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MAGNETIC FLUX AND GAMMA RAY CONJUNCTION IMPACTS WITH *Beauvaria bassiana* (BALSAMO) ON CERTAIN ASSESSMENTS OF *Earias insulana* (BOISD.)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Under laboratory conditions; the spiny bollworm, *Earias insulana* (Boisd.) treated with LC_{50}'s of the fungal compound, *Beauvaria bassiana* (Balsamo) exposed to gamma doses (400 & 700 Gy) and prepared in magnetized water (180 mlt). Assays of toxicity, biological and biochemical of *E. insulana* treated as newly hatched larvae with *B. bassiana* in different six treatments were done. *B. bassiana* + 400 or 700 Gy + magnetized water were the most effective treatments on the most *E. insulana* larvae assays used. While, *B. bassiana* + 400 or 700 Gy without preparing in magnetized water had the lower effect comparing with the two aforementioned treatments. Meanwhile, *B. bassiana* treatment when used singly caused the least effective on *E. insulana* larvae different assays.

At 5- day post treatment, the compound of *B. bassiana* + 700 Gy + magnetized water was the best treatment caused the highly toxicity (LC_{50}: $8x10^2$ IU) on *E. insulana* larvae. Also, the same treatment had drastically biological effect on larval and pupal stages. Addition, total carbohydrate, followed by total lipid and proteins of *E. insulana* had drastically decreased with the mentioned treatment; while, free amino acids increased than untreated. Meanwhile, all the assayed enzymes had the highly decreased compared with untreated, especially aspartate amino transferase (AST/GOT), followed by alanine aminotransferase (ALT/GPT), acetyle cholinesterase, trehalase, invertase and amylase.

So, *B. bassiana* exposed to gamma doses (400 & 700 Gy) and prepared in magnetized water can potentiate the fungal compound used on *E. insulana* larvae to be effective than *B. bassiana* when used singly.

Keywords: *Earias insulana*; *Beauvaria bassiana*; gamma ray; magnetized water; toxicity; biological; biochemistry.

1. INTRODUCTION

Spiny bollworm, *Earias insulana* (Boisd.) (Lepidoptera: Nolidae) is one of the serious lepidopteran pests. Its larvae are the most destructive stage for buds, flowers & bolls of the cotton, okra, corn and many economic crops leading to decrease in quantity and quality of infesting crop [1]. For controlling *E. insulana* on many crops using chemical pesticides, leads to many environmental problems. So, for facing this problem used the alternative methods as entomopathogenic fungi biopesticide [2].

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Beauvaria bassiana (Balsamo), is a pathogenic fungus with a large host range and it is used for insect biological control. *B. bassiana* infects and kills the pest in contact with the fungal spores that attach to the cuticle of insect; they germinate sending out structures (hypha) that penetrate in the body of insect. It may take 3-5 days for insects to die, but infected cadavers may serve as a source of spores for secondary spreading of the fungus. Insects can also spread the fungus through mating [3]. [4] Inclusion of this fungal in the pest control strategy against *Helicoverpa armigera* (Hunner) can be successful step in removing chemical pesticides from environment. For potentiating the fungal activity, it used gamma ray in current work. Gamma ray is important part of the genetic control [5]. Many studies used gamma ray for fungal biopesticide potentiating purposes [6-9]. Meanwhile, the static magnetic fields had an apparent effect on insect egg hatching; the hatching was delayed by the strong static magnetic fields. The larval development in the strong magnetic field was slower than in the geomagnetic field [10].

So, the main purpose of current work is potentiating *Beauvaria bassiana* (Balsamo) activities by exposed to gamma ray doses of 400 & 700 Gy with using magnetized water for investigating the toxicity, biological and biochemical assays of *Earias insulan* (Boisd.) treated as newly hatched larvae.

2. MATERIALS AND METHODS

2.1 Insect Pests

A laboratory strain of the spiny bollworm, *Earias insulana* (Boisd.) (Lepidoptera: Nolidae) was reared at Bollworms Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, on semi-artificial diet as described [11]. Rearing conditions were adjusted at 27±1^{o}C and 65-75% RH.

2.2 Tested Compound

Biover 10% W.P, *Beauvaria bassiana* (Balsamo), 200gm per 100-liter water/Faddan, Special Unit of Producing Bio-insecticides, Plant Protection Research Institute, Agriculture Research Center, Egypt.

2.3 Gamma Radiation Treatment

Fungal compound, *Beauvaria bassiana* (Balsamo) exposed to 400 & 700 Gy to assess its lethal, biological and biochemical of *E. insulana* treated as newly hatched larvae. All irradiations were done by a Cesium^{137} Indian GC Research, National Center for Radiation Research and Technology, delivered at a dose rate of 1.277 K.Gy/h.

2.4 Magnetic Treatment

The apparatus of magnetic field consists of two components: Inside the first one, found the eight magnetic pieces; each piece measured, 30mlli- tesla power was arranged inside a row in an attractive position. Another 8 magnetic pieces similar arranged inside a row represented the second component. The two rows were put together parallels (with 2 cm distance among them) and in repulsion position, that allows the magnetic power to 180 mlt.

The apparatus measured by using mille-tesla meter at faculty of Engineering, Menofiya University.

2.4.1 Magnetized water preparation

The water kept in tubes and it exposed to the magnetic field power (180 mille-tesla) at 60 minutes, between the two components of the apparatus magnetic field.

2.5 Lethal Assays

Two gm of semi artificial diet/Petri-dish (7 cm diameter) were mixed with 1 cm of each *B. bassiana* prepared concentrations (128 X 10^{24}, 64 X 10^{12}, 32 X 10^{6},16 X 10^{3} & 8 X 10^{2} IU). Twenty-five of newly hatched larvae of *E. insulana* to each four replicates/ concentrate/ tested compound were exposed to the compound alone or exposing to gamma doses of 400 & 700 Gy or aforementioned treatments prepared in magnetized water (*B. bassiana*, *B. bassiana* + magnetized water, *B. bassiana* +400 Gy, *B. bassiana* + 700 Gy, *B. bassiana* +400 Gy + magnetized water and *B. bassiana* + 700 Gy + magnetized water). The petri- dish used as untreated was prepared with 1 cm distilled water mixed with 2 gm artificial diet and kept at 27±1^{o}C and 65-75% R.H. Then the larvae were investigated (alive and dead larvae) at 5- day post treatment.

LC_{50}, LC_{90} and slope values were assessed [12,13] by using Ldp-line software (www.Ehabbakr software/ Ldp line).

2.6 Biological Assays

Newly hatched larvae of *E. insulana* treated with LC_{50}'s for different six treatments of *B. bassiana* (*B. bassiana*, *B. bassiana* + magnetized water, *B. bassiana* +400 Gy, *B. bassiana* + 700 Gy, *B. bassiana* +400 Gy + magnetized water and *B. bassiana* + 700 Gy + magnetized water). Also, an untreated newly hatched larvae and magnetized water treatment were

done. The following biological aspects were investigated as follows:

2.6.1 Larval stage

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

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

% Corrected mortality also used [12].

% Corrected mortality = % tested mortality - % untreated mortality/100 - % untreated mortality X 100

2.6.2 Pupal stage

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

Corrected pupal mortality as used before [12].

2.7 Biochemical Assays

The assays were done at the Physiological Department, Plant Protection Research Institute (P.P.R.I.). Eight samples of *E. insulana* treatment larvae (untreated, magnetized water, *B. bassiana*, *B. bassiana* + magnetized water, *B. bassiana* +400 Gy, *B. bassiana* + 700 Gy, *B. bassiana* +400 Gy + magnetized water and *B. bassiana* + 700 Gy + magnetized water) were collected after 14 days from different treatments and homogenized in distilled water. The homogenates were centrifuged at 5000 rpm at 5°C. The supernatants were kept in deep freezer at - 20 °C till use for biochemical assays. The colorimetric determination of total soluble protein, total lipids, and carbohydrate in total homogenate of *E. insulana* that were estimated [14-16]. The method used to determine the digestion of sucrose by invertase, trehalase and amylase enzymes, respectively were described before [17]. In additional; the alanine amino transferase (ALT/GPT) and aspartate amino transferase (AST/GOT) activities were determined according to the method described before [18]. The activity of acetylcholine esterase (AchE) was measured as described before [19].

2.8 Statistical Analysis

All data of biological and biochemical assays of *E. insulana* were analyzed [20] and Duncan's multiple range test [21] at 5% probability level to compare the differences among time means.

3. RESULTS

3.1 Toxicity Assay

Table 1 described the six toxicities of the fungal compound treatments, *Beauvaria bassiana* (Balsamo) singly or exposed to gamma ray doses of 400 & 700 Gy with preparing in magnetized water on the pest of *Earias insulana* (Boisd.) treated as newly hatched larvae. At 5- day post treatment, the compound of *B. bassiana* + 700 Gy + magnetized water was the best treatment caused the highly toxicity (LC_{50}: $8x10^2$ IU) on *E. insulana* larvae, followed by *B. bassiana* + 400 Gy + magnetized water treatment. Meanwhile, the same two mentioned treatments without magnetized water gave the lower toxicity, Although, *B. bassiana* treatment when used alone, gave the less toxicity against *E. insulana* larvae comparing with other tested compounds; but when magnetized water add to *B. bassiana* in preparation enhance from its toxicity on *E. insulana* larvae as well as *B. bassiana* + 700 Gy toxicity.

3.2 Biological Assays

Seven different treatments singly or exposed to gamma ray (400 & 700 Gy) with magnetized water inverse its effects on the biological assays of *E. insulana* comparing with untreated larvae as described in Table 2.

Table 1. Toxicity assays of *B. bassiana* exposed to gamma ray and magnetized water on *E. insulana* newly hatched larvae at 5-days post treatment

Treatments	LC_{50} (IU) ± Confidence limit	LC_{90} (IU) ± Confidence limit	Slope ± SE
B. bassiana	128 X 10^{24}	512 X 10^{96}	0.345± 0.159
B. bassiana+400 Gy	66 X 10^{12}	258 X 10^{48}	0.398±0.167
B. bassiana+ 700 Gy	30 X 10^{6}	126 X 10^{24}	0.412±0.17
B. bassiana+ Magnetized water	30 X 10^{6}	126 X 10^{24}	0.412±0.17
B. bassiana+ 400 Gy + Magnetized water	70 X 10^{4}	280 X 10^{16}	0.493±0.163
B. bassiana+ 700 Gy + Magnetized water	8 X 10^{2}	32 X 10^{8}	0.512±0.179

Table 2. Biological assays of *E. insulana* treated with *B. bassiana* exposed to gamma ray and magnetized water

Treatments	Larval duration (days)	Comparison with untreated	Larval mortality %	Comparison with untreated	Pupal duration (days)	Comparison with untreated	Pupal mortality %	Comparison with untreated
Untreated	16 [c]	-	20 [e]	-	10 [a]	-	15 [c]	-
Magnetized water	17 [bc]	+1	23 [de]	+3	10 [a]	0	19 [c]	+4
B. bassiana	19 [b]	+3	30 [d]	+10	9 [a]	-1	50 [b]	+35
***B. bassiana* +400 Gy**	19 [b]	+3	50 [c]	+30	9 [a]	-1	100 [a]	+85
***B. bassiana* + 700 Gy**	22 [a]	+6	80 [b]	+60	10 [a]	0	100 [a]	+85
***B. bassiana* + Magnetized water**	19 [b]	+3	75 [b]	+55	10 [a]	0	100 [a]	+85
***B. bassiana* + 400 Gy + Magnetized water**	7 [d]	-9	100 [a]	+80	-	-	-	-
***B. bassiana* + 700 Gy + Magnetized water**	5 [d]	-11	100 [a]	+80	-	-	-	-
L.S.D $_{0.05}$	2.370	-	7.813	-	2.935	-	12.75	-

3.2.1 Larval stage

E. insulana treated as newly hatched larvae with LC_{50} of seven treatments for *B. bassiana* singly or exposed to gamma ray of 400 & 700 Gy or with magnetized water compared with untreated one were shown in Table 2. Untreated *E. insulana* larval duration was 16 days. This period increased in all the treatments (Table 2), except for *B. bassiana* treatments exposed to gamma ray of 400 & 700 Gy with magnetized water had drastically decreased reach to 9 & 11 days compared with untreated one.

All tested compounds used had larval mortality percentages of *E. insulana* treated as newly hatched larvae ranged from 23% in magnetized water treatment to reach 100% completed *E. insulana* larval mortality in two treatments of *B. bassiana* + 400 Gy or 700 Gy + magnetized water compared with untreated (20%) as shown in the same table.

3.2.2 Pupal stage

Normal pupal duration of *E. insulana* was 10 days as well as that found in the treatments of *B. bassiana* + 700 Gy and *B. bassiana* + magnetized water. While, pupal duration of *E. insulana* decreased about 1-day only when treated as newly hatched larvae with LC_{50}'s of the treatments of *B. bassiana* and *B. bassiana* + 400 Gy as mentioned in Table 2.

When *E. insulana* treated as newly hatched larvae with LC_{50}'s of *B. bassiana* + 400 Gy, *B. bassiana* + 700 Gy and *B. bassiana* + magnetized water, the *E. insulana* pupal mortality was 100% compared with untreated (15%). Whereas, the magnetized water caused *E. insulana* pupal mortality (19%) with increasing about 4% than untreated. While, *E. insulana* pupal mortality was 50% when the newly hatched larvae treated with LC_{50}'s of *B. bassiana* treatment when used only.

3.3 Biochemical Assays

Inverse the *B. bassiana* mode of action on *E. insulana* larvae was clearly appeared in biochemical assays as described in Tables 3-5.

3.3.1 Total carbohydrates

Normal total carbohydrates of *E. insulana* larvae was 45.7 mg/g.b.wt, the value decreased about 3.7 in *B. bassiana* treatment and reached to 27.73 decreasing in the treatment of *B. bassiana* + 700 Gy + magnetized water compared with untreated value of *E. insulana* larvae (Table 3).

3.3.2 Total proteins

E. insulana larvae treated with *B. bassiana* + 700 Gy + magnetized water had drastically decreased in total protein (3.77 mg/g.b.wt.) compared with untreated *E. insulana* (13.9 mg/g.b.wt.). Moreover, all the treatments used decreased from *E. insulana* total protein ranged from 2.03 decreasing than untreated in magnetized water treatment until reach to 10.13 decreasing in *B. bassiana* + 700 Gy + magnetized water treatment compared with *E. insulana* untreated value as in Table 3.

3.3.3 Total lipids

Treatment of *B. bassiana* + 700 Gy + magnetized water had drastically decreased from lipids of *E. insulana* larvae (6 mg/g.b.wt) that comparing with untreated *E. insulana* larvae (21.9 mg/g.b.wt). While, the rest of treatments used in Table 3 had the lipid decreasing from 0.8 in magnetized water treatment to 15.9 decreasing in *B. bassiana* + 700 Gy + magnetized water compared with normal lipid of *E. insulana* larvae (Table 3).

3.3.4 Free amino acids

Table 3 showed the highly free amino acids in *E. insulana* larvae treated with LC_{50} of *B. bassiana* + 400 Gy + magnetized water (528.3 µg D,L-alanine/g.b.wt) compared with other tested treatments that ranged from 234 µg D,L- alanine/g.b.wt in *E. insulana* treated with magnetized water treatment and 498 µg D,L- alanine/g.b.wt in *E. insulana* treated with LC_{50} of *B. bassiana* + magnetized water comparing with untreated *E. insulana* value of free amino acids that was 299.7 µg D,L- alanine/g.b.wt (Table 3).

3.3.5 Invertase enzyme

Table 4 obvious the invertase enzyme activity of *E. insulana* that is considered one of the carbohydrate hydrolyzing enzymes. All the *B. bassiana* treatments used inverse its effects on the invertase enzyme activity that ranged from 63.3 µg glucose/ min/ g.b.wt. in *E. insulana* larvae treated with LC_{50} of *B. bassiana* + 700 Gy + magnetized water to 170.3 µg glucose/ min/ g.b.wt. in magnetized water treatment comparing with normal invertase activity of *E. insulana* (192.3 µg glucose/ min/ g.b.wt.) as in Table 4.

3.3.6 Trehalase enzyme

Normal activity of trehalase enzyme in *E. insulana* larvae was 252.3 µg glucose/ min/ g.b.wt. that was one of the carbohydrates hydrolyzing enzymes. Different *B. bassiana* treatments used had drastically decreased from trehalase enzyme activity of *E. insulana* treated as larvae with LC_{50} of different treatments, especially in the treatments of *B. bassiana* + 700 Gy + magnetized water that reached the

trehalase enzyme activity of *E. insulana* to 74.9 µg glucose/ min/ g.b.wt. While, magnetized water treatment of *E. insulana* larvae had the highest trehalase activity to reach 231.7 µg glucose/ min/ g.b.wt. compared with other treatment used (Table 4).

3.3.7 Amylase enzyme

Table 4 described one of the carbohydrates hydrolyzing enzyme activity that was amylase enzyme in *E. insulana* larvae. Normal activities of amylase were 119.3 µg glucose/ min/ g.b.wt. in *E. insulana* larvae. Its activity had drastically decreased when *E.insulana* treated as larvae with LC_{50} of different *B. bassiana* treatments used. *B. bassiana* + 700 Gy + magnetized water treatment caused the highly decreased in amylase activity reached to 47 µg glucose/ min/ g.b.wt. While, treatment of *B. bassiana* had the least amylase activity increasing (102.3 µg glucose/ min/ g.b.wt.) in *E. insulana* larvae (Table 4).

3.3.8 Acetylcholine esterase

Activity of acetylcholine esterase was stimulus affected by *B. bassiana* treatments with *E. insulana* larvae compared with untreated acts in enzyme activity decreasing in *E. insulana* larvae treated with LC_{50} of *B. bassiana*, *B. bassiana* + 400 Gy and *B. bassiana* + 700 Gy compared with untreated acetylcholine esterase activity of *E. insulana* larvae (222.7 µg Br /min/ g.b.wt.). Meanwhile, the rest of treatments increased from acetylcholine esterase activity of *E. insulana* larvae ranged from 313 µg Br /min/ g.b.wt. in magnetized water treatment to 889.7 µg Br /min/ g.b.wt. in *B. bassiana* + 400 Gy + magnetized water compared with normal acetylcholine esterase activity (Table 5).

3.3.9 Alanine amino transferase (ALT/ GPT)

ALT and AST have an important role in protein synthesis. Data in Table 5 showed remarkable decreasing in alanine amino transferase (ALT) in *E. insulana* larvae treated with LC_{50} of different *B. bassiana* treatments, especially in *B. bassiana* + 700 Gy + magnetized water that ALT activity reached 792.7 U x 10^3/g.b.wt. compared with normal ALT in *E. insulana* larvae that was 1849 U x 10^3/g.b.wt. Other treatments ranged the ALT activity in *E. insulana* larvae depress from 827.7 U x 10^3/g.b.wt. in *B. bassiana* + 400 Gy + magnetized water to 1708.3 U x 10^3/g.b.wt. in *E. insulana* larvae with magnetized water treatment (Table 5).

3.3.10 Aspartate amino transferase (AST/GOT)

All the *B. bassiana* treatments used caused stimulus depress from aspartate amino transferase enzyme (AST) in treated *E. insulana* larvae compared with untreated value (4063.3 U x 10^3/g.b.wt .) as in Table (5). The least AST activity found in *E. insulana* larvae treated with *B. bassiana* + 700 Gy + magnetized water (1904 U x 10^3/g.b.wt.), (Table 5).

4. DISCUSSION

Generally, it can be concluded that *B. bassiana* treatments classified into three categories according to its effects on the most toxicity, biological and biochemical assays of *E. insulana* treated as 1st instars larvae were used in the current work.

1. *B. bassiana* +700 Gy + magnetized water, followed by *B. bassiana* +400 Gy + magnetized water and *B. bassiana* + magnetized water that had the drastically effects on the most *E. insulana* assays used.
2. *B. bassiana* +700 Gy and *B. bassiana* +400 Gy had the intermediated effect on the most assays used of *E. insulana* treated as larvae.
3. *B. bassiana,* followed by magnetized water had the least effect on *E. insulana* larvae toxicity, biological and biochemical assays.

Results obvious the relationship among the treatments of *B bassiana* and it's exposing to gamma ray or magnetized water. *B. bassiana* that exposed to gamma ray to be genetic modified [22] for becoming the most effective compounds compared with the same compound without exposing to gamma doses. Moreover, in current work when *B bassiana* exposed to gamma ray and prepared with magnetized water become exposing to both effects of gamma ray and magnetized water that caused the highly effects on *E. insulana* larvae. Also, *B bassiana* prepared in magnetized water become active compound on *E. insulana* larvae than uses *B bassiana* alone without magnetized water. Effects of magnetic flux on water studied before with many works [23] that reported when the treated water using magnetic field, the covalent bound will broke leading to absorbed more energy causing to reduce the bounded between water molecules and increasing electrical decay. Also, they mentioned the molecules of a material could be either polar or no polar and it can change it from no polar to polar under influence of a magnetic field, when a no polar molecule becomes polarized, they will be charges, this charge will be pulling them together. Magnet also reduces hydrogen-oxygen bond angle within the water molecule. Meanwhile, [24-26] it was mentioned that water is under the influence of magnetic field, the magnetic force leads to breakage of these bonds and single particles are formed. These particles can easily and efficiently complete the hydration process as compared to normal water and increase the compressive strength.

Table 3. Biochemical assays of *E. insulana* treated with *B. bassiana* exposed to gamma ray and magnetized water

Treatments	Total Carbohydrates (mg/g.b.wt.)	Comparison with untreated	Total protein (mg/g.b.wt.)	Comparison with untreated	Total lipid (mg/g.b.wt.)	Comparison with untreated	Free amino acids (µg D,L- alanine/g.b.wt.)	Comparison with untreated
Untreated	45.7[a]	-	13.9[a]	-	21.9[a]	-	299.7[f]	-
Magnetized water	35.3[b]	-10.4	11.87[ab]	-2.03	21.1[a]	-0.8	234[g]	-65.7
B. bassiana	42[a]	-3.7	8.9[bc]	-5	11[bc]	-10.9	337.3[e]	+37.6
***B. bassiana*+ 400 Gy**	32.5[bc]	-13.2	8.87[bc]	-5.03	13.1[b]	-8.8	406[d]	+106.3
***B. bassiana*+ 700 Gy**	28.7[cd]	-17	6.67[cd]	-7.23	9.7[cd]	-12.2	481.3[b]	+181.6
***B. bassiana*+ Magnetized water**	28.3[cd]	-17.4	6.37[cd]	-7.53	8.07[de]	-13.83	498[b]	+198.3
***B. bassiana*+ 400 Gy + Magnetized water**	24.7[d]	-21	5.07[d]	-8.83	7.27[de]	-14.63	528.3[a]	+228.6
***B. bassiana*+ 700 Gy + Magnetized water**	17.97[e]	-27.73	3.77[d]	-10.13	6[e]	-15.9	445.3[c]	+145.6
L.S.D $_{0.05}$	5.120	-	2.870	-	2.667	-	19.85	-

Table 4. Biochemical assays of *E. insulana* treated with *B. bassiana* exposed to gamma ray and magnetized water

Treatments	Invertase (µg glucose / min/ g.b.wt.)	Comparison with untreated	Trehalase (µg glucose / min/ g.b.wt.)	Comparison with untreated	Amylase (µg glucose / min/ g.b.wt.)	Comparison with untreated
Untreated	192.3[a]	-	252.3[a]	-	119.3[a]	-
Magnetized water	170.3[b]	-22	231.7[a]	-20.6	95.7[b]	-23.6
B. bassiana	169[b]	-23.3	195.7[b]	-56.6	102.3[b]	-17
***B. bassiana* +400 Gy**	152.7[c]	-39.6	192.7[b]	-59.6	79.3[c]	-40
***B. bassiana*+ 700 Gy**	91.7[e]	-100.6	156.7[c]	-95.6	64[d]	-55.3
***B. bassiana*+ Magnetized water**	116.3[d]	-76	148[c]	-104.3	76[c]	-43.3
***B. bassiana*+ 400 Gy + Magnetized water**	75.7[f]	-116.6	95[d]	-157.3	56[e]	-63.3
***B. bassiana*+ 700 Gy + Magnetized water**	63.3[g]	-129	74.9[d]	-177.4	47[f]	-72.3
L.S.D $_{0.05}$	11.12	-	27.02	-	7.861	-

Table 5. Biochemical assays of *E. insulana* treated with *B. bassiana* exposed to gamma ray and magnetized water

Treatments	Acetyle cholin esterase (µg Br /min/ g.b.wt.)	Comparison with untreated	Alanine aminotransferase (ALT/GPT) (U x 10^3/g.b.wt)	Comparison with untreated	Aspartate aminotransferase (AST/GOT) (U x 10^3/g.b.wt)	Comparison with untreated
Untreated	222.7^{e}	-	1849^{a}	-	4063.3^{a}	-
Magnetized water	313^{d}	+90.3	1708.3^{b}	-140.7	2233.7^{c}	-1829.6
B. bassiana	209.3^{ef}	-13.4	1123^{c}	-726	3506^{ab}	-557.3
***B. bassiana* +400 Gy**	195.3^{f}	-27.4	911^{d}	-938	3066^{b}	-997.3
***B. bassiana*+ 700 Gy**	122.7^{g}	-100	860.3^{de}	-988.7	3043.7^{b}	-1019.6
***B. bassiana*+ Magnetized water**	405.7^{c}	+183	862.7^{de}	-986.3	2020^{c}	-2043.3
***B. bassiana*+ 400 Gy + Magnetized water**	889.7^{a}	+667	827.7^{de}	-1021.3	1994.3^{c}	-2069
***B. bassiana*+ 700 Gy + Magnetized water**	744.3^{b}	+521.6	792.7^{e}	-1056.3	1904^{c}	-2159
L.S.D $_{0.05}$	19.602	-	89.11	-	642.5	-

There is relationship among *B bassiana* effecting as a fungal compound with its exposing to both of gamma ray and magnetized water and the mechanism each of toxicity, biological and biochemistry in *E. insulana* treated as larvae. In biochemical analysis, the carbohydrates are considered the vital importance since they can be utilized by the insects' body for production the energy or conversion to lipids or proteins. Metabolism of carbohydrates is controlled mainly by carbohydrate hydrolyzing enzymes. The final product of carbohydrates metabolism is glucose, the increase of these enzymes during the larval stage suggested that these enzymes degrade carbohydrates to glucose for chitin build-up. Also, amylase activity decreasing leads the degradation of carbohydrates decreasing. This leads to disturbance in chitin building and failure of molting process [27]. Therefore, the inhibition of carbohydrate hydrolyzing enzymes recorded in the present study might affect the molting process and subsequently may explain the reason of mortality occurred.

These current results are in agreement with [28] that observed pronounced decrease in the carbohydrate hydrolyzing enzymes especially amylase and invertase when observed after treated of *S. littoralis* 5th instar larvae with sub-lethal concentrations of *B. thuringeinsin* (beta-exotoxin). On the other hand, Consult and Mimic (IGRs) decreased the invertase activity after 5 days of treatment, whereas Consult, Atabron and Cascade exhibited reduction in trehalase and invertase activities in *S. littoralis* [29]. Additionally, the activities of trehalase, invertase and amylase enzymes in *S. littoralis* larvae treated with Tracer (spinosad) and triflumuron were generally decreased than untreated larvae during different tested times [30].

[7] It was tested the 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 radiation at doses of 15, 30 and 60 Gy to increase its activity on toxicity and biological parameters of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) treated as 4th instar larvae. *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 the nearest results among tested bio-agents singly and the same bioagents exposed to gamma doses. Also, 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 in comparison to untreated.

[31] It was showed under laboratory conditions, the spiny bollworm, *Earias insulana* (Boisd.) adult stage field strain was exposed to the two magnetic fields (28.6 & 2.21 mt) to study some aspects of the pest acts in biological, morphological and biochemical assays as affected by the treatments used. Data obtained of *E. insulana* adult female biological aspects revealed that increasing in pre-oviposition period in the most treatments used as well as post-oviposition period; vice versa was happened with oviposition period. The same trend found in adult female longevity that increasing in the most treatments and contrary in male adult longevity. Eggs lying by treated adult female had severed reduction, especially in high magnetic field as well as fertility and fecundity. First generation of treated adults was increasing in larval and pupal duration, reduction and mortalities as well as pre-oviposition period in the most treatments. All biochemical determinations of adult *E. insulana* had reduction of total protein, free amino acids, total lipid, total carbohydrate, Alanine aminotransferase (ALT/GPT), Aspartate aminotransferase (AST/GOT) and phenoloxidase that reflects to depress malformations in different stages especially in temperature at level 30^{o}C. [8] It was exposed the spiny bollworm, *E. insulana* egg stage to two gamma rays (50&500 Gy) and magnetic flux (20&180 mlt) for studying some aspects of the pest act in biological assays as affected by the treatments used. The results showed that gamma ray dose of 500 Gy was the most efficacies on *E. insulana* egg compared with other treatments used. The aforementioned dose caused 19.3% egg hatchability and the larvae were completely dead at 1st or 2nd instar larvae of *E. insulana*. A dose of 50 Gy had a hatchability percentage (75%), but it caused the increasing larval mortality and completely pupal stage death. Meantime, magnetic flux of 180 mlt, followed by 20 mlt had many deleterious actions for biological parameters in *E. insulana* treated as one day old egg, it caused the decreasing hatchability, larval & pupal weights, longevity, sex ratio and no. of egg/female; on the other hand, it caused larval and pupal mortalities increasing. So, gamma-ray doses (50&500 Gy) treatments were the most efficacy against *E. insulana* egg stage than magnetic flux treatments (20& 180 mlt); but the magnetic flux caused severe deleterious on *E. insulana* biological parameters. Addition, [9] It had done a Field experiment at Plant Protection Research Institute Experimental Station, Qaha district, Qalubeiah governorate during 2018 &2019 two cotton seasons. Thirteen compounds related to different groups were used; three of them were exposed to gamma radiation doses of 400 &700 Gy for potentiating purpose. The treatments were *Bacillus thuringiensis* (Kurstaki), *Beauvaria bassiana* (Balsamo), *B. thuringiensis +B. bassiana*, *B.*

bassiana+400 Gy, *B. bassiana*+700 Gy, azadirachtin, azadirachtin+400 Gy, azadirachtin+700 Gy, fertacho, fertacho+400 Gy, fertacho+700 Gy, profenofos and profenofos+amadene. The treatments aforementioned were evaluated against three pests of cotton bolls that were pink bollworm, *Pectinophora gossypiella* (Saund); spiny bollworm, *Earias insulana* (Boisd.) and Cottonseed bug, *Oxycarenus hyalinipennis* (Costa) population and infestation reduction percentages. Profenofos+Amadene was considered the best treatments caused reduction percentages in population and infestations against three pests used, followed by profenofos as well as fertacho+ 700 Gy nearly, azadirachtin +700 Gy, fertacho + 400 Gy, azadirachtin + 400 Gy, fertacho, azadirachtin, *B. bassiana*+700 Gy, *B. bassiana* + 400 Gy, *B. thuringiensis* +*B. bassiana*, *B. thuringiensis* and *B. bassiana*. In addition, the compounds used enhance the most cotton crop parameters act in seed numbers, lint and seed weights during the two cotton seasons 2018 & 2019. So, gamma radiation can potentiate the three compounds of *B. bassiana*, azadirachtin and fertacho to become the most effective compounds on aforementioned three pests and cotton crop parameters compared with the same compounds without exposing to gamma radiation. [32] Isolated a local fungus *Beauvaria bassiana* was tested against the beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) larvae reared on artificial diet. Seven successive increased conidiospore concentrations (2×10^{123}, 2×10^{4}, 2×10^{5}, 2×10^{6}, 2×10^{7}, 2×10, and 2 × 10 spores/ml) were tested against larvae of L3 and L4. Larval mortality increased by increasing the conidiospore concentrations. The larvae of L3 were more susceptible to the treatment with *B. bassiana* conidiospores than larvae of L4. The infected larvae survived the tested low concentrations more than 5 days and died later in the fifth larval instar. The LC_{50} for L3 and L4 were 18.463 (slope 0.414) and 35.990 (slope 0.387) spores/ml; while, the LC_{90} was 37.806 (slope 0.345) and 74.391 (slope 0.387) for the two larval instars, respectively. Applying *B. bassiana* (6×10^{7} conidio spore/ml) for controlling the beet worm, *S. exigua*, in sugar beet fields at Fayoum Governorate, Egypt, resulted to a suppression in its larval populations through 5 applications by 54.5–70% in season 2016/2017 and 66.6–80% in season 2017/2018.

[33] The pest of date stored in Khuzestan Province is *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) treated with *Bacillus thuringiensis* Kurstaki bacteria and *Beauveria bassiana* pathogenic fungi on the population dynamics of *E. kuehniella* date stock pest was investigated. To fulfill this, insectariums were kept in vitro at an average temperature of 27 ± 5°C and RH of 40 ± 5% for duration of 6 months, equivalent to the maximum permitted shelf life of date. Three samples were taken from each insectarium. During 25-week storage period of date treated with *B. bassiana* and B.t.k, 6 outbreaks occurred in the population of *E. kuehniella*. The mechanism of the epidemic is in the way that, once the population has increased to a certain threshold, the incidence of the pathogenic epidemic will be mass-dependent, leading to a decreasing trend in the pest population. Therefore, population reduction was estimated to be at 6 to 32 times that of the untreated. The highest disease mortality rate occurred at the egg and larval developmental stages; so, the pathogens caused the highest mortality before the population entered the reproductive stage. Gradually, from the egg growth stage to the complete insect stage, a reduction was observed in the number of individuals which entered the subsequent growth stage. At all stages, the reduction in the treatment population group was faster than the untreated population. The highest reduction in the number of individuals entering the next developmental stage was associated with the larval developmental stage, followed by developmental stages of the adult, pupal, and egg. Regarding the highest potential of *B. bassiana* and BtK in reducing the stock pest population, it is very possible to exploit this interaction for biocontrol. Moreover, [34] revealed that *M. anisopliae* against *Cx. pipiens* showed maximum larval mortality (88%) with the lowest lethal time (LT_{50}) (22.6 hrs) at 108 spores/ml followed by *B. bassiana* (73.33%) with LT_{50} (38.35 hrs). A reduction in female fecundity, number of hatched eggs, pupation and adult emergence percentage were recorded. The biochemical analysis of the treated larvae revealed different quantitative decrease in total soluble proteins, lipids, and carbohydrate hydrolyzing enzymes compared to control. Histopathological effects of fungal infection upon insect cuticles, muscles, and midgut were investigated.

5. CONCLUSION

The relationship among the *E. insulana* treated as larvae toxicity, biological responses, biochemical assays and the compound of *B. bassiana* exposed to gamma ray (400 & 700 Gy) with preparing in magnetized water that led to completely mortality in the larval stage in the treatments of *B. bassiana* +700 Gy or 400 Gy + magnetized water due to inhibition in metabolism process of the most biochemical assay. Meanwhile, the same compound exposed to gamma ray 400 or 700 Gy without magnetized water used caused depression in all biochemical assays in *E. insulana* larvae lead to completely mortality less, but depress than mentioned treatments. Additionally, *B. bassiana* singly or magnetized water singly caused the

lowest effect on *E. insulana* larvae compared with other treatments used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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