinson 1998). Bean beetles species (Coleoptera: Chrysomelidae: Bruchinae), mainly from the genera *Acanthoscelides*, *Callosobruchus*, *Caryedon* and *Zabrotes*, are important pests of stored dried legumes [beans, peanuts and groundnuts (*Vigna*, *Phaseolus*, *Glycine* etc.)] worldwide (Southgate 1979, Johnson 1981). The Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae), is an important pest of stored dried beans (*Phaseolus vulgaris*) in tropical areas and the Mediterranean region (Southgate 1979, Abate and Ampofo 1996, Tuda 2007). The larvae of the Mexican bean weevil feeds on bean grains (*Phaseolus vulgaris* L.) damaging up to 99.3% of stored beans in warehouses (Barbosa et al. 2000). Currently, there are few registered insecticides to control *Z. subfasciatus* infestations in Brazil. They are aluminum and magnesium phosphide for fumigation and deltamethrin (pyrethroid) as a grain protector (Agrofit 2017). Therefore, it is important to develop control methods that not only kill adults of *Z. subfasciatus* but also prevent its larvae to penetrate bean grains. Studies concerning the use of plant-based insecticides and resistant plant varieties have been performed in order to minimize the damages promoted by such pest's larvae (Ribeiro-Costa et al. 2007, Luethi et al. 2013, Gonçalves et al. 2015, Goncalves et al. 2017). However, currently, synthetic insecticides (pyrethroids and phosphine) are the major adopted tool to control insect-pests of stored products, but unfortunately there are resistant insect-pest populations for some insecticides (Chaudhry 1997, Zettler and Arthur 2000, Pimentel et al. 2010, Boyer et al. 2012). Furthermore, the indiscriminate use of synthetic pesticides has promoted a wide range of harms to human health and environment; what instigated a debate concerning their use, necessity, relevance, improvement and compatibility with other pest management methods (Aktar et al. 2009).

For centuries, agriculture has been structured on polyculture, crop rotation, fallow, and the gradual and unconscious selection of varieties adapted to the specific characteristics of productive microregions (Mazoyer and Roudart 2006) that favor IPM and reduce the dependence on insecticides. In this context, inorganic insecticides (e.g. inorganic sulfur) and insecticidal plants have played an important role in pest control (Oberemok Volodymyr et al. 2015), what can now be recovered for farm systems aiming sustainability. Nonetheless, in order to replace environmentally aggressive molecules of synthetic insecticides by plant-based insecticides, it is necessary to perform bioprospection studies (toxicological bioassays along with chemical separation techniques) in order to isolate and identify new insecticidal compounds from plants.

As a response to the need of discovering new active ingredients and developing new insecticides, the research with botanical insecticides has shown a constant ascendancy in the number of publications since 1980 (Isman and Grieneisen 2014). Considering articles about insecticides, 61 (1.43% of total) publications on botanical insecticides were registered in 1980, and 1207 (21.38%) in 2012 (Isman and Grieneisen 2014). The demand for new plant-based insecticides is already illustrated at the data concerning new active ingredients registered at *Environmental Protection Agency* (EPA); between 1997 and 2010 the new active ingredients of insecticides registered in EPA included 67.9% of synthetics, 21.4% of synthetics derived from natural products, and 10.7% of natural products (Cantrell et al. 2012). Moreover, two plant-based classes of insecticides, neonicotinoids [from *Nicotiana tabacum* (Solanaceae)] and pyrethroids [*Tanacetum cinerariaefolium* (Asteraceae)] represent 27% and 9% of total sales of insecticides in the world, respectively (Cantrell et al. 2012, Sparks and Nauen 2015).

Unfortunately, Earth have suffered a high rate of deforestation in the last few centuries resulting in an immensurable loss of genetic patrimony that could have been explored to discover and develop new synthetic and botanical insecticides (Hansen et al. 2013), therefore, bioprospection can be considered not only the contemporary “gold rush” but also a race against time. In this context, Brazil, as the owner of an enormous plant genetic diversity, with more than 56,000 catalogued plant species (Giulietti et al. 2005), can assume a leading role in such studies. The Solanaceae botanical family includes several species of economic relevance and is widely present in both the temperate and tropical zones, with around 2,300 species distributed in 92 genera (Martins and Barkman 2005). In Brazil, there are 450 species (150 endemic ones) of Solanaceae distributed in 31 genera (Giulietti et al. 2005). Such family presents a great diversity of alkaloids with direct application in the control of agricultural pests, both in the form of botanical insecticides, e.g. nicotine, as well as its synthetic derivatives, e.g. neonicotinoids (Elbert et al. 2008, El-Wakeil 2013). Therefore, the Solanaceae family is a promising source of secondary metabolites (whitanolides, capsinoides, alkaloids and flavonoids) with insecticidal properties suitable for both the formulation of botanical insecticides and synthetic insecticides with novel mechanisms of action (Silva et al. 2003, Veleiro et al. 2005, Luo et al. 2011). Thus, in the present dissertation it was conducted a series of chemical separations and toxicological bioassays in order to evaluate the bioactivity of chemical extracts and fractions from Solanaceae against *Z. subfasciatus*, and identify the classes of chemical compounds responsible for the observed bioactivity.

2. CONCLUSIONS

2.1. Ethanolic Extracts

- Ethanolic extracts from Solanaceae reduced the number of eggs per sample and the egg-adult viability and, consequently, reduced the F_1 progeny and the damages on bean grains promoted by *Z. subfasciatus*, but none of them interfered on sex ratio.

- Ethanolic extracts from leaves of *Solanum lycocarpum* A. St.-Hil and flowers of *Brugmansia suaveolens* (Willd.) Bercht. & J. Presl promoted the most promissory effects on *Z. subfasciatus.*

2.2. *Solanum lycocarpum*

- *S. lycocarpum* presents chemical compound(s) that can kill adults of *Z. subfasciatus*, reduce the number of eggs per sample (due to oviposition deterrence and decrease of fecundity), and reduce F_1 progeny and damages on bean grains, and reduced the fecundity of F_1 progeny.

- Chemical analysis with high-performance liquid chromatography with mass spectrometry (HPLC-MS) with UV detector and their comparison with the database *Dictionary of Natural Products* indicated the possible presence of withanolides and alkaloids in the bioactive fractions SLHexF7Ac-1-A and SLHexF7Ac-1-B.

2.3. *Brugmansia suaveolens*

- Bioactive fractions of *B. suaveolens* expressed their effects mainly by preventing the embryonic development of *Z. subfasciatus* and inhibiting its egg`s chorion to form. Consequently, F_1 progeny and damages on bean grains were completely inhibited.

- Some fractions also killed adults of *Z. subfasciatus*, which demonstrated signs of hyperexcitation, a symptom related to insecticidal compounds interfering on insect nervous system.

- The fraction BSHidAcF1-1 (150 mg Kg^{-1}), for example, killed 56% of adults of *Z. subfasciatus*, promoted eggs deterrence, and drastically reduced egg-adult viability.

- Gas chromatography mass spectrometry analysis performed with the fraction BSHidAcF1-1-C revealed the presence of fatty acids derivatives in this fraction.

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