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Efficacy of gamma irradiated Steinernema carpocapsae BA2 against *Corcyra cephalonica and Ephestia kuehniella*

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ABSTRACT

The effect of different doses of gamma radiation (2, 4, 6, 8 and 10 Gy) on the infective juviniles (IJs) of *Steinernema carpocapsae* has been studied. The results showed that there was a positively relationship between the mortality and the storage period of the infective juviniles. The susceptibility of *Corcyra cephalonica* and *Ephestia kuehniella* to unirradiated and 2 and 3 Gy gamma irradiated *Steinernema carpocapsae* were indicated by the daily mortality rate. It was observed that the larval mortality increased when gamma irradiated *Steinernema carpocapsae* was used as the lower time was needed for larval mobidity. In contrast, the reproductive rate of the *Steinernema carpocapsae* IJs was decreased after the irradiation.

KEYWORDS

Entomopathogenic Nematodes, Control, Gamma Radiation, Moth.

INTRODUCTION

The rice moth, *Corcyra cephalonica* (Staint) is one of the most serious pests in grains and stored products. This insect infests rice, sorghum, cashew nuts, dates, raisin and millet. Damages are caused by larvae. The larvae spin tough silken fibers which web together the kernels, frass

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and moulted skins and generally cause quantitative and qualitative losses reduce the germinability of seed stocks **(Hodges, 1979)**.

The caterpillars of the Mediterranean flour moth, *Ephestia kuehniella* are often found feeding on flour, cereals, macaroni, dried fruit, cocoa, nuts, almonds and other dry grain products in food storage areas. Often, dried fruits or mushrooms and even peat or rotting wood may be less eaten. This pest is wide spread in all Mediterranean countries and damage caused by larvae induces troubles in mills. Means of controlling this pest and suppressing population are very urgent in order to avoid loss in major food.

Stored-product pest management has depended heavily on the use of chemical pesticides, but more emphasis is now being given to alternative control tactics **(Subramanyam and Hagstrum, 1995)**. Management of stored-product insects can be targeted at two general areas: preventing and eliminating infestation of the stored-product, and eliminating sources of infestation. Pest management based on identifying these sources of infestation, and targeting pest management is an important component of less chemically intensive management programs. It is also potentially a better fit for biological control than applications targeted at preventing or eliminating infestations within the stored commodity **(Schőller and Flinn, 2000)**.

Recently many authors investigated and published the non classic control methods, such as: Physical, natural and biological to control the pest in field and store.

Entomopathogenic nematodes are lethal endoparasites to insects **(Gaugler and Kaya, 1990; Gaugler, 2002)**. The term of "entomopathogenic nematode" refers to the nematode's ability to quickly kill hosts (1-4 days, depending on nematode and host species) that is facilitated by their mutalistic association with bacteria in the genus *Xenorhabdus* for *Steinernematidae* and *Photorhabdus* for *Heterorhabditidae*. The infective Juvinile (IJs) carry cells of their bacterial symbion in their intestines. After location in a suitable host, the IJs invade it through natural openings (mouth, spiracles, and anus) or thin areas of the host's cuticle **(Peters and Ehlers, 1994)** and penetrate into the host haemocoel. The IJs release their symbiotic bacteria that adhere to the host haemocytes to reproduce within it, kill the host by septicemia and metabolize its tissues. The nematodes start developing and feed on the bacteria and metabolized host tissues. Entomopathogenic nematodes have proven to be effective against a wide variety of insects in different environments **(Hussein, 2004; Sayed, 2008; Sayed 2012):**.

Recently, The irradiation technique is used as a physical control method that is cheaper, safe and more reliable than chemical methods. According to **Marples and Collis (2008)**, Nematodes could be exposed to modest amounts of gamma radiation, just as other biological creatures. Several research have previously examine the use of gamma-irradiated EPNs to manage various insect pests **(Salem *et al*., 2014; Sayed *et al*., 2015; Sayed and Shairra, 2017; Sayed *et al*., 2018; Salem *et al*., 2020 & 2021; Sayed *et al*., 2022 &2023).**

This study was designed to illustrate the effect of gamma radiation on the vitality, virulence and the production of *Steinernema carpocapsae* BA2 against *Corcyra cephalonica* and *Ephestia kuehniella.*

MATERIALS AND METHODS

Insects:. Maintenance of Cultures

Corcyra cephalonica

Laboratory strain of the rice moth, *C. cephalonica* was obtained from the National Research Center, Giza, Egypt. A reference standard colony has been maintained for three generations under constant laboratory conditions of 26±1°C and 70±5% R.H to be used in the present experiments. Newly emerged adults were allowed to mate and oviposit in inverted

jars with screen tops. The eggs were collected with wire mesh in open petri-dishes. The eggs were transferred to breeding jars containing sterilized whole wheat flour mixed with yeast at a ratio of 40 gm to 1 kg flour. Jars were covered with muslin cloth and fixed with a rubber band and each was contained 20 larvae **(Abdalla, 2004)**. The third larval instar was used in our study.

***Ephestia kuehniella* Zeller**

The Mediterranean flour moth, *E. kuehniella* Zeller used in this study were obtained from well established laboratory strain maintained at the Nationl Research Center, Giza, Egypt. The rearing technique for the stock culture was adopted according to the methods of **Locatelli and Limonta (1998)** , where larvae were reared on flour-yeast media in glass jars 470cc (approximately 50 larvae per jar). Each jar was provided with a mixture of crushed, whole-wheat flour and 5% yeast. The wheat grains were previously heated in an oven set at 60°C for at least 6 hours to eliminate infestation by other pests. Mature larvae were collected for the experiments. Adult moths were offered 10% sugar solution. Jars were maintained under laboratory conditions, at 27±2°C and 65±5% R.H. with 12h photo phase. The third larval instar was used in our study.

Entomopathogenic nematodes

The entomopathogenic nematodes (EPN) were originally obtained from the National Research Center (NRC), Pests & Plant Protection Department. *Steinernema carpocapsae* BA2 was used in our experiments which had been isolated from the Egyptian soil and identified by **Hussein and Abou El Soud (2006)**. All nematodes used in this study were reared *in vivo* according to **Glazer and Lewis (2000)**. Newly emerged infective juveniles (IJ's) were harvested and stored at 15°C for two weeks prior to the bioassay. Their virulence was tested before starting up the experiments.

Radiation Experiments

S. carpocapsae BA2 exposed to gamma irradiation using Gamma Cell Irradiation Unit (caesium, Cs^{137} source) located at the National Center for Radiation Research and Technology (NCRRT)). The dose rate was 0.83084 Rad/sec. In the present study, all results were calculated as a Gray unit (Gy); where Gy= 100 rad.

Bioassay Experiments

Effect of gamma radiation on *S. carpocapsae* BA2 nematode:

The nematode suspension (2000IJs/ml) was irradiated with serial doses of gamma ray 0, 2, 4, 6, 8 and 10 Gy. The percent mortality of the juveniles for each dose was calculated after 1,3 and 5 days of irradiation. Five replicates of each treatment were conducted and stored at 5±2°C during the experiment days.

Virulence of irradiated *S. carpocapsae* BA2 on some stored product pests:

The infective juveniles were irradiated with 2 and 3Gy. Larvae of each insect were treated with irradiated juveniles (30 IJs/ 5 larvae). Five replicates of each treatment were conducted, experiments were held in the laboratory under 30±2°C. Cups were checked and larval mortality were observed over 3 days. The control plots received only water. The treatment repeated after 1 and 2 weeks of juveniles' irradiation.

***In vivo* Production of *S. carpocapsae* BA2:**

In this study, normal and gamma irradiated (2Gy) *S. carpocapsae* BA2 were produced *in vivo* using the techniques of **Woodering and Keya (1988).** This technique is useful for laboratory and small field trials but is not practical for large-scale nematodes production. In vivo culture 30 IJs were used to infection of 5 larvae of *C. cephalonica* and *E. kuehniella*. After 24-48 hours the infected larvae

were placed over white trap **(White, 1927)**. The new progeny of IJs were migrated from the cadaver after 10-12 days according to the strain and temperature and collected daily till no progeny. one ml of collection solution were diluted then counted to calculate the reproductive rate, five replicates were done for each sample count. The mean and standered division were calculated. The collected EPNs were washed three times with water mixed with 0.1% formaline 40% to remove the host tissue and non infective stages.

Statistical Analysis

The data were statistically evaluated by analysis of variance (F) followed by Duncan's multiple range test to examine the significant differences between treatment. The 5% level of probability was used in all statistical tests. The statistical software program **CoStat (1995)** was used for all analyses.

RESULTS

Results in Figure (1) showed the destructive effect of gamma radiation on *St. carpocapsae*. The percent mortality gradually increased with increasing the dose, except at the dose 2Gy; it was zero, as compared with the control. The percentages mortality was increased as the dose of irradiation increased, they were 0, 4.36, 9.67 and 13.88 for the doses of 2, 4, 6, 8 and 10 Gy, respectively. The juveniles' mortality increased as the time of treatment increased with irradiation. For example: after irradiation with 10Gy; the percentages mortality were 13.88, 17.78, 60.55 and 65.55 when they were immediately calculated, 1 day, 3 days and 5 days after irradiation, respectively. The lowest percentages mortality of The gamma radiation doses were 2 and 4 Gy.

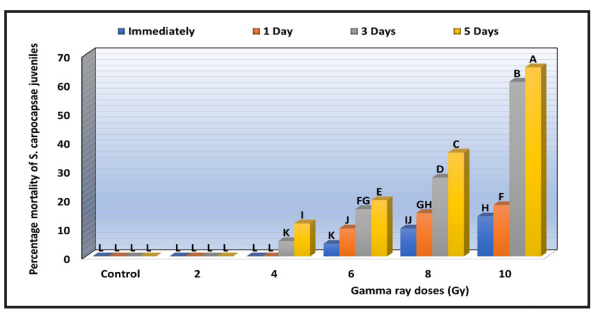

Fig. (1): Effect of gamma radiation doses on Steinernema carpocapsae percentage mortality at different time intervals (days): • Values represent the mean of percentages of 5 replicates for each group.

Data in Tables (1&2) represented the percentage mortality of *Corcera cephalonica* and *Ephestia kuehniella* treated with normal and gamma irradiated *S. carpocapsae*. The obtained results clearly showing that mortality rate was significantly affected with the juveniles' irradiation; the lower time needed for larval morbidity were seen in larvae treated with gamma irradiated *S. carpocapsae*. Treatments after 1 and 2 weeks of the irradiated juveniles' showed similar accumulative mortality inspite of lower response of the larvae at 1st day. Also, the given data show that *S. carpocapsae* irradiated with 3Gy causing lower larval mortality at 1st day of the infection than *S. carpocapsae* irradiated with 2 Gy.

Table (1) : *Effect of gamma irradiated Steinernema carpocapsae on Corcera cephalonica at different intervals time.*

Time (week)	Doses of gamma irradiation (Gy)	Percent mortality (%)		
		Days post infection		
		1 D	2 D	3 D
Control	**0**	0	0	0
Normal *S. carpocapsae*	**0**	40	80	100
immediately	**2**	94.3	100	
	3	80	100	
1	**2**	88.6	100	
	3	77.2	100	
2	**2**	82.9	100	
	3	71.4	100	

• Values represent the mean of percentages of 5 replicates for each group

The data indicated that gamma irradiated *S. carpocapsae* eradicated the larvae of tested pests within 2 days. Infection of *E. kuehniella* larvae with *S. carpocapsae* immediately irradiated with 2Gy caused 100% mortality at 1st day of the infection (Table 2).

Table (2) : *Effect of gamma irradiated Steinernema carpocapsae on Ephestia kuehniella at different intervals time.*

Time (week)	Doses of gamma irradiation (Gy)	Percent mortality (%)		
		Days post infection		
		1 D	2 D	3 D
Control	0	0	0	0
Normal *S. carpocapsae*	0	74	91.4	100
immediately	2	100	-	
	3	80	100	
1	2	94.3	100	
	3	77.2	100	
2	2	88.6	100	
	3	71.4	100	

• *Values represent the mean of percentages of 5 replicates for each group*

Data in Figure (2) indicated that there was a negative relationship between the average production rate of *S. carpocapsae* and gamma irradiation of the juveniles. This means that; the average production of nematode/5 larvae decreased when *S. carpocapsae* were irradiated with 2 Gy.

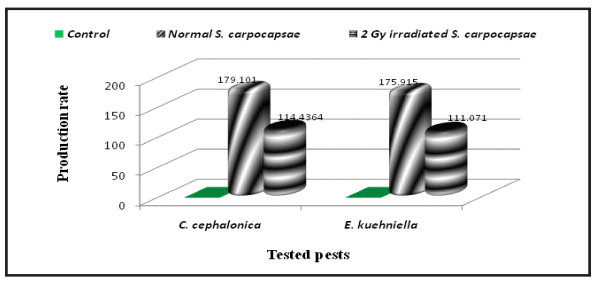

Fig. (2): Reproduction rate of normal and 2Gy gamma irradiated *Steinernema carpocapsae* BA2 in different tested pests.

DISCUSSION

Efficacy of EPNs is dependent on matching the most effictive nematode with the target pest **(Georgis and Gaugler, 1991)**. In this study, the results revealed that the infectivity of *Steinernema carpocapsae* BA2 increased with increasing the concentration against the tested pests (*Corcera cephalonica* and *Ephestia kuehniella*).

From the aforementioned results it obvious that the susceptibility of *E. kuehniella* is more than *C. cephalonica*. This difference in susceptibility may be caused by the nature of larvae as reported by **Abdel-Razek and Abdel-Gawad (2007)** or because of environmental factors that affect the infectivity of tested EPNs as nematode species or strain is perhaps the most critical aspect to achieving an efficacious application **(Gaugler, 1999)**. Also, the exposure time is an important factor in nematodes infection **(El-Bishry *et al*., 2002; El-Khonezy, 2007; Shamseldean *et al*., 2008)**.

Infectivity of steinernematid and heterorhabditid nematodes varied greatly due to some environmental factors or requirements. These environmental requirements could be physical or biotic parameters **(Woodering and Kaya, 1988)**. Some other important factors affect the infectivity for a great extent such as nematode species/strain, exposure period, nematode concentration and period of nematode storage. The degree of infectivity of each of the nematode species/strain for different hosts varied considerably and any species of nematode was not the most infective for all insect species **(Bedding *et al*., 1983)**.

The obtained data revealed that mortality of gamma irradiated *S. carpocapsae* was dose dependant. This agree with the results of **El-Mandarawy *et al*. (2006)** on *S. riboravae, H. bacteriophora* and *H. tayserae*. Also, **Gaugler and Boush (1979)** reported that gamma radiation doses caused harmful effect to *S. carpocapsae* as exposure to 10.000 rad

completely inhibited reproduction, 100.000 rad inhibited maturation and 300.000 rad reduced pathogenicity 50%.

After exposure of *S. carpocapsae* to 2Gy, the pathoginecity increased (for 1 week of irradiatin); showing reduction in time needed to give 100% mortality of *C. cephalonica* and *E. kuehniella* larvae. This result coincide with **Yussef (2006)** who stated that regarding the susceptibility of *Callosobruchus maculatus* to *S. carpocapsae* and gamma irradiation, the lowest doses (2.5, 5 and 10 Gy) were more effective than the higher ones. Moreover, **Sayed and Shairra (2017)** evaluated the effectiveness of 2Gy gamma radiated *S. scapterisci* among *Spodoptera littoralis*, they found the similar outcome. According to **Sayed *et al.* (2018)**, *S. scapterisci* exposed to 2Gy gamma radiation exhibited greater virulence against *Bactrocera zonata* larvae and pupae, as evidenced by lower LC_{50} values compared to unirradiated S. scapterisci. The reason for this rise in pathogenicity could be attributed to low levels of gamma radiation stimulating the symbiotic bacteria to proliferate more, increasing their toxins. The reason of increasing the pathogenicity may be atributed to the effect of low doses of gamma radiation to increase the *X. nematophila* toxins.

CONCLUSION

From the aforementioned disscussion, it could be recommended that gamma irradiated (2Gy) of ***Steinernema carpocapsae*** may contribute to reduction in the application of biological insecticides, which in turn increases the opportunity for natural control of various important stored product pests by entomopathogenic nematodes.

REFERENCES

- **Abdalla, R.S. (2004):** Effect of gamma irradiation and plant substances on certain stored product insects. M.Sc. Thesis, Fac. Agric. Ain Shams Univ.
- **Abdel-Razek, A.S. and Abdel-Gawad, M.M. (2007):** Investigations of efficacy of entomopathogenic nematodes against *Spodoptera littoralis* (Biosd) and *Galleria mellonella* (L.). *Archiv. Phyto. Plant Prot*., 40(6): 414.
- **Bedding, R.A.; Molyneux, A.S. and Akhurst, R.J. (1983):** *Heterorhabditis* spp., *Neoaplectana* spp., *Steinernema kraussei*: interspecific and intraspecific differences in the infectivity for insects. *Exp. Parasitol*., 55: 249.
- **CoStat (1995):** CoStat, user's manual. CoHortSoftware, Minneapolis, MN.
- **El-Bishry, M.H.; El-Assal, F.M. and El-Rahman, R.M.A. (2002):** Factors affecting penetration rate of the entomopathogenic nematodes Heterorhabditis spp. *Egypt. J. Bio. Pest Cont*., 12(1): 25.
- **El-Khonezy, M. (2007):** Purification and charactrization of protease from the infective juveniles of Entomopathogenic nematodes. M.Sc. Thesis, Faculty of Sci., Microbiology Dept. El Mansoura University, Egypt. pp185.
- **El-Mandarawy, M.B.R.; Rizk, S.A. and Abdel Samea, A.A. (2006):** Effect of some entomopathogenic nematodes and gamma irradiation on the Greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). *J. Egypt. Ger. Soc. Zool*., 49(E): 29.
- **Gaugler, R. (1999):** Matching nematodes and insects to achieve optimal field performance. In: Optimal Use of Insecticidal Nematodes in Pest Management. (Ed. Polavarapu, S.), Rutgers university, New Brunswick, New Jersey. PP.9-14.
- **Gaugler, R. (2002):** Entomopathogenic Nematology. CABI Publishing., Wallingford, UK.
- **Gaugler, R. and Boush, G.M. (1979):** effect of gamma radiation on the entomogenous nematode, *Neoaplectana carpocapsae*. *J. Inv. Pathol*., 33: 121.
- **Gaugler, R. and Kaya, H.K., (1990):** Entomopathogenic Nematodes in Biological Control, CRC Press, Boca Raton, Florida, Pp: 365.

- **Georgis, R. and Gaugler, R. (1991):** Predictability in biological control using entomopathogenic nematodes. *J. Econ. Entomol*., 84: 713.
- **Glazer, I. and Lewis, E.E. (2000):** Bioassays for Entomopathogenic Nematodes. In Bioassays for Entomopathogens and Nematodes. Navon A. [Ed.] Kluver Academic Publisher, Holland.
- **Hodges, R.J. (1979):** A review of the biology and control of rice moth ***Corcyra cephalonica*** (Lepidoptera: Galleriidae). Report, Tropical Products Institute No. G. 125, 20pp.
- **Hussein, M.A. (2004):** Utilization of entomopathogenic nematodes for the biological control of some lepidopterous pest entomology (BioControl). Ph.D. Thesis, Fac. Sci., Ain Shams University, Egypt Pp.203.
- **Hussein, M.A. and Abou El-Soud, A.B. (2006):** Isolation and characterization of two Heterorhabditids and one Steinernematid nematodes from Egypt. *Int. J. Nematol*., 16(1):7.
- **Locatelli, D.P. and Limonta,L. (1998):** Development of *Ephestia kuehniella* (Zeller), *Plodia interpunctella* (Hubner) and *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) on kernels and whole meal flours of *Fagopyrum esculentum* (Moench) and *Triticum aestivum* L. *J. Stored Prod. Rese*., 34: 269.
- **Peters, A. and R.U. Ehlers, (1994):** Susceptibility of Leather-jackets (*Tipula paludosa* and *Tipula oleracea*; Tipulidae; Nematocera) to the entomopathogenic nematodes *Steinernema feltiae*. *J. Inv. Pathol*., 63: 163.
- **Salem, H.M.; Hussein, M.A.; Hafez, S.E.; Hussein, M.A. and Sayed, R.M. (2014)**: Ultrastructure Changes in the haemocytes of *Galleria mellonella* larvae treated with gamma irradiated *Steinernema carpocapsae* BA2. *J. Rad. Res. Appl. Sci*., 7(1): 74.
- **Salem, H.M.; Hussein, M.A.; Hafez, S.E.; Hussein, M.A. and Sayed, R.M. (2020)**: Hemocytic studies on the synergistic effect of the entomopathogenic nematode species, *Steinernema carpocapsae* and gamma radiation on the greater wax moth, *Galleria mellonella* (L.) larvae. *Egy. J. Biol. Pest Cont*., 30: 48.
- **Salem, H.M.; Hussein, M.A.; Hafez, S.E.; Hussein, M.A. and Sayed, R.M. (2021):** Influence of Gamma Irradiated *Steinernema carpocapsae* on Some Physiological Aspects of *Galleria mellonella* Larvae. *Biol. Bull*., 48(2): 165.
- **Sayed, R.M. and Shairra, S.A. (2017)**: Enhancing the Efficacy of Entomopathogenic nematodes by Gamma Radiation in Controlling *Spodoptera littoralis* larvae. *J. Rad. Res. Appl. Sci*., 10(2): 97.
- **Sayed, R.M.; El sayed, T.S.; Rizk, S.A. (2023):** Assessing the efficacy of un and gamma-irradiated entomopathogenic nematode, *Steinernema carpocapsae* as an eco-friendly approach to control *Agrotis ipsilon* larvae. *Int. J. Trop. Insect Sci*., 43: 609.
- **Sayed, R.M.; Ibrahim, S.S. and El-Gepaly, H.M.K.H. (2022):** Susceptibility of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), larvae to un-irradiated and gamma-irradiated entomopathogenic nematodes. *Egy. J. Biol. Pest Cont.*, 32: 119.
- **Sayed, R.M.; Khidr, A.A. and Hemat Z. Moustafa. (2015)**: Changes in defense mechanism related to controlling *Spodoptera littoralis* larvae by gamma irradiated *Steinernema carpocapsae* BA2. *J. Entomol. Res*., 39 (4): 287.
- **Sayed, R.M.; Zahran, N.F. and Hamza, A.F. (2018)**: Evaluation of the synergistic effect of gamma irradiated *Steinernema scapterisci* and soil depth in controlling *Bactrocera zonata* Saunders (Diptera: Tephritidae). *J. Rad. Res. Appl. Sci*., 11(1): 18.
- **Schőller, M. and Flinn, P.W. (2000):** Parasites and predators. In: Subramanyam, B., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers. Norwell, MA, pp. 229-271.
- **Shamseldean, M.M.; Ibrahim, A.A.; Zohdi, N.M.; Shairra, S.A. and Ayaad, T.H. (2008):** Effect of Egyptian Entomopathogenic nematodes isolates on some economic insect pests. Egyp. *J. Bio. Pest Cont*., 18(1): 81.

- **Subramanyam, B. and Hagstrum, D.W. (1995):** Resistance measurement and management. In: Subramanyam, B., Hagstrum, D.W.(Eds.), Integrated Management of Insects in Stored Products. Marcel Dekker, New York, 331-397.
- **White, C.F. (1927):** A method for obtaining infective larvae from culture. *Science*, 66: 302.
- **Wiesner, A. (1993):** Die Induktion der Immunabwehr eines Insekts (*Galleria mellonella*, Lepidoptera). Durch Synthetische Materialien und Arteigene Haemolymphfaktoren, Berlin.
- **Woodering, J. L. and Kaya, H. K. (1988):** Steinernematid and heterorhabditid nematodes: A handbook of techniques. Arkansas Agricultural Experiment Station Southern Cooperative Bulletin, 331: 430.
- **Yousef, D.M. (2006):** Biological and biochemical studies on the effect of parasitic nematodes, some plant extracts and gamma radiation on *Callosobruchus maculates* (F). M.Sc. Thesis, Fac. Girls, Ain Shams University, Egypt.