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**MOLECULAR AND BIOCHEMICAL STUDIES ON SLUG- PARASITIC
NEMATODES AND THEIR POTENTIAL AS BIOCONTROL AGENT**

A Thesis

Submitted to the Faculty of Science, Ain Shams University

For Ph.D. in Zoology

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Abstract

Terrestrial gastropods are economic pests of horticultural crops, vegetables and nurseries in Egypt. Biological control using slug parasitic nematodes is one of the alternatives of chemical control to avoid the hazards of pesticides. This thesis describes the finding of new species of nematode parasites in *Limax flavus* slug in Egypt. It was identified as *Rhabdetella axei* using molecular (18s r DNA sequencing) technique, however its nucleotide sequencing exhibited 97.7% homology with this species of nematode. and this sequence was deposited in the Gene Bank under accession number (KU881631). Pathogenicity of this nematode was tested against three species of land gastropods, two land snails, *Monacha cartusiana*, *Eobania vermiculata* and one land slug, *Limax flavus*. The Results revealed that the nematode gave highly significant effect against *Monacha cartusiana* snail, whereas the high concentration of nematodes (12000 IJ/3ml) caused mortality rate that reached 80% after 7 days of treatment, but there was no effect on the other two land gastropods, *Eobania vermiculata* and *Limax flavus*. Also, the results revealed that *Rhabdetella axei* can live a dual life (free living and parasitic life) i.e. they could be pathogenic to only adult species of small size gastropods like *Monacha cartusiana* (parasitic life) and they can live necromenically on large size species of land gastropods. Finally it can be concluded that the nematode *Rhabdetella axei* can be used in integrated pest management as a biological control against *Monacha cartusiana* land snail. *Rhabdetella axei* nematode was then used for subsequent experiments to isolate the symbiotic and associated bacteria. Three associated bacteria were isolated from its gut and identified by biochemical and genetic analyses. The Biochemical characterization of the isolated bacteria showed that, these were *Serratia liquifaciens*, *Achromobacter denitrificans* and *Providencia rettgeri*. However, the molecular identification using 16SrRNA gene sequencing technique of these bacteria proved that, the three species are in consistence homology with genus classification but different at species level (*serratia proteamaculans* 568; 100 % and *Providencia vermicola*; 99.8% and *Alcaligenes faecalis*; 100 %). All these three sequences were deposited in the Gene Bank under accession numbers KU881632, KU881633 and KU881634. This is the first report to isolate those bacterial species from such slug- parasitic nematode. The efficiency of the three bacterial species to kill the land gastropod *Eobania vermiculata* was investigated. The results revealed that 50h culture of all bacteria showed high molluscicidal activity against *Eobania vermiculata* snail whereas the highest concentration of *Serratia proteamaculans* 568(21×10^7 CFU), *Providencia vermicola* (22.5×10^7 CFU) and *Alcaligenes faecalis* (25.5×10^7 CFU) caused mortality rate reaching 80%, 86% and 93% respectively after five days of treatment, But 24h culture has no effect on that snail. All bacteria are gram negative related to family Enterobacteriaceae. So the results showed that 50h culture of bacteria secrete toxic metabolites like endotoxins, extracellular proteases and antibiotic compounds during this period and these toxic metabolites have role to cause mortality to snails. So the bacterial species identified in this study are good candidates for biological control of the destructive mollusc pests as *Eobania vermiculata* in Egypt.

Key words: Biological control, nematodes, Land gastropod species, Molecular identification, bacteria, pathogenicity.



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