Entomotoxic Properties of Chitin-binding Mistletoe (*Viscum album* L.) Protein Against Lepidoptera Pests

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Lepidoptera are the most diverse group of animals in the world (>160,000 species) after the Coleoptera, and they are practically all herbivores. Among the polyphagous Lepidoptera insects the noctuid (Noctuidae) family is one of the largest in the order and includes the most devastating pests on the planet. Rustic Shoulder-knot (*Apamea sordens* Hufn.) and Turnip Moth (*Agrotis segetum* Schiff.) are found in all the major crops and during the immense reproduction cause sowing damage, as well as, are responsible for significant post harvest loss of agricultural production. The distribution of species from these two genera covers all the continents.

Intensive use of agrichemicals for the pest control results in increased pest resistance and subsequent growth of pesticides applied to the fields. Due to the environmental concerns of pesticide use and limited list of effective alternatives it is therefore urgent to develop novel biopesticides from plants and other natural sources that have low mammalian and environmental toxicity for crops, fruits and ornamentals. In this regard, plant agglutinins (lectins), which represent the plant natural defense agents, are promising candidates.

In the present work we isolated and characterized chitin-binding lectin (MChbL) from the European mistletoe (*Viscum album* L.) and determined its toxic effects against *A. sordens* and *A. segetum* (Lepidoptera: Noctuidae) larvae. European mistletoe is considered to be a toxic plant, and its content of toxic lectins lends support to this. Poison centers report toxicity of the whole plant, but especially the berries. MChbL interacted exclusively with chitin and did not react with simple sugars and the oligosaccharides carrying GlyNAc residues. The determined N-terminal amino acid sequence of MChbL showed a high sequence similarity to deduced amino acid sequences of thaumatin protein family. N-terminal amino acid sequencing of MChbL showed homology to osmotin-like protein from *Hevea brasilienisis* and α-amylase/trypsin inhibitor from *Zea mays* with 60% homology between them. MChbL showed anti-nutritive effects against *A. sordens* and *A. segetum* larvae at different stages of development. MChbL produced 40% mortality of larvae when incorporated into an artificial diet at a level of 0.001% (w/w). The rate of adults successfully emerging from pupae fed on MChbL was 33% and 5%. MChbL affected larval gut proteolytic enzymes by decreasing of total midgut protease activity. FITC-labeled casein fluorescence polarization showed 60% decrease of total protease activity of the midgut preparations when incubated with MChbL at a concentration of 0.25μg/μl. MChbL showed no inhibition towards bovin trypsin, indicating the possible digestibility of MChbL by mammalian gut enzymes.

The results obtained demonstrate that mistletoe lectins have obvious anti-nutritive effects on *A. sordens* and *A. segetum* larvae. Apparently, proteinase inhibitory activity and perceptible resistance to proteolytic degradation by the insect digestive enzymes are basic prerequisites for MChbL to exert their deleterious effects on insects. Possible implication of mistletoe chitin-binding lectins as potential entomotoxic biopecticides for the control of polyphagous herbivore Lepidoptera pests is discussed.