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Deleterious effects of *Myracrodruon urundeuva* leaf extract and lectin on the maize weevil, *Sitophilus zeamais* (Coleoptera, Curculionidae)

Thiago Henrique Napoleão [a], Bernardo do Rego Belmonte [a], Emmanuel Viana Pontual [a], Lidiane Pereira de Albuquerque [a], Roberto Araújo Sá [b], Laura Mesquita Paiva [c], Luana Cassandra Breitenbach Barroso Coelho [a], Patrícia Maria Guedes Paiva [a,*]

[a] Departamento de Bioquímica-CCB, Universidade Federal de Pernambuco, Cidade Universitária, 50670-420 Recife, Pernambuco, Brazil
[b] Centro Acadêmico do Agreste, Universidade Federal de Pernambuco, Nova Caruaru, 55002-970 Caruaru, Pernambuco, Brazil
[c] Departamento de Micologia-CCB, Universidade Federal de Pernambuco, Cidade Universitária, 50670-420 Recife, Pernambuco, Brazil

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ABSTRACT

Deterioration and degradation of grains by storage insect pests lead to economic losses of several billion dollars and affect food security. *Sitophilus zeamais* is responsible for pre- and post-harvest damages to maize. The high toxicity of synthetic insecticides and the development of resistance by insects to the chemicals currently used stimulate the investigation of plant-derived insecticides as new alternatives for pest control. In this study, we report the effects of diets containing *Myracrodruon urundeuva* leaf extract (10–150 mg/g) and lectin (MuLL; 3–150 mg/g) on the survival, feeding, and nutrition of the storage pest *S. zeamais*. The digestive enzyme activity in gut extracts from the insects reared on the leaf extract (25 mg/g) or MuLL (15 mg/g) diets was also evaluated. The leaf extract induced mortality (LC_{50}: 72.4 mg/g), while MuLL (30–150 mg/g) exerted strong feeding deterrence. The leaf extract and MuLL promoted the loss of biomass, as reflected in the negative values for relative biomass gain rates and efficiencies in converting ingested food. Protease, trypsin-like, acid phosphatase, and amylase activities in the insects reared on leaf extract or MuLL diets were significantly ($P < 0.05$) lower than those in the control insects. MuLL ingestion also significantly reduced ($P < 0.05$) endoglucanase and alkaline phosphatase activities. In conclusion, the leaf extract and MuLL have the potential for *S. zeamais* control by killing adults and preventing the use of a food source, respectively. The deleterious effects of the extract and lectin on *S. zeamais* may be linked to enzyme inhibition and consequent suppression of digestive processes.

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1. Introduction

Protection of food crops against attack by storage insect pests and pathogens is a major concern for the food industry, farmers, landowners, smallholders, public health organizations, and environmental agencies. Deterioration, degradation, and contamination of grains by insects during storage lead to economic losses of several billion dollars and affect food security. In addition, the grain losses have a great ecological impact, since energy, land, water, and non-renewable resources were used for the production of grains that will never be consumed (FAO, 2009).

The weevil *Sitophilus zeamais* Motschulsky (Coleoptera, Curculionidae) is a cosmopolitan insect pest of maize (*Zea mays*) that can also attack sorghum, rice, wheat, and processed food products like pasta and biscuits (Fazolin et al., 2010). *Sitophilus zeamais*, together with other storage insect pests, causes an estimated 24.5% loss of maize, and the damaged grains have reduced nutritional value and weight, low frequency of germination, and low market value (Yuya et al., 2009; Tefera et al., 2011; Mugo, 2012). The maize weevil is able to infest maize already in the field both pre- and post-harvest (Giles and Ashman, 1971).

Populations of *S. zeamais* developed moderate to high levels of resistance to synthetic chemical insecticides such as lindane (discontinued use since 2004) and malathion in Mexico (Perez-Mendonza, 1999; CEC, 2005), although it is thought that the resistance levels to organophosphates are still low in Brazil (Pereira et al., 2009). However, an increase in the levels of resistance is expected because of the massive use of insecticides, mainly fenitrothion (Braga et al., 2011). Alternative strategies for the control of *S. zeamais* populations include the use of entomopathogenic fungi, plant extracts, essential oils, seed oils, leaf powders, and pressurized carbon dioxide and temperature

* Corresponding author. Tel.: +55 8121268540; fax: +55 8121268576.
E-mail address: ppaivaufpe@yahoo.com.br (P.M.G. Paiva).

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management techniques (Adane et al., 1996; Kehinde and Angela, 2004; Ileleji et al., 2007; Noomhorm et al., 2009; Ukeh et al., 2009; Yuya et al., 2009; Betancur et al., 2010; Nukenine et al., 2010; Coitinho et al., 2011).

Plant lectins (carbohydrate-binding proteins) are biodegradable insecticidal agents that act against insects from several orders of socio-economic importance (Lam and Ng, 2011; Paiva et al., 2011). Plant lectins have deleterious effects on the survival, growth, oviposition, and reproduction of insect storage pests such as the moths *Ephestia (Anagasta) kuehniella* Zeller and *Corcyra cephalonica* Stainton and the weevils *Callosobruchus maculates* Fabricius and *Zabrotes subfasciatus* Bohemann (Zhu-Salzman et al., 1998; Macedo et al., 2002; Sadeghi et al., 2006; Coelho et al., 2007; Macedo et al., 2007; Oliveira et al., 2011). The mechanisms by which lectins kill insects have been studied and can involve interactions with glycoconjugates along the digestive tract, resistance to proteolysis, and binding to digestive enzymes. These effects may result in morphological damage to the digestive tract, disruption of the gut epithelium and peritrophic matrix, and inhibition or stimulation of enzyme activities that promote metabolic imbalance and impair nutrition and feeding behavior (Zhu-Salzman et al., 1998; Carlini and Grossi-de-Sá, 2002; Sauvion et al., 2004; Macedo et al., 2007; Coelho et al., 2009; Napoleão et al., 2012).

Myracrodruon urundeuva (Engl.) Francisco Allemao (Anacardiaceae) is a tree broadly distributed in Brazil and popularly known as "aroeira-do-sertão" or "urundel". The chitin-binding lectin isolated from *M. urundeuva* leaves (MuLL) has shown insecticidal activity against the termite *Nasutitermes corniger* Motschulsky (Isoptera) and the larvae of *Aedes aegypti* Linnaeus (Diptera), the dengue mosquito vector (Napoleão et al., 2011, 2012). The insecticidal mechanisms of MuLL were investigated, and it was reported that the lectin resisted degradation by proteases from the guts of *N. corniger* and *A. aegypti*, killed symbiotic bacteria in the *N. corniger* gut, stimulated α-amylase activity, and inhibited protease and trypsin-like enzymes from the gut of *A. aegypti* larvae (Napoleão et al., 2011, 2012). In addition, it was suggested that the chitin-binding property of MuLL allows its interaction with the peritrophic matrix of both insects (Napoleão et al., 2011, 2012).

The previous reports on insecticidal activity of MuLL stimulated the evaluation of its potential against another insect species. In this study, we evaluated the potential of *M. urundeuva* leaf extract and lectin (MuLL) to affect the survival and feeding of *S. zeamais* adults when incorporated into an artificial diet. In addition, their effects on nutritional (relative consumption rate, relative biomass gain rate, and efficiency in conversion of ingested food) and biochemical (gut protease, trypsin-like, α-amylase, cellulase, and phosphatase activities) parameters were determined.

2. Materials and methods

2.1. Plant material

Leaves of *M. urundeuva* were collected in the State of Maranhão, northeastern Brazil, conditioned in plastic bags and transported to the laboratory. A voucher specimen (identified by Mr. Gonçalo Mendes da Conceição) is deposited under number 054 at the Herbarium Aluisio Bittencourt, *Centro de Estudos Superiores de Caxias*, *Universidade Estadual do Maranhão*, Brazil. The authors have authorization from the *Instituto Chico Mendes de Conservação da Biodiversidade* from Brazilian Ministry of the Environment for plant collection (number 36301-1). The leaves were dried for 7 days (27 ± 2 °C; relative humidity of 70 ± 5%), powdered, passed through 40 mesh screen, and stored at 28 °C. About 1000 g of leaf powder was used to obtain the lectin amount used in this work.

2.2. Insects

Sitophilus zeamais adults originated from colonies maintained at the *Departamento de Micologia* at the *Universidade Federal de Pernambuco*, Recife, Brazil, were reared in the *Laboratório de Glicoproteínas*, *Departamento de Bioquímica* from the same university. The colonies were maintained at 28 ± 2 °C in glass containers (capacity, 1 L) sealed with thin TNT-type tissue to permit aeration. The diet consisted of maize grains (100 g per container), selected according to integrity, sanitary conditions, size, and absence of contamination with other insects. Insects that were in the colony for 1–2 months were used in the assays.

2.3. Chemicals

The chemicals listed in this section were used in the experiments of protein extraction, lectin purification or assessment of enzyme activities described in the following sections Asialofetuin, Avicel, azocasein, *N*-benzoyl-DL-arginyl-ρ-nitroanilide (BApNA), bovine serum albumin, carboxymethylcellulose, chitin powder from shrimp shells, 3,5-dinitrosalicylic acid (DNS), D(+)-glucose, glutaraldehyde, ρ-nitrophenyl-β-D-glucopyranoside, ρ-nitrophenyl phosphate, ρ-nitrophenol, sodium bicarbonate, trichloroacetic acid and Trishydroxymethylaminomethane (Tris) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetic acid, ammonium sulfate, chloridric acid, dibasic sodium phosphate, monobasic sodium phosphate, and sodium chloride were purchased from Vetec (Rio de Janeiro, Brazil). Calcium chloride, sodium acetate, sodium hydroxide, soluble starch, and Triton X-100 were purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade.

2.4. Isolation of MuLL

MuLL was isolated according to the procedure described by Napoleão et al. (2011). Powdered leaves (10 g) were suspended in 0.15 M NaCl (100 mL) and the mixture was homogenized in a magnetic stirrer (16 h at 4 °C), filtered through gauze (Cremer, Blumenau, Brazil) and centrifuged (3000 *g*, 15 min). Next, the supernatant (leaf extract) was treated with ammonium sulfate (60–80% saturation) and the precipitated protein fraction was chromatographed on chitin column. MuLL was eluted with 1.0 M acetic acid and dialyzed (10-kDa cut-off membrane; Sigma–Aldrich, USA) against four changes of distilled water every 2 h using a volume of 2 L for dialysis fluid.

2.5. Protein content

Protein concentration in both plant and insect preparations was determined according to Lowry et al. (1951) using bovine serum albumin (31–500 μg/mL) as standard.

2.6. Hemagglutinating activity

Hemagglutinating assay was carried out in microtiter plates (TPP-Techno Plastic Products, Trasadingen, Switzerland) according to Paiva and Coelho (1992) by incubating 50-μl samples (plant extract, precipitated protein fraction or isolated lectin) with 2.5% (v/v) suspension of human O-type erythrocytes treated with glutaraldehyde for 30 min (Bing et al., 1967). One hemagglutinating unit (titer^{-1}) was defined as the reciprocal of the highest dilution of the sample promoting full agglutination of erythrocytes. Specific hemagglutinating activity (unit mg^{-1}) was defined as the ratio titer to protein concentration (mg/mL). HA inhibitory assay was performed by incubation (45 min) of lectin sample with 0.5 mg/mL asialofetuin solution before erythrocyte suspension addition (Napoleão et al., 2011).

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