BIOINSECTICIDE ACTION OF CYSTATINS ISOLATED FROM SEEDS OF
Clitoria fairchildiana R.A HOWARD

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Cystatins are inhibitors of cysteine proteases that specifically inhibit proteases from the classes of papain and cathepsins B, L and H. These proteins are present in animals and plants. Cystatins inhibit the digestion of phytophagous insects, suggesting biopesticide potential. This study tested the potential of C. fairchildiana seed cystatins against the bruchid Callosobruchus maculatus. Proteins were extracted from cotyledons and the crude extract was boiled (BCE) for 10 min. The BCE was subjected to molecular exclusion chromatography on Sephadex G-100 and three peaks were obtained. Peak 3 had the highest inhibitory activity rate towards papain and it was subjected to an affinity chromatography on CNBr-Sepharose 4B CM-papain. A non retained (NR) and a retained (R) fraction, eluted at pH 11.5, were obtained. All fractions were analysed for protein quantification and inhibitory activity against papain, as well as visualized by tricine-SDS-PAGE.

C. maculatus larvae were fed with artificial seeds containing increasing concentrations (0.05%, 0.1%, 0.5%, 1% and 2%) of EBF and fraction R. Intestinal homogenates from these fed larvae were analysed for cysteine protease activities on SDS-PAGE-gelatin gels. The cystatin purification process achieved an enrichment level of about 10.27 times and a purification yield of 7%. Fraction R was seen to contain an isolated band of ~12 kDa. Larvae fed with 0.05% and 0.1% EBF had 41.7% and 85.86% reduction in their masses, respectively. Larvae fed with 0.05%, 0.1% and 0.5% fraction R had reductions of 14.15%, 38.38% and 69.19% in their masses, respectively. The proteins retained on the affinity chromatography inhibited cysteine proteases from C. maculatus, and it was possible to see that the EBF contains other proteins that also interfere with the larval development of the bruchid.

Keywords: Clitoria fairchildiana, cystatin, bioinsecticide.
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