Characterization and purification of a chitinolytic enzyme active against *Sesamia cretica* (pink borer)

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ABSTRACT

Corn borers are serious insect pests in many corn growing areas in Egypt and are responsible for significant yield losses. The ability of insect chitinases for attacking and digesting insect chitin raises the idea of using it for controlling insects such as corn borers. In this study, insect chitinases have been extracted from molting fluid as well as from the integument of Sesamia cretica (pink borer) and subjected to protein purification techniques, through three steps of precipitations 40, 60 and 80% of ammonium sulfate saturation. Protein Gel electrophoresis of the purified protein showed that the expected 88 kDa chitinase band is obtained with 40% ammonium sulfate precipitation. The purified protein was eluted from the gel and tested against Sesamia cretica. The LC100 was conducted and revealed 5 mg/ ml. The positive reaction of the 88 kDa purified protein in western blot analysis with monoclonal antibody against Manduca sexta (tobacco hornworm) chitinase protein, confirms its identity to be a chitinase. The obtained results indicate the ability of using the pink borer chitinase protein as a toxic biological reagent for controlling the insect.

Key word: *insect chitinases - Manduca Sexta (tobacco hornworm) - chitin.*

INTODUCTION

Billions of dollars are lost every year due to inadequate control of pests. It is evident that the world food supply depends on effective protection of crops, animals and humans from pests. The chemical control of pests was efficacious and attractive during the forties and fifties; however the adverse effects of such chemicals quickly began to show accumulation of chemical pesticides in soil, water, air, agricultural products and animals, and the development of resistance in target organisms which necessitated the use of more selective and environmentally acceptable agents for pest control. The critical need for safe and effective alternatives to chemical pesticides has stimulated considerable interest in using pathogens and predators as biological control agents for agriculturally and medically important pests. For instance, destruxins of the entomopathogenic fungus (*Metarhizium anisopliae*) are being used to perturb crucial biochemical targets related to growth and development in insects such as cuticle