TALISIN, A PLANT RESERVE PROTEIN, INTERFERES ON DIGESTIVE ENZYMES ACTIVITY OF SPODOPTERA FRUGIPERDA

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Introduction
Talisin is a storage protein from Talisia esculenta seeds that presents lectin-like and peptidase inhibitor properties. These characteristics suggest that Talisin may be also involved in plant defense process, making Talisin a multifunctional protein. In this work, we aim to evaluate the insecticidal properties of Talisin on Spodoptera frugiperda larvae, an important agricultural pest worldwide. We evaluated the effects of Talisin intake on larval weight, survival and activity of the main digestive enzymes of S. frugiperda larvae. Material and methods Talisin was purified by gel filtration (Sephadex G-100 column) and incorporated at 0.1, 0.5 and 1% (w/w) to an artificial diet fed to neonate larvae until reach the fifth instar. Larval midgut lumen and frass homogenates were obtained and used to assess trypsin, chymotrypsin and α-amylase activity. We evaluated the sensitivity of trypsin midgut proteases to inhibition by Talisin through an inhibition curve using increasing concentrations of Talisin (0-0.6µg/µL). Zymography in native polyacrylamide gel containing 0.1% casein was also employed for visualization of protease activity. Results and discussion Fifth-instar larvae fed Talisin 0.1, 0.5 and 1% had their weight reduced by approximately 26, 48 and 73%, respectively. In turn, Talisin presented no effect on survival. Talisin-fed larvae (0.5 and 1%) had trypsin, chymotrypsin and α-amylase activities of the midgut lumen reduced significantly, while in frass only trypsin activity was reduced. SDS-PAGE of frass homogenates showed that ingested Talisin is partly excreted in frass. In addition, we verified that Talisin excreted in frass remains active. Zymogram with casein showed no differences on enzymatic profile of control and Talisin-fed larvae. Furthermore, sensitivity of trypsin-like enzymes present in midguts from Talisin-fed larvae was pronouncedly reduced, suggesting a possible adaptation mechanism. Conclusion Talisin causes a decrease in larval weight and interferes with the normal activity of mainly digestive enzymes of S. frugiperda.

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