**Title:**  Insecticidal properties of honeybee chitosan derivatives.

**Author:** Najmeh Sahebzadeh, Mansour Ghaffari-Moghaddam, and Syed-Kazem Sabbagh; Faculty of Agricu;ture, University of Zabol, Iran

**Email:** najmeh.sahebzadeh@gmail.com; n.sahebzadeh@uoz.ac.ir

**Date:** October 2021

**Introduction**

Considering the lack of effective chemical toxins for principled control of plant pests in farms and greenhouses, introducing new bio-based components is presumably a suitable solution for sustainable management of plant pests to save agricultural products. Chitosan and its derivatives have received attention as alternatives to pesticides in agriculture. Insects are a good source for chitosan isolation. In the present study, chitosan was obtained from honey bee (*Apis mellifera* L.; Hymenoptera, Apidae) and dwarf grasshopper (*Chrotogonus trachypterus* Blanchard; Orthoptera, Pyrgomorphidae), and the insecticidal activities of N-alkyl and O-acyl chitosan derivatives were assessed to control some plant pests including warehouse and greenhouse pests (including three aphid species (cabbage aphid, oleander aphid, and spring green aphid), three Coleopteran species (cowpea weevil, rice weevil, and flour beetle), and two mite (two-spotted spider mite and varroa mites) species).

**Methods**

To extract the chitin, insect sources (honey bees and dwarf grasshoppers) were ground into a powder (>200mm). The insect powders were individually demineralized in 1M HCl, filtered off and washed to pH7.0, and vacuum dried. Then, the powder of honey bees and dwarf grasshoppers was used for deproteinization and depigmentation processes. The deproteinization process was carried out at 80 °C in 1M NaOH solution. Products of the deproteinization step were subtracted by filtration, washed to neutrality, and dried. The samples were decolorized by 1% potassium permanganate. The obtained light chitin was rinsed to reach neutrality and was dried (24h, 60°C). The chitin content (W%) was calculated. To prepare chitosans from honey bees and dwarf grasshoppers, their chitins were individually refluxed in 50% NaOH. The samples were rinsed until neutralization and dried (24h, 50°C). For purification, the chitosan structures were dissolved in 2% acetic acid and re-precipitated in 20% NaOH. The chitosan samples were washed with distilled water to obtain a neutral pH. Chitosan yield and the characterization of chitin and chitosan obtained from honey bees and dwarf grasshoppers were determined. Synthesis of N-alkyl and O-acyl chitosan derivatives was done according to conventional protocols. To determine the mortality of the mentioned pests, bioassay tests were carried out at concentration ranges of 200 to1000mg l-1. Under a complete randomized design, the data of pest mortalites were compared by one-way analysis of variance (ANOVA) using SPSS software (version 21.0). Differences between treatment means were established using Student- Newman-Keuls (SNK) test. Probit analysis was used to calculate different lethal concentrations of the selected derivatives of N-alkyl and O-acyl chitosan. P<0.05 was considered statistically significant.

**Results** The results revealed that the dried weight of extracted chitin from dwarf grasshopper and honey bee was 9.1% and 16%, respectively resulting in the 89% and 90% production of chitosan. Our findings suggest that these insects provide a desirable amount of natural chitin as an alternative supply for crustaceans in hot and dry desert ecosystems and are applicable in different fields including agriculture, biotechnology, pharmaceutics, food industries, etc. O-acyl and N- alkyl derivatives of obtained chitosan showed a considerable effect on the different pests that we studied. A concentration of 1000 mg L-1 of O-heptanoyl-chitosan and N-3 phenyl-butyl-chitosan showed over 50% insecticide activity on all three studied pest groups (aphids, coleopteran, mites). The effect of time was only significant on aphids treated by O-acyl- and N-alkyl- derivatives synthesized from honeybee and dwarf grasshopperʼs chitosan. Furthermore, there was not any significant correlation between the chitosan derivatives of honeybee and dwarf grasshopper which confirms that natural chitosan extracted from insects is beneficial in toxicological studies as well as the integrated management of pests.