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The Influence of Short Wavelength Laser on the Pupae Development of the Old World Screw- Worm Fly (OWSWF) *Chrysomya bezziana* Villeneuve (Diptera: Calliphoridae)

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Abstract: The biological effects of pulsed N_2-laser on the old world screw-worm fly, ***Chrysomya bezziana*** Villeneuve in the pupal stage were investigated. Different laser parameters were involved in this work. The old pupae of 1, 2, 3, 4 and 5 days were exposed to laser radiation during 10, 30 and 60 second with repetition rate 10, 20 and 30 pulse/second. The percent of normal adults emergence (female and male) was investigated. The results showed that the adults emergence was highly decreased as the repetition rate and exposure time increased when the pupae irradiated for 1, 2 and 3 days old as compared with 4 and 5 days. The results also indicated that the pupal period was significantly increased of irradiated pupae for 1, 2, 3 and 4 days old, while the 5 days old pupa was decreased.

Introduction

The Old World Screw-Worm Fly (OWSWF), ***Chrysomya bezziana*** is a member of the insect family Calliphoridae and is an obligate parasite of warm blooded animals (Norris and Murray, 1964). Female flies lay their eggs on the edge of wounds or body orifices and the resulting larvae invade the host tissues creating traumatic lesions, which may lead to loss of conditions or death (Humphery *et al*., 1980). Recorded hosts include cattle, sheep, goats, dogs, cats and man (Zumpt, 1965; Spradbery and Vanniasingham, 1980). This investigation describes the incidence of myiasis caused by ***C. bezziana*** in Iraq from September 1996 to March 1998 (Abdul Rassoul *et al.*, 1996; Al-Taweel *et al*., 2000).

Recently, lasers have entered almost all fields of biology (microbiology, entomology, botany, immunobiology, photobiology and genetics) and have made a wide step of progress in many of them (Absten *et al.*, 1988; Goldman, 1982). It replaces, in many cases, the conventional light source (Kandela, 1991). Tissue is very susceptible to energy inputs including laser energy. The effects are dependent upon the character of the energy source, the mode of application, and the nature of the tissue (Chopra and Chawla, 1992).

The present study aims to investigate the influence of short wavelength N_2 laser as a control of ***C. bezziana***.

Materials and Methods

Samples Preparation:

Laboratory rearing of OWSWF Chrysomya bezziana was the first step in the sequences of the present work.

The laboratory colony was reared under laboratory conditions, adequate with growth and development of the stages of the insect. This

was carried out at Iraqi Atomic Energy Commission (IAEC) / Entomology Department.

Laser System:

Pulsed N_2 – laser was used in this study. It is of Molectron UV 24-model. The laser emits radiation in the ultraviolet region of the electromagnetic spectrum, 337.1 nm. It can be operated with repetition rate from 1 to 50 pulses per second. The pulse energy is 1.5 mJ with pulse duration of 10 ns. The samples were exposed to the laser beam through a reflecting prism as shown in Fig. (1)

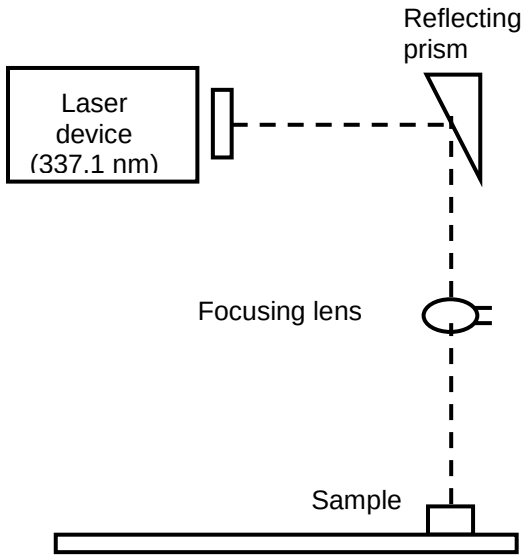

Fig (1)
Set-up of irradiation of samples by laser.

Fifty pupae / replicate, 2 replicate /treatment for each experiment, with different ages (1, 2, 3, 4 and 5) days were taken from the laboratory colony. As mentioned earlier only large and medium sizes were treated.

The posterior dorsal side of the pupae was exposed individually to the laser beam for the periods of 10, 30 and 60 seconds with repetition rates of 10, 20 and 30 pulses per second for each exposure time.

The irradiated pupae were placed individually in a glass vial 2.5 cm in diameter and 5 cm in depth. It contains corncob grits of 2 cm in thickness. The vials were covered with a white or a black organza, which was tighter, by a rubber band to prevent escaping of the emerged adults. Then a sprier was used to spray water. The aim of this procedure is to keep corncob grit humid. The humidity is important for pupae to complete their development to adult. These vials were incubated in adult room under a temperature of 26 ± 2 °C and RH of 60- 70 %.

The adults were counted and classified according to their sex for each replicate. For each experiment, the adults were placed in a cage and were provided with essential food supply such as water, 10 % sugar solution and dry sugar. At the fifth day of the adult cage, they were supplied by a protein source to stimulate them to lay eggs. At the sixth or seventh day, it was provided with a spent medium for egg laying. This should continue until all females die.

The percentage of adult emergence, pupal period, life span of adults and sex ratio were calculated .The unmerged pupae were dissected; also some females were dissected to identify mating status.

Results

Table (1) lists the parameters of the N_2 laser output. These parameters were calculated for each experiment to find the effect of N_2 laser on pupal stages with different ages (1, 2, 3, 4 and 5 days).

Table (1) N_2 laser parameters.

Number of Pulses l/sec	Exposure Time (sec)	Energy Density J /cm^2
10	10	2.12
	30	6.37
	60	12.74
20	10	4.25
	30	12.74
	60	25.48
30	10	6.37
	30	19.11
	60	38.22

The results of irradiating different old pupa with N_2 laser are given in Tables (2 - 6). It is clearly indicated from Tables (2 - 4) that adults emergence is highly affected in comparison with

the results represented in Tables (5) and (6). Means that it when pupa becomes older the effect was lower and this was also reflected on the duration of pupal period and life span of males and females and to some extends on the sex ratio.

In Table (2), the percent of adult emergence was positively reduced from 92 % in the control group to 61 % when 1 day old pupae treated with 6.37 J/cm^2 energy density for 30 seconds. This percent was further reduced to 51 % when 1 day old pupae exposed for 60 seconds to 38.22 J/cm^2 energy density, in comparison with exposing 4 and 5 days old pupae which showed that adult emergence is also affected but the effect was not as in 1, 2 and 3 days old pupae and it was ranged between 97 % in the control group to 89 %.

Furthermore, from Table (2) it is clear that the pupal period is significantly increased from 7.11 days in the control group to 8.48 days when 1 day old pupae treated with 6.37 J/cm^2 energy density for 30 seconds, in comparison with exposing 2 days old pupae which showed that the pupal period is also affected and it was 8.02 days at 30 seconds exposure with 19.11 J/cm^2 energy density as illustrated in Table (3). While in case 5 days old pupae the pupal period was significantly decreased and it was, ranged between 7.11 days in the control group to 5.23 days in the treatment with 6.37 J/cm^2 energy density and 30 seconds exposure time (Table 6).

Moreover the effect was significantly extended to the life span of the produced males and females and this clearly demonstrated in Tables (2- 6).

Table (5) shows also the effect of treatment with 12.74 J/cm^2 energy density for 60 seconds on sex ratio, which was also significantly affected. Furthermore, in Table (6) the results also show significant differences in the sex ratio at 4.25, 19.11 and 38.22 J/cm^2 energy density for 10, 30 and 60 seconds.

Table (2)

Effect of N_2 laser on development of **1- day** old *Chrysomya bezziana* pupae.

Treatment		Adult emergence %	Pupal period range (day)	Pupal period average ± S.D	Life span of female range (day)	Life span of female average ± S.D	Life span of male range (day)	Life span of male average ± S.D	Sex ratio M : F, chi-square
Energy density J/cm^2	Exposure time (sec)								
Control	0	92 f	(6-8)	7.11 ± 0.68b	(7-20)	15.34±3.85e	(7-20)	14.86±3.11b	1:1.14 , 0.19
2.12	10	69 e	(5-7)	5.96 ± 0.82a	(2-9)	6.53 ±2.43cd	(2-9)	6.04±2.40a	1:1.76 ,2.62
6.37	30	61 d	(8-9)	8.48 ±0.49c	(2-9)	6.04 ±2.03bcd	(2-9)	6.20±2.07a	1:1.35 ,0.66
12.74	60	59 cd	(6-8)	7.11 ±0.76b	(3-9)	6.61 ±2.97d	(3-9)	6.49±2.10a	1:1.57 ,1.43
4.25	10	58 bcd	(6-8)	7.23 ±0.88b	(3-9)	6.59 ±2.89cd	(3-9)	6.49±2.80a	1:1.64 ,1.69
12.74	30	57bc	(6-8)	7.33 ±0.77b	(2-9)	5.98 ±2.15bc	(2-9)	5.74±2.18a	1:1.38 ,0.71
25.48	60	57 bc	(6-8)	7.45 ±0.65b	(3-10)	6.28 ±2.45bcd	(2-10)	6.07±2.38a	1:1.59 ,1.48
6.37	10	55 b	(7-8)	7.69 ±0.48bc	(2-8)	5.68 ±2.18ab	(2-8)	5.47±2.93a	1:1.62 ,1.54
19.11	30	61 d	(7-8)	7.66 ±0.46bc	(2-9)	6.19 ±2.26bcd	(2-9)	5.62±2.20a	1:1.35 ,0.66
38.22	60	51 a	(7-8)	7.76 ±0.47bc	(2-8)	5.05 ±2.84a	(2-8)	5.38±2.86a	1:1.68 ,1.65

* Total number of pupae = 100

** Values in the same column followed by the same letter are not significantly different from each other ($P< 0.05$) as determined by Duncan's multiple range test.

Table (3) Effect of N_2 laser on development of **2- day** old *Chrysomya bezziana* pupae.

Treatment		Adult emergence %	Pupal period range (day)	Pupal period average ± S.D	Life span of female range (day)	Life span of female average ± S.D	Life span of male range (day)	Life span of male average ± S.D	Sex ratio M : F, chi-square
Energy density J/cm^2	Exposure time (sec)								
Control	0	91 e	(5-7)	5.10±0.70 a	(5-18)	11.93±3.19 c	(5-18)	12.33±3.78 c	1:1.17 , 0.27
2.12	10	72 d	(5-8)	6.33±1.02 ab	(2-10)	6.03±2.59 a	(2-10)	6.60±2.54 ab	1:1.57 , 1.78
6.37	30	66 cd	(7-9)	7.45±0.76cd	(2-9)	6.29±2.23 a	(2-9)	6.34±2.37 a	1:1.20 , 0.27
12.74	60	61 bc	(6-8)	6.75±0.98abc	(3-10)	6.67±2.04 ab	(3-9)	6.10±2.15 ab	1:1.44 , 0.99
4.25	10	63 bc	(6-8)	6.76±0.95 bc	(3-10)	7.16±2.20 b	(3-10)	7.33±2.31 b	1:1.25 , 0.39
12.74	30	62 bc	(6-8)	7.65±0.77 d	(2-10)	6.48±2.39 ab	(2-10)	6.72±2.34 ab	1:1.70 , 2.07
25.48	60	58 ab	(6-8)	7.39±0.88 cd	(2-11)	6.55±2.86 ab	(2-10)	6.72±2.54 ab	1:1.52 , 1.24
6.37	10	65 bcd	(6-8)	7.39±0.74 cd	(2-10)	6.56±2.19 ab	(2-10)	7.00±2.37 ab	1:1.10 , 0.07
19.11	30	63 bc	(6-8)	8.02±0.58d	(2-10)	6.30±2.35 a	(2-10)	6.63±2.57 ab	1:1.33 , 0.64
38.22	60	53 a	(7-8)	7.72±0.45 d	(2-9)	6.25±2.17 a	(2-10)	6.18±2.27 a	1:1.65 , 1.59

Table (4) Effect of N_2 laser on development of **3-day** old *Chrysomya bezziana* pupae.

Treatment		Adult emergence %	Pupal period range (day)	Pupal period average ± S.D	Life span of female range (day)	Life span of female average ± S.D	Life span of male range (day)	Life span of male average ± S.D	Sex ratio M : F, chi-square
Energy density J/cm^2	Exposure time (sec)								
Control	0	92 e	(5-6)	5.20±0.41 a	(5-18)	11.85±3.77 b	(5-18)	11.60±3.32 c	1:1.14 , 0.20
2.12	10	77 cd	(5-6)	5.47±0.50 a	(2-10)	6.97±2.47 a	(2-10)	7.53±2.94 ab	1:1.75 , 2.86
6.37	30	79 d	(5-6)	5.71±0.46 ab	(2-10)	6.86±2.70 a	(2-10)	6.62±2.86 a	1:1.52 , 1.44
12.74	60	59 a	(5-6)	5.39±0.50 a	(3-10)	8.01±2.30 a	(3-10)	7.92±2.25 ab	1:1.36 , 0.69
4.25	10	71 bc	(5-8)	6.39±0.71 bc	(3-11)	7.70±2.32 a	(3-11)	8.21±2.11 b	1:1.22 , 0.35
12.74	30	68 b	(6-8)	7.09±0.66 c	(2-11)	7.79±2.70 a	(3-11)	7.77±2.61 ab	1:0.88 , 0.16
25.48	60	78 cd	(5-7)	6.65±1.06 c	(2-12)	8.24±3.06 a	(2-11)	7.91±2.51 ab	1:1.23 , 0.41
6.37	10	72 bcd	(5-8)	6.52±0.96 c	(2-12)	8.24±2.85 a	(2-12)	7.99±3.02 ab	1:1.12 , 0.11
19.11	30	75 bcd	(5-8)	6.76±1.04 c	(3-11)	8.12±2.37 a	(2-11)	7.32±2.78 ab	1:1.34 , 0.81
38.22	60	55 a	(5-8)	6.62±1.30 c	(2-11)	7.18±2.85 a	(2-11)	7.50±2.49 ab	1:1.90 , 2.63

Table (5) Effect of N_2 laser on development of **4- day** old *Chrysomya bezziana* pupae.

Treatment		Adult emergence %	Pupal period range (day)	Pupal period average ± S.D	Life span of female range (day)	Life span of female average ± S.D	Life span of male range (day)	Life span of male average ± S.D	Sex ratio M : F, chi-square
Energy density J/cm^2	Exposure time (sec)								
Control	0	92 ef	(5-7)	5.28±0.70a	(6-20)	12.19±3.37f	(4-14)	12.96±3.35e	1:1.27 , 0.61
2.12	10	82 cd	(5-6)	5.35±0.48a	(4-10)	8.36±2.68abc	(7-13)	8.53±2.81ab	1:0.03 , 0.03
6.37	30	76 bc	(6-7)	6.42±0.50bc	(7-13)	10.78±2.98e	(3-13)	10.67±2.15d	1:1.17 , 0.24
12.74	60	76 bc	(6-7)	6.26±0.44b	(3-13)	8.94±3.15bcd	(2-13)	9.16±3.28abc	1:2.42 , 7.69
4.25	10	89 def	(7-8)	7.22±0.41cde	(2-13)	7.77±3.78ab	(3-14)	9.01±3.64abc	1:1.60 , 2.42
12.74	30	91 ef	(7-8)	6.80±0.73bcd	(3-14)	8.83±3.43bc	(3-15)	10.17±3.01cd	1:1.80 , 3.43
25.48	60	84 de	(6-8)	6.85±0.68bcd	(4-14)	9.72±3.41cde	(3-14)	9.91±3.53bcd	1:1.67 , 2.25
6.37	10	72 b	(7-8)	7.69±0.46e	(2-14)	10.47±3.37de	(2-12)	11.04±3.30d	1:1.62 ,2.13
19.11	30	76 bc	(6-7)	6.19±0.39b	(2-12)	8.76±2.94bc	(2-12)	8.47±3.15ab	1:2.00 , 3.17
38.22	60	57 a	(7-8)	7.33±0.48de	(3-12)	6.83±3.05a	(6-20)	8.19±3.17a	1:1.09 ,0.09

* Total number of pupae = 100

** Values in the same column followed by the same letter are not significantly different from each other ($P< 0.05$) as determined by Duncan's multiple range test.

Table (6) Effect of N_2 laser on development of **5-day** old *Chrysomya bezziana* pupae.

Treatment		Adult emergence %	Pupal period range (day)	Pupal period average ± S.D	Life span of female range (day)	Life span of female average ± S.D	Life span of male range (day)	Life span of male average ± S.D	Sex ratio M : F, chi-square
Energy density J/cm^2	Exposure time (sec)								
Control	0	97 e	(6-8)	7.11±0.69d	(7-20)	15.59±3.56d	(7-20)	15.06±3.67e	1:0.98 , 0.01
2.12	10	96 e	(5-7)	5.81±0.75bc	(2-13)	8.82±3.68a	(2-13)	9.68±3.39bc	1:1.02 , 0.01
6.37	30	89 cd	(5-6)	5.23±0.44a	(2-14)	10.94±2.70bc	(2-14)	10.18±3.10c	1:0.65 , 2.03
12.74	60	84 bc	(5-6)	5.72±0.45ab	(6-13)	11.20±2.08c	(5-13)	11.48±2.04d	1:1.33 , 0.86
4.25	10	93 de	(5-6)	5.42±0.49ab	(1-13)	10.12±3.09abc	(2-13)	8.88±3.10ab	1:0.48 , 5.86
12.74	30	85 bc	(5-7)	5.63±0.65ab	(4-14)	10.29±3.67abc	(4-14)	10.19±3.32c	1:0.77 , 0.71
25.48	60	82 b	(5-6)	5.39±0.49ab	(3-19)	14.09±3.87d	(3-19)	14.96±3.38e	1:1.10 , 0.10
6.37	10	87 bc	(5-8)	6.28±0.83c	(3-14)	9.44±3.31ab	(2-14)	9.30±3.67abc	1:1.56 , 2.08
19.11	30	86 bc	(5-6)	5.25±0.44ab	(4-13)	9.59±2.80abc	(3-13)	8.20±3.27a	1:2.44 , 7.54
38.22	60	62 a	(7-8)	7.23±0.42d	(2-13)	8.65±3.86a	(2-14)	8.65±3.87ab	1:2.44 , 5.42

* Total number of pupae = 100

** Values in the same column followed by the same letter are not significantly different from each other ($P< 0.05$) as determined by Duncan's multiple range test.

Discussion

The results showed that the pulsed N_2 laser with 337.1 nm wavelength have a reduction on ***C. bezziana*** stages (pupal) developed to adulthood. The effect of N_2 laser was increased as the number of pulses increased. Also increasing exposure time lead to increasing energy density, and cause the negative effect on survival of eggs stage.

The most probable mechanism of reaction between N_2 – laser and the cells of developed pupa may be explained as follows:

The effect of N_2 laser at 337.1 nm wavelength may be photochemical, due to absorption of laser light at certain wavelength and energy densities by chromospheres namely the NADP which is found within cells of pupa of insect.

If the photon energy of laser light is lower than the bond dissociation energy between atoms and enough to elevate the energy of the system with the excited state, the excitation caused biostimulation, as follows:

$$(A\text{-}B) + h\nu \longrightarrow (A\text{-}B)^*$$

where:

(A-B)= A molecule consists of 2 atoms: A and B.

(A-B)* = The molecule in the excited state.

hν = Photon energy.

On the other hand, if the photon energy is equal or higher than the dissociation energy of the bonds between the atoms, this may lead to bond breaking between atoms, as follows:

$$(A\text{-}B)^* + h\nu \longrightarrow A + B + E$$

It is clear that the photon energy of nitrogen laser is 3.6 eV (only absorbed by NADPH) so this energy is able to cause breaking of bonds which have dissociation energy equal or less than of 3.6 eV and cause a defect in function of NADPH .

The pupae with different ages need higher dosage of radiation than the eggs and larvae for the same insect. The younger pupa was considered to be more sensitive to radiation than the older pupae. The energy density and exposure time effected the survival of pupae (Shirbash, 1995). The decrease in adult emergence (number of normal adult) can be explained as a result of death of most adults inside the pupal sheath or can not be able to emerge from pupal sheath this result was agreed with the results of Zumreoglu et al.,

(1979) who's studied the effect of gamma radiation on fruit fly ***Ceratitis capitata***. Furthermore, the cells in irradiated pupae (1,2 and 3 days old) were still in division, while in 4 and 5 days old pupae the all parts of adult insect will be formed and the cell division were continued only in testes and ovaries (Ricmann and Thorson, 1969).

The irradiation of pupae affected the life span of adults (male and female), total number of egg laid, gradual reduction in the egg hatching percent and increasing pupal and larval periods (Shirbash, 1995).

Conclusions

N_2 laser significantly reduces the adult emergence and life span of males and females when irradiating different ages of pupae. Furthermore when the irradiated pupae of 5 days old pupae with N_2 laser the pupal periods were significantly decreased, while the 1, 2, 3 and 4 days old pupa were increased.

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تأثير ليزر قصير المـوجة على تطور عذارى ذبابة الدودة الحلزونية
Old World Screw- Worm Fly (OWSWF) Chrysomya bezziana Villeneuve (Diptera:Calliphoridae)

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الخلاصة **تمت دراسة التأثيرات الحياتية لليزر النتروجين النبضي على ذبابة الدودة الحلزونية OWSWF *Chrysomya bezziana* Villeneuve والمعرضة بدور العذارى لنبضات وأزمان تعريض مختلفة . درس تأثير ليزر النتروجين على نسبة خروج البالغات (الاناث والذكور) عندما شععت العذارى بعمر 1 و2 و3 و4 و5 أيام على التوالي لنبضات مختلفة 10 و20 و30 نبضة/ثانية وزمن تعريض 10 و30 و60 ثانية. تشير نتائج تشعيع العذارى لمختلف الاعمار ان لاشعة ليزر النتروجين تاثير معنوي في انخفاض نسبة خروج البالغات ومدة حياة الذكور والاناث والنسبة الجنسية وان العذارى كلما تقدمت بالعمر فان التاثير يكون اقل (بعمر 1 و2 و3 ايام) مقارنة مع تشعيع عذارى بعمر 4 و5 ايام . كذلك كان لاشعة ليزر النتروجين تاثير معنوي في زيادة مدة الدور العذري عند تشعيع عذارى بعمر 1 و2 و3 و4 ايام ، بينما عند تشعيع عذارى بعمر 5 ايام فان مدة الدور العذري انخفضت معنوياً .**