

Stimulation of Hatching Efficiency in Capsulated Cysts of *Artemia fransiscana* Using 890 nm Diode Laser

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Abstract:

Artemia fransiscana is one of the most important live food for commercial larval aquaculture.

The aim of this study is to investigate the effects of 890 nm diode laser irradiation on *Artemia* capsulated cysts using (1-10) minutes exposure time, and 2.26×10^{-3} J/cm² Fluence. The *Artemia* samples were obtained from two locations: Dyalaa and Basraa. After irradiation, hatching percentage (H %) and hatching efficiency (HE) of *Artemia* were measured after 24 and 48 hours of incubation.

The results of the effect of laser light on the capsulated cysts from Dyalaa showed that the optimum dose for enhancing (H %) after 24 hours of incubation is using 10 minutes exposure time, while after 48 hours of incubation the (H %) enhancement can be achieved using 6 minutes exposure time. The optimum exposure times for (HE) enhancement after 24 and 48 hours of incubation were 5 and 7 minutes.

The results of the effect of laser light on the capsulated cysts from Basraa showed that after 24 hours of incubation, the optimum exposure times for enhancement (H%) was 9 minutes, while after 48 hours of irradiation the best exposure times was 5 minutes .

Very effective enhancement of (HE) was noticed after 24 hours of irradiation at 3 minutes exposure time using 2.26×10^{-3} J/cm² Fluence. No enhancement was observed after 48 hours of irradiation

In conclusion, 890 nm diode laser irradiation can be used successfully for increasing Hatching percentage (H %) and Hatching Efficiency (HE) of *Artemia* capsulated cysts using certain energy density and certain exposure times.

Key words: Genus *Artemia*, 890 nm diode laser, capsulated cysts, biostimulation.

Introduction:

Artemia are commonly called brine shrimp.[1] Brine shrimp, genus *Artemia*, inhabit inland saline lakes worldwide.[2]. *Artemia* eggs are very high in nutrient value; they contain 52% protein and 27% fat.[3] *Artemia* is an excellent model organism to study the modes of action of probiotic and pathogenic bacteria, as it can easily be cultured under gnotobiotic conditions and can be used as a vector

for transferring probiotics to larvae of target species.[4]

Gurney 1921[5], was the first author that report the presence of *Artemia* in Iraq. After a half-century Al-Uthman, 1971[6] studied some of the ecological factors of *Artemia* habitat. Few years later Khalaf, et al 1976[7] collected *Artemia* from a round Baghdad, while Saker, et al 1977[8], collected the *Artemia* from Basrah area. Sultan and Abdul Sahib,

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1992[9], studied the generation alternation in *Artemia* cultured in laboratory from Basrah area. Recently, Maknoon, 2001[10], studied some biological and ecological aspects of *Artemia* in field (from south and middle of Iraq) and laboratory. Nasiri and Al-Obaydi (2004)[11] found that *Artemia* live in temporal pools on sides of roads and near cultivated lands when there is no drainage system, in middle and south of the country. Pools are small and shallow because of high rate of evaporation around the year. *Artemia* adults can survive for few months when factors are suitable. There are more than 10 types of *Artemia*: one is reproducing bisexually and the other is parathogentic.

Low intensity laser light has unique properties that can influence biological activities under certain conditions.[12] Biostimulation is an important biological effect of low intensity laser that accelerate proliferative processes in irradiated cells [13].It has attracted interest in both clinical and research areas in both veterinary and human medicine.[14]

The aim of this study is to examine the effects of diode laser irradiation (890nm) wavelength on *Artemia fransiscana* capsulated cysts and to determine the optimum conditions for improving Hatching percentage (H %) and Hatching Efficiency (HE).

Materials and Methods:

Cysts collection:

The capsulated cysts were obtained from two geographical locations (Basrah [Bisexually reproductive form] and Dyalaa [Parthenogenetic reproductive form]).

Sample preparation:

~ 0.1- 0.2 mg of mixed *Artemia Sp.* cysts was transferred into (1.5) ml ependrof tubes .1ml of salt (0.3 gm) water was added to each tube.

Samples irradiation:

Pulsed diode laser, (MILTA, Moscoo 2000), 890 nm wavelength, was used in experiments.

The experiment was designed according our preliminary studies to use the laser system with these parameters: (4 W) average power, 2.26×10^{-3} J/cm² Fluence, 1000 Hz frequency and (90-120) η s pulse duration. Samples were irradiated for (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 minute) exposure times.

Samples incubation:

A containers (bottles) of V shaped (500 ml) were filled with clean tap water then locked with cap. The bottle was put inverted in order to allow unhatched cysts, empty shells, and hatched nauplii to be easily removed separately. These conditions were used for hatching *Artemia*: 25-28 °C temperature, small bright lamp 60 watt were put directly over the container, good circulation is maintained to keep the cysts in suspension.[15,16]

The irradiated cysts were added to the V container. After 15-20 hours at 25C, the hatching of cysts to nauplii was observed.

Harvesting:

After 20 hours of adding cysts, the air pump was switched off. After 10 minutes, unwanted empty cysts that float on the surface were and the *Artemia* was harvested from the container.[15]

Enumeration:

The nauplii were enumerated after 24 and 48 hours using four replicates for control and irradiated samples by 20 ml pipette.

Sample calculations:

After nauplii counting, the hatching parameters were calculated including Hatching percentage (H %) and Hatching Efficiency (HE) using the equations:[15]

H % = (number of napulii/number of cyst in 1 gm) X 100.

HE = Average number of naupili/1 gm of cyst.

Cysts number was counted by weighting 1 gm of cysts and was enumerated using Dissecting Microscope (16X).

Statistical analysis:

Statistical Analysis System (SAS) was used for studying effect of different dosage of diode laser on hatching percentage and hatching efficiency of cysts of *Artemia*. The significant differences were compared between means by using less significant differences (LSD).[17]

Results:

Figure (1) shows the effect of 890 nm diode laser on the Hatching percentage (H %) of *Artemia Sp.* (Dyala) using 2.26×10^{-3} J/cm² Fluence. After 24 hours of irradiation, the values of (H %) are fluctuated according to exposure time. The highest value was (74.07 a %) at 10 minutes exposure time in comparison with control group (59.60 abc %). After 48 hours, the highest value of hatching (70.04 a %) was noticed at 6 minutes exposure time. The lowest value was (25.93d %) at 10 minutes exposure time compared with control group which is (40.40 bcd %).

It is clear that the optimum dose for enhancing (H%) after 24 hours of incubation is using 10 minutes exposure time and 2.26×10^{-3} J/cm² Fluence, while after 48 hours of incubation the (H%) enhancement can be achieved using 6 minutes exposure time and 2.26×10^{-3} J/cm² Fluence.

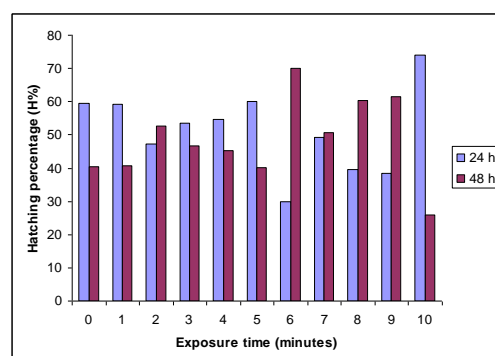


Fig. (1) Effect of 890 nm diode laser on Hatching percentage (H %) of *Artemia Sp.* (Dyala).

Figure (2) illustrates the effect of 890 nm diode laser on the Hatching Efficiency (HE) of *Artemia Sp.* (Dyala) at 2.26×10^{-3} J/cm² Fluence. After 24 and 48 hours of irradiation, fluctuations of (HE) values were noticed in comparison with control group. It was clearly seen that the highest values are significantly increased to (26.333a) and (21.667 a) using 5 and 7 minutes exposure time after 24 and 48 hours respectively.

The optimum exposure times for (HE) enhancement after 24 and 48 hours of incubation were 5 and 7 minutes at 2.26×10^{-3} J/cm² Fluence.

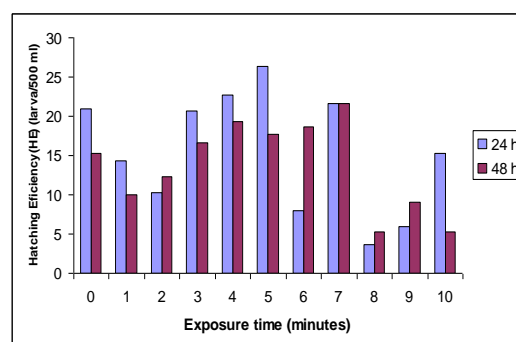


Fig. (2) Effect of 890 nm diode laser on Hatching efficiency (HE) of *Artemia Sp.* (Dyala).

Figure (3) illustrates the effect of 890 nm diode laser on H % of *Artemia Sp.* (Basra) using 2.26×10^{-3} J/cm² Fluence. It is clear that the H % (after 24 hours of irradiation) is highly affected (significantly increased) with

increasing exposure time. The least value of (H %) (45.05e %) was observed at 5 minutes exposure time, while the highest value (86.094 a %) was observed at 9 minutes exposure time in comparison with control group (56.977 d %). After 48 hours of irradiation the (H %) values increased to the highest values: (54.95 a %) and (48.351 ab %) at 5 and 10 minutes exposure time in comparison with control group (43.023 b %).

It was noticed that after 24 hours of incubation, the optimum exposure times for enhancement (H %) was 9 minutes at 2.26×10^{-3} J/cm² Fluence, while after 48 hours of irradiation the best exposure times were 5 and 10 minutes using 2.26×10^{-3} J/cm² Fluence.

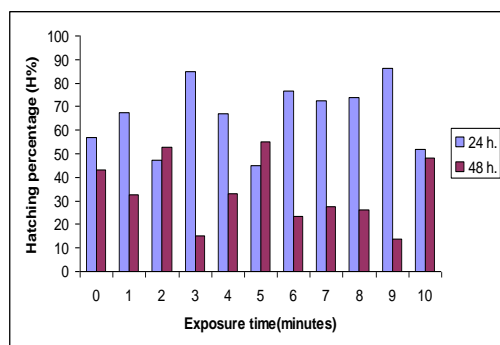


Fig. (3) Effect of 890 nm diode laser on Hatching percentage (H%) of *Artemia Sp.* (Basraa).

Figure (4) illustrates the effect of 890 nm diode laser on HE of *Artemia Sp.* (Basraa) at 2.26×10^{-3} J/cm² Fluence. After 24 hours of irradiation, It is clear that the (HE) is increased significantly to reach to (332.00 a) using 3 minutes exposure time, in comparison with control group (186.33 b). Significant decrease in (HE) values was observed to reach to (32.67 e) and (30.00 e) respectively using 5 and 10 minutes exposure time in comparison with (186.33 b) for control group. After 48 hours of irradiation the HE values were significantly lower than the value of control group for all exposure times.

Very effective enhancement of (HE) was noticed after 24 hours of irradiation at 3 minutes exposure time using 2.26×10^{-3} J/cm² Fluence.

No enhancement was observed after 48 hours of irradiation.

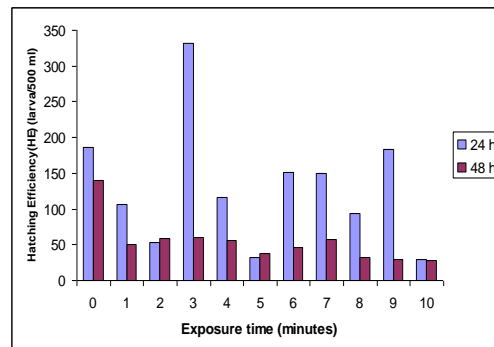


Fig. (4) Effect of 890 nm diode laser on Hatching Efficiency (HE) of *Artemia Sp.* (Basraa).

Discussions:

In this study, there was a variation in response of *Artemia* samples to the laser light according to the location. Using the same fluence, the exposure time that required to enhance (H %) of Dyalaa samples after 24 hours of incubation was 10 minutes, comparing with 9 minutes for Basraa.

Regarding the exposure times that required enhancing the (H %) after 48 hours of irradiation they were, 6 minutes for samples from Dyalaa and 5 and 10 minutes for the samples from Basraa.

The exposure times that enhance (HE) values were also affected according location. The exposure time that required to enhance (HE) of Dyalaa samples after 24 and 48 hours of incubation were 5 and 7 minutes for both, comparing with 3 minutes for Basraa samples after 24 hours of irradiation after irradiation with no enhancement after 48 hours of irradiation. These variations in responses according locations may be explained in term of differences of strains. Strain-specific differences in the light sensor mechanism can also be

expected. The light effect on cyst hatching could be a trigger of hatching metabolism. Indeed various kinds of 'environmental conditions have been reported to effect hatching.

Paul Vanhaecke, A in 1981 found that the light-intensity threshold at which the maximal hatching rate was attained, varied from strain to strain. Differences in light sensitivity between the *Artemia* strains studied can, at least partly, be attributed to variation in chorion characteristics. The hypothesis is discussed that light might act as a dia-pause inhibitor in marine and freshwater.[18]

It was noticed that the stimulation of Hatching percentage and Hatching efficiency of cells were affected by the time after irradiation and exposure time.

In this work positive and constructive aspect of radiations is reported. Low intensity laser light has a unique property that increases the biological activities under certain conditions. It was suggested that the mechanism of low-power laser therapy at the cellular level is based on the absorption of monochromatic visible and NIR radiation by components of the mitochondrial respiratory chain causing changes in their redox properties and acceleration of electron transfer (primary reactions), which are followed by secondary (dark) biochemical reactions of cellular signaling[19].

Karu in 1984 found that laser irradiation does not influence the proliferation of various sub population to the equal degree. In the series of the experiments, the activity of some enzymes was measured in a stimulated cultures , following incubation for 18 hours , the activity of various enzymes was determined .Irradiation caused considerable activation of respiratory chain components (NADH

dehydrogenase) and cytochrome C oxidase .[20]

In our study, certain inhibition was also observed; this can be explained in term of inhibition of certain metabolic molecules in the cells at certain laser doses (certain exposure time and energy density).

Karu in 1991, illustrate the principle that laser wavelength which are appropriately matched to the absorption characteristics of target molecules can not only stimulate but also selectively inhibit specific molecular components in cells . The lack of wavelength specificity can be probably explained by absorption and resulting changes in various molecules in the respiratory chain, the final results being overall inhibition of the electron transfer chain.[21]

In conclusion, 890 nm diode laser irradiation can be used successfully for increasing Hatching percentage (H %) and Hatching Efficiency (HE) of *Artemia franciscana* capsulated cysts using certain energy density and certain exposure times.

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تحفيز نسبة الفقس وكفاءة الفقس للحويصلات المتكسلة للجنس *Artemia* بأستخدام ليزر الدايبود بالطول الموجي 890 نانوميتر

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الخلاصة:

تعتبر *Artemia franciscana* واحدة من اهم الاعلاف للحيوانات المائية، السمك، الروبيان، السرطان و.. الخ خلال ادوارها اليرقية.

الهدف من الدراسة هو البحث في تأثير 890 نانوميتر دايبود ليزر على الحويصلات المتكسلة للجنس *Artemia* بأستخدام (10-1) دقائق زمن تشعيع و $10 \times 2.26 \times 10^{-3}$ جول/سم² كثافة طاقة. عينات الجنس *Artemia* مأخوذة من منطقتين هي ديالى والبصرة. بعد التشعيع، تم قياس كل من نسبة الفقس % وكفاءة الفقس بعد 24 و 48 ساعة من الحضن.

أظهرت نتائج تأثير ضوء الليزر على الحويصلات المتكسلة لمنطقة ديالى بأن الجرعة المثلى لزيادة نسبة الفقس % بعد 24 ساعة من الحضن كانت عند 10 دقائق زمن تعريض، بينما النسبة المئوية للفقس بعد 48 ساعة من الحضن ازدادت عند 6 دقائق زمن تعريض. اما وقت التعريض المثالي لكفاءة الفقس فقد شوهد عند 5 و 7 دقائق زمن تعريض بعد 24 و 48 ساعة حضن بالتعاقب.

أما بالنسبة لحويصلات منطقة البصرة كانت اعلى نسبة فقس لوحظت عند 9 دقائق زمن تعريض بعد 24 ساعة حضن بينما سجلت بعد 48 ساعة حضن افضل نسبة فقس % عند 5 دقائق زمن تعريض. لوحظ زيادة فعالة لكفاءة الفقس بعد 24 ساعة حضن عند 3 دقائق زمن تعريض، لكن لم يلاحظ أي زيادة لكفاءة الفقس لجميع اوقات التعريض للتشعيع بالليزر بعد 48 ساعة اذا ما قورنت مع مجموعة السيطرة.

نستنتج من ذلك يمكن استخدام ليزر الدايبود ذات الطول الموجي 890 نانوميتر لزيادة النسبة المئوية للفقس وكفاءة الفقس للحويصلات المتكسلة ذات الكفاءة الواطنة للـ *Artemia* بأستخدام جرعة ليزر بخصائص مثلى.