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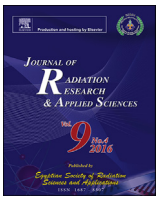

Enhancing the efficacy of entomopathogenic nematodes by gamma radiation in controlling *Spodoptera littoralis* larvae

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ABSTRACT

Nowadays, finding a safe control program is the aim of all researchers. The goal of this work is to investigate the effect of gamma radiation on the Entomopathogenic nematodes, *Steinernema scapterisci* and *Heterorhabditis bacteriophora* Poinar (HP_{88}) efficacy were tested against larvae of cotton leaf worm, *Spodoptera littoralis* (Boisd.) under laboratory conditions. Results showed that 2 Gy irradiated *S. scapterisci* and *H. bacteriophora* were substantially effective in controlling *S. littoralis* larvae, while *H. bacteriophora* was more potent in controlling insect larvae. The results revealed that total protein concentration was significantly decreased ($P < 0.05$) after treatment with normal or irradiated *H. bacteriophora* or *S. scapterisci*. In addition, larvae infected with normal *S. scapterisci* or *H. bacteriophora* showed a significant elevation in phenoloxidase activity and represented significant reduce after treatment with 2 Gy irradiated *S. scapterisci* or *H. bacteriophora* as compared to control group. Also, lysozyme activity was significantly decreased after treatment with irradiated *H. bacteriophora*, but there was no significance with irradiated *S. scapterisci*, when compared with control. LDH activity was significantly high ($p<0.05$) in the haemolymph of larvae treated with normal or irradiated *H. bacteriophora* or *S. scapterisci*, as compared to control group. Furthermore among all treatments, 2 Gy irradiated *H. bacteriophora* was the most potent and efficient in the biomarkers changes. Therefore, it could be concluded that 2 Gy irradiated *S. scapterisci* and *H. bacteriophora* can serve within an integrated pest management (IPM) program in an agroecosystem.

1. Introduction

The Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is one of the most destructive phytophagous insect pests in Egypt, not only to cotton but also to other field crops and vegetables (Shairra & Nouh, 2014). The pest causes serious and considerable economic losses to many crops in both greenhouses and open fields (Abd El-Razik & Mostafa, 2013). Microbial insecticides as biological entomopatogenic agents are valuable. They can be used as alternatives to chemical control where they leave no harmful chemical residues in the environment behind and without inducing resistance in insect hosts (Evans, 1999).

The entomopathogenic nematodes (EPNs) that belong to the families; Steinernematidae and Heterorhabditidae, associated with their symbiotic bacteria *Xenorhabdus*, and *Photorhabdus*, respectively, have been used commercially produced as biocontrol agents of economic insect pests (Gaugler & Kaya, 1990, pp. 365–396). So, EPNs seemed to be the most appropriate weapons for controlling this serious pest.

Insects possess a well-developed innate immune system for protection against pathogens, which exhibited both as cellular and humoral reactions. In cellular response, the pathogens are phagocytosed, nodulated or encapsulated by haemocytes. The humoral response including melanisation, clotting and the production of antimicrobial peptides, either induced (e.g. cecropins) or constitutive (e.g. lysozyme) (Lavine & Strand, 2002). In addition, humoral reactions include activation of enzymic cascades which organize coagulation and melanization of haemolymph (i.e. phenoloxidase), and production of reactive oxygen (ROS) (Mavrouli, Tsakas,

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Theodorou, Lampropoulou, & Marmaras, 2005). Phenoloxidase is a key factor in the insect immune system with a vital role in melanin synthesis, which is responsible for recognition, encapsulation and coagulation of foreign particles in the haemolymph. As well as, melanization and coagulation processes (Cotter, Hails, Cory, & Wilson, 2004).

The use of irradiation technique as a physical control method is cheaper, safe and can integrate with other best control methods. Many studies were done on the activation of entomopathogenic nematodes by gamma radiation (Sayed, 2011; Salem, Hussein, Hafez, Hussein, & Sayed, 2014; Sayed, Khidr, & Moustafa, 2015) on *Steinernema carpocapsae*.

Therefore, this study aimed to evaluate the virulence of gamma irradiated *Steinernema scapterisci* and *Heterorhabditis bacteriophora* against the cotton leaf worm, *S. littoralis* larvae and investigate the associated biochemical changes.

2. Materials and methods

2.1. Breading of the cotton leaf worm (Spodoptera littoralis)

The culture of the cotton leaf worm, *S. littoralis* used in this study, was obtained from a standard laboratory colony, reared at cotton leaf worm Department, Plant Protection Research Institute; Agricultural Research Center (ARC), Giza, Egypt on castor bean leaves and maintained under laboratory conditions of 27 ± 2 °C and R.H. 70 ± 5% according to the method described by El-Defrawi, Toppozada, Mansour, and Zeid (1964).

2.2. Nematodes

Imported nematode species, *Steinernema scapterisci* (*S. scapterisci*) and *Heterorhabditis bacteriophora* Poinar (HP_{88}) (*H. bacteriophora*) were supplied by Dr. El-Sadawy, National Research Centre, Dokki, Giza, Egypt. For nematodes mass culturing, the last instar larvae of the greater wax moth, *Galleria mellonella* were used as hosts according to Shamseldean, Ibrahim, Zohdi, Shairra, and Ayaad (2008).

2.3. Irradiation technique

The third infective juveniles (IJs) of *S. scapterisci* and *Hp.* were irradiated by 2Gy using Gamma Cell Irradiation Unit (caesium, Cs^{137} source) located in the National Centre for Radiation Research and Technology (NCRRT). The dose rate calibration determined for gamma radiation at the time of the experiment was 1Gy/2min and 20 sec..

2.4. Virulence of irradiated S. scapterisci and H. bacteriophora on S. littoralis larvae

4th larval instars were exposed to serial concentrations of *S. scapterisci and H. bacteriophora* in a bioassay technique according to Woodering & Kaya (1988). Ten larvae were placed in 100 cm^3 plastic cups lined with filter paper and wetted by nematodes suspensions (10, 20, 40 and 80 IJ/ml/cup). Three replicates of each treatment were conducted. The control filter paper wetted with 1 ml distilled water. Experiments were held in the laboratory under 30 ± 2 °C. Larval mortalities were recorded daily, and the accumulative percent mortality was also calculated. Percentages of mortalities were corrected according to Abbott's formula (Abbott, 1925). Values of LC_{50} using a software package Ldp-line" a copyright by Ehab, M. Bakr, Plant Protection Research Institute, ARC, Giza, Egypt.

The toxicity index (Ti) was calculated using the equation of Sun (1950) as follows:

$$\mathrm{Ti} = \frac{\mathrm{LC_{50}ofA}}{\mathrm{LC_{50}ofB}} \times 100$$

where; A: the most effective material

B: the other tested material

2.5. Biochemical studies

Due to the faster mortality of the treated larvae with the irradiated *S. scapterisci* and *H. bacteriophora*, the haemolymph was collected from the survived larvae according to Abou El-Ghar, Khalil, and Eid (1996) after 24 h. of the treatment with LC_{50} of the tested nematodes. The total protein was colorimetrically determined in the supernatant by the method according to Slater (1986, p. 269), using kits purchased from Biodiagnostic Compo, Dokki, Giza, Egypt.

Phenoloxidase activity was determined according to a modification of Ishaaya (I971), in a reaction mixture consisting of 0.5 ml phosphate buffer (0.1 M, PH 7), 200 µl enzyme solution and 200 µl catechol solution (2%). Prior to the initiation of the reaction, the substrate and other ingredients of the reaction mixture were separately incubated at the optimum temperature of the reaction (25 °C). The enzyme reaction was initiated by adding catechol solution, then after exactly 1 min, the optical density was determined. Zero adjustment was against sample blank. The phenol oxidase activity was determined as O.D. units × 10^{3} at an absorbency of 405 nm.

The lysozyme activity was determined using the turbidity method according to Azambuja, Garcia, Ratcliffe, and Warthen (1991) with slight modifications. The reaction mixture was formed of asuspension of 0.5 mg/ml of heat killed Micrococcus lysodeikticus (Sigma) in 10 mM sodium phosphate buffer (pH 6), added to 100 µl insect homogenate. Incubate for 30 min at 37 °C. The reaction was stopped by the addition of 0.5 ml 0.5 M sodium carbonate, and the absorbance was read at 450 nm. One unit of enzyme activity is defined as the amount of enzyme that decreases the absorbance reading at a rate of 0.001 absorbance units per min per ul sample in one ml of the reaction described.

Lactate dehydrogenase (LDH) activity was estimated using method derived from the formulation recommended by the German Society for clinical chemistry (DGCK, 1972). Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate; NADH is oxidized to NAD in the process. The rate of decrease in NADH is directly proportional to the LDH activity and is determined photometrically. The reaction mixture (consisted of phosphate buffer; 68 mmol/L, pH 7.5, pyruvate 0.73 mmol/L, and 1.1 mmol/ NADH. 100 µL sample) was mixed with 2.5 ml of the reaction mixture that preincubated at 37 °C. Then they were poured into spectrophotometer cuvette, and the initial absorbance was read. The timer was started simultaneously, and the absorbance was read again after 1, 2 and 3 min. Zero adjustment was against air. LDH activity was calculated according to the following equation:

$$\text{LDH activity} = \text{Factor} \times \Delta\ \text{A 340 nm/min}$$

where:

Factor = 4468 (as recommended by the used kit; Randox, United Kingdom).

Δ A = the change in absorbance/min.

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