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Evaluation of the synergistic effect of gamma irradiated *Steinernema scapterisci* and soil depth in controlling *Bactrocera zonata* Saunders (Diptera: Tephritidae)

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A B S T R A C T

The Peach fruit fly, *Bactrocera zonata* (Saunders) is a serious devastating pest in Egypt. This pest spends in soil from full grown larvae till adult emergence. Therefore, the present study was planned to evaluate the pathogenicity of *Steinernema scapterisci* against larvae and 1 day old pupae (at different soil depths), and to investigate the effect of gamma radiation on its virulence. The results revealed that adult emergence percentage decrease as the soil depth and *S. scapterisci* concentration increase. In contrast, the larval mortality increased with *S. scapterisci* concentration increased. In addition, this study showed that gamma irradiation of *S. scapterisci* juveniles with 2Gy increased its virulence against both larvae and pupae, which presented by lower LC_{50} values than unirradiated *S. scapterisci*. Subsequently, it could be concluded that 2Gy irradiated *S. scapterisci* can serve as a bio-tolerated control method for *B. zonata*.
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1. Introduction

Fruits play a vital role for food security and supply chances for commerce and work as well as a significant source of national income for many developing countries. In the Arab countries *Bactrocera zonata* (Saund.) has become one of the most destructive fruit pests that causing regional problems. It has a world-wide reputation because of its several hosts like; citrus, mango, guava, stone-fruit, olives, vegetable crops and many others. The economic damage is due to larvae that feed on the pulp of the fruit, making it soft and ineligible for human. Also, the damage includes not only the direct loss of yield and increased control costs, but also the loss of export markets and/or the cost of establishing and maintaining phytosanitary measures, (Lysandrou, 2009).

Over the years the insecticides have been important for humans to control the population of harmful insects and the chemical insecticides have been used for this purpose in agricultural and horticultural sectors. But due to the ecosystem pollution, the harmful effects of these insecticides on non-target organisms, and the emergence of resistant insects to the insecticides, it is important to find an alternative integrated control method for *B. zonata*. Entomopathogenic nematodes (EPN) (of the genera *Heterorhabditis* and *Steinernema*) are a welcome natural-enemy specific to insects, environmentally safe, compatible with other biological and chemical agents, can explore pests in soil inhabiting and can be commercially produced in large quantities (Glaser, 1931). Prior studies to estimate the pathogenicity of some steinernematid and heterorhabditid nematodes against *B. zonata* propose that they can be used as a biological control agent for it (Fetoh, Abdel Gawad, Shalaby, & Elyme, 2011; Nouh & Hussein, 2014; Rashad, El-Heneidy, Djelouah, Hassan, & Shaira, 2015).

The use of irradiation technique as a physical control method is cheaper, safe and can be integrated with other control methods. The biological influences of ionizing radiation can vary according to the radiation dose obtained. Marples and Collis (2008) suggest that low-radiation dose can encourage favorable effects on activity. Several reports mentioned that *Steinernema carpocapsea* was activated by 2Gy irradiation of the infective juveniles (Salem, Hussein, Hafez, Hussein, & Sayed, 2014; Sayed, 2011; Sayed, Khidr, & Moustafa, 2015).

Therefore the present study was conducted to assess the ability of using unirradiated or irradiated *Steinernema scapterisci* to control

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B. zonata (Diptera: Tephritidae).

2. Materials and methods

2.1. Rearing of the Peach fruit fly, Bactrocera zonata

The original colony of Peach fruit fly, *B. zonata* was obtained from Natural Products Department, National Center for Radiation Research and Technology, Cairo, Egypt. The insect was reared under laboratory conditions at 25±2 °C and 60 ± 5% relative humidity (Zahran, 2013, p. 111).

2.2. Nematodes

Steinernema scapterisci (*S. scapterisci*) was obtained from Biological Control Department, Agriculture Research Center, Giza, Egypt. For nematodes mass culturing, the last instar larvae of the greater wax moth, *Galleria mellonella* were used as hosts according to Glazer and Lewis (2000).

2.3. Irradiation treatment

The third infective juveniles (IJs) of *S. scapterisci* were irradiated with 2Gy using Gamma Cell Irradiation Unit (Caesium, Cs^{137} source) located at the National Center for Radiation Research and Technology (NCRRT) at dose rate 1Gy/2min (Sayed, 2011).

2.4. Virulence of S. scapterisci on B. zonata

2.4.1. For larval stage

Infectivity of unirradiated and irradiated *S. scapterisci* to the full grown larval instars was examined. Fifteen larvae were placed in 100 cm^3 plastic cups lined with sterile sandy soil and wetted by nematodes suspensions (50, 100, 150 and 200 IJ/ml/cup). Mortalities were recorded daily, and the accumulative percent mortality was also calculated.

2.4.2. For pupal stage

Twenty pupae were placed at 2, 4 and 6 cm depth from the surface in plastic cups (100 cm^3) filled with sterile sandy soil treated with the nematodes suspension (200, 400 and 600 IJs/1ml/cup) and covered with plastic lids. Mortalities were calculated as pupae failed to emerge adults.

The aforementioned assays were conducted for both unirradiated and 2Gy irradiated *S. scapterisci* with 2Gy. All experiments were held in the laboratory under 25 ± 2 °C. Five replicates of each treatment were conducted. The control soil wetted with distilled water. Percentages of mortalities were corrected according to Abbott's formula (Abbott, 1925). Values of LC_{50} were calculated using a software package Ldp-line".

$$\text{Corrected mortality} = \frac{\%\ \text{test mortality} - \%\ \text{contrtol mortality}}{100 - \%\ \text{control mortality}} \times 100$$

2.5. Statistical analysis

The data were statistically evaluated by analysis of variance followed by **Duncan's multiple range test** (Duncan, 1955) to compare the different means at 5% using the statistical software program CoStat (1995).

3. Results

3.1. Effect of unirradiated Steinernema scapterisci at different depths on Bactrocera zonata adults treated as 1-d-old pupae

Data in Table 1 showed the mean numbers of emerged *Bactrocera zonata* adults (1-*d*-old pupae) post treatment with different concentrations of unirradiated *Steinernema scapterisci* at different soil depths. The data showed that the emerged adults from both sexes of *B. zonata* were significantly decreased as the concentration of infective juvenile stage (IJs) and the depth increased. Data indicated that the adults emergence from untreated pupae (without nematode) decreased by increasing the sand depth, the mean numbers of emerged male adults decreased from 8.3 to 7.7 and 6.7 adults at 2, 4 and 6 cm sand depth, respectively. The same pattern was observed in female adults, the mean numbers of emerged adults decreased from 11.3 to 10.3 and 8.7 adult in the same previous sand depths. At the 1st sandy soil depth (2 cm) the emerged male decreased from 8.3 adults in the control (without nematode treatment) to 7.7, 4.0 and 2.7 adults in 200, 400 and 600 IJs/ml, respectively. At the same trend in the other both depths (4 and 6 cm) the emerged male adults decreased from 7.7 adults in the control to be 0.3 adults in the depth of 4 cm with the higher concentration of *S. scapterisci* (600 IJs/ml), while at the last depth (6 cm) the adults failed to emerge at all concentrations of nematode in comparison with 6.7 adults in control.

The present result showed that the mean number of emerged female adults at the 1st depth (2 cm) decreased from 11.3 adults in control to 7.7, 5.7 and 4.7 adults at concentration of 200, 400 and 600 IJs/ml, respectively. At the 2nd depth (4 cm) data showed highly decrease in the emerged female adults in all nematode concentrations, the mean number of adults emerged treated with the higher concentration 600 IJs/ml reached 1.7 adults in comparing with 1.7 adults in control. The obtained results in Table 1 showed that the female adults more successful in emerging than male adults in all nematode concentrations at the 3rd depth (6 cm). The mean number of emerged female adults decreased from 8.7 adults in control to 1.3, 1.0 and 0.3 adults in 200, 400 and 600 IJs/ml, respectively.

3.2. Effect of irradiated Steinernema scapterisci at different depths on Bactrocera zonata adults treated as 1-d-old pupae:

Data in Table 2 represented that *B. zonata* pupae were more susceptible to irradiated *S. scapterisci* than the unirradiated at all tested concentrations and all depths. Both male and female adults at 1st depth (2 cm) decreased from (8.3 and 11.3) at control to (1.7 and 3.7), (1.7 and 2.3) and (0.0 and 0.7) by increasing the concentrations of the nematode (200, 400 and 600 IJs/ml), respectively. The same notice was observed in those emerged from the 2nd depth (4 cm) except in the higher concentration (600 IJs/ml) no adult of both sexes succeeded in emergence from the sand soil. The effect was more potent in the latter cases (6 cm), it could be concluded that the irradiated nematode play an important role in the sensitivity of the emerging adults (male and female) from pupae. The emerged adults reduced from 6.7 (male) and 8.7 (female) at control to 0.0 adults in all concentrations of irradiated nematodes.

Table 3 and Fig. (1) recorded the LC_{50} values of unirradiated and irradiated *Steinernema scapterisci* against *Bactrocera zonata* pupae at different soil depth. It was noticed from the obtained data that the higher toxicity was 6 cm and irradiated nematode (123.88 IJs), While the lowest LC_{50} value was (399.47IJs) at 2 cm and unirradiated nematodes (see Fig. 2).

From Table 4 the results showed that the unirradiated and

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