Effects of *Croton urucurana* Extracts and Crude Resin on *Anagasta kuehniella* (Lepidoptera: Pyralidae)

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ABSTRACT

Hundreds of plant species have been studied in order to find out the active ingredient responsible for their insecticidal activity against the pests of economic importance. To verify the insecticidal activity in the husk of stem of Croton urucurana *Baillon 1864 (Euphorbiaceae) against* Anagasta kuehniella *Zeller 1879 (Lepidoptera: Pyralidae), the methanolic (EMeOH) extract, dichloromethane fraction (FDM), ethyl acetate fraction (FAE) and crude resin, incorporated into an artificial diet were evaluated. EMeOH (0.5, 1.0 and 2.0%) and crude resin (2.0%) interfered with neither the weight nor the survival of fourth instar larvae and other analyzed parameters. FDM (2.0%) fraction caused mortality of 65%, and the artificial diet containing 2.0, 1.0 and 0.5% FAE caused 100, 55 and 68% mortality respectively when compared with the control, confirming the least efficiency rates of food conversion for FDM(2.0%) and FAE(1.0%). The tryptic analysis performed with the midgut fluid of fourth-instar larvae demonstrated that tryptic and chymiotryptic activities for the larvae fed artificial diet containing EMeOH and crude resin were not different.*

Key words: Euphorbiaceae, insecticide, 'sangra d'água', meal moth, toxicity

INTRODUCTION

Given increasing problems with resistance and impact on non-target organisms related to the use of synthetic insecticides, there is an urgent need for the development of safer alternatives, such as insecticide proteins that interfere with the digestive process (Carlini and Grossi-de-Sá, 2002; Montesdeoca et al., 2005) and the use of substances from the secondary metabolism of plants with insecticidal activity (Omar et al., 2000;

Simões et al., 2002; Medina et al., 2003; Akhtar and Isman, 2004).

It is believed that many secondary metabolites found in the plants occurred originally as a result of natural selection during the coevolution between the insects and plants (Mello and Silva-Filho, 2002). Hundreds of plant species have been studied in order to find out the active ingredients responsible for their insecticidal activity against various pests of economic significance. Among the insecticidal plants, Meliaceae,

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Rutaceae, Asteraceae, Labiateae, Piperaceae and Annonaceae are more frequently cited for presenting a broad spectrum of action in relation to both the number of affected insect species and their different modes of action (Akhtar and Isman, 2004). There are not many works available in the literature on the insecticidal activity of Euphorbiaceae species, which present diverse biological activities.

Cis-dehydrocrotonin extracted from *Croton cajucara* bark inhibits the growth of *Heliothis virescens* (Lepidoptera) (Viegas-Junior, 2003). *Jatropha curcas* (Euphorbiaceae), known for its insecticidal properties, affects the insects of various families, and its ingestion inhibits the growth of several Lepidoptera species (Sauerwein et al., 1993). Its contact toxicity was described by Solsoloy (1993). The methanolic extract from *J. curcas* has insecticidal activity against *Helicoverpa armigera* (Lepidoptera) and *Sitophilus zeamais* (Coleoptera) (Solsoloy, 1995), and *Culex quinquefasciatus* (Diptera) (Karmegam et al., 1997).

Although innumerous species of the genus *Croton* (Euphorbiaceae) present several biological activities (Peres et al.,1997 and 1998; Suárez et al., 2003; Anazetti et al., 2004; Fisher et al., 2004), there are no reports for *Croton urucurana* on insecticidal, deterrent, repellent activities or inhibiting action on insects' digestive enzymes.

Anagasta kuehniella (Zeller) (Lepidoptera: Pyralidae), popularly known as Mediterranean flour moth is found worldwide, particularly in the stored grains as a flour and grain feeder: it is often a severe pest in flour mills. Controlling these insects generally requires the use of chemical insecticides that are toxic to the humans and domestic animals and harmful to the environment.

Considering the advancement of researches on the botanical insecticides and their potential for pest control, the objective of this work was to evaluate, under laboratory conditions, the bioactivity of methanolic extract, semipurified fractions and crude resin of *Croton urucurana* Baillon, 1864 (Euphorbiaceae), against *Anagasta kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae).

MATERIALS AND METHODS

Botanical material and methanolic extract and semipurified fractions preparations

Husk of stem and crude resin of *C. urucurana* were collected in May 1994, in the region of Dourados, Mato Grosso do Sul, Brazil. The species was identified by Claudio Conceição, Biology Department, Federal University of Mato Grosso do Sul-UFMS, Campo Grande –MS, and a sample has been deposited (no. 5009) at CGMS Herbarium .

Husk of stem, dried at room temperature and ground, was extracted with methanol. After methanol evaporation in a rotavapor, a solid brown-reddish material, named methanol extract (EMeOH) was obtained. Afterward, the material was percolated on a silica gel 60 (200g) column, using hexane, dichloromethane and ethyl acetate successively. This resulted three semipurified fractions, hexane (FH), dichloromethane (FDM) and ethyl acetate (FAE). Crude resin was collected directly from the trunk.

Insect Biossays

Colonies of *A. kuehniella* were maintained at the Protein Purification and Biological Function Laboratory (LPPFB), Department of Natural Sciences – UFMS, 'Três Lagoas' *Campus* – MS.

To evaluate the effects of EMeOH, FDM, FAE and crude resin of *C. urucurana* on *A. kuehniella* larval development, the moths were kept in plastic boxes, with perforated plastic covers at 28±1°C, 65-77% relative humidity, with a 16-h photophase. An artificial diet of *A. kuehniella* was prepared by mixing the whole-wheat flour, wheat germ and wheat bran (8:1:1), three concetrations (0.5, 1.0 and 2.0%) of EMeOH, and 2.0% of FDM, FAE and crude resin each. A control meal was also prepared without the addition of EMeOH, FDM, FAE and crude resin. Treatments were repeated 16 times with five neonate larvae (n=80) per concentration. After four instars, the weight and larval survival were recorded.

The analyzed biological and biochemical parameters were weight and larval mortality, pupal and larval stages, pupal weight and pupal morphological imperfections, sex ratio, adult emergence and enzymatic activity of fourth instar larvae.

Midgut homogenate preparation

Larval gut homogenates were prepared according to Macedo et. al., (1993). Fourth instar larvae were cold immobilized in 250mM NaCl and dissected. Larval midguts were surgically removed using tweezers. Only actively feeding larvae with guts filled with food were used. The gut portion posterior to the proventriculus and anterior to the malpighian tubules was taken. After removing all the extraneous tissue and freeing the lumen from its contents by rinsing in 0.05M Tris/HCl buffer (pH 8.0), midgut tissues were homogenized, centrifuged at 17,000 rpm for 20 minutes at 4ºC, and the supernatants were collected in a known volume of appropriate buffer and immediately used as enzymes sources for enzymatic assays.
The protein concentration in the extract was determined according to Bradford (1976).

Protease activity assay

The total tryptic activity was determined using BAPNA (N-benzoyl-D,L-arginine-ρ-nitroanilide) as substrate. Midgut and excrements extract (50μg of protein) were incubated in 0.05M Tris-HCl buffer (pH 8.0), to a final volume of 500μL for 10 min before the addition of 1 mL BAPNA substrate. The reaction was allowed to proceed at 37ºC for 20 min then stopped by adding 30% acetic acid (v/v). The resulting absorbance was read at 410nm. One enzyme unit was defined as the amount of protein that catalyzed the cleavage of 1μmol substrate/min.

Chymotryptic activity was determined using SAAP (N succinyl ALA-ALA-PRO-PHE p-Nitranilide) as substrate. Midgut and excrements extract (50μg of protein) were incubated in 0.1M Tris-HCl buffer (pH 8.0), to a final volume of 500μL for 10 min before the addition of 1 mL SAAP substrate dissolved in pure DMSO (dimethylsufoxide). The reaction was allowed to proceed at 37ºC for 20 min, then stopped by adding 30% acetic acid (v/v). The resulting absorbance was read at 405nm. One enzyme unit was defined as the amount of protein that catalyzed the cleavage of 1μmol substrate/min.

Polyacrylamide gel eletrophoresis

SDS polyacrylamide gel (10%) electrophoresis (SDS-PAGE) was performed as described by Laemmli (1990). Proteins were detected by staining with 0.25% Coomassie brilliant blue R-250.

Nutritional Indexes

Food ingestion and utilization by the insects was determined according to Parra (1991).

Statistical Analysis

The data were examined using a one-way analysis of variance (ANOVA). The Tukey's test was used to indicate the means significantly different, $p < 0.05$.

RESULTS

Development of *A. kuehniella* larvae

The effect of *C. urucurana* methanol extract (EMeOH), crude resin, dichloromethane (FDM) and ethyl acetate (FAE) fractions incorporated to the artificial diet on the development of *A. kuehniella* was assessed by determining the number and weight of surviving fourth instar larvae.
The number and weight of surviving *A. kuehniella* larvae fed artificial diet with 0.5, 1.0 and 2.0% EMeOH (Fig. 1 A;B) and 2.0% crude resin of *C. urucurana* (Fig. 1 C;D) did not differ from the control.
The dose response effect of FAE on the mortality of larvae is shown in figure 1G. The artificial diet containing 2.0, 1.0 and 0.5% FAE caused 100, 55 and 68% mortality, respectively (Fig. 1 H) when compared with the control. Larvae fed on 1.0 and 0.5% FAE had 30 and 54% decrease in weight respectively. With FDM, the mortality and weight of *A. Kuenhiella* larvae feeding on control diet were 25% and 6mg, respectively, while a diet containing 2.0% FDM produced an estimated 65% mortality and a 30% decrease in weight (Fig. 1E;F).
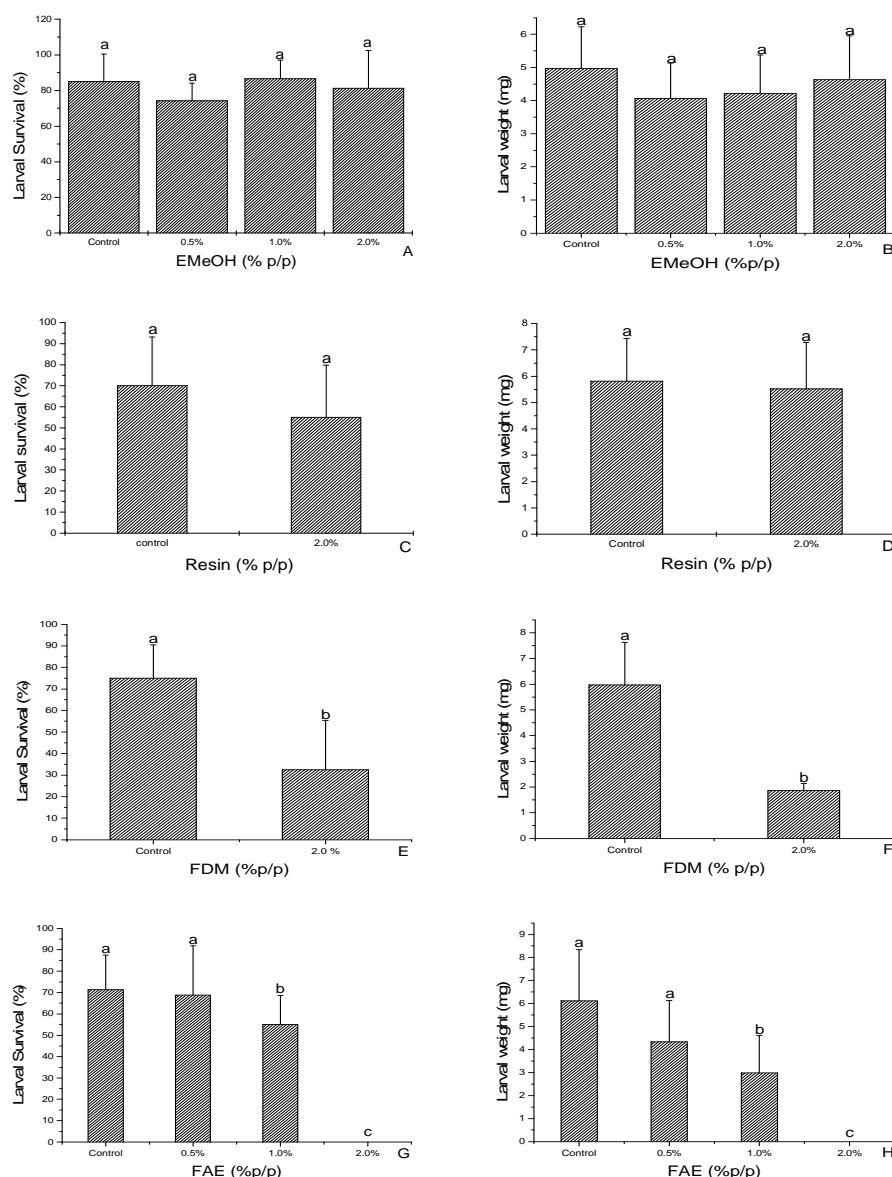
Figure 1 - Larval survival and weight of fourth-instar *A. kuehniella* larvae fed at control and artificial diets containing EMeOH **(A;B)**, crude resin **(C;D)**, FDM **(E;F)** and FAE **(G;H).** Bar with the same letter are not significantly different by the Tukey's test at 5 % probability.

Nutritional Indexes for *A. kuehniella*

The results showed no significant differences for the amount of food ingested and excrements produced, comparing larvae fed with control diet to those fed EMeOH (Fig. 2 A;B) and crude resin (Fig. 2 C;D). There was no reduction in the efficiency of conversion of ingested food (ECI) or in the efficiency of conversion of digested food (ECD) for the larvae fed EMeOH and crude resin compared with the control. The metabolic cost was

not significantly altered in relation to the control (Table 1).

Approximate digestibility (AD), which represented the percentage of ingested food that was effectively assimilated by the insect, was not significantly different (Table 1).

Figure 2 (E;F) showed that with the diet containing FDM food ingestion and excrements produced were reduced, with lower insect weight gain.

The lowest efficiencies of conversion of ingested (ECI) and digested (ECD) food were found in larvae fed the diet containing FDM (2%) and FAE (1%) (Table 1), representing a reduction of approximately 60% and consequently the metabolic cost was 40% higher for the insects fed with these diet. As seen in Table 1, the approximate digestibility (AD) was about 23 % higher for the insects fed with the diet containing FDM and FAE, when compared with the control.
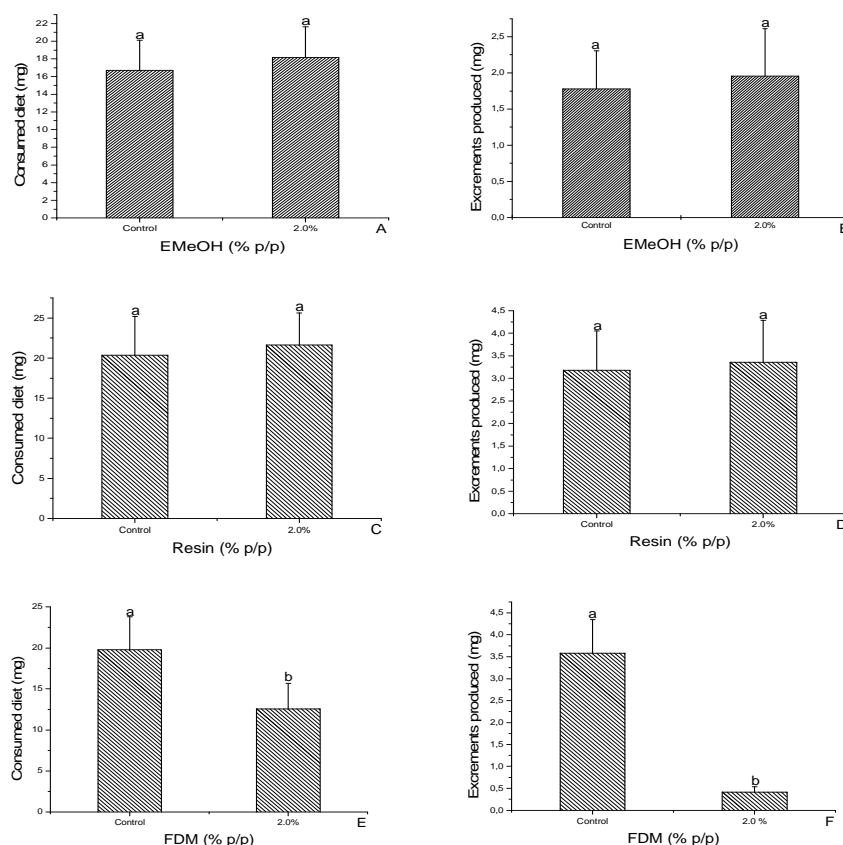

Figure 2 - Diet ingested and excrements produced by fourth-instar *A. kuehniella* larvae fed control diet and artificial diet containing EMeOH **(A;B)**, crude resin **(C;D)** and FDM **(E;F)**. Bar with the same letter are not significantly different by the Tukey's test at 5 % probability.

Table 1 - Efficiency of conversion of ingested (ECI) and digested (ECD) food, approximate digestibility (AD) and metabolic cost (CM) of *A. kuenhiella* larvae fed control diet and artificial diet with 2.0% EMeOH, 2.0% crude resin of *C. Urucurana,* 2.0% FDM and 1.% FAE.

Nutritional Indexes	Control	EMeOH	Crude Resin	FDM	FAE
ECI	27.7 ± 3.9 a	23.42±4.7 a	25 ±5.2 a	11.7 ± 6.0 b	12.3 ± 8.5 b
ECD	33 ± 6.5 a	26.56±6.1 a	30 ± 6.9 a	12 ± 7.3b	13.4 ± 9.3b
AD	86 ± 8.5 a	88.21±3.3 a	84 ± 3.7 a	95 ± 3.1 b	93.7 ± 8.1 b
CM	65 ± 6.5 a	73.43±6.1 a	69 ± 6.9 a	87 ± 7.3 b	83.2 ± 8.3 b

Values followed by the same letter within a row are not significantly different by the Tukey's test at 5 % probability.

Duration of larval stage, pupa weight, duration of pupal stage, adult emergence and sex ratio

There were no significant differences for the treatments with EMeOH and crude resin of *C. urucurana* for the duration of the larval stage. Neither EMeOH nor crude resin reduced the larval viability of *A. kuehniella* (Table 2).

The average pupa weights were 19 mg for the control and 18 mg for the treatments. The results for pupal viability showed no significant differences between the control and the treatments with EMeOH and crude resin (Table 2).

The number of adult *A. kuehniella* emerging after two months from the artificial diet containing EMeOH and 2.0% crude resin did not differ from the number of adults from the control, and the viability was on average 72% of the 50 initial larvae. The sex ratio was similar for both the control and treatments.

Table 2 - Duration and viability of larval and pupal stages and pupa weight of *A. kehniella* fed with control diet and artificial diet containing 2.0% EMeOH and 2.0% crude resin (n= 50) of *C. urucurana*.

Concentration (%)	Larval stage		Pupal stage		
	Duration day	Viability(%)	Duration day	Viability(%)	Pupa weight (mg)
Control	36.8±4.0a	88a	8.7±1.1a	84a	19.2±1.8a
EMeOH 2,0%	38.4±4.7a	90a	8.6±1.2a	88a	17.6±1.8a
Resin 2,0%	36±2.8a	80a	9.0±0.2a	83a	18±1.2a

Values followed by the same letter within a row are not significantly different by the Tukey's test at 5 % probability.

Effects of EMeOH, Crude Resin and FAE on proteolytic activity

Tryptic and chymotryptic activities of *A. kuehniella* larvae with EMeOH., crude resin and FAE of *C. urucurana,* were analyzed through the hydrolysis of BAPNA and SAAP, respectively.

No significant differences were found for the tryptic activity of midguts and excrements in larvae of *C. urucurana* fed EMeOH (Fig. 3 A;B) and larvae receiving the artificial diet with crude resin (Fig. 4 A;B)*,* when compared with the control.

The tryptic activity of the midgut fluid and excrements of larvae fed 2.0% FAE (Fig. 5 A) was reduced by 78.5% and the assays with the excrements homogenate decreased 56.5% when compared with the control (Fig. 5 B).

The chymotryptic activity in the midguts of the larvae fed the diet containing EMeOH (Fig. 3 C;D) and crude resin (Fig. 4 C;D) was similar to that found in the larvae fed the control diet.

However, the chymotryptic assays carried out with the midgut fluid homogenate of larvae fed FAE demonstrated a reduction in the enzyme activity (Fig. 5 C;D).

The eletrophoresis confirmed the data obtained from the midgut triptic activity, in which the larvae treated with EMeOH (Fig. 6 I) and crude resin (Fig. 6 II) presented proteolytic activity similar to the control. The fecal material did not allow the protein bands, corresponding to the enzymes to be well defined (data not shown).

The electrophoresis of gut enzyme activity, confirmed the results obtained for the triptic activity, in which the insects treated with FAE showed a lower trypsin-like proteolytic activity (Fig. 6 III).

The eletrophoresis also showed that the insects treated with EMeOH, crude resin and FAE (Fig.6 I, II and III) presented trypsin-like proteolytic activity; the proteolytic bands were inhibited by *N*-tosyl-L-lysine chloromethyl ketone (TLCK), confirming their tryptic activity.
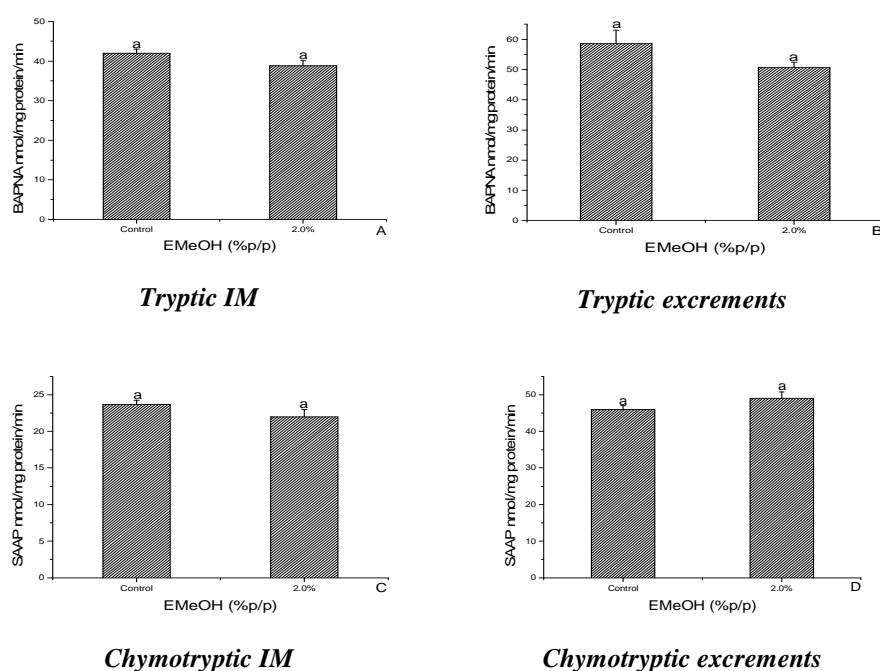
Tryptic IM   *Tryptic excrements*

Chymotryptic IM   *Chymotryptic excrements*

Figure 3 - Tryptic and chymotryptic activity of the midgut fluid and excrements of *A. kuehniella* fourth-instar larvae fed control diet and artificial diet containing EMeOH. Same letters are not significantly different by the Tukey's test at 5 % probability.
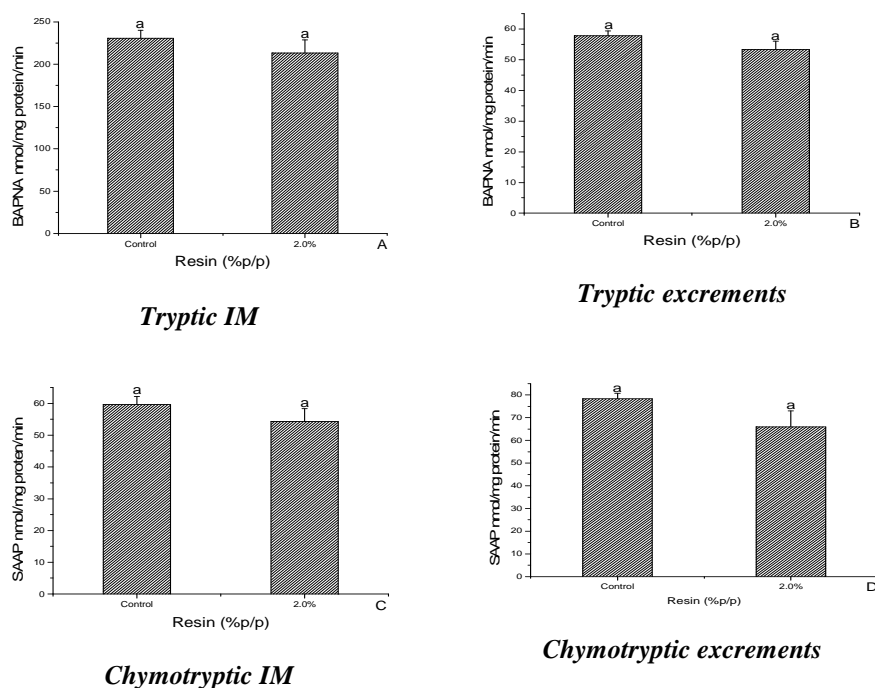

Tryptic IM   *Tryptic excrements*

Chymotryptic IM   *Chymotryptic excrements*

Figure 4 - Tryptic and chymotryptic activity of midgut fluid and excrements of *A. kuehniella* fourth-instar larvae fed control diet and artificial diet containing crude resin. Bar with the same letter are not significantly different by the Tukey's test at 5 % probability.
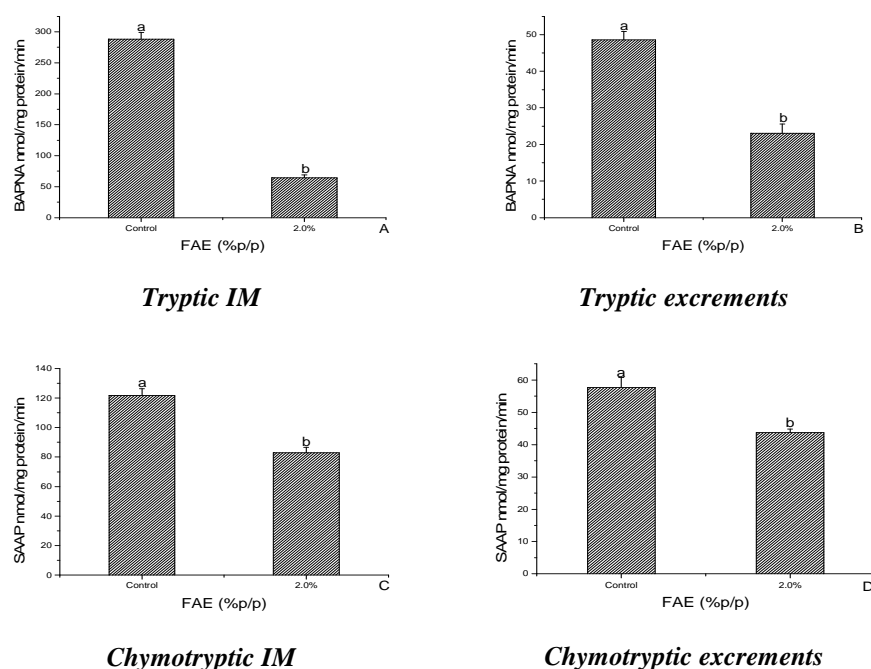
Tryptic IM   **Tryptic excrements**

Chymotryptic IM   **Chymotryptic excrements**

Figure 5 - Tryptic and chymotryptic activity of midgut fluid and excrements of *A. kuehniella* fourth-instar larvae fed control diet and artificial diet containing FAE**.** Bar with the same letter are not significantly different by the Tukey's test at 5 % probability.
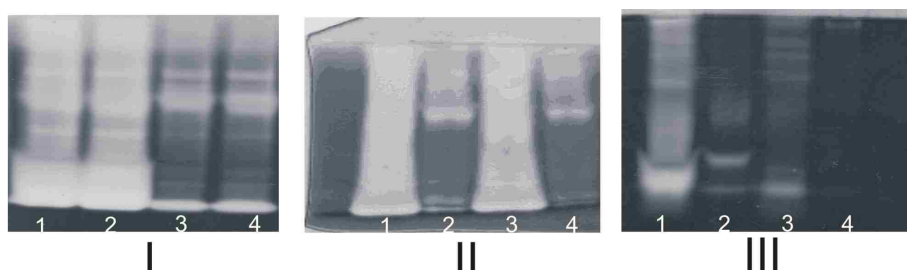

Figure 6 - Responses of *A. kuehniella* to EMeOH, Crude Resin and AE ingestion. Proteinase assays after SDS-PAGE: (**I**) 2.0 % EMeOH **1**-IM control, **2**-IM 2.0% EMeOH, **3**-IM control+TLCK, **4**-IM 2.0% EMeOH +TLCK. (**II**) crude resin. **1**-IM control, **2**- IM control+TLCK, **3**-IM 2.0% resin, **4**-IM 2.0% resin +TLCK. (**III**) FAE. **1**-IM control, **2**- IM control+TLCK, **3**-IM 1.0% FAE, **4**-IM 1.0% FAE +TLCK.

DISCUSSION

The use of plants with insecticidal activity has gained importance over the past few years. Though their utilization is not a novelty, it has been rather common before inorganic insecticides became available. The resurgence as a method of insect control is based on the current demand for the molecules that are useful in pest management without contaminating the environment or remaining as residues in the foods.

The selection of *C. urucuran,* was based on the popular information, its bactericidal activity and chemical studies (Peres et al.*,* 1997, 998). The present work verified the toxicity of *C. urucurana* methanolic extract, semipurified fractions and crude resin on the development of *A. kuehniella.*
The semipurified FDM and FAE fractions caused expressive mortality to *A. kuehniella* larvae, especially FAE (2.0%), reaching 100% mortality rate. Previous chemical studies on this fraction resulted in the isolation of the phenolic compounds

catechin and gallocatechin, which played a role in the insecticidal activity (Peres et al., *1998).*

According to Shirley (2001) condensed tannins, catechin and gallocatechin are phenolic compounds responsible for the astringency of many fruits as well as defense against the pests, as they attach to insects' digestive proteins, becoming an important mechanism for the plant defense against the pathogens (Oliveira et al.,2003). This ability of tannins to interact with the proteins makes this class of substances rather toxic elements to insects, fungi and bacteria.

Studies using histological sections have demonstrated the toxic effect of tannic acid, a hydrolyzable natural tannin on the midgut epitelium of Diptera larvae (Rey et al., 1999). Other investigators, after evaluating the toxic effects of tannins on the fauna associated with Culicidae, suggested that the vegetable tannins can be useful as a complement in control programs of hematophagous species of mosquitoes associated with the human diseases. Studies carried out with the extracts of *Magonia pubescens* (Sapindaceae) provided evidence that the action mechanisms and morphological changes leading to the death in *A. aegypti* were similar to those of tannic acid (Arruda et al., 2003; Pautou et al., 2000; Silva et al., 2003).

Moreover, toxicological studies performed in the rabbits, mice and guinea pigs have proved to be atoxic, according to the rules for plant products. Field tests have confirmed their degradation and atoxicity from the fourth week onward (Silva et al., 2003).

Despite these favorable features, additional toxicological studies on catechinic tannins must be carried out in order to evaluate their impacts, mainly on non-target species, and to identify the formulations that provided stability and easiness of management (Silva et al., 2004).

For the FDM fraction (2.0%), chemical studies led to the isolation of terpenoid compounds (Peres et al., 1998). The 65% mortality rate found for *A. kuehniella* larvae fed on FDM diet may have been caused by diterpenes, such as 12-epi-methylbarbascoate, isolated from this fraction. The chemical class of diterpenes has already been reported as having insecticidal activity (Mancebo et al., 2002; Medina, et al.,2003; Breuer, et al., 2003; Akhtar and Isman, 2004; Boeke et al., 2004).

The use of plants that affect the insect larval development is quite advantageous because when life is interrupted at this stage, there is a reduction in the insect population during the period in which grain damage is being caused (Hernández andVendramim, 1996). The losses caused by the pest attacks during grain storage can reach up to 10% (Obeng-Ofori and Amiteye, 2005).

Lower efficiency of conversion of ingested and digested food was found for the insects fed FDM (2%) and FAE (1%) diet, which meant that in these diets, a smaller quantity of the food (energy) was used to produce the insect biomass. It was likely that in these fractions, a larger amount of metabolic energy was used for the degradation of toxins. This was confirmed by comparing the metabolic costs, which were higher for the larvae fed on FDM and FAE diet. The approximate digestibility was also higher for the larvae fed on FDM and FAE diet, indicating differential activity of the digestive enzymes, therefore the need for the food to remain in the insect's digestive tract for a longer period of time in order to allow the degradation of the secondary compounds of this fraction (Hernández and Vendramim, 1996). According to the same authors, the inverse correlation between the AD and ECD values was probably because when digestibility was high, there was a proportional higher expenditure of digested food on the metabolic energy, which resulted in a lower biomass gain per unit of digested food, corresponding to ECD.

Nathan et al. (2005) reported that limonoids from *Azadiracta indica* A. Juss (azadirachtin, salannin, and acetylgedunin, gedunin), the main representatives of the terpernoid class with insecticidal activity, have significant effect on the larvae and pupae of *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae)*,* causing weight loss and reduction of enzymatic activity. The adult physiology is harmed when the larva is exposed to the limonoids present in this meliaceae.

The methanol (EMeOH) extract and crude resin of *C. urucurana* incorporated into the artificial diet did not interfere with the weight or with the survival of *A. Kuehniella* larvae*.* The larvae that survived EMeOH and crude resin turned into pupae normally, and the pupae derived from these larvae had no significant differences in weight or morphological changes, which demonstrated that the larvae were, to a certain extent, able to overcome the toxic action of substances present either in EMeOH or in the crude resin incorporated in the diet.

The tryptic and chymotryptic assays showed no effect that could impair the development of the insects fed on diet with EMeOH or crude resin.

The tryptic and chymotryptic activities performed with the midgut fluid and excrements presented a significant reduction for the larvae treated with FAE, when compared with the control. Comparing the results of larval weight, it was observed that the significant reduction in the tryptic and chymotryptic activity could have reflected on insect physiological misfit, since the larvae of this treatment had a reduction of about 30% in weight.

Trypsin was present in all the degradation products from insects subjected to EMeOH and crude resin, as band intensity was reduced in the presence of TLCK.

The eletrophoresis of the midgut homogenates from the control larvae and the larvae treated with EMeOH and crude resin showed that there were no changes in the pattern of digestion products of the treated larvae compared to the control.

The results demonstrated the importance of the bioassay-guided isolation research. As shown in the present work, the biological activity was evaluated through the biological and biochemical parameters of the crude methanolic extract, semipurified fractions (Ethyl acetate and Dichloromethane) and crude resin of *C. urucurana*. Although there have been innumerous reports on the insecticidal potential of plants against Lepidoptera, few works used *A. kuehniella* (Macedo et al., 2003 and 2004 a and b; Warchalewski et al., 2002) as model, and no report was found on the insecticidal activity of *C. urucurana*.

Elucidation of the mechanism of action of the extracts and other compounds derived from the plants in the insects of economic importance, and the mechanism that insects developed in order to survive the toxic action of substances present in the diet, would be useful for discovery and synthesis of new insecticidal molecules. Knowledge of the chemical composition of the plant secondary metabolites and their respective biological activities would contribute to an integrated pest management. More studies with purified compounds to determine their mechanism of action are required.

RESUMO

Atualmente centenas de plantas são investigadas para se conhecer os princípios ativos responsáveis pela atividade inseticida contra as diversas pragas de importância econômica. Com o objetivo de verificar a atividade inseticida das cascas do caule de *Croton urucurana* Baillon 1864 (Euphorbiaceae) em relação a *Anagasta kuehniella* Zeller 1879 (Lepidoptera: Pyralidae), avaliou-se o extrato metanólico (EMeOH), fração diclorometano (FDM), a fração acetato de etila (FAE) e a resina *in natura,* os quais foram adicionados à dieta artificial. O EMeOH (0,5, 1,0 e 20,%) e a resina *in natura* (2,0%), não interferiram no peso, sobrevivência das larvas de 4ª ínstar, bem como nos demais parâmetros analisados. A fração FDM (2,0%) causou mortalidade de 65%, e a dieta artificial contendo 2,0, 1,0, e 0,5% de FAE causou 100, 55 e 68% de mortalidade respectivamente quando comparado com o controle, constatando-se as menores eficiências de conversão do alimento para a FDM (2,0%) e FAE (1,0%). Através do ensaio tríptico realizado com o fluido do intestino médio das larvas, foi possível verificar que a atividade tríptica e quimiotríptica para as larvas alimentadas com dieta artificial contendo o EMeOH e a resina *in natura* não apresentaram diferenças significativas.

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