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Suppressive effect of Benzothiophene derivatives and/or gamma radiation on antioxidant response of *Spodoptera littoralis* larvae

Rehab M Sayed[1*], Salwa A Rizk[2], Tammy S El Sayed[3], Ragaa S Abdalla[4], Mohamed M Ghobashy[5]
[1-4] Natural Products Department, National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt
[5] Radiation Research of Polymer chemistry Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt

Abstract
This study was designed to evaluate the effect of the sub sterilizing dose of gamma radiation (70Gy) and LC_{50} of three benzothiophene derivatives on the lipid peroxidation content as an oxidative stress biomarker and on the antioxidants response (glutathione –S-Transferase (GST) activity and glutathione reduced level (GSH) in 4th instar larvae of F_1 and normal *Spodoptera littoralis* larvae. The results revealed that both gamma radiation and the three benzothiophene derivatives induced a significant decline in the GST and GSH which was remarkable in F_1 larvae resulted from irradiated male parent pupae with 70Gy and feed on treated leaves with the first derivative. In contrast, the MDA exhibited a significant elevation which also was pronounced in F_1 larvae resulted from irradiated male parent pupae with 70Gy and feed on treated leaves with the first derivative. Consequently, it could be concluded that the three derivatives and their combination with gamma radiation disturbed the physiological balance *Spodoptera littoralis* which lead to larval mortality and can use them as an integrated control program for *S. littoralis*.

Keywords: *Spodoptera littoralis*, benzothiophene derivatives, gamma radiation, GST, GSH, MDA

Introduction
The heterocyclic compounds such as thiophene derivatives have high attention due to their pesticide and pharmacological importance, dyestuffs, chemical reagents and auxiliaries for high polymers [1-4]. The derivatives five - membered of thiophene heterocyclics have most wide applications fused with five membered aromatic rings made up of one sulfur as heteroatom with formula C8H6S. These compounds can be easily converted to their corresponding acids, esters and amines [5, 6]. The thiophene derivatives heterocyclic being useful as pesticidal compounds and as antibiotics [7-13].
Pesticides exert their biological effects through the generation of reactive oxygen species (ROS) [14] which resulting in adverse effects in different living organisms and a significant damage to cell structures [15], this process is known as oxidative stress [16]. Oxidative stress has been shown to be associated with exposure to several organophosphorous compounds [17] and different classes of pyrethroids [18]. In addition, oxidative stress is defined as an imbalance between higher cellular levels of ROS and the cellular antioxidant defense [19]. Since, ROS cause lipid peroxidation; protein, enzyme, and DNA oxidation; and glutathione (GSH) depletion, leading to oxidative damage in insect tissues [20].
Malonaldehyde (MDA) is one of the indicators of lipid peroxidation, and it also reacts with DNA, protein, enzyme, and other biomolecules, leading to oxidative damage [21].
A suite of biochemical defense mechanisms called the antioxidant defense system is found in different organisms to prevent cellular damage from ROS by the antioxidant enzymes (catalases (CAT), peroxidases (POX) superoxide dismutases (SOD) and glutathione-S-transferase (GST)) and nonenzyme antioxidants (glutathione reduced (GSH),) [15, 22].
The GSTs is an enzyme with critical role in detoxification of both endogenous and xenobiotic compounds and are also involved in physiological processes such as intracellular transport, biosynthesis of hormones and protection against oxidative stress [23]. GSH plays a multifunctional role in antioxidant protection, acting as a cofactor for a number of antioxidant enzymes, and directly scavenges ROS and peroxides [24].
Insects can catabolize toxic or other detrimental chemicals that endogenously produce ROS for survival in a chemically unfriendly environment. Furthermore, insects can express a suite of antioxidant enzymes that protect cells from the damaging effects of oxidative stress [20, 23].
Thus, the aim of the present work was to evaluate the impact of thiophene derivatives on the redox balance of the cotton leaf worm for being used as new safe insecticides.

Material and Methods
The three organic compounds used for this study display in table (1).

Table 1: List of the organic compounds used for this study

The compound	The Formula
Compound A	3-*Chloro*-1-benzothiophene-*2-carbonyl* chloride
Compound B	3 chlorobenzo (b) thiophene-2-carbonyl aspartic acid
Compound C	3 chlorobenzo (b) thiophene-2-carbonyl cysteine (C)

Preparation method

Preparation of 3-*Chloro*-1-benzothiophene-*2-carbonyl* chloride (Comp A)

In (500 ml) three necks round flask (30 gm 0.20 mol) of the trans-cinnamic acid was gradually added to thionyl chloride (20 ml) with stirring. And then, gradually add 16 ml of pyridine to the mixture and heated to 130 oC for 2 hr. The rest of the thionyl chloride (30 ml) was added dropwise over a period of 2 hr. The mixture was stirred at this temperature (130 °C) for an additional 1 hr. the mixture was cooled, and dissolved in 200 ml of n-hexane. The solution was dry in ambient condition and given yellow precipitation of a racemic mixture of 3-Chloro-1-benzothiophene-2-carbonyl chloride (A) and another compound as detected by TLC and M.P. the two compounds could be separated by dissolving the product in chloroform. We found un-dissolving white powder m.p (315-325ºC) and the yellow mother liquor was evaporated given yellow PPT with m.p (112 ºC) should it is pure 3-*Chloro*-1-benzothiophene-*2-carbonyl* chloride (A). Total yield of (A) was 67%.

The preparation of 3 chlorobenzo (b) thiophene-2-carbonyl aspartic acid (Comp B)

In three necks round flask, under heterogeneous conditions (3 g - 0.013 mol) of (A) ether reacts with sodium hydroxide powdered in 20 ml acetone / diethyl ether anhydrous. (3 g - 0.013 mol) of (A) after complete dissolved the homogenous yellow solution was formed. Add to a solution (1.73 g 0.013 mol) of aspartic acid and (0.5 g-0.125 mol) of NaOH. The mixture was heated at 65ºC (bath temperature) for 24 hr ambient temperature for 3 hr, and then filtrate the solution. The crystals are formed after recrystal dissolving the product in hexane given comp (B).

The preparation of 3 chlorobenzo (b) thiophene-2-carbonyl cysteine (Comp C)

In three necks round flask, under heterogeneous conditions (3.1 gm 0.0123) of (A) in 20 ml in acetone / diethyl ether anhydrous. Add to solution (5.33 g 0.013 mol) of cysteine and (0.5 g-0.125 mol) of NaOH. The mixture was heated at 65°C (bath temperature) for 3 hr ambient temperature, and then filtrate the solution. The white crystals are formed of comp (C).

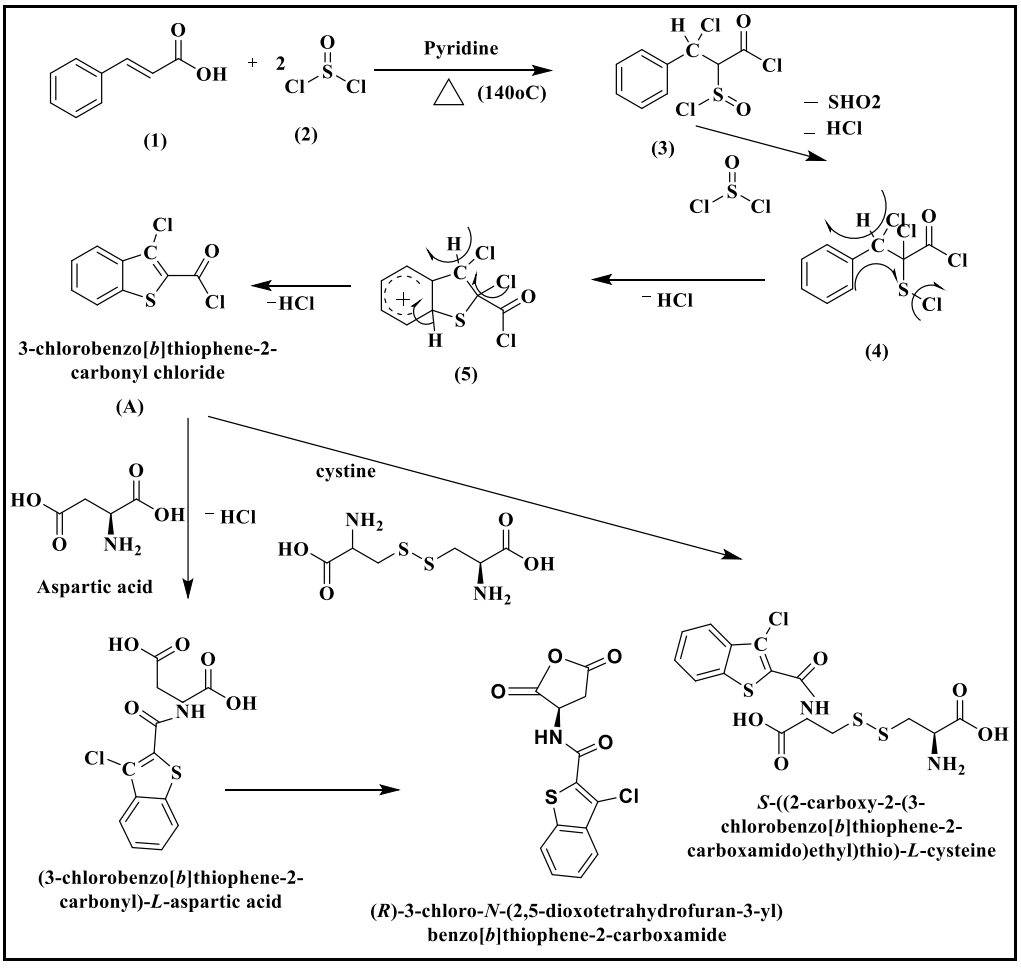

Fig 1: Proposed concentrate elimination cyclization (CEC)

Irradiation Technique

Full grown male pupae of cotton leaf worm *S. littoralis* were irradiated with 70Gy in Cobalt-60 gamma cell. This source is located, at National Center for Radiation Research and Technology, Cairo (NCRRT). The dose rate was 1.269KGy/h. at the time of the present investigation (July, 2018). LC_{50} values were calculated based on the accumulative mortality after 72hrs. using a software package Ldp-line" a copyright by Ehab, M. Bakr, Plant Protection Research Institute, ARC, Giza, Egypt. Full grown male pupae irradiated with 70Gy and the F_1 larvae were used in the experiments.

Biochemical studies

1gm of the samples (larvae treated with LC_{50} of each compound or F1 larvae alone or their combination) were homogenized in a cold glass homogenizer containing phosphate buffer saline at 4000 rpm for 15 minutes at 4ºC, the supernatants were kept frozen at-20 °C till required after 24h [25].

Assay of Glutathione –S-Transferase (GST) activity was estimated using a commercial assay kit obtained from Biodiagnostic (Egypt) based on the method of Habig *et al*. [26].

Estimation of glutathione reduced (GSH) plasma level was carried out according to the method described by Srivastava and Beutler [27] using a commercial assay kit obtained from Biodiagnostic (Egypt). Lipid peroxidation content (Malondialdehyde, MDA) was determined according to Yoshioka *et al*. [28] using a BioDiagnostic kit (BioDiagnostic Co., Egypt).

Statistical Analysis

The data were statistically evaluated by analysis of variance (F) followed by Tukey Pairwise Comparisons test to examine the significant differences between the treatments. The statistical Minitab program was used for all analyses.

Results

Molecular reactivity indices stemming from density functional theory (DFT), such as chemical potential (μ), hardness (η), electrophilicity (ω) and softness (S) were computed from the energies of frontier orbitals (which are represented in scheme1). The results of frontier molecular orbitals FMO such as E_{HOMO}, E_{HOMO-1}, E_{LUMO}, E_{LUMO+1} and HOMO-LUMO energy gap (Eg) of the three moleculea A, B and C are summarized in Fig. (2).

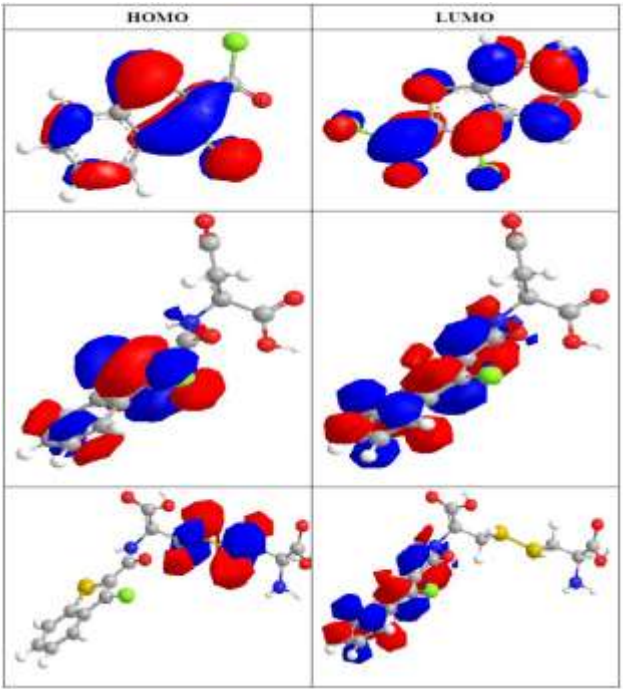

Fig 2: Calculated HOMO, LUMO, Energy Gaps (HOMO-LUMO) and Related Molecular Properties of the three cpds.

A detail of quantum molecular descriptors of three Cpds, within the DFT, these can be approached by the frontier HOMO and LUMO energies. Such as the electron affinity (*A*), ionization potential (*I*), electronic chemical potential (μ), chemical hardness (η) and electrophilicity (ω) are collected in Table 1. As shown in Fig. 1 and Table 2, E_{HOMO} and E_{LUMO} of the three Cpds. As shown in Figure 1, charge transfer takes place within the molecules. The graphic pictures of LUMO and HOMO orbitals show that the HOMO of (A) molecule is localized mainly on thiophene moieties and LUMO is localized mainly on carbonyl chloride. The HOMO → LUMO transition implies an electron density transfer from c thiophene moieties to both carbonyl chloride and benzene ring. For cpd (B) HOMO is localized mainly on thiophene moieties and LUMO is localized mainly on 3-Chloro-1-benzothiophene-2-carbonyl chloride. The HOMO → LUMO transition implies an electron density transfer from c thiophene moieties to 3-Chloro-1-benzothiophene-2-carbonyl chloride molecules. For cpd (C) is different where HOMO is localized mainly on cysteine molecules and LUMO is localized mainly on 3-Chloro-1-benzothiophene-2-carbonyl chloride. The HOMO → LUMO transition implies an electron density transfer from cysteine molecules to 3-Chloro-1-benzothiophene-2-carbonyl chloride molecules.

Table 2: The Energy gap and the electrophilicity ω of the three compounds.

Property	Cpd (A)	Cpd (B)	Cpd (C)
E_{HOMO} (eV)	-9.23	-9.09	-4.13
E_{LUMO} (eV)	-3.822	-2.862	-2.880
E_{HOMO-1} (eV)	-10.019	-9.780	-8.217
E_{LUMO+1} (eV)	+0.289	+0.206	+0.538
Energy gap (E_g) (eV)	5.41	6.23	1.25
Ionization potential ($I=-E_{HOMO}$)(eV)	+9.23	+9.09	+4.13
Electronic affinity ($A=-E_{LOMO}$) (eV)	+3.822	+2.862	+2.880
Chemical potential ($\mu=-(I+A/2)$(eV)	6.526	5.976	3.505
Global hardness $\eta=(I-A/2)$(eV)	2.705	3.115	0.625
Global electrophilicity ($\omega=\mu^2/2\eta$) (eV)	3.83907	55.62234	57.60118
Softness $S = 1/\eta$	1.6	0.321027	0.369686

Fourth instar larvae treated with LC_{50} of each of the three compounds alone showed that GST activity of untreated larvae was 2.69 this decreased in F1 larvae produced from irradiated male parent pupae with 70Gy to become 1.85, GST activity of 4th instar larvae feed on castor leaves treated with LC_{50} of Comp C, Comp B and Comp A were 0.904, 1.022 and 1.52 respectively, but more decrease in GST activity were obtained when F1 irradiated larvae treated with LC_{50} of each of the three compounds to be 0.362, 0.625 and 0.978 respectively.

Statistical analysis showing significant differences in GST between control and different treatments and also between all treatments with each other except between larvae treated with comp c and comp b and F1 irradiated larvae treated with LC_{50} of comp A (Fig. 3).

- Values represent the mean ± S.E of 3 replicates.
- Different letter means statistically significant at $p<0.05$ (Tukey Pairwise Comparisons test).

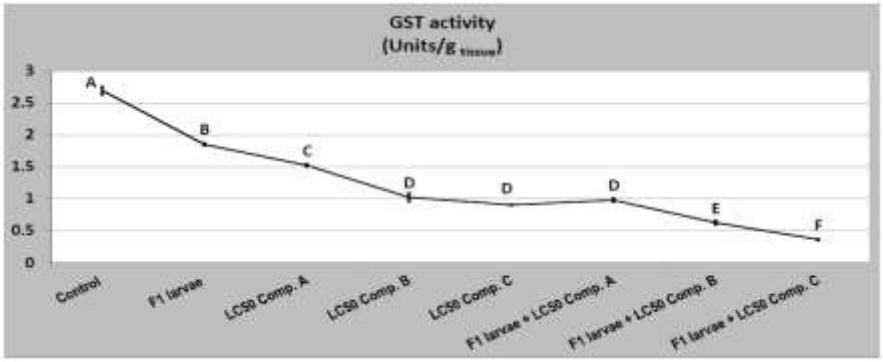

Fig 3: Changes in GST activity as a result of feeding 4th instar larvae on castor leaves treated with three toxic compounds alone or combined with gamma radiation

Data in Fig. (4) displayed that GSH level was 43.61 in untreated larvae (control) and decreased in F1 larvae produced from irradiated male parent pupae with 70Gy to become 37.52, GSH content of 4th instar larvae feed on castor leaves treated with LC_{50} of Comp C, Comp B and Comp A were 15.79, 20.86 and 33.46 respectively, but more depletion in GSH activity was obtained when F1 irradiated larvae treated with LC_{50} of each of the three compounds to reach 8.97, 13.30and 19.62 respectively. Statistical analysis showed a significant difference between GSH level in control and different treated groups.

- Legends as in Fig. 3.

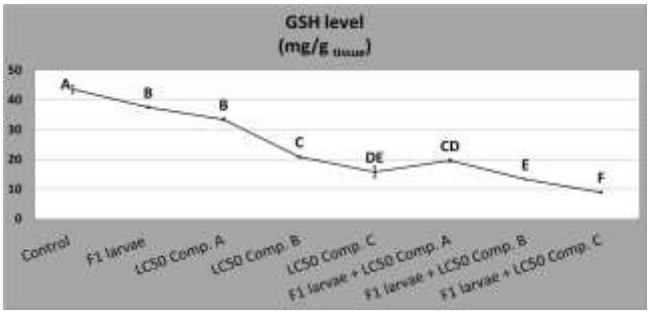

Fig 4: Changes in GSH level as a result of feeding 4th instar larvae on castor leaves treated with three toxic compounds alone or combined with gamma radiation.

Figure (5) declared that MDA content in all treatment groups was higher than that in control one. Comp C was the most effective compound. Treatment the larvae with gamma radiation beside any of the three compounds increased the MDA content more than that in larvae treated with any of the three compounds alone, Comp C record 85.52 compared to 54.37 in unirradiated larvae. Comp B records 64.52 in irradiated larvae compared with 44.22 in unirradiated larvae Also in case of Comp A MDA contents were 29 and 46.67 in unirradiated and irradiated larvae.

- Legends as in Fig. 3.

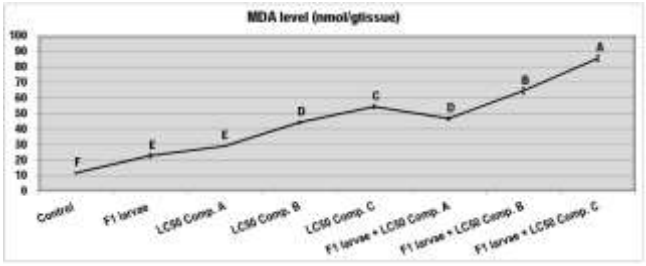

Fig 5: Changes in MDA content in4th instar larvae as a result of feeding on castor leaves treated with three toxic compounds alone or combined with gamma radiation

Discussion

The present data showed that treatment of 4th larval instar of *S. littoralis* with the three derivatives of benzothiophene caused a significant decrease ($p<0.05$) in GST activity and GSH level in larvae feed on treated leaves with LC_{50} of benzothiophene derivative or in F1 larvae produced from irradiated male parent pupae with 70Gy or in F1 larvae feed on treated leaves. Moreover, these reductions were more reliable in F1 larvae feed on treated leaves. The reduction in GST was in accordance to Mukanganyama *et al.* [29] who discovered a reduction in GST activity of *Rhopalosiphum padi* treated with DIMBOA (2,4-dihydroxy-7-methoxy- 1,4-benzoxazine-3-one). Also, Büyükgüzel *et al.* [30] conveyed presence a significant decline in GST activity of *G. mellonella* treated with boric acids. Abu El-Saad [31] discovered a significant lowering in GSH in *Apis mellifera* from polluted environment with organophosphorus insecticides. While, Morales *et al.* [32] stated that there was no significant alterations in GST and GSH of *Cydia pomonella* treated with chlorpyrifos. Moreover, a significant depletion in GSH and GST induced by gamma radiation in *Blaps polycresta* was reported by Kheirallah and Abu El-Samad [33].

Basically, GSH and GST have a vital role in the nonenzymatic and enzymatic antioxidative reactions that guarding cells from oxidative damage by sustaining cellular redox balance. Thus the obtained reduction might be attributed to their exhaustion in the scavenging free radicals that possibly produced by pesticides [34].

The obtained depletion by gamma radiation in GSH and GST might be due to its deleterious impact which could be inherited for next generations [35]. Therefore in our opinion, the remarked decline of GSH and GST in the combination treatment of gamma radiation and LC_{50} of the benzothiophene derivative could be regarded to the synergistic effect of the both tool.

In contrast, the obtained data declared a significant raise ($p<0.05$) in MDA level in larvae feed on treated leaves with LC_{50} of benzothiophene derivative or in F1 larvae produced from irradiated male parent pupae with 70Gy or in F1 larvae feed on treated leaves. Furthermore, these increases were more reliable in F1 larvae feed on treated leaves. A significant elevation in MDA by insecticides was previously reported in *Helicoverpa armigera* treated with pyrethroids, permethrin and fenvalarate [36]. Furthermore, Abu El-Saad [31] exposed a significant increase in MDA of *Apis mellifera* from polluted environment with organophosphorus insecticides. The same finding was found in *G. mellonella* treated with boric acids by Büyükgüzel *et al.* [30]

The rise of MDA can be clarified by the elevation of ROS ($HO^{\cdot}$), which damages polyunsaturated fatty acids of cell membranes and is one of the reasons of the deficiency of both GST and GSH [37].

The obtained results of comp C were more noticeable than those of comp A and B. This may be attributed to the cysteine group added to the benzothiophene in comp C that contains a sulphur molecule. Since, sulphur can deteriorate the enzyme systems and disturb the physiological balance of the living body [38].

Conclusion

From the aforementioned results, three benzothiophene derivatives caused a significant physiological disturbance (oxidative stress and antioxidant alterations) in *Spodoptera littoralis* larvae which lead to larval death like other insecticides. Subsequently, we concluded that three benzothiophene derivatives could be used as an ecofriendly pest control tools especially in combination with gamma radiation.

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