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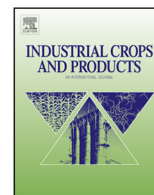

Peptide composition, oxidative and insecticidal activities of nectar from flowers of *Spathodea campanulata* P. Beauv

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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.

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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:

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

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:

$$\textit{Number of dead insectsTotal number of insects} \times 100$$

2.4. Oxidant tests

2.4.1. Albumin degradation oxidative test

The albumin degradation oxidative test was adapted from the method of Hsieh et al. (2005). Briefly, bovine serum albumin (BSA) (2 mg/ml) was diluted in phosphate buffered saline (PBS) (10 mM, pH 7.4) and incubated with Cu^{2+} (2 mM) at 37 °C for 2 d in the presence of 500 μL of gross nectar, denatured nectar, 5 mM hydrogen peroxide solution (positive control) or BSA solution (110 μg/ml) in phosphate buffer (negative control), respectively. Electrophoresis was carried out by SDS-polyacrylamide (12% acrylamide and a 5% stacking gel) gel electrophoresis (SDS-PAGE), according to established techniques (EnCor Biotechnology Inc·, FL, USA). The oxidant activity was evaluated by comparing the treatments with the negative control (PBS + BSA).

2.4.2. Evaluation of oxidation potential by thiobarbituric acid reactive substances (TBARS)

A thiobarbituric acid reactive substances (TBARS) test was performed to quantify the level of lipid peroxidation, according to the method of Boligon et al. (2014). Egg yolks were homogenized (1% w/v) in 50 mM phosphate buffer (pH 7.4) and used as a lipid-rich substrate (Guimarães et al., 2010). The homogenate (0.5 mL) was then sonicated and added to 0.1 mL of gross nectar (1%, 5% and 10%), denatured nectar (1%, 5% and 10%) and 140 $\mu g\,mL^{-1}$ of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid i.e. Trolox (negative control), respectively. Lipid peroxidation was induced by adding 0.1 mL of 2,2′-azobis (2-amidino-propane) hydrochloride (AAPH, 0.12 M) (positive control) and the solutions subsequently incubated in a water bath at 37 °C for 30 min. After cooling, 0.5 mL of trichloroacetic acid (15%) and 0.5 mL of thiobarbituric acid (0.67%) were added and the mixture then heated at 97 °C for 15 min. *n*-Butanol (1 mL) was then added to each solution, centrifuged at 1200 rpm for 10 min, cooled and their respective absorbance values measured at 532 nm. The results were expressed as a percentage of TBARS ($A_{control} - A_{sample} \times 100/A_{control}$), where $A_{control}$: is the absorbance obtained with AAPH and A_{sample}: is the absorbance of the treatment solutions and negative control, respectively.

2.5. Protein characterization

2.5.1. Precipitation of the gross nectar

For improved purification and concentration of the proteins, 1 mL of gross nectar was mixed with 4 mL of 80% acetone, vortexed and stored in a refrigerator at 3 °C for 2 h (Lanças et al., 2003). The supernatant was dried under nitrogen flow, resuspended in PBS (10 mM, pH 7.4) and stored at −10 °C.

2.5.2. Protein content

Quantification of the protein in samples of crude, dialysed, lyophilized and precipitated nectar samples was performed to evaluate the presence of protein content after each processing of the nectar, used the Bradford method (1976) and BSA as standard, protein values were obtained in mg BSA per ml of PBS buffer (10 mM, pH 7.4).

2.5.3. Polyacrylamide gel electrophoresis (SDS-PAGE)

The protein composition of the dialyzed and precipitated samples was detected by SDS-PAGE, according to the method of Laemmli (1970), on 10% separating gel and 3% stacking gel. Prior to electrophoresis, the samples were solubilized in sample buffer (0.5 M Tris buffer, pH 6.8; 10% SDS; β-mercaptoethanol; glycerol; bromophenol blue and distilled water). An aliquot of each sample (15 μL) was loaded onto the electrophoresis gel, which was run under constant voltage (100 V) at room temperature (25 °C ± 2) for 2 h. The gel was subsequently stained with a solution of Coomassie Brilliant Blue R-250.

2.5.4. Two-dimensional electrophoresis (2D-PAGE)

The proteins in lyophilized nectar samples were investigated by 2D-PAGE due to the high protein concentration in these samples and the improved resolution of this method compared to SDS-PAGE. Aliquots of nectar (212 mg) were resuspended in phosphate buffer (0,005 M; pH8) and the total protein concentration then determined using the ToPA kit (Biosciences, PA, USA). Then, 120 μg of total protein was transferred to test tubes and precipitated using the ToPREP kit (Biosciences, PA, USA) to remove sugars and other interfering substances. The precipitated sample was resuspended in 50 μL ToPI-DIGE buffer (Biosciences, PA, USA) for 2D-PAGE. The first dimension was performed using a 24 cm long IPG (immobilized pH gradient) strip of pH 3–10, at 30 V. Then, proteins were focused at 8000 V permitting a total of 65000 V/h. After equilibration of the IPG strips with reduction the proteins were separated in the second dimension using a 12.5% polyacrylamide gel of 24 × 20 cm for approximately 4 h. The gel was fixed overnight and stained with Coomassie Brilliant Blue R-250. The gel was then scanned with a Typhoon digital imager. The image was analyzed using biological variation analysis (BVA), a module of the DeCyder software v 6.5.

2.5.5. Protein digestion and mass spectrometry analysis

After analyzing the 2D gels and selecting the main representative spots by DeCyder BVA software, 13 spots were excised manually (approximately 1 mm^2) with a plastic plunger. The spots were then digested with trypsin and cleaned with Zip Tip C18 (Millipore, Bedford, USA) for MS analysis, according to the method of Silveira et al. (2008).

After obtaining the digested and purified samples, the peptides were loaded onto an LC column using an autosampler, and eluted using a linear acetonitrile (Sigma, St Louis, USA) gradient from 2 to 30% over 60 min, then high and low organic washes for another 30 min into an LTQ XL mass spectrometer (Thermo Scientific, Germering, DE) *via* a nanospray source. The spray voltage was set to 1.8 kV and the ion transfer capillary set at 180 °C. A data-dependent top-5 method was used, which involved a full MS scan from *m*/*z* 400–1500, prior to MS/MS scans of the five most abundant ions. Each ion detectable in the spectrum was further selected for CID to identify the peptide.

Raw data files were searched against the most recent databases from UniProt (http://www.uniprot.org/) using Proteome Discoverer 1.4 (Thermo Scientific) and the SEQUEST HT search algorithm. Oxidation (of Met) was used as a variable modification and the carbamidomethyl (of Cys) was used as a fixed modification. The fixed value PSM (peptide-spectrum match) algorithm was used as PSM validation in the database searches.

The Angiosperm (flowering plants) database was selected for the first search and returned the majority of the match with *Vitis vinifera* proteins. Therefore, the *Vitis vinifera* database was downloaded and a second search conducted.

2.6. Statistical analysis

Statistical analysis was performed using the Shapiro-Wilks normality test and the Levene homogeneity test. The data were normally distributed and the variances were homogeneous. Therefore, the data were analyzed using the parametric ANOVA and Tukey tests ($\alpha = 0.05$). These tests were performed using BioEstat 5.0 software.

Table 1
Mortality index of *Euschistus heros* (Fabr.), *Helicoverpa zea* (Boddie) and *Anticarsia gemmatalis* (Hübner).

Treatments	Mortality[a]		
	E. heros	*H. zea*	*A. gemmatalis*
Gross néctar	12 ± 3a	16 ± 2a	10 ± 1a
Dialysate nectar	7 ± 2b	11 ± 2b	10 ± 1a
Control	4 ± 2c	5 ± 2c	4 ± 2b

[a] Data are expressed as the mean of three replicates ± SD, (n = 20). Means within a column followed by the same letter are not significantly different (Tukey's test, $p < 0.05$).

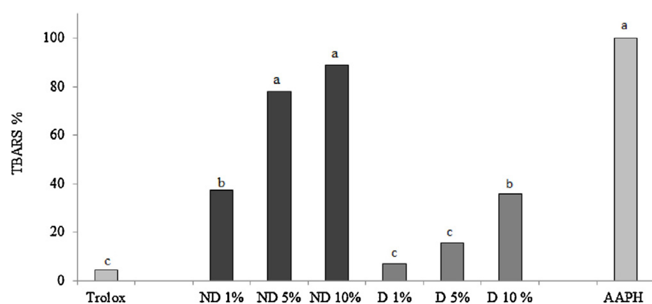

Fig. 1. % TBARS oxidation compared to a positive control (AAPH). Means with the same letter do not differ significantly by Tukey's test (α = 0.05). (ND = non-denatured nect and D = denatured nect).

3. Results

3.1. Protein content

The total protein present in the samples was calculated using a BSA standard curve ($y = 0.012 + 0.027x$; $R^2 = 0.991$). The gross nectar and dialyzed nectar had a total protein content of 95 μg mL^{-1} and 10 μg mL^{-1}, respectively.

3.2. Insecticidal tests

Insecticide tests showed toxic activity against groups treated with gross or dialyzed nectar, independent of the insect species used (Table 1). In assays conducted with *E. heros* the gross nectar showed a mortality of 60%, the dialyzate nectar, 35% and the control, 20%. Similar results were observed for *H. zea*; gross nectar presented the highest mortality (80%), followed by dialyzate nectar (55%) and control (25%). *A. gemmatalis* showed no difference between gross and dialyzate nectar (50% mortality for both). However, both nectar samples were efficient compared to the control (20%).

3.3. Evaluation of oxidation potential by thiobarbituric acid reactive species (TBARS)

There was a tendency for non-denatured nectar (5 and 10%) of *S. campanulata* to show high levels of oxidative activity, by TBARS (Fig. 1). In contrast, no significant oxidative activity was observed using different denatured nectar content. The highest amount (D 10%) presented a similar oxidation percentage as the lowest percentage of non-denatured nectar (ND 1%).

3.4. Albumin degradation oxidative test

Oxidative analysis of albumin degradation in a polyacrylamide gel showed high oxidant activity in non-denatured nectar (Fig. 2). This activity was observed by comparing the bands for the treated group (BSA + Cu + non-denatured nectar) with the positive control (BSA + Cu + hydrogen peroxide) and BSA + Cu. Denatured

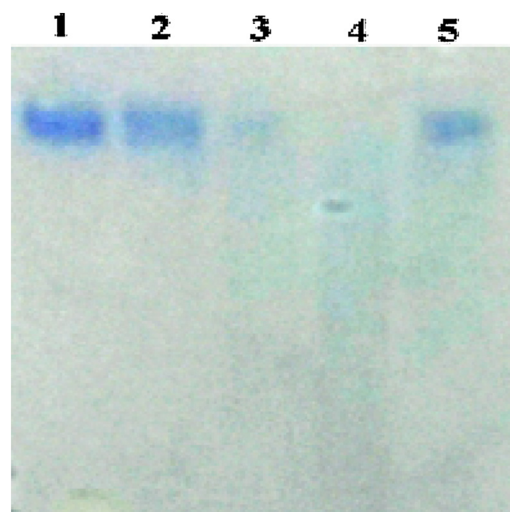

Fig. 2. Oxidative analysis of albumin degradation in a polyacrylamide gel stained by Coomassie Brilliant Blue. 1-BSA (negative control); 2-BSA + Cu; 3-BSA + Cu + hydrogen peroxide (positive control); 4-BSA + Cu + non-denatured nectar; 5-BSA + Cu + denatured nectar.

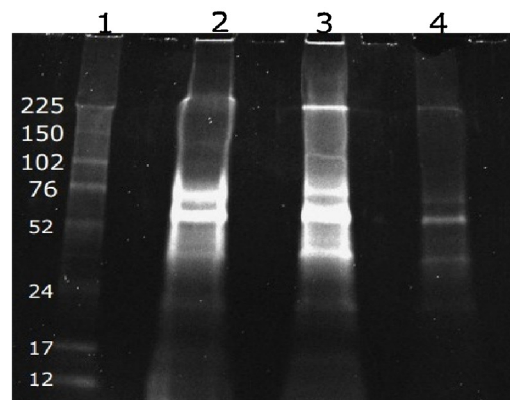

Fig. 3. Electrophoretic analysis of nectar in the polyacrylamide gel stained with Coomassie Brilliant Blue R-250. 1-molecular marker (kDa); 2-lyophilized nectar; 3-precipitated nectar; 4-gross nectar.

nectar + BSA + Cu presented a lower oxidative activity compared to non-denatured nectar.

3.5. Polyacrylamide gel electrophoresis (SDS-PAGE)

Prior to SDS-PAGE, the total protein content of gross, lyophilized and precipitated nectar was determined as 109.2, 784.4 and 412.7 μg mL^{-1}, respectively. SDS-PAGE analysis (Fig. 3) showed that the various nectar preparations had comparable protein profiles. Three major bands with molecular masses of approximately 38 kDa, 60 kDa, 76 kDa and a more intense band at 225 kDa were present in all samples. Based on the results, lyophilized nectar was investigated by 2D-PAGE. The dialyzed nectar was not quantified by SDS-PAGE because it presented low concentration in the determination of total proteins (Section 3.1).

3.6. Two-dimensional electrophoresis (2D-PAGE)

In 2D-PAGE, each identified spot could be considered a possible protein candidate. According to the DeCyder BVA software, 51 spots were detected (Fig. 4). Despite a large number of spots detected, most of the spots were probably artifacts and did not represent proteins. From the 51 spots annotated by the software, 13

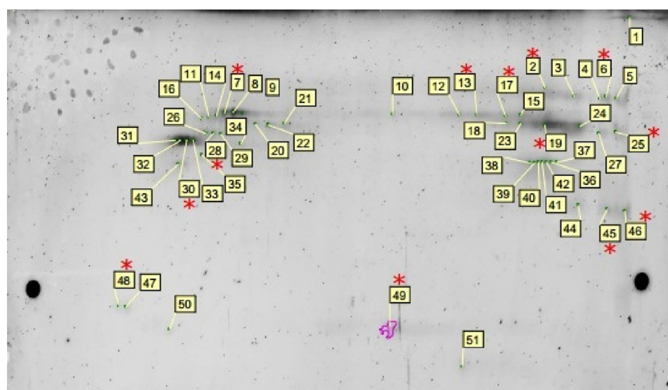

Fig. 4. All 51 spots assigned as possible protein candidates detected by DeCyder BVA software. Spots (red marks) were further analyzed by mass spectrometry. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

spots (marked red in Fig. 4) were compatible with proteins and subsequently submitted to MS analysis.

3.7. Mass spectrometry analysis

From the 13 spots selected in the 2D-PAGE, peptide sequences were identified according to their degree of similarity to sequences in the UniProt database. After peptide identification, those related to insecticidal activity were selected (Table 2). Due to the scarcity of proteomic information concerning the species investigated in this study, it was not viable to isolate the proteins with insecticidal activity by selective fractionation because the protein content of the nectar had not been previously reported. The complete list of peptides found in the 13 spots obtained by 2D-PAGE is presented in the supplementary material.

4. Discussion

Plants have evolved sophisticated defense mechanisms against insects and associated diseases. Nectar, leaves, bark and seeds may accumulate defensive compounds that confer resistance against phytophagous predators and infection by microorganisms. The insecticide tests with *E. heros*, *H. zea* and *A. gemmatalis* demonstrated a toxic effect of gross and dialyzed nectar against all groups. A similar effect has been observed with other insects, such as *Plebeia droryana*, *Tetragonisca angustula*, *Scaptotrigona postica*, *Trigona spinipes* and *Friesella schrottky*, present in the inflorescences of *S. campanulata* (Nogueira-Neto, 1997). In other research related to nectar toxicity, Portugal-Araujo (1963) found 200 dead insects, including ants, bees and flies, in a single inflorescence of *S. campanulata*. In addition to this, scientific studies carried out with different parts of this species (bark, leaves and flowers) only demonstrated the presence of phytochemical compounds of secondary metabolism, mainly with pharmacological activity (Niyonzima et al., 1999). Regarding to the insecticidal activity, although demonstrated in different works, there is no scientific information on the compounds responsible for this activity.

Characterization of plant constituents is essential to the management of pest control, as compounds associated with secondary metabolism and/or classes of peptides and proteins may be responsible for insecticidal effects. These compounds are frequently associated with protection against herbivores, a characteristic described in many plant species producing nectar (Adler, 2000). Cintra et al. (2005) hypothesized that plant-insect interactions, such as between flower and pollinators (nectar action), should

Table 2
List of peptides associated with insecticidal activity identified in nectar of *Spathodea campanulata*.

N° Spot	Acess[a]	Score	N° of peptides[b]	Coverage	MW [kDa][c]	Calculation of Ip[d]	Peptide sequences	Name of protein	Species[e]
2	Q40870	2,17	1	2,95	57,3	7,44	SYRPFSLAGPGSSSR	Legumin-like storage protein	*Picea glauca*
6	I9X2B9	4,03	1	2,60	58,4	9,16	LRPGFPEAGPALVR	Glycosyl transferase family protein	Methylobacterium sp.
46	D7TP64	2,37	1	1,46	96,8	6,48	LEVPSSGAASmDK	Serine/threonine-protein phosphatase	*Vitis vinifera*
46	A0A097PPL1	2,61	1	1,72	108,1	6,10	AMVTLTHGPENVKPQRK	Carbohydrate-binding-like fold protein	*Sorghum bicolor*

[a] Access to database (UniProt).
[b] Number of peptides into specific spot.
[c] Molecular weight (MW [kDa]).
[d] Calculation of the isoelectric point.
[e] Species with the highest similarity for each of the selected proteins.

be studied together and not separately, as many particularities of selection occur in both interactions.

Chemical compounds (secondary metabolites and some classes of proteins) with insecticide effect can confer biochemical protection to plants by inducing the production of ROS that can act negatively on insect metabolism when they come in contact (Doke et al., 1996; Seo et al., 2013). In this study, the results obtained by TBARS quantification and oxidative analysis of albumin degradation suggested that the insecticide action may be due to chemical compounds in the nectar of *S. campanulata*. This active compound may be a protein because denatured nectar was less effective than non-denatured nectar in both tests. Grant and Loake (2000) reported that ROS present in plant tissues may lead to the death of plant pathogens by mechanisms that affect nutrient absorption, respiratory activity and synthesis of proteins and enzymes, among other metabolic functions.

Others studies that support the idea of nectars with insecticidal proteins were carried out with *Nicotiana attenuate* (Naqvi et al., 2005). Seo et al. (2013), studying the nectar of this same species, showed that despite the presence of large amounts of sugars such as sucrose, glucose and fructose, they also contained several classes of proteins. Moghaddam et al. (2016) suggested that nectarines are not involved in attracting pollinators but instead involved in defense against the proliferation of microorganisms in the nectar, mainly by production of strong oxidants, such as hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$) and superoxide radical ($O_2^{\bullet-}$).

The biochemical defense performed by proteins may occur directly or indirectly (Carozzi and Koziel, 1997; Falco et al., 2001). The direct action is performed by the protein itself without activation of other pathways, whereas proteins with indirect action are able to produce or induce the formation of others substances with bioactive activity (Ferreira-da-Silva et al., 2000; Abdollahi et al., 2004). Lectin is an interesting class of protein that can induce an insecticide effect when present in the chemical composition of the plant (Sales et al., 2000; Pereira et al., 2015). This carbohydrate-binding protein is considered an important metabolite and can act in defense mechanisms against insects and pathogens, causing degenerative effects on epithelial cells of the digestive system. Thus, they are able to paralyze insect digestion and cause food deprivation and death (Santos et al., 2009; Tajne et al., 2014). Lectin may be considered a legumin-like storage protein as it is one of the most abundant compounds in legume seeds (Sales et al., 2000). In the current work, the MS analysis revealed the presence of both classes of proteins, carbohydrate-binding-like and legumin-like storage proteins, which may be associated with the insecticidal activity of the nectar.

Proteins that may be involved indirectly in the insecticidal effect of *S. campanulata* nectar, are the glycosyl transferase family proteins and serine-threonine-protein phosphatase (Table 2). The glycosyl transferase family are enzymes that might be involved in the biosynthesis of secondary compounds with insecticidal activity (Ferreyra et al., 2013). An example is maysin, a C-glycosyl flavone predominant in silk tissues of some maize varieties and a natural insecticide against the corn earworm *H. zea* (McMullen et al., 2004). Nevertheless, C-glycosyl flavonoids identified in cucumber leaves were shown to act as phytoalexins in defense against powdery mildew fungi (McNally et al., 2003). In the instance of serine-threonine-protein phosphatase, this enzyme is involved in the biosynthesis of a phytohormone with insecticidal effect, the brassinosteroid. In tests with insects, the effects of brassinosteroid on cell cultures *in vitro* and whole larvae *in vivo* (Sobek et al., 1993; Charrois et al., 1996) and in delayed development of two holometabolous insects from different fly species (*Phornia terranovae*, *Calliphora vicina*) has been reported, revealing a significant antagonistic activity (Richter and Koolman, 1991). Scientists believe its insecticidal activity is based on interference in the various ecdysteroid dependent steps, resulting in disruption or abnormal growth and development of the target pest, helping to overcome resistance to insecticides (Smagghe et al., 2002; Davison et al., 2003).

5. Conclusion

The present study described the peptide composition investigation of the nectar of *S. campanulata* which led to the identification of presence of proteins with insecticidal activity against insect pests of soybean. This action may be promoted by proteins or peptides with pro-oxidant activity that directly or indirectly affect insects. The present preliminary study suggests that *S. campanulata* could have potential as natural insecticide that could be employed in developing new insecticides types.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2016.12.025.

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