Industrial Crops & Products 116 (2018) 81–89

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Sitophilus zeamais adults have survival and nutrition affected by *Schinus terebinthifolius* leaf extract and its lectin (SteLL)

João Ricardo Sá Leitão Camaroti[a], Welton Aaron de Almeida[a,b], Bernardo do Rego Belmonte[a], Ana Patrícia Silva de Oliveira[a], Thâmarah de Albuquerque Lima[a], Magda Rhayanny Assunção Ferreira[c], Patrícia Maria Guedes Paiva[a], Luiz Alberto Lira Soares[c], Emmanuel Viana Pontual[b], Thiago Henrique Napoleão[a,*]

[a] Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil
[b] Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Recife, Brazil
[c] Departamento de Farmácia, Centro de Ciências da Saúde, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil

ARTICLE INFO

Keywords:
Brazilian pepper tree
Pest control
Deterrent effect
Flavonoids
Tannins
Lectin

ABSTRACT

Alternative methods for controlling insect pests are required because of the hazards of synthetic chemicals to people and the environment. Lectins are proteins that have been reported as insecticidal agents; however, only one study on the effects of these proteins on *Sitophilus zeamais* Motsch. (maize weevil) has been performed. In the present study, we evaluated the effects of ingestion of artificial diets containing a saline extract from *Schinus terebinthifolius* Raddi leaves (LE) or its lectin (SteLL, *S. terebinthifolius* leaf lectin) on the survival and nutritional parameters of *S. zeamais* adults. The in vitro effects of LE and SteLL on the activity of insect digestive enzymes were also investigated. In addition to SteLL, the LE contained hydrolysable tannins (including gallic acid at 0.559 g%) and flavonoids. Ingestion of LE (100, 200, and 250 mg of extract per g of *Triticum aestivum* L. flour) impaired the survival of the *S. zeamais* adults, with mortality rates ranging between 94% and 97% after 12 days of incubation. A strong deterrent effect was detected, and the insects lost biomass during the assay. However, more than 60% of the insects in the SteLL (1–5 mg/g) treatments remained alive during the 34 days of the experiment. The lectin did not show a deterrent effect, but the biomass and efficiency in conversion of ingested food decreased in a dose-dependent manner. The LE was able to inhibit in vitro the protease activity of the insect gut, while SteLL inhibited protease activity and stimulated amylase activity. In conclusion, the leaf extract had insecticidal properties against *S. zeamais*, which may be due to starvation induction in consequence of the deterrent effect and interference with proteolytic enzymes. Although SteLL did not cause the mortality of the insects, it may be useful as an additive or synergistic agent that reduces pest fitness by affecting the food conversion into biomass.

1. Introduction

Hundreds of insect species have been reported to be capable of attacking stored products of agricultural and animal origin (Rajendran and Sriranjini, 2008). The infestation of grains by insects during cultivation, storage, and transport can seriously damage production and cause significant economic losses as well as threaten food safety (Tefera, 2012; Kumar and Kalita, 2017). Annual losses of 10–15% in grain production have been estimated to be caused by insect pest attacks (Casini and Santajulia, 2015).

Sitophilus zeamais Motschulsky, known as the maize weevil, is one of the main stored grain pests. It attacks mainly maize but also rice, wheat, barley, oat, cotton, and derived products, and it reduces the weight, nutritional value, germination ability, and market value of the grains (Goñi et al., 2017). Together with other insect pests, *S. zeamais* is responsible for losses in maize production (14–50%) (Yuya et al., 2009; Tefera et al., 2011; Ojo and Omoloye, 2012), which can reach 90% in the case of unprotected grains (Nwosu et al., 2015a,b).

According to the Department of Agriculture of the United States of America, the global production of maize in 2017/2018 will be around 1,043.9 million tons, and Brazil will be responsible for the production of 95,000.000 tons (USDA, 2017). There is great concern about the protection of maize production since it is the source of many human and animal nutritional products as well as it has many industrial uses

* Corresponding author.
E-mail addresses: thiagohn86@yahoo.com.br, thiago.napoleao@ufpe.br (T.H. Napoleão).

https://doi.org/10.1016/j.indcrop.2018.02.065
Received 19 October 2017; Received in revised form 19 February 2018; Accepted 20 February 2018

(Herrera et al., 2017). However, despite all the advances in agriculture techniques and technologies, maize production still faces losses, mainly because of the action of pests (Kumar and Kalita, 2017).

Chemical (insecticides), physical (e.g. heat and radiation) and biological (use of predators, parasitoids, pathogens, or competitors) methods have been used for controlling pest insects (Zhou et al., 2014; Yun et al., 2016; Coelho et al., 2017; Porto et al., 2017; Malaikozhundan and Vinodhini, 2018). The use of conventional synthetic insecticides is effective, but there are several problems such as toxicity to non-target organisms, residual contamination of the products, high toxicity to the manipulators, and emergence of resistant populations due to intensive and indiscriminate application (Camaroti et al., 2017). The resistance of *S. zeamais* to pyrethroids has been described since the 1990s (Ribeiro et al., 2003; Fragoso et al., 2003, 2005, 2007). In Brazil, the resistance of populations of *S. zeamais* to organophosphates such as malathion and fenitrothion has been reported (Guedes et al., 1994, 1995), and an increase in the emergence of resistant populations is expected because of the excessive use of synthetic insecticides to combat this pest (Zhang et al., 2015; Freitas et al., 2016).

The co-evolution of plants and predators/herbivores resulted in the selection of plants that possess the best arsenals of defensive biomolecules produced in response to aggression. Thus, plants may provide potential alternatives to insect control. Plant extracts contain several types of secondary metabolites, bioactive proteins (e.g., lectins and enzyme inhibitors), and essential oils that have been reported to be insecticidal agents (Camaroti et al., 2017). These compounds may affect the survival, nutrition, development, locomotion, and behavior of insect pests (Mouhouche et al., 2009; Wale and Assegie, 2015; Lira et al., 2015; Correa et al., 2015; Herrera et al., 2015). For example, a lectin from the leaves of *Myracrodruon urundeuva* Allemão was found to have a deterrent effect on *S. zeamais* and caused death because of starvation (Napoleão et al., 2013).

Schinus terebinthifolius Raddi (Anacardiaceae) is a plant commonly known as “aroeira-da-praia” in Portuguese or “Brazilian pepper tree” in English. It is known for its medicinal properties such as healing, anti-inflammatory, antioxidant, anticancer, and antimicrobial activities (Queires et al., 2006; Matsuo et al., 2011; Bernardes et al., 2014; Fedel-Miyasato et al., 2014; Costa et al., 2015; Rosas et al., 2015). The bark of this plant is commonly commercialized in public markets in Brazil, mainly for therapeutic use (Miranda et al., 2016). The leaves of this plant contain a chitin-binding lectin (SteLL) with an antimicrobial effect against human pathogenic bacteria and fungus (Gomes et al., 2013). A leaf extract obtained using saline solution was reported to be a larvicidal agent against *Aedes aegypti* Linnaeus, causing damage to the midgut of the larvae and interfering with their development (Procópio et al., 2015). This extract also contained SteLL, but this protein was not active against the mosquito larvae.

In the present study, it was evaluated the effects of ingestion of artificial diets containing the saline extract from *S. terebinthifolius* leaves (LE) or SteLL on the survival and nutritional parameters of *S. zeamais* adults. The in vitro effects of the extract and lectin on the activity of insect digestive enzymes were also investigated.

2. Materials and methods

2.1. Plant material

Leaves of *S. terebinthifolius* were collected from different specimens found in an area (8°02′55.9″S 34°56′48.4″W) of the campus of the *Universidade Federal de Pernambuco* at Recife, Brazil. The leaves were dried for 3–5 days at 28 °C and then powdered using a blender. The powder was stored at −20 °C. The collection of plant material was performed with authorization (36301) from the *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio) of the Brazilian Ministry of Environment. A voucher specimen has been archived in the herbarium of the *Instituto Agronômico de Pernambuco* (IPA), Recife, Brazil, under the registration number 73,431.

2.2. Insects

A colony of *S. zeamais* is maintained at the *Laboratório de Bioquímica de Proteínas* of the *Universidade Federal de Pernambuco* with authorization (36301) of the ICMBio. The insects are reared in glass vessels (capacity of 1 L) containing maize grains (100 g), sealed with unwoven fabric, and maintained in a BOD chamber at 25 °C, relative humidity of 70%, and 12:12 light:dark. The maize grains (non-GMO) were obtained from crops for which agrochemicals were not used. Insects of 30–40 days of age were used in the assays.

2.3. S. terebinthifolius leaf extract (LE)

The LE was prepared in 0.15 M NaCl, since it has been previously reported that saline solution is effective in solubilizing both SteLL and hydrophilic secondary metabolites from *S. terebinthifolius* leaves (Gomes et al., 2013; Procópio et al., 2015). Ten grams of the leaf powder was homogenized for 16 h at 28 °C with 100 mL of 0.15 M NaCl by using a magnetic stirrer. Next, the suspension was passed through a filter paper, centrifuged (3000*g* for 15 min at 4 °C), and dialyzed against distilled water for 4 h (one change of water after 2 h). The extract was then freeze-dried in a lyophilizer (LIOTOP L101; Liobras, São Carlos, Brazil) at −45 °C and vacuum of 300 µm Hg below atmospheric pressure. The material was stored at −20 °C until further use.

2.4. Phytochemical characterization and lectin detection assay

The LE and standards listed in Table 1 were analyzed using thin-layer chromatography (TLC) in 60-F_{254} silica gel plates (Macherey-Nagel®, Germany). The plates were developed in chambers after saturation with the mobile phase (Table 1) for 15 min at 28 °C. After elution, the plates were dried at 28 °C and observed under UV light (254 and 365 nm) and visible light. Next, the plates were analyzed with specific reagents for each metabolite class (Table 1). The bands were compared with the standards.

For high-performance liquid chromatography (HPLC) analysis, LE (5 mg) was transferred to a volumetric flask and diluted in 5 mL of ultrapure water (PureLab Classic UV, Elga). The solution was then placed in an ultrasound bath (Ultracleaner®) for 15 min and then filtered with a 0.45 µm PVDF filter. The extract was analyzed using the HPLC system Ultimate 3000 (Thermo Fisher Scientific, USA) coupled to a photodiode array detector (DAD; Thermo Fisher Scientific) and equipped with a binary pump (HPG-3x00RS; Thermo Fisher Scientific), degasser, and automatic sampler with a 20 µL loop (ACC-3000; Thermo Fisher Scientific). The wavelength was fixed at 270 and 350 nm.

Chromatographic separation was performed at 26 °C in an NST C_{18} column (250 mm × 4.6 mm d.i., 5 µm) equipped with a Phenomenex

Table 1
Elution systems, revealers, and standards used in the phytochemical analysis of the saline extract from *Schinus terebinthifolius* leaves with thin-layer chromatography (TLC).

Metabolite class	System	Reagent	Standard
Hydrolysable tannins	90:5:5	Iron(III) chloride	Gallic acid
Condensed tannins	90:5:5	Chloridric vanillin	Catechin
Flavonoids	100:11:11:27	NEU + PEG	Quercetin and rutin
Cinnamic derivatives	100:11:11:27	NEU + PEG	Caffeic acid
Terpenes and steroids	70:30	Lieberman-Burchard + Δ	β-sitosterol

Systems: 90:5:5, ethyl acetate:formic acid:water; 70:30, toluene:acetate; 100:11:11:27, ethyl acetate:acetic acid:formic acid:water. NEU: Neu’s reagent. PEG: polyethylene glycol. Δ: heating. The analysis was performed according to Wagner and Bladt (1996).