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Physiological Studies on the Effect of Gamma Radiation and some Plant Oils on Greater Wax Moth, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae)

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ABSTRACT

The biochemical changes in the total body weight of *Galleria mellonella* larvae, treated with sublethal concentrations of botanical oils and gamma radiation, were studied. The total proteins and carbohydrates of F_1 progeny 6th instar larvae of ***Galleria mellonella*** treated with (LC_{40}) of various botanical oils or/and (LD_{40}) of gamma radiation decreased in all treatments compared to the control. Both botanical oils (LC_{40} of thyme 41.5623 and LC_{40} of camphor 50.8833) and gamma irradiation (LD_{40} 500.7547 Gy.) caused significant decrease in the activity of both amylase and protease of 6th instar larvae compared to the control. The applications caused the appearance of new protein bands and disappearance of another, meanwhile changes in the intensity of the bands.

KEYWORDS

Galleria Mellonella; Greater Wax Moth; Plant Oils; Gamma Radiation.

INTRODUCTION

Wax is one of the most useful products of honey bees. It is used in the pharmaceutical industry, dentistry and cosmetics. Wax contains many nutrients, pollen and honey, and is therefore attacked by various pests **(Ebadi, 1975)**. The greater wax moth, *Galleria mellonella* (L.), is a lepidopterous insect; it is one of the most devastating and economically important pests of wax in the world **(Chang & Hsieh, 1992 and Haewoon *et al*., 1995).** Enzymes play a role in insect's digestion and play fundamental roles for

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life because they are necessary catalyst to speed up the chemical reaction of metabolite. The enzyme is used to break down complex structure in food. The changes in the midgut carbohydrate enzymatic activity after treatment with some plant extracts and gamma-irradiation have been studied by **(Shoukry *et al*., 2003 and Boshra, 2007)** against a variety of insect pests.

The objective of this work is to evaluate the effect of both plant oils and gamma-irradiation on some digestive enzymes, total carbohydrate and protein on six instar larvae of the greater wax moth ***Galleria mellonella*.**

MATERIALS AND METHODS

Rearing Technique:

The strain of the greater wax moth, *G. mellonella* (L.) was obtained from the (NCRRT) and reared according to **Hussein (2004).**

Irradiation Technique:

Irradiation was conducted by Gamma Cell 220 Irradiation Unit (Co_{60} source) located of the National Center for Radiation Research and Technology, The dose rate of 6.6kGy/h. The LD_{40} of the larvae determined in other search.

Botanical oils used in this study:

1. Two botanical oils (Thyme and Camphor) were purchased from El-captain Company (CAP. PHARM., Egypt) for extracting natural oils, herbs and cosmetics, Cairo, Egypt.

2. To evaluate the toxicity of the chosen botanical oils, ten healthy4th instar *G. mellonella* larvae with (three replicates for each),were left to feed on the plant oils treated bees wax, SiSi-6 treated bees wax, and untreated bees wax as a control. The larvae were fed on treated wax for 24, 48,and 72 hours after each period larval mortality was recorded.

Preparation of concentrations:

Four different concentrations (25%,50%.75% and 100%) of each of the tested oils were prepared from the stock solution by diluting with SiSi-6 (Potassium alkyl sulphonate) as emulsifier (3ml/1literof water) in volumetric flasks to give the necessary concentrations.

Methods of application:

Susceptibility of the 4th instar larvae of *G. mellonella* to the above-mentioned plant oils was tested by the use of leaf spraying technique as follows: Bees wax were discs (2×2cm) and sprayed with different concentrations of botanical oils, then the bees wax were left until dryness before offered to the larvae for feeding on it, and determined LC_{40} of both two botanical oils in other search.

Biochemical determination:

Preparation of insects for analysis:

Larval supernatant were prepared according to **Zaghloul (2004**)

Amylase and protease activity:

Digestive enzymes were determined according to the modification of **Amin (1998)** to the method described by **Ishaaya and Swirski (1976)** using soluble starch as substrates for amylase enzyme. Proteolytic activity was measured as described by **Tatchell *et al.* (1972)**.

Determination of total carbohydrates and protein:-

Total carbohydrates were extracted and prepared for assay according to **Crompton and Birt (1967).** Total protein was determined calorimetrically by the method according to **Slater (1986)**.

Preparation of (SDS-PAGE) gel:

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate was per-

formed as described by **Smith (1976)**using an acrylamide gradient (12%) gel.

Statistical analysis

Data were statistically analyzed and the average percent mortality of the tested larvae were recorded. The method of ANOVA by using SPSS computer program was used and calculated at 5 % level.

RESULTS

Changes in total proteins and total carbohydrates

Total proteins:

Data summarized and illustrated in **Table (1)** showed the changes in the total content of body proteins of 6th instar larvae treated with (LC_{40}) of various botanical oils or/and (LD_{40}) of gamma radiation. The total proteins were 36.60, 35.16, 35.86, 34.23 and 33.00 treated with thyme, camphor, gamma irradiation, thyme + gamma irradiation and camphor + gamma irradiation, respectively compared to the control 42.36. As shown from the results, the total proteins decreased in all treatments compared to the control. There is significant difference between control and all treatments. Significant difference between gamma radiation and both thyme + gamma-irradiation and camphor + gamma-irradiation, also significant difference found between camphor and camphor + gamma- irradiation.

Total carbohydrates:

Data summarized and illustrated in **Table (1)** showed the changes in the total content of body carbohydrates of 6th instar larvae treated with (LC_{40}) of various botanical oils or/and (LD_{40}) of gamma radiation. The total carbohydrates were 17.16, 16.40, 15.76, 12.20 and 11.30 treated with thyme, camphor, gamma irradiation, thyme + gamma irradiation and camphor + gamma irradiation, respectively compared to the control 18.56. As shown from the results the total carbohydrates decreased in all treatments compared to the control. There is significant difference between control and all treatments, also significant difference found between Camphor and both thyme, thyme + gamma-irradiation and camphor + gamma-irradiation.

Table (1) *Changes in the total content of Carbohydrates and total content of proteins of F_1 progeny 6th instar larvae of Galleria mellonella treated with (LC_{40}) of various botanical oils or/and (LD_{40}) of gamma radiation.*

Treatment	Mean ± S.E.	
	Carbohydrates (mg/g.b.wt)	Proteins (mg/g.b.wt)
Control	18.56 ± 0.33^{bcdef}	42.36 ± 0.72^{abcdef}
Thyme	17.16 ± 0.18^{adef}	36.60 ± 0.65^{acef}
Camphor	16.40 ± 0.26^{aef}	35.16 ± 0.51^{abf}
Gamma irradiation	15.76 ± 0.57^{abef}	35.86 ± 0.40^{aef}
Thyme + gamma irradiation	12.20 ± 0.25^{abcdf}	34.23 ± 0.30^{abcf}
Camphor + gamma irradiation	11.30 ± 0.16^{abcde}	33.00 ± 0.45^{abcde}

a: significant different for control at (P<0.05)
b: significant different for (Thyme) at (P<0.05)
c: significant different for (Camphor) at (P<0.05)
d: significant different for (Gamma irradiation) at (P<0.05)
e: significant different for (Thyme + gamma irradiation) at (P<0.05)
f: significant different for (Camphor + gamma irradiation) at (P<0.05).

Characterization of protein by polyacrylamide gel electrophoresis in total body tissue homogenate of G. mellonella larvae:

Table (2) and **Figure (1) showed the electrophoretic protein pattern of total body tissue of 6th instar *G. mellonella*** larvae untreated or treated with (LC_{40}) of various botanical oils (thyme and camphor) alone or combined with (LD_{40}) of gamma radiation. The results indicated that 15 protein bands No (1 – 15) with molecular weights ranging from (133.317 to 18.195 KDa.). In control (untreated larvae) 10 bands with molecular weight ranging from (62.828 to 18.195 KDa.) appeared only and 5 bands disappeared **(In lane, 1)**

Table (2) and **Fig (1)** show that the total protein bands detected in the larvae treated with (LC_{40}) Thyme plant oils resulted in 6 bands with molecular weight ranging from (62.828-23.121KDa) appeared and 9 bands disappeared **(In lane, 2).**

The results obtained in **Table (2)** and **Fig (1)** show that Larvae treated with (LC_{40}) Camphor plant oils resulted in 10 bands with molecular weight ranging from (100.675-18.195) appeared and 5 bands disappeared **(In lane, 3).**

Table (2) and **Fig (1)** show that larvae treatment with (LD_{40}) of gamma radiation resulted in the appearance of 4 bands ranging from (51.656-23.121) and 11 bands disappeared (**In lane, 4).**

From **Table (2)** and **Fig (1)** show that larvae treated with larvae treated by combination of (LC_{40}) of Thyme plant oils with (LD_{40}) of gamma radiation leads to appearance of 14 bands with molecular weights ranging from (133.317-18.195KDa) and 1 band only disappeared **(In lane, 5).**

Larvae treated with Larvae treated by combination of (LC_{40}) of Camphor plant oils with (Ld_{40}) of gamma radiation **Table (2)** and **Fig (1)** leads to appearance of 11bands with molecular weights ranging from (133.317-23.121). Also this treatment leads to 4 bands disappearance **(In lane, 6).**

On general there are 9 polymorphic bands with molecular weight (133.317, 100.675, 91.521, 62.828, 31.824, 24.609,29.609, 26.3,24.596 and 18.195 KDa.), and 4 monomorphic bands with molecular weight (51.656,42.034,38.608 and 23.121 KDa.) and also there are two unique bands with molecular weight (27.834 and 20.222 KDa.), (Table, 3).

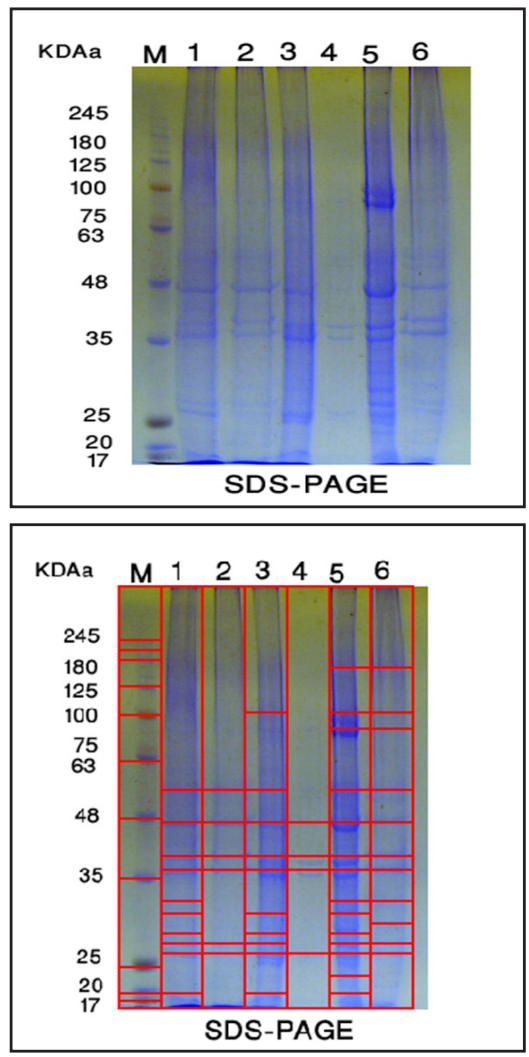

Fig. (1): SDS- polyacrylamide gel electrophoresis of the whole body of the 6th instar larvae of ***Galleria mellonella***. Where: M Marker, 1 = Control larvae 2 = Larvae treated by (Lc_{40}) Thyme plant oils, 3 = Larvae treated by (Lc_{40}) Camphor plant oils , 4 = Larvae treated by (LD_{40}) of gamma radiation, 5 = Larvae treated by combination of (LC_{40}) of Thyme plant oils with (LD_{40}) of gamma radiation, 6 = Larvae treated by combination of (LC_{40}) of Camphor plant oils with (LD_{40}) of gamma radiation.

Table (2) *Molecular weights and relative concentrations of protein fractions of total body tissue of 6th instar larvae of* ***G. mellonella*** *treated with (LC_{40}) of various botanical oils or/and (LD_{40}) of gamma radiation.*

Band number	MW(KDa)	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Polymorphism
1	133.317	0	0	0	0	1	1	Polymorphic
2	100.675	0	0	1	0	1	1	Polymorphic
3	91.521	0	0	0	0	1	1	Polymorphic
4	62.828	1	1	1	0	1	1	Polymorphic
5	51.656	1	1	1	1	1	1	Monomorphic
6	42.034	1	1	1	1	1	1	Monomorphic
7	38.608	1	1	1	1	1	1	Monomorphic
8	31.824	1	0	0	0	1	1	Polymorphic
9	29.609	1	0	1	0	1	0	Polymorphic
10	27.834	0	0	0	0	0	1	Unique
11	26.3	1	0	1	0	1	0	Polymorphic
12	24.596	1	1	1	0	1	1	Polymorphic
13	23.121	1	1	1	1	1	1	Monomorphic
14	20.222	0	0	0	0	1	0	Unique
15	18.195	1	0	1	0	1	0	Polymorphic

Where: Lane1 = Control larvae, Lane 2 = Larvae treated by (LC_{40}) Thyme plant oils, lane3 = Larvae treated by (LC_{40}) Camphor plant oils , Lane4 = Larvae treated by (LD_{40}) of gamma radiation, lane5 = Larvae treated by combination of (LC_{40}) of Thyme plant oils with (LD_{40}) of gamma radiation, Lane6 = Larvae treated by combination of (LC_{40}) of Camphor plant oils with (LD_{40}) of gamma radiation.

Table (3) *A comparison between the polymorphism of electrophoretic protein bands in the 6th instar larvae of G. mellonella larvae.*

Monomorphic bands	4
Unique bands	2
Polymorphic (without Unique)	9
Polymorphic (with Unique)	11
Total number of bands	15
Polymorphism (%)	73.333 %
Mean of band frequency	0.611

Changes in amylase and protease activities:

Amylase activity:

Table (4) showed the changes induced in amylase activity of 6th instar larvae treated with (LC_{40}) of various botanical oils or/and (LD_{40}) of gamma radiation. The amylase activity were 228.33, 214.67, 203.00,168.67 and 168.33 treated with thyme, camphor, gamma- irradiation, thyme + gamma irradiation and camphor + gamma- irradiation, respectively compared to the control 239.33. The results indicated that the amylase activity decreased, gradually, in all treatments compared to the control. There is significant difference between control and all treatments, also significant difference found between gamma radiation and both thyme and Camphor.

Protease activity:

Table (4) showed the changes induced in protease activity of 6th instar larvae treated with (LC_{40}) of various botanical oils or/and (LD_{40}) of gamma radiation. The protease activity were 89.37,86.70,86.07,84.97 and 83.97 treated with thyme, camphor, gamma-irradiation, thyme + gamma- irradiation and camphor + gamma-irradiation, respectively compared to the control 99.53. The results indicated that the protease activity decreased gradually in all treatment compared to the control. There is significant difference between control and all treatments, also found significant difference between thyme and all treatments.

Table (4) *Changes in the activity of amylase and protease enzymes of F_1 progeny 6th instar larvae of* ***Galleria mellonella*** *treated with (LC_{40}) of various botanical oils or/and (LD_{40}) of gamma radiation.*

Treatment	Mean of enzyme activity ± S.E.	
	Amylase µg glucose / min /g.b. wt	Protease µg D,L, alanine / min /g.b. wt
Control	239.33±0.33 bcdef	99.53±1.86 bcdef
Thyme	228.33±3.28 acdef	89.37±0.45 acdef
Camphor	214.67±2.73 abdef	86.70±0.26 abf
Gamma irradiation	203.00±3.61 abc	86.07±0.12 ab
Thyme + gamma irradiation	168.67±0.88 abcd	84.97±0.19 ab
Camphor + gamma irradiation	168.33±0.88 abcd	83.97±0.56 abc

a : significant different for control at (P<0.05)
b: significant different for (Thyme) at (P<0.05)
c : significant different for (Camphor) at (P<0.05)
d : significant different for (Gamma irradiation) at (P<0.05)
e : significant different for (Thyme + gamma irradiation) at (P<0.05)
f : significant different for (Camphor + gamma irradiation) at (P<0.05).

DISCUSSION

Total carbohydrates and proteins:

The results indicated that, total carbohydrates and proteins decreased, gradually, in all treatments compared to the control. Similar reduction in total carbohydrates and proteins of haemolymph of the 6th instar larvae of *A. ipsilon* was recorded, after treatment with acetone extract on *Melia azedarach* by **El-Shiekh (2002). Gabarty (2008)** who found that, both gamma-irradiation (100Gy) and botanical oil tested (LC_{50}) caused a significant (P< 0.05) decrease

in the total content of haemolymph protein, lipids and carbohydrates of F_1 progeny 6th instar larvae males of *A.ipsilon*. **(Al khalaf and Abdel Baki (2013**) studied the effect of gamma irradiation on amino acid contents in different larval stages of *Corcyra cephalonice* (Staint); the influence of gamma radiation on free and protein hydrolysis amino acid contents was more pronounced by increasing the irradiation dose level and time after treatment. Increasing the dose level was accompanied by reducing the free and protein amino acid contents. The changes in total proteins, lipids and carbohydrates of 6th instar larvae of *Corcyra cephalonica* (Staint) resulted from combination of irradiated males and normal females, combination of irradiated females and normal males and combination of irradiated males and irradiated females were studied. The total protein, lipids and carbohydrate levels decreased gradually as the dose of gamma radiation increased **Farghaly *et al*. (2013).**

Characterization of protein by polyacrylamide gel electrophoresis in total body tissue homogenate of G. mellonella larvae:

As shown from the results, the protein bands of 6th instar larvae of ***Galleria mellonella*** due to the treatment of 4th instar larvae treated with (LC_{40}) of various botanical oils(Thyme and camphor) or/and (LD_{40}) of gamma were completely different from those of the control. The applications caused the appearance of new protein bands and disappear another band. In agreement with the present results, **El-Bermawy and Abdel-Fattah (2000)**, found qualitative changes in protein pattern in larvae and pupae of ***T. confusum*** after treating fourth instar larvae with plant oil (Vetiver). Electrophoretic analysis of total proteins, lipoproteins and glycoproteins revealed inhibitory action of the used plant extract of the Myrrh, namely; oil and oleo resin on the protein contents of ***Culex pipiens*** larvae **(Massoud *et al*., 2001).** Also, electrophoretic analysis of total protein showed appearance and disappearance of some protein bands in the treated ***Culex pipiens*** larvae by Lemongrass, Red basil, citronella and peppermint, as compared with control group **(Mohammed *et al*., 2003).** The qualitative variations in the protein bands of different treatments and in different days during the larval life indicated both utilities of the specific proteins as well as the synthesis of new proteins by the insect **Lokesh, *et al.* (2006). Amin (2010)** determined the variation induced in electrophoretic protein pattern of the flesh fly, ***Sarcophaga bullata*** (first and third instar larvae) irradiated with 30, 45, 60 and 75 Gy comparing with un irradiated larvae. SDS- polyacrylamide gel electrophoresis (SDS- PAGE) showed a variable number of 14 electrophoretic protein bands in the whole body tissue of first instar larvae with molecular weight ranged between 80.439 to10.542 kDa. The quantitative analysis also clearly indicated variations in the number as well as intensity of the protein bands.

Changes in amylase and protease activities:

The results indicated that the amylase and protease activity gradually decreased in all treatments, compared to the control. In agreement with the present results, **Abo El-Ghar *et al.* (1995)** noticed a significant decrease in carbohydrates digestive enzymes and a considerable increase in the activity of trehalose after treatment of *Agrotis ipsilon* larvae by *Melia azedrach*. Similar results were reported by **Boshra (2007)** studied the effects of gamma irradiation on the consumption, digestion and utilization of food by 8-18-day-old larvae of *Ephestia cautella* treated as 1-day-old larvae with 80 Gy, and also the activity of protease, amylase and invertase enzymes, were studied. Protease, amylase and invertase enzyme activity were adversely affected in all irradiated larvae. **Gabarty (2008)** found that both gamma irradiation (100Gy) and botanical oil tested (LC50) caused a significant ($P< 0.05$) decrease in the activity of both haemolymph amylase and invertase of 6th instar larvae. Moreover, this decrease was greatly remarkable in larvae resulted from irradiated females crossed with irradiated males of *A.ipsilon*. The

changes in amylase and protease enzymes of 6th instar larvae of *Corcyra cephalonica* (Staint) resulted from combination of irradiated males and normal females, combination of irradiated females and normal males and combination of irradiated males and irradiated females were studied. Changes in amylase and invertase activities in the larvae resulted from the previous combinations showed that the activity of haemolymph amylase and invertase of F1 progeny 6th instar larvae decreased gradually as the dose of gamma radiation increase **Farghaly *et al.* (2013)**.

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مجلة
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دراسات فسيولوجيه عن تأثير أشعة جاما وبعض الزيوت النباتيه على فراشة الشمع الكبرى جاليريا ميلونيلا

دعاء سعد فرغلى[1] - أسماء ذوالهمة الشرقاوى[1] - سلوى عبدة رزق[2] - نجلاء فتحى بدر[1]

اظهرت النتائج ان المحتوى الكلى من الكربوهيدارت والبروتينات ليرقات العمر السادس فى حشرة فراشة الشمع الكبرى المعامله بالجرعه القاتله لـ٤٠٪من اليرقات لكلا من اشعة جاما والزيوت النباتيه معا اومنفردين انخفضت فى كل المعاملات مقارنه باليرقات الغير معامله. تسببت الجرعه القاتله لـ٤٠٪من اليرقات لكلا من اشعة جاما والزيوت النباتيه فى انخفاض نشاط كلا من الاميليز والبروتيز ليرقات العمر السادس فى حشرة فراشة الشمع الكبرى مقارنه باليرقات الغيرمعامله. وأظهرت المعاملات ظهور اشرطة بروتين جديده واختفاء اخريات وايضا ظهور كثافه بعض الاشرطة.

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