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Susceptibility of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), larvae to un-irradiated and gamma-irradiated entomopathogenic nematodes

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Abstract

Background: The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is an endemic destructive pest for several cultivars in America and recently in Africa and Asia. Due to the development of pesticide resistance as well as environmental contamination, chemical control of the fall armyworm is ineffective. Alternatively, entomopathogenic nematodes (EPNs) provide a successful biological control tool sustainably. This study was designed to estimate the virulence of 2 isolates (*Steinernema carpocapsae* (All) and *Heterorhabditis bacteriophora* (HP88)) on 3rd and 5th larval instars of FAW under laboratory conditions. As well, the effect of gamma radiation (with 2 Gy) on the nematodes' pathogenicity was studied.

Results: The results revealed that *S. frugiperda* larvae were sensitive to the 2 tested nematodes which were more apparent to *S. carpocapsae*. The mortality rates presented a significant elevation with the increase in un-irradiated and irradiated nematode concentrations. The highest recorded mortality for the 3rd and 5th larval instars was 100% after 3 and 4 days of treatment at concentration (80 IJs/ml) irradiated *S. carpocapsae* and the recorded death rate for un-irradiated *S. carpocapsae* was 72.2 and 77.8% for the two treated larval instars, respectively, after 4 days of the treatment with the same concentration. However, *H. bacteriophora* caused mortality of 88.9 and 61.1% at irradiated concentration (80 IJs/ml) and 66.7 and 50% at un-irradiated concentration (80 IJs/ml) for the 3rd and 5th larval instars, respectively, after 6 days of treatment. Based on the LC_{50} values, the 3rd instar larvae was more susceptible than the 5th instar larvae. In addition, juveniles' irradiation increased their virulence.

Conclusions: Laboratory studies indicated that *S. carpocapsae* had a high potency among *S. frugiperda* larvae, especially the irradiated juveniles. Therefore, they have the potential to be developed as a biological control agent for *S. frugiperda* after further field studies.

Keywords: Entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *Spodoptera frugiperda*, *Steinernema carpocapsae*, Gamma radiation, Virulence assays

Background

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a polyphagous lepidopteran pest that feeds on the leaves and stems of about 350 host plant species. It causes significant damage to

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maize, rice, sorghum, sugarcane, as well as various vegetable crops. Due to its wide host range, it is considered one of the most destructive pests that attacks annual harvests in tropical areas (Rwomushana 2019). In 2016, FAW was recorded first in Africa (Goergen et al. 2016). In 2018; Food and Agriculture Organization (FAO) announced it as a quarantine pest worldwide. In 2019, it was recorded in Egypt for the first occurrence in maize fields at the Upper Egypt Governorates (Dahi et al. 2020). The high reproduction rate of the FAW, migration behavior, the capability of high spread and the strong fling ability for oviposition are the main reasons to give it a high economic importance (Prasanna et al. 2018). FAO (2019) announced that maize is the favorite host for FAW among the infested countries, as the loss in the maize crop may reach 70% of the yield.

Resistance against most of the used insecticides was reported in case of the FAW (Zhu et al. 2015). Furthermore, these pesticides pose a threat to humans and other organisms. As well they cause environmental pollution (Carvalho 2017). So, it was essential to develop a safe and eco-friendly effective integrated pest management (IPM) strategy for *S. frugiperda* as the insect larvae are sensitive to entomopathogenic microorganisms "nematodes, bacteria, fungi" (Ríos-Velasco et al. 2010).

Entomopathogenic nematodes (EPNs), like other biological control tools, are prospective and auspicious agents for managing insect pests (Lacey and Georgis 2012). EPNs are parasitic nematodes that live in the soil and belong to the families Steinernematidae and Heterorhabditidae (Bedding 1990). The EPNs mode of action was well known and confirmed in many previous studies (Griffin et al. 2005). EPNs enter the insect through the natural openings on the body "such as the mouth, anus, and spiracles" and then regurgitate them symbiotic bacteria which they carry in their gut (*Xenorhabdus* spp. and *Photorhabdus* spp. in the Steinernematidae and Heterorhabditidae, respectively). Due to the bacteria reproduction and production of several metabolites and toxins, the host insect dies by septicemia or toxemia. The virulence of each EPN differs depending on the EPN type; the host insect species and the host stage (Yan et al. 2020). Many researchers in different countries examined the susceptibility of different EPNs strains among *S. frugiperda* (Lalramnghaki et al. 2021). Like other biological organisms, nematodes could be activated by irradiation with low doses of gamma radiation (Marples and Collis 2008). Using 2 Gy gamma-irradiated EPNs to control different insect pests was earlier reported in several studies (Sayed et al. 2018).

Subsequently, the present study targeted to assess the susceptibility of 3rd and 5th larval instars of *S. frugiperda* to un-irradiated and gamma-irradiated EPNs "*S. carpocapsae* and *H. bacteriophora*."

Methods

Rearing of *Spodoptera frugiperda*

FAW were collected from infested maize fields and laboratory-reared in cotton leaf worm Department, Plant Protection Research Institute; Agricultural Research Centre (ARC), Giza, Egypt. Larvae were fed on fresh castor bean leaves and placed in 20 ml plastic cups at 26 ± 2 °C and 70 ± 5% R.H. Once the pupae matured, they were collected and retained in a plastic container inside a rearing cage (30 × 30 × 30 cm). When the adults emerged, they were fed on 10% sugar solution in a rearing cage. Fresh plant leaves were soaked in water and placed inside the chamber for egg laying. When the larvae had reached the appropriate stages, they were transferred for the experiments.

Nematodes

Steinernema carpocapsae (All) and *Heterorhabditis bacteriophora* (HP88) were previously identified and reared at Biological Control Department, Plant Protection Research Institute; Agricultural Research Centre (ARC), Giza, Egypt. The two strains were cultivated on the last larval instar of the greater wax moth, *Galleria mellonella* L. (Bedding and Akhurst 1975).

Irradiation technique

The 3rd infective juveniles (IJs) of the two EPN species were gamma irradiated with 2 Gy (Sayed et al. 2018) using the Gamma Cell Irradiation Unit located in the National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority. The dose rate used cesium unit (Cs^{137}) was 0.613 rad/sec. at the time of irradiation.

Virulence of un-irradiated and irradiated EPNs on *S. frugiperda* larvae

The bioassay was carried out by Woodering and Kaya (1988) method after EPN 24 h. of irradiation. Different concentrations of un-irradiated and irradiated IJs nematode suspension were prepared 10, 20, 40 and 80 IJs/ml for *S. carpocapsae* and 10, 20, 40, 80 and 160 IJs/ml for *H. bacteriophora*. The control consists of the same volume of sterilized distilled water. The 3rd and 5th larval instars were separately tested against each EPN. Six larvae were placed in 100 cm^3 plastic cups (1 ml) from each nematode concentration was sprayed. Each concentration and the control were replicated 3 times under a controlled condition of 25 ± 2 °C. The mortalities were daily counted, and the accumulative mortalities were calculated. The median mortality values (LC_{50}) for the 3rd and 5th larval instars of *S. frugiperda* treated by the un-irradiated and irradiated EPNs was calculated.

Statistical analysis

Minitab program was used to adjust and analyze the obtained results using ANOVA, followed by Tukey Pairwise Comparisons test to examine the significant differences ($P \leq 0.05$) across the means of the treatments. The Ldp-line® software "copyrighted by Ehab, M. Bakr ([http://www.ehabsoft.com/ldpline), Plant Protection Research Institute, ARC, Giza, Egypt" was used to evaluate the values of LC_{50} of the un-irradiated and irradiated EPNs.

Results

Mortality rates of 3rd instar larval of the FAW by irradiated and un-irradiated *S. carpocapsae* at different intervals are recorded in Table 1. Data showed that larval mortality was in parallel correlation with both *S. carpocapsae* concentration and time of exposure increase. After 1-day of irradiated *S. carpocapsae* treatment, the statistical analysis revealed a non-significant increase ($P \leq 0.05$) in the larval mortality between 80 and 40IJs/ml and between 40 and 20IJs/ml. Also, the obtained mortality rates at concentration of 20IJs/ml were non-significant, raised when compared to that of 10IJs/ml after 2-day of treatment and also, at concentrations of 10 and 40IJs/ml post 3 and 4 days of the treatment. When using un-irradiated *S. carpocapsae,* there was a non-significant elevation ($P \leq 0.05$) in the larval death rate between 80 and 40IJs/ml at all times and between 40 and 20IJs/ml at 2, 3 and 4 days post-treatments. Generally, the highest used concentrations recorded the highest mortality. Irradiated *S. carpocapsae* caused 100% mortality of the 3rd larval instar after 3-day of the treatment with a high concentration used (80 IJs/ml). Although when using un-irradiated *S. carpocapsae*, the highest mortality rate (72.2%) was obtained 4-day post-treatment with 80 IJs/ml.

The results revealed that the 5th instar *S. frugiperda* larvae was susceptible to the irradiated and un-irradiated *S. carpocapsae* (Table 2). The overall death rate showed a significant difference ($P \leq 0.05$) with increasing the nematode concentration and the time of exposure. However, on the first-day of treatment with irradiated *S. carpocapsae*, a non-significant increase in larval mortality was reported when using 80, 40 and 20IJs/ml as

Table 1 Accumulative percentage mortality by irradiated and un-irradiated *Steinernema carpocapsae* on 3rd instar *Spodoptera frugiperda* larvae at different interval time

Conc. (IJs)	Time (days)							
	Irradiated *Steinernema carpocapsae*				Un-irradiated *Steinernema carpocapsae*			
	1	2	3	4	1	2	3	4
Control (0)	0^{C}	0^{D}	0^{D}	0^{C}	0^{C}	0^{C}	0^{D}	0^{D}
10	0^{C}	11.1^{CD}	27.8^{C}	44.4^{B}	0^{C}	$5.6B^{C}$	22.2^{C}	33.3^{C}
20	11.1^{BC}	27.8^{C}	44.4^{BC}	55.6^{AB}	$5.6B^{C}$	16.7^{BC}	33.3^{BC}	44.4^{BC}
40	27.8^{AB}	61.1^{B}	66.7^{B}	77.8^{A}	22.2^{AB}	27.8^{AB}	44.4^{AB}	55.6^{AB}
80	50^{A}	94.4^{A}	100^{A}		27.8^{A}	50^{A}	61.1^{A}	72.2^{A}

Values represent the mean of 3 replicates

Means that do not share a letter in the same column are significantly different (Tukey Pairwise Comparisons) ($P \leq 0.05$)

Table 2 Accumulative percentage mortality by irradiated and un-irradiated *Steinernema carpocapsae* on 5th instar *Spodoptera frugiperda* larvae at different interval time

Conc. (IJs)	Time (days)							
	Irradiated *Steinernema carpocapsae*				Un-irradiated *Steinernema carpocapsae*			
	1	2	3	4	1	2	3	4
Control (0)	0^{B}	0^{C}	0^{D}	0^{D}	0^{C}	0^{B}	0^{C}	0^{D}
10	0^{B}	5.6^{C}	27.8^{C}	38.9^{C}	0^{C}	5.6^{B}	22.2^{BC}	33.3^{C}
20	16.7^{AB}	22.2^{BC}	38.9^{BC}	50^{C}	11.1^{BC}	16.7^{B}	33.3^{AB}	55.7^{B}
40	22.2^{AB}	44.4^{B}	55.6^{B}	72.2^{B}	16.7^{AB}	38.9^{A}	44.4^{AB}	61.1^{AB}
80	38.9^{A}	72.2^{A}	88.9^{A}	100^{A}	27.8^{A}	44.4^{A}	55.6^{A}	77.8^{A}

Values represent the mean of 3 replicates

Means that do not share a letter in the same column are significantly different (Tukey Pairwise Comparisons) ($P \leq 0.05$)

compared to control. While at the 2 and 3 days, the non-significant change was between 40 and 20 IJs/ml and between 20 and 10 IJs/ml at the 4-day post-treatment. The results of using un-irradiated *S. carpocapsae* exposed that there was a non-significant elevation in the mortality percentage when using 40 or 80 IJs/ml. In general, the highest concentration of irradiated and un-irradiated *S. carpocapsae* recorded the highest larval mortality and 100% mortality rate was obtained after 4-day of treatment with irradiated 80 IJs/ml.

Based on the calculated LC_{50} values after 2-day of the treatment, irradiated *S. carpocapsae* was more pathogenic to both 3rd and 5th larval instars that recorded a high pathogenicity than those treated by un-irradiated *S. carpocapsae* (Table 3 and Fig. 1). Moreover, the 3rd instar larvae were more susceptible and showed the lowest resistance ratio to irradiated *S. carpocapsae* than the 5th instar larvae.

Data in Table 4 presented that the percentage mortality of 3rd instar larvae of *S. frugiperda* treated with irradiated and un-irradiated *H. bacteriophora* were increased as the IJs number increased at different interval times, which was non-significant ($P \leq 0.05$) among some IJs concentrations. The recorded percentages of mortality caused by 160 IJs/ml irradiated and un-irradiated *H. bacteriophora* at the different tested times showed non-significant raised than those treated by 80 IJs/ml; however, they were significantly variation recorded when the mortalities compared to other used concentrations. Overall, the highest concentration of irradiated and un-irradiated *H. bacteriophora* (160 IJs/ml) produced the highest accumulative percent mortality. Irradiated *H. bacteriophora* caused 88.9% larval death after 6-day treatment with 160 IJs/ml, while 66.7% mortality rate was obtained when

Table 3 Median lethal concentration (LC_{50}) after 2-day of the different treatments of *Steinernema carpocapsae* on *Spodoptera frugiperda* larvae

Treatments	Instar larvae	LC_{50} (lower–upper)	Pathogenicity index	Resistance ratio	Slope
Irradiated *Steinernema carpocapsae*	3rd	29.152 (26.057–32.636)	100	1.00	3.024
Irradiated *Steinernema carpocapsae*	5th	44.856 (38.983–52.776)	64.990	1.539	2.347
Un-irradiated *Steinernema carpocapsae*	5th	81.215 (62.51–121.88)	35.895	2.786	1.580
Un-irradiated *Steinernema carpocapsae*	3rd	82.05 (63.984–119.793)	35.530	2.815	1.695

Pathogenicity index and resistance ratio compared to irradiated *S. carpocapsae* on 3rd instar larvae

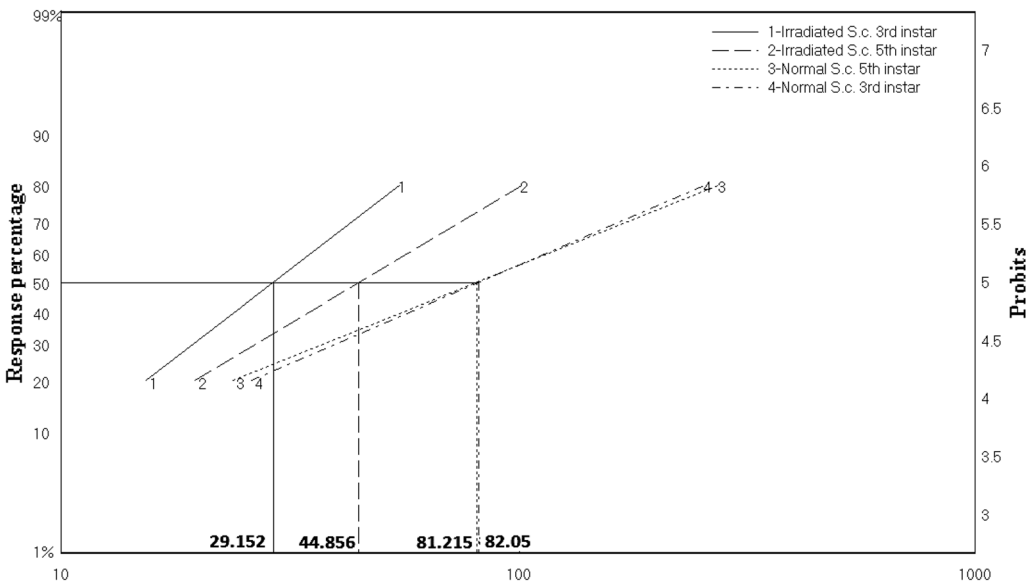

Fig. 1 Median lethal concentration (LC_{50}) after 2-day of the different treatments of *Steinernema carpocapsae* on *Spodoptera frugiperda*

Table 4 Accumulative percentage mortality by irradiated and un-irradiated *Heterorhabditis bacteriophora* on 3rd instar *Spodoptera frugiperda* larvae at different interval time

Conc. (IJs)	Time (days)											
	Irradiated *Heterorhabditis bacteriophora*						Un-irradiated *Heterorhabditis bacteriophora*					
	1	2	3	4	5	6	1	2	3	4	5	6
Control (0)	0^{B}	0^{C}	0^{C}	0^{C}	0^{C}	0^{E}	0^{B}	0^{B}	0^{D}	0^{D}	0^{D}	0^{C}
10	0^{B}	5.6^{BC}	11.1^{CD}	16.7^{BC}	27.8^{BC}	38.9^{D}	0^{B}	0^{B}	5.6^{D}	16.7^{CD}	27.8^{C}	33.3^{B}
20	5.6^{B}	11.1^{BC}	22.2^{BCD}	27.8^{BC}	38.9^{B}	44.4^{CD}	0^{B}	0^{B}	11.1^{CD}	22.2^{BCD}	33.3^{BC}	44.4^{AB}
40	11.1^{AB}	16.7^{BC}	27.8^{BC}	38.9^{B}	44.4^{AB}	61.1^{BC}	5.6^{AB}	16.7^{AB}	22.2^{BC}	33.3^{ABC}	38.9^{ABC}	50^{AB}
80	16.7^{AB}	22.2^{AB}	38.9^{AB}	44.4^{AB}	50^{AB}	66.7^{AB}	11.1^{AB}	27.8^{A}	33.3^{AB}	44.4^{AB}	50^{AB}	55.6^{AB}
160	27.8^{A}	38.9^{A}	55.6^{A}	72.2^{A}	77.8^{A}	88.9^{A}	16.7^{A}	27.8^{A}	44.4^{A}	50^{A}	55.6^{A}	66.7^{A}

Values represent the mean of 3 replicates

Means that do not share a letter in the same column are significantly different (Tukey Pairwise Comparisons) ($P \leq 0.05$)

un-irradiated *H. bacteriophora* at the same concentration and interval time was used.

Data in Table 5 showed the percentage mortality of the 5th instar *S. frugiperda* larvae were susceptible to the irradiated and un-irradiated *H. bacteriophora*. The one-way ANOVA revealed a significant change ($P \leq 0.05$) in the death rate with increasing the irradiated nematode concentration, except between 80 and 160 IJs on all tested days and among 40, 80 and 160 IJs at 1, 2, 5 and 6 days post-treatment. When using un-irradiated *H. bacteriophora*, there was no mortality on the first-day post-treatment, except at 160IJs and after 2-day the larval mortality began at 20 IJs/ml. Generally, the highest concentration of irradiated and un-irradiated *H. bacteriophora* recorded the highest larval mortality rates.

Data in Table 6 displayed the calculated LC_{50} values after 4-day of the treatment with irradiated and un-irradiated *H. bacteriophora*. The results revealed that the 3rd instar larvae were more susceptible to irradiated and un-irradiated *H. bacteriophora* than the 5th instar larvae. As well, the irradiated *H. bacteriophora* was more pathogenic than the un-irradiated for both 3rd and 5th larval instars (Table 6 and Fig. 2).

Discussion

Entomopathogenic nematodes (EPNs) have already proved their efficacy to control under and above ground insect pests (Bhairavi et al. 2021). The infectivity of each of the nematode species/strain for different hosts differs considerably; as well it varies according to the pest habitat and the targeted stage (Bedding et al. 1983).

Previous results showed that FAW larvae were susceptible to both *S. carpocapsae* and *H. bacteriophora* and the mortality rate was in parallel correlation with the EPN concentration increase. This was in accordance with Caccia et al. (2014) who found that increasing the concentrations of *S. diaprepesi* from 50 to 100 IJs increased the *S. frugiperda* larval mortality from 93 to 100% at 144 h post-incubation, respectively.

Table 5 Accumulative percentage mortality by irradiated and un-irradiated *Heterorhabditis bacteriophora* on 5th instar of *Spodoptera frugiperda* larvae at different interval time

Conc. (IJs)	Time (days)											
	Irradiated *Heterorhabditis bacteriophora*						Un-irradiated *Heterorhabditis bacteriophora*					
	1	2	3	4	5	6	1	2	3	4	5	6
Control (0)	0^{B}	0^{B}	0^{C}	0^{D}	0^{C}	0^{C}	0^{A}	0^{A}	0^{B}	0^{C}	0^{C}	0^{B}
10	0^{B}	0^{B}	0^{C}	5.6^{CD}	16.7^{BC}	22.2^{BC}	0^{A}	0^{A}	0^{B}	5.6^{BC}	16.7^{BC}	22.2^{AB}
20	0^{B}	5.6^{B}	16.7^{B}	22.2^{BC}	33.3^{AB}	38.9^{AB}	0^{A}	0^{A}	5.6^{B}	16.7^{ABC}	22.2^{AB}	22.2^{AB}
40	5.6^{AB}	11.1^{AB}	16.7^{B}	27.8^{B}	38.9^{AB}	50^{AB}	0^{A}	5.6^{A}	11.1^{AB}	22.2^{AB}	33.3^{AB}	38.9^{A}
80	11.1^{AB}	16.7^{AB}	27.8^{AB}	33.3^{AB}	44.4^{AB}	55.6^{A}	0^{A}	11.1^{A}	16.7^{AB}	22.2^{AB}	27.8^{AB}	44.4^{A}
160	16.7^{A}	27.8^{A}	38.9^{A}	50^{A}	55.6^{A}	61.1^{A}	5.6^{A}	11.1^{A}	27.8^{A}	33.3^{A}	38.9^{A}	50^{A}

Values represent the mean of 3 replicates

Means that do not share a letter in the same column are significantly different (Tukey Pairwise Comparisons) ($P \leq 0.05$)

Table 6 Median lethal concentration (LC_{50}) after 4-day of the different treatments of *Heterorhabditis bacteriophora* on *Spodoptera frugiperda* larvae

Treatments	Instar larvae	LC_{50} (lower–upper)	Pathogenicity index	Resistance ratio	Slope
Irradiated *Heterorhabditis bacteriophora*	3rd	69.641 (55.124–93.075)	100	1.00	1.178
Un-irradiated *Heterorhabditis bacteriophora*	3rd	139.62 (93.62–275.186)	49.879	2.005	0.846
Irradiated *Heterorhabditis bacteriophora*	5th	161.496 (113.723–280.179)	43.122	2.319	1.075
Un-irradiated *Heterorhabditis bacteriophora*	5th	573.835 (262.748–3293.664)	12.136	8.240	0.766

Pathogenicity index and resistance ratio compared to irradiated *H. bacteriophora* on 3rd instar larvae

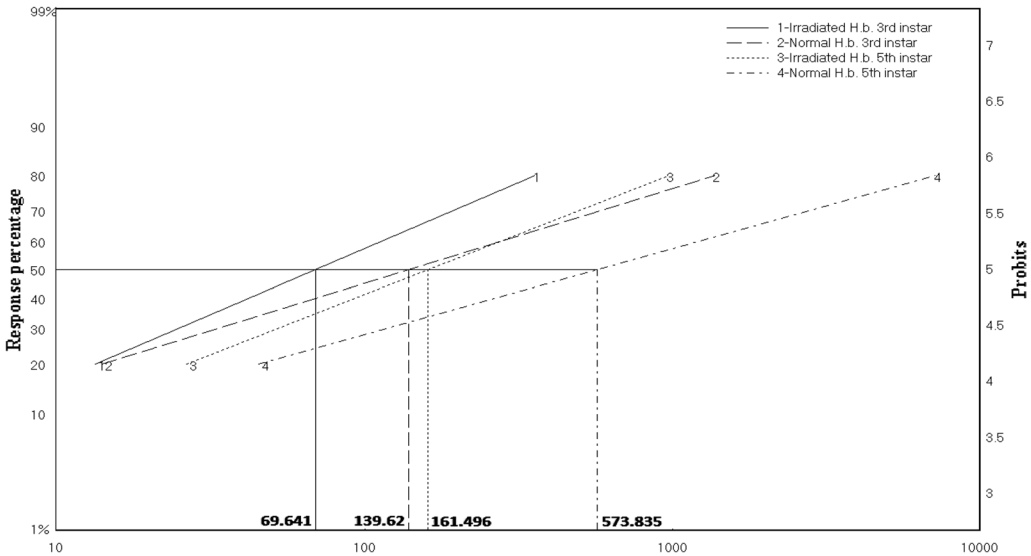

Fig. 2 Median lethal concentration (LC_{50}) after 4-day of the different treatments of *Heterorhabditis bacteriophora* on *Spodoptera frugiperda*

In the present study, the 3rd instar larvae were more susceptible than the 5th instar larvae. This agrees with the finding of Wattanachaiyingcharoen et al. (2021) who found that 2nd larval instar FAW was more susceptible to *H. indica* (AUT 13.2) and *S. siamkayai* (APL 12.3) than the 5th larval instar. Furthermore, Acharya et al. (2020) reported that *H. indica* and *S. carpocapsae* were more pathogenic to younger FAW larvae (1st to 3rd), *S. longicaudum* and *S. arenarium* were more virulent to elder larvae (4th to 6th).

Also, it was noticed that *S. carpocapsae* was more virulent and caused rapid mortality of FAW larvae than *H. bacteriophora*. Some authors have stated that FAW larvae differ in their susceptibility to different EPNs. As Acharya et al. (2020) examined the effect of 7 species of EPN and discovered that only *S. carpocapsae, S. longicaudum, S. arenarium* and *H. indica* were pathogenic to FAW larvae. In addition, Yan et al. (2020) informed that *S. arenarium* was more pathogenic among 3rd and 4th larval instars *S. litura* larvae of than against 2nd instar larvae.

The previous data of LC_{50} values denoted that the 2 Gy gamma-irradiated *S. carpocapsae* and *H. bacteriophora* were more virulent against 3rd and 5th larval instars of FAW. Some researchers studied the pathogenicity of the gamma-irradiated EPN species and they found that gamma-irradiated EPNs were more lethal than un-irradiated ones. For example, Sayed (2011) found that mortality of *Galleria mellonella*, *Corcyra cephalonica* and *Ephestia kuehniella* was faster when treated with irradiated *S. carpocapsae*. Sayed and Shairra (2017) recorded the same results when the 2 Gy gamma radiated *S. scapterisci*-treated *Spodoptera littoralis*. Similarly, Sayed et al. (2018) declared that 2 Gy gamma radiated *S. scapterisci* showed more virulence against both larvae and pupae of *Bactrocera zonata*, which presented by lower LC_{50} values than un-irradiated *S. scapterisci*. That increase in the

pathogenicity may regard in that low doses of gamma radiation activating the symbiotic bacteria to multiply more which increases their toxins.

Conclusions

It was concluded that *Spodoptera frugiperda* larvae were susceptible to the tested EPN isolates. In addition, gamma irradiation of the EPNs increased their pathogenicity. Also, *Steinernema carpocapsae* was more efficient than *Heterorhabditis bacteriophora*. So it could be concluded that 2 Gy gamma-irradiated *S. carpocapsae* may offer an eco-friendly control tool for *S. frugiperda* after several field studies.

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Author contributions
RMS, SSI and HMKHE designed and carried out all experiments, recorded the data, analyzed and interpreted the results. RMS wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data and materials are available if requested.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no conflict of interest.

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