Journal of Stored Products Research 55 (2013) 6–14

Contents lists available at SciVerse ScienceDirect

Journal of Stored Products Research

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

Annona mucosa Jacq. (Annonaceae): A promising source of bioactive compounds against *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae)

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ARTICLE INFO

Article history:
Accepted 4 June 2013

Keywords:
Maize weevil
Allelochemicals
Acetogenins
Alkaloids
Bioinsecticide

ABSTRACT

New control methods are necessary for stored grain pest management programs due to both the widespread problems of insecticide-resistance populations and the increasing concerns of consumers regarding pesticide residues in food products. Thus, this study evaluated the bioactivity of extracts and fractions obtained from different structures (leaves, branches, and seeds) of *Annona mucosa* (Annonaceae) against *Sitophilus zeamais* (Coleoptera: Curculionidae), which is a primary insect pest of stored cereals in tropical conditions. In the screening assay, the most promising treatments were extracts prepared from the seeds of *Annona mucosa* in hexane and dichloromethane (LC_{90} values of 259.31 and 425.15 mg kg^{-1}, respectively) and, to a lesser extent, an extract prepared from the leaves in hexane (LC_{90} of 1047.15 mg kg^{-1}). Based on these results and the chromatographic profile of the bioactive crude extracts, the extract prepared from the seeds in hexane was fractionated by liquid–liquid partitioning. The dichloromethane and hydroalcoholic fractions exhibited insecticidal activity against *S. zeamais*, and no significant difference was observed between these two fractions. The chemical analyses (^{1}H NMR, HPLC, and TLC) showed the presence of alkaloids and acetogenins in the bioactive fractions, which are likely related to the observed bioactivity. Thus, *A. mucosa*, particularly its seeds, is a promising source of compounds that can be used as a prototype model and/or a biorational insecticide for the control of *S. zeamais* in stored cereals.

1. Introduction

In tropical countries, especially those with developing economies, post-harvest grain losses are very significant (Basavaraja et al., 2007; Hodges et al., 2010; Olayemi et al., 2012; Tefera, 2012). Infestations caused by stored grain insect pests is a major cause of these losses; these infestations are mainly due to climatic conditions that favor the development of these biota and the unsatisfactory conditions of the storage infrastructure (Lazzari and Lazzari, 2009), which enable the constant re-infestation of the stored grain and undermine the effectiveness of management programs.

The structural and technological limitations inherent in the management of insect pests of stored grains are usually accompanied by an increased frequency of the use of synthetic insecticide applications, which is the main method for the management of these insect pests. This increase in insecticide use leads to greater selection pressure and an increase in the number of populations that are resistant to the insecticidal active ingredients (Pereira et al., 2009; Pimentel et al., 2009; Rofrano et al., 2009; Araújo et al., 2011; Braga et al., 2011). This effect is compounded by the limited availability of registered insecticides, which complicates the management of insect resistant populations through the alteration of the active ingredients (Boyer et al., 2012). The post-harvest losses are thereby increased, and the investments made in the previous stages of the production process, as well as

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0022-474X/$ – see front matter
http://dx.doi.org/10.1016/j.jspr.2013.06.001

the agricultural sustainability, are jeopardized (Ribeiro et al., 2008).

It is thus necessary to incorporate new substances that meet the requirements of agronomic efficiency, toxicological safety, and low environmental impact (Viegas Júnior, 2003) into programs for the integrated management of the insect pests of stored grains (IPM). The identification of compounds with modes/mechanisms of action that are different from those that are found in the currently used insecticides (which are mostly neurotoxic) is also desirable, especially for the management of resistant populations of these pests in the storage units.

Plants produce a diverse range of allelochemicals that regulate insect–plant interactions, especially in the defense of the plant against herbivory (Van Beek and Breteler, 1993; Cloyd, 2004; Isman, 2006). Thus, these allelochemicals constitute an important source of insecticide molecules that can be applied in an integrated management program (IPM) against the insect pests of stored grain through two methods: the preparation of homemade insecticides from locally available species for direct use on the property, which contributes to the reduction of the technological dependence of farms (this is particularly true for small farms), and as model prototypes for the development of new synthetic insecticides (Vendramim and Castiglioni, 2000).

Annonaceae is one of the largest families of angiosperms; to date, it includes 135 genera and approximately 2500 species (Chatrou et al., 2004). However, despite its great diversity, this family is one of the lesser studied tropical plant families from the phytochemical viewpoint. The studies conducted to date have demonstrated the presence of a large amount of diverse chemical compounds in the different species and structures of this family (Leboeuf et al., 1980; Chang et al., 1998; Kotkar et al., 2001).

Among the Neotropical Annonaceae, *Annona mucosa* Jacq. (formerly grouped in the genus *Rollinia* (Rainer, 2007)) is a native fruit tree of the Amazon and the Atlantic forest that is popularly known as “biribá”. Although Brazil is its place of origin, this species grows well in different habitats (Ferreira et al., 2010). Pharmacological and phytochemical studies have revealed the presence of acetogenins (Pettit et al., 1987; Shi et al., 1996, 1997; Gu et al., 1997; Chávez et al., 1998,1999; Liaw et al., 2003), alkaloids (Caetano and Dadoun, 1987; Chen et al., 1996), amides (Chávez et al., 1999), and lignans (Chen et al., 1996; Figueiredo et al., 1999; Estrada-Reyes et al., 2002) as major constituents of different *A. mucosa* structures. A number of compounds isolated from this species have shown antimicrobial, antifungal, antiprotozoal, and antitumor potential (Caetano and Dadoun, 1987; Shi et al., 1996; Gu et al., 1997; Chávez et al., 1999; Liaw et al., 2003), which has generated interest in the assessment of the potential of *A. mucosa* derivatives as a source of allelochemicals with insecticidal properties that could be used in the management of the pest species of economically important stored grain. Thus, the objective of the present study was to evaluate the bioactivity of organic extracts and fractions obtained from different *A. mucosa* structures against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), which is the main pest species of stored cereals in tropical conditions.

2. Material and methods

2.1. Vegetation samples and preparation of crude extracts

The leaves, branches, and seeds of *A. mucosa* that were used in the study were obtained on January 22, 2010, from a specimen grown in the “Luiz de Queiroz” College of Agriculture/University of São Paulo (ESALQ/USP) in Piracicaba, SP, Brazil (latitude: 22°42′ 26″S; longitude: 47°37′39″W). A voucher specimen was deposited in the herbarium of the “Luiz de Queiroz” College of Agriculture (Herbarium ESA) in Piracicaba, SP, Brazil, under reference number 120985.

To prepare the extracts, the collected plant parts were dried in an oven at 38 °C for 48–72 h. Subsequently, the materials were separately milled in a knife mill to obtain a powder of each plant structure, which was stored separately in sealed glass containers until use.

The organic extracts were obtained by cold maceration using solvents (5:1, v/w) with increasing polarity: hexane (polarity = 0.06), dichloromethane (polarity = 3.4), and ethanol (polarity = 5.2). The extraction in each solvent was performed until exhaustion, after which the solvent with the next higher polarity was used. During each solvent change, the macerate was filtered through filter paper, the solvent was removed from the sample, and the sample was maintained in a rotary evaporator at a temperature of 40 °C and a pressure of −600 mm Hg. After the complete evaporation of the solvent in an air-flow chamber, the extraction yield for each plant structure in each of the different solvents was determined.

2.2. Bioassays

All of the bioassays were conducted at a room temperature of 25 ± 2 °C, with a relative humidity (RH) of 60 ± 10%, a 14-h photoperiod, and an average luminosity of 172 lux. Whole corn grains (hybrid AG 1051: yellow-toothed, semi-hard) were used as the test substrate. The grains were selected manually from a crop grown without the use of insecticides. Before use, the grains were maintained in a climate-controlled room under the conditions described above for a minimum of 30 days to equilibrate the moisture content (grain humidity was approximately 12.5 ± 0.5%).

Prior to the definitive assays, preliminary bioassays (Ribeiro, 2010) were conducted to determine the solution volume that should be used, to verify the uniformity of the application of the extracts in the grains, and to assess the possible effects of the organic solvents used in the resuspension of the plant extracts and fractions on *S. zeamais*.

To identify the extracts that exhibit bioactive effects on *S. zeamais*, bioassays were conducted to determine the insecticidal activity of the extracts and to assess their effect on the F1 progeny of *S. zeamais* and the damage to the corn. The extracts were applied at concentrations of 300 and 1500 mg kg^{-1} (mg of extract per kg of corn), which were defined based on previous studies, on samples of corn grains through the aid of a microatomizer coupled to a pneumatic pump and adjusted to provide a pressure of 0.5 kgf cm^{-2} using a spray volume of 30 L t^{-1}. After the spraying, the grain-extract mixtures were manually collected in 2-L plastic bags, which were then stirred for 1 min and maintained for 2 h in an air-flow chamber to evaporate the solvent that was used for the resuspension of the extracts.

2.2.1. Evaluation of insecticidal activity

In this bioassay, Petri dishes (6-cm diameter and 2-cm high) containing 10-g samples of corn were used. These Petri dishes were treated separately with the respective plant extract or the control growth substrate that consisted of the solvent used in the respective extract resuspension. Each sample unit was then infested with 20 (unsexed) *S. zeamais* adults aged between 10 and 20 days that were obtained from a laboratory-reared population; 10 replicates of each treatment were performed. The adult survival was assessed on the 10th day after the infestation. Those individuals with completely distended wings and that showed no reaction to contact with a brush during 1 min of observation were considered dead.

The same sampling units from the previous test were used in the bioassay to assess progeny production and resulting damage. In this assay, the grains were treated with the respective extracts and