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Bioassay and Life Table Changes in *Spodoptera littoralis* (Boisd.) Treated with Biocontrol Agents Exposed to Gamma Ray

Soheir F. Abd El-Rahman, Yacoub Sh. S., Dalia A. Abdel-Salam and Abd El-Nasser T. Hassan

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

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ABSTRACT

Biocontrol treatments of *Bacillus thuringiensis* (Kurs.), emamectin benzoate and Spinosad were used singly or exposed to gamma ray doses (120 & 480 Gy) to potentiate its efficacy purposes on *Spodoptera littoralis* (Boisd.) treated as 4th instars larvae. Also, biological & life table changes were assessed as a result of *S. littoralis* treated with LC_{50} of mentioned compounds. Emamectin benzoate treatments, especially emamectin benzoate + 480 Gy had the highly efficacy compound with the least LC_{50} values on *S. littoralis* larvae, followed by Spinosad treatments and finally *B. thuringiensis* treatments that was the least efficacy. Also, emamectin benzoate + 480 Gy than other treatments used had the highly changes in the most biological assessments of *S. littoralis* treated as 4th instars larvae with LC_{50} of the treatments used acts in egg, larval, pupal & moth duration and mortality; addition, no. egg/female, fecundity%, sterility (observed & corrected) and control of hatchability were affected by the same treatment. Moreover, emamectin benzoate +480 Gy compared to other treatments effect on the most life table assessments acts in generation time (T) & doubling time (DT) increasing. Also, net reproductive rate (Ro), intrinsic rate of natural increase (r_m), finite rate of increase (e^{rm}), female progeny/female (Mx) and its survival rate (Lx) were decreased in *S. littoralis* treated as 4th instars larvae by LC_{50} of the same compound compared with other treatments and untreated. So, gamma rays used (120 & 480 Gy) can potentiate its biocides action used especially in emamectin benzoate + 480 Gy treatment that considered the highly changes on efficacy, biological & life table assessments of *S. littoralis* treated as 4th instars larvae.

Keywords: Biocontrol, *Spodoptera littoralis*, *Bacillus thuringiensis*, emamectin benzoate, Spinosad, gamma ray.

1. Introduction

Cotton leaf worm, *Spodoptera littoralis* (Boisd.) [Lepidoptera: Noctuidae] is the most important cotton pests in Egypt. Its control strategy based mainly on the synthetic insecticides used but its toxic and persistence for environment, causes carcinogenic and mutagenic to human, animals, birds, predators and benefit insects. To avoid the insecticidal injuries, need to alternative biocontrol agents with new mode of action as *B. thuringiensis,* Emamectin benzoate and Spinosad. Among biocontrol agents is gamma ray as a genetic control method. Genetic pest suppression is unique among biological methods; 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 (2006a,b); Amer *et al.* (2011) and (2012). All treatments increased fiber and seed weights. Addition, Amer *et al.* (2012) exposed the biocide compound, protecto (*B. thuringiensis*) to three gamma doses (150, 250 & 350 Gy) to assess the insecticidal activity on three pests of *P. gossypiella* newly hatched & 4th instar larvae, *S. littoralis* 4th instar larvae and the nymph & adult of *A. craccivora.* It was showed that LC_{50}'s on subjected insects treated with protecto exposed to gamma doses were lower than protecto unexposed to gamma doses. Addition, Amer *et al.* (2020 a) used *B. thuringiensis* with exposing to gamma radiation doses of 160, 320 & 640 Gy for potentiating

Corresponding Author: Soheir F. Abd El-Rahman, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

purpose. Gamma radiation used could potentiate *B. thuringiensis,* especially with 640 Gy to become the most effective compound on *P. gossypiella, E. insulana* & *O. hyalinipennis* and cotton crop parameters (lint & seed weight) compared with the same compounds without exposing to gamma ray during cotton seasons (2018 & 2019). Meanwhile, Amer *et al.* (2020 b) potentiated the lethal effect of emamectin benzoate by exposing to gamma ray (50 & 500 Gy) against *P. gossypiella* treated as newly hatched larvae. Dose of 500 Gy caused the higher larval mortality that reached to complete death in larval stage as well as dose of 50 Gy comparing with emamectin benzoate lethal effect when used singly. Moreover, Mohammed *et al.* (2024) evaluated the effectiveness of three insecticides: lufenuron, emamectin benzoate and *B. thuringiensis* on *S. frugiperda* larvae collected from maize fields and reared under laboratory conditions. Emamectin benzoate displayed the highest toxicity, followed by *B.t* and lufenuron. The variance in efficacy is attributed to varying in chemical compositions and modes of action. Emamectin benzoate led to prolonged larval development, while lufenuron and *B.t* induced prolonged larval development and postponed pupation. Substantially, *B.t* reduced significantly the carbohydrate level; however, both B.t and emamectin benzoate reduced significantly the total protein. Moreover, disparity with significance in the activity of the digestive enzymes activity of amylase and invertase, likewise detoxifying enzymes notably glutathione S-transferase (GST) and acetylcholinesterase (AChE) was apparent in insecticides treated larvae.

So, biocontrol agent exposed to gamma doses (120 & 480 Gy) for potentiating its efficacy against *S. littoralis* is our goal; addition biological and life table changes as a result of biocontrol treatments was done.

2.Materials and Methods

2.1. Target pest

A laboratory strain of the cotton leafworm, *Spodoptera littoralis* (Boisd.) [Lepidoptera: Noctuidae], 4th instar larvae were raised on castor oil leaves, *Ricinus communis* (L.), at the Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt. The insect was reared according to the procedure reported by El-Defrawi et al. (1964). Rearing conditions were modified to 27±1°C and 65-75% RH.

Table 1: Biocontrol agents used

Trade Name	Common name	Application Rate	Product company	Imported Company
Protecto 9.4% WP	*Bacillus thuringiensis* (Kurstaki)	300 g/feddan	Pesticide production unit, Plant Protection Research Institute, Agriculture Research Center, Egypt.	
Andraws L. 1.9% EC.	Emamectin benzoate	150cm^3/feddan	Nanjing Red Sun Co., Ltd, China	Company for Agriculture Chemicals
Master Top 48% SC	Spinosad	15 cm^3/100 L water	Qilu pharmaceutical (Inner Mangolia) Co, Ltd, China.	Star chem Industrial chemicals, Egypt.

2.2. Gamma radiation.

B. thuringiensis, Emamectin benzoate & Spinosad compounds were exposed to gamma radiation doses of 120 & 480 Gy at a dose rate of 0.682 KGy/h by a Cesium Hendy Gamma Cell Research at National Center for Radiation Research and Technology. Nine treatments were used as follows:

1. *B. thuringiensis*
2. *B. thuringiensis* + 120 Gy
3. *B. thuringiensis* +480 Gy
4. Emamectin benzoate
5. Emamectin benzoate + 120 Gy
6. Emamectin benzoate +480 Gy
7. Spinosad
8. Spinosad + 120 Gy

9. Spinosad + 480 Gy.

2.3. Efficacy assays.

Dipping technique was used with castor oil leaves in tested biocontrol agents' six concentrations of 2, 0.5, 0.125, 0.0313, 0.0078 & 0.00196 g/L for *B. thuringiewnsis* (Protecto), *B. thuringiewnsis* +120 Gy & *B. thuringiewnsis* +480 Gy; Concentrations of 0.75, 0.188, 0.0469, 0.0117, 0.00293 & 0.000732 ml/L for Emamectin benzoate (Andraws L.), Emamectin benzoate+120 Gy & Emamectin benzoate +480 Gy and Concentrations of 1.5, 0.375, 0.0938, 0.0234, 0.0059 & 0.00146 ml/L for Spinosad (Master Top), Spinosad + 120 Gy & Spinosad + 480 Gy. Castor oil leaves dipped in water alone served as the control. There were four replicates, concentrations, and tested bio-control agents. After the water evaporated, the leaves were put in 11x22 cm glass jars. 25 larvae of *S. littoralis* in their fourth instar were placed in each jar after they had been starved for approximately four hours and kept at 26±1oC. At 3, 4, 5, 6, and 7 days following treatments, the number of larvae that were alive and dead was counted.

Mortality percentages of *S. littoralis* were corrected by Abbott (1925). LC_{50}, LC_{90} and slope values were assessed according to Finney (1971) using Ldp-line software (www.Ehabbakr software/Ldp line).

The comparing among treatments efficacy (Toxicity index) were according to Sun (1950) formula.

Toxicity index = LC_{50} (LC_{90}) of the compound A/ LC_{50} (LC_{90}) of the compound B X 100

Where A: The most effective compound and B: The other tested compound.

2.4. Biological assessments.

Fourth instars larvae of *S. littoralis* treated with LC_{50} 's by *B. thuringiensis,* Emamectin benzoate and Spinosad treatments. Also, an untreated 4th instars larva was done. The following biological aspects were assessed as follows:

1. Larval stage.

- Larval duration (days).
- Larval mortality percentage.

% Larval mortality= No. dead larvae/ Total tested larvae X 100

% Corrected mortality according to Abott's (1925) formula:

% Corrected mortality = % tested mortality-% control mortality/100-% control mortality X 100.

2. Pupal stage

- Pupal duration (days).
- % Pupation= No. produced pupae/Total tested larvae X100

3. Adult stage

- **%** Moths emergency = No. emerged moth/total tested larvae X100
- Pre-oviposition, oviposition and post-oviposition periods: determined by three replicates. Each one contained 5 pairs of emerged moths in clean glass chimneys (17 cm height and 7-12 cm in diameter) till female moth death.
- **Egg laying rate (total number of eggs per female).** counted from daily deposited eggs on a piece of paper in upper and lower of glass chimneys. Each treatment yielded through the daily egg production and on the differential survival of females.
- **Egg hatchability percentage.** calculated as Zidan and Abdel-Megeed (1987):
- **Control of hatchability percentage.** calculated as Zidan and Abdel-Megeed (1987):
- **Fecundity percentage.** calculated as Crystal and Lachance (1963):
- **Sterility Percentages.**
- **Sterility observed percentage.** Calculated as Zidan and Abdel-Megeed (1987):
- **Sterility corrected percentage.** Calculated as Zidan and Abdel-Megeed (1987):

4. Sex ratio. no of adult female/Total no. of adult female and male X100

5. Life cycle. extended from egg deposition till adult emergence (days).

6. Life span. extended from egg deposition till adult moth death (days).

2.5. Life table assessments.

Data of life table were analyzed by using life 48 basic computer program (Abou-Setta *et al.,* 1986). The program has output data include information for each interval of adult female age: (egg laying rate) (M), number of females alive at age x (L), mean female age at each interval mid-point (X), female progeny per female produced (Mx), survival rate (Lx). In addition, generation time (T), net reproductive rate (Ro), intrinsic rate of natural increase (r_m), finite rate of increase (e^{rm}) and the number of times which the population multiplies in a unit time (doubling time, DT).

2.6. Statistical analysis.

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

3. Results and Discussion

Three biocontrol treatments of *Bacillus thuringiensis* (Kurs.), emamectin benzoate & Spinosad were tested singly and exposing to gamma ray (120 & 480 Gy) to reach nine treatments and untreated for *Spodoptera littoralis* (Boisd.) 4^{th} instars larvae to study some biological and life table changes as affected by aforementioned treating biocides efficacy.

3.1. Biocontrol efficacy.

Table (2) illustrated that *B. thuringiensis* +480 Gy was the best efficacy compound had the least LC_{50} & LC_{90} values on *S. littoralis* 4^{th} instars larvae and the highly toxicity index (100), followed by *B. thuringiensis* +120 Gy and *B. thuringiensis* singly as described from 3-days passed up to 7-days passed.

Emamectin benzoate treatments efficacy on *S. littoralis* 4^{th} instars larvae had the same trend as in Table (3). Emamectin benzoate + 480 Gy had the least LC_{50} & LC_{90} and the highly toxicity index (100) on *S. littoralis* 4^{th} instars larvae compared to emamectin benzoate + 120 Gy and the same compound singly from 3-day passed from treatment until reach to 7-days after treatments (Table, 3).

The bioccontrol compound, Spinosad also had the same sequence efficacy of two biocontrol aforementioned. Spinosad + 480 Gy treatment had the highly efficacy on *S. littoralis* 4^{th} instars larvae and had the lower LC_{50} & LC_{90} compared to Spinosad + 120 Gy and Spinosad singly (Table, 4).

Gamma ray treatments can be potentiating all the tested biocontrol compounds to enhance its efficacy, especially with exposing to 480 Gy, followed by 120 Gy comparing with the same compound singly as described in Tables (2-4). Emamectin benzoate treatments was considered the best treatments efficacy on *S. littoralis* 4^{th} instars larvae, followed by Spinosad and *B. thuringiensis* treatments.

Current results adopted with previous work by Amer *et al.* (2012) exposed the biocide compound, protecto (*B. thuringiensis*) to three gamma doses (150, 250 & 350 Gy) to assess the insecticidal activity on *S. littoralis* 4^{th} instar larvae. It was found that protecto exposed to gamma doses were highly efficacy than protecto unexposed to gamma doses. Also, Amer *et al.* (2015) enhanced the efficacy of *B. thuringiensis* by exposing to gamma doses (15, 30 & 60 Gy) against *S. littoralis* treated as 4^{th} instar larvae. *B.t* exposed to gamma doses had the most efficacy on *S. littoralis* than *B.t* used alone. Addition, Amer *et al.* (2020 a) applied *B. thuringiensis* exposed to 160, 320 & 640 Gy for *P. gossypiella, E. insulana* & *O. hyalinipennis* controlling. Gamma ray used could potentiate *B. thuringiensis,* especially with 640 Gy compared with the same compound alone during cotton seasons (2018 & 2019). Meanwhile, Amer *et al.* (2020 b) potentiated the lethal effect of emamectin benzoate by exposing to gamma ray against *P. gossypiella*. Dose of 500 Gy as well as dose of 50 Gy caused the higher larval mortality comparing with emamectin benzoate singly. While, Mokbel & Huesien (2020) showed that emamectin benzoate proved high toxicity against *S. littoralis*.
chemical compositions and modes of action.

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Table 2: Efficacy of *B. thuringiensis* exposed to gamma ray on *S. littoralis* 4th instars larvae.

Treatments	LC_{50}± Confidence limit	LC_{90}± Confidence limit	Slope± SE	Toxicity index	
				LC_{50}	LC_{90}
3-days passed					
B. thuringiensis	0.539×10^{-5} $0.167\pm3.3301\times10^{-5}$	150.45×10^{-5} $65.975\pm1460.2\times10^{-5}$	0.372± 0.0957	2.78	1.46
***B. thuringiensis* +120 Gy**	0.024×10^{-5} $0.0108\pm0.0548\times10^{-5}$	13.649×10^{-5} $1.1418\pm80.372\times10^{-5}$	0.434± 0.1256	62.5	16.1
***B. thuringiensis* +480 Gy**	0.015×10^{-5} $0.0032\pm0.0493\times10^{-5}$	2.195×10^{-5} $0.523\pm49.846\times10^{-5}$	0.655± 0.1324	100	100
4-days passed					
B. thuringiensis	0.0109×10^{-5} $0.0055\pm0.0518\times10^{-5}$	58.951×10^{-5} $29.475\pm117.9\times10^{-5}$	0.271± 0.0951	28.4	0.32
***B. thuringiensis* +120 Gy**	0.0046×10^{-5} $0.0016\pm0.009\times10^{-5}$	0.2914×10^{-5} $0.0959\pm3.2853\times10^{-5}$	0.649± 0.1412	67.4	64.8
***B. thuringiensis* +480 Gy**	0.0031×10^{-5} $0.0006\pm0.0072\times10^{-5}$	0.1888×10^{-5} $0.0784\pm0.9557\times10^{-5}$	0.793± 0.1447	100	100
5-days passed					
B. thuringiensis	0.0035×10^{-5} $0.001\pm0.0086\times10^{-5}$	1.2451×10^{-5} $0.2591\pm34.689\times10^{-5}$	0.503± 0.1002	14.3	2.09
***B. thuringiensis* +120 Gy**	0.0009×10^{-5} $0.0005\pm0.0018\times10^{-5}$	0.2215×10^{-5} $0.1108\pm0.553\times10^{-5}$	0.535± 0.1768	55.6	11.8
***B. thuringiensis* +480 Gy**	0.0005×10^{-5} $0.0002\pm0.001\times10^{-5}$	0.0261×10^{-5} $0.0094\pm0.1868\times10^{-5}$	0.759± 0.1400	100	100
6-days passed					
B. thuringiensis	0.001×10^{-5} $0.0004\pm0.0021\times10^{-5}$	0.0831×10^{-5} $0.0221\pm1.402\times10^{-5}$	0.522± 0.1304	10	9.63
***B. thuringiensis* +120 Gy**	0.0003×10^{-5} $0.0001\pm0.0012\times10^{-5}$	0.009×10^{-5} $0.002\pm0.2502\times10^{-5}$	0.665± 0.1331	33.3	88.9
***B. thuringiensis* +480 Gy**	0.0001×10^{-5} $0.00005\pm0.0009\times10^{-5}$	0.008×10^{-5} $0.0009\pm2.999\times10^{-5}$	0.668± 0.1356	100	100
7-days passed					
B. thuringiensis	0.0005×10^{-5} $0.000025\pm0.0001\times10^{-5}$	0.0277×10^{-5} $0.0062\pm3.5102\times10^{-5}$	0.645± 0.1825	20	0.72
***B. thuringiensis* +120 Gy**	0.0003×10^{-5} $0.0001\pm0.0007\times10^{-5}$	0.0007×10^{-5} $0.0002\pm0.0154\times10^{-5}$	0.750± 0.1996	33.3	28.6
***B. thuringiensis* +480 Gy**	0.0001×10^{-5} $0.00006\pm0.0009\times10^{-5}$	0.0002×10^{-5} $0.0001\pm0.0122\times10^{-5}$	0.829± 0.2036	100	100

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Table 3: Efficacy of Emamectin benzoate exposed to gamma ray on *S. littoralis* 4th instars larvae.

Treatments	$LC_{50}\pm$ Confidence limit (ppm)	$LC_{90}\pm$ Confidence limit (ppm)	Slope± SE	Toxicity index	
				LC_{50}	LC_{90}
		3-days passed			
Emamectin benzoate	0.0094×10^{-7} $0.002\pm0.0237\times10^{-7}$	1.383×10^{-7} $0.228\pm105.23\times10^{-7}$	0.565± 0.2433	1.06	1.29
Emamectin benzoate +120 Gy	0.0002×10^{-7} $0.00005\pm0.0009\times10^{-7}$	0.0199×10^{-7} $0.0099\pm0.0398\times10^{-7}$	0.591± 0.1792	50	89.5
Emamectin benzoate+480 Gy	0.0001×10^{-7} $0.00003\pm0.0008\times10^{-7}$	0.0178×10^{-7} $0.0068\pm0.0853\times10^{-7}$	0.622± 0.0955	100	100
		4-days passed			
Emamectin benzoate	0.0037×10^{-7} $0.0011\pm0.0073\times10^{-7}$	0.095×10^{-7} $0.041\pm0.609\times10^{-7}$	0.698± 0.1866	2.70	3.26
Emamectin benzoate +120 Gy	0.0002×10^{-7} $0.0001\pm0.0011\times10^{-7}$	0.0047×10^{-7} $0.0009\pm0.6835\times10^{-7}$	0.853± 0.1936	50	65.9
Emamectin benzoate+480 Gy	0.0001×10^{-7} $0.00009\pm0.008\times10^{-7}$	0.0031×10^{-7} $0.0012\pm0.0308\times10^{-7}$	0.910± 0.2062	100	100
		5-days passed			
Emamectin benzoate	0.0013×10^{-7} $0.0005\pm0.0024\times10^{-7}$	0.0253×10^{-7} $0.0115\pm0.1262\times10^{-7}$	0.803± 0.1919	3.85	0.79
Emamectin benzoate +120 Gy	0.0001×10^{-7} $0.0001\pm0.0004\times10^{-7}$	0.0015×10^{-7} $0.0005\pm0.0169\times10^{-7}$	0.943± 0.1984	50	13.3
Emamectin benzoate+480 Gy	0.00005×10^{-7} $0.000025\pm0.0001\times10^{-7}$	0.0002×10^{-7} $0.0001\pm0.0029\times10^{-7}$	1.003± 0.2069	100	100
		6-days passed			
Emamectin benzoate	0.0004×10^{-7} $0.0001\pm0.0007\times10^{-7}$	0.0095×10^{-7} $0.0039\pm0.0662\times10^{-7}$	0.881± 0.1958	10	1.05
Emamectin benzoate +120 Gy	0.00008×10^{-7} $0.00004\pm0.00016\times10^{-7}$	0.0002×10^{-7} $0.0001\pm0.0014\times10^{-7}$	0.922± 0.1983	50	50
Emamectin benzoate+480 Gy	0.00004×10^{-7} $0.00002\pm0.0008\times10^{-7}$	0.0001×10^{-7} $0.001\pm0.0005\times10^{-7}$	1.201± 0.3001	100	100
		7-days passed			
Emamectin benzoate	0.0003×10^{-7} $0.0002\pm0.0004\times10^{-7}$	0.0018×10^{-7} $0.001\pm0.0056\times10^{-7}$	1.351± 0.3233	6.67	3.33
Emamectin benzoate +120 Gy	0.00004×10^{-7} $0.00002\pm0.0009\times10^{-7}$	0.0001×10^{-7} $0.00005\pm0.0004\times10^{-7}$	1.464± 0.3217	50	60
Emamectin benzoate+480 Gy	0.00002×10^{-7} $0.00001\pm0.0007\times10^{-7}$	0.00006×10^{-7} $0.0003\pm0.007\times10^{-7}$	1.621± 0.3366	100	100

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Table 4: Efficacy of Spinosad exposed to gamma ray on *S. littoralis* 4th instars larvae.

Treatments	LC_{50}± Confidence limit (ppm)	LC_{90}± Confidence limit (ppm)	Slope± SE	Toxicity index LC_{50}	Toxicity index LC_{90}
		3-days passed			
Spinosad	0.0212×10^{-6} $0.0017±0.0551x10^{-6}$	$3.3667x10^{-6}$ $0.6649±1712.5x10^{-6}$	0.518± 0.0838	9.91	17.7
Spinosad +120 Gy	$0.0038x10^{-6}$ $0.0011±0.0086x10^{-6}$	$0.6215x10^{-6}$ $0.1701±6.1512x10^{-6}$	0.582± 0.1829	55.3	96.1
Spinosad +480 Gy	$0.0021x10^{-6}$ $0.0007±0.0068x10^{-6}$	$0.5972x10^{-6}$ $0.1805±5.828x10^{-6}$	0.583± 0.1062	100	100
		4-days passed			
Spinosad	0.0152×10^{-6} $0.0053±0.0317x10^{-6}$	$1.2046x10^{-6}$ $0.3685±14.563x10^{-6}$	0.675± 0.1361	7.24	4.58
Spinosad +120 Gy	$0.0013x10^{-6}$ $0.0006±0.0024x10^{-6}$	$0.4315x10^{-6}$ $0.0701±2.1512x10^{-6}$	0.731± 0.1846	84.6	12.8
Spinosad +480 Gy	$0.0011x10^{-6}$ $0.0005±0.0048x10^{-6}$	$0.0552x10^{-6}$ $0.0202±0.3724x10^{-6}$	0.782± 0.1401	100	100
		5-days passed			
Spinosad	0.0036×10^{-6} $0.0006±0.0081x10^{-6}$	$0.1627x10^{-6}$ $0.0598±2.2047x10^{-6}$	0.783± 0.1433	5.56	0.37
Spinosad +120 Gy	$0.0004x10^{-6}$ $0.0002±0.0008x10^{-6}$	$0.0103x10^{-6}$ $0.0043±0.0672x10^{-6}$	0.929± 0.2016	50	5.83
Spinosad +480 Gy	$0.0002x10^{-6}$ $0.0001±0.0005x10^{-6}$	$0.0006x10^{-6}$ $0.0002±0.0034x10^{-6}$	1.003± 0.2069	100	100
		6-days passed			
Spinosad	0.0017×10^{-6} $0.0006±0.0031x10^{-6}$	$0.0351x10^{-6}$ $0.0155±0.1936x10^{-6}$	0.789± 0.2140	4.71	0.57
Spinosad +120 Gy	$0.0001x10^{-6}$ $0.00025±0.002x10^{-6}$	$0.0038x10^{-6}$ $0.0014±0.0392x10^{-6}$	0.845± 0.1948	80	5.26
Spinosad +480 Gy	$0.00008x10^{-6}$ $0.00004±0.0009x10^{-6}$	$0.0002x10^{-6}$ $0.0001±0.0039x10^{-6}$	0.972± 0.2046	100	100
		7-days passed			
Spinosad	0.0002×10^{-6} $0.0001±0.0004x10^{-6}$	$0.0049x10^{-6}$ $0.0023±0.0213x10^{-6}$	0.883± 0.1967	25	1.22
Spinosad +120 Gy	$0.00007x10^{-6}$ $0.00004±0.0009x10^{-6}$	$0.0006x10^{-6}$ $0.0002±0.0035x10^{-6}$	0.935± 0.2036	71.4	10
Spinosad +480 Gy	$0.00005x10^{-6}$ $0.00002±0.0005x10^{-6}$	$0.00006x10^{-6}$ $0.00001±0.0002x10^{-6}$	0.962± 0.2080	100	100

Moreover, Amer *et al.* (2023) evaluated *B. thuringiensis*, Emamectin benzoate and Spinosad exposed or non-exposed to gamma ray doses (120 and 480 Gy) for *S. frugiperda* controllingin maize field trials. Spinosad treatments were considered the best dose lethality for *S. frugiperda* controlling, followed by Emamectin benzoate and finally *B. thuringiensis* treatments. Meanwhile, Emamectin benzoate treatments gave the least time-response lethality than other treatments aforementioned.

Gamma ray dose of 480 Gy, followed by 120 Gy can potentiate the biocide compounds of *B. thuringiensis*, Emamectin benzoate and Spinosad to become lethality more than the same compounds without exposing to gamma doses and saving the dose and the time-response for controlling *S. frugiperda* in maize plants. While, Mohammed *et al.* (2024) evaluated the effectiveness of lufenuron, emamectin benzoate and *B. thuringiensis* on *S. frugiperda* larvae. Emamectin benzoate was the highest toxicity, followed by *B.t* and lufenuron. The variance in efficacy is attributed to varying in

3.2. Biological changes.

S. littoralis 4th instars larvae treated with LC_{50} 's of biocontrol nine treatments (*B. thuringiensis, B. thuringiensis* +120 Gy, *B. thuringiensis* +480 Gy, emamectin benzoate, emamectin benzoate +120 Gy, emamectin benzoate + 480 Gy, Spinosad, Spinosad + 120 Gy & Spinosad + 480 Gy) and untreated to mention some biological changes of *S. littoralis* affected by aforementioned treatments.

1. Larval and pupal stages.

Emamectin benzoate + 480 Gy treatment had the highly increasing effect on larval & pupal duration of *S. littoralis* treated as 4th instars larvae (25.6 & 12.6 days, respectively) compared to normal *S. littoralis* larval & pupal that were 22.3 & 11.5 days, respectively (Table, 5). While, Spinosad treatments had decreased effect on the same stages compared with normal values (Table, 5).

Also, emamectin benzoate + 480 Gy treatment still the highly compound make changes on larval & pupal mortality as described in Table (5), the values reached 54.2 & 39.6%, respectively compared to untreated (7.92 & 9.95%, respectively).

Addition, the same treatment had changes in pupation % by decreasing reached to 45.8% compared with other treatments used and untreated that was 92.1%.

Table 5: Biological changes of *S. littoralis* treated as 4th instars larvae with biocontrol compounds exposed to gamma ray.

Treatments	Larval duration (days)	Larval mortality %	Pupal duration (days)	Pupation %	Pupal mortality %	Life Cycle (days)	Life Span (days)
Untreated	22.3^{bcd}	7.92^{c}	11.5^{abc}	92.1^{a}	9.95^{c}	36.3^{ab}	51.6^{a}
B. thuringiensis	22.2^{bcd}	32.1^{b}	10.2^{de}	67.9^{b}	24.1^{b}	35.65^{ab}	44.05^{ab}
***B. thuringiensis* +120 Gy**	23.1^{b}	33.9^{b}	12.1^{ab}	66.1^{b}	30^{ab}	38.2^{ab}	46.7^{ab}
***B. thuringiensis* +480 Gy**	21.3^{bcd}	34.2^{b}	11.2^{bcd}	65.8^{b}	35.7^{ab}	35^{b}	43.9^{ab}
Emamectin benzoate	22.4^{bc}	26.4^{b}	10.4^{cde}	73.6^{b}	30.4^{ab}	36.3^{ab}	43.1^{ab}
Emamectin benzoate +120 Gy	25.2^{a}	29.02^{b}	11.8^{ab}	72.7^{b}	38.1^{a}	40.5^{ab}	47.2^{ab}
Emamectin benzoate+480 Gy	25.6^{a}	54.2^{a}	12.6^{a}	45.8^{c}	39.6^{a}	42.2^{a}	48.9^{ab}
Spinosad	21.4^{bcd}	24.5^{b}	9.8^{e}	75.5^{b}	35.1^{ab}	35.2^{b}	44.2^{ab}
Spinosad +120 Gy	20.3^{cd}	25.9^{b}	11.9^{ab}	74.1^{b}	27.9^{ab}	35.95^{ab}	45.15^{ab}
Spinosad +480 Gy	20.2^{d}	28.1^{b}	10.4^{cde}	71.9^{b}	35.4^{ab}	34.1^{b}	42.3^{b}
$L.S.D_{0.05}$	1.876	8.815	1.139	8.908	12.18	5.801	7.463

2. Adult stage.

Normal moth emergency was 90.1 %, that values had decreased to 60.4% with emamectin benzoate + 480 Gy treatment that made the changes in *S. littoralis* treated as 4th instars larvae (Table, 6).

Table (6) described the emamectin benzoate + 480 Gy made the changes in sex ratio to 0.4 in *S. littoralis* treated as 4th instars larvae; while, the untreated sex ratio was 0.68.

Also, emamectin benzoate treatments had the drastically changes in male longevity of *S. littoralis* treated as 4th instars larvae (Table, 6) compared to untreated *S. littoralis* (11.7 days).

Addition, female longevity reached to 6.5 days in emamectin benzoate + 480 Gy treatment compared to untreated (15.3 days) as in Table (6).

Female pre-oviposition period increased about one day with Spinosad treatment compared to untreated (2.4 days).

Emamectin benzoate treatments had drastically changes on female oviposition period by decreasing this period compared to other treatments and untreated (Table, 6).

The same table, showed that Spinosad + 120 Gy, followed by *B. thuringiensis* treatments had the most post-oviposition period increased to 2.7 & 2.6 days for *S. littoralis* treated as 4th instars larvae; while, emamectin benzoate treatments had slightly decreased compared to normal *S. littoralis* post oviposition period (2 days) as in Table (6).

Moreover, emamectin benzoate + 480 Gy increased the life cycle about 6- days when *S. littoralis* treated as 4th instars larvae with LC_{50} 's of the compound used compared to normal value (Table, 5).

The same table showed that Spinosad + 480 Gy had the highly decreased from life span period (42.3 days) of *S. littoralis* treated as 4th instars larvae with LC_{50} of the compound; while, normal life span value was 51.6 days (Table, 5).

3. Egg stage.

No. of egg/female *S. littoralis* treated as 4th instars larvae with emamectin benzoate + 480 Gy were the most decreased to reach 237.3 egg/female compared to normal *S. littoralis* no. of egg/female (1261.3 egg) as in Table (6).

That no. egg/ female had 4-days duration in emamectin benzoate + 480 Gy as well as Spinosad treatments when *S. littoralis* as 4th instars larvae treated by LC_{50} of the compounds compared with normal *S. littoralis* egg duration (2.5 days), (Table, 6).

Biocontrol treatment of *B. thuringiensis* + 480 Gy was the lowest *S. littoralis* treated as 4th instars larvae with decreased in egg hatchability percent (654.8%) compared to other treatments used and normal *S. littoralis* (90.5%) as described in Table (6).

Fecundity percentage of *S. littoralis* treated as 4th instars larvae was the least (18.8%) in emamectin benzoate + 480 Gy treatment (Table, 7).

While, *B. thuringiensis* + 480 Gy treatments had the highly changes in observed & corrected sterility by increasing the values to 34.2 & 27.29 % compared to other treatments used and normal sterility (Table, 7).

Control of egg hatchability percentage reach to 84.3% in Emamectin benzoate + 480 Gy treatment with *S. littoralis* treated as 4th instars larvae, followed by Emamectin benzoate + 120 Gy and Emamectin benzoate singly. Spinosad exposed to gamma ray had a highly effect on control of hatchability than Spinosad when used singly as well as happened in *B. thuringiensis* treatments (Table, 7).

Current results matched with results of Amer *et al.* (2015) that potentiated *B. thuringiensis* by gamma doses (15, 30 & 60 Gy) exposing to assess some biological parameters of *S. littoralis* treated as 4th instar larvae. Gamma treatments increased the larval mortality; addition, decreased the percentages of pupation, moths' emergence and fecundity, oviposition period, egg laying, especially with *B.t* + 60 Gy. While, Mokbel & Huesien (2020) showed that LC_5 & LC_{15} of emamectin benzoate prolonged larval period, mean longevity of males & females and pre-ovioposition period of *S. littoralis* compared with untreated. Addition, Ramzan *et al.* (2021) evaluated the entire 31-day developmental phase of *S. littoralis* from egg to adult. Three days was the incubation time, and 90% of the eggs on cabbage were viable. The pupa weighed 300 mg and the fully fledged larva weighed 1000 mg. On cabbage, the maximum adult emergence rate was 90%. Emamectin benzoate was the most hazardous of the studied pesticides, followed by lufenuron and chlorpyrifos. Also, Abdel-Hamid

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Table 6: Biological changes of *S. littoralis* treated as 4th instars larvae with biocontrol compounds exposed to gamma ray.

Treatments	Moth emergency %	Sex ratio	Male Longevity (days)	Female Longevity (days)				No.egg/ Female	Egg duration (days)	Egg Hatchability %
				Pre-oviposition	Oviposition	Post-oviposition	Total			
Untreated	90.1 a	0.68 a	11.7 a	2.4 ab	10.9 a	2 bc	15.3 a	1261.3 a	2.5 b	90.5 a
B. thuringiensis	75.9 ab	0.61 a	6.4 b	2 b	3.8 b	2.6 ab	8.4 b	1230.3 a	3.25 ab	83.5 ab
***B. thuringiensis* +120 Gy**	70 bc	0.58 a	6.8 b	2.8 ab	3.7 b	2.2 abc	8.5 b	952.3 ab	3 ab	79.3 bc
***B. thuringiensis* +480 Gy**	64.4 bc	0.56 a	3 d	3 ab	3.9 b	2.2 abc	8.9 b	810.5 bc	2.5 b	65.8 d
Emamectin benzoate	69.6 bc	0.66 a	2 d	2.3 b	2.7 c	1.8 c	6.8 cd	518.3 cd	3.5 ab	79.3 bc
Emamectin benzoate +120 Gy	61.9 bc	0.66 a	3 d	2.3 b	2.5 c	1.9 c	6.7 d	268.8 d	3.5 ab	78.5 bc
Emamectin benzoate+480 Gy	60.4 c	0.4 b	2.8 d	2 b	2.7 c	1.8 c	6.5 d	237.3 d	4 a	75.5 bc
Spinosad	64.9 bc	0.65 a	4.8 c	3.4 a	3.7 a	1.9 c	9 b	1161.8 a	4 a	82.3 abc
Spinosad +120 Gy	72.03 bc	0.64 a	5.2 c	2.1 b	4.4 b	2.7 a	9.2 b	660.5 bc	3.75 a	75.5 bc
Spinosad +480 Gy	64.6 bc	0.53 ab	4.4 c	2.4 ab	3.9 b	1.9 c	8.2 bc	673.3 bc	3.5 ab	73 cd
L.S.D$_{0.05}$	12.24	0.133	1.081	0.945	0.803	0.574	1.418	294.3	1.071	8.776

et al. (2021) Assessed the toxicity and residual effects of chlorpyrifos, emamectin benzoate, lufenuron, and Spinosad on 2nd and 4th instar larvae of *S. litraltois* from both laboratory and field strains. Results indicated latent effect on 2nd instar larvae, the lowest percentage of pupation were 18.55 and 28.56% when treated larvae with lufenuron whereas, the highest 39.55 and 44.23% with Spinosad for larvae of lab and field strains, respectively and adult emergence was the lowest when treated larvae with chlorpyrifos, lufenuron and Spinosad for lab strains; while, larvae of field strains gave the lowest percentage with treated by lufenuron (14.32%) and the mean of eggs /female were the lowest (155.45 egg/female) when treated larvae with lufenuron for lab & field strains and was the lowest (138.0 egg /female) when treated larvae with Spinosad for field strain.

Table 7: Egg viability changes of *S. littoralis* treated as 4th instars larvae with biocontrol compounds exposed to gamma ray.

Treatments	Fecundity %	Observed Sterility %	Corrected Sterility %	Control of hatchability %
Untreated	- ^{g}	9.5 ^{d}	- ^{e}	-
B. thuringiensis	97.5 ^{a}	16.5 ^{c}	7.735 ^{d}	10^{h}
***B. thuringiensis* +120 Gy**	75.5 ^{b}	20.7 ^{bc}	12.38 ^{cd}	33.84^{f}
***B. thuringiensis* +480 Gy**	64.3 ^{c}	34.2 ^{a}	27.29 ^{a}	53.49^{e}
Emamectin benzoate	41.1 ^{e}	20.7 ^{bc}	12.38 ^{cd}	63.99^{c}
Emamectin benzoate +120 Gy	21.3 ^{f}	21.5 ^{bc}	13.26 ^{bcd}	81.52^{b}
Emamectin benzoate+480 Gy	18.8 ^{f}	24.5 ^{b}	16.57 ^{bc}	84.3^{a}
Spinosad	92.1 ^{a}	17.7 ^{c}	9.061 ^{d}	16.23^{g}
Spinosad +120 Gy	52.4 ^{d}	24.5 ^{b}	16.57 ^{bc}	56.31^{d}
Spinosad +480 Gy	53.4 ^{d}	27 ^{b}	19.34 ^{b}	56.94^{d}
L.S.D$_{0.05}$	8.46	5.081	6.117	2.26

In case of 4th instar larvae of lab strain showed the lowest pupation percentage (31.23%) when treated with Spinosad while the lowest pupation percentage was 40.42% when treated larvae with chlorpyrifos and hatchability percent increase in the field strain compared to lab strain. Addition, Ismail *et al*. (2022) evaluated the field foliar residues (Emamectin benzoate, Indoxicarb, Spinosad, Flubendiamide and *B. thurngiensis*) in tomato plants by feeding *S. littoralis* larvae to study the long-term effects of feeding larvae on the development of *S. littoralis* throughout its life cycle. Residues Spinosad was the highest mortality (87.7 and 83.6%) against 2nd & 4th instars larvae, respectively; followed by Flubendiamide and *B. thurngiensis*; while, Emamectin benzoate and Indoxicarb residues showed the significantly lowest mortality. At vice versa, Emamectin benzoate was the highest initial kill against 2nd and/or 4th instars larvae; while, Spinosad was the longest residual effect among the tested insecticides. Larval duration increasing was observed with the slowed metamorphosis. Treated larvae exhibited lower pupal weights, higher pupal mortality, deformed pupae, and more deformed adults than untreated larvae. All insecticides had a significant effect on larval development time and adult longevity. The males & females development period was about four days lower than the untreated for all tested insecticides. Finally, *S. littoralis* adults treated as 2nd & 4th instars by tested insecticides were affected in their number of eggs laid per female (fecundity). Moreover, Mohammed *et al.* (2024) evaluated of lufenuron, emamectin benzoate and *B. thuringiensis* on *S. frugiperda* larvae. Emamectin benzoate led to prolonged larval development, while lufenuron and *B.t* induced prolonged larval development and postponed pupation.

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3.3. Life table changes.

Changes in life table assessments of *S. littoralis* treated as 4th instars larvae by nine treatments used and untreated *S. littoralis* was illustrated in Table (8).

Emamectin benzoate + 480 Gy treatment had the first arrange made changes in all the life table assessments of *S. littoralis* treated as 4th instars larvae compared to other treatments used (Table, 8).

1. Generation time (T).

Time of generation was reached to 45.79 days for *S. littoralis* treated as 4th instars larvae with emamectin benzoate + 480 Gy (Table,8); while, *S. littoralis* normal generation time was 41.44 days as tabulated in the same table.

2. Net reproductive rate (Ro).

Normal *S. littoralis* net reproductive rate (Ro) was 782.3 female's daughter progeny/females in one generation, this value had drastically decreased to 73.9 female daughter progeny/ female when *S. littoralis* treated as 4th instars larvae with LC_{50} of emamectin benzoate + 480 Gy than other treatments used (Table, 8).

3. Intrinsic rate of natural increase (r_m).

Table (8) showed the normal value of *S. littoralis* intrinsic rate of natural increase (r_m) that means ability to inherit increase was 0.161 individual/ female/ day; the value decreased in all the treatments up to reached 0.094 individual/ female/ day when *S. littoralis* treated as 4th instars larvae with LC_{50} of emamectin benzoate + 480 Gy (Table, 8).

4. Finite rate of increase (e^{rm}).

Also, emamectin benzoate + 480 Gy had drastically decreased effect (1.098 individual/ day) on *S. littoralis* finite rate of increase (e^{rm}) when treated as 4th instars larvae compared to *S. littoralis* normal daily population that was 1.174 individual/ day (Table, 8).

5. Doubling time (DT).

Untreated *S. littoralis* generation that can be twice or doubling time (DT) was 4.311 days. While, *S. littoralis* doubling time was 7.375 days when treated as 4th instars larvae with emamectin benzoate + 480 Gy (Table, 8).

Table 8: Life table assessments of *S. littoralis* treated as 4th instars larvae with biocontrol compounds exposed to gamma ray.

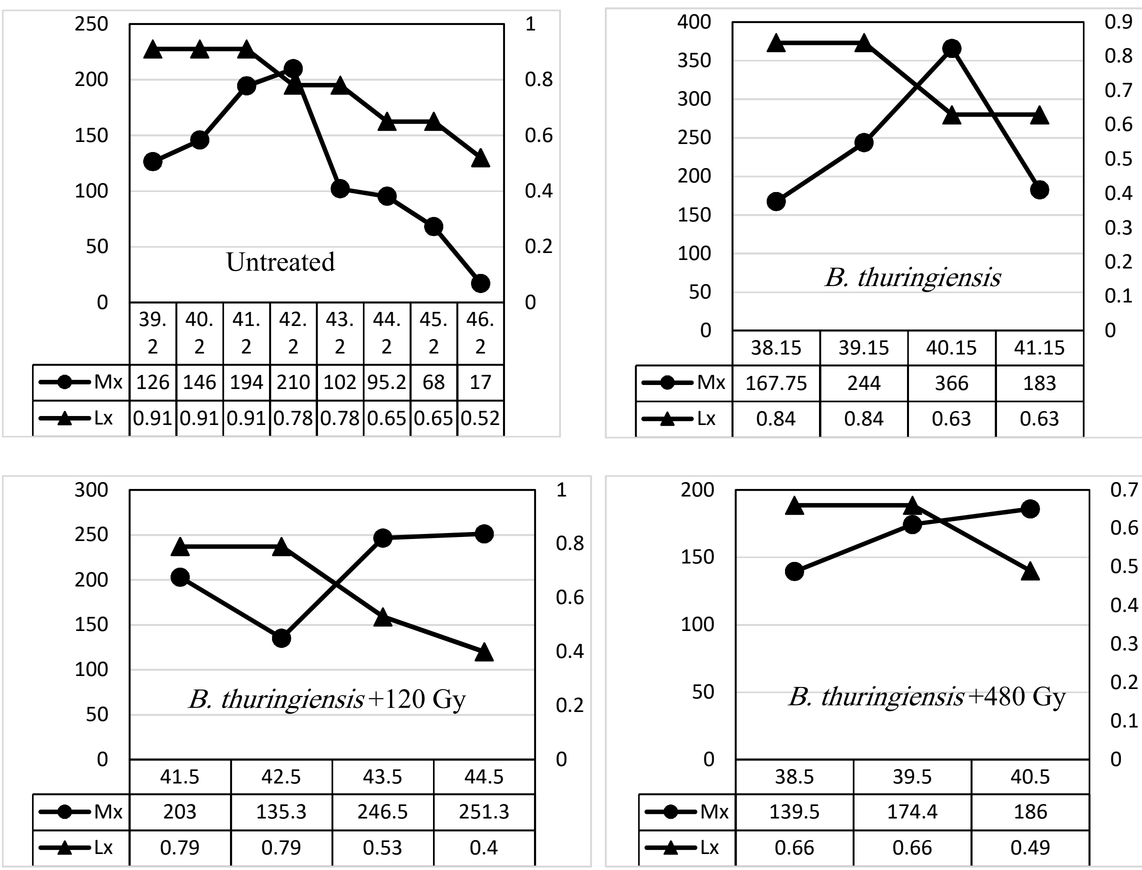
Treatments	T (days)	Ro	Increase rate		DT (days)
			r_m	e^{rm}	
Untreated	41.44 a	782.3 a	0.161 a	1.174 a	4.311 a
B. thuringiensis	39.53 a	691.7 a	0.165 a	1.179 a	4.19 a
***B. thuringiensis* +120 Gy**	42.75 a	498.3 b	0.145 a	1.156 a	4.771 a
***B. thuringiensis* +480 Gy**	39.46 a	298.3 c	0.144 a	1.155 a	4.799 a
Emamectin benz oate	40.46 a	170.1 de	0.127 a	1.135 a	5.459 a
Emamectin benzoate +120 Gy	44.07 a	140.5 e	0.112 a	1.119 a	6.177 a
Emamectin benzoate+480 Gy	45.79 a	73.9 e	0.094 a	1.098 a	7.375 a
Spinosad	40.18 a	444.5 b	0.152 a	1.164 a	4.568 a
Spinosad +120 Gy	40.09 a	270.9 cd	0.139 a	1.149 a	4.96 a
Spinosad +480 Gy	38.57 a	252.9 cd	0.143 a	1.154 a	4.83 a
$L.S.D_{0.05}$	9.266	105.3	0.080	0.542	3.094

(T) = Generation time (Ro) = Net reproductive rate (r_m) = Intrinsic rate of natural increase
(e^{rm}) = Finit rate of increase (DT) = Doubling time

6. Female progeny/ female (Mx) and survival rate (Lx).

Figure (1&2) clear that emamectin benzoate treatments, especially when exposed to 480 Gy had drastically decreased in female progeny daughter/ female (Mx) that ranged from 26.67 to 12 and its survival rate (Lx) ranged from 0.75 to 0.5 when *S. littoralis* treated as 4th instars larvae. Followed by Spinosad treatments exposed to gamma ray (120 & 480 Gy). While, *B. thuringiensis* treatments that had increased from female progeny/ female (Mx), but decreased from its survival rate comparing with normal *S. littoralis* (Mx) that ranged from 126.3 to 0.17 female progeny/ female and its survival rate from 0.91 to 0.52 (Figures, 1&2).

Amer (2006b) treated the newly hatched larvae of *P. gossypiella* with Dipel-2x (*B. thuringiensis*) exposed to 5, 10, 20, 40 & 80 Gy gamma doses in addition to Spinosad and Dipel-2x + Spinosad. Treatment of Dipel-2x + 80 Gy had decreased the female progeny/female (Mx), rate of survival (Lx), net reproductive rate (Ro), increase rate (intrinsic rate of natural increase (r_m) and finit rate of increase (e^{rm})) & sex ratio. Also, it caused increasing the doubling time (DT), followed by treatments of Dipel-2x + Spinosad, Spinosad, Dipel-2x +40 Gy, Dipel-2x + 20 Gy, Dipel-2x + 10 Gy, Dipel-2x + 5 Gy and Dipel-2x alone compared with the control values. While, Amer *et al.* (2015) exposed the *B. thuringiensis* to gamma doses (15, 30 & 60 Gy) to enhance its activity on life table parameters of *S. littoralis* treated as 4th instar larvae. Life table parameters of net reproductive rate (Ro), generation time (T), intrinsic rate of natural increase (r_m), finite rate of increase (e^{rm}), female progeny/female (Mx) and rate of survival (Lx) were decreased compared to control. Meanwhile, doubling time (DT) was increased compared to control affected by different treatments, especially with *B.t* + 60 Gy. Addition, Mokbel & Huesien (2020) showed that emamectin benzoate prolonged the mean generation time (T), doubling time (DT); while decreased the net reproductive rate (Ro), intrinsic rate of increase, finite rate, gross reproductive rate of *S. littoralis* compared to control.



Mx= Female progeny/female Lx= Survival rate

Fig. 1: Female progeny/ female (Mx) and survival rate (Lx) of *S. littoralis* treated as 4th instars larvae with *B. thuringiensis* exposed to gamma ray.

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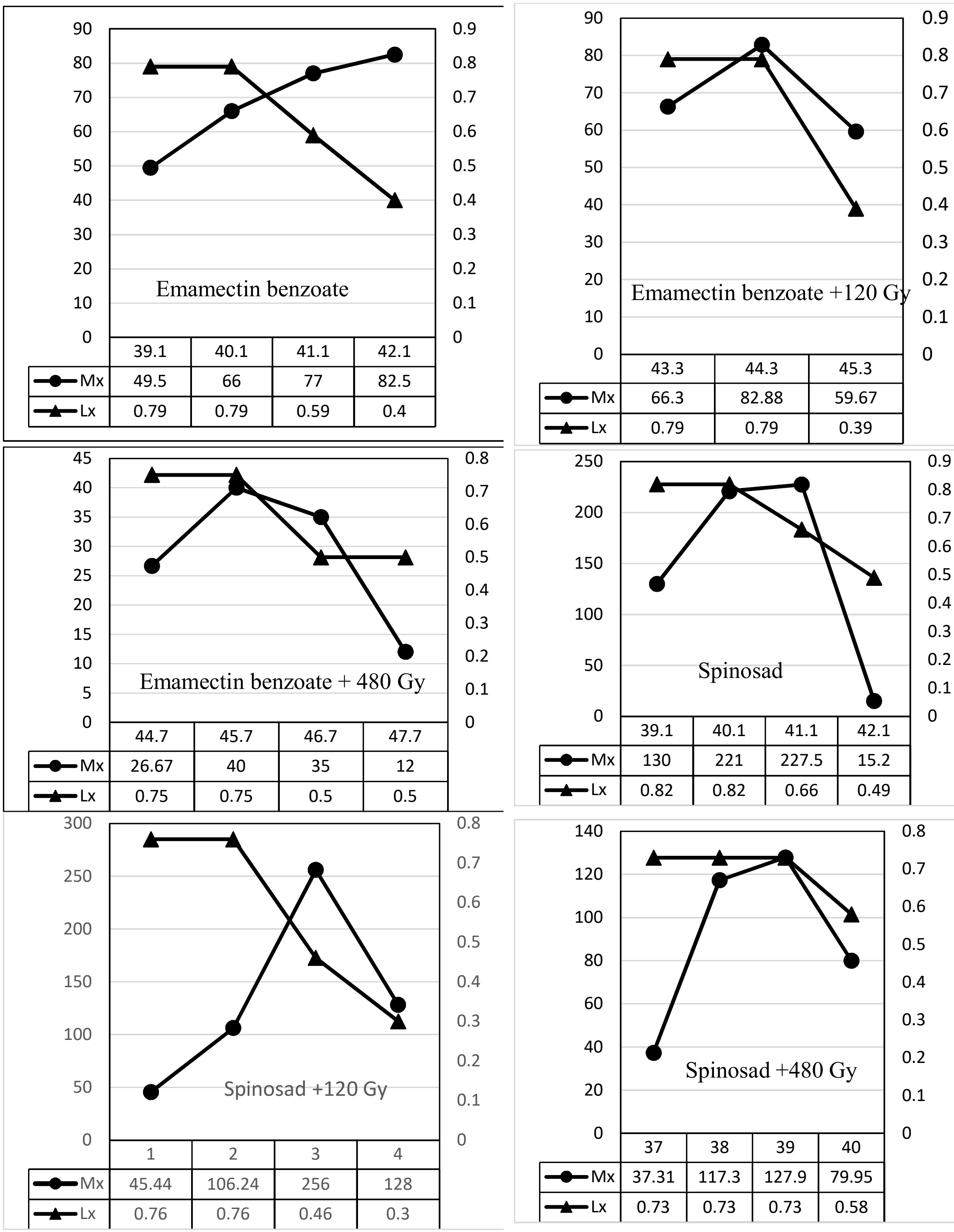

Mx= Female progeny/female   Lx= Survival rate

Fig. 2: Female progeny/ female (Mx) and survival rate (Lx) of *S. littoralis* treated as 4th instars larvae with Emamectin benzoate and Spinosad exposed to gamma ray.

So, it can be concluded that gamma ray doses of 120 & 480 Gy can potentiated and enhanced the biocontrol agent compounds used efficacy of *B. thuringiensis,* Emamectin benzoate and Spinosad on *S. littoralis* than compounds when used singly. Also, the same compounds exposed to ray made the highly changes on the biological and life table assessments of *S. littoralis* compared to unexposed compounds and untreated. Thus, it can be classified the biocontrol compounds according to its efficacy, biological and life table changes of *S. littoralis* on three categories; the first the compounds exposed to 480 Gy; the second the compounds exposed to 120 Gy and the third, compounds without exposing to gamma ray that have the least effect on *S. littoralis.*

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