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MUTAGENIC EFFECT OF DIOD LASER RADIATION ON *Tribolium castaneum* (HERBST)

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ABSTRACT

The mutagenic effect of Diod Laser Radiation were examined on *Tribolium castaneum* (Herbst), measuring to determine the sub-lethal doses (LD_{30}), (LD_{50}), lethal dose (LD_{99}) and to investigate DNA alteration for polymorphic number of genetic bands using RAPD-PCR primers comparing with control. Results strongly suggest that Diod Laser Radiation causes mutagenic effect on *T. castaneum* insect.

INTRODUCTION

The rust red flour beetle, *Tribolium castaneum* (Herbst): Tenebrionidae (Coleoptera) is a secondary insect pest of a wide range of cereals; cereal products, legumes, oilseeds, cakes, nuts, spices and animal products. It is regarded as extremely successful omnivores particularly abundant in warm and often dry conditions (Abdel-Fattah, 2012). Also Quinones secreted by adults which produce an unpleasant musty taint under high population densities. The economic losses caused by stored-product pests are about 2.5 billion dollars annually in the United States, the red flour beetle; *T. castaneum* may cause an allergic response. Also, the presence of excrement and insect fragments reduces the quality of food products (Flinn *et. al*., 2007). Controlling of pests in stored products, principally cereal grains, by use of chemicals, a common strategy for post-harvest loss avoidance and leads to apparition of many problems like environmental pollution, human toxicity, and emergence of resistant strains of insects. The low cost of laser light treatment and the results obtained, suggest that the Laser light (as a new potential method for pest control in preserved foods) may become a practical method of pest control with great value in the future (Elordy, 2010).

The term Laser is an acronym for light amplification by stimulated emission of radiation; Laser radiation affected mortality, induced sterility, inhibited or prevented reproduction, enhanced or reduced longevity and affected physiology and biochemical processes. Also, the development of better methods of insect detection in grain, seeds, fruits, and processed food is a very important problem throughout the world. Molecular biology techniques as Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) used for extraction and detection proved for large-scale analysis of multiple samples (Nowaczyk *et al.,* 2009). In addition, denaturation of the DNA helix, cross linking and base modifications occurs. These radiation induced genetic variation in DNA fragments could be a basis for detection of irradiation treatment in both animal and plant, Geard (1983); Ishihara *et*. *al*. (2000); Ikeda (2007). A sensitive technique to detect DNA fragmentation is the micro gel electrophoresis of single cells commonly called 'DNA Comet Assay' The shape, length and intensity of comets indicate the

degree of DNA damage, which in turn is an indication of the radiation dose applied. Irradiated cells with a large number of DNA strand-breaks will show considerably longer comets than non-irradiated cells. This DNA Comet Assay has in the meantime been standardized on European level (Khan *et. al*., 2005).

The purpose of this study is to evaluation of bioassay and mutagenic effect of LASER radiation against *T. castaneum* larvae using Random Amplified Polymorphic DNA assay method (RAPD-PCR).

MATERIALS AND METHODS

Treated and untreated *Tribolium castaneum* (Herbest) larvae were used in this study. They were obtained from laboratory of Grains and Stored Product Pest Research Department, Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation in Dokki, Giza-Egypt. The insect was reared for several generations without any insecticidal exposure. Eggs exposed to different Laser Irradiation doses: 9.54, 14.31, 19.10 and 23.85 Energy dose (J/cm^2) which carried out at Department of Environmental, Chemical and Agricultural Applications, National Institute of Laser Enhanced Sciences, Cairo University, Giza, Egypt.

Type of laser and irradiation technique

Type of laser radiation used in this study was solid state diod pump (SSDP) laser with wave length of 532 nanometers. The laser beam coming from the used instrument was dropped vertically by hand on Petri dish containing either 0-1-day old eggs of *T. castaneum*.

In order to study the effect of laser radiation on different stages, 0-1 day old eggs were exposed to different energy doses of laser radiation to evaluate the sub-lethal doses (LD_{30}) and (LD_{50}) and lethal dose (LD_{99}). According to Abd el-Fattah (2012):

The energy (joules) = power (milli watt) x time (second)

Where power density = P/ A, $A = \pi r^2$ P=power of laser instrument, A= area of radiation field (circle). π = constant = 3.14, r = radius of circle.

Power of used SSDP instrument was 250 mw and times which applied in this study were 30, 45, 60 and 75 seconds for eggs stage a primary experiment to record the mortality rates. The result data were analyzed by computer program (probit program) to calculate the time required to the sub-lethal doses (LD_{30}) and (LD_{50}) and lethal dose (LD_{99}).

Computer program probit analysis was used to calculate the times LT_{30}, LT_{50} and LT_{99} to determine LD_{30}, LD_{50} and LD_{99} of each insect by tested laser rays.

RAPD-PCR Analysis of *T. castaneum* DNA

The work of DNA analysis was carried out in Center for Genetic Engineering of Agriculture Faculty, Cairo University in Giza, Egypt. By molecular technique, Random Amplified Polymorphic DNA- Polymerase Chain Reaction (RAPD-PCR).

DNA extraction and amplification

Isolation of genomic DNA was performed from treated and untreated 15-day-old larvae irradiated as 0-1 day old eggs with LD_{30}. DNA isolation was carried out by the CTAB method, Rogers and Bendich (1985) & Doyle and Doyle (1990). Amplification of DNA was performed in 10mg reaction mix containing 20ng of genomic DNA, 0.5 unit Tag polymerase (Promega, USA), 200µg each of d ATP, d CTP, d GTA and d TTP, 5 p mole random primer (Operon Tech. Inc., USA) and appropriate amplification buffer.

The mixture was assembled on ice, overlaid with a drop of mineral oil. On the other hand, Amplification was performed for 45 cycles, using UNO thermal cycler (Biometra, Germany) as follows: One cycle at 92C° for 3 min, 45 cycles at 92 °C for 30 sec., 35 °C for 60 sec and 72 °C for 2 min. The reaction was finally incubated at 72 °C for 10 min. further 10 min. at 63 °C. Electrophoresis was done in 2% agarose gel (1% Nusieve GTG, 1% Seakam L.E., FMC Bioproducts) in TAE buffer (0.04 M Tris – acetate, 1 m M EDTA, pH8) .RAPD products were stained in 0.2 µg /ml ethidium bromide and photographed under UV-light. Results were documented with Gel Doc 2000 (Bio RAD).

Table (1): The primer sequences used in the RAPD-PCR of the tested insect:

Primer name	Sequences 5`→ 3`
OPE A-07	5`- GAAACGGGTG- 3`
OPE B-05	5`- TGCGCCCTTC - 3`
OPE B-07	5`- GGTGACGCAG- 3`
OPE P-09	5`- GTGGTCCGCA- 3`
OPE A-10	5`- GTGATCGCAC- 3`

RESULTS AND DISCUSSION

1. Effect of different laser doses on 0-1 day old eggs

Effect of diod pump laser with wave length (532 nanometer) on 0-1 day old eggs with 23.85, 19.1, 14.31 and 9.54 J/cm^2 doses of radiation were occurred against *T. castaneum*. Survival rate of egg and its successive stages resulted from irradiated eggs are presented in Table (2).

Results showed that Hatchability percentages of irradiated *T. castaneum* it was decreased as the dose increased; corresponding eggs hatchability were 0.0, 19.6, 61.6 and 80.3 % for energy doses of 23.85, 19.10, 14.31 and 9.54 J/cm^{2,} respectively compared with control as 93.6 %, respectively. Pupation ratios resulted from irradiated eggs were 0.0, 8.3, 51.0 and 77.3 % compared with control as 90.6 %.

On the other hand, adult's emergence resulted from irradiated eggs gradually increased as the dose decreased which being 0.0, 4.60, 18.6 and 74 % compared with control as 89.3 % for *T. castaneum*, respectively. On the other hand, Malformation ratios of adult stage were 0.0, 30.9, 16 and 10.2 % compared with control as 1 % for *T. castaneum*, respectively.

The lethal dose that causes 99, 50 and 30 % mortality rates were 31.23 (LD_{30}), 14.06 (LD_{50}) and 11.74 (LD_{99}) J/cm^2 doses, respectively for *T.*

castaneum. These results are going in line with those obtained by many investigators who studied the effect of different sources of laser radiation on some insects species i.e. Khalifa (2002) and Abdel-Fattah (2012).

Table (2): Effect of different doses of Diod Laser Radiation (532 nm) on survival rates of insects as result of irradiated 0-1 day old eggs of *T. castaneum*.

Energy dose (J/cm^2)	Egg hatchability %	Pupation %	Adult	
			% emergence	% malform.
23.85	0.0	0.0	0.0	0.0
19.10	19.6	8.3	4.6	30.9
14.31	61.6	51.0	18.6	16.0
9.54	80.3	77.3	74.0	10.2
Control	93.6	90.6	89.3	1.0

2. RAPD-PCR DNA Analysis of *T. castaneum* larvae:

Genetic variation in DNA fragments after LD_{30} diod laser radiation treatment on 15 days old larvae treated as 0-1 day old eggs (*T. castaneum*) was detected by RAPD-PCR DNA analysis. The generated bands of five primers were scored as present (+) and absent (-) as indicated in Figs (1- 5) and illustrated in Table (4). The primers OPE A-07 gave three generated bands with size (510, 700 and 1000 b.p.) for treatment and control without any polymorphic bands (Fig. 1, Tables 3 & 4). While, both two primers i:e: OPE B-05 and OPE P-09 induced five generated bands with size (200, 250, 500, 650 and 1000 b.p.) and (350, 420, 510, 750 and 950 b.p.) respectively after laser radiation treatment without monomorphic bands compared with control (Figs 2 & 4, Tables 3 & 4).

On the other hand, OPE B-07 primer produced six generated bands (250, 300, 350, 510, 650 and 1000 b.p.) but diod laser radiation treatment caused disappearance of one DNA band size of 1000 b.p. with 16.6 % polymorphism compared with control (Fig. 3, Tables 3 & 4) . While the OPE A-10 primer produced three generated bands (200, 250 and 500 b.p.) with two polymorphic bands, one of them (200 b.p.) was absent in the control while the other (250 b.p.) was absent in treatment with 66.6 % unique and polymorphism (Fig. 5, Table 3 & 4).

Table (3): Polymorphic bands and polymorphism % of five primers used for *T. castaneum* larvae after LD_{30} of diod laser radiation (532 nm) treatment.

Primer name	generated bands	polymorphic bands	Polymorphism %	band size range (b.p.)
A-07	3	0	0	510-1000
B-05	5	0	0	200-1000
B-07	6	1	16.6	250-1000
P-09	5	0	0	350-950
A-10	3	2	66.6	200-500
Total	22	3	13.6	-

Table (4): RAPD profile alteration in DNA bands as detected with five primers in *T. castaneum* larvae after LD_{30} of diod laser (532 nm) radiation

Primer name	Sequences 5`→ 3`	Size of polym. Bands (b.p.)	Treatments	
			Control	Diod Laser
OPE A-07	5`- GAAACGGGTG- 3`	510	+	+
		700	+	+
		1000	+	+
OPE B-05	5`- TGCGCCCTTC - 3`	200	+	+
		250	+	+
		500	+	+
		650	+	+
		1000	+	+
OPE B-07	5`- GGTGACGCAG- 3`	250	+	+
		300	+	+
		350	+	+
		510	+	+
		650	+	+
		1000	-	+
OPE P-09	5`- GTGGTCCGCA- 3`	350	+	+
		420	+	+
		510	+	+
		750	+	+
		950	+	+
OPE A-10	5`- GTGATCGCAC- 3`	200	-	+
		250	+	-
		500	+	+

RAPD-PCR based analysis assays are very important as genome wide DNA variation strategy, toxicant induced genotoxic effects, DNA variation, DNA damage, genetic instability and mutagenic effects which have been evaluated with RAPD analysis successfully by pervious work.

RAPD assay has proved useful to detect genomic instability manifested such as point mutation, genetic, chromosomal rearrangements, deletion and insertions, Baeshin *et. al*.,(2009). RAPD can be used to detect genomic instability as the newly growing and developing cells will produce a clone of dividing daughter cells. Thus, the proportion of cells presenting the same genomic instability is high and easy to detect. In the field of genetic toxicology most RAPD studies describe changes such as differences in band intensity, as well as gain/loss of RAPD bands, defined as diagnostic RAPD (Guzin *et. al*., 2010). In conclusion RAPD-PRC method can be used as an investigation tool for laser induced genomic alterations. So, the present results suggest that RAPD_PCR finger printing together provided with physiological parameter can be a powerful strategy for assessing levels of diod laser radiation (532 nm) exposure. OPE B-07 and OPE A-10 primers were informative for detecting laser induced specific genomic alterations. From the obvious results we concluded that diod laser radiation (532 nm) have a mutagenic effect in *T. castaneum* larvae. The obtained results are in agreement with many authors on different insect pests after radiation treatments, Emery *et. al*. (2000); Ciabatti *et. al*. (2006) and Lessard&Pronier (2006).

Figures(1-5) : RAPD profiles of genomic DNA of *Tribolium castaneum* after Laser treatment by using five primers (pr.).

M: DNA marker, C: control, T: laser treatment with dose of LD30.

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تأثير أشعة الديود ليزرعلي الإختلافات الجينيه في حشرة خنفساء الدقيق الكستنائية

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تم دراسة تأثير أشعة الليزر علي حشرة الترابيوليم كاستينيم (خنفساء الدقيق الكستنائية) وذلك لمعرفة الجرعات تحت المميته و المميته. أظهرت نتائج الدراسه أن الجرعات الخاصة بكل من LD99 ,LD50, LD30 هى٣١.٢٣ و ١٤.٠٦ و ١١.٧٤ J/cm^2 علي الترتيب. علي الصعيد الأخر تم دراسة تأثير الجرعة تحت المميته LD30 علي التغيرات الوراثيه للحشره وقدرته علي احداث طفرات في الحامض النووى DNA (التأثير المطفر) باستخدام طريقة البلمرة المتسلسل RAPD- PCR. واظهرت النتائج أن اشعة الليزر لها القدرة علي احداث تغيرات في الحامض النووى DNA للحشره بالمقارنه بالكنترول ولذلك نقترح من الدراسه إمكانية استخدام أشعة الليزر في مكافحة خنفساء الدقيق الكستنائيه بهذه الجرعات.