Effect of Nitrogen laser on the pupae of *Drosophila melanogaster*

Ayad G Anwer
Institute of laser for post graduate studies, University of Baghdad, Baghdad, Iraq

Abstract:
Using 337.1nm wavelength Pulsed nitrogen laser, the pupae of ***Drosophila melanogaster*** were irradiated with laser beam with pulses repetition rates 5,10 pulses/sec for exposure periods: 5, 10, 15, 20, 30,45 and 60 minutes for each repetition rate . The results showed that the effect is directly proportional to the exposure time and pulses repetition rates . The likely effect of laser is may be based on photochemical mechanism due to absorption of laser light at certain wavelength by endogenous chromophores.

Introduction:
The invention of the laser in the early 1960s introduced a variety of applications in many fields. The distinguishable properties of the laser light were the corner stone in these achievements. The applications of laser in biology began to be reported during the period 1963-1964(1).
The effect of ultraviolet radiation on insects has been studied from many perspectives; the use of 254nm radiation to kill insects and the mutagenic effect of such radiation. The lethal effect of UVC radiation (190-290nm) radiation varies for different insects and different stages of life cycle in early development it has been attributed to transmission of UV radiation into animals with DNA damage, which could also be the mechanism of mutagenesis(2).
The absorption of UV radiation through the abdominal wall of *Drosophila melanogaster* has been studied and the data suggested transmission from 240 to 440 nm (3). An interesting observation is that 253.7 nm radiation has a repellent action on the Ammerican cockroach(4).
Biological and genetical effects of visible laser radiation on the fig moth ***Ephestia cautela*** was observed (5). The effect of the low-intensity impulse laserr radiation (LILR) on the life span of Drosophila melanogaster has been studied. The flies at various stages of their life (larvae and imago) were exposed to LILR. The estimation of the effect of LILR was carried out on the basis of the analysis of the basis parameters of aging. The direction of the effect depends on parameters of radiation, stage of development of irradiated insect(6)
The present work is an attempt to study the outcome of exposing pupae of *Drosophila melanogaster* to nitrogen laser radiation .The aim is to observe the transformation percents of pupae to adults and the effects of laser on the first generation of insects.

Material And Methods:
Drosophila melanogaster were brought up in the laboratory under the optimum experimental conditions in media composed of (100gm sugar, 100gm flour, 100gm agar, 20 gm yeast, 5 drops of propionic acid) were dissolved in 1000 ml of distilled water and sterilized in autoclave at 1.5 b/inch for 15 min. The media then poured in 20 ml capacity vials with the presence of filter paper pieces in each vial (7).

Pulsed nitrogen laser (Molectron UV24) was used in the irradiation experiments, this type of laser emits light In the ultraviolet region of the electromagnetic spectrum at 337.1nm wavelength, the system is operated with repetition rates between (1-50) pulse/second ,with 1mili joule pulse energy and 10 nanoseconds pulse width.

The laser beam was focused to (0.8) cm on the tube that contains the pupae in means of a lens with a focal length 5 cm using a mask. (Rj-7610, Laser precision crop, England) joulemeter was used for measurement of laser pulse energy before each experiment. The fluence was constant in all irradiation experiments. It was calculated as the following Fluence = Pulse energy/ Area (J/cm^2).

Diagram (1) illustrates the setup of irradiation. The laser beam was applied on the sample vertically using flat mirror. The beam was passed through a quartz lens to get certain diameter of the irradiated area equal to 0.8cm.

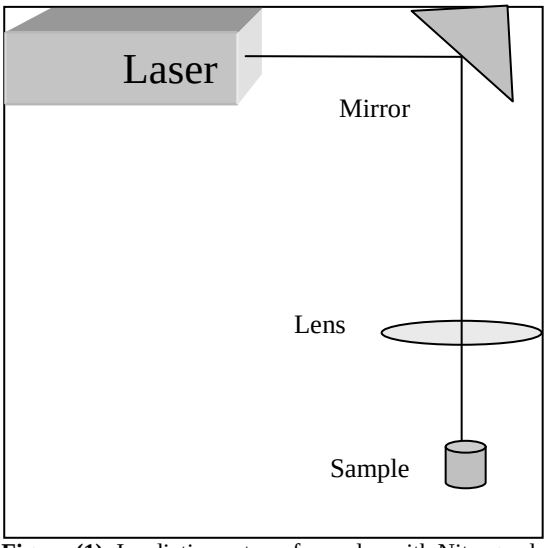

Figure (1): Irradiation setup of samples with Nitrogen laser.

Three males and three females of wild type of insect were brought up in each vial and incubated at 25 C. After laying the eggs by females, the eggs hatched to larvae and developed to pupae. The pupae were isolated at 1 day age from media and subdivided into subgroups. Each group composed of 15 pupae were irradiated with laser beam with repetition rates 5, 10 pulses/sec for exposure periods: 5, 10, 15, 20, 30 ,45 and 60 minutes for each repetition rate . The irradiated pupae were returned to the breading vials and incubated at 25C.The number of adults that produced from irradiated pupae were calculated every 12 hours for four days.
Statistical analyses were done using binomial test and Chi –square test to evaluate the effect of nitrogen laser on the hatching of adults from the irradiated pupae.

Results and discussion:
Table (1) illustrates the effect of nitrogen laser on the number of adults that hatched from the irradiated pupae

using 5 pulses/second repetition rate $2x10^{-3}$ J/cm^2 fluence. Binomial statistical comparisons were done between control group and groups of pupae that irradiated by nitrogen laser.

Table (1) The number of adults that hatched from the irradiated pupae after irradiation with nitrogen laser (5 pulses/second repetition rate) .

Period / Group	24 hours after irradiation	36 hours after irradiation	48 hours after irradiation	60 hours after irradiation	72 hours after irradiation	84 hours after irradiation	96hours after irradiation
Control Without irradiation	3	8	8	13	14	15	15
5 minutes Irradiation	3	5	7	14	14	15	15
10minutes Irradiation	2	2	9	12	14	15	15
15 minutes Irradiation	3	5	7	13	13	13	15
20 minutes Irradiation	2	4	4	9	10	12	14
30 minutes Irradiation	0	2	3	10	10	13	13
45 minutes Irradiation	0	0	4	11	11	12	13
60 minutes Irradiation	0	0	4	8	8	10	10

As it is shown in the table, in the control group, the adults were hatched gradually from the pupae and the complete hatching occur after 96 hours (four days).

No significant effect of laser on the number of adults that hatched was noticed at 5, 10 ,15 minutes exposure time .While the significant decrease in the number of adults appear at 20 minutes exposure time. At 60 minutes exposure time the number of adults was 10 from 15 pupae and 5 pupae failed to transform to adults after 4 days in comparison with control group.

Using 10 pulses/second repetition rates, $2x10^{-3}$ J/cm^2 fluence, table (2), the significant decrease in the number of adults that hatched from pupae was noticed at 15 minutes exposure time. Also the number of adults decrease with increase the exposure time to reach to 7 adults from 15 pupae and 5 pupae failed to transform to adults after 4 days in comparison
with control group.

Table (2) The number of adults that hatched from the irradiated pupae after irradiation with nitrogen laser (10 pulses/second repetition rate) .

Period / Group	24 hours after irradiation	36 hours after irradiation	48 hours after irradiation	60 hours after irradiation	72 hours after irradiation	84 hours after irradiation	96hours after irradiation
Control Without irradiation	3	8	8	13	14	15	15
5 minutes Irradiation	3	4	7	12	12	14	15
10minutes Irradiation	2	2	7	12	13	15	15
15 minutes Irradiation	3	3	7	11	13	13	13
20 minutes Irradiation	2	3	4	9	10	10	10
30 minutes Irradiation	0	2	3	7	7	8	10
45 minutes Irradiation	0	0	4	6	7	7	7
60 minutes Irradiation	0	0	4	4	6	7	7

Chi –square test were done between groups that irradiated by nitrogen laser with 5 pulses / second and groups of pupae that irradiated with 10 pulses / second, to evaluate the role of increasing the pulse repetition rate on the hatched adults.

No significant differences were noticed between the groups that that irradiated with 5 and 10 pulses / second, for 5, 10 and 15 minutes exposure times.

The significant differences appeared at 20, 30, 45 and 60 minutes exposure time.

With increasing the exposure time and pulse repetition rate , the dose of the laser radiation increases , since the dose equal to: fluence (energy density) for certain exposure time at certain pulse repetition rate.

The most probable mechanism of interaction of the nitrogen laser light with the living cells is a photochemical interaction, taking into account the term of absorbing chromophore or Photoacceptor that have high absorbance at the wavelength of the applied laser light.

A 193 nm laser that penetrates only 2-5 micron(s) was able to induce a response but unable to kill the larvae. These results suggest a photochemical reaction occurs in the cuticle which produces free radicals that stimulate the nerves and muscle which are present immediately below the epidermis(8)

Electromagnetic radiation from frequency at 900 MHz, decreases the reproductive capacity of the insect Drosophila melanogaster by 50%–60%,. The insects were exposed for 6 min per day during the first 2–5 days of their adult lives.

Results suggest that this field-radiation decreases the rate of cellular processes during gonad development in insects (9)

It have been demonstrated that the double-strand break (DSB) in chromosomal DNA -induced phosphorylation of histone variant H2Av in *Drosophila melanogaster* occur after irradiation with 365 nm UV light for one minute. (10)

The proteins and carbohydrates components of the cuticle become cross-linked by catecholamines which are derived from tyrosine ; the two major ones are N-acetyldopamine (NADA) AND N-B- alanyldopamine (NBAD) (11)

The chemistry of the reactions has been studied extensively and involves quinons as intermediates. Catecholic compounds are present in the cuticle of flies at different stages of life cycle in order to cross-linked the proteins and carbohydrates. These molecules might give rise to free radicals following irradiation with UV light (12).

A possible target molecule for the UV laser radiation is a catecholic compound secreted into the cuticle in the late stage of larvae, which is responsible for the transformation of cuticle into the puparial case by cross-linking of and protein(13)

A mechanism for the UV radiation effect could be the absorption of radiation by a catacholic compound with the generation of free radicals .These can diffuse and readily reach the muscles and nerves which are attached immediately below the epidermis.

The conclusion that nitrogen laser with 337.1 nm wave length effect significantly on the percentage of hatched adults from pupae, the severity of the effect at certain energy density depends mainly on the exposure time and then on the pulse repetition rate.

References:

1. Goldman,L. (1982). Applications of laser .CRC . Press.Inc. PP:155-77.

1-Beard. RL.(1971): Lethal action of UV irradiation on insects.J Econ Entomol 65: 650-654

2-Durand. E, Hollaender. A, Houlahan.MB(1981):Ultraviolet absorbtion spectrum of the abdominal wall of Drosophila melanogaster .J Hered 32: 51-56.

3-Wharton. DR. (1971):Ultraviolet repellent and lethal action on the American cockroach. J Econ Entomol 64: 252-255.

4-Zuhair S. Al-Hakkak, Abdul-Mageed A.k. Al-Sufi & Alya M.B. Murad. (1985): Biological and genetical effects of visible laser radiation on the fig moth Ephestia cautela.J.Biol.Sci.Res. Vol.19(1).pp: 95-107.

5-Chernova GV, Vorsobina NV.(2002) Effect of low intensity pulsed laser radiation of basic parameters of aging in Drosophila melanogaster. Radiats Biol Radioecol. May-Jun;42(3):331-6.

6-G.Jardener. & T.R.Mertenz. (1985) : Genetics experiments ,the culture of drosophila pp: 11-18.

7-Baden HP, Kollias N, Anderson RR, Hopkins T, Raftery L.(1996). Drosophila melanogaster larvae detect low doses of UVC radiation Arch Insect Biochem Physiol.;32(2):187-96.

8-Dimitris J. Panagopoulos, (2004) Effect of GSM 900-MHz Radiation on the Reproductive Capacity of Drosophila melanogaster .Electromagnetic Biology and Medicine. Volume 23, Number 1.pp29 – 43

9-James P. (2002)DNA double-strand break-induced phosphorylation of Drosophila histone variant H2Av helps prevent radiation-induced apoptosis Nucleic Acids Research, , Vol. 30, No. 17 3698-3705.

10- Anderson.SO(1991):Sclerotisation. In Bennington K,Retnakaran A(eds): Physiology of the insect epidermis.Canberra, Australia Publications,Inc.,pp 123-140.

11- Land.EJ , (1986): Photochemistry of melanin precursors. Photochem Photobiol 44: 697-702.

12- Sugumaran. M(1991):Molecular mechanism s of sclerotisation. In Bennington K,Retnakaran A(eds): Physiology of the insect epidermis.Canberra, Australia: CSIRO Publications, Inc.,pp 141-168.

تأثير أشعة ليزر النتروجين على عذارى حشرة ذبابة الفاكهة

أياد غازي أنور

معهد الليزر للدراسات العليا، جامعة بغداد، بغداد، جمهورية العراق

الملخص:

باستخدام ليزر النتروجين النبضي بالطول ألموجي ٣٣٧,١ نانومتر، تم تشعيع عذارى حشرة ذبابة الفاكهة بتكرارية نبضات: ٥ و ١٠ نبضة /ثانية و بأزمان تشعيع ٥،١٠،١٥،٢٠،٣٠،٤٥،٦٠ دقيقة لكل تكرارية نبضات . اضهرت النتائج بان تأثير أشعة الليزر يتناسب مع زمن التشعيع و تكرارية النبضات، و إن التأثير المحتمل لليزر هو كيميائي ضوئي نتيجة امتصاص الطول الموجي لضوء الليزر بواسطة متحسسات داخلية للضوء.