Impact of Gamma Irradiation and Phenol on the Biological Activity of the Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.)

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ABSTRACT

Gamma irradiation and Simple Phenol could potentially be used as a new approach to control the cotton leaf worm, *Spodoptera littoralis* (Boisd.). Inherited sterility (IST) technique using sub-sterilizing doses of gamma irradiation and/or different concentrations of phenol were tested on the biological activity of *S. littoralis*. The biological aspects included egg hatchability; larval mortality, pupation, survival, adult emergence, sex ratio and longevity were tested. It was noticed that the phenol concentrations (2.5 and 5%) and sub-sterilizing dose of gamma irradiation 100Gy were the most promising for combination treatment. The results indicated that the combined treatments drastically decreased the percentages of pupation and survival more than separate treatments, to reach 3.0 and 2.0%, respectively in the case of 100Gy+ 5%. Also, the deleterious effect of combined treatments on growth inhibition, relative growth index, growth index was sharply decreased in F₁ generation than each treatment. The reduction of F₁ progeny in combined treatments was higher than could be expected from the sum of the two treatments. It was concluded that insect suppression was more pronounced in the case of 100Gy+ 5% phenol than in the case of 100Gy+ 2.5% phenol. These findings indicate that the IS technique might be considered as an environmentally compatible alternative or supplement to the phenol for *Spodoptera littoralis* pests management.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Boisd.) is one of the main important harmful insect pests around the world (¹). It has a wide range of host, feeding on 112 species worldwide, of which 100 species are known in Egypt (²).

The intensive uses of chemicals to control this pest lead to many problems to the environment or damage to non-target species (biological enemies), in addition to enhancing insect resistance. Using gamma irradiation to release sterilized insects is one of the successful new pest control technology and environmental safety (³, ⁴). Inherited sterility (IST) offers significant advantages over classical sterile released method for lepidopteran insects (⁵, ⁶). IS technique depends on using substerilizing doses of gamma irradiation to produce sexually offspring which are nearly sterile; thus, reproduction is suppressed in at least two generations with only one release.

Previous studies also demonstrated that some plants have been shown as sources of insect toxicity. These plants contain some compounds, which are useful for plants and toxic for insects. This includes phenols which occur in the leaves and stems of plant to prevent water loss and protect the plant from sunlight. Another ecological role of the phenolic components is related to defense against phytophagous insects, as other compounds like flavonoids (⁷), triterpenoids (⁸), gedunin (⁹) and phytoecdysteroids (¹⁰). These plant compounds are not widely used in pest management as they are less effective than organic pesticides and to avoid using organic solvents needed for dissolving which are environment unfriendly and expensive. The toxicological properties of pheolic compound 2,6-diiodophenol; 4-chloro-2,6-diiodophenol and; 2,6-dichloro-4-iodophenol was evaluated on *Aedes aegypti* (¹¹) and indicated that the compound 4-chloro-2,6-diiodophenol is the most toxic.
Interest in the application of phenolic components for insect pest management is basically due to its availability, reasonable cost and low mammalian toxicity. Moreover, the use of these components leads to avoiding deleterious effects produced by synthetic pesticides on the environment.

On the other hand, any insect control measure that will reduce population densities before the application of an IS technique will enhance the effectiveness of both procedures. Thus, phenol might be effectively used with IST program. The response of irradiated Lepidoptera insects to insecticides or plant extracts was studied by several authors (12, 13, 14). Thus, the aim of this study is to investigate a new approach for using a combination treatment of IS with simple phenol against Spodoptera littoralis. Thus, the larvicidal activity and certain biological aspects of P₁ and F₁ generations of combined treatment were studied.

2. MATERIALS AND METHODS

2.1. Insect rearing

The culture of the cotton leaf worm S littoralis was reared and maintained for several generations in the laboratory at 25 ± 2 °C and 65% R.H. Larvae were fed on fresh castor oil plant leaves, Ricinus communis.

Irradiation Technique

Full-grown pupae of S. littoralis (male and female) were irradiated using Cobalt 60 gamma cell. This source is located at the cyclotron project, Nuclear Research Center, Atomic Energy Authority, Egypt. The dose rate of irradiation source was 2.0 Gray/second. The emerged parental moths (P₁) were allowed to mate with opposite unpredicted sex to obtain the F₁ generation. The biological aspects of P₁ and F₁ were studied.

Chemical Assays

The phenol reagent (C₆H₅OH) 95% was obtained from El-Gamhoria Company. The newly molted fourth instars larvae of S. littoralis were fed on fresh leaves of castor bean that treated with tested phenol dissolved in distilled water at concentrations of (10, 5, 2.5, 1.25 and 0.625) using the leaf dipping technique (15).

Combination Treatments

The experiments of combined treatment were set up to evaluate the biological effects of Phenol on the F₁ adults produced from irradiated pupae with 100 Gy at parent generation. Groups of F₁ larvae were treated with 2.5 and 5% Phenol dissolved in water. The produced F₁ male moths from male line were then crossed with the opposite sex of un-irradiated adults. The F₁ female progeny from female line were crossed with un-irradiated male moths. Parallel groups of separately treatments Irradiation treatments and Phenol treatments were used for comparison between separate and combination treatments. In addition, a control group of non-irradiated one was used. The biological activity was measured according to the following equations (16).

- The growth inhibition (GI) = number of surviving larvae/total larvae used.
- Relative growth inhibition (RGI) = GI treatment/ GI control
- Percentages of reduction in F₁ progeny = (Number of adults emerged in control – Number of adults in treatment)/ No. of adults in control
- Growth index = % of adult emergence / Total period.
RESULTS AND DISCUSSION

3.1. Effect of Phenol on the Biological Aspects

Fig. (1) shows that the percentages total mortality till adult stage was increased (0, 36.0, 45.0, 48.0, 61.0 and 95.0 %,) with the concentration increase of 0, 0.625, 1.25, 2.5, 5 and 10%, respectively, while the percentages of survival and pupation significantly decreased as the concentration increase to reach 5 and 8% at 10%, respectively. The mortality, pupation and survival can be used to determine the effective toxic concentrations of phenol. On the other hand, the data of the same table demonstrate that the percentages of adult emergence were insignificantly different in the tested range of phenol concentrations, while the percentages were significantly decreased as compared with control treatment.

![Graph showing percentages of mortality, pupation, adult emergence and survival](image)

**Fig. (1):** Percentages of mortality, pupation, adult emergence and survival of phenolic treated 4th instar larvae of *Spodoptera littoralis*

The obtained results of mortality, pupation and survival indicated that, at low phenol concentrations from 0.625 to 2.5%, the relations were linear as compared with control treatment. Above 2.5% the toxicity was too high and increased by increasing the phenol concentration.

Fig. (2) shows the average developmental period that 4th instar larvae surviving to pupae greatly prolonged and the average larval period were (20.20, 20.38, 20.04, 19.46 and 19.20 days) in phenol concentration (10%, 5, 2.5, 1.25 and 0.625%), consequently as compared with control treatment. Regarding adult longevity (Fig. 2), a significant reduction was found with the phenol concentrations in comparison to the control treatment. The results in Fig. (2) also indicated that there were no significant differences in pupal duration as compared with the control.

![Graph showing larval duration, pupal duration and adult longevity](image)

**Fig. (2):** Average larval, pupal duration and adult longevity of phenolic treated 4th instar larvae of *Spodoptera littoralis*
In general, the results confirm that phenol treatment may be attributed to the insect growth regulating effect of phenol in the treated larvae. The toxicity bioassay of pheolic compounds was studied by Catelan et al. (11) on Aedes aegypti, where it was found that the compound 4-chloro-2,6-diiodophenol showed a high toxicity and larvaicidal activity, as well as hatching was 100% inhibited by exposure to the compound 4-chloro-2,6-diiodophenol, 4- hydroxyl-3,5-diiodacetophenone and 2,6- dichloro-4-iodophenol. Also, that pheolic compounds gallic acid and salicylic acid were toxic to Spodoptera frugiperda (7). Many authors obtained similar results for the mortality and survival of lepidopteran insects as a result of plant extracts treatment containing phenolic components, (17) on Spodoptera frugiperda, (18) on Agrotis ipsilon and (19) on Spodoptera litura.

3.2. Irradiation Treatment

The data given in Fig. (3) indicates that the percentages of larvae survived to adults were obviously reduced from 76% in the control to 52, 34 and 0% in the treatments of 100, 150 and 300Gy respectively, and the same trend was observed in the percentages of egg hatch and pupation, while, irradiation doses 100 and 150Gy did not clearly affect the percentages of adult emergence, however the percentage gradually reduced to reach 0% at the dose 300Gy.

![Gamma irradition (Gy) vs (%) Insects](image)

**Fig. (3): (%) mortality, pupation, adult emergence and survival of gamma irradiated pupae of Spodoptera littoralis**

The results confirm the finding that the sterility (expressed as un-hatched eggs and the reduction of larvae and pupae) was clearly observed at the dose 300Gy and this could be the sterile dose., However it significantly increased at the doses 100 and 150gy in comparison with the un-irradiated control treatment, as well as the difference in sterility between the two doses was highly significant.

The obtained results in (Fig. 4) shows that the adult longevity of F1 adults was sharply decreased (13.2, 11.9 and 10.1 days) as the dose increased (0, 100 and 150Gy), respectively, while the significant prolonged of F1 larval duration was recorded (21.0, 23.4 and 25.8) at the doses (0, 100 and 150Gy), respectively, the same trend was observed in the pupal duration (11.0, 12.3 and 13.8) at the doses (0, 100 and 150Gy), respectively.

![Gamma irradition (Gy) vs Days](image)

**Fig. (4): (Av) larval duration, pupal duration and adult longevity of gamma irradiation effects on the 4th instar larvae of Spodoptera littoralis**
The reduction was more pronounced in F₁ generations. Similar results were reported by authors working on the lepidopterous insects, on Carpocapsa pomonella, on Cactoblastis cactorum, on Spodoptera litura and on Helicoverpa armigera respectively. These authors studied the effect of gamma radiation on the developmental inhibition of all stages and insects biological aspects, which were reduced throughout the F₁ generation descendant from irradiated P₁ males when compared with the control.

According to the above mentioned results, as gamma irradiation effect on the sterility at P₁ and F₁ generations, the low sub-sterilizing dose 100 Gy can induce the sterility as similar to the levels of sterility that are suggested for IS programme. On the other hand the lower concentrations of phenol 2.5 and 5% were effective toxic concentrations for suppression of S. littoralis population suppression.

3.3. Combination Treatment of Gamma Radiation and Phenol:

The data presented in Table (1) demonstrate that, the growth inhibition and relative growth index were significantly decreased by either the separate or combined treatments as compared to the control treatment, and as the most delayed effect were occurred in treatments of 100Gy+2.5 and 100Gy +5 % Phenol.

In the same table, the mean of larval weight was clearly decreased (0.150 and 0.130g) in 5% phenol and 100Gy +5 % Phenol, respectively. While, the reduction of average pupal weight was recorded (0.225 and 0.223) in the combined treatments of 100Gy of gamma with (2.5 and 5 %) phenol respectively.

Table (1): Growth inhibition, relative growth index, larval weight, pupal weight, pupation and survival of subjected to combined treatment of phenol and gamma irradiation of the 4th instar larvae of Spodoptera littoralis

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>GI</th>
<th>RGI</th>
<th>(AV.) larval weight (g)</th>
<th>(AV.) pupal weight (g)</th>
<th>(%) Pupation</th>
<th>(%) Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.920</td>
<td>1.000</td>
<td>0.300</td>
<td>0.258</td>
<td>92.00</td>
<td>88.0</td>
</tr>
<tr>
<td>100 GY</td>
<td>0.860</td>
<td>0.930</td>
<td>0.280</td>
<td>0.230</td>
<td>86.00</td>
<td>75.00</td>
</tr>
<tr>
<td>Phenols (2.5%)</td>
<td>0.610</td>
<td>0.660</td>
<td>0.230</td>
<td>0.280</td>
<td>61.00</td>
<td>52.00</td>
</tr>
<tr>
<td>Phenols (5%)</td>
<td>0.49</td>
<td>0.530</td>
<td>0.150</td>
<td>0.250</td>
<td>49.00</td>
<td>39.00</td>
</tr>
<tr>
<td>100GY+ Phenols (2.5%)</td>
<td>0.380</td>
<td>0.413</td>
<td>0.200</td>
<td>0.225</td>
<td>38.00</td>
<td>29.00</td>
</tr>
<tr>
<td>100GY+ Phenols (5 %)</td>
<td>0.03</td>
<td>0.33</td>
<td>0.130</td>
<td>0.223</td>
<td>3.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

The data presented in Table (1) demonstrates also that the percentage of pupation of subsequent F₁ generation was reduced at combined treatments compared with treatment of phenol or substerilizing dose 100 Gy separately and the lowest percentage (3.%) was recorded in 100Gy +5 % Phenol.

A similar trend was observed in the percentage F₁ larvae that survived to the adult stage which was severely decreased among the combined treatment of 100Gy with 2.5, 5 % phenol in comparison with their control treatments or with 2.5, 5 % Phenol when used alone to reach its maximum 20.8 at 100Gy + phenol (5%). These results agree with those obtained by (24),(13) and(14) who found that combined treatment of S. littoralis with the extracts of Eucalyptus camaldulensis and gamma irradiation increased the mortality percentage of larvae and pupae as compared to the control.
The data in Table (2) indicates that the adult longevity of the male moths was longer than female moths at all treatments. The adult longevity decreased with increasing the concentration and doses treatments. Males and females longevities of F1 generation at the combined treatments of 100Gy with 2.5, 5 % phenol were decreased to (7.2, 7.00 and 6.00, 6.50 day) in comparison with the control (11.4 and 11.00).

Accordingly, the sex ratio of F1 generation was more declined in favor of male at the treatments of 100Gy combined with 2.5 and 5 % phenol, it was around 1.2:1.3 when compared with other treatments where it was around 1:1 in the control treatment.

**Table (2):** (AV.) sex ratio, (AV.) adult longevity, Growth index and (%) reduction in F1 progeny subjected to combined treatment of phenol and gamma irradiation of the *Spodoptera littoralis*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>(AV.) Sex ratio</th>
<th>(AV.) adult longevity (days)± S.E.</th>
<th>Growth index</th>
<th>(%) Reduction in F1 progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Control</td>
<td>1.3</td>
<td>1</td>
<td>11.40±0.05</td>
<td>11.00±0.06</td>
</tr>
<tr>
<td>100GY</td>
<td>1.2</td>
<td>1</td>
<td>08.11±0.03</td>
<td>07.80±0.04</td>
</tr>
<tr>
<td>Phenols (2.5%)</td>
<td>1.2</td>
<td>1</td>
<td>08.16±0.06</td>
<td>08.40±0.04</td>
</tr>
<tr>
<td>Phenols (5%)</td>
<td>1.1</td>
<td>1</td>
<td>07.50±0.05</td>
<td>08.40±0.07</td>
</tr>
<tr>
<td>100GY+ Phenols (2.5%)</td>
<td>1.3</td>
<td>1</td>
<td>07.20±0.03</td>
<td>07.00±0.02</td>
</tr>
<tr>
<td>100GY+ Phenols (5 %)</td>
<td>1.2</td>
<td>1</td>
<td>06.00±0.02</td>
<td>06.50±0.02</td>
</tr>
</tbody>
</table>

Table (2) indicates the growth index of F1 generation was obviously reduced. The reduction was more pronounced among the treatment 100Gy with 2.5 and 5 % phenol (2.04 and 1.87) in comparison with other comparable treatments (3.6, 2.75 and 2.44, 2.41), respectively. In the same table the reduction in F1 progeny was increased with increasing the treatment dose or concentration. The reduction at the combined treatment of 100Gy with 2.5, 5% phenol was increased, it was (76.05 and 97.73 ) in comparison with control and other treatment when used separately. The results coincided with those of Hazza et al. (25) who stated that the growth index in *S. littoralis* of F1 progeny resulting from irradiated male parents and treated with certain Tafla extracts was obviously decreased than control treatment. (26) and (27) reported that gamma irradiation and plant extract in combination treatment increase the efficacy of F1 sterility technique with synergistically effect on the reduction of insect growth and reproduction.

In conclusion, using this low dose of gamma irradiation combined with low concentrations of phenol makes advantage for successful of *S. littoralis* population suppression.
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REFERENCES