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Peptide composition, oxidative and insecticidal activities of nectar from flowers of *Spathodea campanulata* P. Beauv

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ABSTRACT

The growing demand for food has intensified the search for compounds of plant origin to protect field crops from predators and pathogens, as these compounds have less environmental impact and are considered healthier than synthetic compounds. Among plant species with insecticidal activity, *Spathodea campanulata* has been identified as a potential source of insecticidal compounds. Therefore, in this study we verified the insecticidal effect of nectar from *S. campanulata* against three different insects. In addition, the oxidant activity of nectar and proteomic assay were conducted to identify the insecticide potential. Both gross and dialyzate nectar showed a promising toxic effect against *Euschistus heros* (Fabr.), *Helicoverpa zea* (Boddie) and *Anticarsia gemmatalis* (Hübner) insects. According to oxidant tests, non-denatured nectar showed a higher oxidant activity than denatured nectar, in both albumin degradation and TBARS tests. SDS-PAGE and 2D-PAGE were used to characterize the nectar proteins, revealing 13 spots that were compatible to either proteins or peptides. The most relevant spots were analyzed by mass spectrometry, confirming the presence of proteins associated with insecticidal activity. In conclusion, it is hypothesized that *S. campanulata* nectar has insecticidal effects and this activity is linked to the classes of pro-oxidant proteins or peptides present in its chemical composition.

1. Introduction

The search for compounds able to control and protect against plant pests and consequential diseases is one of the main challenges faced by the agricultural industry. Prior to pesticides, farmers solved their phytosanitary problems using natural insecticides extracted from plant leaves, bark, flowers or nectar. However, with the advance of agricultural technology these natural insect control practices have been abandoned. Conversely, the widespread use of pesticides has triggered countless phytosanitary problems, including the development of insecticide resistance, mortality of non-target species, ecosystem damage, and residual insecticide accumulation in foods with toxic effects on humans and other organisms (Hernández-Lambraño et al., 2014). In addition to phytosanitary problems, there is a considerable increase in acquisition costs and application of pesticides in affected crops (Tavares et al., 2009).

Insect pests are capable of evolving into biotypes that can adapt to new situations and overcome the effect of toxic materials or natural plant defenses, resulting in extensive destruction of field crops. Soybean is one of the most important crops in the world, covering approximately 32 million hectares in Brazil, but is constantly affected by insect pests (Conab, 2015), such as *Anticarsia gemmatalis*; *Helicoverpa zea* and *Euschistus heros A. gemmatalis* is the major soybean insect pest, occurring mainly in the growing regions of North and South America (Macrae et al., 2005). Substantial infestation of *A. gemmatalis* is responsible for high levels of defoliation and meristem damage (Crialesi-Legor et al., 2014). Larval stage *H. zea* is a major polyphagous agricultural pest, consuming a wide

Abbreviations: 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; AAPH, 2,2′-azobis (2-amidino-propane) hydrochloride; BSA, bovine serum albumin; BVA, biological variation analysis; PBS, phosphate buffered saline; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances.

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variety of crops, such as cotton, tomato, corn and soybean (Reay-Jones and Reisig, 2012). *E. heros* feeds on various plant structures, mainly fruits and immature seeds, affecting their quality, development and maturation (Timbó et al., 2014). Due to the number of soybean pests, companies working with the crop have supported studies to control insect pests, especially by genetic improvement, biological control or plant-derived insecticidal compounds (Santos et al., 2015).

Plants produce a great arsenal of compounds as a strategic defense mechanism against insects. These compounds may be involved in the normal development of plants or act exclusively in an insecticidal protection system (Ibanez et al., 2012). Natural chemical compounds are generally considered less harmful to human health and the environment and have less adverse effects caused by the uncontrolled application of agrochemicals (Vasconcelos et al., 2006). Many proteins have been tested for their repellent, deterrent, or lethal effects against insect pests (Carlini and Grossi-De-Sá, 2002; Vandenborre et al., 2011; Ibanez et al., 2012). Some of these proteins exert their insecticidal effect by oxidative mechanisms, forming reactive oxygen species (ROS) and free radicals in the insect digestive tract (Barbehenn et al., 2008). However, it is still unclear whether the toxic activity can be directly attributed to proteins or to the induction of oxidative stress.

The plants investigated to control insects and weeds are species with the potential capacity to produce substances from primary and/or secondary metabolism that affect the growth and development of herbivores (Rice, 1984). Within the group of primary metabolites with potential insecticide activity, there are a few notable classes of proteins, such as inhibitors of α-amylase, lectins and proteinase inhibitors (Falco et al., 2001). These compounds can generate several injuries on contact with insects, such as the inhibition of carbohydrate catalytic enzymes in the digestive tract and modification of the growth rate and development of pests (Peumans and Van Damme, 1995; Iulek et al., 2000). Others studies have shown that ROS can be generated in response to these natural substances and cause severe injuries to the organism, leading to death. One of the best-known modes of action is by interaction with lipids in cell membranes and generation of a destructive process known as lipid peroxidation (Grant and Loake, 2000; Abdollahi et al., 2004).

Spathodea campanulata P. Beauv (Bignoneaceae), popularly known as African tulip tree, is native of West African tropical forests. It has been widely introduced as an ornamental plant in several regions of tropical America. In Brazil it is frequently used in urban forestry. Portugal-Araujo (1963) and Trigo and Santos (2000) reported the occurrence of different species of dead insects (bees, flies and ants) on flowers of an inflorescence of *S. campanulata*. Different authors suggest that the insecticidal action is related to the presence of toxic compounds present in the mucilage of flowers and young shoots that would be dissolved in the nectar, and are responsible for the insects death (Alarcón-Noguera and Penieres-Carrillo, 2013; Queiroz et al., 2014; Franco et al., 2015). The presence of toxic compounds in the nectar is partially explained because the pollinators of this plant are birds, mainly hummingbirds, they in addition to consuming the nectar also feed on the dead insects present in the flowers, representing an extra attraction for the pollinators (Zaheer et al., 2011).

In this study, a possible oxidative, insecticidal activity associated with the natural compounds present in *S. campanulata* nectar was analyzed against insect pests of soybean. Additionally, the proteins present in the nectar were quantified and identified by electrophoresis (SDS-PAGE and 2D-PAGE) and mass spectrometry (MS).

2. Materials and methods

2.1. Collection and preparation of nectar

The nectar was collected in fully opened flowers from *S. campanulata* grown in the urban perimeter region of Bela Vista do Paraíso city, Paraná state (23°00′57.3″S, 51°11′28.4″W), Brazil. Plants were randomly selected 7 days prior to collection and their inflorescences packed to protect them from wind, rain and herbivores. From nine plants, using a Pasteur pipette, approximately 0.5 mL of nectar per flower was harvested, totaling 40 flowers per individuals in the morning period. The period of collection was established after determination of sugars concentration by refractometry, establishing the period of greatest concentration throughout the day. The nectar samples were stored in plastic bottles refrigerated (4 °C) and taken to the laboratory. Soon after the nectars were collected (approximately 180 mL), frozen and lyophilized, yielding 9.82 g of dried nectar.

2.2. Gross nectar dialysis and denaturation

Dialysis was performed in deionized water using a 25 × 16 mm membrane (InLab, USA) with a 10 kDa molecular weight cut-off, for 24 h. After, the dialyzate was frozen and stored for insecticidal activity assays. Gross nectar was denatured at 80 °C in a water bath, for approximately 20 min.

2.3. Insecticidal tests

2.3.1. Insecticidal activity against Euschistus heros (Fabr.)

E. heros nymphs, in the 3rd–5th instar, were provided by the Agronomic Institute of Campinas (IAC) and were fed with *Phaseolus vulgaris* L. (jack bean) plants sprayed with gross nectar, dialyzate nectar or distilled water (control). After, 20 nymphs were put into polyethylene experimental units (10 × 11 cm boxes), with food (i.e. jack bean, as abovementioned) and kept under controlled luminosity (12 h light/dark photoperiods), temperature (25 ± 1 °C) and humidity (60 ± 10%). The test was conducted in a randomized design with four replications for each of the experimental groups, including the control. Nymph mortality was assessed daily for up to 15 days. The experimental groups received 30 μL of gross nectar or dialysate nectar and negative control 30 μL of distilled water.

Percentage nymph mortality was calculated using the formula:

$$\frac{Number\ of\ nymphs\ dead}{Total\ number\ of\ nymphs} \times 100$$

2.3.2. Insecticidal activity against Helicoverpa zea (Boddie) and Anticarsia gemmatalis (Hübner)

Twenty caterpillars of *A. gemmatalis* and *H. zea* were randomly assigned to individual Petri dishes (60 × 15 mm) and fed with the diet proposed by Greene et al. (1976) and Navarro (1987). The food was sprayed with gross nectar, dialyzate nectar or distilled water (control) prior to using as feed and replaced every 2 days. Insect mortality index was assessed every 2 days for up to 30 days. The experimental groups received 30 μL of gross nectar or dialysate nectar and negative control 30 μL of distilled water.

Percentage insect mortality was calculated using the formula:

$$Number\ of\ dead\ insects Total\ number\ of\ insects \times 100$$