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Genotoxic studies of selected plant oil extracts on *Rhyzopertha dominica* (Coleoptera: Bostrichidae)

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Abstract

This study was conducted to compare the genotoxic effects of various concentrations of plant oils from *Eruca sativa* (Brassicaceae), *Zingiber officinale* (Zingiberaceae) and *Origanum majorana* (Lamiaceae) to the conventional organophosphate insecticide (Chlorpyrifos) against *Rhyzopertha dominica* Fabricius. The *R. dominica* population was reared for several generations without exposure to any insecticide. Wheat grains were sterilized at 55 °C for 6 h in order to eliminate any hidden infestation, treated with serial dilutions of Chlorpyrifos and plant oil extracts, and subsequently fed to *R. dominica* for 1, 2, 3, 6 and 8 days. The results indicated that the LC_{50} values of oils from *E. sativa*, *Z. officinale* and *O. Majorana* were 0.14, 0.23 and 0.32%, respectively, after 2 days. Genetic variations in DNA fragments after treatment with LC_{50} and LC_{25} concentrations of *E. sativa*, *Z. officinale* and *O. majorana* were detected by RAPD-PCR analysis using five primers. The results exhibited distinct DNA polymorphisms or alterations in DNA bands. These alterations varied depending on the substance being examined. Chlorpyrifos causes the highest level of DNA alterations (based on the appearance and disappearance DNA bands) followed by *E. sativa*, *Z. officinale* and *O. majorana*. These results were in direct correlation with the differences in mortality rates between extracts. It could be concluded that the plant oil extracts can be used as one of the integrated pest management tools to control *R. dominica* in stored products, as they are safer than chemical insecticides.

Keywords: *Eruca sativa*; *Zingiber officinale*; *Origanum majorana*; Plant oils; RAPD-PCR

1. Introduction

The lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae), is one of the most serious pests of stored grain worldwide due to the quantitative and qualitative losses that it causes [1]. *R. dominica* is a major pest of the stored grains in the world. Insects in immature stages may develop inside the grain. Larvae or adults feeding on grain kernels may leave behind dust, and thin brown shells or a musty odor are often associated with the infestations of this insect [2]. However, residues from synthetic insecticides are a potential hazard for mammals and insect resistance to synthetic insecticides is an ongoing challenge [3]. Therefore, there is a need to search for natural organic sources of insecticide that are available, affordable, less toxic

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to mammals and less detrimental to the environment [4]. Chemical insecticides are effective tools against this pest. However, the extensive use of chemical insecticides may cause such problems as mutation and carcinogenesis in non-target organisms or pest resistance [5]. Thus, it is very important to find other safer compounds that provide protection against pests without harmful effects to the non-target organisms. Recently, new approaches to control stored-product pests using materials of natural origin are being pursued because of their lower environmental hazard risk and mammalian toxicity [6]. Stored product protection methods involve mixing grains with plant oils [7]. However, many studies aiming to estimate the toxicity and mortality of plant essential oils against *R. dominica* used plants belonging to Lamiaceae and another plant families because of their constituents, such as monoterpenoids, sulphur compounds and thiocyanates [8]. Additionally, many reports have concluded that monoterpenoids cause insect mortality by inhibiting acetylcholinesterase enzyme activity [9]. Methylthiobutyl-isothyiocyanate, which is the main bioactive component in *Eruca sativa* (salad rocket), has high toxicity against insects and low mammalian toxicity compared to other active ITCs [10]. The most toxic essential oils were extracted from *Origanum majorana* and *Thymus serpyllu*, which contain Isoprenoids and phenylpropanoids [11]. Additionally, the essential oil contents of *Zingiber officinale* (α-zingibereno, geranial, neral and α-farneseno) are safe to mammals but harmful to insects [12].

Currently, many studies use a specific technique to measure the genotoxic effect of plant products on multiple systems: random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). This technique is used for large-scale analysis of multiple samples for measuring the effects of genotoxic chemicals [13]. RAPD is a PCR-based technique that amplifies random DNA fragments of genomic DNA with single short primers with arbitrary nucleotide sequences under low annealing conditions [14] and [15]. The RAPD-PCR technique was used recently to check the genotoxicity of xenobiotic agents in biological systems by studying genomic DNA damage and mutation [16–18]. Polymorphisms are detected by RAPD profiles through disappearance of a normal band and appearance of a new band in comparing RAPD profiles [19,20].

The object of this study is to evaluate the toxic effect, as well as genotoxic hazards, of three plant oils and a recommended insecticide (Chlorpyrifos) against *R. dominica* adults using RAPD-PCR.

2. Materials and methods

2.1. Plant material

Wild-grown plants were collected in the Sinai region. The plant materials were collected during 2015 and cleaned, dried, ground into a powder and kept in tightly closed containers under laboratory conditions. Natural herbs from different families were used; Salad rocket – *E. sativa* (Brassicaceae), Ginger – *Z. officinale* (Zingiberaceae) and Marjoram – *O. majorana* (Lamiaceae).

2.2. Preparation of the plant oil extracts

Plant samples (100 g from each plant) were subjected to hydro-distillation for 2 h using the Clevenger apparatus according to the method of Clevenger [21]. Oil extracts were stored in clean dark glass bottles at 4 °C for the experimental trials.

2.3. Insecticide

Chlorpyrifos was obtained from local nursery in Saudi Arabia and its chemical purity was 99.8%. $^{13}C_2$-^{15}N-Chlorpyrifos.

2.4. Chemicals

All chemicals used in the present study were obtained from Sigma, except the following: Taq DNA polymerase (Promega, USA), dNTPs (Boehringer Mannheim), DNA extraction reagents, agarose gel (Qiagen), oligonucleotides as random primers (Operon Tech. Inc., USA), DNA Maker for agarose gel electrophoresis (Gibco BRL) and loading dye solution (Fermentas, Lithuania).

2.5. Tested insect and toxicological assay

R. dominica adults used in this study were reared for several generations in the Grains and Stored Product Pests Department, Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation in Dokki, Giza-Egypt.

The insects used in this study were reared for several generations without exposure to any insecticide. Wheat grains given as food for the insect pests were sterilized at 55 °C for 6 h in order to eliminate any hidden infestation, then mixed with serial concentrations of *E. sativa*, *Z. officinale* and *O. majorana* oils (1.0, 0.5, 0.25, 0.125 and 0.0625%, w/w) or Chlorpyrifos (0.1, 0.08, 0.06, 0.04 and 0.02%). The treated wheat

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