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

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

Piracicaba
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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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I dedicate this work to my mother, Maria de Fátima Padoan,
who always supported me in my decisions.

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SUMMARY

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.

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2. LITERATURE REVIEW

2.1. Botanical insecticides

For centuries botanical insecticides (composed by insecticidal phytochemicals) have been used for pest management of insect-pests in the form of plant extracts, vegetable powders and essential oils (Pavela 2016). They interact with insects through residual contact, ingestion and fumigation, and promote various effects on insects, such as mortality, repellency, disturbances in larval development, and reduction in adult fecundity and fertility (Isman 2006, Cantrell et al. 2012, Ansante et al. 2015, Ansante et al. 2017, Gonçalves et al. 2017). One of the earliest records of the use of botanical insecticides for pest control is the powder of chrysanthemum flowers, *Tanacetum cinerariaefolium* (Asteraceae), used in Persia around 400 B.C. to control human ectoparasites (Pavela 2016). The insecticidal effect of this powder is due to the presence of pyrethrins with neurotoxic action destabilizing the ion exchange in the sodium channels in the insect axiom nerve (Cantrell et al. 2012, El-Wakeil 2013). In ancient Rome aromatic plants such as rosemary (*Rosmarinus officinalis*), mirra [resin extracted from *Commiphora* (Burseraceae)] and juniper [*Juniperus* (Cupressaceae)] species) were used for insect-pest management (Pavela 2016). In the seventeenth century began the use of tobacco extract for insect-pest control due to the presence of nicotine. Nicotine, an alkaloid from the Solanaceae *Nicotiana tabacum*, *Nicotiana glauca* and *Nicotiana rustica* binds to acetylcholine receptors on the postsynaptic neuron promoting hyperexcitation and loss of muscle coordination by the insect (El-Wakeil 2013). In the nineteenth century, around 1850, the isoflavonoid rotenone began to be used in pest control (Pavela 2016). Coming from the roots and rhizomes of *Derris* spp. (Fabaceae) and *Lonchocarpus* spp. (Fabaceae), rotenone binds to NADH:Q oxireductase and prevents the oxidation of $NADH_2$ in the electron transport chain of the Complex I of the mitochondria, thus blocking insect respiration (Yamamoto 1970). From 1939, when the Swiss chemist Paul Müller discovery the insecticidal properties of DDT, synthetic insecticides became the main tool used to control agricultural pests (Oberemok et al. 2015). Consequently, insecticides from plant sources were largely replaced and reallocated to a secondary position in chemical control of major agricultural pests; their use remained mainly to control insect-pests in organic agriculture, greenhouses, gardens and repel insects vectors of diseases (Schmutterer 1990, Isman 2006). However, due to the negative effects promoted by the inappropriate and excessive use of synthetic insecticides, there was a resurgence of interest

in insecticides less aggressive to human health, more selective to non-target organisms, and capable of controlling resistant pest populations (Aktar et al. 2009).

Since the second half of the twentieth century, other botanical insecticides have been discovered and formulated, and there are currently several commercial products registered in different countries for pest control, in addition to those already mentioned above. The main ones are: (i) neem, *Azadirachta indica* (Meliaceae), a botanical insecticide with several commercial products in different countries containing several limonoids (tetranortriterpenoides) such as azadirachtin that is capable of promoting food deterrence and interference on the synthesis and release of ecdysteroids by insects (Schmutterer 1990). (ii) Sabadilla, which comes from the seeds of *Schoenocaulon*, mainly from *Schoenocaulon officinale* (Melanthiaceae), presenting the neurotoxic alkaloids cevadine and veratridine (El-Wakeil 2013). It is annually applied in avocado and citrus fields by organic farmers in California (Isman 2006). (iii) *Ryania speciosa* (Salicaceae), a shrub native to South America whose roots and branches are used to produce a botanical insecticide containing rianodine, an alkaloid, which binds to the calcium channels of the endoplasmic reticulum interfering with the flow of calcium ions into the cells (Nauen 2006). (iv) Garlic, *Allium sativum* (Liliaceae), presenting repellent sulfur compounds (Katz et al. 2008, Pavela 2016). (v) The pongam oil from *Pongamia pinnata* (Fabaceae) presenting karanjin that acts as growth regulator, sterilant and repellent (Pavela 2012, Pavela 2016). (vi) Two commercial botanical insecticides formulated in India from Annonaceae species: Anosom™, from seed extracts of *Annona squamosa* and *Annona reticulata* with 1% squamocin, and Bio Rakshak™, produced from leaves of *A. squamosa* (Ribeiro de Souza et al. 2009, Isman and Seffrin 2014). Finally, several essential oils are used in formulations of botanical insecticides, especially *Rosmarinus officinalis*, *Mentha* spp., *Cymbopogon schoenanthus*, *Thymus vulgaris*, *Syzygium aromaticum*, and *Citrus* spp. (Pavela 2016).

2.2. Secondary phytochemicals with insecticidal activity

Throughout their evolutionary process plants were selected to present sophisticated defense mechanisms against herbivores and pathogens expressed biophysically (cutin, wax and suberin) and biochemically (allelochemicals from their secondary metabolism) (Wink 2003). Secondary phytochemicals can affect insect-herbivore behavior, biology and physiology by acting as repellents, food deterrents, oviposition deterrents, reducing digestibility of plant tissue, reducing adult fertility, interfering in molting process, and promoting morphological

deformations and mortality (Hartmann 2007). The research and literature concerning insecticidal compounds from plants is constantly increasing (Isman and Grieneisen 2014); however, it has been reported in literature, at least, 119 different chemical compounds from plants that present insecticidal activity (Boulogne et al. 2012). Most of them are terpenoids (37%), alkaloids (30%) and phenolic compounds (20%), followed by lipids, sulfur compounds, aldehydes, proteins, amides, aromatic hydrocarbons, inorganic acids and polyacetylenes, consecutively (Boulogne et al. 2012).

The secondary metabolites are separated into three chemically distinct groups according to their biosynthesis: terpenes, phenolic compounds, and nitrogen compounds. The terpenes with insecticidal action are pyrethroids, limonoids (triterpenes), essential oils (mixtures of monoterpenes and volatile sesquiterpenes), phytoecdysteroids (steroids capable of disrupting insect ecdysis), cardenolides, and saponins (steroids and glycosidic triterpenes) (Bennett and Wallsgrove 1994, Mithofer and Boland 2012). The main phenolic compounds that negatively affect insects are lignins (phenolic polymers) that help in the mechanical support of the plant and protection against herbivores, tannins (phenolic polymers) that reduce the palatability, digestibility and attractiveness of the plant to herbivores, and isoflavonoids (rotenoids with insecticidal action) (Bennett and Wallsgrove 1994, Mithofer and Boland 2012). Last but not least, nitrogen compounds include a wide variety of chemical compounds with insecticidal activity, such as alkaloids (compounds with heterocyclic rings containing N and C), cyanogenic glycosides (releasing hydrocyanic acid), and glucosinolates (present in the Brassicaceae family) (Bennett and Wallsgrove 1994, Mithofer and Boland 2012).

2.3. Secondary phytochemicals in Solanaceae

A recent review listed that 656 plant species reported with insecticidal action were distributed in 110 different botanical families (Boulogne et al. 2012). Lamiaceae, a botanical family including many aromatic plants, accounts for 28% of plant species with insecticidal activity; and it is followed by the families Fabaceae, Asteraceae, Apiaceae and Solanaceae, consecutively (Boulogne et al. 2012). The Solanaceae botanical family is widely present in both the temperate and tropical zones producing a big diversity of secondary phytochemicals that might be used as active ingredients for insecticides (Martins and Barkman 2005). The classic example is nicotine, an alkaloid from *N. tabacum* that was used as a botanical insecticide and as model molecule for synthesizing analogous compounds (Pavela 2016). In addition, the

nornicotine and anabasin alkaloids are found in *Nicotiana glutinosa* and *N. glauca* (Rosell et al. 2008). These compounds served as prototypes for developing neonicotinoids, a major insecticide class presenting systemic and translaminar action with excellent efficacy against insect pests, acting as nicotinic acetylcholine receptor agonists (Elbert et al. 2008). Solanaceae family also produces steroidal alkaloids, glycosylated alkaloids like solasonin present in *Solanum crinitum*, tropane alkaloids such as hyoscyamine and scopolamine in *Datura stramonium*, and glycoalkaloids (solamargine and solasonin) with antifungal activity identified in *Solanum asperum* (Shonle and Bergelson 2000, Alves et al. 2003, Pinto et al. 2011, Ohyama et al. 2013).

This family has a big diversity of flavonoids, flavones and flavonols (Alves et al. 2003) such as the flavonoid tyrosine identified in *S. asperum* (Pinto et al. 2011), and more than 300 cataloged withanolides that may present anti-inflammatory, cytotoxic and immunomodulatory effects. The withanolides have already been identified in South American Solanaceae genera such as: *Dunalia*, *Deprea*, *Exodeconus*, *Jaborosa*, *Salpichroa*, *Vassobia* and *Withania* (Anjaneyulu et al. 1998, Veleiro et al. 2005). The withanolides are steroidal lactones such as vitaferin A, first isolated from *Withania somnifera*, and nicandrenone present in species of the genus *Nicandra* as *Nicandra physalodes* (Nalbandov et al. 1964). Finally, species of the genus *Capsicum* (peppers) present capsaicinoids and capsinoids with various pharmacological effects such as antiinflammatory, analgesic, antioxidant, gastrointestinal and cardiovascular benefits, plus weight loss and cancer prevention (Luo et al. 2011).

2.4. Bioactivity of Solanaceae extracts, fractions and chemical compounds on insects

Derivatives from different plant structures of Solanaceae promote lethal and sublethal effects on species belonging to different orders of insects. Tarmadi et al. (2014) verified that the hexane fraction of the methanolic extract of *Brugmansia candida* applied at 3% and 4% killed all individuals of *Coptotermes gestroi* Wasmann (Isoptera: Rhinotermitidae) and *Cryptotermes cynocephalus* Light (Isoptera: Kalotermitidae), respectevily. Zouiten et al. (2006), testing the effect of four extracts (hexane, dichloromethane, ethyl acetate and methanol) from fruit peels of *Solanum sodomaeum* on fifth instar nymphs of *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae), observed a phagodeterrent effect of methanol, ethyl acetate and dichloromethane extracts which also exerted mortality of 50, 50 and 37.5%, respectively, due to the presence of alkaloids and saponins. In addition, the extracts extended the fifth instar

of *S. gregaria* and interfered with its morphological development (resulting in distorted legs and antennae).

Concerning the effect of Solanaceae on Diptera, it was verified that the aqueous extract of the leaves of *Cestrum parqui*, incorporated into an artificial diet, promotes lethal effects on adults (LC_{50}: 0.9%) and neonatal larvae (0.6%) of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), whereas lower concentrations prolonged larval development and reduced the formation of pupae (Zapata et al. 2006). Another report of the effect of solanaceous derivatives on Diptera was done by Vinayaka et al. (2010), who evaluated the insecticidal effect of methanolic extracts of leaves and fruits of *Capsicum frutescens* var. *longum* against second and third instar larvae of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) (Diptera: Culicidae) using different concentrations. They detected the presence of tannins, alkaloids, steroids and glycosides in both extracts. In addition, the acetone extract from *Solanum trilobatum* applied at 100 ppm killed 100% of eggs of *Culex quinquefasciatus* Say (Diptera: Culicidae) and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae) (Rajkumar and Jebanesan 2004). Furthermore, Chowdhury et al. (2007) observed the toxicity of extracts in petroleum ether (LC_{50}: 645.75 ppm), absolute alcohol (LC_{50}: 321.89 ppm), benzene (LC_{50}: 204.30 ppm), acetone (LC_{50}: 107.66 ppm) and chloroform:mehtanol (1:1 v/v) (LC_{50}: 39.19 ppm) from leaves of *Solanum villosum* against *C. quinquefasciatus* after 24 hours of exposition. In addition, Rawani et al. (2010) verified that extracts from leaves of *Solanum nigrum* in petroleum ether (LC_{50}: 54.11 ppm), absolute alcohol (LC_{50}: 59.81 ppm), benzene (LC_{50}: 27.95 ppm), acetone (LC_{50}: 72.91 ppm) and chloroform:methanol (1:1 v/v) (LC_{50}: 32.69 ppm) also promoted mortality of *C. quinquefasciatus*.

Concerning to Lepidoptera, the ethyl acetate extract from seeds of *Solanum pseudocapsum*, applied at 5 mg L^{-1}, presented phagodeterrent and insecticidal activity on fourth instar caterpillars of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), *Spodoptera litura* Fabricius (Lepidotera: Noctuidae) (Jeyasankar et al. 2012), and *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae), and its chemical analyzes showed the presence of triterpenoids, flavonoids and alkaloids such as quinine (Jeyasankar et al. 2012). The aqueous and acetone extracts of the fruits of *Solanum viarum* exert lethal effects on fourth instar larvae of *S. litura* when fed with treated castor leaves (Ramesh et al. 2013), and the aqueous extract from leaves of *Solanum cernuum*, *Datura suaveolens* and *N. tabacum* promoted oviposition deterrence on *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Medeiros et al. 2005). Capsaicin from fruits of *Capsicum* sp. can promote growth inhibition of *Earias insulana* (Boisduval)

(Lepidoptera: Noctuidae) (Weissenberg et al. 1986); and solanin from leaves of *Lycopersycum esculetum* (= *Solanum lycopersicon*) has antifeedant activity against *E. insulana* (Weissenberg et al. 1986).

The steroidal glycosides laxumin A (LC_{50} 4.3 μM) and laxumin B (LC_{50} 6.1 μM) from leaves of *Solanum laxum* promotes mortality of *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) when they were incorporated in artificial diet (Soule et al. 1999). Evaluating the effect of aqueous extracts from many Solanaceae species against *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae), Lovatto et al. (2004) observed that *Brugmansia suaveolens*, *Solanum* spp., *Capsicum annuum* and *N. tabacum* promoted toxicity on nymphs (24 h old) when sprayed at 10% on cabbage leaves; whereas *N. tabacum* var. *virginia*, *S. fastigiatum* var. *acicularium*, *S. diflorum* and *C. annuum* var. *variegated* promoted repellence when sprayed at 5%. Moreover, under field conditions, the aqueous extract of *S. fastigiatum* var. *acicularium* applied at 10% (20 ml $plant^{-1}$) on cabbage seedlings (*B. olereaceae* var. *acephala*) reduced 45% of *B. brassicae* infestation (Lovatto et al. 2010).

2.5. Bioactivity of Solanaceae chemical extracts and fractions against insect-pests of stored products.

There are few reports of the activity of chemical derivatives of the Solanaceae family on stored grain pests; however, the effect of such derivatives extends from mortality to the reduction of the F_2 progeny of such insects. The ethanolic extract from *Datura stramonium* promoted mortality [LC_{50} (3.94 mg L^{-1}) and LC_{90} (15.37 mg L^{-1})] of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) through residual contact bioassay, and also a fagodeterent effect (Abbasipour et al. 2011); whereas its acetone extract promotes mortality (LC_{50}: 564.62 ppm and LC_{90}: 3.60 ppm) of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) (Abbasipour et al. 2011). Freire et al. (2016) observed the lethal and repellent effects of *Solanum melongena* and *Capsicum annuum* against *C. maculatus*. Investigating the effect of aqueous and methanolic extracts of medicinal plants on *T. castaneum*, Padin et al. (2013) found that the methanolic extract of *Solanum sisymbriifolium*, tested at 1,000 ppm, promoted 21% of mortality by topical application after 48 hours exposure. Testing the effect of aqueous suspension, aqueous extract and ethereal extract of roots, leaves, flowers and fruits of *Solanum surratense* on oviposition of *Callosobruchus chinensis* (Linnaeus) (Coleoptera: Chrysomelidae) at different concentrations (1, 2.5, 5 and 10%), Srivastava and Gupta (2007) found a reduction in the number of eggs in a dose-dependent manner. Finally, the acetone

extract from *Datura alba* through residual contact bioassay, applied at 2.5%, promoted mortality of 3.5 and 45% of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae), respectively, and reduced the number of individuals in both F_1 and F_2 progeny (Ali et al. 2012).

2.6. Taxonomic and bioecological aspects of *Zabrotes subfasciatus*

The subfamily Bruchinae includes more than 1,700 species distributed in 6 tribes and 64 genus, with the genera *Zabrotes* (Amblicerini), *Caryedon* (Pachymerini), *Acanthoscelides*, *Bruchus* and *Callosobruchus* (Bruchini) including pests of stored legumes (Johnson 1981, Tuda 2007).

The Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae), is distributed in Central and South America, Africa, the Mediterranean region and India (Southgate 1979, Abate and Ampofo 1996, Tuda 2007). It is a major pest of stored dried beans (*Phaseolus vulgaris* L.) able to damage up to 99.3% of stored bean grains of some varieties after 150 days of storage (Barbosa et al. 2000). The damages are a consequence of its larvae penetration and feeding inside grains, causing weight loss and reduction of their nutritional value; and excrements, eggs and fragments of insects contaminate stored grains. In addition, its attack can reduce the germination power of bean grains by 100% (Rego et al. 1986).

Morphologically, the larvae of *Z. subfasciatus* presents a fine tegument with white to yellowish coloration, whereas during its adult phase, females of *Z. subfasciatus* present four spots of cream color in the elytra, contrasting with the bright dark color of the body, while males are smaller with brown coloration. Females of *Z. subfasciatus*, at 30ºC and 70% relative humidity, oviposit, on average, during 5.93 days, with fecundity of 38.13 eggs per female, with 72.9% of the eggs being viable, generating 50.4% females (Sari et al. 2003). These same authors also verified that the average longevity of the adults is of 9.4 days for the females and 13.3 days for the males, being the egg-adult cycle of 28.9 days. At a temperature of 27ºC and 70% R.H., females of *Z. subfasciatus* ovulate, on average, 55 eggs, with an average viability of 80%, and 75% of hatched larvae reach the adult stage and complete the egg-adult cycle after 34 days on average (Dendy and Credland 1991).

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3. BIOACTIVE ETHANOLIC EXTRACTS FROM SOLANACEAE AGAINST *Zabrotes subfasciatus* (BOHEMAN)

ABSTRACT

Insecticidal secondary phytochemicals can promote lethal and sublethal effects on insects. Not coincidentally, they have been used to control insect pests since ancient times. During green revolution plant-based insecticides were largely replaced by synthetic insecticides, however, in the last twenty years there have happen a resurgence of botanical insecticides. The research regarding bioprospection of insecticidal compounds from plant has grown and the number of commercial formulated botanical insecticides as well. Therefore, in the present study it was performed a screening with 25 ethanolic extracts from 17 Solanaceae species in order to evaluate their bioactivity against the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae: Bruchinae). The bioactivity of Solanaceae ethanolic extracts (2,500 mg Kg^{-1}) was tested with residual contact bioassays. Adults of *Z. subfasciatus* were exposed to treated bean grains, and adult mortality, oviposition, F_1 progeny and damages on grains were quantified. Most of the 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*; thereby they were selected for bioguided fractionations in order to identify their insecticidal compounds.

Keywords: Insecticidal plants; Secondary metabolites; *Phaseolus vulgaris*.

3.1. Introduction

Plant species present a complex defense mechanism characterized by the presence of physical structures and chemical compounds (allelochemicals) with adverse action on the physiology and behavior of phytophagous insects (Wink 2003). Plant allelochemicals can promote mortality, repellency, phagodeterrence, inhibition of oviposition, reduction of body mass intake, prolongation of larval or nymphal development, morphological deformations, and reduction of fertility and fecundity in adults (Kubo et al. 1983, Hummelbrunner and Isman 2001, Dayan et al. 2009, Baskar and Ignacimuthu 2012, Ansante et al. 2015, Gonçalves et al. 2017). In addition, some plant allelochemicals can attract and improve the performance of natural enemies (parasitoids and predators) of herbivorous insects by releasing attractive volatiles (chemical signal indicating the presence of the herbivorous insect) (Gatehouse 2002).

Indubitably, plant allelochemicals play important roles in ecological interactions of tritrophic chains (plant + phytophagous insect + natural enemy) that can be manipulated to favor crop development and production (Ode 2006, Miresmailli and Isman 2014). They can be considered in breeding projects of resistant plant varieties presenting antibiosis or antixenosis, but they can also be identified, isolated and have their effects on insects characterized to formulate insecticides that promote both lethal and sublethal effects on insect-pests (Wink 1988, Rattan 2010, Oberemok et al. 2015). In the second case, they can be applied as botanical insecticides or synthetic insecticides with identical or similar chemical structure to the natural ones. Currently, there are insecticidal products that exemplify both cases. Many botanical insecticides such as neem oil, pogam oil, rotenone and essential oils have been used worldwide, and two major classes of insecticides, pyrethroids and neonicotinoids, are based on plant insecticidal chemical compounds (Pavela 2016, Sparks et al. 2017).

The use of botanical insecticides can be a suitable alternative for pest control for less intensive crop systems that cannot rely on synthetic insecticides. There are two ways of acquiring botanical insecticides, they can be commercialized as formulated products by a company or they can be prepared by farmers using available local insecticidal plants and simple extraction techniques with solvents (water or ethanol) (Miresmailli and Isman 2014, Pavela 2016). Due to its diverse flora, in Brazil there are opportunities for both methods. Solanaceae is a promissory botanical family to discover new insecticidal molecules. It is widely present in both the temperate and tropical zones with about 2,300 species presenting secondary metabolites (flavonoids, alkaloids, whitanolides, capsinoids etc.) that can promote effects on insect pests (Shonle and Bergelson 2000, Alves et al. 2003, Martins and Barkman 2005, Veleiro et al. 2005b, Luo et al. 2011, Pinto et al. 2011, Ohyama et al. 2013).

Therefore, a screening with ethanolic extracts of Solanaceae species from different genera was performed in order to evaluate their bioactivity using the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae: Bruchinae), as a model insect for toxicological bioassays. Bruchinae beetles are important pests of stored beans. *Z. subfasciatus* together with *Acanthoscelides obtectus* Say, 1831 (Coleoptera: Chrysomelidae: Bruchinae) are major pests of *Phaseolus vulgaris* L. whereas *Callosobruchus maculates* Fabricius, 1775 (Coleoptera: Chrysomelidae: Bruchinae) and *Callosobruchus chinensis* Linnaeus, 1758 attack *Vignia* spp. (Southgate 1979, Abate and Ampofo 1996, Tuda 2007). These beetles can promote high levels of damages on bean grains when they are not controlled, mainly at warehouses of developing countries in Sub-Saharan Africa and Latin America (Southgate 1979, Abate and Ampofo 1996, Tuda 2007).

3.2. Material and Methods

3.2.1. Collecting plant material and preparing ethanol extracts

The list of plants used in the present study and their respective localization, collecting date and voucher are listed in Table 1. The selection of Solanaceae species used in the present study were based on (i) scientific data regarding their chemical diversity and biological effects on different organisms, and (ii) focus on including species from different genera (8 in total). Ethanolic extracts were tested for selecting the most bioactive Solanaceae species. The process adopted to obtain ethanolic extracts was cold maceration in ethanol solvent (analytical grade, 99.5%). All plant parts were separately drilled in a knife mill to produce a powder. These plant powders were separately immersed in ethanol, stirred for 10 minutes, stored at 25ºC for 72 hours to finally be filtered with qualitative filter paper (80 g m^{-2}, the porosity of 3 μm). Such process was repeated three times with each plant powder. Afterwards, the remaining solution (ethanol with extracted compounds from plant powders) was placed in a rotary evaporator at 50°C and -600 mmHg in order to eliminate the ethanol solvent and acquire the plant extract (with paste and/or oil consistence).

In the present research, 25 ethanolic extracts from 17 Solanaceae species were studied. They were the ethanolic extracts of the leaves (9.84% yield) and stems (2.82%) of *Acnistus arborescens* (L.) Schltdl.; flowers (17.31%) of *Brugmansia suaveolens* (Willd.) Bercht. & J.Presl; leaves (11.38%) of *Brunfelsia uniflora* (Pohl) D. Don; leaves (6.25%) of *Cestrum intermedium* Sndtn.; leaves (12.96%) of *Cestrum nocturnum* L.; leaves (16.96%) of *Lycianthes asarifolia* (Kunth & Bouché) Bitter; leaves (8.16%) of *Lycianthes rantonnei* (Carrière) Bitter; leaves (14.19%) of *Nicotiana longiflora* Cav.; leaves (9.32%) of *Nicandra physaloides* (L.) Gaertn; leaves (11.07%) and stems (5.12%) of *Solanum americanum* Mill.; leaves (5.61%) of *Solanum cernuum* Vell.; leaves (12.01%), fruits (18.14%) and stems (5.74%) of *Solanum granulosoleprosum* Dunal; leaves (8.64%) of *Solanum lycocarpum* A. St.-Hil; leaves (8.49%) of *Solanum paniculatum* L.; leaves (8.7%), fruits (14.9%) and stems (4.09%) of *Solanum scuticum* M. Nee.; leaves (9.86%) and fruits (9.96%) of *Solanum seaforthianum* Andrews, and leaves (17.57%) and stems (1.86%) of *Solanum viarum* Dunal.

3.2.2. Toxicological bioassays with ethanolic extracts from Solanaceae

3.2.2.1. Colony of *Zabrotes subfasciatus*

The colony of *Z. subfasciatus* was initiated with individuals acquired from warehouses of Piracicaba municipality, São Paulo, Brazil. They were maintained in the laboratory under controlled conditions (25±2°C, 60±10% R.H. and a photoperiod of 14 L: 10 D hours) in glass containers (2.6 L) with *Phaseolus vulgaris* grains cv. Bolinha as substrate for female adults to lay eggs and their larvae feed.

3.2.2.2. Bioassays using *Z. subfasciatus* as model insect

Residual contact toxicological bioassays using adults of *Z. subfasciatus* [five couples aging 24 hours per sample unit (100 insects per tretment)] consisted of samples of bean grains treated with Solanaceae ethanolic extracts [10 repetitions (Petri dishes dimensions of 6.5 cm diameter × 2 cm high) with 10 g of bean cv. Bolinha per sample] in a completely randomized design. For each treatment 100 g of beans were placed inside a plastic bag (2 L) and sprayed with an ethanolic extract using a microatomizer pistol coupled to a pneumatic pump adjusted to provide a spray pressure of 0.5 kgf cm^{-2} and a volume of 30 L t^{-1} [3 mL of solution (solvent + Solanaceae derivative) per each 100 g of beans]. After applying ethanolic extracts (2,500 mg Kg^{-1}), treated beans were softly shaken during one minute to homogeneously distribute Solanaceae extracts on grains surface. They were kept in an airflow chamber during two hours for solvent evaporation before insects (five couples of *Z. subfasciatus*) were inserted in treated bean samples (10 g of beans). The concentration (2,500 mg Kg^{-1}) used to apply ethanolic extracts in toxicological bioassays was defined based on preliminary tests. For each experiment a negative control with acetone:methanol (1:1, v/v) was included. In addition, the botanical insecticide Azamax® 1.2EC {azadiractin A/B [12 $g.L^{-1}$ (1,2% m/m)]} was included in bioassays to compare its activity with the ethanolic extracts. Azamax® 1.2EC, a botanical insecticide registered in Brazil to control many insect pests, presents limonoids (azadiractin A and B) that can cause phagodeterrence and hormonal disbalance on insects. Azamax® 1.2EC was applied adopting the same concentration (2,500 mg Kg^{-1}) used for spraying ethanolic extracts.

The mortality of adults and number of eggs per sample were assessed five days after infestation (adults were withdrawn from sample units), and insects were considered dead when they did not react to a brush touch after one minute. Moreover, the F_1 progeny and damages on grains were assessed after 56 days of infestation.

3.2.3. Data analysis

The data from bioassays with Solanaceae ethanolic extracts were analyzed with the software "R", version 3.3.1. It was used Generalized Linear Models with quasibinomial or quasipoisson family distribution together with a Half-Normal Probability Plot with Simulation Envelope to verify Generalized Linear Models' fit (Nelder and Wedderburn 1972, Demétrio and Hinde 1997, Hinde and Demetrio 1998). In the case of significant differences among treatments, a multiple comparison test (Tukey's test, $p<0.05$) was performed to identify such differences.

3.3. Results

Some Solanaceae ethanolic extracts affected *Z. subfasciatus* mainly by reducing the number of eggs per sample and, consequently, reduced the F_1 progeny and the damages on bean grains (Tables 2, 3, 4, 5 and 6). Species from different genus promoted statistically significant effects on *Z. subfasciatus*, but at different intensities. None of the ethanolic extracts interfered on sex ratio, but many of them reduced egg-adult viability (Tables 2, 3, 4, 5 and 6).

The ethanolic extract of *S. lycocarpum* leaves was the most promissory among all extracts; it almost reduced the number of eggs per sample to zero and completely protected bean grains against damages resulting from *Z. subfasciatus* larvae feeding (Table 4). In addition, the ethanolic extract from flowers of *B. suaveolens* provided a good protection to beans grains against *Z. subfasciatus*. It promoted an expressive decrease in the number of eggs per sample (11.00), the F_1 progeny (6.60), egg-adult viability (57.6%) and damaged grains (15.61%) (Table 3). Therefore, the ethanolic extracts from *S. lycocarpum* (leaves) and *B. suaveolens* (flowers) were selected for a set of chemical fractionations based on toxicological results using *Z. subfasciatus* as model insect.

In addition, only the ethanolic extract of *Solanum paniculatum* (leaves) promoted significant mortality on *Z. subfasciatus* adults comparing to the negative control, but it did not expressively reduce oviposition, F_1 progeny and damages on grains comparing to other extracts (Table 2). However, some extracts [*S. viarum* (stems) and leaves of *S. seaforthianum*, *S. scuticum*, *S. cernuum*, *S. americanum* and *L. asarifolia*] intensively reduced both the number of eggs per sample and F_1 progeny, and also promoted a moderate toxicity to *Z. subfasciatus* eggs expressed by the reduction in egg-adult viability (Tables 5 and 6). Some ethanolic extracts such as *A. arborescens* (leaves), *N. physalodes* (leaves), *S. granuloso-leprosum* (stems), *S. cernuum* (leaves) and *S. americanum* (leaves) promoted a good reduction of damages caused

by *Z. subfasciatus* larvae on bean grains, but less intensevely than *S. lycocarpum* and *B. suaveolens* (Tables 2, 3, 4 and 5). Interestingly, the ethanolic extract from *N. physalodes* (leaves) promoted an intense reduction of eggs per sample indicating a possible egg deterrence effect (Table 2).

3.4. Discussion

In the present study, some of the 25 ethanolic extracts from 17 different Solanaceae species promoted significant interference on *Z. subfasciatus* oviposition, F_1 progeny or egg-adult viability resulting in less damaged grains (Tables 2-6). It demonstrates that Solanaceae species can be a rich source of insecticidal compounds to control *Z. subfasciatus* that are able to affect its biology by different ways. The bioactivity of Solanaceae chemical extracts against Bruchinae beetles is already reported in literature. The acetone extract from *Datura stramonium* promotes mortality (LC_{50}: 564.62 ppm and LC_{90}: 3.60 ppm) of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) (Abbasipour et al. 2011); and chemical extracts from *Solanum surratense* can reduce the number of eggs of *Callosobruchus chinensis* (Linnaeus) (Coleoptera: Chrysomelidae) (Srivastava and Gupta 2007). Such bioactivity is probably due to the production of a diverse range of chemical compounds such as flavonoids, alkaloids, whitanolides and capsinoids (Veleiro et al. 2005a, Luo et al. 2011, Pinto et al. 2011).

Many of the toxicological studies with insecticidal plants to control *Z. subfasciatus* are focused in terpenes such as limonoids (azadirachtin) and essential oils (Weaver et al. 1994, Silva et al. 2008, Morais et al. 2015). However, in the present study, three of the most bioactive species tested against *Z. subfasciatus* produce alkaloids. The tropical soda apple, *S. viarum*, presents the steroidal alkaloid solasodine (Mola et al. 1997, Shakirov et al. 2012); *B. suaveolens* (flowers) produces the alkaloids hyoscyamine, atropine and scopolamine (Andreola et al. 2008). Scopolamine promotes insecticidal effects on *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Alves et al. 2007, Roesler et al. 2007); and *S. lycocarpum* (leaves) has the steroidal alkaloids solasonine e solamargine, two defensive allelochemicals (Miranda et al. 2012, Al Sinani and Eltayeb 2017). Moreover, two tested species produce withanolides, *N. physalodes* produces nicandrenone with insecticidal properties and *A. arborescens* produces citotoxic withanolides (Nalbandov et al. 1964, Adam et al. 1976, Roumy et al. 2010).

Alkaloids tend to produce neurotoxicity on insects maybe because they share equal amino acid precursors with insect neurotransmitters what may enable them to act as agonist or antagonists by interacting with neurotransmitters' molecular biding sites (Wink and Schimmer

2010). Unfortunately, other animals also present neurotransmitters like acetylcholine making alkaloids toxic to many of them, what can be considered an disadvantage for them (Jank and Rath 2017). However, alkaloids played an important role in crop protection against insects. *Nicotiana* spp. (Solanaceae) present nicotine, an agonist alkaloid acting at nicotinic acetylcholine receptors of insects that was largely used as a botanical insecticide (tobacco extract) since the 17th century (Matsuda et al. 2001, Pavela 2016); and nicotine was also a model for the synthesis of neonicotinoid insecticides, a major class of insecticides worldwide (Matsuda et al. 2001).

Brazil is one of the greatest consumers of pesticides in the world. In 2015, it was applied 395,646 tons of pesticides in Brazil (tons of active ingredients) including 71,663 tons of insecticides (FAO 2017). Botanical insecticides can reinforce an image of environmental responsibility and healthier products for specific niche markets, e.g., organic products or gourmet products without synthetic pesticides. Exploring local resources (flora) to protect crops from pests reduces the dependence on extern resources to produce food. Moreover, botanical insecticides can be compatible with other methods of controlling insects. Some resistant bean varieties to *Z. subfasciatus*, due to the presence of protein arcelin in their grains, can be used with botanical insecticides (Barbosa et al. 2002, Mazzonetto and Vendramim 2002) to reduce even more the use of synthetic pesticides for small farmers in developing countries in Africa, Asia and Latin America.

The Normative Instruction nº 46/2011, of the Brazilian organic food law (Federal law nº 10.831/2003 which is regulated by the Federal decree nº 6.323/2007), establishes the technical regulation for organic systems in general, including the substances for pest and disease management. In such Normative Instruction, the Annex VII includes the use of "*plant extracts*" in specific conditions, "*plant oils*", "*essential oils*" and "*natural waxes*" without needing registration. This legal exemption provides great commercial opportunities for developing botanical insecticides based on Brazilian flora to attend organic market. The organic food market is constantly growing worldwide. In 2014, there was an area of 43.7 million hectares occupied with organic agriculture worldwide resulting in a business of U$ 80 billion, while at Brazil it was 705.233 hectares (Willer and Lernoud 2016). Despite these incentives, market opportunities and the rich Brazilian flora, the Brazilian Ministry of Agriculture, Livestock and Food Supply has few plant-based insecticides registered to use in Brazilian agriculture, such as an ant-bait insecticide based on leaf extract of *Tephrosia candida* containing rotenoids as active ingredients, and an *Azadirachta indica* A. Juss. (Meliaceae) oil product with azadirachtin A and

B as active ingredients for use in many cultures against different insect-pests (Agrofit 2017). Unfortunately, none of them is registered to control the Mexican bean weevil, *Z. subfasciatus*, which is a major pest for stored beans. That is why it is important to discovery a suitable sources of insecticidal molecules in Brazilian flora to produce a botanical insecticide for the management of Bruchinae pests.

Therefore, based on the results from toxicological bioassays, extraction yielding and plant material availability the species *B. suaveolens* and *S. lycocarpum* were selected for bioguided chemical fractionations in order to identify their chemical compounds responsible for the observed bioactivity. In the next two chapters it will be presented the results regarding the lethal and sublethal effects of chemical fractions from *S. lycocarpum* (Chapter II) and *B. suaveolens* (Chapter III) against *Z. subfasciatus*.

Table 1. Identification of Solanaceae species used in the present study including their voucher number, localization and date of collecting.

Species	Collecting local	Collecting date	Voucher number
Acnistus arborescens (L.) Schltdl.	Campus CENA/USP, Piracicaba, SP (22°42'30.2"S 47°38'38.2"W)	16/11/15	D. S. Gissi 46
Brugmansia suaveolens (Willd.) Bercht. & J.Presl	Campus ESALQ/USP, Piracicaba, SP (22°42'27.4"S 47°37'46.0"W)	31/10/15	G. L. P. Gonçalves 01
Brunfelsia uniflora (Pohl) D. Don	Campus ESALQ/USP, Piracicaba, SP 22°42'34.0"S 47°37'53.4"W	04/08/15	F. Rocha 02
Cestrum intermedium Sndtn.	Campus ESALQ/USP, Piracicaba, SP (22°42'27.4"S 47°37'46.0"W)	22/08/15	D. S. Gissi 274
Cestrum nocturnum L.	Louveira, SP (23°04'48.0"S 46°57'00"W)	10/08/15	F. Rocha 03
Lycianthes asarifolia (Kunth & Bouché) Bitter	Campus ESALQ/USP, Piracicaba, SP (22°42'54.4"S 47°37'50.5"W)	16/11/15	D. S. Gissi 47
Lycianthes rantonnei (Carrière) Bitter	Campus ESALQ/USP, Piracicaba, SP (22°42'27.4"S 47°37'46.0"W)	17/08/15	A. F. Lima 03
Nicandra physaloides (L.) Gaertn	Campus ESALQ/USP, Piracicaba, SP (22°42'28.9"S 47°37'48.9"W)	02/09/15	D. S. Gissi 36
Nicotiana longiflora Cav.	Campus ESALQ/USP, Piracicaba, SP (22°42'27.4"S 47°37'46.0"W)	05/01/15	D. S. Gissi 274
Solanum americanum Mill.	Campus ESALQ/USP, Piracicaba, SP (22°42'27.4"S 47°37'46.0"W)	16/11/15	A. F. Lima 04
Solanum cernuum Vell.	Campus ESALQ/USP, Piracicaba, SP (22°42'28.9"S 47°37'50.4"W)	17/11/15	D. S. Gissi 38
Solanum granulosoleprosum Dunal	Campus ESALQ/USP, Piracicaba, SP (22°41'59.4"S 47°37'59.8"W)	05/01/16	D. S. Gissi 271
Solanum lycocarpum A. St.-Hil	Sitio Retiro, Lavras, MG (21°12'08.3"S 45°09'52.2"W)	31/12/15	A. F. Lima 02
Solanum paniculatum L.	Campus ESALQ/USP, Piracicaba, SP (22°42'28.9"S 47°37'48.9"W)	10/09/15	D. S. Gissi 35
Solanum scuticum M. Nee.	Campus ESALQ/USP, Piracicaba, SP (22°41'59.5"S 47°37'59.8"W)	05/01/16	D. S. Gissi 270
Solanum seaforthianum Andrews	Campus ESALQ/USP, Piracicaba, SP 22°42'36.1"S 47°37'58.5"W	16/11/15	F. Rocha 01
Solanum viarum Dunal	Campus ESALQ/USP, Piracicaba, SP (22°42'28.9"S 47°37'48.9"W)	16/11/15	D. S. Gissi 37

Table 2. Bioactivity (mean ± SE) of ethanolic extracts from Solanaceae (tested at 2,500 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	Mortality (%) [1]	Nº eggs/ sample [2]	F_1 progeny [2]	Viability (%) (egg-adult) [1]	Sex ratio [1]	Damaged grains (%) [1]
Solanum paniculatum (leaves)	11.0±5.86 c	35.8±4.71 d	30.1±3.84 d	84.5±1.49 b	0,54±0.03	57,2±5.96 e
Cestrum nocturnum (leaves)	7.0±2.13 bc	26.2±3.74 c	22.7±3.34 c	88.5±3.51 ab	0,48±0.04	42,3±4.96 cd
Brunfelsia uniflora (leaves)	6.0±2.21 bc	29.8±3.92 cd	23.7±3.48 c	78.9±3.86 c	0,52±0.02	45,7±6.55 cd
Lycianthes rantonnei (leaves)	4.0±3.06 ab	31.3±5.00 cd	24.6±4.20 cd	77.6±2.43 c	0,51±0.05	47,0±6.21 d
Cestrum intermedium (leaves)	3.0±2.13 ab	25.7±6.43 c	23.0±5.95 c	79.2±9.61 a	0,49±0.09	36,3±8.87 c
Nicandra physalodes (leaves)	1.0±1.00 a	13.9±3.05 b	12.6±2.95 b	86.1±4.45 a	0,58±0.08	25,1±6.70 b
Control (acetone:methanol (1:1))	4.0±1.63 ab	82.0±4.16 e	70.0±3.91 e	85.3±1.71 b	0,51±0.02	88,6±2.49 f
Azamax® (2,500 mg kg^{-1})	28.0±6.29 d	0.8±0.42 a	0.5±0.22 a	30.0±13.33 **	0,30±0.15**	1,6±0.66 a
F	5.087	27.930	25.934	2.469	0.366	19.606
p value	<0.0001	<0.0001	<0.0001	0.03317	0.8977^{ns}	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p < 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p < 0.05$);
** Not analyzed due to small sample unit;
[ns] Not significant ($p > 0,05$).

Table 3. Bioactivity (mean ± SE) of ethanolic extracts from Solanaceae (tested at 2,500 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	Mortality (%) [1]	Nº eggs/ sample [2]	F_1 progeny [2]	Viability (%) (egg-adult) [1]	Sex ratio [1]	Damaged grains (%) [1]
Brugmansia suaveolens (flowers)	8.0±2.00 b	11.0±3.22 b	6.6±1.80 d	57.6±11.23 a	0.45±0.09	15.6±3.82 b
Acnistus arborescens (stems)	4.0±2.67 a	26.1±5.29 d	20.9±4.04 b	76.3±9.29 c	0.52±0.07	37.8±6.58 d
Acnistus arborescens (leaves)	3.0±1.53 a	16.6±3.23 c	11.2±2.38 c	66.0±5.87 b	0.57±0.07	23.2±3.92 c
Control (acetone:methanol (1:1))	7.0±2.13 b	67.1±8.48 e	55.4±6.94 a	83.0±1.88 d	0.52±0.01	83.9±6.05 e
Azamax® (2,500 mg kg^{-1})	21.0±6.23 c	2.4±1.11 a	0.3±0.21 e	5.3±3.69**	0.05±0.05**	0.8±0.57 a
F	4.686	28.237	42.652	5.742	1.071	37.265
p value	0.003	<0.0001	<0.0001	0.00266	0.3748 [ns]	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
** Not analyzed due to small sample unit;
[ns] Not significant ($p>0,05$).

Table 4. Bioactivity (mean ± SE) of ethanolic extracts from Solanaceae (tested at 2,500 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	Mortality (%) [1]	Nº eggs/ sample [2]	F_1 progeny [2]	Viability (%) (egg-adult) [1]	Sex ratio [1]	Damaged Grains (%) [1]
Solanum granuloso-leprosum (stems)	5.0±2.24 b	24.1±5.32 b	15.5±3.52 b	63.5±4.78 a	0.51±0.06	25.0±6.2 a
Solanum granuloso-leprosum (fruits)	4.0±1.63 b	50.4±6.68 c	40.9±5.47 c	80.6±3.01 c	0.51±0.03	60.9±5.5 c
Nicotiana longiflora (leaves+stems)	4.0±2.21 b	28.0±8.11 b	19.6±3.58 b	79.1±13.75 ab	0.52±0.04	39.7±6.1 b
Solanum granuloso-leprosum (leaves)	1.0±1.00 a	25.9±4.89 b	18.0±3.67 b	62.6±7.14 b	0.46±0.03	34.6±6.4 b
Solanum lycocarpum (leaves)	1.0±1.00 a	0.1±0.10 a	0.0±0.0*	0.0±.0.0*	0.0±0.0*	0.0±0.0*
Control (acetone:methanol)	0.0±0.00*	66.0±7.01 d	53.8±5.79 d	81.7±1.05 c	0.52±0.02	81.7±4.3 d
Azamax® (2,500 mg kg^{-1})	36.0±10.46 c	0.7±0.70 a	0.5±0.50 a	7.1±7.14**	0.06±0.06**	0.0±0.0*
F	10.915	26.565	25.455	4.485	0.349	13.837
p value	0.0001	0.0001	0.0001	0.003984	0.843 ns	0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
* Not included in the analysis (null variance);
** Not analyzed due to small sample unit;
ns Not significant ($p>0,05$).

Table 5. Bioactivity (mean ± SE) of ethanolic extracts from Solanaceae (tested at 2,500 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	Mortality (%) [1]	Nº eggs/ sample [2]	F_1 progeny [2]	Viability (%) (egg-adult) [1]	Sex ratio [1]	Damaged Grains (%) [1]
Solanum scuticum (leaves)	10.0±2.98 c	15.8±5.58 a	11.6±4.02 b	46.1±13.0 b	0.36±0.10	19.8±6.97 ab
Solanum scuticum (fruits)	7.0±2.13 bc	30.8±7.12 b	24.6±5.88 cd	70.8±8.5 bd	0.44±0.06	37.3±8.18 cd
Solanum cernuum (leaves)	5.0±2.69 ab	16.2±4.02 a	12.5±3.05 b	69.3±8.6 bc	0.43±0.06	23.7±6.31 ab
Solanum scuticum (stems)	4.0±2.21 ab	24.6±5.88 b	19.4±4.68 c	64.6±11.2 bd	0.37±0.08	28.3±7.29 bc
Lysianthes asarifolia (leaves)	4.0±2.21 ab	30.0±5.44 b	6.7±1.45 a	21.3±4.7 a	0.59±0.10	16.6±3.74 a
Solanum americanum (leaves)	2.0±2.00 a	14.8±4.93 a	12.1±4.19 b	55.7±12.4 cd	0.37±0.08	19.4±6.51 ab
Solanum americanum (stems)	0.0±0.00*	32.1±4.70 b	26.9±4.44 d	82.2±2.9 d	0.51±0.06	44.5±4.64 d
Control (acetone:methanol)	2.0±1.33 a	61.2±4.58 c	49.8±4.13 e	81.1±2.9 cd	0.52±0.02	75.8±4.43 e
Azamax® (2,500 mg kg^{-1})	27.0±5.97 d	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*
F	5.733	5.957	8.728	26.529	0.864	8.390
p value	0.0001	0.0001	0.0001	0.0001	0.5399 ns	0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
* Not included in the analysis (null variance);
** Not analyzed due to small sample unit;
ns Not significant ($p>0,05$).

Table 6. Bioactivity (mean ± SE) of ethanolic extracts from Solanaceae (tested at 2,500 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	Mortality (%) [1]	Nº eggs/ sample [2]	F_1 progeny [2]	Viability (%) (egg-adult) [1]	Sex ratio [1]	Damaged Grains (%) [1]
Solanum viarum (stems)	12.0±3.27 b	5.6±1.74 b	2.9±0.98 b	46.8±11.72 b	0.23±0.10	6.1±1.8 b
Solanum viarum (leaves)	11.0±4.07 ab	10.4±3.47 c	6.6±2.25 c	56.8±18.86 c	0.37±0.10	12.3±3.9 c
Solanum seaphortianum (leaves)	10.0±4.47 ab	13.1±3.74 c	1.0±0.39 a	4.6±1.67 a	0.12±0.06**	2.2±0.8 a
Solanum seaphortianum (fruits)	7.0±3.35 a	56.2±6.56 d	38.1±4.17 d	68.3±2.25 d	0.50±0.02	56.4±5.5 d
Control (acetone:methanol)	12.0±2.91 b	76.3±5.70 e	57.9±5.13 e	75.4±1.98 e	0.52±0.02	83.5±2.6 e
Azamax® (2,500 mg kg^{-1})	42.0±4.42 c	2.1±0.89 a	1.2±0.59 a	21.6±10.02**	0.16±0.08**	2.4±1.1 a
F	7.716	47.224	76.596	44.158	0.324	81.068
p value	0.0001	0.0001	0.0001	0.0001	0.8595 [ns]	0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
** Not analyzed due to small sample unit;
[ns] Not significant ($p>0,05$).

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4. INSECTISTATIC EFFECTS OF *Solanum lycocarpum* A. ST.-HIL (SOLANACEAE) CHEMICAL FRACTIONS AGAINST THE MEXICAN BEAN WEEVIL

ABSTRACT

Insect-pests have attacked stored grains (cereals and legumes) for hundreds of years in warehouses. Currently, Bruchinae beetles are an important problem for stored beans (*Phaseolus* and *Vigna*) that spread over the world due to the international trade of beans. Their control relies mainly on synthetic insecticides; however, due to risks to human health and the presence of resistant pest populations it is necessary to discover new insecticidal active ingredients. Solanaceae plants are a promissory source of insecticidal compounds such as withanolides, flavonoids, capsaicinoids and alkaloids that can be applied to control Bruchinae beetles in warehouses. Thus, in the present study it was performed chemical fractionations of the ethanolic extract from leaves of *Solanum lycocarpum* A. St.-Hil (Solanaceae) in order to identify insecticidal compounds able to interfere on the biology of *Zabrotes subfasciatus* Boheman, 1833 (Coleoptera: Chrysomelidae: Bruchinae). The results from toxicological bioassays with chemical fractions demonstrated that *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 also reduced the fecundity of F_1 progeny. Based on chemical analysis and their comparison with the database *Dictionary of Natural Products* apparently there may be withanolides and alkaloids in the bioactive fractions SLHexF7Ac-1-A and SLHexF7Ac-1-B.

Keywords: *Zabrotes subfasciatus*; Secondary phytochemicals; Withanolides

4.1. Introduction

Archaeological evidences demonstrate that insect-pests of Coleoptera order have promoted damages on stored grains since antiquity (Panagiotakopulu and Buckland 1991) and that fragrant resins produced from plant species have been used to control them (Levinson and Levinson 1998). In recent decades, international trade of agricultural products facilitated the dispersion of invasive species of the subfamily Bruchinae (Coleoptera: Chrysomelidae) (Tuda 2007). In Europe, 56% of the species of alien beetles introduced were from the subfamily Bruchinae (Kenis and Branco 2010). Of these, 27.3% came from South America, and probably the international trade of beans favored the introduction of *Zabrotes subfasciatus* (Boheman, 1833), *Aconthoscelides obtecus* Say, 1831, *Bruchus* spp. and *Callosobruchus* spp. which attack seeds of the botanical subfamily Papilionoideae (*Phaseolus*, *Lathyrus*, *Pisum*, *Vicia*) (Kenis and

Branco 2010). At Africa, stored beans suffer from the attack of *Z. subfasciutus*, *A. obtectus*, *Callosobruchus rhodesianus* (Pic), *Callosobruchus maculatus* (Fab.) and *Callosobruchus chinensis* (L.) (Abate and Ampofo 1996). In tropical regions, *Z. subfasciatus* is an important pest of stored beans (*Phaseolus vulgaris*) causing serious economic losses due to larvae penetration and feeding of bean grains resulting in weight loss and reduction of the nutritional value and degree of hygiene of the product due to the presence of eggs and fragments of insects.

Formerly, organochlorine and organophosphorous pesticides were adopted to control insect-pests of stored products, then they were replaced by pyrethroids such as deltamethrin (Boyer et al. 2012). Specifically to fumigation, phosphine is the most important insecticide worldwide; however, its use is threatened due to concerns regarding its risks to the environment, human health and resistant insect-pest populations (Chaudhry 1997, Zettler and Arthur 2000, Pimentel et al. 2010, Boyer et al. 2012). Due to such issues, insecticidal compounds of plant origin were reconsidered as a potential source for new active ingredients to control insect-pests in warehouses (Rajendran and Sriranjini 2008, Phillips and Throne 2010).

Insecticidal plants are applied in the forms of powders, oils and extracts able to act through contact, ingestion and fumigation, and promote mortality, repellency, phagodeterrence, inhibition of oviposition, disturbances in larval development and reduction of fertility of insects (Rattan 2010, Miresmailli and Isman 2014, Ansante et al. 2015, Giongo et al. 2016, Gonçalves et al. 2017). However, after the advent of synthetic insecticides, botanical insecticides have lost their importance in the world scenario of insect pest control in agriculture (Isman 2008). Nonetheless, the indiscriminate use of synthetic insecticides resulted on many direct and indirect negative effects on human health and environment. It promotes intoxication of humans due to food residues and it contaminates soil, underground water and streams (Joy et al. 2005, Damalas and Eleftherohorinos 2011, Beduk et al. 2017). Thereby, pesticides reduce biodiversity in both natural and agricultural ecosystems as well as they bioaccumulate in the trophic chain (Desneux et al. 2007, Aktar et al. 2009, Katagi and Tanaka 2016). Inevitably, all these negative aspects induced a negative perception of society regarding synthetic insecticides.

In such context, botanical insecticides can be a technical and economically promissory alternative to managed insect-pest populations in agriculture (Isman 2015). A demand for new active ingredients also stimulated the rise of scientific studies on bioprospection of insecticidal compounds from plants in the last three decades. In 1980 only 1.43% (61) of scientific publications on insecticides was related to botanical insecticides whereas in 2012 this number was 21.38% (1207) (Isman and Grieneisen 2014). Moreover, insecticidal plant compounds can be directly used for: (i) preparing homemade botanical insecticides at small farms in developing

countries; (ii) formulating botanical insecticides for organic agriculture; and (iii) synthesizing active ingredients with new modes of action against resistant insect-pest populations for conventional agriculture.

The Solanaceae botanical family includes several species of economic relevance and it is widely present in both the temperate and tropical zones, with around 2,300 species distributed in 92 genera (Martins and Barkman 2005). It is a promissory source of insecticidal compounds presenting a wide range of metabolites from different classes such as withanolides, flavonoids and capsaicinoids (Silva et al. 2003, Chen et al. 2011, Luo et al. 2011). *Solanum* is the biggest genus in Solanaceae family with approximately 1,500 species, including many economically important species for agriculture (Weese and Bohs 2007). Nonetheless, wild *Solanum* species might be a promissory source of insecticidal compounds to be applied in the management of insect pests from subfamily Bruchinae.

Solanum lycocarpum St. Hill, a small tree (2-7 meters) species used in Brazilian popular medicine, naturally occurs in "*Cerrado"* biome, in areas of forest regeneration and degraded pastures (Bueno et al. 2002). It is commonly known as "lobeira" or "fruta-do-lobo" (wolf´s fruit) due to the fact that it is an important food source to *Chrysocyon brachyurus* (Illiger, 1815) (Mammalia: Canidae), the Guará wolf, mainly during the dry season (Bueno et al. 2002). Most of the bioprospection studies conducted with *S. lycocarpum* focused in the bioactivity and identification of chemical compounds of its fruits. The fruits of *S. lycocarpum* present steroidal saponins and alkaloids such as solasosine and solamargine (Nakamura et al. 2008, Munari et al. 2014). The alkaloid extract (acid-base extraction) from *S. lycocarpum* fruits and their most abundant steroidal alkaloids solasonine and solamargine present toxicity against adult worms of *Schistosoma mansoni* (Miranda et al. 2012); and a mixture of them promoted leishmanicidal activity against *Leishmania amazonensis* (Miranda et al. 2013). Moreover, solasonine and solamargine from *S. lycocarpum* fruit present antiproliferative activity against different tumor cell lines (Munari et al. 2014) and it also promotes cytotoxicity on Chinese hamster lung fibroblasts (V79 cells) (Munari et al. 2014). Meanwhile, it is reported the larvicidal activity of different extracts, fractions, oils, fatty acids and methyl esters from *S. lycocarpum* fruits against *Culex quinquefasciatus* Say (Diptera: Culicidae) larvae demonstrating the potential of finding insecticidal compounds in this plant species (Pereira et al. 2014, Silva et al. 2015).

Thereby, in the present study it was performed a series of bioguided chemical fractionations with the ethanolic extract (leaves) from *S. lycocarpum*. Chemical fractionations

were based on toxicological bioassays using *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae) as a bioindicator.

4.2. Material and methods

4.2.1. Bioassays procedures using *Z. subfasciatus* as model insect

4.2.1.1. Insects used in experiments

Individuals of *Z. subfasciatus* were reared under laboratory controlled conditions (25±2°C, 60±10% RH and a photoperiod of 14 L: 10 D hours) in glass containers (2.0 L) containing *Phaseolus vulgaris* grains cv. Bolinha as substrate for larvae feeding. The colony was initiated with *Z. subfasciatus* specimens collected in warehouses of Piracicaba municipality, São Paulo, Brazil.

4.2.1.2. Bioassays

Completely randomized residual contact bioassays were composed of bean grain samples [10 Petri dishes (6.5 cm diameter × 2 cm high) with 10 g of bean cv. Bolinha per sample (100 g per treatment)] treated with *S. lycocarpum* fractions and infested with five couples of adults of *Z. subfasciatus* aging between 0-24 hours (100 insects per treatment). A microatomizer pistol coupled to a pneumatic pump adjusted to provide a spray pressure of 0.5 kgf cm^{-2} with a volume of 30 L t^{-1} [3 mL of solution (solvent + Solanaceae derivative) per each 100 g of beans] was used to spray chemical fractions on beans samples (100 g) placed inside plastic bags (2 L) which were softly shaken during one minute to homogeneously distribute them on grains surface. These treated beans stayed during two hours in an airflow chamber for solvent evaporation before insects (5 couples of *Z. subfasciatus*) were inserted in treated bean samples.

For all experiments, a negative control (beans treated with the organic solvent used to solubilize fractions of *S. lycocarpum*) was included. In addition, the botanical insecticide Azamax® 1.2EC {azadiractin A/B [12 $g.L^{-1}$ (1,2% m/m)]} was included in bioassays to compare its activity with the fractions. Azamax® 1.2EC, a botanical insecticide registered in Brazil to control many insect pests, presents limonoids (azadiractin A and B) that can cause phagodeterrence and hormonal disbalance on insects. Azamax® 1.2EC was applied adopting the same concentrations used for spraying chemical fractions.

The number of dead adults and eggs was assessed five days after infestation (adults were withdrawn from sample units). Due to the fact that *Z. subfasciatus* adults have the habit of pretending to be dead, insects that did not react to a brush touch after one minute were considered dead. The number of individuals in F_1 progeny and damages on grains was assessed after 56 days of infestation.

4.2.1.3. Effect of *S. lycocarpum* fractions on progeny F_1 fecundity and progeny F_2 development

In this experiment it was evaluated the effects of *S. lycocarpum* fractions on progeny F_1 fecundity and progeny F_2 development of *Z. subfasciatus*. To do so, five couples of *Z. subfasciatus* adults (aging 0-24 hour), emerged from beans previously treated with *S. lycocarpum* fractions, were placed inside Petri dishes containing 10 g of bean grains (without any treatment). The experimental design was completely randomized and it was used 10 repetitions per treatment. The same variables and parameters evaluated in item 4.2.1.2 were assessed in this experiment.

4.2.1.4. Oviposition deterrent effect of fractions SLHexF7Ac-1-A and SLHexF7Ac-1-B on *Z. subfasciatus* females

A free-choice bioassay was performed with 10 acrylic arenas (square-shape) presenting one central chamber and four peripheral chambers containing 5 g of beans in each one of them. Two chambers presented treated beans and two with untreated ones. Arenas were arranged using a completely randomized design and infested with 10 couples of *Z. subfasciatus*. After five days, the number of eggs laid on both treated and untreated bean grains was assessed.

4.2.2. Fractionation of *S. lycocarpum* ethanolic extract

4.2.2.1. Liquid-liquid partitioning

The ethanolic extract from the leaves of *S. lycocarpum* was submitted to a liquid-liquid partitioning using organic solvents hexane and methanol. For each gram of *S. lycocarpum* ethanolic extract it was added 100 ml of methanol:water (1:3, v/v) for its solubilization in a

separation funnel where hexane was added three times (100 ml for each gram of extract) to separate chemical compounds into a hexane fraction (SLHex) and the remaining hydroalcoholic phase (SLHid). Both the hexane fraction (26% yield) and the remaining hydroalcoholic phase (68% yield) had their solvents eliminated in a rotary evaporator (at 50°C and -600 mmHg). Fractions were applied on grains surface using acetone:methanol (1:1, v/v).

4.2.2.2. First Solid Phase Extraction

A Solid Phase Extraction (SPE) with silica as stationary phase (Strata-Phenomenex, 10 g) was conditioned using a solvent proportion of 9 hexane:1 dichloromethane (mobile phase). An amount of 0.94 g of the previous hexane fraction (SLHex) was applied into the silica cartridge using organic solvent (9 hexane:1 dichloromethane). A series of organic solvent combinations with crescent polarity was used to separate the chemical compounds present in the hexane fraction of *S. lycocarpum*. Therefore, it was consecutively applied 100 mL of 9hex:1dcm (9 hexane: 1 dichloromethane), 8hex:2dcm, 7hex:3dcm, 6hex:4dcm, 5hex:5dcm, 4hex:6dcm, 3hex:7dcm, 2hex:8dcm, 1hex:9dcm, 1 methanol:1 dichloromethane, and finally 100% methanol inside the Sep-Pak Silica cartridge. These 11 fractions were grouped into seven different fractions according to their chemical profile similarities of silica thin layer chromatography. The fractions and their respective yields were: SLHexF1 (10.11%), SLHexF2 (10.27%), SLHexF3 (7.62%), SLHexF4 (4.80%), SLHexF5 (9.43%), SLHexF6 (8.27%) and SLHexF7 (40.71%). Fractions were applied on grains surface using acetone:methanol (1:1, v/v).

4.2.2.3. Second Solid Phase Extraction

In this step, 0.5 g of SLHexF7 fraction was diluted in acetone and applied inside a Sep-Pak Silica Classic Cartridge (Strata-Phenomenex, 10 g). Afterwards 150 mL of acetone and 150 mL of methanol were applied, respectively. Therefore, the fraction SLHexF7 was separated into two fractions, one in acetone (SLHexF7Ac) (43.12% yield) and other in methanol (SLHexF7Met) (51.8% yield). Fractions were applied on grains surface using acetone:methanol (1:1, v/v).

4.2.2.4. Third Solid Phase Extraction

The SLHexF7Ac fraction (0.2 g) was diluted using a proportion of 9 hexane:1 ethyl acetate and placed inside a Sep-Pak Silica Classic Cartridge (Strata-Phenomenex, 2 g). It was consecutively applied inside the cartridge 100 mL of 9dcm:1EtAc (9 dichloromethane:1 ethyl acetate), 8dcm:2EtAc, 7dcm:3EtAc, 6dcm:4EtAc, 5dcm:5EtAc, 4dcm:6EtAc, 3dcm:7EtAc, 2dcm:8EtAc, 1dcm:9EtAc, acetone, and finally 100% methanol in the Sep-Pak Silica column. The eleven fractions produced were than grouped into five fractions according to their chemical profile similarities of silica thin layer chromatography. The fractions and their respective yields were: SLHexF7Ac-1 (10.55%), SLHexF7Ac-2 (12.80%), SLHexF7Ac-3 (42.12%), SLHexF7Ac-4 (20.49%) and SLHexF7Ac-5 (13.65%). Fractions were applied on grains surface using ethyl acetate.

4.2.2.5. Fourth Solid Phase Extraction

The fraction SLHexF7Ac-1 (0.05 g) was separated in six fractions based on thin layer chromatography profiles, SLHexF7Ac-1-A (13.9% yield), SLHexF7Ac-1-B (10.7%), SLHexF7Ac-1-C (17.5%), SLHexF7Ac-1-D (11.5%), SLHexF7Ac-1-E (37.6%), SLHexF7Ac-1-F (5.4%). This fraction was solubilized in dichloromethane (mobile phase) and applied in a Sep-Pak Silica Classic Cartridge (Strata-Phenomenex, 2 g). In order to separate its chemical compounds, 100 mL of dichloromethane, 95dcm:5AcEt (95 dichloromethane:5 ethyl acetate), 90dcm:10AcEt, 85dcm:15AcEt, ethyl acetate and methanol were consecutively applied. Fractions were applied on grains surface using ethyl acetate.

4.2.3. High-performance liquid chromatography with mass spectrometry (HPLC-MS) with UV detector

The fractions SLHexF7Ac-1-A and SLHexF7Ac-1-B were submitted to High Performance Liquid Chromatography (Waters ACQUITY) attached to UV diode array detectors and a mass spectrometer XEVO-TQS with scan from 96 to 1963 Da, resolution of 2.8/14.6, energy ion 0.4, capillary (kV) 3.2, cone voltage of 30, font (adapter) temperature of 150 °C, and gas flow of 150 l h^{-1}. It was used a column Ascentis Express C_{18}, with water gradient (acidificated at 0.1% with formic acid) in acetronitrila adopting a flow of 0.45 mL min^{-1} during 33 minutes.

4.2.4. Statistical analysis

All parameters and variables evaluated from bioassays with *Solanaceae* ethanolic extracts and *S. lycocarpum* fractions were analyzed with the software "R", version 3.3.1, considering Generalized Linear Models with quasibinomial or quasipoisson family distribution. In order to verify the quality of Generalized Linear Models' fit, a Half-Normal Probability Plot with Simulation Envelope of the hnp package was used (Nelder and Wedderburn 1972, Demétrio and Hinde 1997, Hinde and Demetrio 1998). Multiple comparisons tests (Tukey's test, $p<0.05$) were performed using the glht function of the multicomp package to identify the differences between treatments.

The Deterrence Index (DI) of fractions SLHexF7Ac-1-A and SLHexF7Ac-1-B was calculated using the following formula: $= \frac{2G}{G+P}$, where G is the % of eggs in treated unit samples, and P is the % of eggs in control. Classification Interval (CI) was estimated using the following formula: $CI = 1 \pm t\ (\frac{SD}{\sqrt[2]{n}})$, where *t* is the Student's t distribution value (n-1; α: 0,05), SD the standard deviation of DI values, and *n* the number of repetitions. Treatments are considered neutral when the DI and CI values overlap, stimulant when DI values are superior to CI values, and deterrent when DI values are lower than CI values.

4.3. Results

4.3.1. Bioassays

The ethanolic extract of *S. lycocarpum* leaves presented the most promissory performance among all extracts; it almost reduced the number of eggs per sample to zero and completely protected bean grains against damages from *Z. subfasciatus* larvae feeding as presented in Chapter I. Therefore, the ethanolic extract of *S. lycocarpum* leaves was selected for liquid-liquid partitioning to produce a hexane fraction (SLHex) and hydroalcoholic (SLHid) remaining phase. None of them, applied at 2,500 mg Kg^{-1}, promoted significant mortality; however, the hexane fraction promoted an elevated reduction in eggs per sample, F_1 progeny and damaged grains (Table 1). The botanical insecticide (Azamax®) almost reduced the number of eggs and damages on bean grains to zero (Table 1). The hexane fraction was divided into seven fractions using a silica cartridge, and among them, the fraction SLHexF7 (1,000 mg Kg^{-1}) was the most active, promoting 46% mortality of the adults of *Z. subfasciatus* (Table 2). In addition, it drastically reduced the number of eggs deposited on the grains, reduced egg-adult viability, and consequently damages on grains (Table 2). Some of the other fractions also

interfered negatively in the development of *Z. subfasciatus*, however, to a lesser extent than fraction SLHexF7. Therefore, the fraction SLHexF7 was selected for another chemical separation using a silica cartridge, and this time it was divided into two fractions, one in acetone (SLHexF7Ac) and another in methanol (SLHexF7Met). Interestingly, none of them promoted mortality of the adults of *Z. subfasciatus* as occurred with the fraction SLHexF7, from which they were derived. In spite of this, the acetone fraction promoted an intense reduction of the number of eggs on bean grains and, consequently, the damages on them (Table 3). The fraction SLHexF7Ac was subjected to a further Solid Phase Extraction, and it was subdivided into five fractions, SLHexF7Ac-1, SLHexF7Ac-2, SLHexF7Ac-3, SLHexF7Ac-4, SLHexF7Ac-5 and SLHexF7Ac-5. The results of the bioassays with these fractions did not allow the selection of the most efficient fraction at a concentration of 500 mg Kg^{-1}, because both the fraction SLHexF7Ac-1 and SLHexF7Ac-2 equally reduced the number of eggs, the F_1 progeny and egg-adult viability (Table 4). However, testing their effect on F_1 progeny fecundity and F_2 progeny development, only the fraction SLHexF7Ac-1 reduced the number of eggs laid by *Z. subfasciatus* females and individuals in F_2 progeny (Table 5). Thus, this fraction was divided into six fractions, SLHexF7Ac-1-A, SLHexF7Ac-1-B, SLHexF7Ac-1-C, SLHexF7Ac-1-D, SLHexF7Ac-1-E, SLHexF7Ac-1-F and tested at 100 mg Kg^{-1}. Both the fractions SLHexF7Ac-1-A and SLHexF7Ac-1-B significantly reduced the number of eggs per sample, F_1 progeny and damages on grains (Table 6); however only the fraction SLHexF7Ac-1-B promoted oviposition deterrence effect on females of *Z. subfasciatus* (Table 7).

4.3.2. Chemical analysis

The fraction SLHexF7Ac-1-A and SLHexF7Ac-1-B presented similar chromatographic bands (Figure 2) and compounds with the same retention times: 23.1, 23.6 and 24.3 minutes. Therefore, this indicates the possible presence of same compounds in both fraction, but demonstrating that the chromatographic method employed for their separation was not quite efficient. These fractions showed several different minor compounds among themselves, but since both fractions promoted similar effects on *Z. subfasciatus*, the focus was on the similarities among their chemical profiles.

The mass spectra of compounds from SLHexF7Ac-1-A and SLHexF7Ac-1-B fractions with the same retention time (23.1, 23.6 and 24.3 min) showed similar mass/load (m/z) values, which may indicate a similarity between their chemical structures (Figure 2, 3, 4 and 5). Thus,

considering the values of molecular ions in their mass spectra (Figure 3, 4 and 5) as probable values of their molecular masses (Da), a search was made in the *Dictionary of Natural Products* (DNP) database adopting Solanaceae as a biological source in order to identify candidates (chemical compounds) with similar mass fragmentation pattern.

For compounds with retention time at 23.6 min, considering a molecular weight between 652 and 654 Da, it was found two compounds: the withanolide withanoside I ($C_{40}H_{48}N_2O_6$) (6,7-Epoxy-1,3,5-trihydroxy-1-oxowitha-24-enolide, 3-O-b-D-glucopyranoside) from *Withania somnifera* (Matsuda et al. 2001); and also a petuniasterone [24,25-epoxy-7,22-dihydroxyergosta-1,4-dien-3-one ($C_{34}H_{52}O_{12}$)] which is an ergostane-type steroid found in *Petunia hybridia* (Solanaceae) (Elliger et al. 1988). For the retention time 23.1 min, considering a molecular weight of 829-832 Da and Solanaceae as biological source, it was found only the alkaloid withanine ($C_{44}H_{80}N_2O_{12}$). Withanine is the major alkaloid of *Withania somnifera* (Kalra and kaushik 2017). Finally, for the retention time of 24.3 min, considering the range of 292-294 Da, it was found seven different compounds reported in literature for Solanaceae, with emphasis to capsaicin ($C_{17}H_{27}NO_3$) (7-Methyloctanoyl analogue and nonanoyl analogue). Capsaicin is an alkaloid found in fruits from the genus *Capsicum* presenting many biological activities (Srinivasan 2016).

4.4. Discussion

In the first three chemical fractionations of the ethanolic extract from *S. lycocarpum* leaves, bioactive fractions drastically reduced the number of eggs, individuals in F_1 progeny and damages on grains. The fraction SLHexF7 even killed 46% of *Z. subfasciatus* adults (Table 2). However, as applied concentrations reduced (from 2,500 ppm to 100 ppm) the intensity of observed effects on *Z. subfasciatus* biology reduced as well. Moreover, in the fourth fractionation, bioactivity dissipated into two fractions, SLHexF7Ac-1 and SLHexF7Ac-2. Both of them similarly reduced the number of eggs, the F_1 progeny and egg-adult viability, but only the fraction SLHexF7Ac-1 affected the fecundity of F_1 progeny females and individuals in F_2 progeny. Therefore, *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 also reduced the fecundity of F_1 progeny.

The fraction SLHexF7Ac-1 reduced the number of eggs deposited by F_1 progeny females and the number of individuals in F_2 progeny demonstrating that bioactive compounds

present in *S. lycocarpum* were able to interfere on fecundity of *Z. subfasciatus* females. Similarly, an acetone extract from leaves of *Datura alba* (Solanaceae) (applied at 2.5% (25,000 ppm) through residual contact) reduced the number of individuals on F_1 and F_2 generations of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Ali et al. 2012). Moreover, the fraction SLHexF7Ac-1-B promoted an oviposition deterrence effect on females of *Z. subfasciatus* (Table 7). Insect females present tarsal and abdominal contact chemoreceptors that mediate host selection for oviposition (Klijnstra and Roessingh 1986). Therefore, the interaction of chemoreceptors with stimulant and deterrent chemical compounds directly interferes on females' choice to lay eggs or not (Jermy and Szentesi 1978, van Loon and Schoonhoven 1999). Thus, probably, some chemical compound in fraction SLHexF7Ac-1-B may be producing negative stimuli for oviposition. Anyway, the oviposition deterrence effect combined with the reduction of fecundity in females of *Z. subfasciatus* can decrease the rate of population growth of *Z. subfasciatus* in stored beans. Based on the chemical analysis of fractions SLHexF7Ac-1-A and SLHexF7Ac-1-B it was not possible to identify their major chemical compounds (Figures 2, 3, 4 and 5). However, comparisons with the database from the *Dictionary of Natural Products* indicate that the withanolide withanoside I, a petuniasterone, and the alkaloids withanine and capsaicin are possible similar chemical compounds to those present in *S. lycocarpum* fractions.

The insecticidal properties of withanolides are relatively well reported in scientific literature. Some withanolides, such as salpichrolides A, C, and G isolated from fresh leaves and stems of *Salpichroa origanifolia* (Solanaceae), promote larval mortality and prolong the development of *Tribolium castaneum* (Coleoptera, Tenebrionidae) when incorporated into wheat flour (Mareggiani et al. 2002). In addition, withanolides extracted from *S. origanifolia* (Solanaceae) and their chemically modified analogues promote high mortality and prolong time development of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Bado et al. 2004). Testing sixteen withanolides from *Iochroma gesnerioides* (Solanaceae) regarding their effects as agonists and antagonists of ecdysteroids on cell lines of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), it was found that none of them promoted agonistic effects, but several did act as antagonists (Dinan et al. 1996).

Ecdysteroid hormones play an important regulatory role in inducing molting and metamorphosis, controlling vitellogenesis and egg maturation in insects (Riddiford and Truman 1993, Parthasarathy et al. 2010, Roy et al. 2018). There are reports in literature about the sterilizing effects of ecdysteroids and their analogues. The hormones 20-hydroxyecdysone and

ponasterone A, and a synthetic ecdysone analog (Δ7-5β-cholestene-2β,3β,14α-triol-6-one) inhibited ovarian maturation and egg production of *Musca domestica* (L.) (Diptera: Muscidae) fed with artificial diet containing such compounds (Robbins et al. 1968). Moreover, ecdisone analogues were capable of inhibiting egg production in *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) fed with them (Earle et al. 1970). Interestingly, withanolides and insect ecdysteroids present similarities between their chemical structures (Figure 6). Therefore, it is possible to assume that the former could bind on molecular sites of the latter interfering on egg production by *Z. subfasciatus* females and, consequently, reducing their fecundity. It could help explaining why females laid less eggs on beans treated with fraction SLHexF7Ac-1, for example, and also why their offspring produced less eggs (Tables 4 and 5). Nowadays, diacylhydrazines are the only registered insecticides acting as ecdysone-receptor agonists; however, there is no insecticide acting as an antagonist of ecdysteroid receptors (Sparks and Nauen 2015). Therefore, if withanolides actually present such mode of action, they could be a new efficient insecticide to manage resistant insect pest populations.

Currently, the distribution of insecticides sales according to their mode of action reveals a high use of products acting on nerve and muscles (85% of sales) followed by growth regulators (9%), energy metabolism interferers (4%), disruptors of midgut membranes (1%), and unknown modes of action (1%) (Dayan et al. 2009, Sparks and Nauen 2015). Thus, finding insecticidal compounds with new modes of action is a key factor to control resistant populations of insect pests and reduce negative effects on beneficial organisms. The Solanaceae botanical family already has some plant species used as botanical insecticides in the world. Capsaicinoids, widely present in the genus *Capsicum*, integrates commercial insecticide formulations based on *Capsicum annum* (Solanaceae) presenting the active ingredient capsaicin that can promote repellence, and negatively interfere on metabolism and nervous system (Pavela 2016). Beyond its insecticidal properties, capsaicin can act as a synergist for organophosphate insecticides against the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), increasing its mortality up to 73% when compared to organophosphate tested alone (Maliszewska and Tęgowska 2012). Moreover, nicotine from *Nicotiana tabacum* (Solanaceae) is an important alkaloid for insect-pest management both in the form of a botanical insecticide and as a prototype-molecule for neonicotinoids synthesis (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam) (Elbert et al. 2008, El-Wakeil 2013). It is possible that fractions SLHexF7Ac-1-A and SLHexF7Ac-1-B also present alkaloids similar to capsacin and withanine, but alkaloids present a neurotoxic mode of action on insects and are not quite selective to natural enemies (Wink and Schimmer 2010).

Botanical insecticidal extracts can be a technically and economically feasible tool to control bean pests of *Phaseolus vulgaris* (Fabaceae) in both fields and warehouses for small farmers. The leaves' powder (15 g Kg^{-1}) from *Cupressus lusitanica* var. *benthamii* (Cuperaceae), *Tagetes minuta* (Asteraceae), *Azadirachta indica* (Meliaceae) and *Chenopodium ambrosioides* (Chenopodiaceae) promoted mortality on *Z. subfasciatus* and *A. obtectus*, reduced their eggs and inhibited their F_1 progeny (Paul et al. 2009). Moreover, in farm warehouses experiments, stored beans treated with *C. lusitanica*, *T. minuta*, *A. indica* and *C. ambrosioides* powders presented less damages and insects during five months than untreated beans (Paul et al. 2009). Below it is provided another example of the efficacy of botanical insecticidal extracts to control bean pests, and how botanical insecticides based on local plants can valorize the native flora and show farmers the importance to preserve native vegetation.

The botanical extracts from four common weeds [*Lippia javanica* (camphor, camphene, α-pinene, eucalyptol etc.), *Tithonia diversifolia* (sesquiterpene lactone tagitinin A), *Tephrosia vogelii* (rotenoid deguelin), and *Vernonia amygdalina* (saponin vernonioside C)] generated a better economic marginal rate of return (USD/ha) than synthetic insecticide Karate® (lambda-cyhalothrin, pyrethroid) (Mkenda et al. 2015). The areas treated with *T. vogelii* and *T. diversifolia* extracts presented more lady beetles and spiders and produced more beans (higher number of bean pods per plant) than all other areas, maybe due to the preservation of natural enemies and pollinators (Mkenda et al. 2015). However, botanical extracts did not controled insect pests (*Aphis fabae*, *Ootheca mutabilis*, *O. bennigseni*, *Epicauta albovittata* and *E. limbatipennis*) as much as Karate®. Therefore, botanical insecticides cannot achieve the same level of control than a synthetic insecticide under field conditions, but they can promote some benefits and be more economically interesting for small bean producers. A plant species to be considered a good source for a botanical insecticide needs to (i) provide plant material during most part of the year, (ii) be easy to harvest and to extract its active compound, which also needs to be constitutively produced by the plant. *S. lycocarpum* presents some of such characteristics. It is a widely present plant species in Brazilian "*Cerrado"* biome, specifically in areas of vegetation regeneration and degraded pastures that can be harvested by farmers to produce an ethanolic (a cheap safe solvent) extract able to reduce to zero the damages on grains produced by *Z. subfasciatus*. Therefore, *S. lycocarpum* can be a good source of plant raw material for small farmers to produce their own botanical insecticide in order to protect stored beans against the attack of the Mexican bean weevil, *Z. subfasciatus*; and it can be a promissory source of chemical compounds to be used as prototype-molecules for synthetic products.

Table 1. Lethal and sublethal effects (mean ± SE) of *Solanum lycocarpum* leaf fractions (tested at 2,500 mg kg^{-1}) to *Zabrotes subfasciatus* through residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
Hexane (SLHex)	6.0±3.40	5.8±2.49 b	3.5±1.59 b	38.8±14.46 b	0.24±0.10	8.2±3.75 b
Hydroalcohol (SLHid)	7.0±3.96	57.9±6.23 c	38.2±4.57 c	66.2±3.37 c	0.45±0.06	42.5±6.28 c
Control (acetone:methanol)	2.0±1.33	92.8±5.34 d	67.4±4.13 d	72.6±1.56 d	0.49±0.02	79.3±2.63 d
Azamax® (2,500 mg kg^{-1})	13.0±3.35	3.7±1.94 a	0.4±0.31 a	2.6±1.93 a	0.0±0.0	0.7±0.44 a
F	2.078	68.888	98.302	10.884	1.576	66.422
p value	0.1203 [ns]	<0.0001	<0.0001	<0.0001	0.2223 [ns]	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[ns] Not significant ($p>0,05$).

Table 2. Lethal and sublethal effects (mean ± SE) of *Solanum lycocarpum* leaf fractions (tested at 1,000 mg kg^{-1}) to *Zabrotes subfasciatus* through residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
SLHexF7	46.0±6.53 d	16.3±3.45 a	7.3±1.93 a	42.1±9.42 ab	0.56±0.11 a	16.7±3.47 a
SLHexF6	8.0±2 c	42.3±7.96 b	36.5±8.04 b	81.2±4.74 f	0.47±0.02 e	48.8±7.41 b
SLHexF5	6.0±2.21 bc	66.5±4.23 cd	54.6±4.1 d	81.7±2.52 ef	0.54±0.02 c	60.3±5.78 c
SLHexF4	4.0±1.63 ab	43.5±6.31 b	34.3±5.59 b	77.9±2.71 de	0.50±0.01 de	51.3±5.17 b
SLHexF3	2.0±1.33 a	59.3±5.9 c	49.2±4.99 cd	82.9±2.08 ef	0.47±0.03 e	62.3±2.94 c
SLHexF2	4.0±1.63 ab	62.5±4.9 c	40±3.88 bc	63.5±2.43 c	0.54±0.02 cd	57.2±3.52 bc
SLHexF1	2.0±1.33 a	75.1±2.66 d	31.8±2.76 b	42.1±2.98 a	0.56±0.02 b	55.5±1.41 bc
Control (acetone:methanol)	4.0±1.63 ab	77.5±7.34 d	58.8±7.15 d	73.7±3.61 d	0.49±0.01 e	72.8±2.05 d
Azamax® (1,000 mg kg^{-1})	8.0±2.49 c	17.7±5.52 a	9.9±3.02 a	56.6±9.22 bc	0.49±0.06 bc	16.7±5.61 a
F	17.646	15.758	15.175	21.070	4.486	17.139
p value	<0.0001	<0.0001	<0.0001	<0.0001	0.00016	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);

Table 3. Lethal and sublethal effects (mean ± SE) of *Solanum lycocarpum* leaf fractions (tested at 2,000 mg kg^{-1}) to *Zabrotes subfasciatus* through residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
Acetona (SLHexF7Ac)	11.0±3.48 b	9.9±2.15 b	6.5±1.50 b	65.4±3.69	0.48±0.05	11.1±2.38 b
Metanol (SLHexF7Met)	11.0±3.48 b	47.6±6.52 c	38.5±4.31 c	77.7±3.02	0.55±0.03	64.2±4.39 c
Control (acetone:methanol)	1.0±1.00 a	56.6±2.53 d	44.0±1.91 d	78.1±2.46	0.48±0.01	67.3±2.20 d
Azamax® (2,000 mg kg^{-1})	19.0±4.07 c	0.8±0.25 a	0.5±0.27 a	30.0±15.28**	0.30±0.15**	2.5±0.94 a
F	6.343	84.418	102.750	1.671	2.374	117.420
p value	0.0014	<0.0001	<0.0001	0.2076 [ns]	0.1138 [ns]	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
** Not analyzed due to small sample unit.
[ns] Not significant ($p>0,05$).

Table 4. Lethal and sublethal effects (mean ± SE) of *Solanum lycocarpum* leaf fractions (tested at 500 mg kg^{-1}) to *Zabrotes subfasciatus* through residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
SLHexF7Ac-1	4.0±1.63	38.5±4.70 b	27.0±2.91 b	72.4±2.86 ab	0.52±0.01	46.5±4.31 b
SLHexF7Ac-2	1.0±1.00	36.3±2.14 b	26.8±1.54 b	74.4±2.84 b	0.49±0.03	48.1±3.02 b
SLHexF7Ac-3	3.0±1.53	47.3±3.37 c	38.0±2.94 c	80.4±2.41 c	0.53±0.02	69.8±5.34 d
SLHexF7Ac-4	2.0±1.33	58.7±3.57 d	48.4±3.46 d	81.8±2.05 c	0.52±0.02	74.9±3.07 e
SLHexF7Ac-5	2.0±1.33	47.3±3.26 c	38.2±2.87 c	80.5±1.72 c	0.48±0.01	58.3±3.91 c
Control (Ethyl acetate)	2.0±1.33	51.5±2.03 c	42.0±2.00 c	81.6±1.89 c	0.52±0.02	68.6±1.90 d
Azamax® (500 mg kg^{-1})	2.0±1.33	15.5±2.58 a	10.4±1.59 a	72.4±4.96 a	0.43±0.05	19.0±3.14 a
F	0.452	19.987	26.591	5.515	0.808	26.814
p value	0.8412 [ns]	<0.0001	<0.0001	0.00012	0.5672 [ns]	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[ns] Not significant ($p>0,05$).

Table 5. Effects (mean ± SE) of *Solanum lycocarpum* leaf fractions (tested at 500 mg kg^{-1}) on progeny F_1 fecundity and progeny F_2 development of *Zabrotes subfasciatus* through residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**F_1 fecundity (nº eggs/sample)** [2]	**F_2 progeny** [2]	**F_2 viability (%) (egg-adult)** [1]	**F_2 sex ratio** [1]	**Damaged grains (%)** [1]
SLHexF7Ac-1	62.9±3.55 a	51.6±2.53 a	82.5±1.64 ab	0.52±0.02	68.1±2.35 a
SLHexF7Ac-2	94.1±5.89 c	75.4±4.45 b	80.6±1.78 a	0.53±0.02	85.4±2.64 c
SLHexF7Ac-3	89.7±5.49 bc	77.8±4.46 b	87.1±1.42 d	0.48±0.03	88.0±1.45 d
SLHexF7Ac-4	86.7±5.27 b	77.0±4.20 b	89.1±0.69 c	0.53±0.03	83.6±2.62 bc
SLHexF7Ac-5	106.3±4.18 d	88.8±2.87 c	83.7±0.74 b	0.48±0.02	88.6±1.72 d
Control (Ethyl acetate)	101.1±5.87 d	86.6±4.05 c	86.2±1.72 d	0.49±0.01	82.2±1.40 b
Azamax® [3]	--	--	--	--	--
F	9.386	12.858	5.354	0.585 [ns]	10.832
p value	<0.0001	<0.0001	0.00045	0.7114	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[ns] Not significant ($p>0,05$).
[3] The positive control (Azamax) did not have sufficient number of individuals (F_1 progeny) to infest unit samples (10 g beans), thus it was not included in this experiment.

Table 6. Lethal and sublethal effects (mean ± SE) of *Solanum lycocarpum* leaf fractions (tested at 100 mg kg^{-1}) to *Zabrotes subfasciatus* through residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
SLHexF7Ac-1-A	1.0±1.00 a	78.3±7.67 b	52.0±5.75 b	68.5±5.90 bc	0.50±0.02	70.6±6.17 b
SLHexF7Ac-1-B	6.0±2.67 b	85.9±2.83 b	58.0±3.13 b	67.2±2.00 cd	0.51±0.02	72.3±3.42 b
SLHexF7Ac-1-C	8.0±2.49 b	137.9±4.36 cd	86.8±1.76 c	63.4±1.81 b	0.50±0.01	90.8±1.47 c
SLHexF7Ac-1-D	6.0±1.63 b	127.5±13.23 c	85.8±9.54 c	66.4±1.37 cd	0.54±0.01	93.4±3.28 cd
SLHexF7Ac-1-E	0.0±0.00	145.7±4.99 d	101.8±3.22 e	70.0±0.90 ce	0.50±0.01	95.6±1.47 d
SLHexF7Ac-1-F	2.0±1.33 a	137.9±3.54 cd	89.6±1.47 cd	65.5±2.35 bd	0.50±0.02	94.2±1.44 cd
Control (Ethyl acetate)	1.0±1.00 a	134.7±4.18 cd	98.4±3.02 de	73.2±1.23 ae	0.52±0.02	95.0±0.61 d
Azamax® (100 mg kg^{-1})	2.0±1.33 a	54.1±6.01 a	41.4±7.25 a	72.2±5.25 a	0.42±0.04	59.6±4.99 a
F	2.565	32.768	17.175	2.329	1.713	18.755
p value	0.02754	<0.0001	<0.0001	0.03362	0.1194 [ns]	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[ns] Not significant ($p>0,05$).

Table 7. Egg deterrence of fractions (tested at 50 mg kg^{-1}) from leaves of *Solanum lycocarpum* on *Zabrotes subfasciatus* through residual contact bioassay in free-choice arenas. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Nº eggs/sample (±SE)**	**Deterrence Index (±SD)** [1]	**Classification Interval** [2]	**Classification**
SLHexF7Ac-1-A	62.2	0.88±0.11	1±0.07	Neutral
Control	78.2			
SLHexF7Ac-1-B	41.2	0.60±0.08	1±0.05	Deterrent
Control	96.6			

[1]Deterrence Index: $= \frac{2G}{G+P}$, where G is the % of eggs in treated unit samples, and P is the % of eggs in control. SD: standard deviation.

[2]Classification Interval: $CI = 1 \pm t\ (\frac{SD}{\sqrt[2]{n}})$, where *t* is the Student's t distribution value (n-1; α: 0,05), SD the standard deviation, and *n* the number of repetitions.

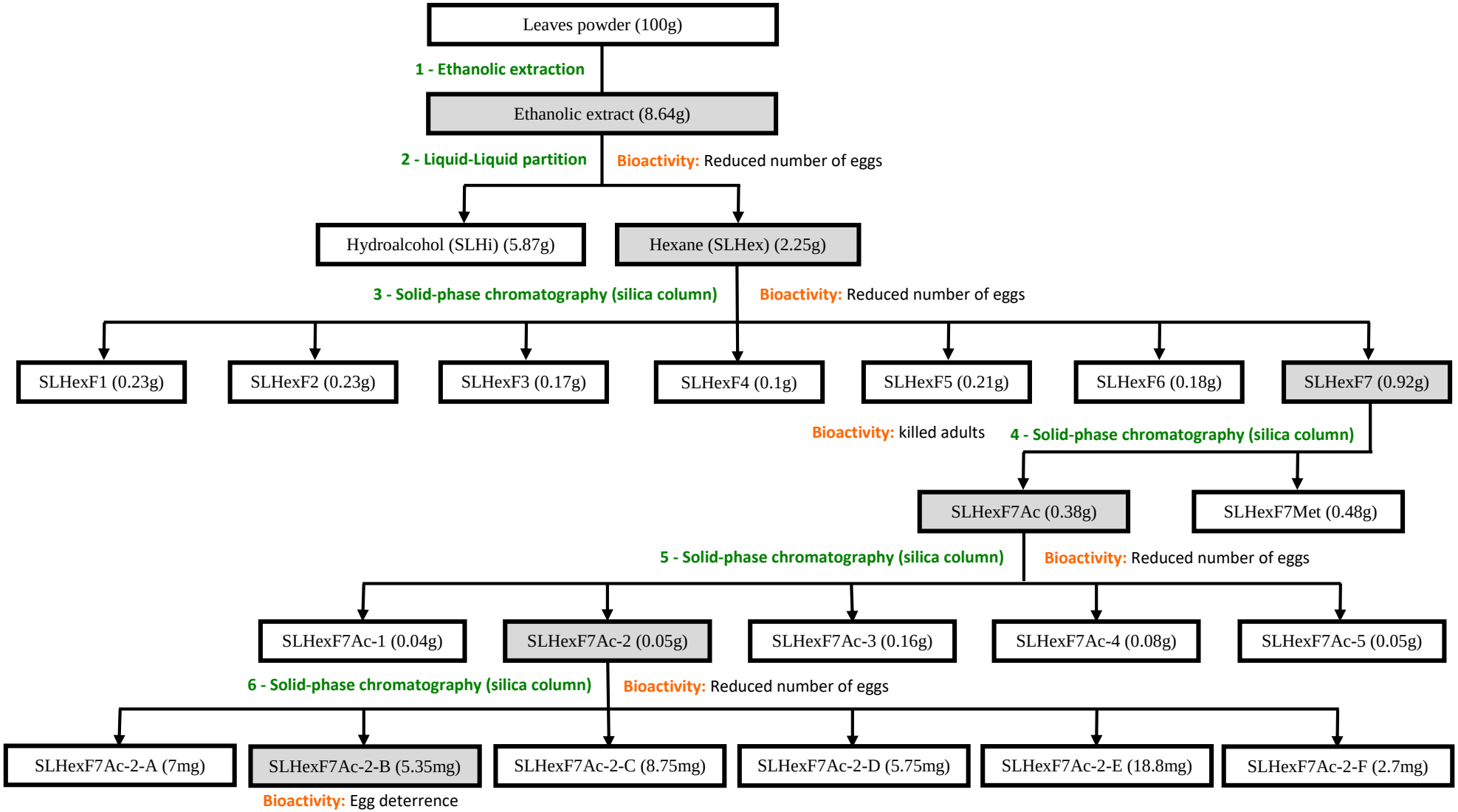

Figure 1. Scheme of chemical separations and fractions of the ethanolic extract from leaves of Solanum lycocarpum. In green: chemical separation procedures. In orange: bioactivity promoted.

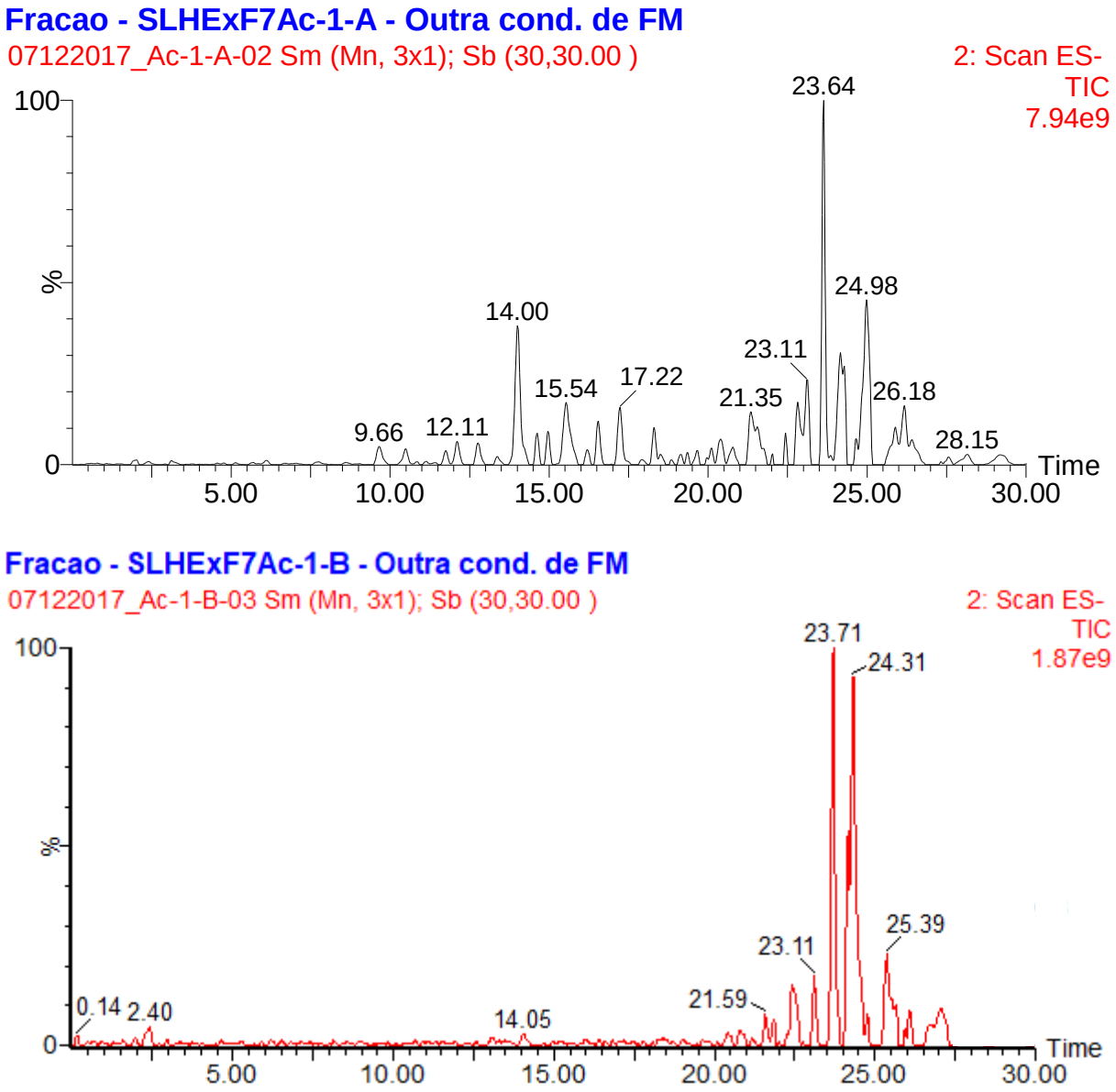

Figure 2. Chromatogram UPLC-MS in negative mode of the fractions SLHexF7Ac-1-A (above) and SLHexF7Ac-1-B (below).

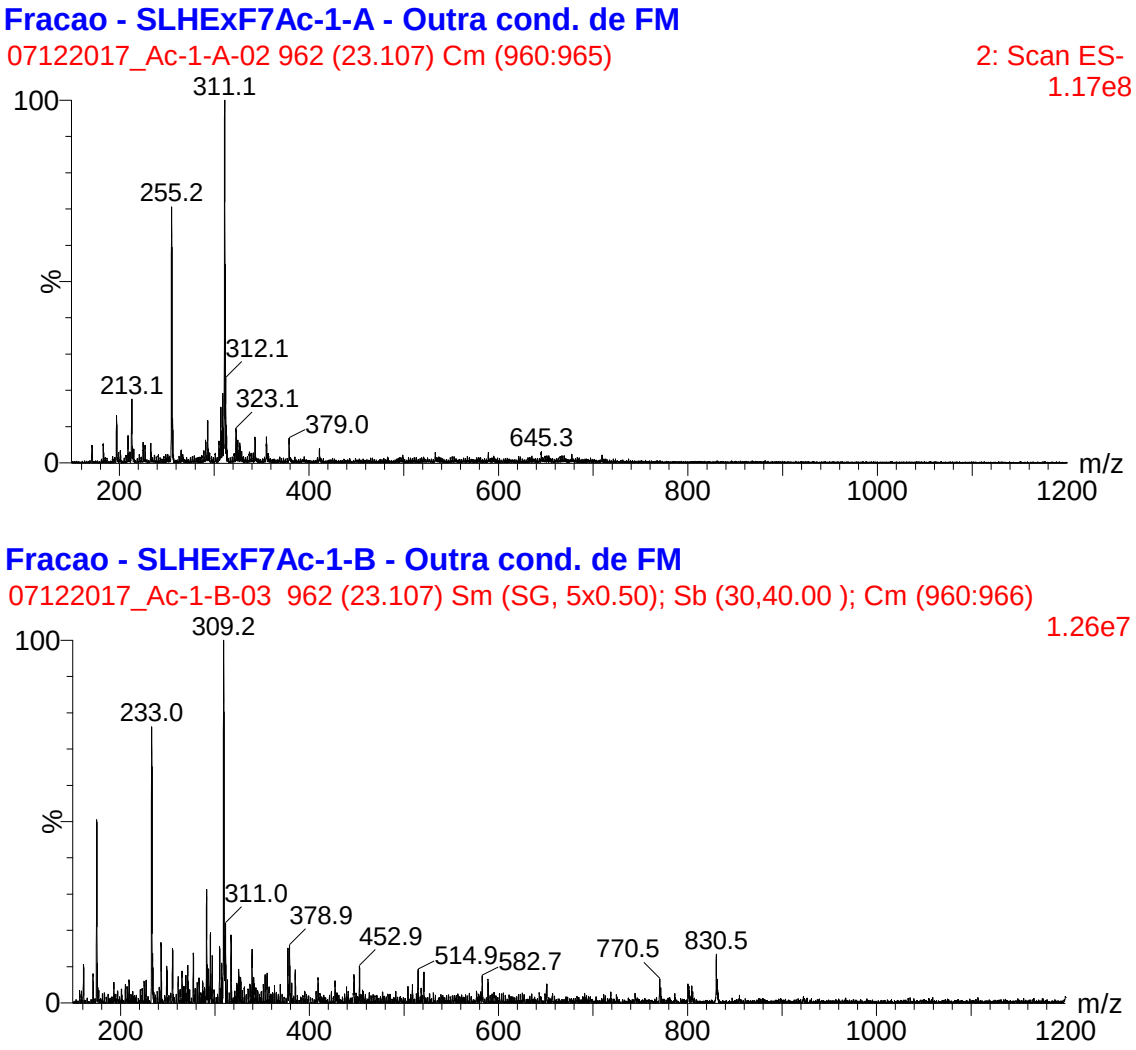

Figure 3. Mass spectrum of a major compound from fraction SLHexF7Ac-1-A (retention time 23.11 min) and one from fraction SLHexF7Ac-1-B (retention time 23.11 min).

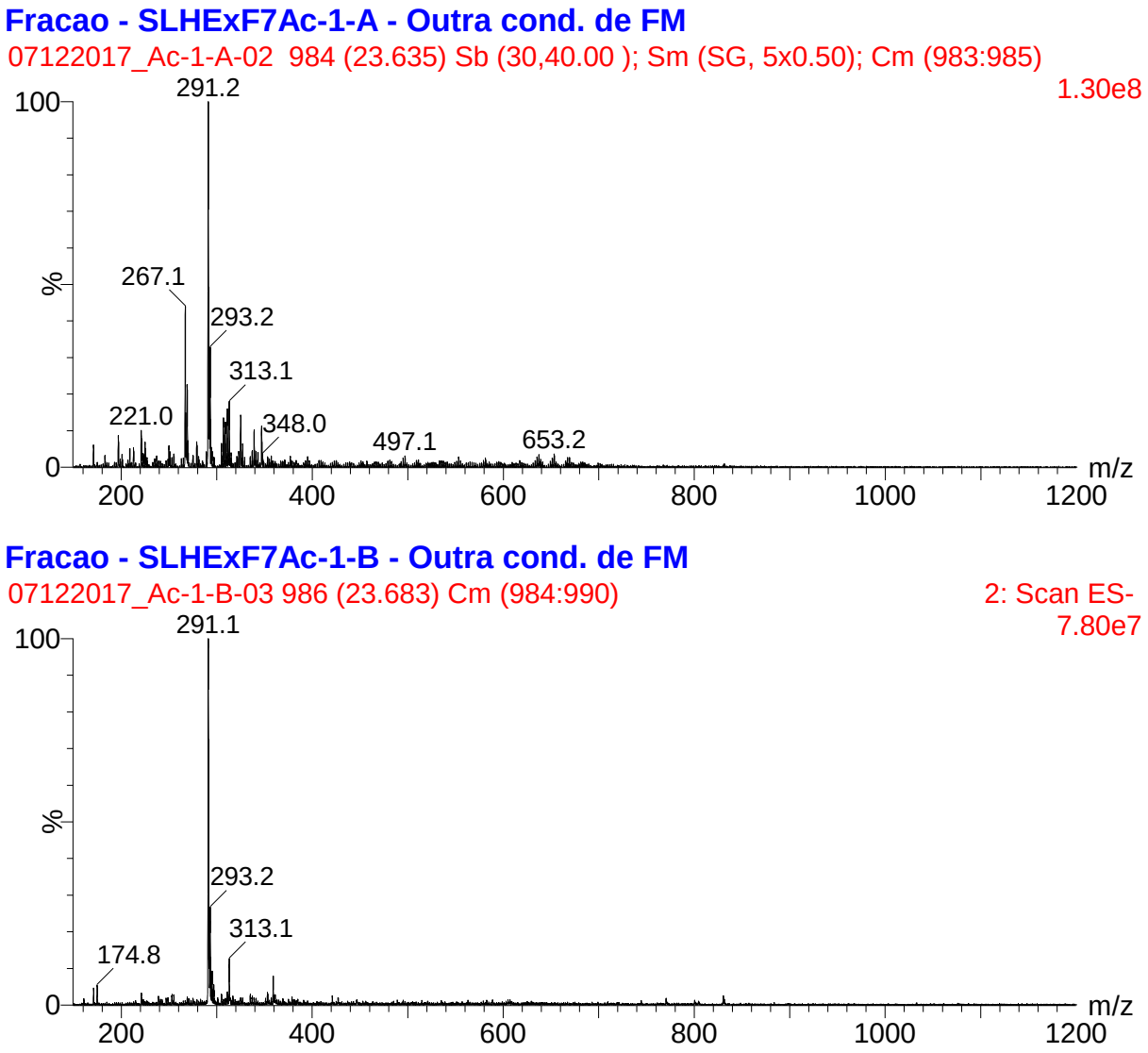

Figure 4. Mass spectrum of a major compound from fraction SLHexF7Ac-1-A (retention time 23.64 min) and one from fraction SLHexF7Ac-1-B (retention time 23.71 min).

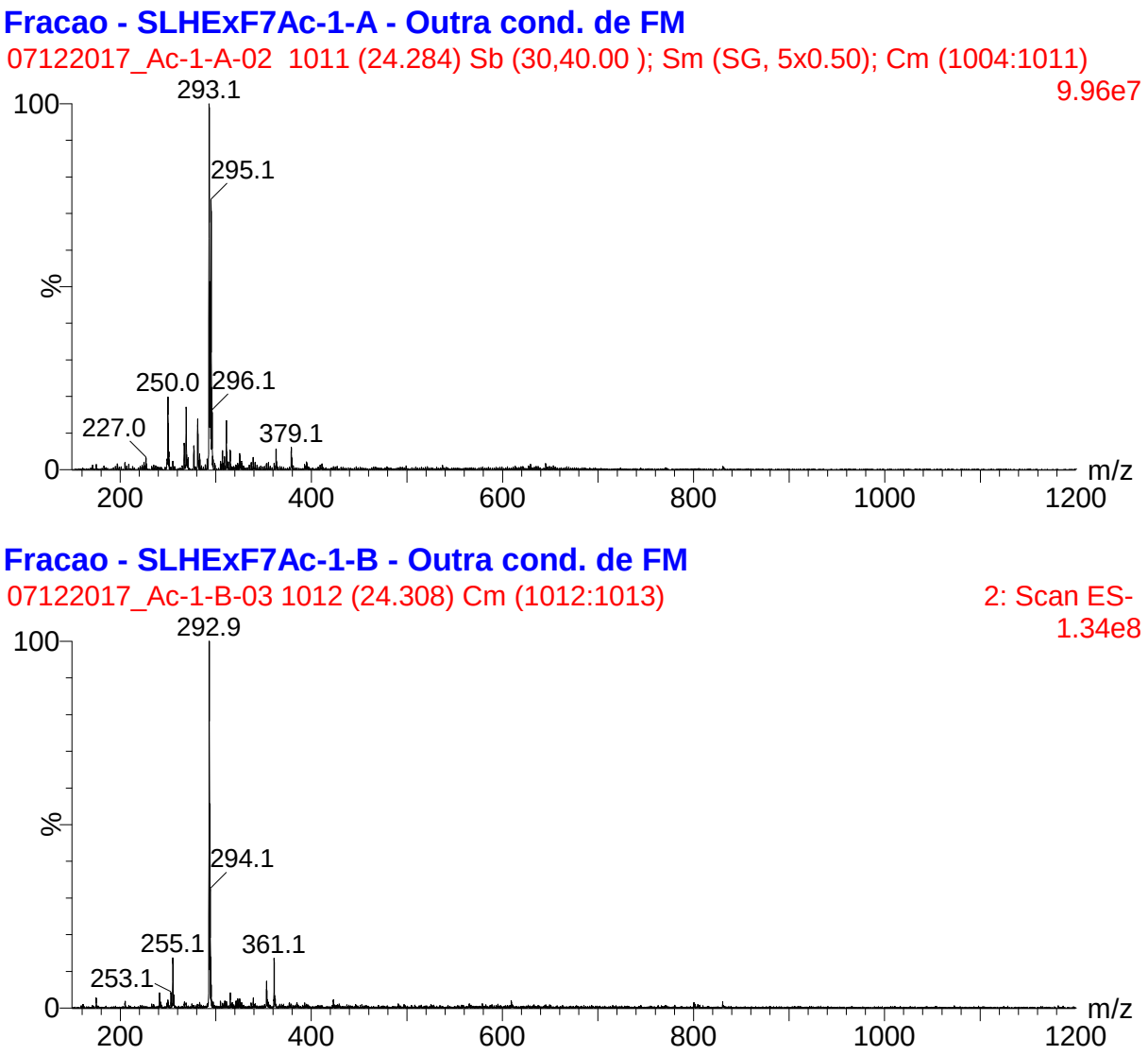

Figure 5. Mass spectrum of a major compound from fraction SLHexF7Ac-1-A (retention time 24.3 min) and one from fraction SLHexF7Ac-1-B (retention time 24.3 min).

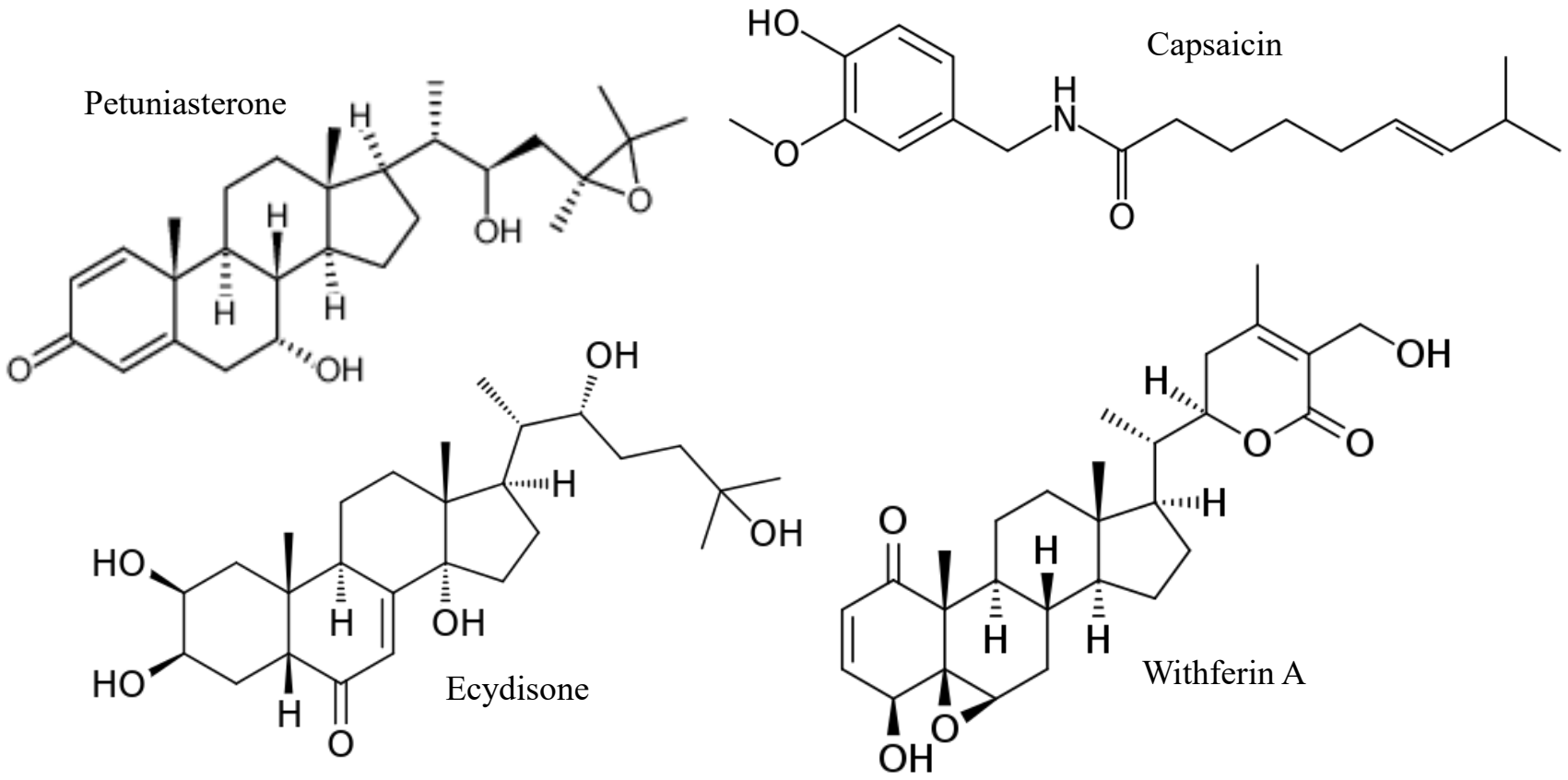

Figure 6. Chemical structures of capsaisin, a petuniasterone and a whitanolide (Whitaferin A) that occur on Solanaceae species. It is also presented the chemical structure of ecydisone, an steroid insect hormone that controls molting process.

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5. INSECTICIDAL FATTY ACID DERIVATIVES TO PROTECT STORED BEANS AGAINST *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae: Bruchinae)

ABSTRACT

The high costs of developing new insecticide molecules combined with stringent regulatory laws for pesticide registration have encouraged more research with insecticidal compounds of plant origin. Moreover, insecticidal plant compounds may be less harmful to humans and beneficial to non-target organisms, and present new mode of actions to control insect pests resistant to insecticides currently sprayed. Therefore, bioguided chemical fractionations of the ethanolic extract from flowers of *Brugmansia suaveolens* (Willd.) Bercht. & J.Presl (Solanaceae) were performed in order to identify insecticidal chemical compounds able to protect stored beans against Bruchinae beetles. The chemical fractionations using silica column chromatography were based on results from toxicological bioassays (residual contact) using *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae: Bruchinae) as a model insect. During the bioguided chemical fractionations, bioactive fractions expressed their effects mainly by preventing the embryonic development of *Z. subfasciatus* and its egg`s chorion was not formed. Consequently, F_1 progeny and damages on bean grains were completely inhibited. Moreover, 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. In order to verify the chemical class of insecticidal compounds in *B. suaveolens* flowers, a Gas Chromatography Mass Spectrometry Analysis was performed with the fraction BSHidAcF1-1-C. The results revealed the presence of fatty acids derivatives in this fraction.

Keywords: *Brugmansia suaveolens*; Botanical insecticides; Fatty acids.

5.1. Introduction

The development of stringent regulatory laws and requirements for pesticide registration at Governmental Regulatory Agencies in response to the negative effects of pesticides has eliminated many active ingredients from the market and has restricted the use of many others (Handford et al. 2015). A total of 370 different pesticide active ingredients were banned in one or more countries until 2017, with emphasis on Brazil accumulating the highest number of bans, 76 in total (Pan International 2017). These restrictions for pesticides registration based on their efficacy, environmental security, and health security were indubitably beneficial for society. However, they hindered and increased the costs of developing new synthetic products with new modes of action that attend to all the following

criteria: more selective to non-target organisms, effective against resistant pest populations and less harmful to human health and environment. The total cost of researching, developing and registering a new plant protection product was $ 152 million in 1995 and increased to $ 286 million in 2010-2014 (McDougall 2016).

On the other hand, worldwide there are some legislations and regulations to facilitate the use of low risk pesticides. In the United States of America (USA) there is a Minimum Risk Exemption regulation [40 CFR 152.25(f)] that exempt active and inert ingredients from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (Handford et al. 2015). The Environmental Protection Agency of USA regulates and publishes lists (list 25b) of active and inert ingredients that can be used in pesticides without needing further registration (EPA 2015, 2016). These lists include plant products such as castor oil, cinnamon oil, citronella oil, rosemary oil, sesame oil, thyme oil, citric acid, kaolin etc. (EPA 2015). In addition, the European Union performed a Thematic Strategy on Sustainable Use of Plant Protection Products by means of the Framework Directive 2009/128/EC including the registration of low risk pesticides and the promotion of Integrated Pest Management programs to reduce the negative effects of pesticides (Villaverde et al. 2014). The Brazilian pesticide legislation (Law Nº 7.802, from July 11th, 1989; Federal Decree nº 4.074 of January 4th, 2002, specifically at the Art. 10-D, Art. 11 and Art. 12) also includes mechanisms to facilitate and prioritize the registration of pesticides to organic agriculture and allows the use of homemade plant-based insecticides at the farm.

Thereby, new sources of active ingredients to develop pesticides become commercially attractive, what is already reflected in the profile of new active ingredients registrations at the *Environmental Protection Agency* (EPA) of the United States of America. Considering the new active ingredients of conventional biopesticides and pesticides registered at EPA between 1997 and 2010, 30.7% were in the Synthetic (S) category; 35.7% in Natural Products (NP); 27.4% in Biological (B) and 6.1% in Synthetic Natural Product Derivatives (NDS) (Cantrell et al. 2012). Specifically for insecticides, they were 67.9% (S), 21.4% (NDS) and 10.7% (NP) (Cantrell et al. 2012). Moreover, natural active ingredients and synthetic active ingredients derived from natural ones represent 33% of pesticides modes of action (Gerwick and Sparks 2014); and in 2012 natural products (fungicides, herbicides, and insecticides) plus natural-based synthetic pesticides accounted for 30% of international pest control sales (Gerwick and Sparks 2014). Curiously, the two most relevant classes of insecticides for the global market, neonicotinoids (27% of total sales) and pyrethroids (9% of total sales) are derived from plant natural compounds from *Nicotiana tabacum* L. (Solanaceae) and *Tanacetum cinerariaefolium* L.

(Asteraceae), respectively (Dayan et al. 2009, Sparks and Nauen 2015). Moreover, many botanical insecticides with different modes of action are used in the world to control insect-pests from different orders in a variety of crops (Isman 2006, 2008, Rattan 2010, Miresmailli and Isman 2014, Pavela 2016).

Worldwide, beans present a huge economic and nutritional importance. In 2014 30,612,842 ha were cultivated with beans which produced 26,529,580 tons of grains (0.867 ton ha^{-1}), featuring Brazil (3,294,586 ton, 1.034 ton ha^{-1}) and Mexico (1,273,957 tons, 0.758 ton ha^{-1}) as major producers. In Brazil, the common bean, *Phaseolus vulgaris* L. (Fabaceae), has a huge food importance with a per capita consumption estimated at around 17.44 kg/inhabitant/year (FNP 2016). This crop has a high economic expression in Brazilian agribusiness, being cultivated in more than 3 million hectares, with a production of 3,116,300 tons in the 2014/2015 harvest (FNP 2016). Unfortunately, the occurrence of Bruchinae beetles such as the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae), at warehouses is a very important issue for stored beans because these pests can promote high quantitative losses on store beans as well as losses in product quality. In Brazil, there are only three registered active ingredients to control *Z. subfasciatus* infestations in warehouses, including aluminum phosphide and magnesium phosphide (inorganic phosphine precursor) for fumigation and deltamethrin (pyrethroid) for grain protection (Agrofit 2017). Nonetheless, Brazil has a huge botanical biodiversity to be chemically explored in order to discover promissory insecticidal molecules to compose formulations of botanical insecticides or synthetic insecticides to support Integrated Pest Management in both conventional and organic agriculture (Giulietti et al. 2005).

Therefore, in the present study, it was performed chemical fractionations in order to isolate and identify bioactive chemical compounds from *Brugmansia suaveolens* (Humb. & Bonpl. ex. Willd.) Bercht. & C. Presl (Syn. *Datura suaveolens*) (Solanaceae) to control *Z. subfasciatus*. This species is a native shrub from South America commonly known as angel's trumpet largely used in landscape projects for ornamental purposes due to its beautiful white or pink blossoms. Regarding to chemical composition, a diverse range of secondary metabolites are present in *B. suaveolens*, including tropane alkaloids, pyrrolizidine alkaloids and kaempferol glycosides isolated from its leaves (Geller et al. 2014). The alkaloids hyoscyamine, atropine and scopolamine occur in *B. suaveolens* flowers (Andreola et al. 2008), and its white blossoms are predominantly composed by the terpenes 1,8-cineole (72.1%), (*E*)-nerolidol (11.7%) and a-terpineol (5.3%) (Anthony et al. 2009).

5.2. Material and Methods

5.2.1. Insects used in bioassays

In all bioassays, it was used individuals of *Z. subfasciatus* from laboratory colonies established with specimens collected in warehouses of Piracicaba municipality, SP, Brazil. The laboratory colonies were maintained in glass containers (2.6 L) containing *Phaseolus vulgaris* grains cv. Bolinha and kept in acclimatized room (25±2°C, 60±10% RH and a photoperiod of 14 L: 10 D hours).

5.2.2. Bioassay procedures

The effects of *B. suaveolens* on *Z. subfasciatus* were verified by evaluating different variables. It was accounted the number of dead insects (insects were considered dead if they did not respond to a brush touch after 1 minute) and the number of eggs deposited on bean grains surface after five days of *Z. subfasciatus* exposition to treated bean samples (adults were withdrawn from sample units). After 56 days from the infestation, it was verified the number of insects in F_1 progeny (males and females) as well as the respective damage caused by them on bean grains.

Residual contact bioassays were conducted under controlled conditions (25±2°C, 60±10% RH and a photoperiod of 14 L: 10 D hours) with a completely randomized experimental design. Treatments (chemical fractions) were composed by 10 repetitions consisted of bean samples (10 g) placed in Petri dishes (6.5 cm diameter × 2 cm high) infested with five couples of *Z. subfasciatus* (aging 0-24 hours after emerging from beans). For each bioassay, a negative control (solvent used for suspension of *B. suaveolens* fractions) was used. In addition, the botanical insecticide Azamax® 1.2EC {azadiractin A/B [12 $g.L^{-1}$ (1,2% m/m)]} or K-Obiol 2P® [deltamethrin 0.2% (m/m)] were included in bioassays to compare their activity with the fractions. They were applied adopting the same concentration used for spraying fractions of *B. suavelens*. Azamax® 1.2EC, a botanical insecticide registered in Brazil to control many insect pests, presents limonoids (azadiractin A and B) that can cause phagodeterrence and hormonal disbalance on insects. The synthetic insecticide K-Obiol 2P® presents the pyrethroid deltamethrin acting as a modulator of voltage gated sodium channels in the neuron.

The bean's grains were sprayed with fractions from *B. suaveolens*. Fractions were solubilized using organic solvents and applied on samples of 100 g of beans (10 replicates with

10 g) per treatment placed inside plastic bags (2 L). A microatomizer pistol attached to a pneumatic pump adjusted to provide a spray pressure of 0.5 kgf cm^{-2} with a volume of 30 L t^{-1} [3 mL of solution (solvent + chemical fraction) per each 100 g of beans] was used to spray fractions on bean grains surface. After this, the grains were softly shaken inside their plastic bags to promote a more homogeneous adherence and distribution of chemical fractions on grains' surface. Afterwards, treated beans were placed in an airflow chamber during two hours for solvent evaporation.

5.2.2.1. Oviposition deterrence bioassay

The oviposition deterrence effect of the fraction BSHidF1Ac-1 was evaluated in a choice bioassay with completely randomized design with 10 repetitions. An acrylic choice-arena (square-shape) containing five interconnected chambers, one central and four in the corners, was used. Inside each corner chamber 5 g of bean grains were placed, two opposite chambers with treated bean grains (BSHidF1Ac-1) and two controls (ethyl acetate). Insects were introduced at the central chamber and five days after their infestation the number of eggs on grains was assessed.

5.2.3. Bioguided fractionations of the ethanolic extract of *B. suaveolens*

5.2.3.1. Liquid-liquid partitioning

The ethanolic extract from flowers of *B. suaveolens* was selected for a liquid-liquid partitioning to produce fractions presenting different chemical affinities, one with more polar compounds [methanol:water (1:3, $v.v^{-1}$)] and other with less polar compounds (hexane). The ethanolic extract was solubilized in methanol:water (1:3, $v.v^{-1}$), adding 100 mL for each gram of extract. To perform the liquid-liquid partitioning it was added 100 mL of hexane for each gram of ethanolic extract in the separation funnel for three times. The hexane fraction (BSHex) and the remaining hydroalcoholic phase (BSHid) were both concentrated in a rotary evaporator (50°C and -600 mmHg).

The hexane (52.67% yield) and hydroalcoholic (39.67% yield) fractions from the flowers of *B. suaveolens* were tested against *Z. subfasciatus* adopting the bioassay procedures

described in item 2.2. They were applied at a concentration of 2,500 mg kg^{-1}, the same one used in the bioassay with crude extracts.

5.2.3.2. Solid Phase Extraction with with silica cartridges

5.2.3.2.1. First Solid Phase Extraction

The previous hydroalcoholic fraction from *B. suaveolens* flowers was separated using a Sep-Pak Silica Classic Cartridge (Strata-Phenomenex, 10 g). A mass of 0.5 g of the hydroalcoholic fraction was solubilized in acetone and inserted in the silica cartridge, and it was applied 150 mL of acetone and 150 mL of methanol. The acetone fraction (BSHidAc) yielded 43.12% and the methanol fraction (BSHidMet), 51.8%. They were applied on bean grains surface using acetone:methanol (1:1, v:v).

5.2.3.2.2. Second Solid Phase Extraction

The fraction BSHidAc (0.5 g) was submitted to another separation in a Sep-Pak Silica Classic Cartridge (Strata-Phenomenex, 10 g). In this step it was used a set of organic solvent combinations with crescent polarity in order to separate bioactive compounds from *B. suaveolens* flowers. It was successively applied inside the cartridge 50 mL of 9hex:1EtAc (9 Hexane: 1 Ethyl Acetate), 8hex:2 EtAc, 7hex:3EtAc, 6hex:4EtAc, 5hex:5EtAc, 4hex:6EtAc, 3hex:7EtAc, 2hex:8EtAc, 1hex:9EtAc, acetone, and finally 100% methanol in the silica cartridge producing 11 fractions. Based on the similarities of their chemical profiles of silica thin layer chromatography, they were grouped in 7 fractions: BSHidAcF1 (15.12% yield), BSHidAcF2 (9.71%), BSHidAcF3 (4.32%), BSHidAcF4 (9.21%), BSHidAcF5 (1.78%), BSHidAcF6 (15.48%) and BSHidAcF7 (39.26%). The organic solvent ethyl acetate was used to apply these fractions on bean grains.

5.2.3.2.3. Third Solid Phase Extraction

Based on toxicological bioassays results, the fraction BSAcF1 (0.41 g) was selected to the next chemical separation in a Sep-Pak Silica Classic Cartridge (Strata-Phenomenex, 10 g). A range of organic solvents was sequentially used to separate chemical compounds in fraction BSHidAcF1. The solvents used were: 100 mL of 8dcm:2hex (8 dichoromethane:2hexane), 100

mL of dichloromethane, 100 mL 98dcm:2EtAc (96 dicloromethane:4 ethyl acetate), 100 mL 96dcm:4EtAc, 100 mL 94dcm:6EtAc, 100 mL 92dcm:8EtAc, 100 mL of ethyl acetate, 100 mL of acetone, and finally 100 mL of methanol. Based on the similarities and differences of their chemical profiles using thin layer chromatography, they were grouped in five fractions, BSHidAcF1-1 (30.43% yield), BSHidAcF1-2 (17.87%), BSHidAcF1-3 (30.91%), BSHidAcF1-4 (14.52%) and BSHidAcF1-5 (4.78%). The organic solvent ethyl acetate was used to apply these fractions on bean grains.

5.2.3.2.4. Fourth Solid Phase Extraction

The bioactive fraction BSHidAcF1-1 (0.144 g) was separated in five different fractions using a Sep-Pak Silica Classic Cartridge (Strata-Phenomenex, 2 g) with different organic solvents. The solvent combinations (50 mL) sequentially applied were: 9hex:1dcm (9 hexane:1 dichloromethane), 8hex:2dcm, 7hex:3dcm, 6hex:4dcm, 5hex:5dcm, 4hex:6dcm, 3hex:7dcm and methanol. Based on chemical profiles of thin layer chromatography they were grouped in 5 fractions, BSHidAcF1-1-A (7.22% yield), BSHidAcF1-1-B (18.40%), BSHidAcF1-1-C (17.92%), BSHidAcF1-1-D (48.19%) and BSHidAcF1-1-E (3.82%). The organic solvent ethyl acetate was used to apply these fractions on bean grains.

5.2.3.2.5. Fifth Solid Phase Extraction

Due to its bioactivity, the fraction BSHidAcF1-1-C (0.083 g) was selected for one more chemical separation using a Sep-Pak Silica Classic Cartridge (Strata-Phenomenex, 2 g). It was sequentially applied 30 mL of each solvent combination inside the cartridge. Firstly, it was used dichoromethane:hexane (1:9) followed by dichoromethane:hexane (2:8). From this combination the proportion between dichoromethane and hexane was gradually changed, every 0.05, from dichoromethane:hexane (205:795) until dichoromethane:hexane (500:500). To finish the process, it was used 100% ethyl acetate and 100% methanol. It was produced six different fractions based on their thin layer chromatography profiles, BSHidAcF1-1-C-1 (21.79% yield), BSHidAcF1-1-C-2 (26.39%), BSHidAcF1-1-C-3 (12.71%), BSHidAcF1-1-C-4 (23.12%), BSHidAcF1-1-C-5 (13.08%), BSHidAcF1-1-C-6 (2.91%). The organic solvent ethyl acetate was used to apply these fractions on bean grains.

5.2.4. Gas chromatography coupled to mass spectrometry (GC-MS)

The analyzes of the fraction BSHidAcF1-1-C was performed on a Shimadzu QP2010Plus system (Shimadzu Corporation, Kyoto, Japan), equipped with AOC-20i automatic injector and electron ionization source (EI-EM) operating at 70 eV. Chromatographic separation was performed on a fused silica Rtx5-MS (Restek) capillary column (30 m x 0.25 mm i. d., 0.25 μm film), composed of 5% diphenylsiloxane and 95% dimethylpolysiloxane. Helium gas (99.999%) was used as the entrainment gas at a constant flow of 1.03 mL min^{-1}. The temperature of the injector and the ion source was 250ºC. Samples were prepared in spectroscopic grade ethyl acetate (J.T.Baker brand) at a final concentration of 1.0 mg mL^{-1}, and the sample injection volume was 1.0 μL. The oven temperature was programmed from 60°C to 300°C at a rate of 10°C min^{-1}. The mass spectra were recorded with a scan interval of 0.5 s in the mass range of 40 to 700Da. The chemical structures were compared with those found in the Wiley 7, NIST 08 and FFNSC 1.2 spectral libraries of the GC-MS data system, as well as their fragmentation patterns.

5.2.5. Statistical analysis

5.2.5.1. Lethal and sublethal effect bioassays

The data from bioassays with ethanolic extracts and *B. suaveolens* fractions was analyzed using the software "R", version 3.3.1. Generalized Linear Models with quasibinomial or quasipoisson family distribution were applied, and a Half-Normal Probability Plot with Simulation Envelope of the hnp package was applied to verify the model´s fit quality (Nelder and Wedderburn 1972, Demétrio and Hinde 1997, Hinde and Demetrio 1998). In the instance of significant differences between treatments (ethanolic extracts and fractions), multiple comparisons tests (Tukey's test, $p<0.05$) were executed using the glht function of the multicomp package.

5.2.5.2. Egg deterrence bioassay

The Deterrence Index (DI) was calculated using the formula $= \frac{2G}{G+P}$, where G is the % of eggs in treated unit samples, and P is the % of eggs in control. Based on DI values for each repetition and their Standard Deviation (SD) a Classification Interval (CI) was calculated using

the following formula $CI = 1 \pm t\ (\frac{SD}{\sqrt[2]{n}})$, where *t* is the Student's t distribution value (n-1; α: 0,05), SD the standard deviation, and *n* the number of repetitions. Treatments are considered neutral when the DI and CI values overlap, stimulant when DI values are superior to CI values, and deterrent when DI values are lower than CI values.

5.3. Results

5.3.1. Bioassays

The ethanolic extract from *B. suaveolens* flowers was selected for a set of chemical fractionations based on results from toxicological bioassays using *Z. subfasciatus* as model insect. The scheme of chemical separations performed with the ethanolic extract from flowers of *B. suaveolens* is represented in Figure 1.

The first chemical fractionation, a liquid-liquid partition, divided *B. suaveolens* ethanolic extract in two fractions, one in hexane (BSHex) and a remaining hydroalcoholic (75% H_2O + 25% methanol) phase (BSHid). Neither hexane nor hydroalcoholic fractions from *B. suaveolens* flowers promoted significant mortality of *Z. subfasciatus* adults (Table 1). Nevertheless, both of them interfered on *Z. subfasciatus* development, mainly the hydroalcoholic one. It reduced females' oviposition on beans surface, F_1 progeny, egg-adult development and damages on grains (Table 1). The BSHid fraction was separated in two fractions using column chromatography, one in acetone (BSHidAc) and other in methanol (BSHidMet). When tested at 1,000 mg kg^{-1}, the BSHidAc fraction slightly reduced the number of eggs on beans, but it completely blocked egg development, thereby inhibiting the F_1 progeny and damages on grains (Table 2). The fraction BSHidMet also reduced the number of eggs and F_1 progeny but less intensely than BSHidAc (Table 2). It was observed that the eggs deposited on grains treated with the BSHidAc fraction did not acquire the specific white coloration of healthy eggs. This promissory fraction was also tested at 2,500 mg kg^{-1} in order to verify if adults of *Z. subfasciatus* would die. However, it was not observed a lethal effect on adults, but a higher concentration reduced even more the number of eggs per sample (Table 3).

Thus, the BSHidAc fraction was fractionated in seven fractions (BSHidAcF1, BSHidAcF2, BSHidAcF3, BSHidAcF4, BSHidAcF5, BSHidAcF6 and BSHidAcF7) using a silica cartridge with organic solvents of different polarities, and they were applied at 250 mg Kg^{-1}. The compound(s) that previously inhibited egg development were exclusively separated

to the fraction BSHidAcF1 as can be observed in Figure 2. This fraction further reduced the number of eggs than the others and killed all eggs resulting in no damage to bean grains (Table 4). The fraction BSHidAcF3 also promoted a good reduction of *Z. subfasciatus* eggs while others did not (Table 4). Therefore, the fraction BSHidAcF1 was submitted to another column chromatography in silica resulting in five fractions.

The bioactivity was concentrated only in the less polar fraction BSHidAcF1-1. This fraction, applied at 150 mg Kg^{-1}, killed 56% of adults of *Z. subfasciatus* and completely inhibited female oviposition resulting in no damage on bean grains (Table 5). With this fraction, the adults of *Z. subfasciatus* presented signs of muscle hyperexcitation followed by difficult to move and paralysis. The surviving insects reacted with spasms and tremors when touched by a brush, and presented great difficulty of moving coordinately. Moreover, this fraction applied at 25 mg Kg^{-1} promoted an oviposition deterrent effect in free-choice bioassays (Table 6). Thus, the fraction BSHidAcF1-1 was separated into five fractions, only the fraction BSHidAcF1-1-C totally killed eggs of *Z. subfasciatus* and completely inhibited the F_1 progeny demonstrating that the chemical separation procedures adopted were quite adequate to separate bioactive compounds from flowers of *B. suaveolens* (Table 7). The fraction BSHidAcF1-1-C was divided into six fractions. Both the fraction BSHidAcF1-1-C-2 and BSHidAcF1-1-C-5, applied at 40 mg kg^{-1}, significantly killed a small percentage of *Z. subfasciatus* adults, but only the fraction BSHidAcF1-1-C-2 completely inhibited eggs development and, consequently, damages on grains (Table 8).

5.3.2. Chemical analysis of the fraction BSHidAcF1-1-C

In the spectrum produced by Gas Chromatography Mass Spectrometry Analysis, it was observed nine main peaks in the fraction BSHidAcF1-1-C. These compounds and their retention (minutes) times were: compound 1 (21.929 minutes), compound 2 (25.577), compound 3 (23.570), compound 4 (24.165), compound 5 (24.238), compound 6 (24.400), compound 7 (25.949), compound 8 (26.077) and compound 9 (27.411) (Figure 5). The molecular fragmentation pattern of such compounds was compared with data from libraries NIST (National Institute of Standards Technology) and WILEY. It was verified that their fragmentation pattern similar to fatty acids, and only for peak nine it was similar with compounds presenting benzenic ring in their structure (Figures 6-14). However, none of the molecular fragmentation pattern of fatty acid derivatives in fraction BSHidAcF1-1-C completely matched other compounds from library data (Figures 6-14). The following is an

example of a chemical compound between brackets with a fragmentation pattern similar to compounds present in fraction BSHidAcF1-1-C. For compound 1 (n-hexadecanoic acid methyl ester), compound 2 (hexadecanoic acid, ethyl ester), compound 3 (methyl 10-trans,12-cis-octadecadienoate), compound 4 (n-propyl 9,12-octadecadienoate), compound 5 (ethyl linoleolate), compound 6 (methyl 17-methyl-octadecanoate), compound 7 (ethyl 9-hexadecenoate) and compound 8 (ethyl n-heptadecanoate) similarity was close to fatty acids (Figures 6-13). Whereas compound 9 (1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester) had a similar fragmentation pattern to compounds presenting benzenic rings (Figure 14).

5.4. Discussion

The CG analysis of the fraction BSHidAcF1-1 from flowers of *B. suaveolens* revealed the presence of fatty acid derivatives, and some compounds with probably benzenic rings (Figure 5). Interestingly, flowers of *T. cinerariifolium* that present insecticidal pyrethrins also have fatty acids with insecticidal effects against Bruchinae species (Head 1968, Don-Pedro 1989, Perumalsamy et al. 2015). The major fatty acids extract from *Pyrethrum* flowers were palmitic, linoleic, linolenic, oleic and stearic (Head 1968). Moreover, the n-hexane fraction (methanolic extract) from flowers of *Azadirachta indica* A. Juss (Meliaceae), an important insecticidal plant, presented sesquiterpenoids, steroids and fatty acid esters (Siddiqui et al. 2009). The insecticidal effects of fatty acids are reported for few stored insect pests. A triglyceride fraction from African palm oil promoted lethal and sublethal effects on *Z. subfasciatus*, that is, oleic acid killed adults while linolenic and arachidonic acids only reduced oviposition of *Z. subfasciatus* (Hill and Schoonhoven 1981). The cyclopropene fatty acid, (2n-octylcycloprop-1-enyl)-octanoic acid, promotes lethality on three stored insect pests *Sitophilus oryzae* (L.), *Callosobruchus chinensis* (L.), and *Tribolium castaneum* (Herbst) through residual contact (Rani and Rajasekharreddy 2010).

The chemical fractions from flowers of *B. suaveolens* killed adults and eggs of *Z. subfasciatus*, reduced the number of eggs on bean grains, and completely inhibited the F_1 progeny and the damages on bean grains. It was the inhibition of egg development that completely inhibited F_1 progeny of *Z. subfasciatus* and damages on bean grains. As *Z. subfasciatus* is an oviparous species, the onset of its embryonic development is linked to the deposition of the egg on bean grains; thereby inhibiting its embryonic development is a key-factor to avoid its larvae to penetrate grains. The females of *Z. subfasciatus* lay their eggs

individually in a transparent gelatinous droplet previously deposited on the surface of bean grains and, thus, the larvae penetrates directly into the grain. Therefore, only the restricted contact of the egg with the treated surface of the grain is enough to prevent the embryonic development of *Z. subfasciatus* and its penetration in bean grains. It was observed that the egg's protective layer, the chorion, was not formed in treated beans (Figure 2, 3 and 4). This is a desirable aspect because it is hard to control *Z. subfasciatus* larvae after they penetrate grains, remaining in this case the option of using extreme temperatures, modified atmosphere (saturated with CO_2) and radiation, which are more expensive and inaccessible methods for most farmers (Boyer et al. 2012, Zaugg et al. 2013). Nonetheless, it is possible to associate the use of chemical fractions from *B. suaveolens* with resistant bean varieties which is a cheap and accessible control method (Eduardo et al. 2016).

Depending on its concentration, the supposed insecticidal fatty acid derivative(s) can kill not only eggs but also adults of *Z. subfasciatus* (Tables 5 and 8), what is a great characteristic, but using a concentration that only kill its eggs can provide some advantages. Killing only the eggs require a lower amount of insecticide compound than killing adults, consequently, it is consumed less active ingredient resulting in a cheaper product to formulate, and it produces fewer residues on beans resulting in shorter interval between insecticide application and bean consumption. In addition, killing beetle adults in the bean grain mass is a problem because their presence is considered a contaminant that reduces the quality and value of the final product. Therefore, another advantage of not killing adults is the possibility to retrieve them from warehouses before processing and packing stored beans. This can be done by different methods; the two technically and environmentally suitable would be the use of sex pheromones or plant repellent/attractants.

The sex pheromones of Bruchinae adult beetles could be used to attract males and virgin females to traps with insecticides or entomological sticker. Some formulated pheromones are already available for stored product pests, but not yet for Bruchinae beetles, and specifically to *Z. subfasciatus*, its sex pheromone is not identified yet (Moreira et al. 2005). Therefore, a feasible and accessible method to avoid the presence of dead *Z. subfasciatus* adults within bean grains would be to combine traps containing volatile secondary attractant/repellent phytochemicals (essential oils) together with compounds from *B. suaveolens* flowers that inhibit egg development. The bioactive chemical compounds from *B. suaveolens* avoid the establishment and damages of *Z. subfasciatus* larvae and essential oils can repel adults of *Z. subfasciatus* when they are found in warehouses. In addition, it was verified that fraction

BSHidAcF1-1 (25 mg Kg^{-1}) promotes a deterrence of oviposition reducing in half the number of eggs deposited on bean grains (Table 6).

The fraction BSHidAcF1-1-C (75 mg Kg^{-1}) was better than Azamax and BSHidAcF1-1-C-2 (40 mg Kg^{-1}) was better than K-obiol 2P to protect bean grains from *Z. subfasciatus* (Table 7 and 8), and it is important to highlight the low concentration required to do so. In the last bioassay performed, the fraction BSHidAcF1-1-C-2 at a concentration of 40 mg Kg^{-1} killed 100% of *Z. subfasciatus* eggs and 14% of adults whereas of bean grains treated with K-Obiol 2P suffered 10.5% of damage (Table 8). It is lower than the concentrations recommended for the insecticide K-obiol 2P (0.5-1.0 g Kg^{-1} grains), Kaolin (4 g Kg^{-1} grains), and neem oil (2 g kg^{-1} grains) to control *Z. subfasciatus* (Barbosa et al. 2002, Costa et al. 2014, Agrofit 2017).

K-obiol 2P (a.i. 0.02% deltamethrin), a pyrethroid, is the only registered insecticide in Brazil able to avoid infestations of *Z. subfasciatus* in stored beans and provide a longer protection than fumigant insecticides (Agrofit 2017). Pyrethroids, such as deltamethrin, are modulators of voltage gated sodium channels in the neuron axon of insects provoking hyper excitation on them (Sparks and Nauen 2015). On the other hand, fatty acids can reduce gas exchange and O_2 levels inhibiting respiratory metabolism of insect eggs and, consequently, its embryonic development. Groundnut oil applied on bean grains promoted a slowly gradual reduction on the respiratory activity of eggs from *C. macullatus* resulting in death by anoxia because of a physical oil barrier blocking gas exchange (it covers the mycropila and egg tegument) (Don-Pedro 1989). This was indicated by a lower CO_2 emission from eggs treated with groundnut oil comparing to control as well as insect embryos not completely developed (Don-Pedro 1989). It is also hypothesized that they can penetrate insect eggs and coagulate eggs proteins, disrupt water balance, or obstruct larvae hatching (Don-Pedro 1989). Furthermore, fatty acids can interfere on insect nervous system. They can inhibit acetylcholinesterase activity and interfere on octopaminergic system increasing cAMP levels (Perumalsamy et al. 2015). However, depending on their chemical structure, fatty acids can act on both cholinergic and octopaminergic systems or on only one of them (Perumalsamy et al. 2015). In the present study, the fatty acids derivatives from *B. suaveolens* flowers may be inhibiting the activity of acetylcholinesterase in insect eggs blocking their embryonic development; and adults of *Z. subfasciatus* presented symptoms of hyper-excitation which is a characteristic symptom of insecticides that inhibit acetylcholinesterase such as carbamates and organophosphates (Sparks and Nauen 2015). Thus, fatty acids present the advantage of having more than one mode of action, and they are all different from the K-obiol 2P one.

A very pertinent aspect to highlight is that *B. suaveolens* produces atropine and scopolamine in its flowers (Geller et al. 2011, 2014). There are some reports of human intoxication due to the exposure to plants containing tropane alkaloids such as hyoscyamine, atropine and scopolamine (Adamse et al. 2014). Scopolamine presents insecticidal action against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Roesler et al. 2007), but scopolamine and atropine can intoxicate human beings promoting loss of body coordination, muscular paralysis, hallucinations and respiratory distress (Smith et al. 1991). Off course, their toxicity depends on its concentration applied, time and form of exposition, and the target-organism; the average fatal dose for atropine is 50 mg, but its therapeutic dose is 1-3 mg (Smith et al. 1991, Eddleston et al. 2008). Curiously, atropine is also used to treat poisoning with organophosphates (Eddleston et al. 2008) that are inhibitors of acethylcholinesterase, the same mode of action described for fatty acids in insects (Perumalsamy et al. 2015, Sparks and Nauen 2015). This demonstrates how complex is the interaction of bioactive compounds with live organisms, but anyway it is important to formulate botanical insecticides with minimum risks to humans despite the general public perception regarding the safety of botanical insecticides.

In general, people tend to have an imprecise concept of "*natural*" and a mistaken perception that everything "*natural*" is good for health or at least less harmful than synthetic products. This notion is expanded to insecticides; synthetic insecticides are considered extremely dangerous while botanical insecticides are considered safe. This assumption can be true for many cases and circumstances; however, complex chemical mixtures from plants can present dangerous toxins. A good example is the alkaloid strychnine extracted from *Strychnos nux-vomica* L. (Loganiaceae) that is an efficient rodenticide, but it is very toxic to humans and other mammals (Flood 1999, Witmer et al. 2017). Therefore, it is important to perform toxicological studies in order to evaluate potential risks of plant-toxins to non-target organisms. Another important aspect is that using purified chemical fractions or isolated compounds can provide some advantages such as avoiding antagonisms among active ingredients, removing contaminants and noxious toxins to non-target organisms. Moreover, it ensures a standard concentration of active ingredients that is essential for registering an insecticide commercial product.

Fortunately, a great advantage of insecticidal fatty acids is their low probability to be toxic to humans what can strongly facilitate their registration in official pesticide regulatory agencies. Plant fatty acids are widely present in human diet providing us many health benefits including prevention of cardiovascular diseases (Hu 2003). Some fatty acids, such as oleic and linoleic acids, can be synthetically produced opening perspectives for a synthetic insecticide

formulation of fatty acids derivatives to protect stored beans against *Z. subfasciatus* (Noller and Bannerot 1934, Raphael and Sondheimer 1950, Yamamoto et al. 2015). Many plant oils containing free fatty acids such as castor, corn, cottonseed, sesame and soybean oils are exempt from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) in United States of America (EPA 2015, 2016). In addition, the Brazilian Organic Farming Law (Federal Law nº 10.831/2003; Federal Decree nº 6.323/2007, annex VI) allows the use of plant oils and their derivatives for pest control.

The results obtained in the present study are an important step to identify an insecticide compound from *B. suaveolens* flowers that can be applied in agriculture to control Bruchinae beetles in stored beans, however further studies are needed to isolate and chemically characterize it. More chemical fractionations and chemical analysis are being performed in order to isolate and identify the chemical compound(s) from flowers of *B. suaveolens* that is responsible for the observed bioactivity on *Z. subfasciatus*. Not only research with insecticidal plant compounds has been growing in recent years but also legislative and bureaucratic regulations to facilitate its registration and use for pest management (Isman and Grieneisen 2014, Handford et al. 2015, Villaverde et al. 2016). This legislative trend can open many opportunities for developing commercial botanical insecticides and synthetic insecticides based on plant chemical compounds. Therefore, Brazilian scientists can play an important role in bioprospecting insectistatic compounds to develop safer and more efficient insecticides due to the huge botanical biodiversity in Brazil`s territory (Giulietti et al. 2005).

Table 1. Bioactivity (mean ± SE) of fractions from *Brugmansia suaveolens* (tested at 2,500 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
Hydroalcohol (BSHid)	6.0±2.67	33.5±6.21 b	20.6±3.98 b	56.8±7.91 b	0.43±0.08	30.8±5.38 b
Hexane (BSHex)	2.0±1.33	68.7±8.21 c	48.4±6.37 c	70.6±2.98 c	0.51±0.03	61.5±6.71 c
Control (acetone: methanol (1:1))	3.0±1.53	87.5±6.21 d	63.0±5.64 d	71.3±2.38 c	0.51±0.02	78.6±3.36 d
Azamax® (2,500 mg kg^{-1})	5.0±2.24	28.4±5.45 a	4.0±0.77 a	5.4±2.34 a	0.10±0.07	7.2±1.42 a
F	0.896	16.522	43.641	52.049	0.268	43.266
p value	0.4527 [ns]	<0.0001	<0.0001	<0.0001	0.8482	<0.0001

1Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
2Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[ns] Not significant ($p>0,05$).

Table 2. Bioactivity (mean ± SE) of fractions from *Brugmansia suaveolens* (tested at 1,000 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
Acetone (BSHidAc)	1.0±1 a	63.1±5.47 b	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*
Methanol (BSHidMet)	4.0±2.21 b	86.0±6.55 c	64.8±5.88 b	74.8±2.14	0.51±0.01	81.2±3.74 b
Control (acetone: methanol (1:1))	4.0±1.63 b	109.8±3.06 d	43.8±3.12 c	79.1±1.04	0.50±0.01	90.0±1.88 c
Azamax® (1,000 mg kg^{-1})	18.0±6.63 c	6.5±1.8 a	4.4±1.26 a	54.8±9.13	0.42±0.10	9.6±2.73 a
F	3.027	101.800	143.320	3.3712	0.265	121.020
p value	0.0419	<0.0001	<0.0001	0.05005 [ns]	0.7693 [ns]	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[*] Not included in the analysis (null variance);
[ns] Not significant ($p>0,05$).

Table 3. Bioactivity (mean ± SE) of fractions from *Brugmansia suaveolens* (tested at 2,500 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
Acetone (BSHidAc)	13.0±4.73 b	14.7±2.57 b	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*
Methanol (BSHidMet)	11.0±3.79 b	36.7±5.16 c	23.0±3.05 b	63.34±3.57 b	0.47±0.02 a	49.4±5.35 b
Control (acetone: methanol (1:1))	3.0±1.53 a	69.6±6.86 d	56.6±5.35 c	71.3±2.38 c	0.54±0.02 b	79.7±5.75 c
Azamax® (2,500 mg kg^{-1})	17.0±4.23 c	4.8±1.20 a	0.6±0.27 a	9.8±4.59 a	0.20±0.13**	2.2±0.82 a
F	3.0272	50.547	106.990	27.513	8.361	65.517
p value	0.0419	<0.0001	<0.0001	<0.0001	0.0097	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
* Not included in the analysis (null variance);
** Not analyzed due to small sample unit;
ns Not significant ($p>0,05$).

Table 4. Bioactivity (mean ± SE) of fractions from *Brugmansia suaveolens* (tested at 250 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
BSHidAcF1	2.0±1.33	18.3±2.58 b	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*
BSHidAcF2	2.0±1.33	33.3±2.92 cd	27.0±2.25 c	81.3±1.28 bc	0.50±0.01	41.0±2.31 b
BSHidAcF3	1.0±1.00	28.3±4.15 c	22.6±3.45 b	78.8±2.35 bc	0.50±0.04	43.8±5.71 b
BSHidAcF4	1.0±1.00	41.7±6.32 ef	28.8±4.23 cd	72.5±5.85 e	0.51±0.01	54.5±7.48 c
BSHidAcF5	1.0±1.00	29.7±1.94 cd	25.0±1.53 bc	85.1±2.92 b	0.51±0.02	55.0±3.76 c
BSHidAcF6	1.0±1.00	34.9±3.06 de	27.0±2.02 c	78.1±1.60 cd	0.52±0.02	57.3±5.39 c
BSHidAcF7	5.0±2.69	35.1±2.39 de	28.0±2.64 cd	79.5±3.60 cd	0.48±0.01	54.5±5.05 c
Control (ethyl acetate)	1.0±1.00	44.1±5.42 f	32.4±2.58 d	77.3±4.58 de	0.50±0.13	61.8±3.74 c
Azamax® (250 mg kg^{-1})	1.0±1.00	6.3±1.38 a	1.3±0.60 a	17.8±6.02 a	0.28±0.13**	2.9±1.41 a
F	0.773	13.602	21.210	5.930	0.082	16.971
p value	0.6272 [ns]	<0.0001	<0.0001	0.000183	0.9977	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
* Not included in the analysis (null variance);
** Not analyzed due to small sample unit;
[ns] Not significant ($p>0,05$).

Table 5. Bioactivity (mean ± SE) of fractions from *Brugmansia suaveolens* (tested at 150 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
BSHidAcF1-1	56.0±5.42 b	0.1±0.01 a	0.0±0.00*	0.00±0.00*	0.0±0.00*	0.0±0.00*
BSHidAcF1-2	1.0±1.00 a	15.8±2.47 c	13.4±2.14 b	83.4±2.45 bc	0.61±0.03 a	23.1±2.31 b
BSHidAcF1-3	1.0±1.00 a	35.4±4.40 ef	30.6±4.50 d	84.6±1.85 b	0.54±0.03 c	47.2±3.61 d
BSHidAcF1-4	1.0±1.00 a	40.6±1.54 f	33.2±1.42 d	81.8±1.73 c	0.45±0.03 d	56.8±1.50 e
BSHidAcF1-5	1.0±1.00 a	29.8±4.77 d	25.2±4.74 c	83.5±5.80 bc	0.53±0.06 b	41.5±5.65 c
Control (ethyl acetate)	1.0±1.00 a	34.0±3.38 de	25.0±3.26 c	72.0±2.92 d	0.52±0.04 c	44.0±2.09 cd
Azamax® (150 mg kg^{-1})	2.0±1.33 a	7.4±2.90 b	3.4±1.48 a	35.0±12.47 a	0.18±0.08**	8.0±2.78 a
F	34.399	29.728	14.719	6.420	3.181	26.602
p value	<0.0001	<0.0001	<0.0001	<0.0001	0.02199	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p < 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p < 0.05$);
* Not included in the analysis (null variance);
** Not analyzed due to small sample unit.

Table 6. Egg deterrence of BSHidAcF1-1 fraction from *Brugmansia suaveolens* (tested at 25 mg kg^{-1}) on *Zabrotes subfasciatus*, by residual contact bioassay in free-choice arenas. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Nº eggs/sample (±SE)**	**Deterrence Index (±SD)** [1]	**Classification Interval** [2]	**Classification**
BSHidAcF1-1	58.1±3.34	0.7±0.09	1.0±0.05	Deterrent
Control	108.4±3.46			

[1]Deterrence Index: $= \frac{2G}{G+P}$, where G is the % of eggs in treated unit samples, and P is the % of eggs in control. SD: standard deviation.

[2]Classification Interval: $CI = 1 \pm t\ (\frac{SD}{\sqrt[2]{n}})$, where *t* is the Student's t distribution value (n-1; α: 0,05), SD the standard deviation, and *n* the number of repetitions.

Table 7. Bioactivity (mean ± SE) of fractions from *Brugmansia suaveolens* (tested at 75 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
BSHidAcF1-1-A	3.0±1.53 b	78.5±7.94 b	61.4±6.21	78.4±2.06 a	0.50±0.02 bc	79.3±3.70 a
BSHidAcF1-1-B	1.0±1.00 a	85.9±3.71 bc	67.8±4.14	78.6±2.53 a	0.47±0.02 b	81.5±2.86 ab
BSHidAcF1-1-C	6.0±2.21 c	61.3±6.53 a	0.0±0.00*	0.0±0.00*	0.00±0.00*	0.0±0.00*
BSHidAcF1-1-D	2.0±2.00 ab	83.5±3.38 b	68.4±3.02	82.0±1.83 c	0.53±0.02 a	83.5±2.83 bc
BSHidAcF1-1-E	0.0±0.00	95.5±4.36 d	74.2±3.46	77.8±1.70 a	0.50±0.01 c	93.1±1.54 d
Control (ethyl acetate)	1.0±1.00 a	86.1±2.81 bc	68.4±1.96	79.6±1.36 b	0.52±0.01 a	86.2±2.75 c
Azamax® (75 mg kg^{-1})	3.0±1.53 b	92.1±5.47 cd	65.2±5.11	70.4±2.77 a	0.53±0.01 a	81.4±2.68 ab
F	1.148	4.575	0.968	3.342	2.763	3.682
p value	0.3466	0.0006512	0.4456	0.01057	0.02706	0.006121

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p < 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p < 0.05$);
* Not included in the analysis (null variance);
ns Not significant ($p > 0,05$).

Table 8. Bioactivity (mean ± SE) of fractions from *Brugmansia suaveolens* (tested at 40 mg kg^{-1}) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

Treatment	**Mortality (%)** [1]	**Nº eggs/ sample** [2]	**F_1 progeny** [2]	**Viability (%) (egg-adult)** [1]	**Sex ratio** [1]	**Damaged grains (%)** [1]
BSHidAcF1-1-C-1	2.0±1.33 a	103.3±6.75 b	90.2±5.77 bc	87.5±2.14 c	0.51±0.01	87.5±2.52 c
BSHidAcF1-1-C-2	14.0±4.00 b	71.9±3.77 a	0.0±0.00 *	0.0±0.00 *	0.0±0.00 *	0.0±0.00 *
BSHidAcF1-1-C-3	0.0±0.00 *	130.9±8.06 de	115.2±7.08 de	88.0±1.11 c	0.49±0.01	88.5±2.01 c
BSHidAcF1-1-C-4	2.0±1.33 a	121.9±7.62 cd	104.0±6.10 d	85.6±1.60 c	0.52±0.02	91.5±1.05 c
BSHidAcF1-1-C-5	12.0±2.91 b	119.3±11.84 cd	101.8±12.64 cd	82.0±4.15 c	0.46±0.01	91.1±3.23 c
BSHidAcF1-1-C-6	4.0±1.63 a	116.3±14.98 bd	84.2±16.91 b	61.6±9.61 b	0.43±0.05	73.3±11.00 b
Control (ethyl acetate)	2.0±1.33 a	143.7±5.11 bc	123.8±4.61 e	86.1±0.96 c	0.51±0.02	89.5±1.09 c
K-Obiol 2P® (40 mg kg^{-1})	4.0±1.63 a	109.7±10.62 e	4.1±0.98 a	3.4±0.88 a	0.41±0.11 **	10.5±2.43 a
F	4.912	5.682	30.023	71.893	1.735	30.883
p value	0.000354	<0.0001	<0.0001	<0.0001	0.1422 [ns]	<0.0001

[1]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[2]Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey's *post hoc* test, $p< 0.05$);
[*] Not included in the analysis (null variance);
** Not analyzed due to small sample unit.
[ns] Not significant ($p>0,05$).

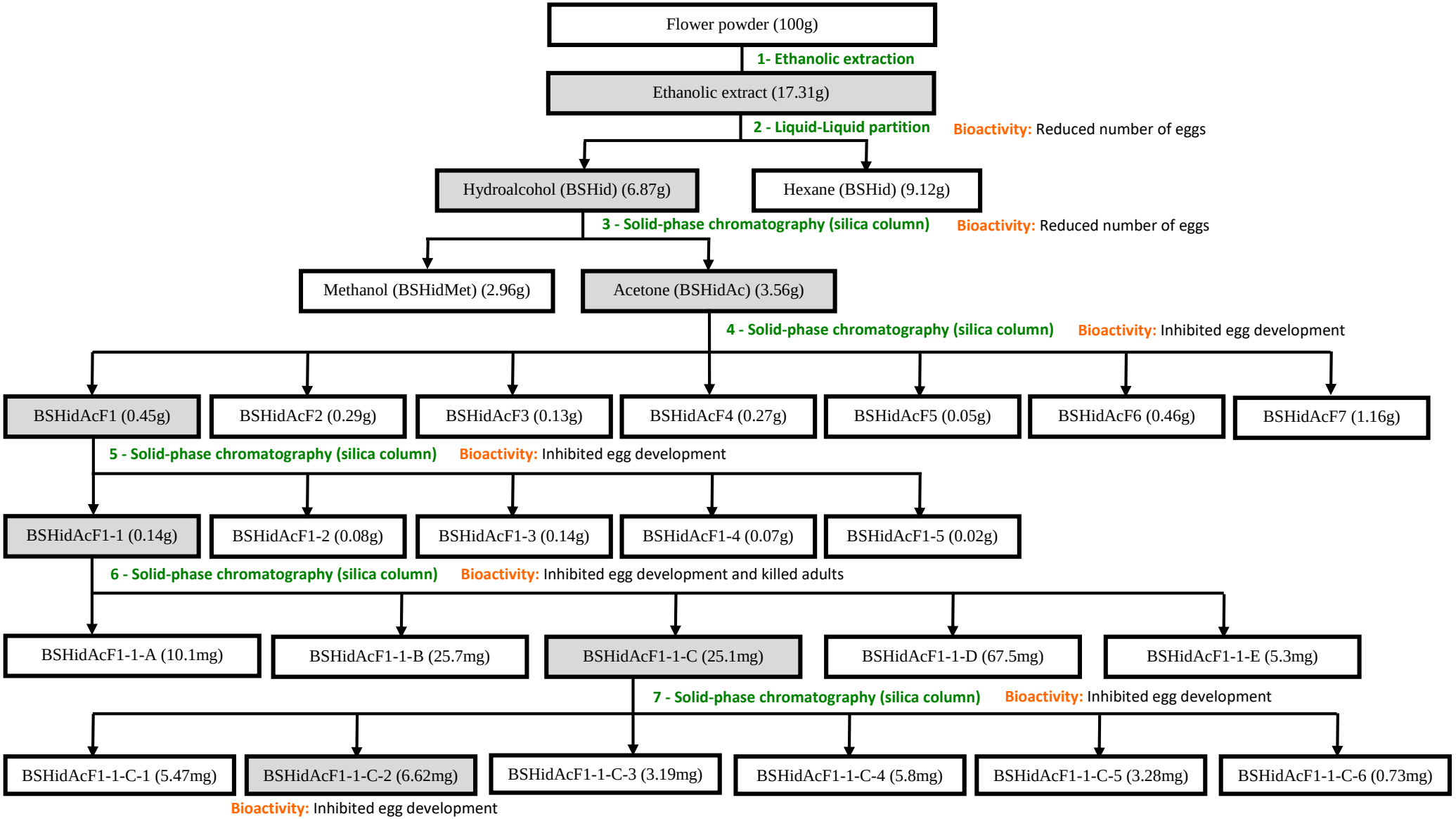

Figure 1. Scheme of bioguided fractionations of the ethanolic extract from flowers of *Brugmansia suaveolens* using *Zabrotes subfaciatus* as bioindicator. In green: chemical separation procedures. In orange: bioactivity promoted.

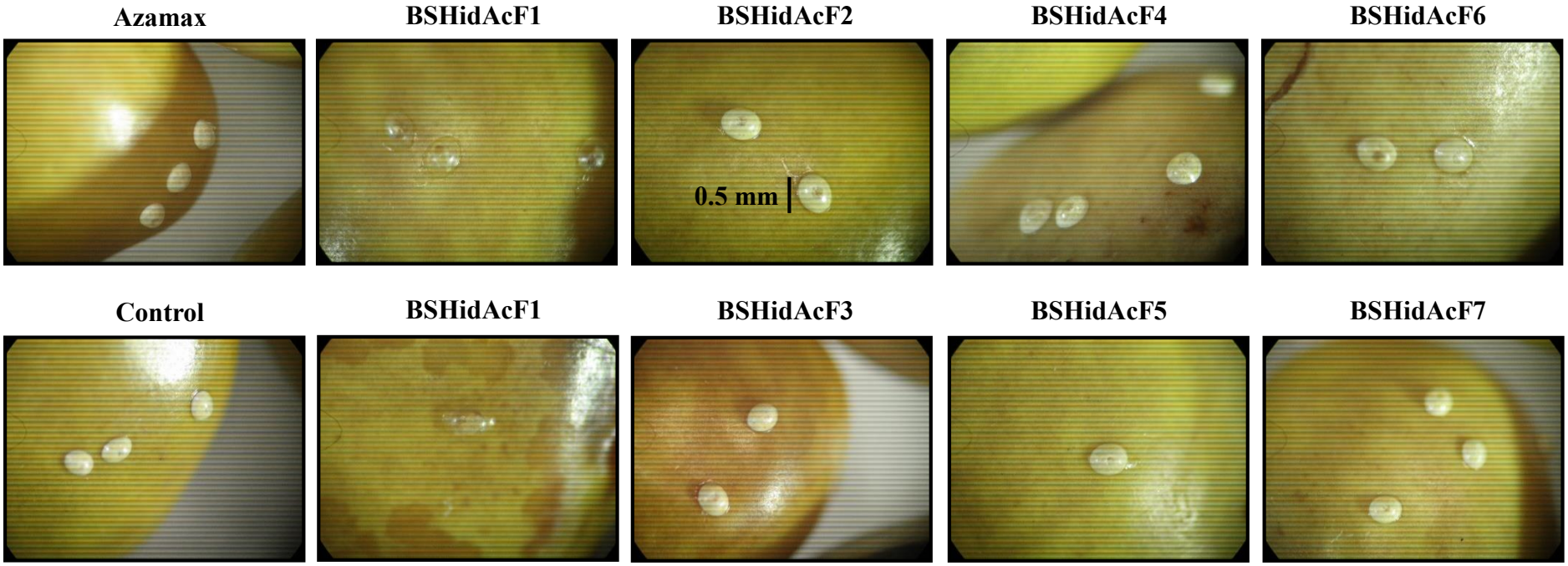

Figure 2. Eggs of *Z. subfasciatus* laid on beans cv. Bolinha treated with different chemical fractions from flowers of *Brugmansia suaveolens*. Not viable eggs: fraction BSHidAcF1; viable eggs: Azamax®, control (ethyl acetate), and fractions BSHidAcF2, BSHidAcF3, BSHidAcF4, BSHidAcF5, BSHidAcF6 and BSHidAcF7.

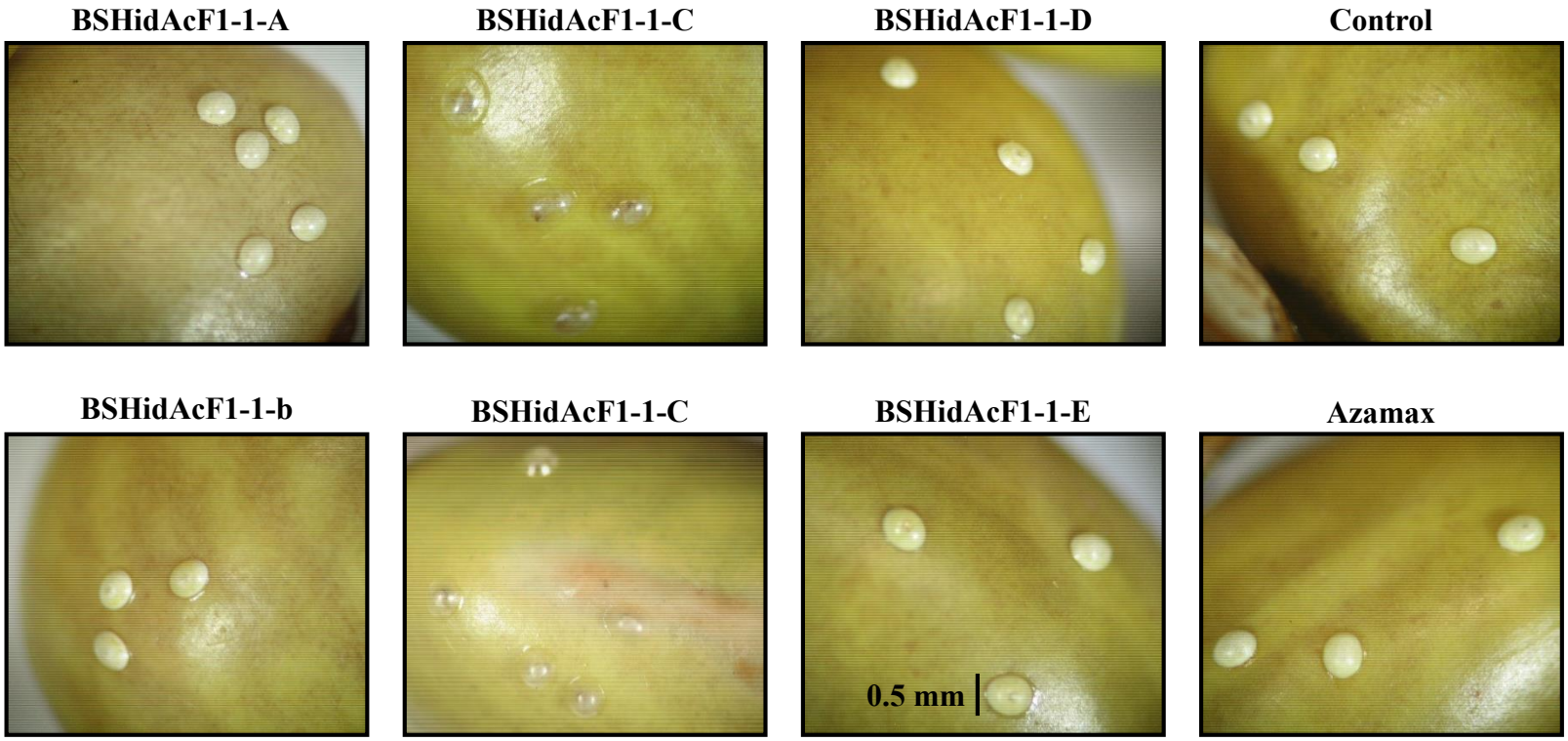

Figure 3. Eggs of *Z. subfasciatus* laid on beans cv. Bolinha treated with different chemical fractions from flowers of *Brugmansia suaveolens*. Not viable eggs: fraction BSHidAcF1-1-C; viable eggs: Azamax®, control (ethyl acetate), and fractions BSHidAcF1-1-A, BSHidAcF1-1-B, BSHidAcF1-1-D, BSHidAcF1-1-E.

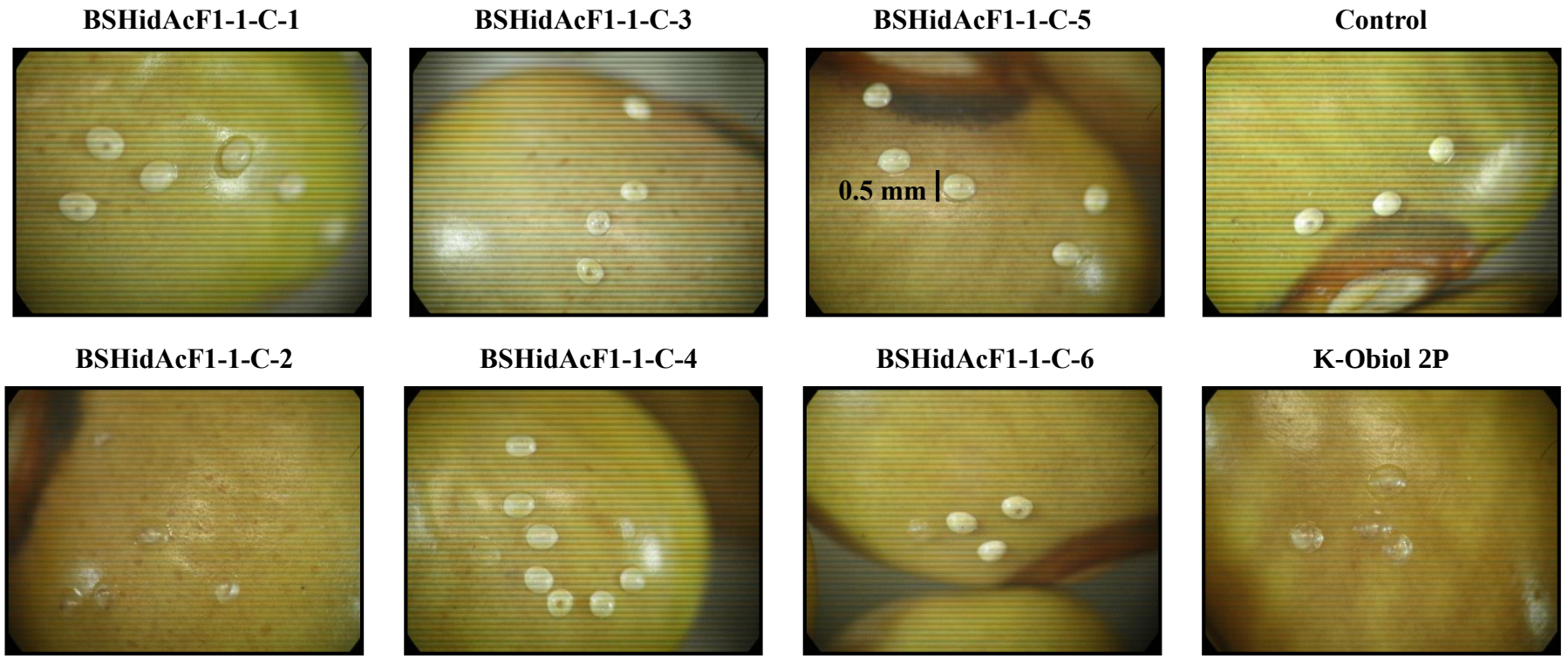

Figure 4. Eggs of *Z. subfasciatus* laid on beans cv. Bolinha treated with different chemical fractions from flowers of *Brugmansia suaveolens*. Not viable eggs: fraction BSHidAcF1-1-C-2 and K-Obiol 2P; viable eggs: Control (ethyl acetate), and fractions BSHidAcF1-1-C-1, BSHidAcF1-1-C-3, BSHidAcF1-1-C-4, BSHidAcF1-1-C-5, BSHidAcF1-1-C-6.

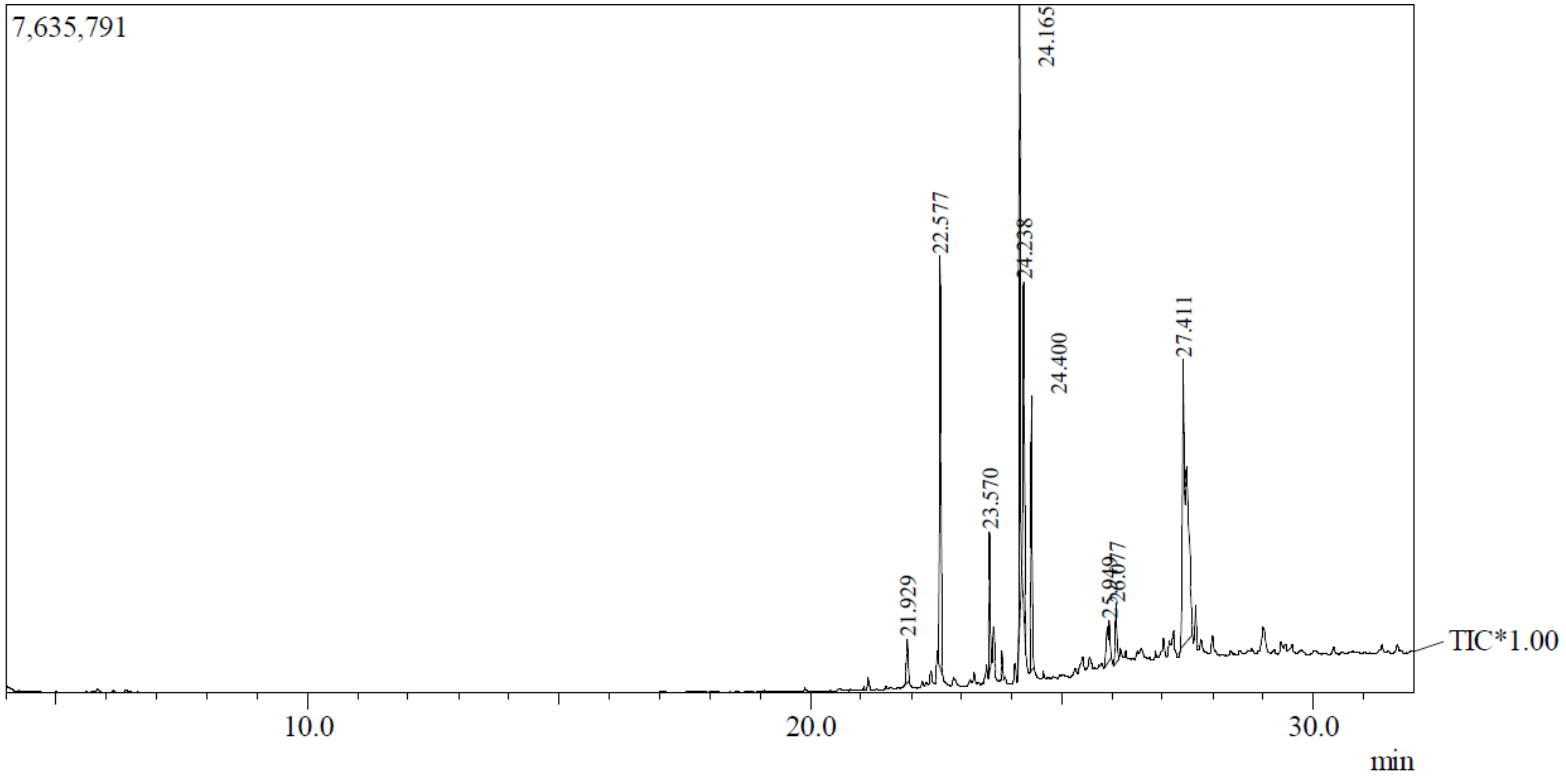

Figure 5. Gas-Chromatography chromatogram of the fraction BSHidAcF1-1-C from flowers of *Brugmansia suaveolens*.

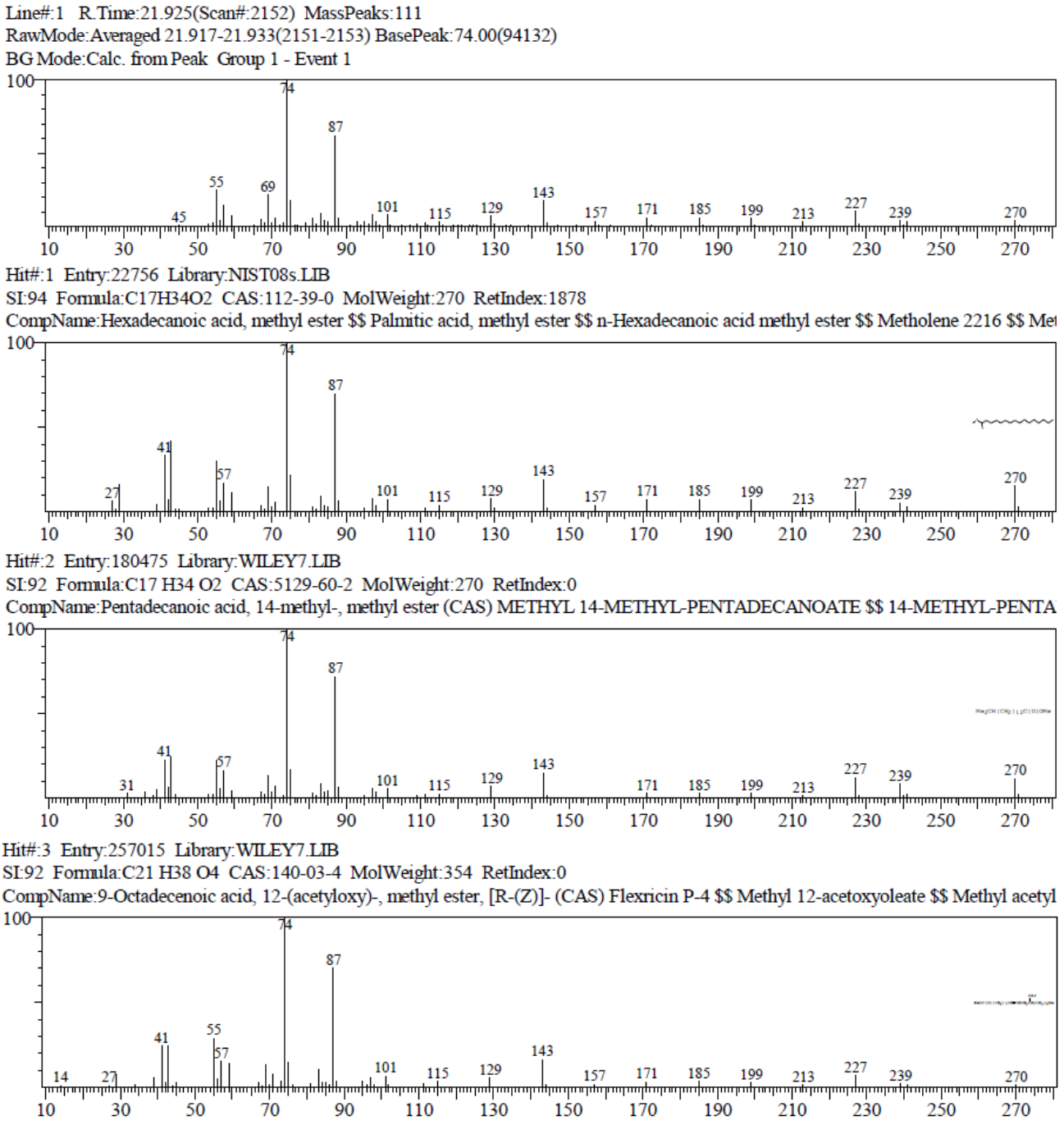

Figure 6. Mass spectrum of compound 1 (Line#1) from the Gas-Chromatography chromatogram of the fraction BSHidAcF1-1-C from flowers of *Brugmansia suaveolens*. Fragmentation pattern of compound 1 (Retention time 21.929 min) is compared with data (Hit#1, Hit#2 and Hit#3) from libraries NIST (National Institute of Standards Technology) and WILEY.

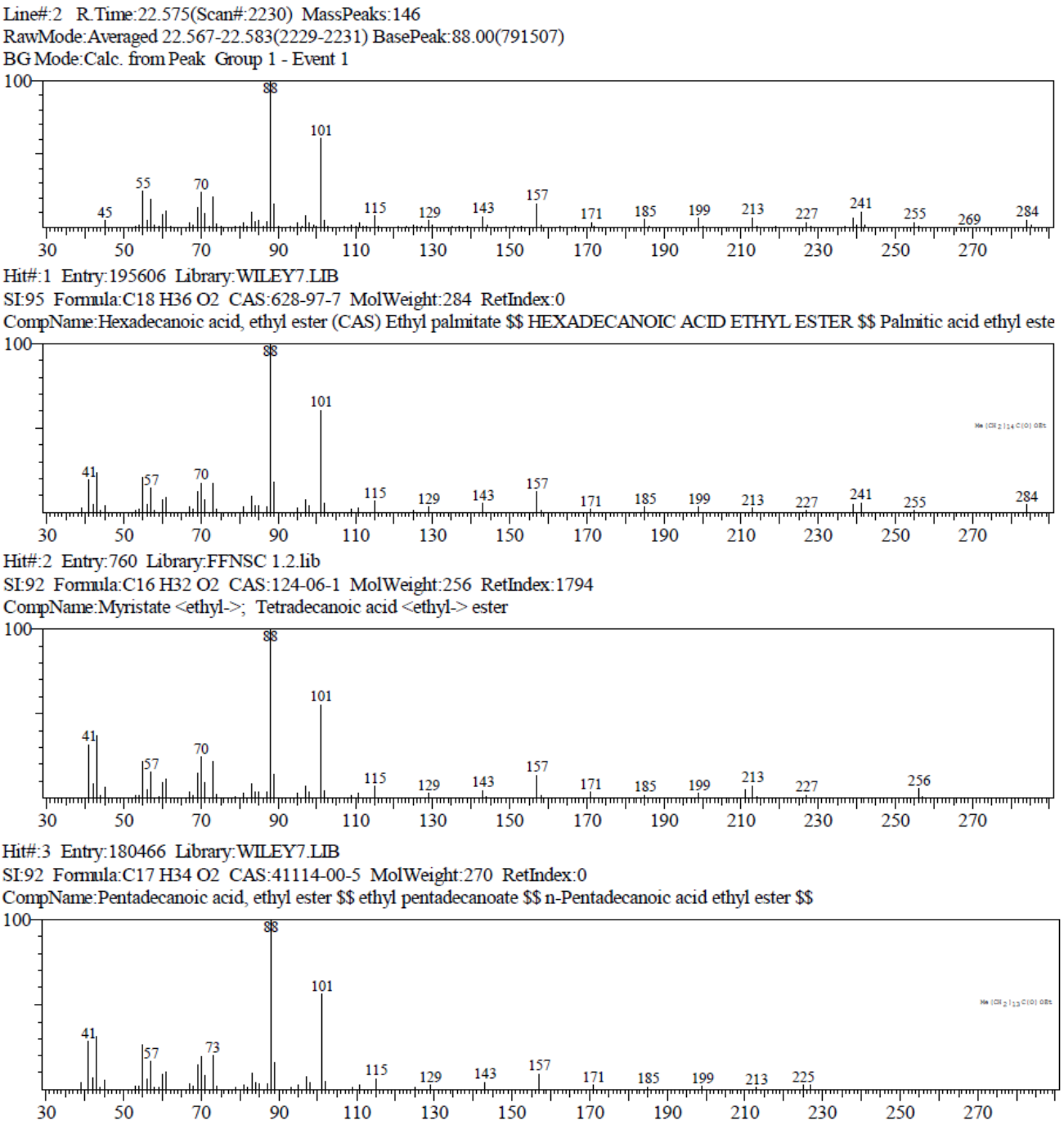

Figure 7. Mass spectrum of compound 2 (Line#2) from the Gas-Chromatography chromatogram of the fraction BSHidAcF1-1-C from flowers of *Brugmansia suaveolens*. Fragmentation pattern of compound 2 (Retention time 22.577 min) is compared with data (Hit#1, Hit#2 and Hit#3) from libraries NIST (National Institute of Standards Technology) and WILEY.

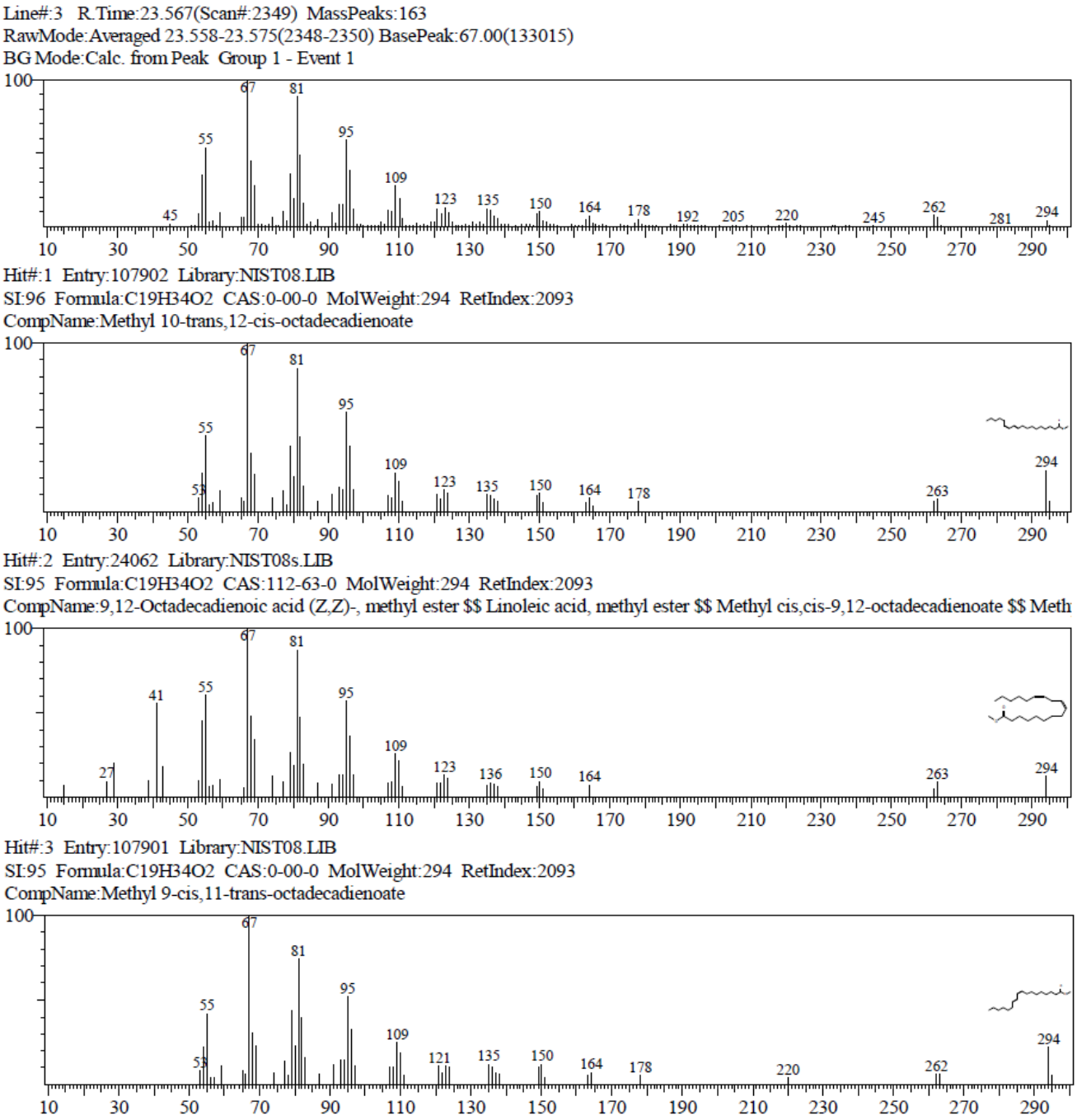

Figure 8. Mass spectrum of compound 3 (Line#3) from the Gas-Chromatography chromatogram of the fraction BSHidAcF1-1-C from flowers of *Brugmansia suaveolens*. Fragmentation pattern of compound 3 (Retention time 23.570 min) is compared with data (Hit#1, Hit#2 and Hit#3) from libraries NIST (National Institute of Standards Technology) and WILEY.

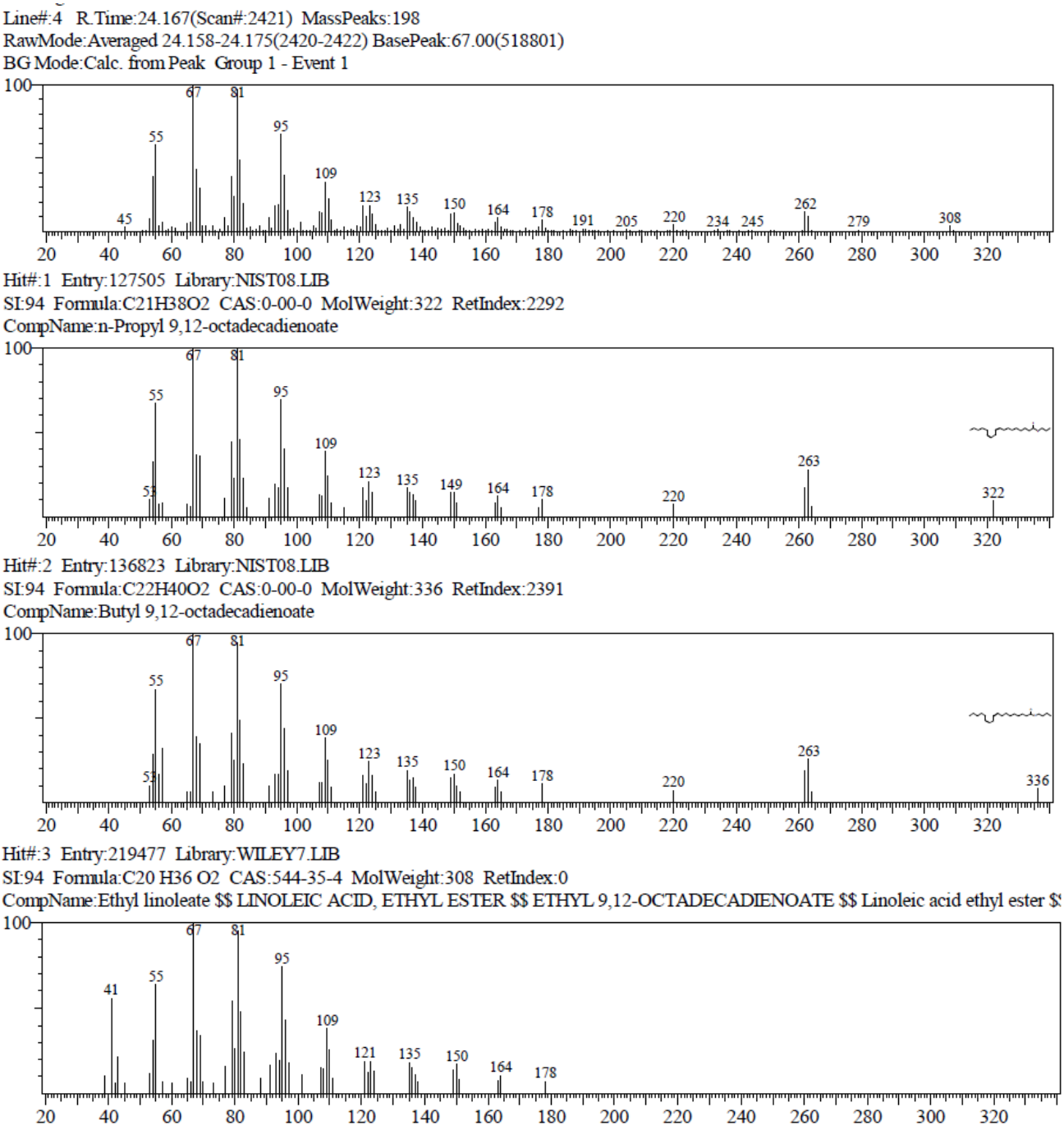

Figure 9. Mass spectrum of compound 4 (Line#4) from the Gas-Chromatography chromatogram of the fraction BSHidAcF1-1-C from flowers of *Brugmansia suaveolens*. Fragmentation pattern of compound 4 (Retention time 24.165 min) is compared with data (Hit#1, Hit#2 and Hit#3) from libraries NIST (National Institute of Standards Technology) and WILEY.

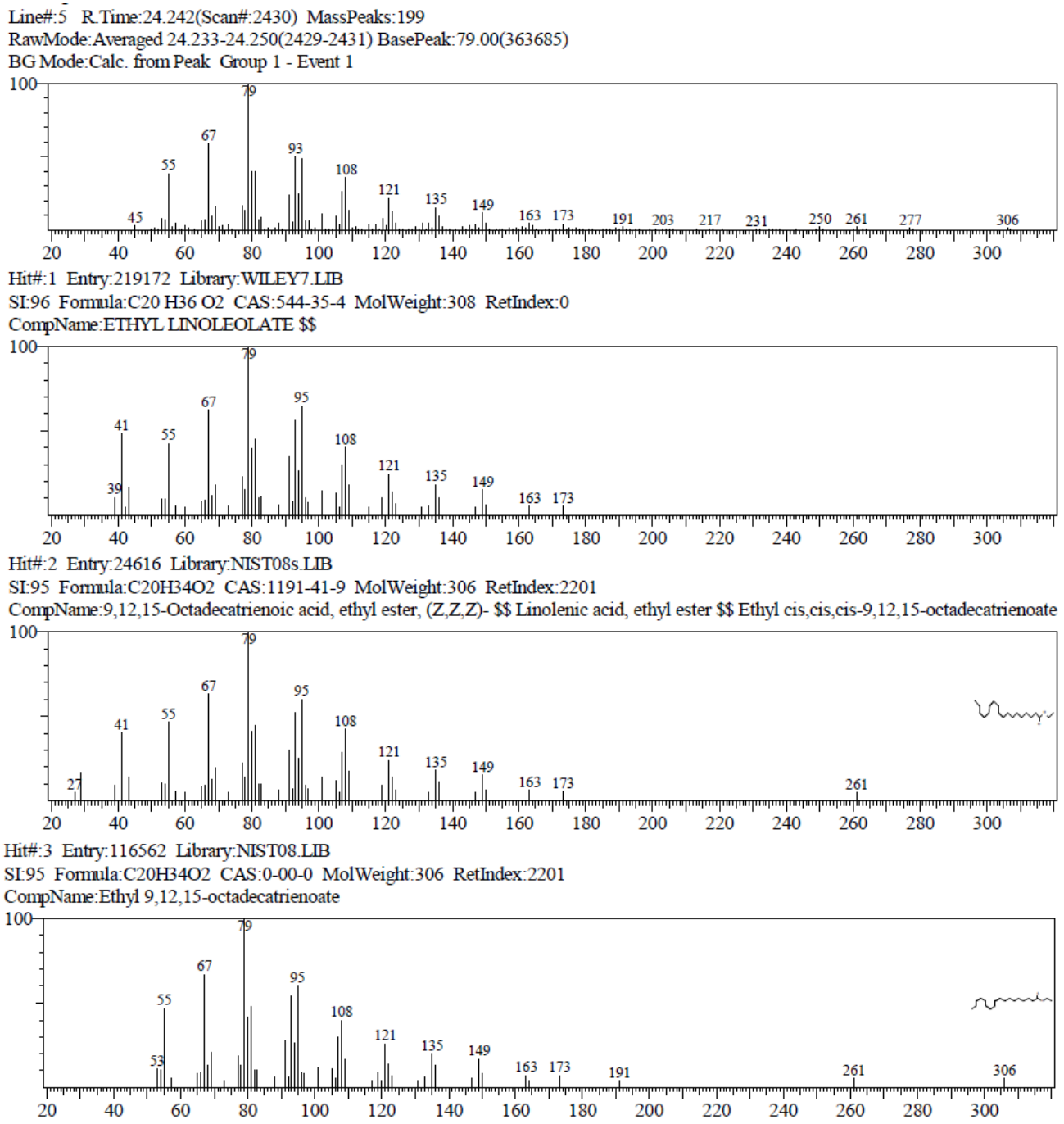

Figure 10. Mass spectrum of compound 5 (Line#5) from the Gas-Chromatography chromatogram of the fraction BSHidAcF1-1-C from flowers of *Brugmansia suaveolens*. Fragmentation pattern of compound 5 (Retention time 24.238 min) is compared with data (Hit#1, Hit#2 and Hit#3) from libraries NIST (National Institute of Standards Technology) and WILEY.

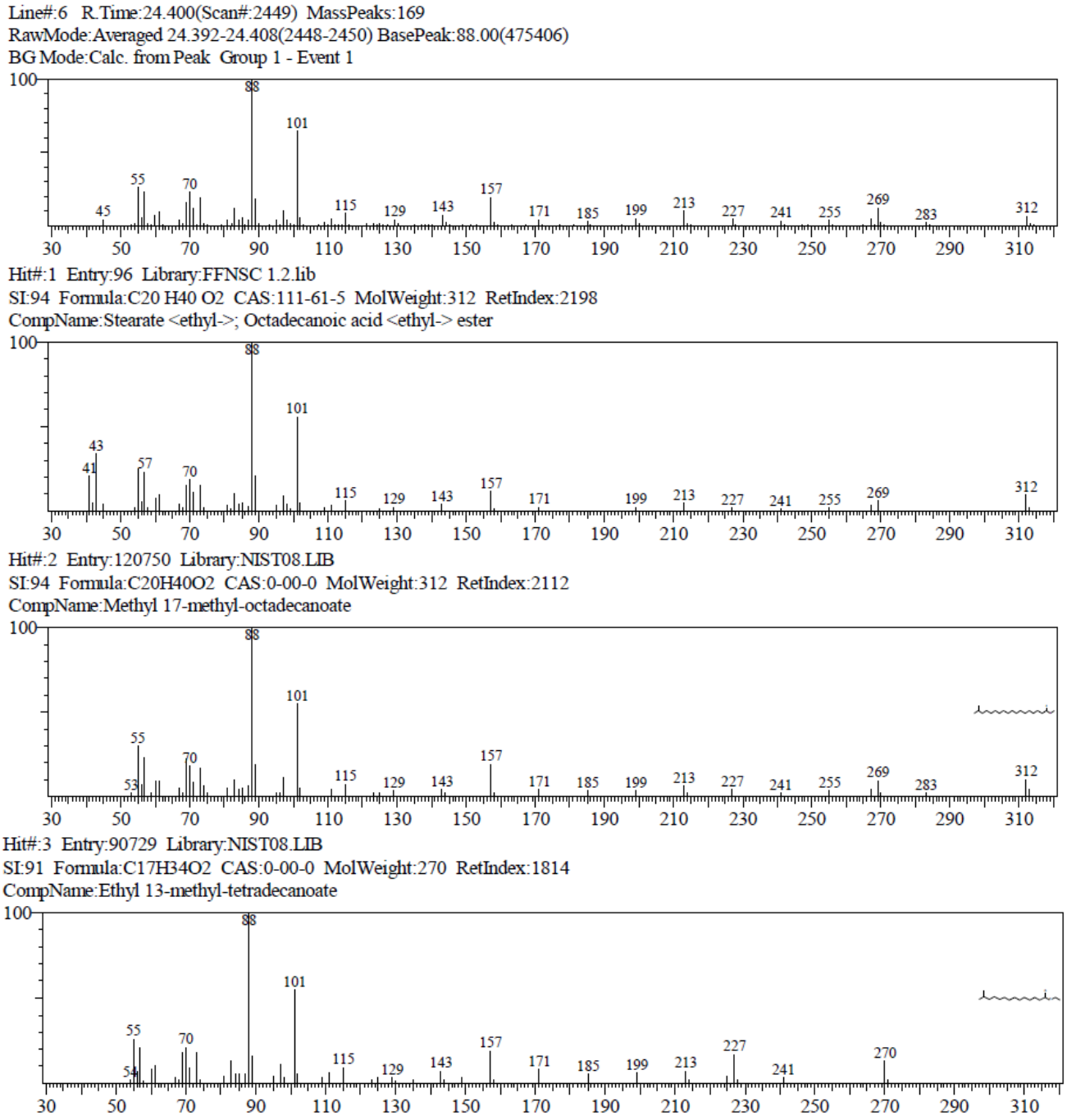

Figure 11. Mass spectrum of compound 6 (Line#6) from the Gas-Chromatography chromatogram of the fraction BSHidAcF1-1-C from flowers of *Brugmansia suaveolens*. Fragmentation pattern of compound 6 (Retention time 24.400 min) is compared with data (Hit#1, Hit#2 and Hit#3) from libraries NIST (National Institute of Standards Technology) and WILEY.

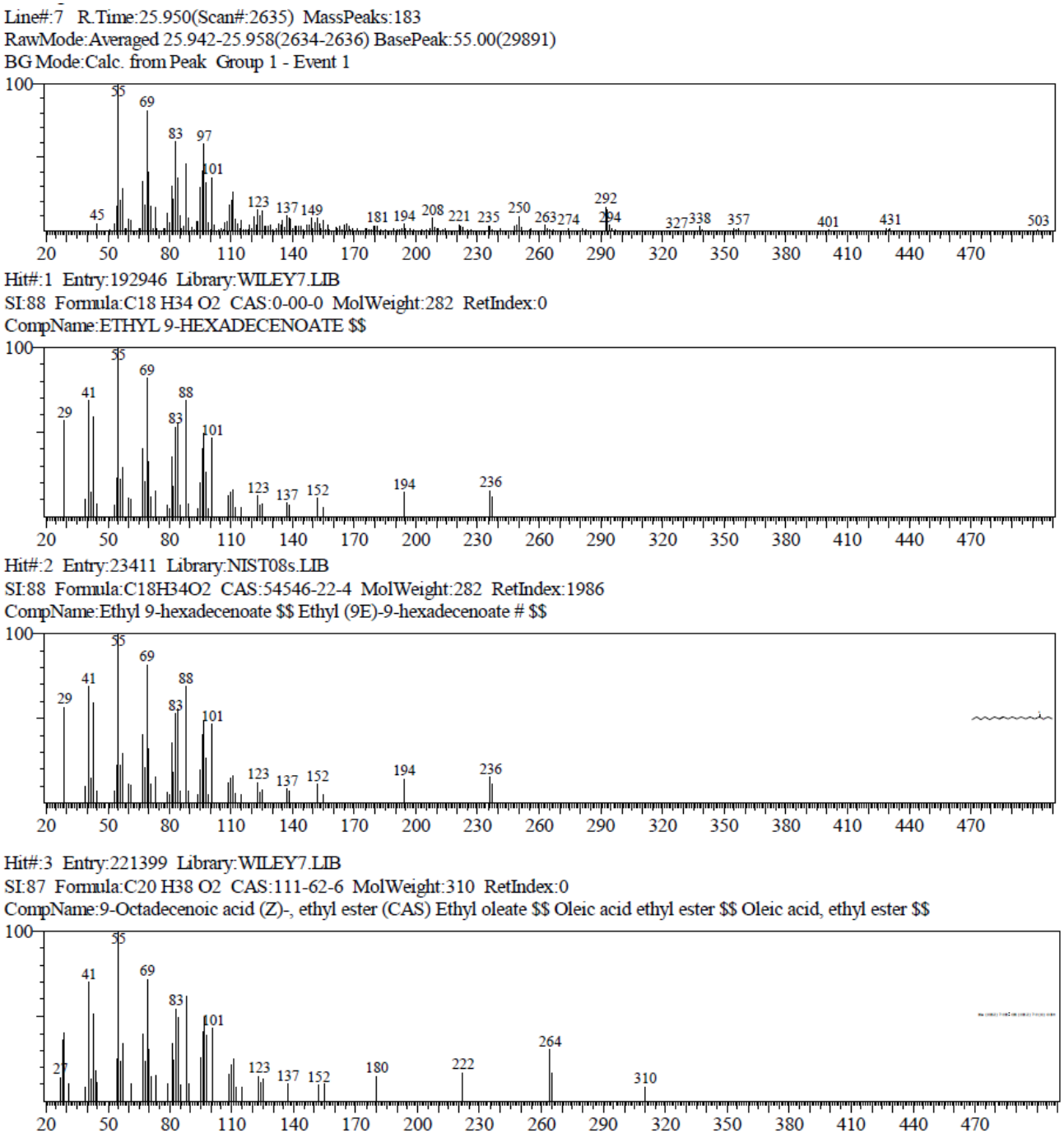

Figure 12. Mass spectrum of compound 7 (Line#7) from the Gas-Chromatography chromatogram of the fraction BSHidAcF1-1-C from flowers of *Brugmansia suaveolens*. Fragmentation pattern of compound 7 (Retention time 25.949 min) is compared with data (Hit#1, Hit#2 and Hit#3) from libraries NIST (National Institute of Standards Technology) and WILEY.

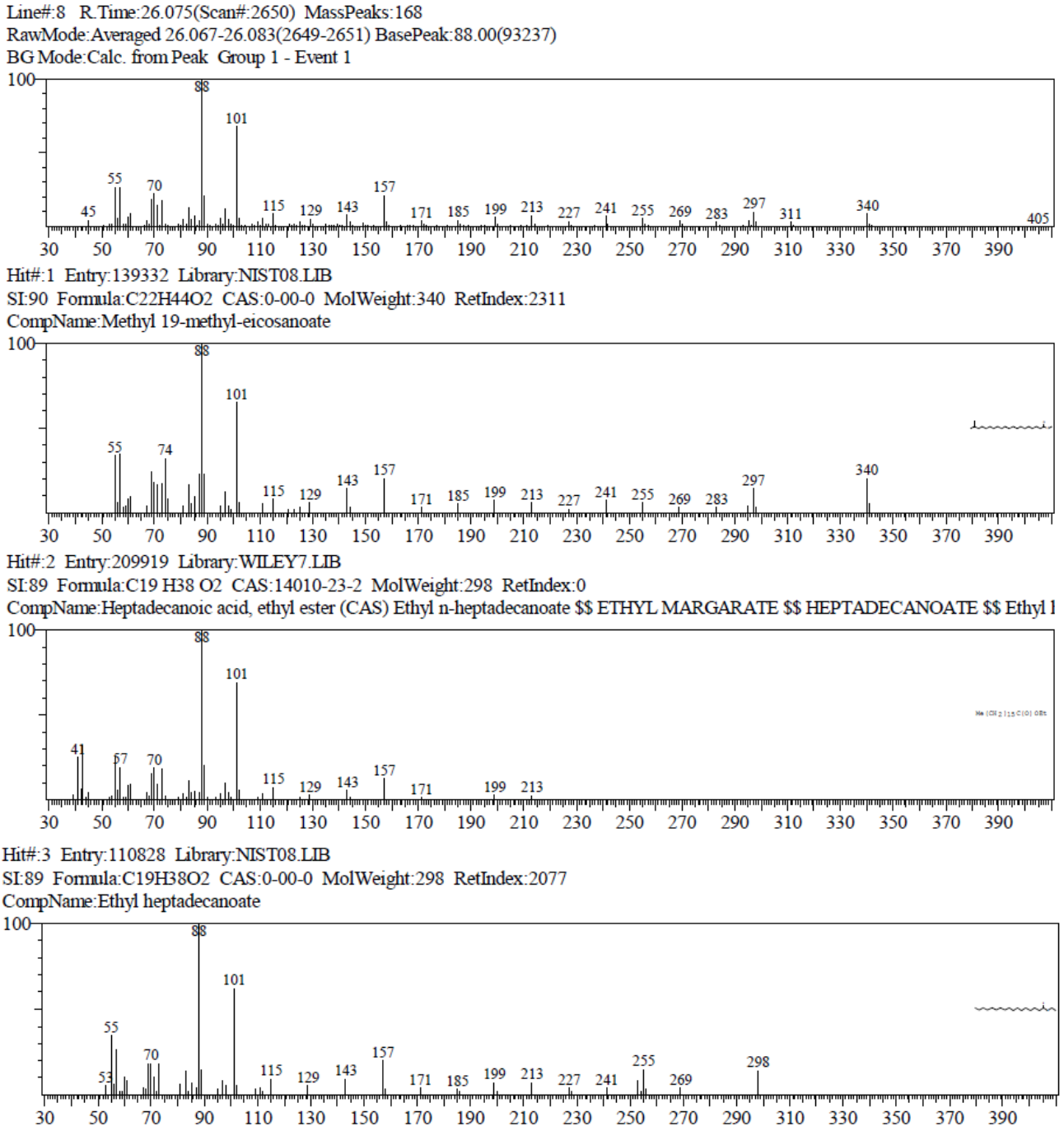

Figure 13. Mass spectrum of compound 8 (Line#8) from the Gas-Chromatography chromatogram of the fraction BSHidAcF1-1-C from flowers of *Brugmansia suaveolens*. Fragmentation pattern of compound 8 (Retention time 26.077 min) is compared with data (Hit#1, Hit#2 and Hit#3) from libraries NIST (National Institute of Standards Technology) and WILEY.

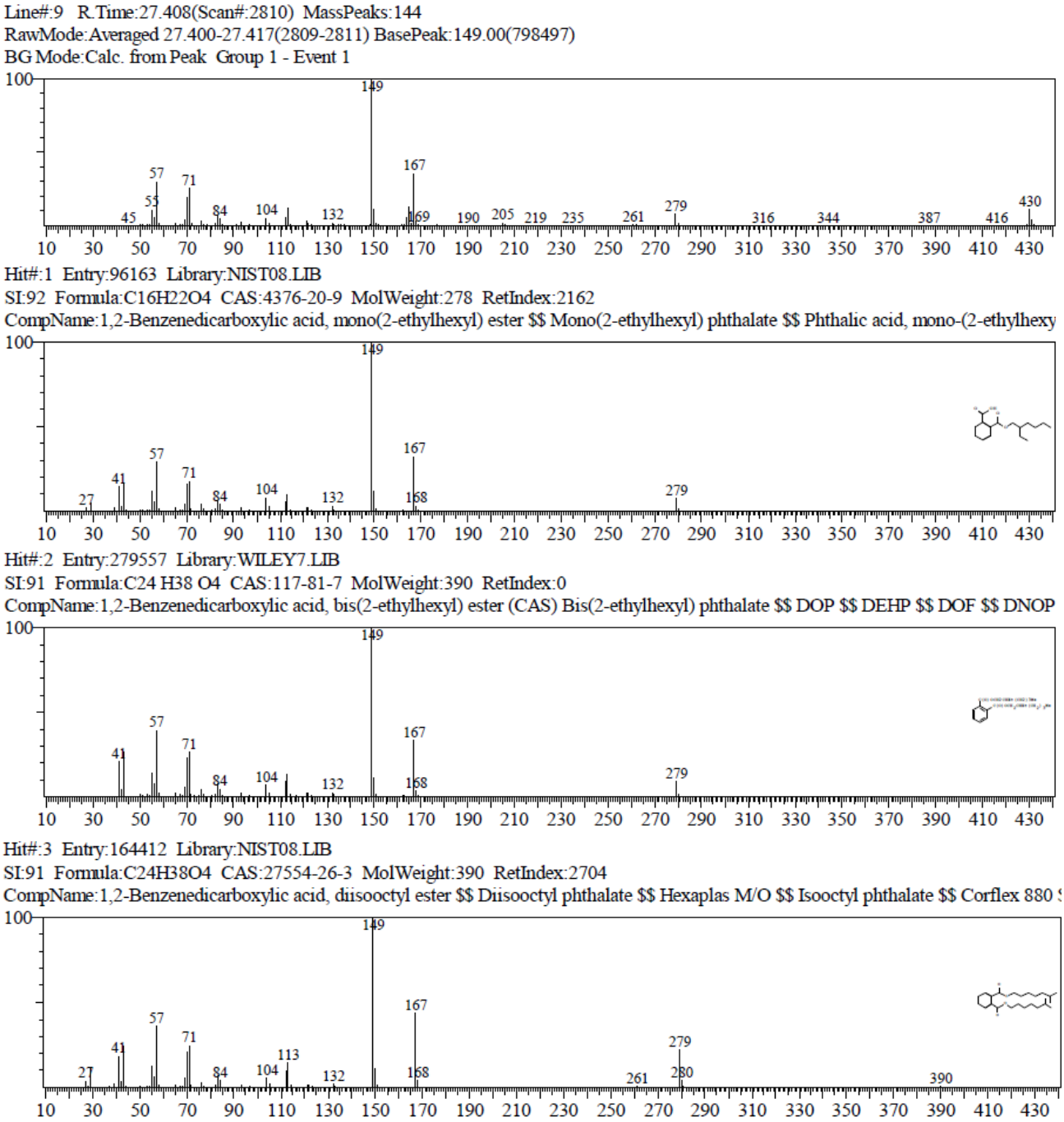

Figure 14. Mass spectrum of compound 9 (Line#9) from the Gas-Chromatography chromatogram of the fraction BSHidAcF1-1-C from flowers of *Brugmansia suaveolens*. Fragmentation pattern of compound 9 (Retention time 27.411 min) is compared with data (Hit#1, Hit#2 and Hit#3) from libraries NIST (National Institute of Standards Technology) and WILEY.

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6. FINAL CONSIDERATIONS

6.1. 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.*

6.2. *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.

6.3. *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.