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Gamma Irradiation to Potentiate Some Bio-agent Compounds against the Cotton Leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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ABSTRACT

Efficiency of four bio-agent compounds; *Bacillus thuringiensis (Bt),* kurs.; *Metarhizium anisopltiae,* Metsch.; *Heterorabditis bacteriophora*, Poinar; *Steinernema carpocapsae*, Weiser and chitosan (biopolymer), exposed to gamma irradiation at doses of 15, 30 and 60 Gy to increase its activity on toxicity, biological and life table parameters of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) treated as 4th instar larvae was tested. *Bt* exposed to gamma doses of 15, 30 & 60 Gy had potentiating efficacy on *S. littoralis* than *Bt* used alone without exposing to gamma doses. *M. anisopltiae* and chitosan had nearest results among tested bio-agents singly and the same bioagents exposed to gamma doses. Opposite with *H. bacteriophora* and *S. carpocapsae* had antagonism effect against *S. littoralis* when exposed to aforementioned gamma doses. *Bt* was the most efficient bio agent compound as compared to other treatments on most biological parameters of *S. littoralis,* in addition to increasing larval mortality %. Also, the same treatment decreased pupation, moths' emergence and fecundity percentages, ovipositional period, egg laying rate, especially when treated with *Bt* irradiated with gamma doses of 60 Gy. Other treatments of *M. anisopltiae* and chitosan had effect on biological parameters of *S. littoralis* but the result was nearly from those of the same compounds when exposed to gamma doses. Life table parameters affected by different treatments, especially with *Bt* exposed to gamma dose of 60 Gy. Female progeny/female (Mx), survival rate (Lx), generation time (T), net reproductive rate (Ro), intrinsic rate of natural increase (r_m) and finite rate of increase (e^{rm}) were decreased in comparison to control. Meanwhile, doubling time (DT) was increased compared to control.

Key words: *Spodotera littoralis,* Gamma irradiation, *B. thuringiensis, M. anisopltiae,* chitosan, *H. bacteriophora, S. carpocapsae,* efficiency, life table parameters.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Boisd.) is one of the most important cotton pests in cotton fields in Egypt (Hosny and Isshak, 1967). Its control strategy based mainly on use of synthetic insecticides but they are toxic to animals and human beings, due to their persistence in environment, where numbers of them have carcinogenic and mutagenic effects on human, domestic animals, birds and predators. In order to avoid the insecticidal hazards, there is a great need to develop alternative control agents with new mode of action. Among these agents is the sterile insect technique using gamma irradiation as a genetic control method. Genetic pest suppression is unique among biological methods in it involves the release of genetically modified insects to control the same species (Soon, 1986). Inherited effects of gamma irradiation doses were studied by many authors as Sallam and Ibrahim (1993), Amer (2006 a), Amer, *et al.* (2011) and (2012).

Infectivity of the three entomopathogenic nematodes, *Steinernema feltiae, Steinernema riobrave* and *Heterorhabditis bacteriophora* to *S. littoralis* was studied where *H. bacteriophora* appeared to be more pathogenic than the others to *S. littoralis* larvae (Ahmed *et al.*, 2014).

A biopolymer, chitosan and its derivatives are very attractive for agriculture applications. Chitosan compound might be used as alternative pesticide because it might possess insecticidal activity and non toxic effect to vertebrates and humans (Badawy *et al.*, 2005). The insecticidal activity of chitosan was reported against *S. littoralis* by (Rabea *et al.*, 2003), *Helicoverpa armigera, Plutella xylostella* and aphids (Badawy and El- Aswad, 2012). Also, El-Gendy *et al.* (2014) evaluated toxicity and biochemical effects of chitosan against the peach fruit fly *Bactrocera zonata* (Saund.). Chitosan effected on female and male adults after 24 and 48 hours. Also, it caused inhibition in AChE and ATPase.

The life table parameters can be used as a guide to investigate the pest population development. This is a valid method for assessment of the efficacy of the compounds used (Mohamed, 1987) and to clarify the effect of external factors on growth, survival, reproduction, intrinsic and finite rate of increase of the pest population (Abou-Setta *et al.*, 1986). Life table parameters as affected by bio-agent compounds was also studied by Amer (2006 b), and Amer and El-Nemaky (2008).

The aim of the present study was to evaluate the combined effects of the bio-agents *Bacillus thuringiensis,* kurs.; *Metarhizium anisopltiae,* Metsch.; *Heterorabditis bacteriophora*, Poinar; *Steinernema carpocapsae*, Weiser and the biopolymer chitosan exposed to gamma irradiation at doses of 15, 30 and 60 Gy on toxicity, biological and

life table parameters of the cotton leaf worm, *S. littoralis* treated as 4th instar larvae.

MATERIALS AND METHODS

Bio-agents used

1- Bactericide: *Bacillus thuringiensis* subsp. *Kurstaki (Bt)*: (Biotect) 9.4% WP (32000 IU/mg), produced by Organic for biotechnology company. Dose rate: 300 gm/feddan (2400 IU/ml).

2- Fungicide: *Metarhizium anisopltiae* (Metsch.): (Bio Magic) 1.75% WP ($1x10^8$ CFU·S/gm). Manufacturer Company: M/S. T. Stanes Company Limit- India. Import Company: Gaara Establishment, Import & Export. Dose rate: 10gm/ L Water ($1x10^6$ CFU·S/ml).

3- Nematicides:

a- *Heterorhabditis bacteriophora* (Poinar) (strain BA1) was isolated form a soil sample collected from Nubaryia district (Egypt) and identified by Hussein and Abou El-Soud (2006).

b- *Steinernema carpocapsae* (Weiser) (strain All) was received from Ramon Georgis, Boisys, Palo Alto California, USA. Mass culturing of both nematode species occurred *in vivo* using larvae of *Galleria mellonella L.* as a host Woodring and Kaya (1988)

Both strains were reared *in vivo* on the full-grown larvae of the greater wax moth, *G. mellonella.* Rearing of entomopathogenic nematode, using larvae of *G. mellonella* as a host, was achieved following the methods of Dutky *et al.* (1964).

4- Chitosan (Biopolymer): Chitocare 2.5%, product of Egypt Chemical Company (E.C.C.). Rate dose: 1L/feddan for crop or vegetable fields.

All the bio-agents used were exposed to gamma irradiation doses of 15, 30, & 60 Gy. All irradiations were done by a Cesium^{137} Hendy Cell Research, National Center for Radiation Research and Technology, delivered at a dose rate 0.75/rad/sec.

Target pest

Laboratory strain of the cotton leaf worm, *S. littoralis* 4th instar larvae was reared at Cotton Leaf Worm Department, Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt on castor oil leaves, *Ricinus communis* (L.). Rearing of the insect was conducted following the technique described by El-Defrawi *et al.* (1964). Rearing conditions were adjusted at 27±1°C and 65-75% RH.

Effect of gamma irradiation doses and Bio-agent compounds on *S. littoralis*

Twenty five *S. littoralis* 4th instar larvae, provided with castor oil leaves in Petri-dishes, were exposed to gamma irradiation doses of 15, 30 & 60 Gy. Four replicates for each gamma dose were used beside the control. Larval mortality rate was recorded daily after treatments. Dipping technique was used as the castor oil leaves were dipped in tested bio-agent compounds concentrations of $16x10^8$, $8 x10^8$, $4 x10^8$, $2 x10^8$ & $1 x10^8$ IU/L of *Bt* (Biotect), *Bt* +15 Gy, *Bt* +30 Gy and *Bt* + 60 Gy. Concentrations of $30 x10^8$, $15 x10^8$, $7.5 x10^8$, $3.75 x10^8$& $1.875 x10^8$ CFU·S/L of *M. anisopltiae* (Bio magic), *M. anisopltiae* +15 Gy, *M. anisopltiae* +30 Gy and *M. anisopltiae* +60 Gy. Concentrations of 50, 25, 12.5, 6.25 &3.125 ml/L of Chitosan (Chitocare), Chitosan + 15 Gy, Chitosan + 30 Gy and Chitosan +60 Gy. The control was done by castor oil leaves dipped in water only. Four replicates/ concentration/ tested bio-agent were used. The leaves were left until water evaporated and placed in glass jars (11x22 cm). Each jar received 25 4th instar larvae of *S. littoralis* after starving the larvae for about 4 hours and maintained under 26±1°C. Numbers of alive and dead larvae were counted 3, 5 and 7 days after treatments.

Both *H. bacteriophora* and *S. carpocapsae* nematodes were exposed to gamma irradiation doses of 15, 30 & 60 Gy. Treatments took place in plastic cups (15-9-7cm), filled with sterile sandy soil and covered with plastic lids. The nematodes suspensions (*H. bacteriophora*, *H. bacteriophora* +15 Gy, *H. bacteriophora* +30 Gy, *H. bacteriophora* +60 Gy, *S. carpocapsae*, *S. carpocapsae* +15 Gy, *S. carpocapsae* +30 Gy and *S. carpocapsae* +60 Gy) were poured in vials and mixed with the soil at 5 concentrations of 4000, 2000, 1000, 500 and 250 IJs/cup. Five replicates were used for each concentration. Ten 4th instar larvae were placed into 1cm depth from the surface and then treated with each of the tested nematode species. Numbers of dead larvae were recorded after one week post treatment. The experiments were carried out at 25°C±2 and 55-60±2% R.H., water content in the soil was kept always at 20%. Mortality percentages as a result of gamma irradiation doses exposure were corrected by Abbott (1925).

LC_{50}; LC_{90} and slope values were assessed according to Finney (1971) by using Ldp line software (www.Ehabbakr software/Ldp line). Efficiency of different insecticides could be measured by using Sun's equation (1950) as follows:

Toxicity index = LC_{50} (LC_{90}) of the compound A/ LC_{50} (LC_{90}) of the compound BX100

Where A: is the most effective compound.
B: is the other tested compound.

Biological parameters

Fourth instar larvae of *S. littoralis* treated with

LC_{50}'s of all bio-agents except for nematode treatments because the alive larvae resulted from nematode treatment did not contain infection that affects physiology of the pest; so, *S. littoralis* was considered as well as normal larvae (control). The following biological parameters investigated were as follows:

1- Larval, pupal and moths durations (in days)

2- Pupation and moths emergency percentages

% Pupation= No. produced pupae/Total tested l arvae X100

% Moths emergency = No. emerged moth/total tested larvae X100

3- Larval mortality percentage: Larval mortalities were corrected according to Abbott's formula (1925).

4- Pre-oviposition, oviposition and post-oviposition periods: were determined by three replicates. Each one contained 5 pairs of emerged moths in a clean glass cages (17 cm height and 7-12 cm in diameter) till death of female moth.

5- Egg laying rate: total number of batches per female. Each batch of eggs was counted by using a binocular was calculated from daily counts of deposited eggs on piece of paper in glass cages. Each treatment data yielded through the daily egg production and on the differential survival of females.

6- Fecundity percentage: % Fecundity= No. eggs per treated female/ No. eggs per untreated female X 100

9- Life span: This period extended from egg deposited until moths death (in days).

All biological parameters of *S. littoralis* were analyzed using Costat statistical program software, 1990 and Duncan's multiple range test (Duncan, 1955) at 5% probability level to compare the differences among time means.

Life table parameters

Changes in life table parameters of *S. littoralis* after treatment as 4th instar larvae with gamma irradiation doses; 15,30&60 Gy, in addition to the LC_{50} values of *Bt*, *M. anisopltiae* and Chitosan and their combinations with gamma doses; 15,30&60 Gy were studied. Data of life table were analyzed by a computer program developed by (Abou-Setta *et al.*, 1986).

RESULTS AND DISCUSSION

Larval mortality rates depended on gamma doses. Dose of 60 Gy had the highest larval mortality, followed by 30 and 15 Gy, respectively (Table 1).

Amer (2006 a) mentioned that gamma irradiation treatments did not differ significantly in larval mortality of the cotton pink bollworm *Pectinophera gossypiella*; where it ranged between 39.04 – 48.44 % for 5 -80 Gy. This may be due to its effect on the acetyl cholin esterase according to feeding of the newly hatched larvae on the irradiated media. Also, Amer, *et al.* (2011) found that gamma irradiation doses of 100, 200 and 300 Gy increased larval mortality of *S. littoralis* treated as 4th instar larvae that was hundred percent at 12-day post treatment. In addition, Amer, *et al.* (2012) showed that tested γ-irradiation doses of 150, 250 and 350 Gy increased larval mortality of *S. littoralis* treated as 4th instar larvae reaching 100% at 10th day after treatment.

Table (1): Effect of gamma irradiation doses on larval mortality of *S. littoralis* treated as 4th instar larvae

Gamma Doses (Gy)	% Larval mortality after			
	4-day	6-day	8-day	10-day
Control	0^{c}	0^{c}	0^{d}	0^{c}
15	20^{b}	30^{b}	42^{c}	60^{b}
30	23^{b}	32^{ab}	52^{b}	63^{b}
60	28^{a}	35^{a}	58^{a}	69^{a}
$LSD_{0.05}$	3.26	2.46	4.15	3.87

Means in the same row followed by the same letter are not significantly different at $p<0.05$.

Table (2) shows that LC_{50} of *Bt* was 1133 $x10^{6}$ IU/L against 4th instar larvae of *S. littoralis*. On the other hand, when *Bt* was exposed to gamma irradiation, it showed potentiating efficacy, where LC_{50} was decreased to 810.2 $x10^{6}$ IU/L (*Bt* + 15 Gy), 337.9 IU/L (*Bt* + 30 Gy) and 163.9 $x10^{6}$ IU/L (*Bt* + 60 Gy) 3 days post larval treatments. The LC_{50} value of *Bt* decreased as time increased up to 7- day from treatment. Also, *Bt* + 60 Gy was considered the most efficacious compound against 4th instar larvae, followed by *Bt* + 30 Gy, *Bt* + 15 Gy and then *Bt* non-irradiated that had the least efficacy compared to the same compounds exposed to gamma radiation.

Obtained results were confirmed previously by Amer (2006 a) who mentioned that the combination of gamma irradiation with Dipel2x activated the spores of biocide compound and caused a potentiating effect on *P. gossypiella* larvae ingestion. Also, Amer *et al.* (2012) showed that LC_{50} value of *S. littoralis* treated with Protecto (*Bt)* exposed to gamma doses were lower than those of un-irradiated Protecto.

The fungus, *M. anisopltiae* effected 4th instar larvae (LC_{50}: 62.23 $x10^{8}$ CFU·S/L), but when *M. anisopltiae* was exposed to gamma doses, it had little increase in its efficacy compared to *M. anisopltiae* when it was applied alone (Table 3). In addition, after 5- days from treatment, the LC_{50} of *M. anisopltiae* decreased until 7- day that reached the least LC_{50} (Table 3). Present results are nearly those of Amer, *et al.* (2011) who reported that gamma irradiation doses

Table (2): Efficacy of *B. thuringiensis* exposed to different gamma doses against *S. littoralis* treated as 4th instar larvae

Treatments	LC_{50} (IU/L) 95%Confidence limits	LC_{90} (IU/L) 95%Confidence limits	Slope	Toxicity index	
				LC_{50}	LC_{90}
		3- days post treatment			
B. thuringiensis	1133 x10^6 965.1 x10^6 ± 1551x10^6	2719x10^6 1934 x10^6±4497x10^6	1.33	14.5	23.8
B. thuringiensis + 15 Gy	810.2 x10^6 581.8 x10^6±1257x10^6	2247x10^6 1639x10^6±3165x10^6	1.76	20.2	28.8
B. thuringiensis + 30 Gy	337.9 x10^6 136.9 x10^6±643.8 x10^6	1682x10^6 877.1x10^6±2669x10^6	1.88	48.5	38.5
B. thuringiensis + 60 Gy	163.9 x10^6 29.7 x10^6±484.2 x10^6	647.7 x10^6 391.7 x10^6±1748x10^6	1.98	100	100
		5- days post treatment			
B. thuringiensis	6 x10^8 463 x10^6±904.3 x10^6	2417 x10^6 1606x10^6±5145 x10^6	1.29	18.3	64.1
B. thuringiensis + 15 Gy	454.7 x10^6 144.7 x10^6±909.1 x10^6	2196 x10^6 1262 x10^6±2781 x10^6	1.33	24.1	70.6
B. thuringiensis + 30 Gy	259.9 x10^6 65.31 x10^6±553.9 x10^6	1869 x10^6 1006 x10^6±2578 x10^6	1.57	42.1	82.9
B. thuringiensis + 60 Gy	109.5 x10^6 4.832 x10^6±272.4 x10^6	1549 x10^6 583.7x10^6±2206 x10^6	1.98	100	100
		7- days post treatment			
B. thuringiensis	159.4 x10^6 114 x10^6±219.9 x10^6	479.4 x10^6 332.2x10^6±803.8x10^6	1.96	5.06	28.3
B. thuringiensis + 15 Gy	124.5 x10^6 3.872 x10^6±260.5 x10^6	327.4 x10^6 49.92x10^6±559.4x10^6	2.12	6.48	41.5
B. thuringiensis + 30 Gy	36.54 x10^6 0.672 x10^6±352.6 x10^6	242.9 x10^6 29.54x10^6±656.9x10^6	2.14	22.1	55.9
B. thuringiensis + 60 Gy	8.064 x10^6 1.76 x10^6±46.43 x10^6	135.8 x10^6 28.1 x10^6±533.4 x10^6	2.24	100	100

Table (3): Efficacy of *M. anisopltiae* exposed to gamma doses against *S. littoralis* treated as 4th instars larvae

Treatments	LC_{50} (CFU·S /L) 95%Confidence limits	LC_{90} (CFU·S /L) 95%Confidence limits	Slope	Toxicity index	
				LC_{50}	LC_{90}
		3 days post treatment			
M. anisopltiae	62.23 x10^8 32.55x10^8±90.38x10^8	950.40 x10^8 340.2x10^8±1192.3x10^8	0.5	96.8	99.7
M. anisopltiae +15 Gy	62.1 x10^8 32.56x10^8±90.42x10^8	950.3 x10^8 340.2x10^8±1191.3x10^8	0.52	97.0	99.7
M. anisopltiae +30 Gy	61.41 x10^8 30.42x10^8±89.58x10^8	949.4 x10^8 320.9x10^8±1189.4x10^8	0.56	98.0	99.8
M. anisopltiae +60 Gy	60.22 x10^8 30.12x10^8±87.87x10^8	947.3 x10^8 310.4x10^8±1177.7x10^8	0.62	100	100
		5 days post treatment			
M. anisopltiae	6.213 x10^8 3.213x10^8±12.24x10^8	240.36 x10^8 120.2 x10^8±390.3 x10^8	0.45	84.4	95.4
M. anisopltiae +15 Gy	6.112 x10^8 3.013x10^8±11.11x10^8	238.2 x10^8 116.6 x10^8±290.2 x10^8	0.48	85.8	96.2
M. anisopltiae +30 Gy	5.987 x10^8 1.568x10^8±10.88x10^8	232.4 x10^8 108.8 x10^8±284.4 x10^8	0.51	87.6	98.6
M. anisopltiae +60 Gy	5.243 x10^8 1.223 x10^8±8.89 x10^8	229.2 x10^8 102.3 x10^8±279.7 x10^8	0.54	100	100
		7 days post treatment			
M. anisopltiae	4.212 x10^8 1.212x10^8±8.231x10^8	47.365 x10^8 26.36x10^8±140.35x10^8	0.86	71.7	89.7
M. anisopltiae +15 Gy	4.112 x10^8 1.123 x10^8±7.45 x10^8	46.58 x10^8 25.4 x10^8±139.9 x10^8	0.89	73.5	91.2
M. anisopltiae +30 Gy	3.895 x10^8 0.983x10^8±5.483x10^8	45.21 x10^8 21.12 x10^8±133.4 x10^8	0.90	77.6	94.0
M. anisopltiae +60 Gy	3.021 x10^8 0.783x10^8±6.63 x10^8	42.50 x10^8 18.18 x10^8±119.9 x10^8	0.91	100	100

Table (4): Efficacy of Chitosan compound exposed to gamma doses against *S. littoralis* treated as 4th instars larvae

Treatments	LC_{50} (ml/L) 95%Confidence limits	LC_{90} (ml/L) 95%Confidence limits	Slope	Toxicity index	
				LC_{50}	LC_{90}
		3 days post treatment			
Chitosan	24.41 18.88 ±40.28	50.81 32.21 ±80.56	3.1	77.1	81.9
Chitosan +15 Gy	21.22 15.46±35.38	47.98 30.35±73.87	3.1	88.7	86.7
Chitosan +30 Gy	20.45 13.25±33.54	43.68 25.45±73.75	3.2	92.0	95.2
Chitosan +60 Gy	18.82 10.89±30.98	41.59 21.98±70.70	3.2	100	100
		5 days post treatment			
Chitosan	20.42 13.32±38.89	41.42 21.21±53.22	2.8	53.8	84.2
Chitosan +15 Gy	18.75 11.81±35.53	40.59 20.32±50.40	2.81	58.6	85.9
Chitosan +30 Gy	14.88 8.898±31.98	37.88 18.89±47.79	2.82	73.8	92.0
Chitosan +60 Gy	10.98 5.895±23.32	34.86 15.56±42.65	2.82	100	100
		7 days post treatment			
Chitosan	12.34 8.895±29.98	29.99 16.65±40.21	2.4	71.9	68.2
Chitosan +15 Gy	11.56 6.341±25.54	27.98 14.53±36.40	2.41	76.7	73.1
Chitosan +30 Gy	9.988 4.983±20.20	23.39 12.28±34.30	2.45	88.8	87.6
Chitosan +60 Gy	8.868 1.486±18.89	20.45 10.46±32.98	3.1	100	100

Table (5): Efficacy of *H. bacteriophora* and *S. carpocapsae* nematodes exposed to gamma doses against *S. littoralis* 4th instar larvae after one week

Treatments	LC_{50} (IJs/Cm^2) 95%Confidence limits	LC_{90} (IJs/Cm^2) 95%Confidence limits	Slope	Toxicity index	
				LC_{50}	LC_{90}
		H. bacteriophora			
H. bacteriophora	720.8 614.7 ±846.4	3263.7 2410.5 ±5105.3	1.95	100	100
H. bacteriophora + 15 Gy	837.9 728.1±960.3	3317.6 2672.8±4393.5	2.14	86.02	98.4
H. bacteriophora + 30 Gy	1030.1 887.6 ± 1196.6	4765.6 3678.9 ±6732.3	1.92	69.9	68.5
H. bacteriophora + 60 Gy	1117.83 964.39 ±1300.3	5141.3 3950.5±7312.6	1.93	64.5	63.5
		S. carpocapsae			
S. carpocapsae	765.97 664.93±875.68	3039.8 2480.6±3943.2	2.14	100	100
S. carpocapsae +15 Gy	906.95 788.37±1040.8	3647.8 2919.5 ±4877.1	2.12	84.5	83.3
S. carpocapsae +30 Gy	1047.3 904.44±1214.4	4715.7 3657.7±6612.4	1.96	73.1	64.5
S. carpocapsae +60 Gy	1379.3 861.83 ±2467.7	5135.9 4302.5±18526.6	2.24	55.5	59.2

Table (6): Effect of tested bio-agents on some biological parameters of *S. littoralis* treated as 4th instar larvae

Treatments	Larval Duration (days)	Larval Mortality %	Pupal Duration (days)	% Pupation	Moths duration (days) ♂	Moths duration (days) ♀	% Moths emerged
Control	20 b	1 g	11 abc	99 a	14 a	20 a	97 a
15 Gy	22 ab	60 ef	15 a	40 bc	5 c	15 bcde	30 c
30 Gy	22 ab	63 de	15 a	37 cd	13 b	13 de	27 c
60 Gy	22 ab	69 c	15 a	31 c	13 b	8 f	18 fgh
B. thuringiensis	22 ab	66 cd	10 bc	34 de	14 b	20 a	24 cd
B. thuringiensis + 15 Gy	23 ab	80 b	13 abc	20 f	14 b	20 a	15 h
B. thuringiensis + 30 Gy	23 ab	80 b	10 bc	20 f	14 b	20 a	10 i
B. thuringiensis + 60 Gy	22 ab	87 a	14 ab	13 g	15 b	19 ab	8 i
M. anisopltiae	22 ab	58 f	11 abc	42 b	14 b	20 a	34 b
M. anisopltiae +15 Gy	20 b	68 c	10 bc	32 c	13 b	18 abc	22 de
M. anisopltiae +30 Gy	20 b	78 b	10 bc	22 f	13 b	16 abcd	17 gh
M. anisopltiae +60 Gy	19 b	58 f	10 bc	42 b	20 a	14 cde	30 c
Chitosan	19 b	68 c	9 c	32 c	12 b	17 abcd	20 fg
Chitosan +15 Gy	22 ab	68 c	11 abc	32 c	11 b	16 abcd	20 fg
Chitosan +30 Gy	22 ab	68 c	11 abc	32 c	11 b	14 cde	18 fgh
Chitosan +60 Gy	25 a	68 c	11 abc	32 c	13 b	11 ef	15 h
LSD 0.05	4.56	6.86	3.65	4.89	2.98	3.54	4.65

Table (7): Effect of tested bio-agents on some biological parameters of *S. littoralis* treated as 4th instar larvae

Treatments	Longevity of female moths (days) Pre-oviposition period	Longevity of female moths (days) oviposition period	Longevity of female moths (days) Post-oviposition period	No. of batches (No. of egg/ female)	Fecundity %	Life span (days)
Control	2 a	13 a	5 cdef	5 (850) a	100 a	51 bcd
15 Gy	2 a	8 bc	5 cdef	2 (200) j	23.5 i	50 bcde
30 Gy	2 a	5 cd	6 bcde	1 (100) k	11.8 j	53 ab
60 Gy	2 a	3 d	3 ef	1 (40) l	4.71 k	50 bcde
B. thuringiensis	2 a	11 ab	7 abcd	3 (492) e	57.9 bc	52 bc
B. thuringiensis + 15 Gy	2 a	10 ab	8 abc	3 (450) g	52.9 f	53 ab
B. thuringiensis + 30 Gy	2 a	9 b	9 ab	2 (300) i	35.3 h	56 a
B. thuringiensis + 60 Gy	2 a	8 bc	9 ab	2 (100) k	11.8 j	56 a
M. anisopltiae	2 a	8 bc	10 a	5 (780) b	91.8 b	50 bcde
M. anisopltiae +15 Gy	2 a	10 ab	6 bcde	5 (750) c	88.2 c	47 e
M. anisopltiae +30 Gy	2 a	8 bc	6 bcde	3 (375) h	44.1 g	48 de
M. anisopltiae +60 Gy	2 a	8 bc	4 def	2 (300) i	35.3 h	53 ab
Chitosan	2 a	10 ab	5 cdef	5 (750) c	88.2 c	53 ab
Chitosan +15 Gy	2 a	9 b	5 cdef	2 (600) d	70.6 d	49 cde
Chitosan +30 Gy	2 a	7 bc	5 cdef	2 (475) f	55.9 ef	43 f
Chitosan +60 Gy	2 a	7 bc	2 f	2 (375) h	44.1 g	48 de
LSD 0.05	0	3.12	3.56	12.56	10.36	4.65

Table (8): Life table parameters of *S. littoralis* treated as 4th instar larvae with gamma doses and LC_{50} of tested bio-agents

Treatments	T (days)	(Ro)	Increase rate r_m	Increase rate e^{rm}	DT (days)	Sex ratio
Control	37.21 cde	425 i	0.31 a	1.36 a	2.24 h	0.5 a
15 Gy	43.34 a	100 j	0.22 f	1.25 f	3.15 g	0.5 a
30 Gy	42.99 ab	50 k	0.217 f	1.242 f	3.19 f	0.45 a
60 Gy	42.99 ab	20 m	0.185 g	1.203 g	3.75 e	0.6 a
B. thuringiensis	38.77 bcd	250 e	0.275 bcd	1.316 c	2.52 k	0.59 a
B. thuringiensis + 15 Gy	39.97 abc	226 f	0.28 bc	1.317 c	2.48 i	0.53 a
B. thuringiensis + 30 Gy	42.5 ab	140 i	0.25 de	1.28 de	2.77 i	0.43 a
B. thuringiensis + 60 Gy	42.3 ab	50 k	0.23 ef	1.26 ef	3.01 h	0.5 a
M. anisopltiae	36.52 cde	385 a	0.29 ab	1.34 ab	2.39 m	0.46 a
M. anisopltiae +15 Gy	35.29 de	270 d	0.29 ab	1.362 a	2.39 m	0.38 a
M. anisopltiae +30 Gy	37.60 cde	190 h	0.276 bcd	1.318 bc	2.51 ki	0.52 a
M. anisopltiae +60 Gy	39.17 abcd	140 i	0.260 cd	1.297 cd	2.67 j	0.43 a
Chitosan	42.32 ab	370 b	0.149 h	1.161 h	4.65 b	0.48 a
Chitosan +15 Gy	39.58 abcd	300 c	0.162 gh	1.175 h	4.28 c	0.5 a
Chitosan +30 Gy	34.18 e	250 e	0.179 g	1.197 g	3.87 d	0.62 a
Chitosan +60 Gy	39.09 abcd	200 g	0.119 i	1.127 i	5.82 a	0.56 a
LSD 0.05	4.32	5.89	0.025	0.022	0.036	0.207

(T) = The generation time (Ro) =The net reproductive rate (r_m) = The intrinsic rate of natural increase
(e^{rm}) = The finit rate of increase (DT) = The doubling time.

of 100, 200 and 300 Gy had antagonism effect on biover efficacy against cotton leaf worm 4th instar larvae and gamma doses used had sub lethal doses higher than untreated biover.

Biopolimer, chitosan had efficacy on 4th instar larvae and LC_{50} was 24.41 m/L. When chitosan was exposed to gamma doses 15- 60 Gy, its efficacy had medium increase reaching 18.82 m/L after 3- days from treatment, in case of chitosan +60 Gy. The same effect appeared clearly at 5- 7 days after treatments. El-Gendy, *et al.* (2014) stated that chitosan gave inhibition of Ach.E and ATPase activities of *B. zonata*.

Table (5) clearly showed that LC_{50} of *H. bacteriophora* and *S. carpocapsae* was increased and the efficacy of entomopathogenic effect decreased with exposing to gamma doses against *S. littoralis* treated as 4th instar larvae one week post treatment. Toxicity index recorded 100% in both entomopathogens used singly compared to the same exposed to gamma doses of 15, 30 & 60 Gy. Gouge *et al.* (1998) studied the interactions between F1 progeny of *P. gossypiella* adults irradiated in the pupal stage and entomopathogenic nematodes. Both sexes of the pupae were exposed to 4, 8, 12 or 16 krad sub sterilizing radiation doses. The F1 larvae were tested in a small bioassay for susceptibility to *S. riobravis*, *S. carpocapsae* and 2 strains of *H. bacteriophora*. Numbers of infecting nematodes were counted after 48h. Increasing parental radiation dose significantly increased F1 larval susceptibility to *S. riobravis* and *H. bacteriophora*, but decreased susceptibility to *S. carpocapsae*. Nouh and Hussein (2013) studied the efficacy of Egyptian strain (BA1) and exotic European strain (Hb1-3) of entomopathogenic nematode; *H. bacteriophora* against full grown larva of *G. mellonella* and concentration dependent. Saleh *et al.* (2015) found that two Egyptian isolates of EPNs; *H. bacteriophora* BA1 and *S. carpocapsae* BA2 in laboratory, semi-field and corn field bioassays against larvae of *S. littorallis* and *Agrotis ipsilon* that infesting corn plants. Tested adjuvant had no adverse effects against nematode survival or infectivity. Some adjuvant significantly improved the performance of the tested nematodes in both semi-field and field experiments. Combinations of more than one adjuvant were more efficient than single adjuvant.

Biological parameters of *S. littoralis* treated with bio-agents

1-Larval, pupal and moth durations

Table (6) shows that larval duration (from newly hatched until pupation) increased when *S. littoralis* treated as 4th instar larvae increased as compared to the control (20 days) with those treated by LC_{50}'s of most tested bio-agents. While, *M. anisopltiae* and chitosan treatments decreased the larval duration about one day than control, the value was 19 days for both treatments (Table 2). Treatments of *M. anisopltiae* +15 Gy and *M. anisopltiae* + 30 Gy had the same values of control. In addition, larval duration increased about 2-3 days compared to control at the three tested gamma doses and *Bt* or chitosan un-irradiated and with exposing each to the different gamma doses. The same trend was observed for pupal duration of *S. littoralis* that increased, especially in 15, 30 & 60 Gy treatments which had the highest increase (15 days), followed by *Bt* + 60 Gy (14 days) and then *Bt* + 15 Gy (13 days). Meanwhile, *Bt* + 30 Gy, *M. anisopltiae* +15 Gy, *M. anisopltiae* +30 Gy and *M. anisopltiae* +60 Gy (10 days) and chitosan (9 days) compared to the control value that was 11 days as *M. anisopltiae*, Chitosan +15 Gy, Chitosan +30 Gy and Chitosan +60 Gy (11 days) as in (Table 6). Treatments with *Bt*, *Bt* + 15 Gy, *Bt* + 30 Gy and *M. anisopltiae* had the same recorded data for male and female moth longevity as those of control value of 14 days for males and 20 days for females of *S. littoralis* treated as 4th instar larvae. Other treatments caused sometimes increase or decrease in male and female moth durations compared to control.

2- Pupation and moth emergency percentages

Pupation percentages were affected by the different compound treatments (Table 6). The pupation percentage decreased, especially with *Bt* + 60 Gy treatment (13%), followed by *Bt* + 30 Gy as *Bt* + 15 Gy (20%) and then *M. anisopltiae* (22%) as compared to normal pupation in the control (99%). In addition, other treatments decreased the pupation between 31 and 42%. Moth emergency percentage was affected where *Bt* + 60 Gy was the most effective treatment (8%), followed by *Bt* + 30 Gy (10%); while, *Bt* + 15 Gy and chitosan +60 Gy had the same effect (15%). Other compounds affected moths' emergence by 17 to 34%, compared to control (97%).

3- Larval mortality percentage

Bt + 60 Gy was the (87%), followed by *Bt* + 15 Gy as well as *Bt* + 30 Gy (80%) and then 78% larval mortality in *M. anisopltiae* +30 Gy treatment (Table 6).

Ovipositional period of normal *S. littoralis* was 13 days; this value was decreased to about 10 days in the females initiated from 4th instars larvae treated by 60 Gy, followed by 30 Gy. Treatments with chitosan +30 Gy as of chitosan +60 Gy (7 days); while, the treatments of 15 Gy, *Bt* + 60 Gy, *M. anisopltiae*, *M. anisopltiae* +30 Gy and *M. anisopltiae* +60 Gy had the same ovipositional period (8 days). Other treatments had decreased the ovipositional period by 9-11 days compared to control (13 days). Gamma dose of 60 Gy alone or when exposed to chitosan gave nearly result for the post-ovipositional period. The

values were 3 and 2 days, respectively, decreased about 2-3 days than the control (5- days) (Table 7). Dose of 15 Gy resulted the same postovipositional period (5-days) as of control value. Other treatments caused increasing in postovipositional period ranged from 6 to 10 days compared to control.

5- Egg laying rate

The egg laying rate of *S. littoralis* normal females in control recorded 850 eggs/ female as shown in table (7). This value was decreased to 40 eggs/female for those treated as 4th instar larvae by dose of 60 Gy, followed by dose of 30 Gy as well as *Bt* + 60 Gy (100 eggs/female). Also, other treatments decreased egg laying rate/ female from 200 to 780 eggs/female compared to control.

6- Fecundity percentage

Dose of 60 Gy had the lowest fecundity percent (4.71%), followed by the dose of 30 Gy similar to *Bt* + 60 Gy gave fecundity percentage (11.8%) when the adult moths were initiated from treated *S. littoralis* 4th instar larvae as shown in (Table 7) compared to control value (100%). Other treatment caused fecundity percentages ranged from 23.5 to 91.8%.

7- Life span

The normal *S. littoralis* life span was 51 days. This period decreased in most treatments (47–50 days), especially in chitosan +30 Gy treatment (43 days). Opposite was in treatments of gamma dose of 30 Gy, *Bt*, *Bt* + 15 Gy, *Bt* + 30 Gy, *Bt* + 60 Gy, *M. anisopltiae* +60 Gy and chitosan where it increased from 52 to 56 days. Significant difference appeared among treatments in most biological parameters, especially with gamma doses alone or combined with *Bt* treatments and *M. anisopltiae* or chitosan combined with 60 Gy, while, other treatments showed low significant difference. Results obtained agree with those of Amer (2007) who mentioned that Dipel-2x exposed to gamma doses of 5 to 80 Gy increased the pupal and adult longevity, life cycle and the percentages of larval and pupal mortality and sterility. On the other hand, it decreased egg laying and egg hatchability of *P. gossypiella* treated as newly hatched larvae.

Life table parameters of *S. littoralis* treated by bio-agents

1-Female progeny/female (Mx) and rate of survival (Lx)

Figures (1&2) showed that female progeny/female (Mx) of untreated *S. littoralis* ranged between 17.5 to 416.67, while the last values drastically decreased in treated females, especially by dose of 60 Gy; as it ranged between 6 to 34 female progeny/female, followed by *Bt* + 60 Gy (Mx: 3.75 to 50 female progeny/female) and chitosan +60 Gy (Mx: 14-67 female progeny/female). Moreover, it ranged between 9 to 77.5 females progeny/female in 30 Gy treatment, followed by dose of 15 Gy treatment (Mx: 6.75-100 female progeny/female), *Bt* + 30 Gy (Mx: 71.5-129 female progeny/female) and *M. anisopltiae* +60 Gy (Mx: 8.60-129 female progeny/female). Other treatments had medium effect on (Mx) female progeny/female initiated from *S. littoralis* 4th instar larvae at different treatments compared to control. The (Lx) parameter (rate of survival) ranged between 14.79 to 100 times in *S. littoralis* untreated females (Figure 1). The Lx of females treated as 4th instar larvae by gamma doses of 15, 30 & 60 Gy ranged from 50-100 times. While, in *Bt* treatments, Lx ranged from 17.5-100 times. On the other hand, in *M. anisopltiae* treatments, survival rate ranged from 8.33 to 100 times that could be considered the most decreased compared with other different treatments. Also, survival rate of females developed from 4th instar larvae of *S. littoralis* treated by chitosan had survival rate ranged between 18 to 90 times.

2-Generation time (T)

S. littoralis, treated as 4th instar larvae by gamma dose of 15 Gy, spent a generation time of 43.34 days as in table (8), followed by the treatments of gamma doses of 30 & 60 Gy, *Bt* +30 Gy, *Bt* +60 Gy and chitosan (nearly to 42 days). Also, treatments of *Bt*, *Bt* +15 Gy, *M. anisopltiae* +60 Gy, chitosan +15 Gy and chitosan +60 Gy had generation time ranged from 38.77 to 39.97 days. While, treatments of chitosan +30 Gy, *M. anisopltiae* +15 Gy and *M. anisopltiae* caused reduction in generation time days compared to control. Treatment of *M. anisopltiae* + 30 Gy (37.60) had generation time nearly as the same result of control (37.21 days).

3- Net reproductive rate (Ro)

The tested gamma irradiation, chitosan and bio-agents caused high reduction in female capacity to increase the population in each generation when *S. littoralis* was treated as 4th instar larvae as shown in table (8), especially in gamma doses treatments (Ro: 20, 50 & 100 females/ female in one generation for gamma doses of 60, 30 & 15 Gy, respectively), followed by *Bt* treatments that had net reproductive rate ranged from 50 to 250 females/ female in one generation. While, *M. anisopltiae* and chitosan treatments had nearest results ranged from 140-385 females/ female in one generation compared to the untreated *S. littoralis* (425 females/female).

4- Increase rate

4.1- Intrinsic rate of natural increase (r_m)

Table (8) shows that intrinsic rate of natural increase (r_m) where the ability of inheriting increase of *S. littoralis* untreated female was 0.31 times/female/day. While, the females treated as 4th instar larvae with chitosan and irradiated dose of

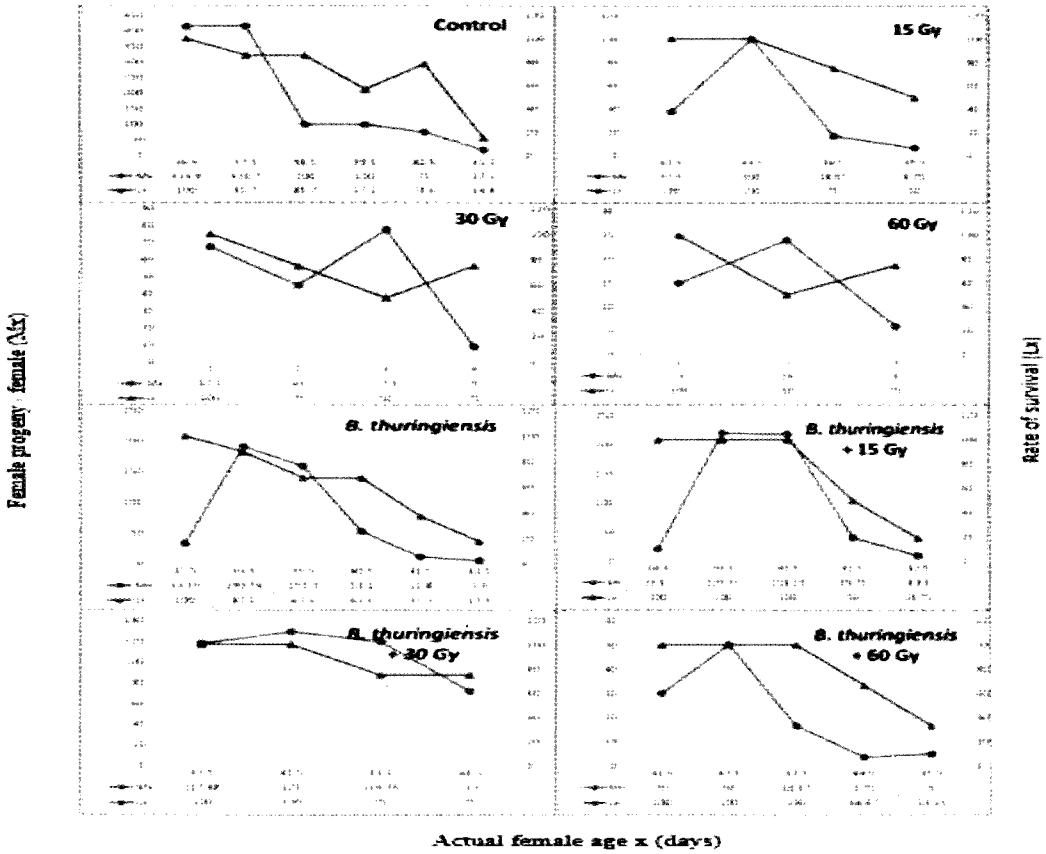

Fig. (1): Effect of gamma doses and bio-agents on female progeny/ female (Mx) and survival rate (Lx) of *S. littoralis*.

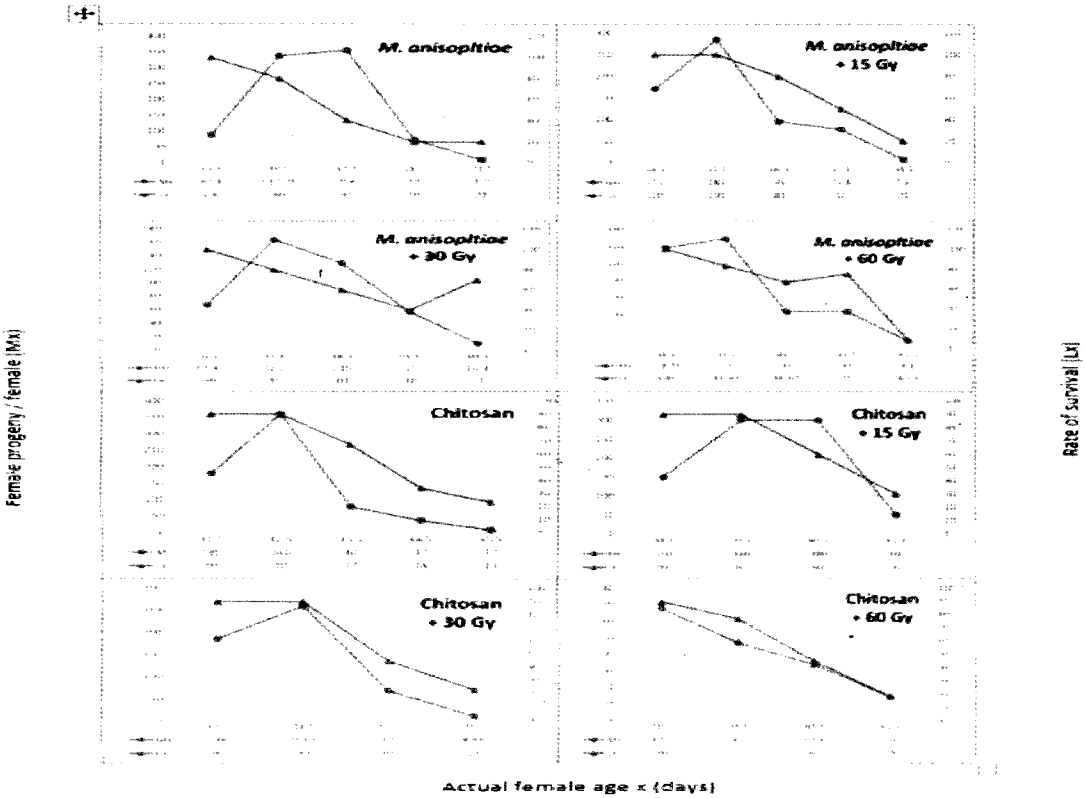

Fig. (2): Effect of gamma doses and bio-agents on female progeny/ female (Mx) and survival rate (Lx) of *S. littoralis*.

60 Gy reduced r_m values ranged from 0.119-0.185 times/female/day. On the other hand, other treatments had the least reduction in intrinsic rate from 0.22 to 0.29 times/ female/ day.

4.2- Finit rate of increase (e^{rm})

Daily population of untreated *S. littoralis* had increased to 1.36 times/female/day as represented in table (8). Also, the females developed from 4th instar larvae treated with *Bt, Bt* + 15 Gy and *M. anisopltiae* had capacity ranged from 1.316 to 1.362 times/female/day close to the control. The opposite was in chitosan treatments that had decreased in finit rate of increase ranged from 1.127 to 1.197 times/female/day. Other treatments had intermediate reduction from 1.203 to 1.297 times/female/day.

5- Doubling time (DT)

The time for population to become twice, that mean doubling time (DT) depends on the intrinsic rate of natural increase (r_m) which could be affected by many factors as the rate of survival, generation time, female in progeny and fecundity. *S. littoralis* in the control (non-treated) had populations that multiply every 2.24 days as in table (8). These days increased from 4.28 to 5.82 days when *S. littoralis* treated as 4th instar larvae by chitosan treatments, followed by the exposure to gamma doses that had ranged from 3.15 to 3.75 days. While, *Bt* and *M. anisopltiae* treatments had the least increase where ranged from 2.48 to 2.77 days that had values close to the control time (2.24 days) to multiply.

6- Sex ratio

Sex ratio was calculated as females/ total. In control (non-treated), it was 0.5. This ratio in case of the cotton leaf worm treated as 4th instar larvae with gamma dose of 60 Gy and chitosan + 30 Gy, increased compared to control. The opposite occurred for sex ratio after the treatment of gamma doses of 30 Gy, *Bt* +30 Gy, *M. anisopltiae*, *M. anisopltiae* +15 Gy, *M. anisopltiae* +60 Gy and chitosan where it decreased than control value that ranged from 0.38 to 0.47, while, other treatments had sex ratio close to control value. There were significant differences among treatments in the most life table parameters, especially with gamma doses used alone or in combination with *Bt* treatments and *M. anisopltiae* or chitosan when combined with 60 Gy, while, other treatments presented low significant differences. The aforementioned result agreed with those of Amer (2006b) who reported that Dipel-2x (*Bt kurstaki*) decreased rate of survival (Lx) and r_m. On the other hand, it increased generation time of the pink bollworm. In addition, Amer and El-Nemaky (2008) reported that Protecto + Biover had potentiated effect in most life table parameters of the pink bollworm than each biocide alone.

Generally, *Bt* when exposed to gamma doses of 15, 30 & 60 Gy, it showed potentiated effect especially with dose of 60 Gy than other doses used against *S. littoralis* treated as 4th instar larvae at different efficiency tests, the most parameters of biological and life table. On the other hand, *M. anisopltiae* had little potentiate effect against *S. littoralis* when exposed to gamma irradiation doses; whereas, it was changes in most biological and life table parameters. Exposure of biopolymer compound, chitosan exposed to gamma doses had medium potentiating effect on *S. littoralis* and it become effective on the biological and life table parameters. On contrary, the exposed entomopathogenic nematodes, *H. bacteriophora* BA1 and *S. carpocapsae* BA2 to gamma doses had antagonism effect against *S. littoralis* 4th instar larvae to become less effective when exposed to gamma doses resulting lower efficacy than when it used singly on *S. littoralis* larvae.

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