ISOLATION OF THE ASTACIN-LIKE METALLOPROTEASE CODING GENE (ASTL) AND ASSESSMENT OF ITS INSECTICIDAL ACTIVITY

By

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THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY
In

Agricultural Sciences
(Genetics)

Department of Genetics
Faculty of Agriculture
Cairo University
EGYPT

2020

Format reviewer **Vice Dean of Graduate studies**

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Title of Thesis: Isolation of the Astacin-like metalloprotease coding gene (ASTL) and assessment of its insecticidal activity.
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ABSTRACT

Biopesticides are considered ecofriendly alternatives to the chemical pesticides for controlling agricultural pests. Spider venoms are a cocktail of thousand peptide toxins, and could have insecticidal activity against a wide range of insect orders. The present investigation was conducted with the main goal of assessing the efficacy of spider-based toxin peptides for pest control and to determine the probability of using these toxins to produce a new biopesticide that is friendly to the environment. Twenty five fragments from genes coding for putative toxins were amplified from five spider species. Sequence analysis revealed that out of the twenty five sequences, only one fragment showed high similarity with the astacin-like metalloprotease toxin gene. Using the barcoding technology, the spider species of this fragment was identified as *Hasarius adansoni*. The full length of *astl* cDNA was cloned from this spider species using the RACE technology. Sequencing of the cloned Ha-*astl* cDNA proved that its full length includes 802 bp with 714bp open reading frame encoding for 238 amino acids.The predicted molecular weight of the encoded protein is 27.33 kDa with two disulfide bridges between cysteine residues at positions 87-238 and 108-128 and three zinc binding sites at histidine residues at positions 136,140 and 146. A 486bp of the catalytic domain was cloned and expressed by the yeast expression system *Pichia pastoris*. In an attempt to enhance the insecticidal activity of the toxic protein, the astacin like metalloprotease toxin was fused to the GNA snowdrop lectin in the same frame and expressed in *Pichia pastoris*. The insecticidal activity of the Ha-astl and the Ha-astl/GNA proteins was determined towards two species of agricultural insects from two different orders, cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) and rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). The bioassay was performed using three concentrations (100,500 and 1000 µg/ml) for four days for *S. littoralis* and 14 days for *S. oryzae*. In *S. littoralis*, the highest insecticidal effect was recorded for the larvae fed for 4 days on the fused protein at the highest concentration (1000 µg/ml). The mortality

percentages were 78.6%± 4.16 and 71.66% ±3.51for first and second larval instars, respectively. This was followed by the larvae fed on astl protein after four days as 69.3%±2.51 and 65%±2.64 mortality for the first and second larval instars, respectively. In addition , growth retardation and different types of larvae abnormalities were noticed. While in *S. oryzae* the mortality ratio induced by the Ha-astl protein was 46.6%±0.5, 48%±1.7 and 64%±3,0 for the three concentrations 100, 500, 1000 µg/ml, respectively. In addition, lower mortality ratios were observed for the adults treated with the three fused protein (Ha-astl/GNA) concentrations ,i.e., 41.3%±2.0, 42.6%±2.8 and 49.3%±2.0, respectively.

Key words: Zinc metalloproteases , astl , GNA , Fusion technology. Insect control, *Spodoptera littoralis*, *Sitophilus oryzae*.