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Diflubenzuron and lufenuron exposed to magnetic flux for *Earias insulana* (Lepidoptera: Noctuidae) suppression

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Abstract:

Two compounds of diflubenzuron and lufenuron were exposed to magnetic flux (180 millitesla) for potentiating purposes to study the toxicity and some biological aspects of the spiny bollworm *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae) treated as newly hatched larvae. Half lethal concentrations were 10.6 and 7.39 ppm for *E. insulana* newly hatched larvae treated with diflubenzuron and lufenuron exposed to magnetic flux compared with the same compounds without exposure to the magnetic flux that had LC_{50} 21.36 and 12.62 ppm. *E. insulana* treated as newly hatched larvae had larval mortalities of 61 and 62% for diflubenzuron and lufenuron without exposure to magnetic compared with 72 and 67% larval mortality for the same compounds exposed to magnetic flux; whereas, the untreated value was 5%. The larval duration was also affected by the elongation in the treatments exposed to magnetic flux compared to those without exposure. In addition, pupal duration and mortalities were affected by compounds used with magnetic flux to become 11.3 and 10.7 days and 45.4 and 53.9% pupal mortality for diflubenzuron and lufenuron exposed to magnetic flux compared with non-exposing compounds that were 9.6 and 9 days and 30 and 41.1% pupal mortality. The adult stage was also affected by reducing its emergency and the malformation was increasing as affected by the treatments exposing to magnetic flux compared with the same compounds without exposure.

Introduction

The spiny bollworm (SBW) *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae) is the key pest of the cotton and a wide range of crops in various parts of Egypt. The larvae feed on fruiting parts of the cotton plant, especially the terminal buds (Khan *et al.*, 2007). It is exposed to many insecticides during controlling some pests from these compounds; chitin synthesis inhibitor (CSI) or Insect growth inhibitor (IGR) groups.

The chitin synthesis inhibitor (CSI) as diflubenzuron or Insect growth regulator (IGR) as lufenuron are widely used in the cotton crop until now, because it was a potent compound against the arthropod, especially, different stage larvae of Lepidoptera. Most CSI or IGR are primarily used for the larval stage that develops until molting but fails to molt due to inhibiting the new cuticle synthesis (Kandil *et al*., 2013). Diflubenzuron or Lufenuron were used to control some lepidopterous (*E. insulana*,

Pectinophora gossepeilla (Saund.) (Lepidoptera: Gelechiidae), and *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) larvae, also recorded high increased mortality and caused the elongation of larval and pupal duration stages of *P. gossepeilla*, or *E. insulana* (Kandil *et al*., 2013).

The magnetic flux was considered one of the environmental factors that had a high effect on some biological systems; also, some trials are continually in the laboratory to knowledge the effects of MFs on some biological aspects of various insects. Pandir *et al.* (2013) reported the effects of MFs on egg hatching that was delayed and the hatching rate when exposing the *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) adults to levels of MFs. Said *et al.* (2017) studied the interaction of some magnetic flux with some biological aspects of *P. gossepeilla* . In addition, Kandil *et al.* (2018a) demonstrated that magnetic Ferro- solution had a high effect on behavior and reduced the fecundity and fertility of *P. gossypiella*. But, up to now, no trials used compound magnetization for controlling the insects in the open field. Moreover, Kandil *et al.* (2018b) mentioned that a magnetic field of 28.6 millitesla (mlt) was affected adult *E. insulana* more than 2.21 mlt on the most toxicity, biological, life table, and biochemical parameters. Meanwhile, Yan, *et al.* (2021) investigated the developmental and behavioral effects of rearing *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) in a near-zero magnetic field (<500 nT) compared to the local geomagnetic field (approximately 50 μT).

So, the current work aims to study the effect of magnetic flux on diflubenzuron and lufenuron potentiation for toxicity and some biological aspects of *E. insulana* treated as newly hatched larvae.

Materials and methods

1. Insect:

Spiny bollworm (SBW) *E. insulana* first instar larvae laboratory strain used in this work was obtained from the laboratory of Bollworms Research Department, Plant Protection Research Institute, that reared on an artificial diet described by Amer (2015).

2. Compounds:

2.1. Diflubenzuron (Benzoy phenyl urea); Dimilin 48%; application rate is 200 ml / 200 L.

2.2. Lufenuron: (Match 5% EC); application rate is 160 ml/feddan.

3. Adjusting and creating the magnetic flux:

Different concentrations of the tested compounds were prepared as follows:

3.1. Diflubenzuron: 120, 60, 30, 15, 7.5, 3.75 and 1.875 ppm.

3.2. Lufenuron: 40, 20, 10, 5, 2.5 and 1.25 ppm.

Each compound is allowed to be slowly passed through a narrow plastic tube (5ml diameter) between the main two magnetic poles of the (Static Magnetism Device) with 180 millitesla magnetic flux. The device was designed and measured in the Faculty of Engineering; Menofia University as described in Figures (1-3). The compounds exposed to magnetic flux were prepared at the same concentrations mentioned.

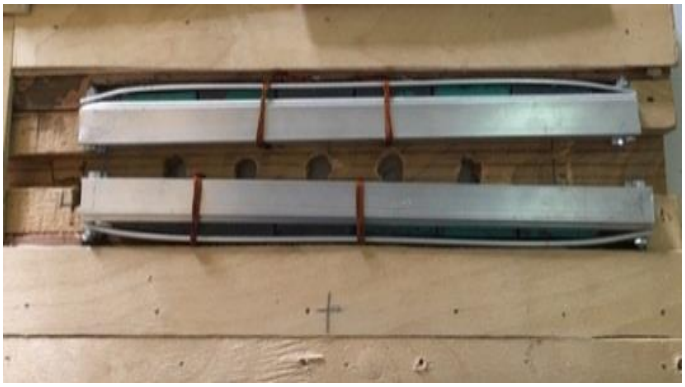

Figure (1): Apparatus consists of 2 rows inside each row 8 magnetic pieces.

Figure (3): Compounds exposed to magnetic flux.

Figure (2): Apparatus of Mille Tesla meter.

4. Bioassay:

Toxicity of tested compounds; diflubenzuron and lufenuron (non-exposing or exposing to magnetic flux) suppress 1st instar larvae of *E. insulana* were tested. The concentrations were sprayed on the surface of an artificial diet in Petri dishes. Three replicates; each replicate 30 newly hatched larvae of *E. insulana* were allowed to feed (3 days) on the treated diet. The untreated (Water only) was done. All treatment was kept under constant conditions of 26 ± 1°C and 65±5 % RH. After 3 days, the dead larvae were counted to represent the acute toxicity of the two tested compounds. Analysis was conducted to estimate LC_{25}, LC_{50}, LC_{90}, and slope values by Probit (Proban) analysis software according to Finney (1971). In addition, the efficiency of different compounds was measured according to Sun's equation (Sun, 1950) by comparing the tested compounds with the most effective ones using the toxicity index (TI).

$$\text{Toxicity index} = \frac{\text{LC}_{25} \text{ or } \text{LC}_{50} \text{ or } \text{LC}_{90} \text{ of the most toxic compound}}{\text{LC}_{25} \text{ or } \text{LC}_{50} \text{ or } \text{LC}_{90} \text{ of the tested compound}} \times 100$$

5. Biological aspects:

The newly hatched larvae of *E. insulana* were treated by LC_{50}'s of the two tested compounds (Exposing to magnetic flux and non-exposing) to study some biological aspects. In three replicates, 50 individuals were used for each replicate. The survival larvae for each treatment were transferred individually (after three days from treatment) by hairbrush to the diet tubes (2 × 7.5 cm) each containing about 3 gm of artificial diet. Another group used as untreated. The tubes were capped with cotton and kept under the previous conditions in an incubator and

investigated daily until pupation. Some biological aspects such as larval or pupal duration and mortalities %; also, adult malformations, and emergency percentages were done.

6. Statistical Analysis:

All biological aspects of *E. insulana* data values recorded were corrected by Abbott's formula (1925) and statistically analyzed, using Costat Statistical Software (1990) and then Duncan's Multiple Range Test (Duncan, 1955) at 5 % probability level to compare the differences among means.

Results and discussion

1. Bioassay:

Effect of diflubenzuron and lufenuron that exposing to magnetic flux (180 mlt) or non-exposing on the susceptibility of *E. insulana* newly hatched larvae after 3- days from treatment was presented in Table (1).

Table (1): Susceptibility of *Earias insulana* newly hatched larvae towards two compounds exposed to magnetic flux.

Compounds	LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope ±SE	Toxicity index		
					LC_{25}	LC_{50}	LC_{90}
Diflubenzuron	6.79	21.39	189.01	1.334 ±0.113	32.4	34.5	38.7
Diflubenzuron +180 mlt	3.51	10.6	86.55	1.405 ±0.121	62.8	69.7	84.6
Lufenuron	4.068	12.62	108.82	1.286 ±0.114	54.2	58.6	67.3
Lufenuron + 180 mlt	2.203	7.39	73.22	1.372 ±0.11	100	100	100

Based on LC_{50} values the two compounds exposed to magnetic flux were more effective against larvae of *E. insulana* than without exposure. The LC_{50} values were 10.6 and 7.39 ppm for diflubenzuron and lufenuron exposed to magnetic flux compared with non-exposing (Nearly equal to two times) which were 21.39 and 12.62 ppm.

2. Biological aspects:

2.1. Larval stage:

Results showed that exposure of both compounds to the magnetic flux at a level of 180 mlt caused increased mortality and malformation in the larval stage than the same compounds without exposure to magnetic flux and untreated (Table 2). Data presented in Table (2) showed that larval durations were increased to 18.3 and 17.6 days for *E. insulana* treated with LC_{50} values of diflubenzuron and lufenuron, respectively; whereas, the same compounds had elongation to 21.3 and 19.9 days, respectively when exposed to magnetic flux (180 mlt), compared with 14.9 days of *E. insulana* untreated larvae. Results were in agreement with Kandil *et al.* (2018b) who tested two levels of magnetic field (MF); 28.6 and 2.21 mlt on *E. insulana* larval duration. Also, the obtained data are in agreement with authors that tested different IGRs against *P. gossypiella* (Kandil *et al.* 2013 and Said *et al*., 2017).

Table (2): Effect of two compounds exposed to magnetic flux on *Earias insulana* larval stage.

Compounds	Duration (days)	Comparing with untreated	mortality %	Comparing with untreated	Malformed %	Comparing with untreated
Diflubenzuron	18.3^{b}	+3.4	61^{b}	+56	11^{b}	+9
Diflubenzuron +180 mlt	21.3^{d}	+6.4	72^{d}	+67	19^{e}	+17
Lufenuron	17.6^{b}	+2.7	62^{b}	+57	13^{c}	+11
Lufenuron + 180 mlt	19.9^{c}	+5	67^{c}	+62	16^{d}	+14
Untreated	14.9^{a}	0	5^{a}	0	2^{a}	0
$LSD_{0.05}$	1.533		3.140		1.054	

The larval percent mortality of *E. insulana* was 61 and 62% when treated with diflubenzuron and lufenuron. On the other hand, when the two compounds were exposed to magnetic flux (180 mlt), the larval mortality percent increased to 72 and 67 %, respectively, compared with 5% mortality in normal *E. insulana*. Meanwhile, percentages of malformed larvae were estimated by 11 and 13% when treated with diflubenzuron and lufenuron. While, it's was increased to 19 and 16% malformed larvae, when the same compounds exposing to magnetic flux, compared with 2% normal malformation. These results agree with Pandir *et al.* (2013) that showed the effects of MFs on the survival of *E. kuehniella*. The highest level of MF (10 mt) had completed mortality for *E. kuehniella*. Also, Matar *et al*. (2018) found that magnetic power (10 and 24 mt) had a highly significant effect on mortality and malformed larval and pupal stages of *E. insulana*.

2.2. Pupal stage:

Data presented in Table (3) obvious that *E. insulana* pupal duration treated as newly hatched larvae were 9.6 and 9 days when treated as newly hatched larvae with diflubenzuron and lufenuron and it was elongated to11.3 and 10.7 days for *E. insulana* treated with diflubenzuron and lufenuron exposing to magnetic flux, compared with 7.6 days in untreated. Kandil *et al.* (2018b) studied the adult stage field strain of *E. insulana* when exposed to the magnetic field (28.6 mt); the magnetic field caused an increase in pupal stages.

Table (3): Effect of two compounds exposed to magnetic flux on *Earias insulana* pupal stage.

Compounds	Duration (days)	Comparing with untreated	mortality %	Comparing with untreated
Diflubenzuron	9.6^{b}	+2	30^{b}	+27
Diflubenzuron +180 mlt	11.3^{d}	+3.7	45.4^{d}	+42.4
Lufenuron	9^{b}	+1.4	41.1^{c}	+38.1
Lufenuron + 180 mlt	10.7^{c}	+3.1	53.9^{e}	+50.9
Untreated	7.6^{a}	0	3^{a}	0
$LSD_{0.05}$	1.354		2.57	

The exposure compounds to magnetic power (180 mlt) affected *E. insulana* pupal mortality percent treated as newly hatched larvae. The effects were obviously 45.4 and 53.9% as treated by diflubenzuron and lufenuron exposed to magnetic flux. While, it decreased to 30 and 41.1% mortality when treated with diflubenzuron and lufenuron without exposure to magnetic flux, compared with 3 % mortality untreated.

2.3. Adult stage:

At the same trend, the adults treated as newly hatched larvae by the two compounds exposing to magnetic flux showed a reduction in the percentage of adult emergence by 51 and 48% with malformed 9 and 6% and 4 and 7% for adult female and male with diflubenzuron and lufenuron exposing to magnetic flux comparing with 67 and 69% adults' emergence with malformation of 6&3% and 7&2% for adult female and male treated as newly hatched larvae with diflubenzuron and lufenuron without exposing to magnetic flux. While normal adult emergency was 89% with 1.0 female and 2.0% male malformed in untreated (Table 4). Chun *et al.* (2014)

stated that adults of *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantriidae) were highly affected when they were exposed to the electromagnetic field.

Table (4): Effect of certain compounds exposed to magnetic flux on *Earias insulana* adult stage.

Compounds	Emergency %	Comparing with control	Malformed %		Comparing with control	
			♂	♀	♂	♀
Diflubenzuron	67^{c}	-22	7^{c}	6^{c}	+5	+5
Diflubenzuron +180 mlt	51^{b}	-38	4^{b}	9^{d}	+2	+8
Lufenuron	69^{c}	-20	2^{a}	3^{b}	0	+2
Lufenuron + 180 mlt	48^{a}	-41	7^{c}	6^{c}	+5	+5
Control	89^{d}	0	2^{a}	1^{a}	0	0
$LSD_{0.05}$	4.133		0.58	1.47		

Current data indicated that there was a positive effect of tested magnetic power (180 mlt) on the two compounds used (Diflubenzuron and lufenuron) that were exposed for 15 minutes; it increased the potency of the compounds and increased the toxicity that lead to an increase the mortality with malformed and a high prolongation the larval & pupal stages and a high reduction in adults emergency. Current results agree with many studies that showed that MF had a high affected on survivor, longevity, viability and fertility of *E. kuehniella* (Pandir *et al.*, 2013). In addition, Kandil *et al.* (2018b) exposed *E. insulana* adult stage to magnetic fields (28.6 & 2.21 mlt) lead to an increase in pre-oviposition, post-oviposition period, female longevity; while reducing was happened in oviposition period, male longevity, egg laying rate, fertility, and fecundity. Also, life table parameters were affected with two magnetic fields used by decreasing female progeny/ female (Mx), survival rate (Lx), net reproductive rate (Ro), intrinsic rate of natural increase (r_m), finite rate of increase (e^{rm}) and sex ratio; whereas, increasing was happened in generation time (T) and doubling time (DT) compared to untreated value.

On the other hand, all biochemical determination for *E. insulana* as affected by two magnetic fields used had reduced total protein, free amino acids, total lipid, total carbohydrate, alanine aminotransferase (ALT/ GPT), aspartate aminotransferase (AST/ GOT) and fenoloxidase. Moreover, Amer *et al.* (2019) tested the effects of magnetic flux (20 and 180 mlt) for *E. insulana* treated as an egg. The magnetic flux of 180 mlt, followed by 20 mlt had many deleterious actions on biological parameters such as decreasing hatchability, larval and pupal weights, longevity, sex ratio, and no. of egg/ female; on the other hand, it caused larval and pupal mortalities increasing. In addition, life table parameters of *E. insulana* were affected; female progeny/ female (Mx), survival rate (Lx), net reproductive rate (Ro), intrinsic rate of natural increase (r_m), finite rate of increase (e^{rm}) were decreasing in both treatments.

Meanwhile, generation time (T) and doubling time (DT) were increased as affected by magnetic flux treatments as a 1-day old egg compared with

untreated *E. insulana* egg. Meanwhile, Yan *et al.* (2021) investigated the developmental and behavioral effects of rearing *M. separata* in a near-zero magnetic field (<500 nT) compared to the local geomagnetic field (approximately 50 μT). The near-zero magnetic field produced by a Helmholtz coil system significantly lengthened larval and pupal development durations increased male longevity, and reduced pupal weight, female reproduction, and the relative expression level of the vitellogenin (Vg) gene in newly emerged females.

Moreover, the near-zero magnetic field had a considerable negative effect on the mating ratio of *M. separata* adults. In addition, the moths in the near-zero magnetic field displayed less flight activity late in the night than those in the Earth's normal geomagnetic field, indicating that the flight rhythm of *M. separata* may be affected by the near-zero magnetic field. Reduction in magnetic field intensity may have negative effects on the development and flight of oriental armyworm, with consequent additional effects on its migration.

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