Study the Effect of Nd:YAG Laser on Cowpea Beetle 
(*Callosobruchus maculates*(Fab))

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**Abstract.** In this study we examined the effect of pulse Nd:YAG laser radiation on the cowpea beetle (*Callosobruchus maculates*(Fab)) in terms of changes in the external appearance of this insect (shape and color), and calculated the percentage of death rate due to Nd:YAG laser irradiation of wavelength (1064 nm) with energies: (300, 360, 420 & 480) mJ and exposure times (10, 20 & 30) sec for each energy with (5 pulse/sec), when the distance was (10 cm) between the source of laser and the sample. The results showed an increase in the percentage of death of the cowpea beetle in addition to increasing distortions where the energy of laser increased and the exposure time increased, where the results of this laser treatment effect were taken in time stages: after (12 hours) and then note after pass (24 hours) and then (48 hours). The results generally showed the thermal effects of laser on tissues where thermal propagation caused damage to structures and thus increased death rates.

1. **Introduction**

Biophysics is a vast cross-disciplinary subject encompassing the fields of biology, physics and computational biology etc. in microbes, plants, animals and human being. The contributions of scientists and experts from different places of the world focus on the main aspects of biophysics, which led to the development of technologies and applications in this field [1]. The most important technologies in this field is laser [2] for its extensive applications in the fields of industrial [3], medical, biological, economic and agricultural [2]. Since the invention of laser, it has been extensively used for various biological purposes. Some fundamental aspects of laser-tissue interaction is discussed, that includes the rate of varying happening in tissue with varying optical parameters, and how temperature distribution occurs within the tissue based on this varying rate [4].

The food stored in general, cereals and beans are of particular economic importance to many countries of the world and maintain strategic supplies, enough for several months to cope with natural disasters and the acute shortage of annual production [5]. Legumes are one of the largest and most widespread plant families [6]. The cowpea beetle insect is an important economic insect that affects the seeds of many legumes such as cowpea, chickpeas and others, causing weight loss during the storage period and spread in most countries of the world [7,8]. The researchers used many methods to combat the cowpea beetle insects, including the use of chemical pesticides commonly used for the speed of their impact, as well as the use of many extracts and powders of plants with toxic effect, in addition to the use the method of vacuum to protection of seeds when stored [9]. Despite the use of insecticides to protect crops and materials stored against harmful insects, there are problems related to the negative impact of pesticides on humans and animals and their adverse impact on the environment and pollution, as well as problems related to pest resistance to pesticides, which prompted researchers in the control of grain pests and stored materials to think and search for modern means of protecting insecticide-containing materials [10], the development of non-toxic, safe and effective alternatives was needed, leading to various advanced experiments and tests of vital effectiveness against invasion of
2. Methodology

2.1. Theoretical

The transmission of laser radiation in tissues is related to its wavelength. Infrared laser radiation shows a higher penetration into tissues than the laser light of the red region of the visible spectrum. Therefore, the latter has proved useful in treatment of skin and mucosal disorders. The stimulatory effects of low-power laser irradiation at the cellular and molecular levels have been shown by many studies [12]. Nd:YAG laser is widely used in a variety of applications, including material treatment during manufacture, laser range determination and laser surgery [13], it has a wavelength of (1064 nm), has a long extinction length and penetrates tissue [14]. In recent years, huge improvements have been achieved in laser technology by study the interaction between intensity of electromagnetic pulses and matter in a completely new physical regime [15]. Laser light affects the mitochondrial respiratory chain by changing the electric potential of cell membranes and, consequently, their selective permeability for sodium, potassium and calcium ions, or by increasing the activity of certain enzymes such as cytochrome oxidase and adenosine triphosphates. It also increases DNA synthesis, collagen and pro-collagen production, and may increase the cell proliferation or alter locomotory characteristics of cells [12]. The effects light has on tissue depend not only on tissue properties but also on the characteristics of the laser source. Besides laser wavelength some of the basic parameters are power, irradiation time, and spot size for continuous wave (CW) lasers, and intensity, pulse width, repetition rate, spot size, and number of pulses for pulsed lasers [16]. Depending on the duration and peak value of the tissue temperature achieved different effects like coagulation, vaporization, carbonization and melting may be distinguished, and ‘figure 1’ showed the location of laser thermal effects inside biological tissue [17].

![Figure 1. The location of laser thermal effects inside biological tissue.](image-url)

When the laser beam is directed towards the skin, the light penetrates the surface of the skin and is absorbed by the tissue and a fraction of it is reflected or dispersed. The photon focuses its strength and ability on the target, and then the ability turns into heat distributed on the neighboring tissue by transport or radiation in cells. Thus, cell proteins begin to melt, as well as RNA, DNA, cell walls and their contents [18].

The thermal reactions of laser with the material is divided into several types, either an interaction that results in the heating or fusion or evaporation or it occurs plasma, and all these reactions occur as a
result the ability of the material to absorb the light of the laser and the extent of change that can be
cased by the characteristics of thermal and temperature melting and evaporation, where the process of
laser interaction with the material contains heating the material and then absorption and distribution
the energy [19]. Laser work does not require vacuum technology or large space, the process is done
without contact with the material, there are no specific specifications for the materials that can be
treated by laser, and laser beam can reach places where other techniques are not accessible [20].
‘Figure 2’ shows the interaction mechanism which includes reflection, refraction, absorption, and
beam propagation within the tissue [17].

2.2. Experimental
Samples of cowpea seeds infected with the insect were collected from local markets. The adult beetles
were collected from the infected cowpea and preserved at approximately (25 °C) and relative humidity
(70%). Laboratory farms were prepared and glass bottles were cleaned and sterilized. The insect adults
were placed with the cowpea to preserve their feeding environment with 10 pairs per flask. The bottles
were covered with a sterile piece of gauze and the nozzle was sealed with rubber belts and placed in
the incubator.

The pulse Nd:YAG laser (1064 nm) of irradiation distance (10 cm) were used in this work to irradiate
the samples collected and reared to observe the effect of changing some laser parameters by changing
the laser energy as well as the time period for exposing the samples to laser radiation, where the
samples were placed in petri dishes and exposed to radiation. The petri dishes containing the samples
were divided into four groups with three plates per group, the first group of them was exposed to laser
irradiation with (300 mJ) and with three time periods of exposure: (10 sec) for the first dish and (20
sec) for the second dish and (30 sec) for the third dish. The second group was exposed to the radiation
energy (360 mJ) with these three exposure periods, then the third group was exposed to radiation
energy (420 mJ) and with the same periods of exposure. And then the fourth group was exposed to
laser energy (480 mJ) as well as the same time periods for exposure to radiation.

After that the external appearance of all samples which were exposed to laser; they were tested by
using a camera filmed type (Sony) strongly enlarge (12 mega pixel). All the samples tested after (12
hour) after exposure to laser, then tested after (24 hour), and then after (48 hour).

3. Results and Discussion
The changes in the external appearance of the beetle ‘figure 3’ were studied, as well as the calculation
of death rates due to laser exposure at specific energies and time durations.
3.1. Effect of laser on the external appearance of cowpea beetle
The study showed clear deformities in the whole insect after exposed to laser radiation for all energies in general, especially at the highest energy and the highest radiation exposure time. The changes were obvious when exposed to laser energy at (360 mJ) for (20 sec) period time, where ‘figure 4’ shows the roasting of the cuticle and distortion in the sensor horns. ‘Figure 5’ shows a tear to one of the wings and scratches in the area near the head at (420 mJ) of laser energy and the exposure time (30 sec). When the exposure energy was increased to (480 mJ), the changes were very clear, such as blackened lining layer of paunch and the shortness of legs and contraction when the exposure time was (10 sec) as shown in ‘figure 6-a’, and an extortion in the body for the back area compared to the normal size of the insect at the exposure time (20 sec) as shown in ‘figure 6-b’ and then the whole body is completely destroyed with clear deformities of the head area, wings and legs at the exposure period (30 sec) as shown in ‘figure 6-c’.

![Figure 3. Cowpea beetle insect.](image)

![Figure 4. Cowpea beetle exposed to laser energy (360 mJ) at time period (20 s).](image)

![Figure 5. Cowpea beetle exposed to laser energy (420 mJ) at time period (30 s).](image)

![Figure 6. Cowpea beetle exposed to laser energy (480 mJ) at time periods: (a): (10 s), (b): (20 s), (c): (30 s).](image)
3.2. Effect of laser on cowpea beetle's life

(Table 1) shown the death percentage rates of cowpea beetle treated by laser with energies (300, 360, 420 & 480)mJ and exposure durations (10, 20 & 30)sec.

**Table 1.** Death percentage rates of cowpea beetle treated by laser.

| Irradiation energy (mJ) | Duration of irradiation (sec) | The percentage of insects death after 12 hours % | The percentage of insects death after 24 hours % | The percentage of insects death after 48 hours % |
|-------------------------|-------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 300                     | 10                            | 7.33                                          | 20                                            | 80                                            |
| 360                     | 10                            | 7.33                                          | 40                                            | 86.66                                         |
| 420                     | 10                            | 16.64                                         | 68.87                                         | 90                                            |
| 480                     | 10                            | 22.22                                         | 70                                            | 100                                           |
| 300                     | 20                            | 10.33                                         | 29.55                                         | 86.66                                         |
| 360                     | 20                            | 12.5                                          | 48.11                                         | 93.33                                         |
| 420                     | 20                            | 16.64                                         | 77.77                                         | 96                                            |
| 480                     | 20                            | 39.33                                         | 85.55                                         | 100                                           |
| 300                     | 30                            | 16.64                                         | 33.33                                         | 90                                            |
| 360                     | 30                            | 18.66                                         | 55.55                                         | 93.33                                         |
| 420                     | 30                            | 20                                            | 81.11                                         | 100                                           |
| 480                     | 30                            | 42.22                                         | 93.33                                         | 100                                           |

3.2.1. The death percentage of cowpea beetle after (12 hour) of laser treatment

The percentages in (Table 1) indicate that there are significant differences in the effect of laser on the life of cowpea beetle after irradiation at duration times (10, 20, 30)sec. The results show that the death rates increase slightly at low energies and less exposure time, then the increase in death rates is clear at high energies, especially when the exposure time is increased as shown in ‘figure 7’.

**Figure 7.** Percentage of cowpea beetle death after (12 hour) treated by laser.

3.2.2. The death percentage of cowpea beetle after (24 hour) of laser treatment

The results of (Table 1) show that the percentage of death after (24 hour) of laser treatment of cowpea beetle is higher than that for the (12 hour) case, and the results showed that the death rates were clear for all energies and times of exposure, as shown in ‘figure 8’.

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3.2.3. The death percentage of cowpea beetle after (48 hour) of laser treatment

The results showed the highest death rates for cowpea beetle as shown in (table 1). It can be seen that the passage of (48 hour) after exposure to laser is sufficient to reach the death percentage of the beetle to (100%) at (480 mJ) and for all exposure times as shown in ‘figure 9’.

The results obtained by laser treatment are similar to those obtained by previous research and studies, in which inactive powders were used, such as silicium oxide coal furnace and others, which led to high rates of killing in the cowpea beetle, in addition to that the laser was effective faster, especially at high energies. In general, the results showed that the death rates of cowpea beetle increased by increasing the exposure time of laser radiation after the calculated hours with stable energy of the exposure, it increased by increasing the energy to be record its highest level when the energy reaches (480 mJ), as it shown in ‘figures 10-a, b, c, d’.
Figure 10. Percentage of cowpea beetle death treated by laser with different energies.

Exposure to laser energy leads to a rise in the temperature of the exposed area. The results obtained for the death percentage of the insect can lead to sharp changes in temperature due to increased exposure time or increased laser energy, which have direct effects on the insect, such as loss of water from the body and the imbalance of gases within the tissues of the insect body followed by a rapid breathing, causing high exchange of energy. The increase in exposure time and energy and the close distance of the source of laser to the insect (10 cm) leads to an excessive increase in laser energy per unit area where the increase in energy efficiency for the unit area (surface of the insect) led to high heat in a very short time which in turn led to significant damage to the structure and appearance of the insect.

4. Conclusions
Laser can be used to make changes to the external appearance of the beetle in (color, shape and appearance of some deformities) and increase the percentage of death of this insect, it can be said that the laser can be used as an alternative to toxic pesticides, it is a safe, efficient and economical source of high temperature in its area without need for high energy to operate. Laser beam excitation was great because of close distance where the laser energy is distributed on a small area of the surface and therefore the effect is significant.

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