Combined effect of gamma radiation and an entomopathogenic nematode on some stored product pests

A Thesis Submitted for the Degree of Ph. D. in Entomology

By

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“FIRST & FORMOST, GRATITUDE AND THANKS ARE DUE TO ALLAH, THE MOST BENEFICENT & MERCIFUL OF ALL”

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<td>Corcyra</td>
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<tr>
<td>De:</td>
<td>Desmosomes</td>
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<td>Dg:</td>
<td>Dense granules</td>
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<td>DN:</td>
<td>Distorted nucleus</td>
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<td>dL:</td>
<td>100ml</td>
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<td>E.:</td>
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<td>Rer:</td>
<td>Rough endoplasmic reticulum</td>
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Pests infesting stored foods are one of the most common economic problems. Stored product pests can have a large economic impact on stored bulk grain and processed commodities (Hagstrum and Flinn, 1995). These insects can survive on small amounts of food that accumulate in inaccessible places, such as cracks and crevices, under perforated floors, and inside machinery, and may move from these refuges into packaged and bulk stored products (Campbell et al., 2004).

The economic importance of stored product pests and health concerns are:

- Stored product pests contaminate food far more than they consume.
- They cause loss in the mass and quality of food.
- They often cause serious damage to stored foods.
- An entire stock may be lost if control measures are not employed.
- Visible damage resulting from their feeding activity will cause food to be refused by consumers. In addition, seeds became invalid for planting.
- The presence of large numbers of pests will cause food to be rejected by consumers.
- The activity of stored product pests may lead to the growth of fungi which may be harmful to consumers.
- Some insects produce benzoquinones (odour chemicals) which may cause persistent disagreeable odour in commodities.
The greater wax moth, *Galleria mellonella* (L.), is one of lepidopterous insect; its larval stage, feeds on wax and pollen stored in combs of active honey bee colonies (*Milam, 1970*). It can also destroy stored honey combs by spinning silk-lined tunnels through the cell wall and over the face of the comb, which prevent the bees to emerge by their abdomen from their cell, so they die by starvation as they unable to escape from their cell. They also eat out a place to spin their cocoons in the soft wood of the combs.

The rice moth, *Corcyra cephalonica* (St.) is one of the most serious pests in grains and stored products. This insect infests rice, sorghum, cashew nuts, dates, raisin and millet (*Hodges, 1979*). Damages are caused by larvae. The larvae spin tough silken fibers which web together the kernels, frass and moulted skins and generally cause quantitative and qualitative losses reduce the germinability of seed stocks (*Senguttuvan et al., 1995*).

The Mediterranean flour moth, *Ephestia kuehniella* (Zell.) is one of the most destructive Lepidopterous pests for wheat flour and also, dried fruits. The caterpillars 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.

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 (Schöller and Flinn, 2000).

The availability, effectiveness, or desirability of chemical pesticides that target insects in these cryptic locations is declining due to changes in government regulation (e.g., Food Quality Protection Act (FQPA), Montreal Protocol), development of resistance (Subramanyam and Hagstrum, 1995), and growing concern about chemical residues, worker safety, and shifting consumer demands that favor the adoption of more environmentally favorable management tools for stored-product pests.

For the chemical pollution, it was necessary to find out more safe alternative methods to control these pests and to be included in integrated pest management (IPM) programs including the use of different methods to minimize its damage with minimum risk to the environment and human health.

Entomopathogenic nematodes are biological insecticide similar to Bacillus thuringiensis – based insecticides. They are lethal endoparasites to insects (Gaugler and Kaya, 1990 and 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 mutualistic association with bacteria in the genus Xenorhabdus for Steinernematidae and Photorhabdus for Heterorhabditidae. The infective Juviniles (IJs) carry cells of
their bacterial symbiont 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. After the bacterial symbionts released into the insect haemocoel, they produce a variety of metabolites including toxins and hydrolytic exoenzymes including lipases, phospholipases, chitinases, phosphatases and proteases that are responsible for the death and bioconversion of the insect cadavers into a nutrient soap that is ideal for nematode growth and reproduction inside the insect cadavers. Proteases represent an important part of the extracellular enzymes produced (Bowen et al., 2003; Cabral et al., 2004 and Marokhazí et al., 2004 & 2007) and play an important role in insect death.

Entomopathogenic nematodes exhibit different search strategies to increase the probability of finding a host. Foraging strategies used by steinernematids vary from an ambush strategy to cruise foraging, with many intermediate types (Lewis et al., 1992 & 1993; Campbell and Gaugler, 1993 & 1997; Grewal et al., 1994 and Campbell and Kaya, 2002). Cruisers move actively in search of hosts while ambushers exhibit more of a "sit and wait strategy". Adoption of a foraging strategy has implications for other aspects of parasite ecology, behavior, physiology, and anatomy and thus influences how parasites interact with hosts (Campbell and Lewis, 2002). So entomopathogenic nematodes have proved to be effective against a wide variety of insects in different environments.
The use of irradiation technique as a physical control method is cheaper, safe and can be used to activate the nematodes.

Insect immune response consists of interactive cellular and humoral actions. The haemocytic mechanisms comprising phagosytosis, nodule formation and cellular encapsulation. The humoral response including melanotic encapsulation and the production of antimicrobial peptides, either induced (e.g. cecropins) or constitutive (e.g. lysozyme). The immediate response against nematodes is encapsulation and against bacteria is phagosytosis, or nodulation in the case of a large load. Also, injury and microbe infection induce the production of antibacterial peptides. However, the details of the response vary with the species of insect and pathogen and their physiological states (Dunphy and Thurston, 1990 and Dushny and Bourchyier, 1992).

Insect haemocytes were identified by a combination of morphological, antigenic, and functional characteristics (Lanot et al., 2001 and Jung et al., 2005). Although haemocytes have similar functions in immunity across all insects, the naming of different haemocytes types varies somewhat among species and taxa (Strand, 2008).

The most common types of haemocytes reported are prohemocytes, granular cells (granulocytes), plasmatocytes, spherule cells (spherulocytes), and oenocytoids. These haemocytes have been described from species in diverse orders including Lepidoptera, Diptera, Orthoptera, Blattaria, Coleoptera, Hymenoptera, Hemiptera, and Collembola (Sonawane and More, 1993 and Joshi and Lambdin, 1996). The other haemocytes
described from Lepidoptera are non adhesive spherule cells, oenocytoids and prohemocytes. Spherule cells have been suggested to transport cuticular components (Sass et al., 1994), while oenocytoids contain cytoplasmic phenoloxidase precursors that likely play a role in melanization of haemolymph (Jiang et al., 1997). Prohemocytes are hypothesized to be stem cells that differentiate into one or more of the aforementioned haemocytes types (Lavine and Strand, 2002).

The high interest in biological means of controlling insects intensifies the need for investigating the response of insects to disease organisms. The haemolymph and haemocytes, in particular offer a readily accessible criterion of this response.

Therefore, it was great interest to investigate the ultra structure response of the haemocytes to entomopathogenic nematode infection.

The objectives of these studies are:

- To study the effect of gamma radiation on entomopathogenic nematode.
- To evaluate the virulence of irradiated entomopathogenic nematode against some stored product pests.
- To investigate the effect of non-irradiated and irradiated entomopathogenic nematodes on some biochemical aspects of the total body tissue homogenate of G. mellonella larvae.
- To study the haemocytes response to entomopathogenic nematode treatment under electron microscope level.
2.1. Effect of entomopathogenic nematodes on some insect pests:

Nasr (1991) reported that *Steinernema feltiae* caused mortality of *Spodoptera littoralis* caterpillar. Hundred percent of (100%) mortality of caterpillar was recorded after 96, 90, 48 and 36 hrs post infection using dosages 50, 100, 200, 500 and 1000 nematodes, respectively.

El-Kifl and Ameen (1991) stated that when *Spodoptera littoralis* were inoculated with *Heterorhabditis heliothidis*, the 4th, 5th and 6th larval instar of the host were the most vulnerable to infection. The inoculums' level, larval stage and exposure period were the main factors affecting larval mortality, 100% mortality occurring after 4 days using 400 Dsj/ml water sprayed on castor leaf.

Mohamed (1995) studied the effect of *Steinernema carpocapsae* irradiated with 2.5, 5, 10, 20, 40, 80 120 and 160 Gy on *Phthorimaea operculella* larvae to. She found that the mortality percent of the host decreased at the high doses of gamma radiation.

Henneberry *et al.* (1995) investigated the susceptibility of the second, third and last stage larvae of pink bollworm (PBW), *Pectinophora gossypiella* to be infected by 3rd infective juveniles of *S. riobravis* Cabanillas, Poinar and Raulston and the Kapow nematode strain of *S. carpocapsae* (Weiser). Third and last instar PBW larvae were more susceptible than second instar larvae, the LC$_{50}$ (1.84 IJs) for last instar PBW larvae treated with *S. riobravis* was significantly less than the LC$_{50}$ (3.07 IJs) for PBW second
instar larvae with *S. carpocapsae*. *S. riobravis* induced higher percentages of larval mortality than *S. carpocapsae* in bioassay containers with 10, 50, or 100 nematodes per 10 PBW larvae and in each case at densities of 1.5 and 10 PBW larvae per bioassay container. Small percentages (4-23) of PBW larvae were found containing infective juvenile nematodes after 1h. exposure to either species.

**Tahir et al. (1995)** investigated the effect of increasing dosages of *S. riobravis, S. carpocapsae* (All) and a *Heterorhabditis sp.* on the last instar larvae of the African bollworm, *Helicoverpa armigera* (Heliothis), and the spotted bollworm of Asia, *Erias vittella*. There was a linear relationship between the dosage of nematodes applied and the number of nematodes established in the two hosts. *H. armigera* was more susceptible to *S. riobravis* than *S. carpocapsae* and *Heterorhabditis sp.* but against *E. vittella*, *Heterorhabditis sp.* was the most effective.

**Shamseldean et al. (1996)** tested the efficacy of four entomopathogenic nematodes (*H. bacteriophora EASD98, H. indicus EAS59, S. riobravi and H. bacteriophora HP88*) against *Spodoptera littoralis*. All the tested nematodes attained almost 100% mortality at 4, 10, and 25°C but at 35°C *H. bacteriophora HP88* achieved 64% mortality.

**Ratnasinghe and Hague (1997)** studied the efficacy of *S. carpocapsae, S. riobravis, S. riobravae and S. feltiae* nematodes against different stages in the life cycle of *Plutella xylostella*. In larvae, the LT$_{50}$ was less than 3 hrs. for all 3 nematodes, where as in *S. carpocapsae* was the most virulent killing for larvae after 6 hrs. exposure. Pre-pupae were very susceptible to nematode treatment and *S. carpocapsae* caused 40% mortality in immature and mature pupae. More infective juveniles of *S. carpocapsae* established in larvae than the other nematodes tested.

**Ben-Yakir et al. (1998)** evaluated the potential of entomopathogenic nematodes in biological control of the
European corn borer (ECB), *Ostrinia nubilalis* (Hubner). *S. carpocapsae* All, *S. carpocapsae* Mexican and *H. bacteriophora* Poínar "PH88" were compared in both dose response assays (5, 50 and 500 infective juveniles [IJ] per Petri dish containing five 5th instar ECB and eggs; 72 hrs. of incubation) and exposure time assays (3, 6 and 8 hrs. of incubation). In the dose response assays the highest rates of ECB killing results from infestation with the Mexican strain of *S. carpocapsae*. While in the exposure time assays there were no significant differences between the killing rates of the three nematode strains. Sweet corn plants (*Zea mays* var. saccharata) grown in a screenhouse, were infected with ECB neonates and 4 days after sprayed with a suspension of the Mexican strain of *S. carpocapsae* (50,000 IJ per plant).

**Shinde and Singh (2000)** studied eight entomopathogenic nematode species/strains, *S. glaseri* (Steiner), *S. carpocapsae* (Weiser), *S. feltiae* (Filipjev), *S. sp. Ecomax strain*, *H. bacteriophora* (Pioner), *Heterorhabditis sp. Ecomax strain*, two locally isolated strains called as JFC and TFC against the late instar larvae of diamond back moth, *Plutella xylostella* (L.). All nematodes were found pathogenic. However, *H. bacteriophora* was considered the most pathogenic tested nematodes.

**Abou El-Sooud et al. (2001)** tested the infectivity of four isolates (BAI, ESI, GF and SA2) belonging to the insect nematode species *H. bacteriophora* against the housefly larvae, *Musca domestica* L. Data revealed that the most infective isolate at a dosage level of 5 IJs/ml per larva was SA2 isolate, causing 40% mortality after 72 hr, followed by ES1, GF and BA1 isolates which caused 32.5, 30.0 and 25.0% mortality after 96 hours, respectively. When SA2 isolate was used at different concentrations against *M. domestica* larvae at different ascending doses, mortality rate was dose-dependent. SA2 isolate was most virulent at 25°C than at 15, 20, 35 and 30°C. The recorded mortality rates at a dose level of 50 IJs/larva were 92, 0, 60, 46 and 36% for the five degrees, respectively. Fly pupae were more resistant than larvae. Pupal mortality was 6, 8, 12, 16, 22 and 26%
at dosage levels of 5, 10, 20, 50, 100 and 200 IJs/pupa, respectively. SA2 isolate by using cotton and fibertex methods caused 100% mortality of housefly adults when treated at 20, 50, 100, 200 IJs/adult after 48 hours of exposure. Also, the same values of mortality were registered when adults were treated with the aforementioned dosages on the filter paper after 96 hrs.

**Shamseldean and Atwa (2001)** evaluated the pathogenicity of some entomopathogenic nematodes (three nematodes were isolated from USA such as *S. glaseri* (N.C.) from North Carolina, *S. riobravis* from Texas and *S. carpocapsae* (All) from Georgia. One was isolated from France such as *S. feltiae* (SN). Two strains were isolated from Egypt, a semiarid region, such as *H. bacteriophora* (EGB2O) and *S. sp.* (EGB4). Two were isolated from the Arabian gulf, an arid region, they both belong to *H. spp.* The strain 0B5 was isolated from the Sultanate of Oman, while the strain EFF was isolated from the United Arab Emirates against the greater wax moth, *G. mellonella*, the Egyptian cotton leafworm, *Spodoptera littoralis*, the greasy cutworm, *Agrotis ipsilon* and the desert locust, *Schistocerca gregaria*. Results indicated that differences in susceptibility between all four tested insect species were mainly due to high levels of internal resistance against invading nematodes rather than defense mechanisms against nematode invasion. In addition, differences in the production of IJs were due to differences in host size, weight and food reserves.

**Farag (2003)** studied the biological control of *Deudorix livia* the most dangerous insect infesting the green pods and *Pseudopachymerus lallemanti* infesting the dry pods of *Acacia farnesiana* by entomopathogenic nematodes *S. feltiae* and *S. carpocapsae*. Both insect species are susceptible to nematodes infection. The mortality rate and the infestation intensity of them increase with increasing the initial dosage of nematodes. The tested nematodes were able to complete their life cycle inside the two insect species.
El-Mandarawy (2005) studied the efficacy of *H. bacteriophora* "HP88" and *H. taysearae*, "SI" and "All" strain *S. carpocapsae*, against *Phthorimaea operculella* larvae and pupae in filter paper and sand barrier assay. Exposure time assays of *H. bacteriophora* were 3, 6, 12, 24 and 36 hrs. from inoculation to larval mortality and nematode production was studied. Results showed that *H. bacteriophora* "HP88" caused the maximum mortality, 60-100, 40-80 and 20-60% after 36 hrs. in the filter paper, sand barrier larval assays and sand barrier pupal assays, respectively. Filter paper assayed larvae showed the lowest LC$_{50}$ value by different nematode species inoculum, while sand barrier gave the highest LC$_{50}$ for the inoculated pupae. The time required to obtain 50% (LT$_{50}$) mortality was decreased by increasing the concentration of different nematode species in each bioassay studied. Mortality of *Phthorimaea operculella* larvae exposed to 6, 12 and 24 hrs., while the reproduction of nematode in larvae showed negative correlation with the time of exposure.

Hussein (2005) determined the virulence of seven strains of entomopathogenic nematodes against 4th instar larvae of the cotton leafworm *Spodoptera littoralis* at two constant temperatures of 15 and 25°C with three concentrations of 100, 50 and 25 IJs/dish. The seven strains showed that not all of entomopathogenic nematodes were pathogenic to *Spodoptera littoralis* larvae, inspite of their ability to invade the larvae at the two different temperatures. At the highest concentration of 100 IJs/dish, three species (*S. kraussei* strain-69, *S. carpocapse* strain NCR, and *S. feltiae* strain ehrovice) where they caused higher mortalities of 100, 100 and 93.3% among *Spodoptera littoralis* larvae respectively than did *S. feltiae* strain Holovousy (60%), *S. kraussei* strain-D (53%), *S. glaseri* (46.7%), and *S. cubanum* (40%). At 25°C, the mortality never reached 100%, where, *S. cubanum* achieved the highest (86.7%) mortality while other nematode strains caused mortalities ranged between 33.3 and 73.3%. At lower concentrations of 50 and 25 IJs, the host mortality proportionally decreased for each nematode strain.
Rodríguez et al. (2006) studied the pathogenicity of genus *Steinernema* as biological control agents of stored-product pests by determining their pathogenicity to some of the major stored-product pest species (*P. interpunctella, E. kuehniella, Oryzaephilus surinamensis, Tenebrio molitor, Tribolium castaneum, Trogoderma variabile, Sitophilus oryzae* and *Rhyzopertha dominica*). They found that *S. riobrave* was either the most pathogenic or of similar pathogenicity compared to *S. carpocapsae* and *S. feltiae*. A dose of 10 infective juveniles of *S. riobrave* caused 80% or higher mortality against larvae of *P. interpunctella, E. kuehniella, T. castaneum*, and *O. surinamensis*, pupae of *T. castaneum* and *T. molitor*, and adults of *T. molitor* and the two moth species. All stages of *Trogoderma variabile* exhibited 70% or higher mortality. Adults of *Sitophilus oryzae* and *R. dominica* exhibited low susceptibility with 15% and 35% mortality, respectively.

Lee et al. (2006) evaluated the biological control effect of Korean entomopathogenic nematodes (EPN), *S. carpocapsae Pocheon strain, S. sp. GSNUS-4strain, S. sp. GSNUS-14 strain, S. sp. GSNUS-16 strain, Heterorhabditis sp. GSNUH-2 strain, and Heterorhabditis sp. GSNUH-3 strain* on *Pryeria sinica Moore* (Lepidoptera: Zygaenidae). They found that Nematode species, strain, and concentration influenced infectivity of Korean EPNs against 3rd instar of *Pryeria sinica* larvae and *S. carpocapsae Pocheon* strain was the most effective nematode.

Shoeb et al. (2006) compared the pathogenicity of entomopathogenic nematodes; *S. abbasi* and *H. bacteriophora* to the 4th larval instars of the black cutworm, *Agrotis ipsilon* Hubn., the Egyptian alfalfa weevil, *Hypera brunneipennis* Boh. and the American bollworm, *Helicoverpa armigera* Hb., also to pre-pupae and 3-days old pupae of *A. ipsilon*. Both *S. abbasi* and *H. bacteriophora* were highly pathogenic to the 4th instar larvae of all tested insects. They found that the concentration of 200 IJs of all nematode species/larva caused 93.3% mortality in *H. armigera*
and *A. ipsilon* and 100% in *H. brunneipennis* larvae. The highest percentage mortality among the treated pre-pupae was 100% at the concentrations of 100 and 200 IJs/cm² of soil surface 72 hours post infection. The two nematode species were found to be virulent to 3- days old pupae, causing 100 mortality at concentrations of 100 and 200 IJs/cm².

Rodríguez et al. (2007) evaluated the efficacy of *S. riobrave* on larvae, pupae and adult of *Tribolium castaneum* and *P. interpunctella*. They found that it reduces the survival of both pests and the larval instars were the most susceptible to *S. riobrave* for both insect species with *P. interpunctella* larvae having 99% mortality and *T. castaneum* larvae having 80% mortality.

Salem et al. (2007) investigated that *Pieris rapae* larvae were more susceptible than *S. littoralis* and *Plutella xylostella* larvae to all tested nematodes (*St. carpocapase* All, *S. caprocapsae* S2, *H. indicus* SAA2, *H. bacteriophora* HP88 and *H. S1*). For *Pieris rapae* larvae, *S. carpocapase* All and *S. caprocapsae* S2 were more virulent to the 2nd larval instar than 5th one; but *H. indicus* SAA2 and *H. bacteriophora* HP88 were the most virulent heterorhabditids to 5th larval instar than 2nd one. As for *S. littorals*, *S. carpocapsae* All and *S. carpocapsae* S2 were the most virulent and fastest in action especially against the younger instar larvae; while all *Heterorhabditis sp.* showed valuable efficiency in virulence and time required for killing the tested pest larvae as indicated by values of lethal mortality concentrations and lethal time required. As well, *S. carpocapsae* All and *S. carpocapsae* S2 showed more efficiency in virulence and faster in action to the 2nd and 3rd instar larvae of *Plutella xylostella*. On the other hand, the Egyptian *H. S1* and *H. indiccus* SAA2 were more effective than *H. bacteriophara* HP88 to the 3rd and 4th instar larvae.

Bruck and Walton (2007) determined the susceptibility of *Cydia latiferreana* to three species of entomopathogenic
nematodes (*H. marelatus* Pt. Reyes, *S. carpocapsae* All and *S. kraussei* L137). They found that all three nematode species were infective in laboratory bioassays. Infectivity ranged from 73-100% and the field results were similar to those found in the laboratory.

Soliman (2007) studied the pathogenicity of *H. bacteriophara* (native isolate), and *S. riobravis* Cabanillas to Peach fruit fly, *Bactrocera zonata* (Saund.) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). She decided that both insects were more susceptible to *H. bacteriophara* than *S. riobravis*.

Ibrahim (2007) studied the efficacy of the two nematode strains; *S. riobravae* and *Heterorhabditis sp.* (ISK-2) (Egyptian isolates) on the fourth larval instars of the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Data indicated that *S. riobravae* was more effective than *Heterorhabtidies sp.* (ISK2) and the concentration of 75 IJs /I gave highest mortality percentages compared with the other concentrations tested.

Mahmoud and Osman (2007) determined the efficiency of the entomopathogenic nematode *S. feltiae* Cross N33 against second and third instar larvae and 1, 4 and 6 days old pupae of the peach fruit fly *Bactrocera zonata*. Mortality rates after 3 exposure times ranged from nil to 24%, nil to 40% and 8 to 56% for 2nd instar larvae and 8 to 72%, 28 to 84% and 32 to 88% for 3rd instar larvae, whereas mortality rates of pupae ranged from 4 to 56% for 1 day old pupae, nil to 32% for 4 day old pupae and nil to 20% for 6 days old pupae. Slope, LC$_{20}$, LC$_{50}$, and LC$_{90}$ were estimated. Slope values were 1.25 and 1.44 for 2nd instar and 3rd instar larvae and 1.6, 1.1 and 0.97 for 1, 4 and 6 days old pupae, respectively. Results demonstrated that 3rd instar larvae and 1 day old pupae of *B. zonata* were significantly more susceptible to nematode infection than 2nd instar larvae and 4, 6 days old pupae at all tested concentrations.
Ali et al. (2008) evaluated the susceptibility of three lepidopteran pests, namely, gram pod borer, *Helicoverpa armigera*, greater wax moth, *G. mellonella*, and rice moth, *C. cephalonica*, to two recently described species, *S. masoodi*, *S. seemae*, and three indigenous *S. carpocapsae*, *S. glaseri* and *S. thermophilum* entomopathogenic nematodes (EPN). Among the five species of EPN, *S. masoodi*, *S. seemae* and *S. carpocapsae* were found most pathogenic to *C. cephalonica*, causing mortality within 24 h, followed by *H. armigera* (36, 38 and 48 h, respectively) and *G. mellonella* (30, 36 and 48 h, respectively). The other species of EPN, viz., *S. glaseri* and *S. thermophilum* was the least pathogenic, which killed the larvae of *C. cephalonica* in 29 and 36 h, respectively.

Ibrahim et al. (2009) studied the pathogenicity of four nematode species (*S. glaseri, S. carpocapsae, H. bacteriophora* onand *H. megdis*) on third larval instars of white grub (*Pentodon bispinosos*). They stated that the percentage mortality increased with increasing the nematode concentration.

2.2. Effect of entomopathogene on some biochemical aspects of insect pests:–

Ghally et al. (1988) studied the effect of *S. feltiea* on total protein content and total lipid of treated *Ceratitis capitata*. They found that the total protein of infected insects were higher than control after 4 and 18 hrs. post infection with 100 and 500 nematodes per insect. While the total lipids were lower than the uninfected once after 18 hrs. of infection.

Mohamed (1995) stated that the total proteins, total lipid and total carbohydrate contents of *Phthorimaea operculella* larvae increased after infection with *S. carpocapsae*, while they decreased as a response of infection to gamma irradiated *S. carpocapsae*. 
Xia et al. (2000) studied the effect of *Metarhizium anisopliae* on the acid phosphatase activity of the desert locust, *Schistocerca gregaria*. Results showed that acid phosphatase activity increased in the haemolymph of the 3rd day after inoculation with the entomopathogenic fungus *M. anisopliae var acridum*.

Abdel-Razek et al. (2004) evaluated the effect of *S. carpocapsae* and *H. bacteriophora* on the total protein and total lipid of the red palm weevil, *Rhynchophorus ferrugineus* larvae. They showed a decrease in their amount after the infection with both nematodes.

Serebrov et al. (2006) stated that acid phosphatase increased in *G. mellonella* larvae treated with *Metarhizium anisopliae*.

El-Sadawy et al. (2009) investigated the effect of *H. bacteriophora* on the haemolymph of *Parasarchofaga aegyptica* larvae and *Argas persicus* adult. They found a dramatically declined in total protein and total lipids in both *P. aegyptica* and *A. persicus*.

Shehata (2010) studied the effect of some Egyptian nematodes, *Photorhabdus sp.* and *Xenorhabdus sp.* (*H. indica* (IB), *H. bacteriophora* (4), *H. brevicadus* (1), *S. glaserii* (Sg) and *S. carpocapsae* All (5)) against total protein content of *G. mellonella*. The results showed that *Photorhabdus sp.* (4, 1 & IB) caused higher reduction in total protein than *Xenorhabdus sp.* (5 & Sg), where the activity of tested isolates were ranged as follows: *H. bacteriophora* > *H. brevicadus* > *H. indica* ≥ *S. carpocapsae* All > *S. glaserii*. 
2.3. Effect of entomopathogenic nematodes on protein pattern of insect pests:

Ayaad et al. (2001) studied the effect of 20 and 40 infective juveniles (IJs) of entomopathogenic nematode, *H. bacteriophora* on the haemolymph protein profile of *Parasarcophaga surcoufi*. They found that the electrophoretic haemolymph protein profile was time-dependent as indicated by appearance and disappearance of protein bands. After 40 hrs. post-injection with the nematode all the protein bands were replaced by new ones (probably containing immune protein).

Yussef (2006) stated that the protein bands of *Callosobruchus maculatus* treated with *S. carpocapsae* showed absence of some bands and reduction in the intensity of the others.

El-Sadawy and Abou El-Dobal (2009) studied the changes in protein haemolymph (HL) of engorged female, *Hyalomma dromedarii* infected with *S. sp. SII, S. carpocapsae DD136, H. TWF* and *H. bacteriophora Hp88*. They reported that quantitative and qualitative changes were detected in SDS-PAGE electrophoresis protein separation. Parasitism caused HL protein lyses as shown by changes in number of fractions. *H. bacteriophora Hp88* was the most effective species in engorged female.

Ibrahim et al. (2009) investigated the effect of four nematode species (*S. glaseri, S. carpocapsae, H. bacteriophora* and *H. megdis*) on the haemolymph protein of the third larval instars of white grub, *Pentodon bispinosos*. The nematode infection induced some additional bands, while disappearance of other bands was recorded.

2.4. Identification of insect's haemocytes:

Neuwirth (1973) identified four hemocyte types in the late last larval instar of *G. mellonella*. Plasmatocytoids are round to
spindle shaped cells, 10-20µ long and 5-10µ wide. The cytoplasm contains no distinguishing inclusions. Golgi complexes, rough endoplasmic reticulum and free ribosomes are abundant. Granular hemocytes are oval shaped cells, 10-20µ long and 5-10µ wide. The granules, their most characteristic feature, have a diameter of 0.2µ, a microtubular sub-structure, and are made up of acidic mucosubstances. Lipid droplets may be present in these cells at some stage of development. These cells appear to be phagocytic. Spherule cells are oval shaped, 15-20µ long and 5-10µ wide. The spherules, approximately 2µ in diameter, have a highly ordered substructure and are made up of acidic mucosubstances. Oenocytoids are the largest cells, 20 by 40µ. The cytoplasm contains mostly free ribosomes and microtubules.

Ribeiro and Brehe'lin (2006) mentioned that four main circulating haemocyte types have been found in Drosophila. These are Plasmatocytes, Granular Haemocytes, Oenocytoids and Spherule Cells.

2.4. Effect of entomopathogen on haemocytes of some pests:-

Götz (1986) stated that in Lepidopterous insects plasmatocytes form nodules around masses of bacteria. In these formations, plasmatocytes synthesize numerous desmosomes and contain large amounts of microtubules in their cytoplasm.

Parka et al. (2005) reported that morphological changes of Spodoptera exigua hemocytes after the X. nematophilus infection similar to cell changes during apoptosis (hemolymph septicemia due to the induction of the programmed cell death). At 4-8 hrs. post-infection, the cell membrane blebbing and apoptotic vesicles were observed and the nuclear membrane was broken apart. At 12 hrs. post-infection, the overall cell shape was lost externally. Also, vacuolation of the endoplasmic reticulum, cell swelling, and cell death by colloid-osmotic lyses. This lead to pores created by toxin on macrophage and blood cell plasma membrane increases with toxin concentration, which leads to a rapid cell lysis.
Abd El-Aziz and Awad (2010) found that five types of haemocytes were observed in the fourth larval instar of the black cutworm, *Agrotis ipsilon*: prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherule cells (SPs) and Adipohaemocytes (ADs). Ultrastructural alterations and malformations have been observed in circulating haemocytes of *A. ipsilon* larvae treated with *B. thuringiensis*. 
3.1 Materials
3.1.1 Insects: Maintenance of Cultures
3.1.1.1: Galleria mellonella (Linnaeus):

The strain of the greater wax moth, *G. mellonella* L. was obtained from the National Research Center (NRC), Giza, Egypt and reared according to Hussein (2004). The greater wax moth *G. mellonella* larvae were reared on media developed from Wiesner (1993). This media consists of:

i. 22% corn groats (polenta)
ii. 22% wheat-flour (full corn) or brushed-grain wheat
iii. 11% milk powder (skim-milk)
iv. 11% honey
v. 11% glycerol
vi. 5.5% yeast powder (brewer’s yeast)

The larvae were originally obtained from bee hives and transferred to transparent plastic rearing jars (17 x17 x 27 cm), containing 250gm from the previous prepared media, closed with a lid of muslin for aeration and incubated at 28± 2°C with a photoperiod (L:D) 8:16 and relative humidity 65± 5% in the insect rearing chamber. When larvae grown to the pupal stages and then to the adult moths, a piece (15 x 15cm) of paper tissue was folded and placed in the container to promote egg laying. Eggs were laid on the lid and on paper tissue. These eggs were gently removed and transferred to other rearing jar containing 250gm media,
closed tightly with double muslin layer to prevent the escape of neonatal larvae, and incubated. Add fresh food frequently (1-2) times per week. The fifth larval instar was used in our study.

3.1.1.2 *Corcyra cephalonica* (*Staint)*:

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.

3.1.1.3 *Ephestia kuehniella* (*Zeller)*:

The Mediterranean flour moth, *E. kuehniella* Zeller used in this study were obtained from well established laboratory strain maintained at the National 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 470cm³ (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 hrs. 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 12hrs. photo phase. The third larval instar was used in our study.

3.1.2 *Entomopathogenic nematodes:*

The entomopathogenic nematodes (EPN) were originally obtained from the National Research Center (NRC), Pests & Plant Protection Department. *S. 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.

3.1.3 *Radiation:*

- Irradiation of *S. carpocapsae* BA2 was carried out using Gamma Cell Irradiation Unit (cesium, Cs\textsuperscript{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.

3.2 *Bioassay Experiments:*

3.2.1 *Effect of S. carpocapsae nematode on larval mortality of some stored product pests:*

Larvae of each insect were exposed to serial concentrations of nematodes in a bioassay technique according to *Woodring and Kaya (1988).* Five larvae of *G. mellonella, C. cephalonica* and *E. kuehniella* were placed in 100cm\textsuperscript{3} plastic cups filled with filter paper and moistened with 15% water (v/w). Nematodes suspensions were prepared in serial concentrations of 5, 10, 15,
20, 25 and 30 infective juveniles (IJ/ml/cup. As many as 5 replicates of each treatment were conducted.

Experiments were held in the laboratory under 30±2°C. Control plots received only water. Cups and larval mortality were checked over 4 – 10 days, as well as the accumulative percent mortality was also calculated.

3.2.2 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.

3.2.3 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 of the larvae with the irradiated juveniles repeated after 1 and 2 weeks.

3.2.4 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 & Kaya (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 infect 5
larvae of *G. mellonella, C. cephalonica* and *E. kuehniella*. After 24-48 hrs. 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 daily collected till no progeny. One ml of the collected solution were diluted then counted to calculate the reproductive rate, five replicates were done for each sample count. The mean and standered deviation were calculated. The collected EPNs were washed three times with water mixed with 0.1% formaline to remove the host tissue and non infective stages.

### 3.2.5 Biochemical studies:

Fifth larval instar of *G. mellonella* was exposed to gamma irradiated *S. carpocapsae* BA2 with 2 Gy and stored at -20 °C after 12 and 18hrs. of infection to be used in this study.

The samples were homogenized in a cold glass homogenizer containing saline phosphate buffer, the contents were transferred to a new Eppendorf tube, and centrifuged at 4000 rpm for 10 minutes at room temperature, the supernatant was transferred to a new Eppendorf tube and kept frozen at-20 °C till required (Zaghloul, 2004).

The larvae were divided into 5 groups:
1- Group 1: normal 5th larval instar (control)
2- Group 2: larvae after 12hrs. treatment with normal *S. carpocapsae.*
3- Group 3: larvae after 18hrs. treatment with normal *S. carpocapsae.*
4- Group 4: larvae after 12hrs. treatment with irradiated *S. carpocapsae.*
5- Group 5: larvae after 18hrs. treatment with irradiated *S. carpocapsae.*
3.2.5.1 Determination of total lipids in total body tissue homogenate of *G. mellonella* larvae:

Total lipids were colorimetrically determined by the method according to Van Handel (1985), using kits purchased from (Biodiagnostic Compo, Dokki, Giza, Egypt).

**Principle:**

Lipids react with sulfuric, phosphoric acids and vanillin to form pink colored complex.

**Reagents:**

1. Standard 1000 mg/dL
2. Color Reagent:-
   - Phosphoric acid 14 mol/L
   - Vanillin 10 mmol/L
3. Sulfuric acid Conc.

**Procedure:**

1. The total lipids concentration was estimated in sample by adding 0.025 ml of larval supernatant and 1.0 ml of conc. Sulfuric acid {solution (I)}.
2. A standard total lipid was measured by adding 0.025 ml of standard to 1.0 ml of sulfuric acid conc. {solution (2)}.
3. Then all test tubes were mixed well, cover the tubes with glass bead, and let stand in boiling water-bath for 10 min. then cool and:
4. From the aforementioned solutions, (1) 0.05 ml was pipetted into dry test tubes and 1.5 ml of color reagent was added.
5. From the aforementioned solutions (2) 0.05 ml was pipetted into dry test tubes and 1.5 ml of color reagent was added.
6. 0.05 ml of conc. sulfuric acid and 1.5 ml of color reagent was added as blank test.
All test tubes were mixed well, let stand at room temperature for 30 minutes. in dark. Pour into dry cuvettes. Read the absorbance of the sample \(A_{\text{sample}}\) and standard \(A_{\text{standard}}\) against reagent blank at 545nm.

**Calculation:**

The level of total lipids was calculated using the following equation:

\[
\text{Total Lipids Concentration (mg/dl)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 1000
\]

Where:
- \(A_{\text{sample}}\): Absorbance of sample.
- \(A_{\text{standard}}\): Absorbance of standard.

### 3.2.5.2 Determination of total protein in total body tissue homogenate of *G. mellonella* larvae:

The total protein was colorimetrically determined by the method according to Slater (1986), using kits purchased from Biodiagnostic Compo, Dokki, Giza, Egypt. The method is dependent on Biuret reaction.

**Principle:**

In the presence of an alkaline cupric sulfate, the protein produces a violet color, the intensity of which is proportional to their concentration.

**Reagents:**

1. Standard Albumin 5 g/dL
2. Biuret Reagent:-
   - Cupric sulfate 6 mmol/L
   - Sodium potassium tartrate 21 mmol/L
• Sodium hydroxide 750 mmol/L
• Potassium iodide 6 mmol/L

Procedure:
1. The total protein reaction mixture was determined in sample by taking 0.025 ml of larval supernatant then adding 1.0 ml of working reagent (Biuret Reagent).
2. Standard of total protein mixture was measured by adding 0.025 ml of standard Albumin to 1.0 ml of working reagent (Biuret Reagent).
3. 1 ml of working reagent (Biuret Reagents blank).

Then all test tubes were mixed well, incubate for ten minutes at 37°C or 30 minutes at room temperature. Read the absorbance of the sample \(A_{\text{sample}}\) and standard \(A_{\text{standard}}\) against reagent blank at 550 nm.

Calculation:
The level of total protein was calculated using the following equation:

\[
\text{Total protein Concentration (g/dL)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 5
\]

Where:
\(A_{\text{sample}}\): Absorbance of sample. \(A_{\text{standard}}\): Absorbance of standard.

3.2.5.3 Determination of total carbohydrates in total body tissue homogenate of \textit{G. mellonella} larvae:

Total carbohydrates contents of the total body homogenation were colorimetrically determined according to \textit{Singh and Sinha} (1977), as follows:
Reagent:

**Anthrone reagent:**- Prepared by addition of 72ml Sulfuric acid (B.D.H) to 28ml distilled (dist.) water, while this mixture was still warm, 50mg of anthrone were added with a vigorous shaking.

Procedure:

1. To determine the total carbohydrate content, 0.1 ml of larval supernatant was diluted with 1.9 ml distilled water and from this aqueous sample taken 0.1 ml, which introduced into a test tube and diluted with 1.0 ml distilled water, then treated with 5ml freshly prepared anthrone.

2. Blank was prepared by adding 5ml of anthrone to 1.1ml distilled water.

All test tubes of unknown and blank were placed in boiling water-bath for 10 minutes then it was left to cool for 15 minutes at room temperature with a dark place. Read the absorbance of the sample ($A_{\text{sample}}$) and standard ($A_{\text{standard}}$) against reagent blank at 620nm.

Calculation:

Total carbohydrate concentration (gm/100ml) = $\frac{A_{\text{sample}}}{A_{\text{standard}}} \times n$

Where:-

$A_{\text{sample}}$: Absorbance of the sample multiplies in dilution factor (20).

$A_{\text{standard}}$: Absorbance of standard.

n: concentration of standard.

Preparation of standard curve of total carbohydrate:

The standard curve was prepared by adding 20 mg of
glucose, to 100 ml of distilled water and from further dilutions were prepared serial concentrations of glucose solution as follows:

- 0.1 ml from glucose solution/0.9 ml distilled water.
- 0.2 ml from glucose solution/0.8 ml distilled water.
- 0.3 ml from glucose solution/0.7ml distilled water.
- 0.4 ml from glucose solution/0.6ml distilled water.
- 0.5 ml from glucose solution/0.5ml distilled water.
- 0.6 ml from glucose solution/0.4 ml distilled water.
- 0.7 ml from glucose solution/0.3ml distilled water.

All test tubes were placed in boiling water-bath for 10 minutes, and then it was left to cool for 15 minutes at room temperature with a dark place. Reading was spectrophotometrically recorded at 620nm.

The optical densities are plotted against concentration, thus a curve can be constructed.

3.2.5.4 Determination of urea concentration in total body tissue homogenate of G. mellonella larvae:-

The urea concentration was colorimetrically determined by the method according to Fawcett and Soctt (1960), using kits purchased from Biodiagnostic Compo, Dokki, Giza, Egypt. The method is dependent on Urease-Berthelot method.

**Principle:**

The method is based on the following reaction:

\[ \text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_3 + \text{CO}_2 \]

The ammonium ions formed are measured by the Berthelot reaction. The blue dye indophenol product reaction absorbs light between 530 nm and 560 nm proportional to initial urea concentration.
Reagents:

1. Standard
   - 50 mg/dL
   - 8.3 mmol/L

2. Buffer – Enzyme:
   - Phosphate buffer
     - 50 Mmol/L
   - Urease
     - >10000 u/L

3. Color Reagent:
   - Phenol
     - 100 mmol/L
   - Sodium nitroprussid
     - 0.2 mmol/L

4. Alkaline Reagent:
   - Sodium hydroxide
     - 150 mmol/L
   - Sodium hypochlorite
     - 15 mmol/L

Procedure:

1. The urea concentration was determined in sample by taken 0.01ml of larval supernatant then adding 0.2 ml of reagent 2.
2. Standard of urea concentration was measured by adding 0.01 ml of standard to 0.2 ml of reagent 2.
3. 0.2 ml of reagent 2 was placed as blank.

Then all tubes were mixed well, incubate for 5 minutes at 37°C.

4. Add 1ml of reagent 3 to the all tubes.
5. Add 1ml of reagent 4 to the all tubes.
6. All the tubes were mixed well; incubate for 10 minutes at 37°C.
7. Read the absorbance of the sample ($A_{\text{sample}}$) and standard ($A_{\text{standard}}$) against reagent blank at 550nm.

Calculation:

The urea concentration was calculated using the following equation:
Urea concentration = \[ \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Standard conc.} \]

Where:
- \( A_{\text{sample}} \): Absorbance of sample.
- \( A_{\text{standard}} \): Absorbance of standard.

### 3.2.5.5 Determination of acid phosphatase activity in total body tissue homogenate of *G. mellonella* larvae:

**Principle:**
Phenyl phosphate \( \xrightarrow{\text{Acid phosphatase}} \) phenol + phosphate (pH 4.9)

The liberated phenol is colorimetrically measured in the presence of 4-aminophenazone and potassium ferricyanide activity according to **Kind and King (1954)**.

**Reagents:**
1. Standard phenol 319 umoll L
2. Substrate 20 mmol/L
3. Buffer pH 4.9 50 mmol/L
4. 4-Aminophenazone 50 mmol/L
5. Color Reagent:
   - Potassium ferricyanide 200 mmol/L
   - Enzyme stabilizer (Acetic Acid) 3 mol/L

**Procedure:**
1. Mix equal volumes of reagent 2 and 3 (Working reagent).
2. In the sample tubes add 0.1ml of larval supernatant then adding 0.5 ml of the working reagent.
3. Standard of acid phosphatase was measured by adding 0.1ml of the standard to 0.5 ml of the working reagent.
4. 0.5 ml of the working reagent used as a blank.
5. All tubes were incubated at 37°C for 30 minutes
6. Add 0.5 ml of reagent 4 and mix well and then add 0.5 ml of reagent 5.

Mix well and let the tubes stand for 10 min. at room temp. in the dark. Read the absorbance of sample \((A_{\text{sample}})\) and standard \((A_{\text{standard}})\) against reagent blank at 510 nm. The color is stable for one hour.

**Calculation:**

\[
\text{Acid phosphatase activity (U/L)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 10
\]

Where:

\(A_{\text{sample}}\): Absorbance of sample.
\(A_{\text{standard}}\): Absorbance of standard.

### 3.2.5.6 Determination of chitinase activity in total body tissue homogenate of *G. mellonella* larvae:

**Preparation of Insect Chitinase**

The crude enzyme solution was prepared from the *G. mellonella* larva by the method of Kimura (1981). In brief, the larval body was homogenized in 50 mM citric acid - \(\text{Na}_2\text{HPO}_4\) buffer (pH 5.0). The homogenates were centrifuged (at 10,000 g for 20 min) and the supernatant was mixed with ammonium sulfate to give 70% saturation (472 gm/L). After centrifugation, the precipitate was dissolved with the same buffer at the concentration of 1.5 larvae per ml, and the solution thus obtained was stored at -80°C and used in the chitinase assay as the enzyme solution.

**Chitinase Assay:**

**Principle:**

The method is based on the incubation of the enzyme with the colloidal chitin suspension (as a substrate) for 18 hrs. at 37°C
and the librated N-acetylglucoseamine (NAG) determined spectrophotometrically (Rössner, 1991).

**Reagents:**

**3,5-dinitrosalicylic acid reagent:**
Dissolving 1gm of 3,5-dinitrosalicylic acid in 20ml of 2N NaOH (8gm NaOH/100 ml dist. Water) and 50ml of dist. water aid of magnetic stirring. Thirty gm Potassium sodium tartrate was added, and magnetic stirring was continued until a clear solution was obtained. Dist. water was added to bring the final volume to 100 ml.

**Colloidal chitin:**
Colloidal chitin was prepared from chitin powder (Sigma Co.) according to the method described by Reid and Ogrydziak (1981). Five grams of chitin powder suspended in 50 ml of 85% phosphoric acid (H₃PO₄) and stored at 4°C for 24hrs., then blended in 2 liter of distilled water using a warring blender. The suspension was centrifuged. This washing procedure was repeated several times till the colloidal chitin suspension was adjusted to pH 7.0 with (1N) NaOH and re-centrifuged. The colloidal chitin pellet was stored at 4°C until used.

For using: 4% w/v aqueous suspension of the colloidal chitin.

**Citric acid - Na₂HPO₄ buffer (pH 5):**
Add 25.7ml of 0.2 M dibasic sodium phosphate (3.5 gm Na₂HPO₄/ 500ml dist. Water) and 24.3 ml of 0.1 M citric acid (4.8 gm citric acid/500ml dist. Water) (Pearse, 1980).
Procedure:

1. The chitinase activity was estimated in sample by adding 0.3 ml of substrate, 0.6 ml of 50 mM citric acid - Na$_2$HPO$_4$ buffer (pH 5.0) and 0.1 ml of the enzyme sample.
2. Blank tube was prepared by adding 0.4 ml dist. Water and 0.6 ml of 50 mM citric acid - Na$_2$HPO$_4$ buffer (pH 5.0)
3. The all test tubes were incubated for 18 hrs. at 37°C.
4. 1ml dinitrosalicylic acid was added to stopped the reaction.
5. All the tubes were placed in boiling in water bath for 10min.

The solutions were centrifugated and then read the absorbance of the sample supernatant ($A_{\text{sample}}$) against blank at 540nm.

Calculation:

The level of Chitinase was calculated using the following equation:

\[
\text{Chitinase activity (gm N-acetyleglucoseamine /min./ml total body) = } \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \frac{1}{\text{Incubation time (min)}} \times \frac{1}{\text{sample volume (ml.)}}
\]

Where:

$A_{\text{sample}}$: Absorbance of sample.
$A_{\text{standard}}$: Absorbance of standard

Preparation of standard curve of chitinase:

The standard curve was prepared by adding 500 mg of N-acetylglucoseamine (NAG), to 50 ml of distilled water and from further dilutions were prepared serial concentrations of N-acetylglucoseamine solution as follows:

- 0.1 ml from NAG solution/0.9 ml distilled water.
- 0.2 ml from NAG solution/0.8 ml distilled water.
- 0.3 ml from NAG solution/0.7ml distilled water.
• 0.4 ml from NAG solution/0.6 ml distilled water.
• 0.5 ml from NAG solution/0.5 ml distilled water.
• 0.6 ml from NAG solution/0.4 ml distilled water.
• 0.7 ml from NAG solution/0.3 ml distilled water.

1. In the standard tubes add 0.3 ml of substrate (colloidal chitin), 0.6 ml of 50 mM citric acid - Na₂HPO₄ buffer (pH 5.0) and 0.1 of the NAG sample.
2. Blank tube was prepared by adding 0.4 ml dist. Water and 0.6 ml of 50 mM citric acid - Na₂HPO₄ buffer (pH 5.0)
3. The all test tubes were incubated for 18 hrs. at 37°C.
4. 1 ml dinitrosalicylic acid was added to stopped the reaction.
5. All the tubes were placed in boiling in water bath for 10 min.

The solutions were centrifugated and then read the absorbance of the (A_{standard}) against blank at 540 nm. The optical densities are plotted against concentration, thus a curve can be constructed.

3.2.6 Characterization of protein by polyacrylamide gel electrophoresis in total body tissue homogenate of G. mellonella larvae:

All solutions must be sterilized at autoclave except monomer filtrate and store in dark conditions at 4°C.

Preparation of (SDS-PAGE) gel:

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate was performed as described by Smith (1976) using an acrylamide gradient (12%) gel. Electrophoresis was proved to be a useful tool for the separation of protein subunits and the determination of their molecular weight.

Protein dissociates into subunits with rod like shape, in
which the diameter of rods is thought to be constant. While the long axis varies with the molecular weight (MW). The later value can be determined by comparing the relative electrophoretic mobility (Rm) of unknown proteins with the mobility of known protein marker.

**Reagents:**

1- Treatment buffer: 2.5 ml of Tris (0.5M, pH6.8) were mixed with 4 ml of SDS (10%), 1 ml 2-mercaptoethanol and 2 ml glycerol. The mixture was completed to 10 ml with distilled water and kept at 4 °C till used.

2- Tank buffer (pH 8.3): 2.4 gm Tris, 11.52 gm of glycine, 8 ml SDS (10%), 200 ml of water were added until dissolving occur and the mixture was completed to 800 ml with distilled water.

3- Separating gel solution (12%): 10 ml of distilled water were added to 8.3 ml 30 % acrylamide, 6.3 ml of Tris (1.5M, pH8.8), 250 µl of 10 % ammonium persulphate, 250 µl of 10 % SDS, and 10 µl of TEMED were added and the mixture was mixed well.

4- Stacking gel solution (4%): 750 µl of Tris (0.5M. pH 6.8), 1 ml of 30% acrylamide and 4.1 ml distilled water were mixed then 60 µl of 10 % ammonium persulphate, 60 µl of 10 % SDS and 6 µl TEMED were added to the mixture and mixed well.

5- Staining solution: 62 ml of stain stock (1 % Commasie blue-R-250) were mixed with 250 ml of methanol then 50 ml of acetic acid were added and the mixture was completed to 500 ml with distilled water. Insoluble materials were removed through several filtrations. The stain was kept in brown bottle for 6 months.
at room temperature

6- Destaining solution: 500 ml of methanol were mixed with 100 ml of acetic acid and then completed to 1 liter with distilled water.

Procedure:

1. 5µl of protein of each sample (total body tissue of the larvae) were mixed with 20µl of treatment buffer and boiled on boiling water bath for 5 min. then a drop of tracking dye was added.

2. 25µl of protein marker were boiled for 5 min. and then loaded side by side on the gel.

3. The electric current was adjusted to 15 mA for 10 min., then increased to 30 mA for 3 hrs.

4. The electrophoresis was terminated and the gel was placed in staining solution for 12-18 hrs. then rinsed in destaining solution until the background became colorless except blue protein bands. The gel was preserved in 7 % glacial acetic acid.

Protein Marker:
Molecular weight determination of the protein bands:

A plot of log polypeptide molecular weight versus relative mobility ($R_f$) revealed a line relationship. $R_f$ refers to the mobility of the protein measured with reference to the tracking dye.

$R_f = \frac{\text{distance migrated by protein}}{\text{distance migrated by dye}}$.

The blotted line of the molecular weight standard can be then used to estimate the molecular weight of the unknown peptides. The optical denisty (represent amount of protein) of different bands were measured using BIO-RAD model GS-700 Imaging Densitometer.

3.2.7 Ultrastructure studies of the haemocytes of G. mellonella larvae:

Preparation of the haemolymph pellet:

Haemolymph of at least 15 larvae of the same age was collected from a punctured abdomen and pulled into an Eppendorf tube containing the insect anticoagulant solution. The haemocyte pellet was obtained by centrifugation at 6500 rpm for 2 min (Falleiros et al., 2003).

Reagents:

Anticoagulant solution:
62 mM NaCl, 100 mM glucose, 10 mM EDTA, 30 mM trisodium citrate, 26 mM citric acid (Mead et al., 1986).

0.2M phosphate buffer:

$$39\text{ ml } (28.39\text{ gm } \text{Na}_2\text{HPO}_4 \text{ [dibasic sodium phosphate]}/1000\text{ ml dist. water}) + 11 \text{ ml } (31.21\text{ g } \text{NaH}_2\text{PO}_4.2\text{H}_2\text{O} \text{ [monobasic sodium phosphate]}/1000\text{ ml dist. water}).$$
5% glutaraldehyde in 0.1 M phosphate buffer saline:
    20ml glutaraldehyde (25%) + 30ml dist. water + 50ml 0.2M phosphate buffer.

1% osmium tetroxide (Os 04):
    Break an ampoule containing 1gm of Os O₄ (previously cleaned by detergent and dist. water) in 50 ml dist. water in dark brown bottle. Left over night in the dark, and then add 50 ml of 0.2M buffer.

Resin:
    Epon 812 resin, 20 ml; DDSA (Dodecenyl succinic anhydride) 22 ml; NMA (Nadic methyl anhydride) 5 ml and DMP-30 (accelerator) (2, 4, 6-tri [dimethylaminethyl] phenol) 0.77 ml; mixed thoroughly by gentle shaking and stored in water-free acetone-cleaned brown glass bottles at 4°C.

Reynolds lead citrate (Reynolds, 1963):
    1.33 gm lead nitrate (Pb[N0₃]₂) and 1.76 gm sodium citrate (Na₃[C2H507]) were mixed with 30 ml distilled water in a 100 ml volumetric flask. The mixture was shaken for 1min. followed by intermittent shaking for further 30 min. to give a cloudy white solution. To this solution, 8 ml carbon dioxide-free IN NaOH was added and mixed by inversion till the solution became clear. Distilled water was added up to a final volume of 50 ml. The stain was stored in stoppered volumetric flask at 4 °C.

Uranyl acetate:
    A saturated aqueous solution of uranyl acetate was used as the stain: 5 g of uranyl acetate is added to 100 ml dist. water in dark brown bottle. Centrifuge or filtrate before use.
Procedure:

1. Haemolymph pellet of *G. mellonella* was fixed in 5% glutaraldehyde in 0.1M phosphate buffer saline (PBS) for 2h. (PH 7.3).

2. Washed three times by dist. water.

3. Fixed in 1% osmium tetroxide in 0.1M buffer.

4. Washed three times by dist. water.

5. Dehydrated through a graded ethanol series.

6. Dehydrated specimens were then embedded in an epoxy resin

7. Dehydrated specimens were then infiltrated with the resin mixture through a graded series in glass vials with polypropylene caps.

8. Semi-thin sections (1µm thickness) were cut with an ultra-microtome and the sections floated onto water. Then picked up using an eye-lash to a slide and stained by toluidine blue dye to examine light microscopically.

9. Ultra-thin sections were then cut, floated onto water and picked up on grids.

10. Sections were double stained in uranyl acetate followed by lead citrate.

11. Stained sections were examined with the Jeol JEM-1010(BU) Transmission Electron Microscope located at the Regional Center for Mycology and Biotechnology – Al-Azhar University.

3.3 Statistical Analysis:

The data were statistically evaluated by analysis of variance (F) followed by *Duncan's multiple range test (1955)* 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

4.1 Effect of *Steinernema carpocapsae* on larval mortality of some stored product pests:

Results in Tables (1 - 3) and Figures (1- 3) represent the percentages mortality and the accumulative effects on the different insects treated with normal *S. carpocapsae* BA2.

4.1.1. *Galleria mellonella* L.

The obtained data in Table (1) clearly show that the percentage mortality increased with the increasing of juveniles concentrations; as well as increasing of the infection days when compared to control (0%).

The means of percentage mortality for the 1\textsuperscript{st} day were 0, 0, 0, 6.67, 20, 13.33, 20 and 33.33. While in the 2\textsuperscript{nd} day, the percentage mortality was 0, 0, 20, 20, 20, 26.67, 40 and 40 for *S. carpocapsae* concentrations 0, 5, 10, 15, 20, 25, 30 and 35 IJs, respectively. In case of the 3\textsuperscript{rd} day, the mortalities were 0, 13.33, 6.67, 13.33, 13.33, 26.67, 20 and 26.67% for the concentrations 0, 5, 10, 15, 20, 25, 30 and 35 IJs, respectively.

The accumulative percentage mortality after the 3 days was 0, 13.33, 26.67, 40, 53.33, 66.67, 80 and 100 for *S. carpocapsae* concentrations 0, 5, 10, 15, 20, 25, 30 and 35 IJs, respectively (Figure 1).
Table (1): Percentage mortality of *G. mellonella* larvae at different interval times (days) as affected with *S. carpocapsae* nematodes:

<table>
<thead>
<tr>
<th>Cons. of Nematode/IJs</th>
<th>% Mortality after 1 Day</th>
<th>2 Days</th>
<th>3 Days</th>
<th>Accumulative percentage mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>13.33</td>
<td>13.33</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>20</td>
<td>6.67</td>
<td>26.67</td>
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<tr>
<td>35</td>
<td>33.33</td>
<td>40</td>
<td>26.67</td>
<td>100</td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 replicates for each group.

Figure (1): Accumulative percentage mortality of *G. mellonella* larvae treated with *S. carpocapsae*.
4.1.2. *Corcyra cephalonica* (St.)

Data in **Table (2)** and **Figure (2)** indicated that there was a positive relationship between the percent mortality and the *S. carpocapsae* concentrations. This means that; the accumulative percentages mortality of *C. cephalonica* larvae increased as the juveniles’ concentration increase.

The average mortality in the 1<sup>st</sup> day was 0, 0, 20, 13.33, 13.33, 40 and 40%. While in the 2<sup>nd</sup> day, the larval mortality was 0, 20, 13.33, 26.67, 26.67, 20 and 40%. At the 3<sup>rd</sup> day, the percentage mortality was 0, 0, 0, 13.33, 26.67, 20 and 20 for *S. carpocapsae* concentrations 0, 5, 10, 15, 20, 25 and 30 IJs, respectively.

**Figure (2)** showed the accumulative larval mortality after the 3 days, they were 0, 20, 33.33, 53.33, 66.67, 80 and 100 % for *S. carpocapsae* concentrations 0, 5, 10, 15, 20, 25 and 30 IJs, respectively.
Table (2): Percentage mortality of *C. cephalonica* larvae at different interval times (days) as affected with *S. carpocapsae* nematodes:

<table>
<thead>
<tr>
<th>Cons. of Nematode/IJs</th>
<th>% Mortality after 1 Day</th>
<th>2 Days</th>
<th>3 Days</th>
<th>Accumulative percentage mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>13.3</td>
<td>3</td>
<td>33.33</td>
</tr>
<tr>
<td>15</td>
<td>13.3</td>
<td>26.6</td>
<td>7</td>
<td>53.33</td>
</tr>
<tr>
<td>20</td>
<td>13.3</td>
<td>26.6</td>
<td>7</td>
<td>66.67</td>
</tr>
<tr>
<td>25</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
<td>40</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 replicates for each group.

Figure (2): Accumulative percentage mortality of *C. cephalonica* larvae treated with *S. carpocapsae*. 

\[ R^2 = 0.996 \]
4.1.3. *Ephestia kuehniella* (Zell.)

The obtained results in Table (3) show that the average mortality increased with increasing *S. carpocapsae* concentrations.

The means of percentage mortality for the 1\textsuperscript{st} day were 0, 20, 33.33, 33.33, 20, 40 and 46.67. While in the 2\textsuperscript{nd} day, the percentage mortality was 0, 0, 20, 20, 33.33 6.67 and 33.33 for *S. carpocapsae* concentrations 0, 5, 10, 15, 20, 25 and 30 IJs, respectively.

In the 3\textsuperscript{rd} day, the mortalities were 0, 0, 0, 6.67, 20, 33.33 and 20 \% for the concentrations 0, 5, 10, 15, 20, 25 and 30 IJs, respectively.

**Figure (3)** illustrated the accumulative larval mortality after the 3 days, they were 0, 20, 53.33, 60, 73.33, 80 and 100 \% for *S. carpocapsae* concentrations 0, 5, 10, 15, 20, 25 and 30 IJs, respectively
Table (3): Percentage mortality of *E. kuehniella* larvae at different interval times (days) as affected with *S. carpocapsae* nematodes:

<table>
<thead>
<tr>
<th>Concentrations of Nematode (IJs)</th>
<th>% Mortality after 1 Day</th>
<th>% Mortality after 2 Days</th>
<th>% Mortality after 3 Days</th>
<th>Accumulative percentage mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>33.33</td>
<td>20</td>
<td>0</td>
<td>53.33</td>
</tr>
<tr>
<td>15</td>
<td>33.33</td>
<td>20</td>
<td>6.67</td>
<td>60</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>33.33</td>
<td>20</td>
<td>73.33</td>
</tr>
<tr>
<td>25</td>
<td>40</td>
<td>6.67</td>
<td>33.33</td>
<td>80</td>
</tr>
<tr>
<td>30</td>
<td>46.67</td>
<td>33.33</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 replicates for each group.

Figure (3): Accumulative percentage mortality of *E. kuehniella* larvae treated with *S. carpocapsae*.
4.2 Effect of gamma radiation on *Steinernema carpocapsae*:

Results in Table (4) and Figure (4) showed the destructive effect of gamma radiation on *S. carpocapsae*. The percent mortality gradually increased with increasing the dose, except at the dose 2 Gy; it was zero, as compared with the control.

The percentage mortality was increased as the dose of irradiation increased; they were 0, 0, 4.36, 9.67 and 13.88 for the doses of 0, 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 10 Gy; 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 percentage mortality of *S. carpocapsae* juveniles was recorded with gamma radiation doses 2 and 4 Gy.
Table (4): Effect of gamma radiation doses on *S. carpocapsae* percentage mortality at different interval times (days):

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Gamma doses (Gy)</th>
<th>Control</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.36</td>
<td>9.67</td>
<td>13.88</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9.67</td>
<td>15</td>
<td>17.78</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0</td>
<td>0</td>
<td>5.29</td>
<td>16.4</td>
<td>27.33</td>
<td>60.55</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0</td>
<td>0</td>
<td>11.41</td>
<td>19.48</td>
<td>36</td>
<td>65.55</td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 replicates for each group.

Figure (4): Percentage mortality of *S. carpocapsae* after gamma irradiation.
4.3 Virulence of irradiated *Steinernema carpocapsae* BA2 against some stored product pests with:

Data in Tables (5-7) and (Figures 5a-7b) represented the percentage mortality of *G. mellonella*, *C. cephalonica* and *E. kuehniella* treated with normal and gamma irradiated *S. carpocapsae*.

The obtained results clearly show 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 obtained 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.

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 8).
Table (5): Effect of gamma irradiated *S. carpocapsae* on *G. mellonella* at different interval times:

<table>
<thead>
<tr>
<th>Types of Treatments</th>
<th>Doses of gamma irradiation (Gy)</th>
<th>Percentage mortality Days post infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Immediately after <em>S. carpocapsae</em> irradiation</td>
<td>2</td>
<td>91.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>86</td>
</tr>
<tr>
<td>1 week after <em>S. carpocapsae</em> irradiation</td>
<td>2</td>
<td>88.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>74</td>
</tr>
<tr>
<td>2 weeks after <em>S. carpocapsae</em> irradiation</td>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>74</td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 replicates for each group.
Figure (5a): Percentage mortality of *G. mellonella* infected with irradiated *S. carpocapsae* after 1 day of infection.

Figure (5b): Percentage mortality of *G. mellonella* infected with irradiated *S. carpocapsae* after 2 days of infection.
### Table (6): Effect of gamma irradiated *S. carpocapsae* on *C. cephalonica* at different interval times:

<table>
<thead>
<tr>
<th>Types of Treatments</th>
<th>Doses of gamma irradiation (Gy)</th>
<th>Percentage mortality Days post infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Immediately after <em>S. carpocapsae</em> irradiation</td>
<td>2</td>
<td>94.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>1 week after <em>S. carpocapsae</em> irradiation</td>
<td>2</td>
<td>88.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>2 weeks after <em>S. carpocapsae</em> irradiation</td>
<td>2</td>
<td>82.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 replicates for each group.
Figure (6a): Percentage mortality of *C. cephalonica* infected with irradiated *S. carpocapsae* after 1 day of infection.

Figure (6b): Percentage mortality of *C. cephalonica* infected with irradiated *S. carpocapsae* after 2 days of infection.
Table (7): Effect of gamma irradiated *S. carpocapsae* on *E. kuehniella* at different interval times:

<table>
<thead>
<tr>
<th>Types of Treatments</th>
<th>Doses of gamma irradiation (Gy)</th>
<th>Percentage mortality Days post infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>0</td>
<td>74 91.4 100</td>
</tr>
<tr>
<td>Immediately after <em>S. carpocapsae</em> irradiation</td>
<td>2</td>
<td>100 -</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>80 100</td>
</tr>
<tr>
<td>1 week after <em>S. carpocapsae</em> irradiation</td>
<td>2</td>
<td>94.3 100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>77.2 100</td>
</tr>
<tr>
<td>2 weeks after <em>S. carpocapsae</em> irradiation</td>
<td>2</td>
<td>88.6 100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>71.4 100</td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 replicates for each group.
Figure (7a): Percentage mortality of *E. kuehniella* infected with irradiated *S. carpocapsae* after 1 day of infection.

Figure (7b): Percentage mortality of *E. kuehniella* infected with irradiated *S. carpocapsae* after 2 days of infection.
4.4. *In vivo* Production of *S. carpocapsae* BA2:

Data in Table (8) and Figure (8) 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.

The highest production was 198.543 IJs from *G. mellonella* infected with normal *S. carpocapsae*, while the lowest production for normal *S. carpocapsae* was 175.915 IJs from *E. kuehniella*.

The production rates of gamma irradiated *S. carpocapsae* were 156.976, 114.436 and 111.071 IJs from *G. mellonella, C. cephalonica* and *E. kuehniella* larvae, respectively.
Table (8): Reproduction rate of *S. carpocapsae* BA2 in different pests' larvae infected by 30IJs:

<table>
<thead>
<tr>
<th>Type of treatments</th>
<th><em>G. mellonella</em></th>
<th><em>C. cephalonica</em></th>
<th><em>E. kuehniella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>198.543 ± 0.559&lt;sup&gt;a&lt;/sup&gt;</td>
<td>179.101 ± 0.454&lt;sup&gt;a&lt;/sup&gt;</td>
<td>175.915 ± 0.089&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2 Gy irradiated <em>S. carpocapsae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>156.976 ± 1.181&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>114.436 ± 0.338&lt;sup&gt;b&lt;/sup&gt;</td>
<td>111.071 ± 0.198&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

- Letters indicate the variance between the means (Duncan's multiple range test).
- Each value represents the mean of 5 replicates for each group ± S.E.

Figure (8): Reproduction rate of normal and 2Gy gamma irradiated *S. carpocapsae* BA2 in different tested pests.

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4.5 Biochemical studies:

Data summarized and illustrated in Tables (9-14) and Figures (9-16) showing the changes in the total body content of lipids, proteins, carbohydrates, urea, acid phosphatase and Chitinase activity.

4.5.1. Determination of total lipid concentration in total body tissue homogenate of *G. mellonella* larvae:

Data in Table (9) showed a negative correlation between the total lipids concentration and the increase in time of *S. carpocapsae* infection, and also a significant difference was found between treatments (Table 9).

The obtained results in Figure (9) show that the total lipid percent was gradually decreased with increasing the time of infection. At normal *S. carpocapsae* treatment the percentages change were -26.6 and -44.2 at 12 and 18 hrs., respectively. While in irradiated *S. carpocapsae* treatment the changes were more as they being -36.6% and -51% at 12 and 18 hour, respectively as compared to control (0).
Table (9): Effect of normal and gamma irradiated S. carpocapsae on total lipid concentration of G. mellonella:

<table>
<thead>
<tr>
<th>Types of treatments</th>
<th>Total Lipid Concentrations (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>349.65±9.7\textsuperscript{a}</td>
</tr>
<tr>
<td>12 Hrs. Normal S. carpocapsae</td>
<td>256.50±5.3\textsuperscript{b}</td>
</tr>
<tr>
<td>Irradiated S. carpocapsae</td>
<td>221.41±6.1\textsuperscript{bc}</td>
</tr>
<tr>
<td>18 Hrs. Normal S. carpocapsae</td>
<td>195.09±9.2\textsuperscript{cd}</td>
</tr>
<tr>
<td>Irradiated S. carpocapsae</td>
<td>171.20±3.5\textsuperscript{d}</td>
</tr>
</tbody>
</table>

- Letters indicate the variance between the means (Duncan's multiple range test).
- Each value represents the mean of 5 replicates for each group ± S.E.

Figure (9): Percentage change of total lipid in G. mellonella treated with normal and gamma irradiated S. carpocapsae.
4.5.2. Determination of total protein concentration in total body tissue homogenate of *G. mellonella* larvae:

Data given in Table (10) indicated the changes in the total body content of protein. As shown from the results a highly significant decrease in the protein content in the larvae treated with irradiated *S. carpocapsae* as compared to the larvae treated with normal *S. carpocapsae* and the normal control.

The protein concentrations were 1 and 0.81 g/dL after 12 hrs. treatment with normal and irradiated *S. carpocapsa*, respectively. While after 18 hrs. of the treatment, the protein concentration becam 0.71 and 0.48 g/dL for normal and irradiated *S. carpocapsa*, respectively.

It was noticed that a great percentage change -25.5 and -39.6 in total protein induced by normal and irradiated *S. carpocapsae* after 12 hour, respectively. Meanwhile, after 18 hour of the infection, the changes were -47% and -64.17% induced by normal and irradiated *S. carpocapsae*, respectively (Figure, 10).
Table (10): Effect of normal and gamma irradiated *S. carpocapsae* on total protein concentration of *G. mellonella*:

<table>
<thead>
<tr>
<th>Types of treatments</th>
<th>Total protein Concentrations (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.34 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 Hrs.</td>
<td></td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>1.00 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated <em>S. carpocapsae</em></td>
<td>0.81 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>18 Hrs.</td>
<td></td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>0.71 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated <em>S. carpocapsae</em></td>
<td>0.48 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Letters indicate the variance between the means (Duncan's multiple range test).
- Each value represents the mean of 5 replicates for each group ± S.E.

Figure (10): Percentages change of total protein in *G. mellonella* treated with normal and gamma irradiated *S. carpocapsae*. 
4.5.3. Determination of total Carbohydrates concentration in total body tissue homogenate of G. mellonella larvae:

Table (11) demonstrates the changes in the total body content of carbohydrate of the total of G. mellonella larvae treated with normal and gamma irradiated S. carpocapsae. The carbohydrate content was higher in normal G. mellonella larvae and began to decrease after infection with S. carpocapsae. Treatment with irradiated S. carpocapsae causing significant decrease in Carbohydrate concentration; giving 50.22 gm/100ml and 26.22 gm/100ml after 12 and 18 hrs. of infection; respectively, as compared to control (61.33 gm/100ml).

Figure (12) represented the percentage change in the carbohydrates content. The results showed a significant reduction in carbohydrate content in the larvae treated with irradiated S. carpocapsae more than the larvae treated with normal S. carpocapsae as compared to the control.

Figure (11): Standard curve of total carbohydrate.
Table (11): Effect of normal and gamma irradiated *S. carpocapsae* on total carbohydrate concentration of *G. mellonella*:

<table>
<thead>
<tr>
<th>Types of treatments</th>
<th>Total carbohydrate concentrations (gm/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61.33 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 Hrs.</td>
<td></td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>55.99 ± 1.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated <em>S. carpocapsae</em></td>
<td>50.22 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>18 Hrs.</td>
<td></td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>38.22 ± 3.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated <em>S. carpocapsae</em></td>
<td>26.22 ± 1.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Letters indicate the variance between the means (Duncan's multiple range tests).
- Each value represents the mean of 5 replicates for each group ± S.E.

Figure (12): Percentage change of total carbohydrate in *G. mellonella* treated with normal and gamma irradiated *S. carpocapsae*.  

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4.5.4. Determination of urea concentration in total body tissue homogenate of *G. mellonella* larvae:

Table (12) indicated the changes in urea concentration of the larvae treated with normal and irradiated *S. carpocapsae*. The results clearly show that urea concentration was negatively correlated to the infection with normal or gamma irradiated *S. carpocapsae*. Significant decrease in urea concentration was induced in larvae treated with irradiated *S. carpocapsae* as compared to the control or infection with normal *S. carpocapsae*.

After treatment with normal *S. carpocapsae*, the urea concentrations were 2.752 and 1.467 gm/100ml after 12 and 18 hrs. of the treatment, respectively. While irradiated *S. carpocapsae* decreased the urea concentrations to 2.538 and 1.039/100ml after 12 and 18 hrs. of the treatment, respectively as compared with control (5.47 gm/100ml).

Figure (13) illustrated the percentage change of urea in the total body content of *G. mellonella* treated with normal and gamma irradiated *S. carpocapsae*. The most marked change was - 81% after 18 hrs. treatment with irradiated *S. carpocapsae* regarding to the control (0%).
Table (12): Effect of normal and gamma irradiated *S. carpocapsae* on urea concentration of *G. mellonella*:

<table>
<thead>
<tr>
<th>Types of treatments</th>
<th>Urea concentrations (gm/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.47±0.32^a</td>
</tr>
<tr>
<td>12 Hrs.</td>
<td></td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>2.752±0.32^b</td>
</tr>
<tr>
<td>Irradiated <em>S. carpocapsae</em></td>
<td>2.538±0.4^b</td>
</tr>
<tr>
<td>18 Hrs.</td>
<td></td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>1.467±0.18^c</td>
</tr>
<tr>
<td>Irradiated <em>S. carpocapsae</em></td>
<td>1.039±0.06^c</td>
</tr>
</tbody>
</table>

- Letters indicate the variance between the means (Duncan's multiple range tests).
- Each value represents the mean of 5 replicates for each group ± S.E.

Figure (13): Percentage reduction of urea in *G. mellonella* treated with normal and gamma irradiated *S. carpocapsae*.
4.5.5. Determination of acid phosphatase activity in total body tissue homogenate of *G. mellonella* larvae:

Data assembled in Table (13) showed the effect of normal and gamma irradiated *S. carpocapsae* on acid phosphatase activity in *G. mellonella* total body tissue. The results implied a highly significant increase in acid phosphatase activity with time increase; they were 21.54 U/L and 22.38 U/L for 12 and 18 hrs. of treatment with normal *S. carpocapsae*, respectively. While; treatment with irradiated *S. carpocapsae* causing increase in acid phosphatase activity to 26.35 U/L and 34.59 U/L for 12 and 18 hour of treatment, respectively as compared to the control (17.30 U/L).

Figure (14) clarified the percentage increase of acid phosphatase. Irradiated *S. carpocapsae* causing a hundred percent increase after 18 hrs. of the treatment.
Table (13): Effect of normal and gamma irradiated *S. carpocapsae* on acid phosphatase activity of *G. mellonella*:

<table>
<thead>
<tr>
<th>Types of treatments</th>
<th>Acid phosphatase activity (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.30±0.3e</td>
</tr>
<tr>
<td>12 Hrs.</td>
<td></td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>21.54±0.23d</td>
</tr>
<tr>
<td>Irradiated <em>S. carpocapsae</em></td>
<td>26.35±0.23b</td>
</tr>
<tr>
<td>18 Hrs.</td>
<td></td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>22.38±0.06c</td>
</tr>
<tr>
<td>Irradiated <em>S. carpocapsae</em></td>
<td>34.59±0.36a</td>
</tr>
</tbody>
</table>

- Letters indicate the variance between the means (Duncan's multiple range tests).
- Each value represents the mean of 5 replicates for each group ± S.E.

Figure (14): Percentage increase of acid phosphatase activity in *G. mellonella* treated with normal and gamma irradiated *S. carpocapsae*. 
4.5.6. Determination of chitinase activity in total body tissue homogenate of *G. mellonella* larvae:

Table (14) demonstrated the change of chitinase activity in *G. mellonella* total body tissue treated with normal and gamma irradiated *S. carpocapsae*.

The results indicated a significant increase after 18 hour of the treatment, it was 11 (gm NAG/min./ml total body) and 18.51 (gm NAG/min./ml total body) for normal and irradiated *S. carpocapsae*, respectively as compared to control (3.17 gm NAG/min./ml total body).

The obtained results represented in Figure (16) show the percentage increase of chitinase activity in *G. mellonella* total body tissue. Twelve hour of the treatment causing increase in chitinase concentration to 31.8% and 43.1% for normal and irradiated *S. carpocapsae*, respectively. The percentages increase were 247 and 483.9 at18 hour treatment with normal and irradiated *S. carpocapsae*, respectively as compared to control.

![Figure (15): Standard curve of chitinase.](image1.png)
Table (14): Effect of normal and gamma irradiated *S. carpocapsae* on chitinase activity of *G. mellonella*:

<table>
<thead>
<tr>
<th>Types of treatments</th>
<th>Chitinase activity (gm NAG/min./ml total body)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.17 ±0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>12 Hrs.</strong></td>
<td></td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>4.18 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated <em>S. carpocapsae</em></td>
<td>4.54 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>18 Hrs.</strong></td>
<td></td>
</tr>
<tr>
<td>Normal <em>S. carpocapsae</em></td>
<td>11 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated <em>S. carpocapsae</em></td>
<td>18.51 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Letters indicate the variance between the means (Duncan's multiple range tests).
- Each value represents the mean of 5 replicates for each group ± S.E.

![Chitinase Percentage Increase](image)

Figure (16): Percentage increase of chitinase activity in *G. mellonella* treated with normal and gamma irradiated *S. carpocapsae*. 
4.6. Characterization of protein by polyacrylamide gel electrophoresis in total body tissue homogenate of *G. mellonella* larvae:

Table (15) and Figures (17-23) presented the electrophoretic protein profile of total body tissue of *G. mellonella* after 12 and 18 hrs. infection with normal and gamma irradiated (2 Gy) *S. carpocapsae*.

The results indicated that 13 protein bands with molecular weights ranging from 118.325 - 26.097 KDa were electrophoretically separated from the whole body tissue of normal *G. mellonella*. Protein band of 65.966 KDa was detected as dominant band with high density in all treatments.

The bands after 12 and 18 hrs. of infection with normal *S. carpocapsae* were in accordance with those of the control.

After 12 hrs. of infection with gamma irradiated *S. carpocapsae*, the number of bands became 14 bands as 1 band disappeared (27.72 KDa) and 2 new bands appeared (52.597 and 37.094 KDa).

While after 18hrs. of infection with gamma irradiated *S. carpocapsae*, the number of bands decreased to 12 bands as 4 bands disappeared (118.325, 106.579, 27.72 and 26.097 KDa) and only 3 new bands were appeared (121.068, 99.316 and 36.748 KDa). In addition, all the bands appeared in low intensity than the other bands.
Table (15): Molecular weights and relative concentrations of protein fractions of total body tissue of *G. mellonella* infected with normal and 2 Gy gamma irradiated *S. carpocapsae*:

<table>
<thead>
<tr>
<th>Band No.</th>
<th>M.W (KDa)</th>
<th>Control</th>
<th>Bands after infection with normal <em>S. carpocapsae</em></th>
<th>Bands after infection with irradiated <em>S. carpocapsae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 hrs.</td>
<td>18 hrs.</td>
</tr>
<tr>
<td>1.</td>
<td>121.068</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>118.325</td>
<td>7.718</td>
<td>5.975</td>
<td>9.276</td>
</tr>
<tr>
<td>3.</td>
<td>106.579</td>
<td>6.666</td>
<td>6.57</td>
<td>11.051</td>
</tr>
<tr>
<td>4.</td>
<td>99.316</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>65.966</td>
<td>106.888</td>
<td>111.071</td>
<td>112.988</td>
</tr>
<tr>
<td>6.</td>
<td>55</td>
<td>2.683</td>
<td>5.291</td>
<td>2.599</td>
</tr>
<tr>
<td>7.</td>
<td>52.597</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>46.269</td>
<td>8.68</td>
<td>8.98</td>
<td>10.436</td>
</tr>
<tr>
<td>10.</td>
<td>41.155</td>
<td>9.298</td>
<td>8.492</td>
<td>7.859</td>
</tr>
<tr>
<td>11.</td>
<td>39.141</td>
<td>32.028</td>
<td>29.536</td>
<td>25.116</td>
</tr>
<tr>
<td>12.</td>
<td>37.094</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>36.748</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14.</td>
<td>35.461</td>
<td>30.415</td>
<td>32.133</td>
<td>9.905</td>
</tr>
<tr>
<td>15.</td>
<td>32.394</td>
<td>35.125</td>
<td>29.531</td>
<td>12.865</td>
</tr>
<tr>
<td>16.</td>
<td>29.592</td>
<td>18.459</td>
<td>15.736</td>
<td>6.853</td>
</tr>
<tr>
<td>17.</td>
<td>27.72</td>
<td>5.68</td>
<td>3.446</td>
<td>0.64</td>
</tr>
<tr>
<td>18.</td>
<td>26.097</td>
<td>2.531</td>
<td>2.37</td>
<td>1.587</td>
</tr>
</tbody>
</table>

|          |          |         | 12 hrs.                                           | 18 hrs.                                             |
| 1.       | 121.068  | -       | -                                                 | -                                                   |
| 2.       | 118.325  | 7.718   | 5.975                                             | 9.276                                               |
| 3.       | 106.579  | 6.666   | 6.57                                              | 11.051                                              |
| 4.       | 99.316   | -       | -                                                 | -                                                   |
| 5.       | 65.966   | 106.888 | 111.071                                           | 112.988                                             |
| 6.       | 55       | 2.683   | 5.291                                             | 2.599                                               |
| 7.       | 52.597   | -       | -                                                 | -                                                   |
| 9.       | 46.269   | 8.68    | 8.98                                              | 10.436                                              |
| 10.      | 41.155   | 9.298   | 8.492                                             | 7.859                                               |
| 11.      | 39.141   | 32.028  | 29.536                                            | 25.116                                              |
| 12.      | 37.094   | -       | -                                                 | -                                                   |
| 13.      | 36.748   | -       | -                                                 | -                                                   |
| 14.      | 35.461   | 30.415  | 32.133                                            | 9.905                                               |
| 15.      | 32.394   | 35.125  | 29.531                                            | 12.865                                              |
| 16.      | 29.592   | 18.459  | 15.736                                            | 6.853                                               |
| 17.      | 27.72    | 5.68    | 3.446                                             | 0.64                                                |
| 18.      | 26.097   | 2.531   | 2.37                                              | 1.587                                               |

Total no. of bands: 13, 13, 13, 14, 12
Figure (17): Electrophoretic pattern of proteins of total body tissue of *G. mellonella* infected with normal and 2 Gy gamma irradiated *S. carpocapsae*. 
Figure (18): Lane 1 profile (Marker lane).

Figure (19): Lane 2 profile (Normal *G. mellonella*).
Figure (20): Lane 3 profile (G. mellonella total body tissue after 12 hrs. infection of normal S. carpocapsae).

Figure (21): Lane 4 profile (G. mellonella total body tissue after 18 hrs. infection of normal S. carpocapsae).
Figure (22): Lane 5 profile (*G. mellonella* total body tissue after 12 hrs. infection of 2 Gy gamma irradiated *S. carpocapsae*).

Figure (23): Lane 6 profile (*G. mellonella* total body tissue after 18 hrs. infection of 2 Gy gamma irradiated *S. carpocapsae*).
4.7. Ultrastructure studies of the haemocytes of *G. mellonella* larvae:

4.7.1. Normal haemocytes:

In the present study, five types of haemocytes were identified in 5th larval instar of *G. mellonella*: Prohaemocytes, Plasmatocytes, Granulocytes, Oenocytoids and Spherulocytes (Figure 24).

**Prohemocytes** are small rounded cell with variable sizes. Plasma membrane was generally smooth, and nucleus (N) was large, centrally located, almost filling up the whole cell. The cytoplasm was basophilic with scattered chromatin and evident nucleoli (Nu) showing a large amount of free ribosome, but only small rough endoplasmic reticulum cisternae (Rer). Few mitochondria (M) and rare Golgi complex (G) was observed.

**Plasmatocytes** are oval and variable in size. The elongate or lobate nucleus exhibit variable sizes and is centrally localized, showing scattered chromatin masses and up to two nucleoli. The cytoplasm showed well-developed rough endoplasmic reticulum, Golgi system, mitochondria and vacuoles (V).

**Granulocytes** were the most frequently observed cell type in larvae and were spherical cells. The nucleus was round, centrally located, with scattered chromatin masses and nucleolus. Two types of membrane bound granules were observed: dense granules (Dg), containing electron-dense and homogenous content and structured granules (Sg), with crystalloid content. Vacuoles of variable sizes and shapes were also present. The developed Rer,
the Golgi apparatus, mitochondria and glycogen particles were dispersed in the cytoplasm.

**Oenocytoids** were a rounded cell. The cellular membrane was smooth. The nucleus was small, eccentric, and showed a distribution pattern with alternate condensed and discondensed chromatin. The cytoplasm was homogenous with rounded structured granules (low electron-dense). Dense mitochondria, generally ring-shaped was observed.

**Spherulocytes** were characterized by their inclusions and membrane-bound spherules took up almost all the cytoplasm. The cellular surface was homogenous but exhibit cytoplasmic protrusion corresponding to the spherules. The nucleus was small, eccentric, mostly deformed by the spherules. The spherules contained moderate electron-dense and flocculent material, with a quite electron-dense core region. Besides the spherules, the cytoplasm contained few organelles around the nucleus, such as Rer and few mitochondria.
Figure (24): Ultrastructure of normal haemocytes of 5th larval instar of *G. mellonella* (TEM mag. = 12000x, bar: 2 microns)

(A): Prohaemocytes  
(B): Plasmatocytes  
(C): Granulocytes  
(D): Oenocytoids  
(E): Spherulocytes

N: nucleus  
Nu: nuclei  
G: Golgi complex  
M: mitochondria  
V: vacuoles  
Rer: Rough endoplasmic reticulum  
Sg: structured granules  
Dg: dense granules
4.7.2. Ultrastructure changes of the haemocytes of *G. mellonella* larvae after infection with normal and irradiated *S. carpocapsae*:

The infection of *G. mellonella* with *S. carpocapsae* induced several pathological detriments.

During infection, Oenocytoids and Spherulocytes vanished from the haemolymph, and the other haemocytes undergo considerable structural changes.

1-Prohaemocytes:

After 12 hrs. of the infection with *S. carpocapsae*, the prohaemocyte was enlarged or take elongate shape. The cell membrane forming thin pseudopodia and there was also evidence of vacuoles (Figure 25).

The rough endoplasmic reticulum cisternae, mitochondria and Golgi complex were disappeared from prohaemocyte.

Prohaemocytes vanished from the haemolymph under the effect of irradiated *S. carpocapsae* and after 18hrs. of the infection with normal and irradiated *S. carpocapsae*. 
Figure (25): Ultrastructure of 5th larval instar of *G. mellonella* prohaemocyte after 12 hrs. infection with normal *S. carpocapsae* (TEM mag. = 12000x, bar= 2 microns).

N: nucleus  
Nu: nuclei  
Ps: pseudopodia  
V: vacuoles
2- Plasmatocytes:

After 12 hrs. of the infection with *S. carpocapsae*, the plasmatocyte became irregular in shape. The cell membrane forming thin pseudopodia with appearance of vacuoles (Figure 26 A&B).

Plasmatocytes showed notable changes in their shape after 12 hrs. of the infection with irradiated *S. carpocapsae*:

- Plasmatocytes that have phagocytized the bacteria (*X. nematophilus*) tend to adhere to one another to contact, and to form aggregations (Figure 27 A). These unstructured aggregations may later be encapsulated by other haemocytes, or by cells that may be released from the aggregations.
- Plasmatocytes synthesized numerous desmosomes (De) and contain numerous microtubules in order to form capsule and nodule (Figure 27 B).

More destructive effects were observed in the plasmatocytes after 18 hrs. of the infection with *S. carpocapsae*. The most changes observed were enlarged shape, destruction of the cell membrane and presence of numerous vacuoles (Figure 28 A). The treatment of irradiated *S. carpocapsae* caused completely lysis of the cell membrane and destruction of the nucleus (Figure 28 B).

The rough endoplasmic reticulum, mitochondria and Golgi complex were disappeared.
Figure (26): Ultrastructure of 5th larval instar of *G. mellonella* plasmatocyte after 12 hrs. infection with normal *S. carpocapsae* (TEM mag. = 12000x, bar A&B= 2microns).

N: nucleus  Ps: pseudopodia  V: vacuoles
Figure (27): Ultrastructure of 5th larval instar of *G. mellonella* plasmatocytes after 12 hrs. infection with irradiated *S. carpocapsae* (TEM mag. = 12000x, bar A&B= 2microns).

N: nucleus  
De: desmosomes  
V: vacuoles
Figure (28): Ultrastructure of 5th larval instar of *G. mellonella* plasmatocytes after 18 hrs. infection with:
(A) normal *S. carpocapsae* and (B) irradiated *S. carpocapsae*
(TEM mag. = 12000x, bar A= 500nm; B= 2microns).

N: nucleus  
Lm: lysed membrane  
DN: distorted nucleus  
V: vacuoles
3- Granulated cell:

After 12 hrs. of the infection with *S. carpocapsae*, granulated cell enlarged with increase in it’s granules size (**Figure 29 A**). While After 12 hrs. of the infection with irradiated *S. carpocapsae*, granulated cell released it’s granular content to come into contact with a foreign body at the beginning of capsule/nodule formation (**Figure 29 B**).

In the late stage of *S. carpocapsae* infection (After 18 hrs.); the infected granulated cell showed enlarged cytoplasmic granules, and lysed cell membrane (Lm) (**Figure 30 A**).

More destructive effects were observed in the haemocytes of *G. mellonella* infected with irradiated *S. carpocapsae*. The cell increased in size with enlarged granules, the cytoplasm seem to swell giving the cells a vacuolated appearance and the cell membrane was completely lysed (**Figure 30 B**).

After irradiated *S. carpocapsae* treatment, the symbiotic bacteria adhere to the haemocyte in order to reproduce within it (**Figure 31 A**) causing damage to the haemocytes in their re-emergence into the hamolymph (**Figure 31 B**).
Figure (29): Ultrastructure of 5th larval instar of *G. mellonella* granulated cell after 12 hrs. infection with: (A) normal *S. carpocapsae* and (B) irradiated *S. carpocapsae* (TEM mag. = 12000x, bar A&B = 2microns).

Dg: dense granules    Sg: structured granules
Figure (30): Ultrastructure of 5th larval instar of G. mellonella granulated cell after 18 hrs. infection with:
(A) normal S. carpocapsae and (B) irradiated S. carpocapsae
(TEM mag. = 12000x, bar A & B = 2microns).

Dg: dense granules    Sg: structured granules    Lm: lysed membrane
Figure (31): Ultrastructure of 5\textsuperscript{th} larval instar of \textit{G. mellonella} haemocytes and the symbiotic bacteria (\textit{X. nematophilus}) interaction (A: TEM mag. = 12000x, bar: = 2microns; B = TEM mag. = 10000x, bar: = 2microns).
Over the years the insecticides have been important for human to control the population of harmful insects and the chemical insecticides have been used for this purpose in agricultural sector. Synthetic insecticides, owing to their various side effects, have been widely replaced by biological insecticides.

The entomopathogenic nematodes (EPNs) are safe bio-control agents (Ehlers and Hokkaen, 1996 and Hazier et al., 2003). EPNs are currently marketed worldwide for using in biological control as recorded by Gaugler and Kaya, (1990); Shapiro-Ilan et al. (2002 & 2004) and Dillon et al., 2008.

The entomopathogenic nematodes used in the present study are non toxic to vertebrates as well as they are widely produced in vivo, the present study showed high bioactivity of gamma irradiated *S. carpocapsae* BA2 against some stored product pests, *G. mellonella*, *C. cephalonica* and *E. kuehniella*. Such results may offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides.

According to the integrated pest management programs, the entomopathogenic nematodes (*H. bacteriophora* BA1 and *S. carpocapsae* BA2) and gamma radiation were conducted to control *G. mellonella* larvae. The mortality rate of the 4th larval instar (resulted from irradiated male parent pupae mated with normal female) increased by increrasing the radiation doses and the nematode concentrations. *G. mellonella* larvae were more
susceptible to *S. carpocapsa* BA2 than *H. bacteriophora* BA1. (Sayed, 2008).

In the present study, we would like to reveal the effect of gamma irradiated *S. carpocapsa* BA2 as a tool for pest control.

### 5.1 Effect of *St. carpocapsae* on larval mortality:

Efficacy of EPNs is dependent on matching the most effective nematode with the target pest (Georgis and Gaugler, 1991).

Infectivity of steinernematid and heterorhabditid nematodes varied greatly due to some environmental factors or requirements. These environmental requirements could be physical or biotic parameters (Woodring and Kaya, 1988).

Some other important factors affect the infectivity for a great extent such as nematode species/strain, exposure period, nematode concentrations 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).

In this study, the results revealed that the infectivity of *S. carpocapsae* BA2 increased with increasing the concentration against all the tested pests (*G. mellonella, C. cephalonica* and *E. kuehniella*).

From the aforementioned results it is obvious that the susceptibility of the tested pests to *S. carpocapsae* may be
arranged in ascending order as follows: \textit{E. kuehniella} > \textit{C. cephalonica} > \textit{G. mellonella}.

This difference in susceptibility may be caused by the difference in the nature of larvae as reported by Abdel-Razek and Abdel-Gawad (2007) or because of environmental factors that affect the infectivity of the tested nematode species or strain (Gaugler, 1999). Also, the exposure time is an important factor in nematodes infection (\textit{El-Bishry et al., 2002; El-Khoney, 2007 and Shamseldean et al., 2008}).

The obtained data reveal that mortality of gamma irradiated \textit{S. carpocapsae} was dose dependant. This agree with the results of \textit{El-Mandarawy et al. (2006)} on \textit{S. riboravae, H. bacteriophora} and \textit{H. tayserae} nematodes. Also, Gaugler and Boush (1979) reported that gamma radiation doses caused harmful effect to \textit{S. carpocapsae} as exposure to 10,000 rad completely inhibited the reproduction, 100,000 rad and inhibited maturation and 300,000 rad reduced pathogenicity to 50%.

In this study, the results revealed that gamma irradiated \textit{S. carpocapsae} has a high virulence against the tested larvae than normal \textit{S. carpocapsae}; showing reduction in time needed to achieve 100% mortality of \textit{G. mellonella, C. cephalonica} and \textit{E. kuehniella} larvae. This result coincide with Yussef (2006) who stated that regarding the susceptibility of \textit{Callosobruchus maculatus} to \textit{S. carpocapsae} and gamma irradiation, the lowest doses (2.5, 5 and 10 Gy) were more effective than the higher ones.

In our study the results indicated that; after gamma irradiated IJs storage for 1 and 2 weeks, their pathogenicity was the same for using them immediately after irradiation (giving 100% mortality within 2 days).
Our results indicated that the average production of nematodes decreased when *S. carpocapsae* were irradiated. The reason of reduction was explained by Kaya and Koppenhofer (1996) who reported that when the number of IJ's penetrating into a host exceeds an optimal level, exploitative interaspecific competition occurs among the developing nematodes, which reduces the total number of progeny emerging from the cadaver.

The obtained data reveal that *G. mellonella* larve yielded the best average production of *S. carpocapsae* juviniles. This is due to that *G. mellonella* is the conventional host for *in vivo* multiplication of entomopathogenic nematodes (Costa et al., 2007).

5.2 Biochemical studies:
Althought the first site of action of *S. carpocapsae* is the haemocytes, but many biochemical changes have been reported to occur in different metabolism pathway. Therefore, in this study we would like to investigate the changes in the metabolic products (proteins, lipids, carbohydrates and urea) and enzymatic activity (acid phosphatase and chitinase) of *G. mellonella* treated with normal and 2Gy gamma irradiated *S. carpocapsae*.

5.2.1 Changes in the metabolic products of total body tissues of *G. mellonella* larvae treated with normal and gamma irradiated (2Gy) *S. carpocapsae*:

Proteins are fundamental components of all living cells and include many substances, such as enzymes, hormones, and antibodies, that are necessary for the proper functioning of an organism (Fagan et al., 2002).
Lipids are the most suitable materials for storage of energy reserves compared to carbohydrates, lipids can supply as much as eight times more energy per unit weight (Beenakkers et al., 1985).

After the bacterial symbionts released into the insect haemocoel, they produce a variety of metabolites including toxins and hydrolytic exoenzymes to enable them and associated nematodes to colonize and reproduce in the insect host. Proteases represent an important part of the extracellular enzymes produced (Bowen et al., 2003; Cabral et al., 2004 and Marokhazi et al., 2004 & 2007) and play an important role in insect cell death because the protease activity lead to breakdown of total proteins (Bowen et al., 2000). This result has been supported by Bowen et al. (2003) when hypothesized that alkaline metalloproteases from Photorhabdus temperata strain K122 and Photorhabdus luminescens akhurstii strain W14 have a role in the bioconversion of the insect cadavers into a nutrient soap that is ideal for bacterial and nematode development (Burnell and stock, 2000; Cabral et al., 2004; Joyce et al., 2006 and Crawford et al., 2010).

In our study, the total body tissues of the 5th larval instar of G. mellonella after 12 and 18 hrs. of the treatments with 30IJs of normal and gamma irradiated (2Gy) S. carpocapsae was used. The results indicated that S. carpocapsae caused a significant decrease in the total body concentration of proteins, lipids, carbohydrates and urea. However, this decrease was greatly in larvae infected with gamma irradiated S. carpocapsae, as compared with the normal S. carpocapsae.
Similar reduction in total protein of *G. mellonella* haemolymph was recorded after infection with *Photorhabdus* sp. and *Xenorhabdus* sp. of some Egyptian nematodes (*H. indica* (IB), *H. bacteriophora* (4), *H. brevicadus* (1), *S. glaserii* (Sg) and *S. carpocapsae* All (5)) by Shehata (2010).

Concerning the studies which have been done by many authors on the effect of *S. carpocapsae* on aspects of the biology of different pests, similar findings were also obtained by Yussef, (2006), who reported a reduction in total protein content of *C. maculates* infected with *S. carpocapsae* was reduced. Also, Mohamed (1995) recorded a decrease in total proteins, total lipid and total carbohydrate contents of *Phthorimaea operculella* larvae infected with *S. carpocapsae*.

Also, Ghally et al. (1988) studied the effect of *S. feltiae* and associated bacteria on total lipids of infected *Ceratitis capitata*. They found that the lowest level in total lipid was after 18 hrs. post infection.

The reasons of total lipid and protein reduction could attributed to the effect of symbiotic bacteria, *X. nematophilus* which released into the insect haemocoel, to produce virulence factors that suppress host immune responses, including toxin complexes, proteases, lipases and lipopoly-saccharide (Forst et al., 1997 and Owuama, 2001). Among other potential virulence factors, there is a complex set of extra cellular enzymes, including lecithinases, chitinases, and phosphatases (Forst et al., 1997).

The reduction in total lipid concentration is explained by Milstead (1979) who studied the pathophysiological influence of *H. bacteriophora* complex on the 7th instar of *G. mellonella,*
reported that shortly after the nematode penetration into the haemocoel of the larvae begins feeding upon the fat body.

The observed reduction in the total lipids of *G. mellonella* may be due to the lipases secreted from the symbiotic bacteria (*Boemare and Akhurst, 1988* and *Forst et al., 1997*). This result is in agreement with *Richards and Blair (2010)* who stated that *X. nematophilus* produces several secreted enzymes, including hemolysins, lipases, and proteases, which are thought to contribute to virulence or nutrient acquisition for the bacterium and its nematode host in vivo. *X. nematophilus* has two lipase activities with distinct in vitro specificities for Tween and lecithin.

Entomopathogenic nematodes exhibit different search strategies to increase the probability of finding a host. They can be categorized as cruising Heterorhabditidae, which are highly mobile and responsive to volatiles (carbon dioxide) released by the host and ambushing Steinernematidae, which tend to stand on their tails in a rigid position allowing them to attack pests (*Campbell and Kaya, 2002*).

*Lewis et al. (1997)* stated that in some cases, *Steinernema* species change their foraging strategy for infecting a host, from ambushers to cruisers (active seekers for host). It seems that nematode juveniles during the ambushing state depend mainly upon carbohydrates as energy reserves because they, actually, stay motionless, waiting for passing by hosts. When they change their searching strategy to be cruisers, where richer sources of energy are needed, they begin to utilize their lipid content. The types of nematodes differ in their energy reserves and their biological activity, such as reproduction and penetration rate, virulence and survival alive in the soil until they find the host.
In this study the reduction in total carbohydrate concentration may be due to the effect of the symbiotic bacteria *X. nematophilus* in order to provide the energy needed to *S. carpocapsae* multiplication. This coincide with Gordon and Webster (1971) who reported that the total carbohydrate in the haemolymph of infected locusts was severely depleted during the active growth period of the nematode *Mermis nigrescens* and the possible utilization of these carbohydrates by the nematode.

Urea is a water-soluble compound, CO(NH$_2$)$_2$, that is the major nitrogenous end product of protein metabolism and is the chief nitrogenous component of the urine in mammals and other organisms. Also, called carbamide (Huchcroft *et al.* 2010).

The obtained data reveal that urea concentration decreased after *S. carpocapsae* treatments. This is due to the reduction in the protein concentration.

### 5.2.2 The enzymatic level activity of acid phosphatase (AcP) and chitinase in total body tissue homogenate of *G. mellonella* larvae infected with normal and gamma irradiated (2Gy) *St. carpocapsae*:

The present study deal with the changes in activities of acid phosphatase and chitinase of 5th larval instar of *G. mellonella* after 12 and 18 hrs. of infection with 30IJs of normal and gamma irradiated (2Gy) *S. carpocapsae*.

AcP is hydrolytic enzyme, which hydrolyse acid phosphomonoesters (Etebari *et al.*, 2005).

The study of invertebrate haemocyte cell death mechanisms is fragmentary; lysozyme (an enzyme that degrades bacterial cell
walls (Shairra, 2000) has been well studied but there is little information on other hydrolases (Zachary and Hoffmann, 1984). Recently, there have been a number of reports on the cytochemistry of the blood of oysters and mussels (Alvarez et al., 1995). The few studies on insects have concentrated on the characterization of naive haemolymph (Miranpuri et al., 1991). Phagocytosis is known to stimulate production of lysosomal enzymes of which acid phosphatase (AcP) is a key component. AcP has been found in insect haemocytes and shown to be released into the plasma.

Cheng (1983) reported hypersynthesis of acid phosphatase by haemocytes of the mollusc Biomphalaria glabrata during phagocytosis. The enzyme was subsequently released into the plasma where its role is unknown though alteration of surface properties of foreign particles by the enzyme may aid immunological recognition though a direct role of AcP in cell death can not be ruled out.

Ayvali (1989) stated that the ultracytochemical localization of acid phosphatase was observed particularly in the plasmatocytes and granular cells of Agrotis ipsilon. In the cytoplasm of granular cells, several lysosome-like vacuole series which include varying electro-dense acid phosphatase reaction products appear. Spherule cells and Oenocytoids have the vacuoles a few in number indicating at least weakly acid phosphatase reaction products.

The results indicated that acid phosphatase activity of G. mellonella larvae was increased after 12 and 18 hrs. of infection with S. carpocapsae. This fact was consistent with that obtained
by Xia et al. (2000) using *M. anisopliae var acridum* to control *Schistocerca gregaria*.

Chitinases are enzymes that catalyze the hydrolysis of beta-1,4-N-acetyl-d-glucosamine linkages in chitin polymers. There are various chitinase enzymes present in many living organisms from bacteria to man, with roles in cell wall modification, carbon source degradation and defense against pathogens (Fuhrman, 1995).

Chitinases serve a variety of functions in nature including morphogenesis, nutrition and pathogenesis (Gooday, 1994).

Chen et al. (1996) reported that *X. nematophilus* (three strains), *X. bovienii* (one strain), and *Photorhabdus luminescens* (one strain) showed both exo- and endo chitinase on PAGE gel. Variation in exo-and endochitinase activity among different species and strains was detected with the strongest activity in *X. nematophilus* and the weakest in *Ph. luminescens*.

The increase in chitinase activity after infection with *S. carpocapsae* is attributed to the chitinase released from it’s symbiotic bacteria *X. nematophilus* to digest the chitinous nutrient or to hydrolyze chitinous cell wall in insect (Boemare and Akhurst, 1988 and Forst et al., 1997).

5.2.3 Characterization of protein by polyacrylamide gel electrophoresis in total body tissue homogenate of *G. mellonella* larvae:

This study focused with the changes in total body tissue protein profile during the different interval times (12 and 18 hrs.) following infection of *G. mellonella* larvae with 30IJs of normal and gamma irradiated (2Gy) *S. carpocapsae*. 

Throughout the nematode life cycle, the symbiotic bacteria and the nematode produce a variety of metabolites to enable them to colonize and reproduce in the insect host. These metabolites often have overlapping functions, a strategy that is likely to contribute to the success of the nematode-bacteria association against a variety of insect hosts. The metabolites produced help to avoid the insect immune system, enzymes such as proteases, lipases and phospholipases to maintain a food supply during reproduction (Thaler et al., 1998) an antifungal and antibacterial agents to prevent degradation or colonization of the insect, carcass, while bacteria and nematode reproduce (Akhurst and Dunphy, 1993).

Comparing the protein bands at different time intervals for all tested treatments used revealed the absence of some bands and changes in the intensity of the others, this may be attributed to the virulence materials and proteolytic enzymes which released from X. nematophilus and hydrolyzing protein of G. mellonella larvae, these results coincide with Abdel-Kawy (1981), El-Sadawy (1994), Yussef (2006) and El-Sadawy and Abou El-Dobal (2009).

Also, it is generally believed that protease activity is involved in the breakdown of insect proteins there by providing nutrition for bacterial and nematode growth (Schmidt et al., 1988).

Presence of additional bands at some time intervals may be as a result of the capability of the host to release a protein as a type of defence against the parasite, this agree with Andreadis and Hall (1976), or may be due to protien of the S. carpocapsae
and its symbiotic bacteria invading the host and this is in accordance with Andreeva (1990).

5.3 Ultrastructure of the haemocytes of *G. mellonella* larvae after infection with normal and irradiated *S. carpocapsae*:

Insect larvae usually respond to bacterial or parasite infections with humoral and cellular immune reactions (Götz and Boman, 1985; Hoffmann et al., 1996 and Loker, 1994). Several types of circulating haemocytes carry out the cellular immune responses to pathogens and parasites. These responses include phagocytosis, encapsulation and nodule formation (Götz and Boman, 1985; Götz, 1986 and Gupta, 1991); the type and intensity of the reactions depend on the size, number, and characteristics of the foreign bodies (Dunphy and Thurston, 1990 and Rohloff et al., 1994).

Phagocytosis is the internalization of microorganisms and/or cellular debris by haemocytes (Ling and Yu, 2006). It is considered to be the primary response of haemocytes to small foreign particles and bacteria (Gillespie et al., 1997).

Encapsulation refers to the binding of usually melanizing granular cells to large targets like parasitoids, nematodes and chromatographic beads, materials larger than the haemocytes (Peters and Ehlers, 1997; Pech and Strand, 2000 and Hernandez-Martinez et al., 2002), which are subsequently covered by an overlapping sheath of granular cells and plasmatocytes around a target (Lavine and Strand, 2002).

Nodules are formed by aggregation of haemocytes that entrap the microorganism in an extracellular matrix. This mass
may then undergo melanization that kills the trapped microorganism (Christensen and Severson, 1993).

Most investigators agree that plasmatocytes form the bulk of capsules around foreign bodies too large to be phagocytosed, or nodules around masses of bacteria and necrotic melanised material, in vivo. Capsule and nodule formations look identical at the cytological level (Ratcliffe and Gagen 1976 & 1977 and Lavine and Strand, 2002). In these formations, plasmatocytes synthesize numerous desmosomes and contain large amounts of microtubules in their cytoplasm (Götz, 1986). The role of plasmatocytes in phagocytosis is disputed.

Granular haemocytes have also been shown to be the first cells to come into contact, in small numbers, with a foreign body at the beginning of capsule/nodule formation. When in contact with the foreign body, they release their granular content (Akai and Sato, 1973; Ratcliffe and Gagen, 1977 and Schmit and Ratcliffe, 1977). According to most authors, this exocytosis of typical inclusions by granular haemocytes serves to attract plasmatocytes (Gillespie et al., 1997) or at least help plasmatocytes to build the capsule or nodule (Pech and Strand, 1996). This exocytosis of opsonin-like material is another main function of granular haemocytes.

Neuwirth (1973) found four haemocyte types in the late last larval instar of G. mellonella: (1) plasmatocytes [round to spindle shaped cells, 10-20μ long and 5-10μ wide with cytoplasm containing no distinguishing inclusions, and an abundance of Golgi complexes, rough endoplasmic reticulum and free ribosomes], (2) granular cells [oval shaped cells, 10-20 μ long and 5-10 μ wide, made up of acidic mucosubstances and contain lipid droplets at some stage of their development], (3) spherule cells
[oval shaped, 15-20 µ long and 5-10 µ wide and made up of acidic mucosubstances] and (4) oenocytoids [the largest cells, 20 by 40 µ, with the cytoplasm containing an eccentric nucleus free ribosomes and microtubules].

In the present study, six types of haemocytes were identified in 5th larval instar of *G. mellonella*; Prohaemocytes, Plasmatocytes, Granulocytes, Oenocytoids and Spherulocytes.

The infection of *G. mellonella* with *S. carpocapsae* induced several pathological deformations. During infection, the haemocytes undergo considerable structural changes. The contents of the granules seem to swell giving the cells an extremely vacuolated appearance. Haemocytes that have phagocytized bacteria and/or other foreign particles tend to adhere to one another to contact, and to form aggregations. These unstructured aggregations may later be encapsulated by other haemocytes, or by cells that may be released from the aggregations.

The late stage of bacterial infection, showed infected haemocyte with enlarged cytoplasmic granules having pre-melanosome-like structure in granulocyte. Also, cell membrane is completely lysed and nucleus is severely distorted. Phagocytosing haemocytes contained intracellular *X. nematophilus* and attached bacteria were also observed.

Similar observations were reported by Brayner *et al.* (2007) who found that granular haemocytes, plasmatocytes and Oenocytoids presented morphological alterations indicative of innate immunological activation in mosquitoes infected with *W. bancrofti*. Similarly, Faraldo *et al.* (2008) reported that at 24-h post-injection of *Saccharomyces cerevisae* yeast cells to *Chrysomya megacephala* cell debris and some free yeast cells
were surrounded by granules and electron dense plasmatocytes which were probably initiating the nodulation process.

Also, Vey et al. (2002) investigated that when G. mellonella were injected with toxin secreted by the entomopathogenic fungus *Metarhizium anisopliae var. anisopliae*, the nucleus of granular haemocytes and plasmatocytes showed a pycontic evolution and their cytoplasm was moderately vacuolated, and a few granular haemocytes exhibited dilations of RER and vesicle appeared as partly granulated.

The reason of haemocytes vaculations was explained by Ribeiro et al. (2003) who report that *X. nematophilus* exhibits different cytotoxic activities on insect (*Spodoptera littoralis*) haemocytes. They purified a cytotoxin and called it (α_Xenorhabdolysin). Also, they show that plasma membrane of insect haemocytes is the first target of this toxin. Electrophysiological and pharmacological approaches indicate that the initial effect of α_Xenorhabdolysin on macrophage plasma membrane is an increase of monovalent cation permeability, sensitive to potassium channel blockers. As a consequence, several events can occur intracellularly, such as selective vacuolation of the endoplasmic reticulum, cell swelling, and cell death by colloid-osmotic lysis.

Once the symbiotic bacteria appeared in the haemolymph, they adhere to the haemocyte in order to reproduce within it and in parallel with their re-emergence into the haemolymph; the bacteria induce damage to the haemocytes of Lepidoptera (Dunphy and Thurston, 1990; Dunphy, 1994 & 1995).
In the present study, the ultrastucture studied of the haemocytes show the damage of the haemocytes after *X. nematophilus* reproduce and re-emergence into the haemolymph.

In general, it may be concluded that *S. carpocapsae BA2* used against the pests (*G. mellonella, C. cephalonica* and *E. kuehniella*) and biological activity represented in causing 100% mortality within 3 days. Moreover, the gamma irradiation (2Gy) of *S. carpocapsae* increased the rate of pests mortality to reach 100% within 2 days.

*S. carpocapsae BA2* showed biochemical changes represented in causing reduction in total concentration of proteins, lipids, carbohydrates and urea of *G. mellonella* total body tissue, and the reduction was more in larvae infected with gamma irradiated (2Gy) of *S. carpocapsae*. In contrast, the acid phosphatase and chitinase activity were increased according to the treatments.

Ultrastructural alternations and malformations have been observed in circulating haemocytes of *G. mellonella* larvae treated with normal and gamma irradiated (2Gy) *S. carpocapsae*.

Moreover, gamma radiation is a valuable tool to increase the efficiency of the entomopathogenic nematodes and its symbiotic bacteria.

From the aforementioned discussion, it could be recommended that gamma irradiated (2Gy) of *S. carpocapsae* may contribute in the application of bio- insecticides, which in turn increases the opportunity for natural control of various important stored product pests by entomopathogenic nematodes.
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Summary

The present study deals with the biological effect of gamma irradiated entomopathogenic nematodes on some stored product pests (*G. mellonella, C. cephalonica* and *E. kuehniella*). In addition to, the biochemical changes in the total body tissue of *G. mellonella*.

The obtained results can be summarized as follows:

1. **The biological effect of gamma irradiated entomopathogenic nematodes on *G. mellonella, C. cephalonica* and *E. kuehniella***:

   - The percentage mortality of full grown larvae of the target pests increased with increasing *S. carpocapsae* BA2 concentration and increasing in the time of treatment (giving 100% mortality within 3 days).

   - When the IJs of *S. carpocapsae* BA2 irradiated with 2, 4, 6, 8 and 10 Gy, the percentage mortality gradually increased with the dose increasing and the IJs storage, except at the dose 2 Gy; it was zero, as compared with the control.

   - When full grown larvae of *G. mellonella, C. cephalonica* and *E. kuehniella* treated with 2 and 3Gy gamma irradiated *S. carpocapsae*, the 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* (2 days).
• Larval treatments after 1 and 2 weeks of *S. carpocapsae* irradiation showed similar accumulative mortality (100% mortality after 2 days).

• Treatment of *E. kuehniella* larvae with *S. carpocapsae* immediately irradiated with 2Gy caused 100% mortality at 1st day of the infection.

• *In vivo* production of *S. carpocapsae* decreased when *S. carpocapsae* were irradiated with 2 Gy.

2. The biochemical changes in the total body tissue of *G. mellonella* treated with *S. carpocapsae*:

• The results indicated that *S. carpocapsae* caused a significant decrease in the total body content of proteins, lipids, carbohydrates and urea of 5th larval of *G. mellonella*. Moreover, this decrease was greatly remarkable in larvae treated with gamma irradiated *S. carpocapsae*.

• The results revealed that *S. carpocapsae* caused a significant increase in the acid phosphatase and chitinase activity of the total body of 5th larval of *G. mellonella*. Moreover, this increase was greatly remarkable in larvae treated with gamma irradiated *S. carpocapsae*.

• Protein bands at different time intervals for all tested treatments revealed the absence of some bands, appearance of new bands and changes in the intensity of the bands.
3. **Ultrastructure of G. mellonella haemocytes after infection with normal and irradiated S. carpocapsae:**

- In the present study, five types of haemocytes were identified in 5\(^{th}\) larval instar of *G. mellonella*; Prohaemocytes, Plasmatocytes, Granulocytes, Oenocytoids and Spherulocytes.

- During treatments, Oenocytoids and Spherulocytes vanished from the haemolymph, and the other haemocytes undergo considerable structural changes.

- The prohaemocytes was enlarged or take elongate shape.

- The cell membrane forming thin pseudopodia.

- The contents of the cytoplasm seem to swell and showing an extremely vacuolated cell appearance.

- Haemocytes that have phagocytized the bacteria (*X. nematophila*) tend to adhere to one another to contact, and to form aggregations.

- The cell membrane granular haemocytes were lysed led to the release of the granular content to attach with the foreign bodies at the beginning of capsule/nodule formation.

- Plasmatocytes synthesized numerous desmosomes and contain numerous of microtubule in order to form capsule and nodule.

- More destructive effects were observed in the haemocytes of *G. mellonella* infected with irradiated *S. carpocapsae*. 
الملخص العربي

تعتبر آفات الحبوب المخزونة من أشد الآفات التي تشارك الإنسان غذاؤه والتي لها أثر كبير على اقتصاديات البلاد بما تحدثه من خسائر كبيرة على الحبوب والمواد الغذائية المخزونة وقد تبلغ الخسائر السنوية في العالم نتيجة الاصابة بهذه الآفات بخمسة مليون طن من الحبوب سنوياً.

ونظراً للعديد من المشاكل التي تسببها الطرق المستخدمة حالياً في مكافحة الآفات (المبيدات) والتي من بينها التلوث البيئي والتأثير الضار على صحة الإنسان والحيوان. فضلاً عن تطور المقاومة والمقاومة للآفات المعاملة فقد أظهرت الإتجاهات الحديثة عناية فائقة في التوسع في استخدام طرق أمنة وغير ملوثة للبيئة والتي من بينها استخدام أشعة جاما كتقنية فزيائية لمكافحة الآفات والمكافحة البيولوجية عن طريق استخدام النيماتودا الممرض للحشرات مثل نيماتودا جنس شتينرنيما كاروبكاسي والتي أثبتت فاعلية عالية في مكافحة العديد من الآفات نظراً لكثرته عوائها وقدرتها على التأقلم مع الظروف المتغيرة.

تتناول هذه الدراسة إلقاء الضوء على التأثير البيولوجي للنيماتودا جنس شتينرنيما كاروبكاسي (بي أ 2) المشعة باشعة جاما على بعض آفات المواد المخزونة (جاليريا ميلونيليا، كورسيرا سيفالونيكا واوفستيا كيوتيليا). وكذلك دراسة التغييرات الكيميائية الحيوية في نسيج الجسم الكلي لليرقات جاليريا ميلونيليا.

وكانت النتائج المت الحصول عليها كما يلي:

أولاً: التأثير البيولوجي للنيماتودا جنس شتينرنيما كاروبكاسي (بي أ 2) المشعة باشعة جاما على يرقات بعض آفات المواد المخزونة (جاليريا ميلونيليا، كورسيرا سيفالونيكا واوفستيا كيوتيليا):

- أظهرت الدراسة زيادة نسبة موت اليرقات كاملة النمو للآفات الثلاثة وذلك بزيادة تركيز شتينرنيما كاروبكاسي المستخدم وزادت فترة العدوى (أعطت نسبة إمالة 100 % خلال 3 أيام).
- عند استخدام أشعة جاما بالجرعات 2، 4 و 10 جراماً وجد تزايد تدريجي في نسبة موت الطور المعدي لشتينرنيما كاروبكاسي وذلك بزيادة الجرعة.
الإشعاعية وفترة إجراء التجربة أيام حفظ النيماتودا، ماعدا في الجرعة 3.
- في حالة إصابة البقات كاملة النمو للأفات الثلاثة بشتيرنيما كاروبوكاسي المشععة بالجرعتين 2 و 3 جراي فإنها نشأت في إحداث تأثيرها وذلك بوجود إنخفاض في عدد الأيام اللازمة لموت البقات لتصبح يومين.
- لم يكن هناك تأثير لحفظ أطوار النيماتودا المعدة لمدة أسبوع وإسبوعين من التشبع على عدد الأيام اللازمة لإماتة كل البقات (أعطت نسبة إماتة 100% بعد يومين).
- أدى استخدام شتيرنيما كاروبوكاسي المشععة بالجرعة 2 جراي إلى إحداث نسبة إماتة 100% بعد يوم من العدوى لبقات إفستيا كيبتيلية.
- أدى استخدام الجرعة 2 جراي إلى إنخفاض معدل إنتاج الأطوار المعدة لشتيرنيما كاروبوكاسي.

ثانيا: التغييرات الكيميائية الحيوية في نسيج الجسم الكلي لبقات جالبيريا ميلونيلا المعاملة بشتيرنيما كاروبوكاسي (30 طور معدة/ 5 بقات):
- أظهرت النتائج إنخفاض معنوي في محتوى البروتين، الدهون، الكربوهيدرات والبيوريا في البقات المعدة بشتيرنيما كاروبوكاسي. كما لوحظ زيادة عظمى عند استخدام شتيرنيما كاروبوكاسي المشععة باشعة جاما. كما لوحظ زيادة في إنخفاض النسبة وذلك بزيادة عدد ساعات العدوى.
- كشفت النتائج زيادة ذات دلالة إحصائية في نشاط كل من الإنزيم المحلل للفوسفات الحمضي والإنزيم المحلي لللكتيتين في بقات جالبيريا ميلونيلا المعدة بشتيرنيما كاروبوكاسي. علامة على ذلك، كانت هذه الزيادة كبيرة جدا في البقات المعدة بشتيرنيما كاروبوكاسي المشععة باشعة جاما. وكذلك ازداد نشاط الإنزيمين بزيادة عدد ساعات العدوى.
- عند دراسة تأثير النيماتودا شتيرنيما كاروبوكاسي على انماط شرائط البروتين في بقات جالبيريا ميلونيلا وذلك بعد 12 و 18 ساعة من العدوى بالنيماتودا الغير المشععة والمشععة بالجرعة 2 جراي. أظهرت النتائج أن الاصابة تسببت في احتواء بعض الشرائط وظهور شرائط جديدة أخرى، كما لوحظ تباين في شدة الشرائط نتيجة مختلف المعاملات.
ثالثًا: الترکیب الدقیق لخلايا دم برقات جالیریا میلونیلا بعد العدوى بواسطة شتینرینیما كاربکابسی:

• تم تعيین خمسة أنواع للخلايا في دم برقات الطور الخامس جالیریا میلونیلا وهم:
  1. الخلايا الأولیة
  2. الخلايا البلازمیة
  3. الخلايا الحبیبة
  4. الخلايا الأنوسیة
  5. الخلايا الكرویة

أظهرت الدراسة اخفاء كل من الخلايا الأنوسیة والخلايا الكرویة وحدوث تغيیرات في باقي أنواع الخلايا نتيجة العدوى بالنيماتودا شتینرینیما كاربکابسی:

• ازدياد حجم مع استطالة الخلايا الأولیة.
• تكوین أقدام كاذبة في جدار الخلیة للمساعدة في عملية تحویل النیماتودا.
• تضخم المحتوى السيتوبلازمی مما أعیض الخلايا مظهر مفرغ.
• التصاق وتجمع خلايا الدم الملتزمة للبکتريا (زنورابدا نیماتوفیلا).
• تحلل جدران الخلايا الحبیبة مما أدی إلى خروج محتوياتها للعروف والتصاق بالأجسام الغريبة في بداية تكوین الحويصلات والعقد.
• تخلیق الخلايا البلازمیة للعديد من الیدمومسوم التي تحتوی على قنوات دقيقة لتكوين الكبسولات والعقد حول أفراد النیماتودا.

عند دراسة خلايا دم برقات جالیریا میلونیلا المصابة بالینتیرینیما كاربکابسی المشععة وجد نفس التغيیرات التي تحدثا شتینرینیما كاربکابسی غير مشععة ولكن بنسبة زائدة.
رسالة: دكتوراه الفلسفة في العلوم
اسم الطالبة: رواب محمود سيد علي

عنوان الرسالة: التأثير المشترك لأشعة جاما والنيماتودا المميتة للحشرات على بعض المواد المخزنة

اسم الدرجة: دكتوراه الفلسفة في العلوم

لجنة المناقشة

الأستاذ الدكتور/ عبد العزيز أبو العلا عبد العزيز خضر
أستاذ علم الحشرات - معهد بحوث وقاية النباتات - مركز البحوث الزراعية.

الأستاذ الدكتور/ محمد سالم عبد الواحد
أستاذ علم الحشرات - قسم وقاية نباتات - كلية الزراعة - جامعة عين شمس.

الأستاذ الدكتور/ محمد عادل حسين
أستاذ علم الحشرات - قسم علم الحشرات - كلية العلوم - جامعة عين شمس.

الأستاذ الدكتور/ هداية الله محمود سالم
أستاذ علم الحشرات - رئيس شعبة التكنولوجيا الحيوية - المركز القومي لبحوث و техنولوجيا
الإشعاع - هيئة الطاقة الذرية.

أجوزت الرسالة بتاريخ: ٢٠١١/٦/٢
اسم الطالبة: رحاب محمود سيد علي

عنوان الرسالة: التأثير المشترك لأشعة جاما والنيمتوودا المرضية للحشرات على بعض آفات المواد المخزونة

الدرجة: دكتوراه الفلسفة في العلوم (علم الحشرات)

المشرفون:
1. أ.د./ محمد عادل حسين
2. أ.د./ سريا الطنطاوي حافظ
3. أ.د./ هدياء الله محمود سالم
4. أ.م.د./ مهدي أحمد حسين

الممتحنون:
1. الأستاذ الدكتور/ عبد العزيز أبو العلا عبد العزيز خضر
2. الأستاذ الدكتور/ محمد سالم عبد الواحد
3. الأستاذ الدكتور/ محمد عادل حسين
4. الأستاذ الدكتور/ هدياء الله محمود سالم

تاريخ البحث: 
أجيزة الرسالة: 
مجلس قسم: 
مجلس الكلية: 
مجلس الجامعة: 

جامعتي عين شمس
كلية العلوم
قسم علم الحشرات
اسم الطالبة: رحاب محمود سيد علي
الدرجة: دكتوراه الفلسفة في العلوم
سنة التخرج:
بكالوريوس علوم – علم الحشرات – ٢٠٠١
ماجستير العلوم – علم الحشرات – ٢٠٠٨
القسم التابع له: علم الحشرات
اسم الكلية: كلية العلوم
الجامعة: جامعة عين شمس
شكر وتقدير

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1. أ.د/ محمد عادل حسين
2. أ.د/ سريا الطنطاوي حافظ
3. أ.د/ هداية الله محمود سالم
4. أ.م.د/ مَنَى أحمد حسين

شكر للسادة الذين ساهموا في العمل:

1. قسم بحوث المنتجات الطبيعية – المركز القومي لبحوث وتكنولوجيا الإشعاع.
2. وحدة إنتاج النباتات للمركز القومي للبحوث.
التأثير المشترك لأشعة جاما والنيماتودا الممرضة للحشرات
على بعض آفات المواد المخزونة

رسالة مقدمة لنيل درجة دكتوراه الفلسفة في علم الحشرات
من الطالبة
رحاب محمود سيد علي

بكالوريوس علوم (علم حشرات) - كلية العلوم - جامعة عين شمس - 2001
ماجستير العلوم (علم حشرات) - كلية العلوم - جامعة عين شمس - 2008

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أستاذ النيماتودا المساعد - قسم آفات ووقاية النبات - المركز القومي للبحوث

قسم علم الحشرات
كلية العلوم
جامعة عين شمس
2011