Notas Científicas

Mortality of *Plutella xylostella* larvae treated with *Aspidosperma pyrifolium* ethanol extracts

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Abstract – The objective of this work was to assess the effects of *Aspidosperma pyrifolium* ethanol extracts on cabbage moth (*Plutella xylostella*) larvae. The ethanol extracts of the stem bark, fruits and roots of *A. pyrifolium* were obtained by classical phytochemical methods, and the resulting subfractions were tested on *P. xylostella*, using 4 and 5 mg L^{-1}. The crude ethanol extract of the stem bark was more lethal. The alkaloid-rich aqueous subfraction derived from the stem bark extract caused 100% larval mortality at 4 mg L^{-1}. Insecticidal activity was associated with the presence of the monoterpenoid indole alkaloids aspidofractinine, 15-demethoxypyrifoline, and *N*-formylaspidofractinine. These alkaloids presented excellent insecticidal properties against *P. xylostella*.

Index terms: *Brassica oleracea* var. *acephala*, alkaloids, cabbage moth, insecticidal activity, organic extracts, pereiro-do-sertão.

Mortalidade de larvas de *Plutella xylostella* tratadas com extratos etanólicos de *Aspidosperma pyrifolium*

Resumo – O objetivo deste trabalho foi avaliar os efeitos dos extratos etanólicos de *Aspidosperma pyrifolium* sobre lagartas da traça-das-crucíferas (*Plutella xylostella*). Os extratos etanólicos da casca do caule, do fruto e da raiz de *A. pyrifolium* foram obtidos pelos métodos fitoquímicos clássicos, e as subfrações resultantes foram testadas contra *P. xylostella*, nas dosagens 4 e 5 mg L^{-1}. O extrato bruto etanólico da casca do caule foi mais letal. A subfração aquosa rica em alcalóides, derivada do extrato da casca do caule, causou 100% de mortalidade larval a 4 mg L^{-1}. A atividade inseticida foi associada à presença dos alcalóides monoterpenóides indólicos aspidofractinina, 15-demetoxipirifolina e *N*-formilaspidofractinin. Estes alcalóides apresentaram excelente propriedade inseticida contra *P. xylostella*.

Termos para indexação: *Brassica oleracea* var. *acephala*, alcalóides, traça-das-crucíferas, atividade inseticida, extratos orgânicos, pereiro-do-sertão.

Plutella xylostella (Linnaeus 1758) (Lepidoptera: Plutellidae), commonly known as the diamondback or cabbage moth, is one of the main pests affecting Brassicaceae plants worldwide (Herbison-Evans & Crossley, 2004).

The control of this pest is mainly through the use of chemical insecticides (pyrethroids and organophosphates) (Castelo Branco & Gatehouse, 2001). The application of these insecticides contributes to production costs and can be detrimental to the environment and to the end consumer by virtue of residual contamination. The application of plant extracts in pest management is an attractive alternative since their use can minimize residual contamination (Isman, 2006). Amongst the various classes of natural products that may have application as natural pesticides are the indole alkaloids, which are typically found within the families Loganiaceae, Apocynaceae and Rubiaceae (Henriques et al., 2001). Monoterpenoid indole alkaloids are abundant amongst these families, especially within the genus *Aspidosperma* (Apocynaceae) (Deutsch et al., 1994).

A number of species of *Aspidosperma* are well known for the excellent quality of their wood, which is commonly employed in construction and furniture manufacture (Deutsch et al., 1994). Insecticidal

properties (specifically against *P. xylostella*) (Torres et al., 2001, 2006) and medicinal activities (Deutsch et al., 1994) have been reported for *Aspidosperma pyrifolium*, a species that is widely distributed in the Caatinga of northeastern Brazil (known as "pereiro-do-sertão").

The objective of this work was to assess the effects of *A. pyrifolium* ethanol extracts on *P. xylostella* larvae.

The experiments were carried out in Laboratório de Ecologia e Comportamento de Insetos and Laboratório de Pesquisas em Recursos Naturais, Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, Maceió, AL, Brazil, in January 2004.

A population of *P. xylostella* was maintained at 26±2°C, 60±10% relative air humidity and 12-hour photoperiod, according to the methods of Medeiros et al. (2003), and was fed with kale (*Brassica oleracea* L. var. *acephala*, a Brassicaceae).

Stem bark, roots and fruits of *A. pyrifolium* were collected in São José da Tapera, AL, Brazil, in October 2001. A voucher specimen was deposited in the herbarium of the Universidade de Brasília (Brasília, DF, Brazil), under the number JEP 3686 (UB). The separated plant parts were air-dried at 40°C for 48 hours, triturated in a Nogueira forage grinding mill (Nogueira S.A. Máquinas Agrícolas, Itapira, SP, Brazil) to yield a fine, uniform powder (mesh size 2.5 mm), and stored at 4±2°C.

Samples of stem bark (3 kg), roots (2 kg) and fruits (600 g) were extracted separately with 90° ethanol (5.5, 3 and 1 L, respectively) for 72 hours in a Soxhlet extractor. The excess solvent was removed under reduced pressure; and the crude residues were placed in a desiccator to remove any remaining water and stored at 4±2°C. The amounts (and percentage yields) of the dried crude residues obtained from stem bark, roots and fruits were, respectively, 150 g (5%), 115 g (5.75%), and 29 g (5%)

The dried and powdered stem bark of *A. pyrifolium* (3 kg) was extracted exhaustively with ethanol (5.5 L) in a Soxhlet extractor for 72 hours; and the ethanolic solution was concentrated under reduced pressure to yield 150 g of a brownish viscous liquid. This crude ethanol extract was dissolved in methanol (300 mL); water (450 mL) was added, and the resulting mixture partitioned against ethyl acetate (5x350 mL). The ethyl acetate layer was concentrated under reduced pressure to yield 91 g of a residue that was retained for further studies; and the hydromethanolic phase was lyophilized producing 55 g of an alkaloid-rich extract. An aliquot (2 g) of this extract was submitted to column chromatography of Sephadex LH-20 (20 cm, MeOH) (Pharmacia AB, Uppsala, Sweden) and eluted with methanol to afford 43 fractions of 10 mL each. The bulk of the alkaloids were present in fractions 8–14 (as determined by thin layer chromatography analysis – TLC, silica gel; CH_2Cl_2:MeOH 90:10 – with detection by Draggendorf reagent), and these were combined and concentrated under vacuum to yield 1 g of a crude alkaloid mixture. The enriched alkaloid fraction was purified and identified previously by Araújo Júnior et al. (2007).

Stock solutions of the ethanol extracts from stem bark, roots and fruits of *A. pyrifolium* were prepared by dissolving each residue (5 g) separately in dimethylsulphoxide (DMSO; 10 mL) and completing to volume (1,000 mL) with dechlorinated distilled water. The final concentration of each extract was 5 mg mL^{-1} in 1% DMSO. Stock solutions of the ethyl acetate and aqueous fractions obtained from the crude ethanol extract of stem bark were prepared in a similar manner, except for the final concentration that was 4 mg mL^{-1} in 1% DMSO. Subfractions 8–14 deriving from column chromatography of the aqueous fraction were also submitted to bioassay at a concentration of 4 mg mL^{-1} in 1% DMSO.

Discs (8 cm in diameter) cut from cabbage leaves including midrib were immersed separately for 30 s in the different stock solutions for leaf-eating larvae, and control discs were immersed in 1% DMSO in water. The discs were left to dry at room temperature for 2 hours, after which they were placed in individual Petri dishes together with 12 newly hatched larvae. The dishes were sealed with plastic film and maintained at room temperature (27±1°C). Since the cabbage discs became yellow, or were consumed by the larvae, they were replaced daily by newly treated discs. Larval mortality was evaluated on the third day of the experiment, when larvae left the leaf mines, and daily until the 12nd day. The experiment was carried out in a completely randomized design, with nine treatments (ethanol extracts of stem bark, roots and fruits, and control; aqueous and ethyl acetate fractions of steam bark ethanol extract and control; the combined subfractions 8–14 of aqueous fraction of steam bark ethanol extract and a control); and five replicates, with 12 larvae per treatment for each replicate.

Data were submitted to analysis of variance and means were compared by Tukey test, at 5% of probability with Sanest version 3.0 software (Machado & Zonta, 1991).

The crude ethanol extracts of the stem bark, roots and fruits of *A. pyrifolium* induced higher *P. xylostella* larvae percentage mortalities than the control (Table 1). The stem bark extract was four to five-fold more active than extracts from the other plant parts. The aqueous fraction of the partition of methanol + water (4 + 6 by volume) was significantly more active against *P. xylostella* larvae than the ethyl acetate fraction. TLC analysis showed that this aqueous fraction was rich in alkaloids isolated previously from the highly active subfractions of the ethanol extract of *A. pyrifolium* stem bark. These alkaloids were identified as monoterpenoid indole alkaloids aspidofractinine, 15-demethoxypyrifoline and *N*-formylaspidofractinine (Araújo Júnior et al., 2007). Column subfractions 8 to 14 contained the bulk of the alkaloids present in the aqueous fraction. These subfractions were combined and the bioassay showed that at 4 mg mL^{-1} these fractions were 100% lethal to *P. xylostella*.

Torres et al. (2001) reported that a 10% aqueous solution of the wood bark of *A. pyrifolium*, collected in Belém de São Francisco, PE, Brazil, produced 100% mortality against *P. xylostella* larvae. Such activity appears to be greater than that observed in the present work, probably due to a greater concentration of the active compounds in the aqueous extract.

Table 1. Mortality of *Plutella xylostella* larvae treated with *Aspidosperma pyrifolium* (stem bark, fruits and roots) ethanol extracts and partially purified fractions of the stem bark ethanol extract[1].

Ethanol extract [2]	Mortality(%)
Stem bark	51.7±11.6a
Root	13.3±2.0b
Fruit	11.7±3.3c
Control	1.7±1.7c
Aqueous fraction of stem bark ethanol extract[3]	65.0±8.1a
Ethyl acetate fraction of stem bark ethanol extract[3]	15.0±6.1b
Control	0.0±0b
Subfractions 8–14 of aqueous fraction of stem bark ethanol extract[3]	100.0±0a
Control	0.9±1.7b

[1]Means (±standard error) (n = 5) followed by the same letters, in the columns, do not differ significantly by Tukey test, at 5% of probability. [2]Sample assayed at a concentration of 5 mg L^{-1} in 1% dimethylsulphoxide (DMSO). [3]Sample assayed at a concentration of 4 mg L^{-1} in 1% DMSO.

In the present work, a large number of larvae died during molting, since they could not fully cast off the old exoskeleton, which typically remained linked to the posterior part of the abdomen giving rise to a dark appearance. Moreover, numerous larvae died in the early stages of the experiment, because they were very small and could not penetrate the leaf to eat. These morphological alterations may have been caused by the effect of alkaloids. Mordue (Luntz) & Nisbet (2000) have observed similar symptoms in larvae submitted to different concentrations of azadirachtin, from the neem tree (*Azadirachta indica*), and attributed the symptoms to the reduction in the concentration of ecdysone (a steroidal prohormone of the major insect molting hormone 20-hydroxyecdysone) or to its delayed release into the circulatory fluid.

Banaag et al. (1997) reported that alkaloid fractions from the yam (*Dioscorea hispida* Dennst.) have feeding deterrent activity and toxicity effects against *P. xylostella*.

The alkaloid-rich aqueous fraction obtained from the ethanol extract of *A. pyrifolium* had excellent insecticide properties against cabbage moth larvae. Although many plant extracts were tested for their insecticidal activities, purification and identification of the active principles is essential to evaluate the toxicity of the compounds, to prepare standardised solutions with consistent bioactivity, to control the quality of the preparations, and to estimate the cost involved in employing plant extracts in pest control strategies in comparison with the use of synthetic products (Banaag et al., 1997; Hermawan et al., 1997; Simmonds et al., 2001; Abdelgaleil & Nakatani, 2003).

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