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# New Biocompatible Technique Based on the Use of a Laser to Control the Whitefly *Bemisia tabaci*

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**Abstract:** The whitefly *Bemisia tabaci* is among the most important agricultural pests in the world and one of the world's top 10 most invasive insect pests. *Bemisia tabaci* is associated with severe yield and quality losses, mainly due to the transmission of plant viruses, as in the case of common beans (*Phaseolus vulgaris* L.). Reducing insecticide applications is a research priority, e.g., developing innovative and clean tools such as electromagnetic waves. The present work aims to determine the effective parameters of laser to reduce the *Bemisia tabaci* population in common beans. Preliminary assays were conducted by manually irradiating continuous-wave laser beams with different wavelengths (444 nm, 527 nm, and 640 nm) and optical intensities directly on the insects. Among these, the most effective wavelength was 444 nm. Later, we repeated the experiments using a homemade automated system to control the exposure time ($t_1$ = 1 s, $t_2$ = 2 s, $t_3$ = 3 s and $t_4$ = 4 s) of whiteflies to the incident beam at different optical intensities ($I_1 \approx 10$ Wcm$^{-2}$, $I_2 \approx 4$ Wcm$^{-2}$, $I_3 \approx 2$ Wcm$^{-2}$). We have achieved 100% insect mortality by irradiating 454 nm laser wavelength on the 3rd instar nymphs of *Bemisia tabaci*, with the following parameters: $I_1(t_1)$, $I_2(t_3)$ and $I_3(t_4)$. Moreover, the laser irradiation test did not affect plant yield and development, revealing that our preliminary results present a photonic technique that could control whiteflies without harming the plants' development.

**Keywords:** integrated pest management; Hemiptera; agriphotonics; photonics; laser applications; *Phaseolus vulgaris*



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## 1. Introduction

The whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype MEAM1 is one of the most relevant insect pests of crops worldwide due to many reasons: its broad geographic coverage, present in all continents; strong performance as a vector of plant viruses; ability to colonize plants belonging to many plant families; high adaptability to different environments; and rapid selection of insecticide-resistant populations. As a result, whiteflies are a threat to food security, especially in developing countries [1].

The common bean (*Phaseolus vulgaris* L.) is a staple food in developing countries of Latin America, Africa, and Asia [2–4]. In Brazil, this crop is produced in a variety of systems, in three cropping seasons (year-round), ranging from family farmers with low use of technology to large business areas using high-tech solutions. Even so, losses of up to 100% have been reported in common bean yield due to damage associated with viruses transmitted by the whitefly [5]. The main control method for plant viruses transmitted by the whitefly has been the application of pesticides to control the vector, thus relying on chemical or biological control processes. However, the intense use of the same molecules, often not associated with other management techniques, has rapidly selected whitefly populations resistant to most of the commercially available insecticides, thus limiting the efficiency of chemical control. In recent years, there have been no records of new insecticides to control this pest, which indicates a limitation in developing new chemical molecules. Furthermore, the excessive use of synthetic pesticides poses a risk to human health and the environment, contributing to the carbon footprint of agricultural production. Pesticides are extremely C-intensive, and their use is increasing rapidly worldwide, but especially in India, China, Brazil, and other emerging economies [6–11]. Reports of 20 applications per common bean crop season are usually a standard procedure [5], as well as in cotton and tomato. Reducing the need to use insecticides has been a research priority for developing a more sustainable production system, ensuring food security for low-income populations.

In addition to chemical and biological control methods, insect control methods based on physics principles have been studied recently. An example, still only a little explored, is using laser to damage insect tissues, causing insect mortality or delayed insect development. Laser devices at several wavelengths have been tested, causing significant mortality to a wide range of insects, such as ants, stored grain beetles, aphids, leaf miners, spider mites, mosquitoes, *Drosophila melanogaster*, the cabbage whitefly *Aleyrodes proletella*, and even larger insects, such as domestic cockroaches [12–22]. These results suggest that electromagnetic radiation, such as the laser, can contribute to the management of the whitefly *B. tabaci* as well. However, as most reports showed that insect mortality is dose and insect-stage dependent, it is necessary to study the appropriate laser wavelength and amount of dose to achieve significant insect mortality without harming the plants. Here, we report the most effective parameters for a new biocompatible photonic technique able to control whitefly nymphs without harming the host plants.

## 2. Materials and Methods

### *2.1. Insect Colonies*

The whitefly *Bemisia tabaci* MEAM1 biotype used in the experiments originated from a colony on common bean (*Phaseolus vulgaris*, cv. Pérola), kept under screen house conditions at Embrapa Arroz e Feijão, Santo Antônio de Goiás, GO, Brazil (16°28′00′′ S, 49° 17′00′′ W; 823 m asl), as previously described [23,24]. To obtain age-synchronized adult insects, plants containing whitefly eggs laid for 2 h were isolated in insect cages, after removing the adults, until reaching the third larval instar. Then, insects were irradiated with laser beams at different larval stages in the preliminary screening.

### *2.2. Laser Sources and Physical Parameters*

The laser sources used in this work were continuous-wave (CW) diode lasers (DL) emitting in the visible (VIS) spectral range. The CW-DL models acquired from the manufacturer Wicked Lasers, Shanghai, China, are S3 Inferno Series (635 nm), S3 Krypton Series (520 nm), and S3 Artic Series (445 nm). The model DM-B5000 (454 nm) was acquired from Sunshine Electronics, Guangzhou, China. All four CW-DL are direct-diode type lasers and can be considered low-cost DL with an average optical power ranging from approximately 408 mW (S3 Krypton Series) to approximately 800 mW (DM-B5000). A thermopile power sensor (Coherent, PowerMAX, Santa Clara, CA, USA) measured the average optical power. The values differ from both manufacturers, as one can verify in Tables S1–S4 in the Supplementary Materials (SM). The DL's spectral emission was obtained by filtering down the average optical power using a set of neutral density filters before the beam collection by an optical fiber ($\phi$ = 20 μm) connected to the optical spectrometer (slit of 25 μm) to avoid CCD saturation. All results are in good accordance with the manufacturer's announcement and can be found in Tables S1–S4 and Figures S1–S4 on the SM.

Whether a DL employs a traditional monolithic design or an external cavity configuration, the laser light must pass through the diode's PN junction via a ridge waveguide. Commonly, these types of structures have an optical cavity with an asymmetric geometry, resulting in a high beam divergence more significant along one axis (known as the fast axis), leading to an elliptical beam shape [25]. DLs also have a highly divergent beam compared to other lasers, e.g., Nd:YAG (neodymium-doped yttrium aluminum garnet). As a result, it needs to be collimated with additional optics on the output facet to manage these types of asymmetric laser beams. The manufacturer provides such lenses, particularly in assembled DL models, such as the ones used in this work. As a result, a low-cost DL beam, after propagating through the set of lenses, typically presents an elliptical spatial profile in the far field. A homemade automated system based on the well-known knife-edge technique [26] was used to characterize the transversal spatial laser beam of the DL models used in this work. Figures S1B–S4B in the SM show the laser transversal spatial profiles. As one can verify, all spatial profiles are approximately elliptical, and the fluctuation intensity profile recorded is directly related to the quality of the laser cavity, which in this case, does not represent a drawback once the purpose is only to irradiate the physical system plant–insect, on the contrary, once our goal is to show that affordable lasers can be used for pest control.

The irradiation area was determined using the knife-edge parameters, i.e., by recording the length of the vertical and horizontal laser beam cross-sections and considering an elliptical area, as shown in Table 1. The models used for the laser wavelength screening were Inferno, Krypton, and Artic. Their optical intensity was intentionally kept at the same values for the three lasers by controlling the average optical power with a set of Glan-laser calcite polarizers (Thorlabs, GL10, Jessup, MD, USA) and an achromatic half-wave plate (ThorLabs, AHWP05M-580, Jessup, MD, USA) placed on the beam optical path immediately after the laser's output. On the other hand, the irradiation made with the DM-B5000 model did not use the previous apparatus to control the optical intensity. We have electronically set the average optical power to 660 mW and utilized a 12,5 cm focal biconvex lens to record the beam area at 20 cm (Spot 1), 25 cm (Spot 2), and 35 cm (Spot 3) from the focal point. The area of each spot was determined by knife-edge parameters, as mentioned before, and presented in Table 1.

**Table 1.** Physical parameters of the lasers used in this work to irradiate the physical system plant–insect.

| CW-DL Model | Beam Area [$cm^{-2}$] | Avg. Optical Power [W] | Optical Intensity [$W\ cm^{-2}$] |
|---|---|---|---|
| Inferno (640 nm) | $0.088 \pm 0.009$ | $0.530 \pm 0.032$ | $6.000 \pm 0.702$ |
| Krypton (527 nm) | $0.057 \pm 0.006$ | $0.345 \pm 0.021$ | $6.049 \pm 0.708$ |
| Artic (444 nm) | $0.065 \pm 0.006$ | $0.395 \pm 0.024$ | $6.014 \pm 0.704$ |
| DM-B5000 (454 nm) Spot 1 | $0.065 \pm 0.006$ | $0.660 \pm 0.040$ | $10.021 \pm 1.173$ |
| DM-B5000 (454 nm) Spot 2 | $0.109 \pm 0.011$ | $0.660 \pm 0.040$ | $6.037 \pm 0.706$ |
| DM-B5000 (454 nm) Spot 3 | $0.242 \pm 0.024$ | $0.660 \pm 0.040$ | $4.074 \pm 0.477$ |

The uncertainty associated with the optical intensity was calculated considering the relative experimental error of average optical power (6%) and beam area calculation (10%). The associated error of the average optical power is mainly due to the laser beam fluctuations, which surpass the calibration uncertainty of the equipment ($\pm$1%). Therefore, considering the propagation of errors, we can estimate that the optical intensity's uncertainty is 11.7% of the measured value.

Another essential physical parameter is dose; in fact, the term "dose" is a concept primarily associated with ionizing EM radiation, commonly used in dosimetry applications [27] expressed in units of millisievert (mSv) or Gray (Gy). For example, Gy measures the amount of EM radiation energy absorbed by a substance, while mSv measures the potential health risk associated with that EM radiation exposure. However, in our work in which we use non-ionizing EM radiation, we will define the physical quantity "dose" as the product of optical intensity (irradiance) with time exposure, commonly called radiant exposure, in the radiometry field. Thus, subsequently, the dose (D) will represent the value that can be assessed to determine insect mortality, expressed in units of Joule per area (J $cm^{-2}$).

*2.3. Laser Wavelength Screening*

Preliminary assays were conducted by manually irradiating the physical system plant–insect with CW-DL models Inferno (640 nm), Krypton (527 nm), and Artic (444 nm) continuous-wave (CW). Because the primary purpose was to identify which wavelength would be more effective on the insect's mortality, we have kept the optical intensity of these irradiations at approximately 6.0 $Wcm^{-2}$, as described in Section 2.2. The manufacturer of such DL models provided an expansion kit of lenses to shape the laser beam. One of those shapes can be described as a line laser beam or a thin laser beam. It is characterized by its long and narrow shape, with a rectangular or elongated cross-section. The estimated optical intensity for this line laser beam was below 0.08 $Wcm^{-2}$, which did not cause any mortality to the whiteflies *Bemisia tabaci.* Therefore, we have conducted the screening wavelength with the beam shapes presented in SM (Figures S1B–S3B).

The laser beams were applied directly on the whitefly nymphs and eggs on common bean leaves to screen the most effective laser parameters for insect control without damaging the plants. Common bean plants cv BRS FC401 RMD with the primary leaves fully expanded (7 days after sowing, DAS) were infested with whitefly adults for oviposition for 1 h. Then, the adult insects were removed from the plants. When the eggs developed into 3rd instar nymphs, the irradiation started within the abaxial surface of the primary leaves (where insects were located) (Figure 1A,B), gently fixed to a sheet of paper using masking tape. Nymph size was about 440 µm × 260 µm (Figure 1C). Each replicate consisted of at least three irradiated areas, containing variable numbers of nymphs. Finally, using a stereoscopic microscope, insect mortality, i.e., the proportion of dead nymphs in relation to the total number of insects evaluated, was evaluated after the laser beam exposure. Dead nymphs are identified by a drier appearance, darker color, and reduced volume, in comparison with the live nymphs (Figure 1D,E)).

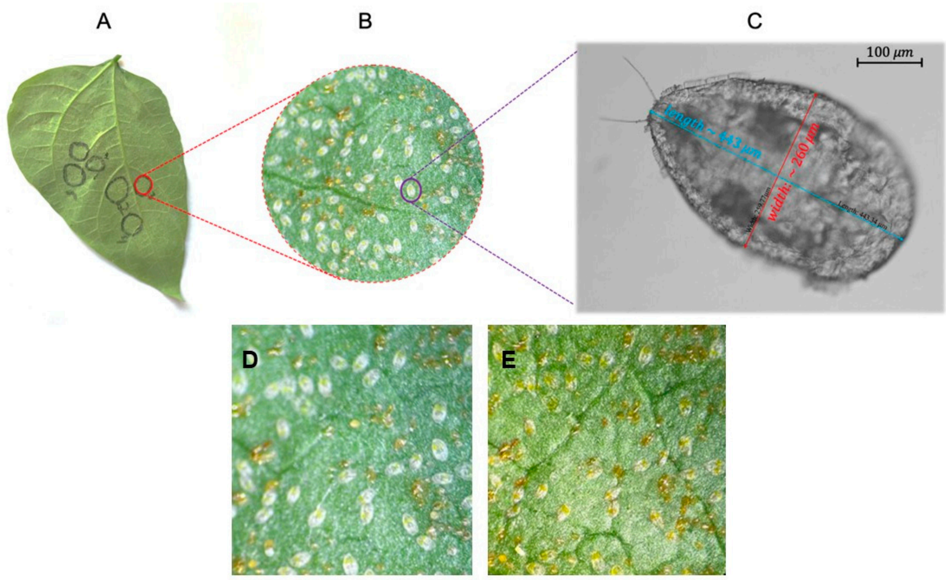


**Figure 1.** Common ben leaf containing whitefly nymphs and eggs. (**A**) View of the entire leaf, marked with a permanent marker in spots where the number of insects was counted. (**B**) Highlighted area showing whitefly nymphs and eggs at a 10× magnified scale, under stereoscopic microscope. (**C**) Whitefly nymph dimensions. (**D**) Live and (**E**) dead whitefly nymphs observed at a 10× magnified scale, under stereoscopic microscope after leaves' irradiation with laser. Dead nymphs have a dry appearance, darker color, and reduced volume.

*2.4. Effect of Laser Application on Plant Development*

To evaluate whether the laser irradiance could impair plant development, common bean plants cv BRS FC401 RMD with primary leaves fully expanded (7 DAS) were exposed to the same laser wavelengths and intensities used in the preliminary assays, except for the 640 nm laser, which did not cause significant insect mortality. Plants were set up with the abaxial surface of their leaves exposed to the laser irradiation as previously described, i.e., the same optical and time exposure parameters used in Section 2.2. Plants were irradiated 2 more times at 14 and 21 DAS, in the first and second leaflets, respectively. Controls were not exposed to the laser beam. To assess a possible thermal effect, thermal images of the leaves were taken at the laser application and immediately afterward, using a FLIR® thermal camera. The thermal camera's characteristic and calibration is reported in SM (Section S2).

Plants were kept in a greenhouse, following all the recommended management, until the end of their cycle to evaluate plant development and yield. The number of pods per plant, number of grains per plant and seed viability (germination rate) were evaluated. To evaluate seed germination rate, seeds were placed on Germitest® paper, previously soaked in water, and then taken to a germination chamber, with a controlled temperature of 28 °C for four days. Three replicates per treatment were performed, each containing 6–10 seeds. The proportion of viable and non-viable seeds in each of the treatments was determined. For the statistical analysis, tests of the normality of residues and homogeneity of variances were performed, followed by Tukey's HSD to compare the treatments.

*2.5. Automated Homemade System for Reliable Time Exposure Laser Irradiation*

After analyzing the most suitable wavelength to achieve the insect's mortality, we have decided to increase the reliability of the experiments. For this purpose, we have used the CW-DL DM-B5000 model (454 nm). The DM-B5000 model showed reasonable stability during the tests, as shown in Figure 2, in which the average optical power variation is below 6%. Furthermore, we built a simple but efficient homemade system that is fully automated to control the exposure time of whiteflies to the incident laser beam. With this homemade system, which also electronically controls the average optical power, we achieved the desirable reliability, guaranteeing the effectiveness of the results presented in this work.

The homemade system has a straightforward construction, as depicted in the schematics of Figure 3A. The working principle is based on controlling the DL, i.e., turning it "ON" and "OFF" and controlling the average optical power assuring the optical intensity used for the insect's irradiation. Still, the most crucial part of this homemade system is the shutter which allows a reliable insect's time exposure. The shutter unblocks the beam when the "ON" plateau is stable (inset of Figure 2). Because this system also intends to be low-cost, an Arduino UNO has been used as a data acquisition (DAQ) device. The Arduino's Pulse Width Modulation (PWM) port was connected to the DL driver to control both on-off and average optical power. Moreover, a metal plate coupled to a low-cost servo motor was used to block the DL beam, which served as a beam shutter. We also developed a graphical user interface (GUI), allowing a non-technical user to perform the irradiation experiments, as shown in Figure 3B.

In this automated assay, four exposure times were used ($t_1 = 1$ s, $t_2 = 2$ s, $t_3 = 3$ s and $t_4 = 4$ s) at different optical intensities ($I_1 \approx 10$ Wcm$^{-2}$, $I_2 \approx 4$ Wcm$^{-2}$, $I_3 \approx 2$ Wcm$^{-2}$). The plant's irradiation procedure was similar to the manual assay, i.e., detached common bean leaves containing whitefly nymphs were fixed to a piece of paper and exposed to laser irradiation (Figure 3C). The control experiment was performed in two ways: (i) using CW-DL Inferno (640 nm); (ii) with no laser irradiation. Both control experiments showed zero percent mortality in the automated assay. The insect mortality rate evaluation was made using a stereoscopic microscope (Figure 1D,E). Finally, all the described experiments were repeated in triplicate.

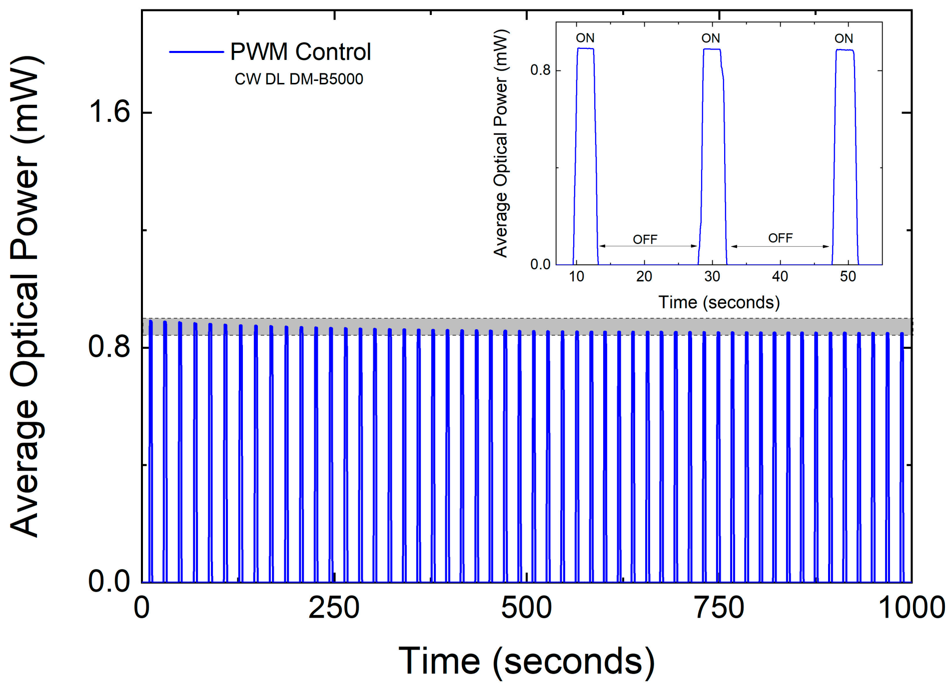


**Figure 2.** Maximum optical power stability during approximately 16 min revealed an uncertainty (highlighted in gray) of about 6% by using the "ON-OFF" automated procedure via PWM.

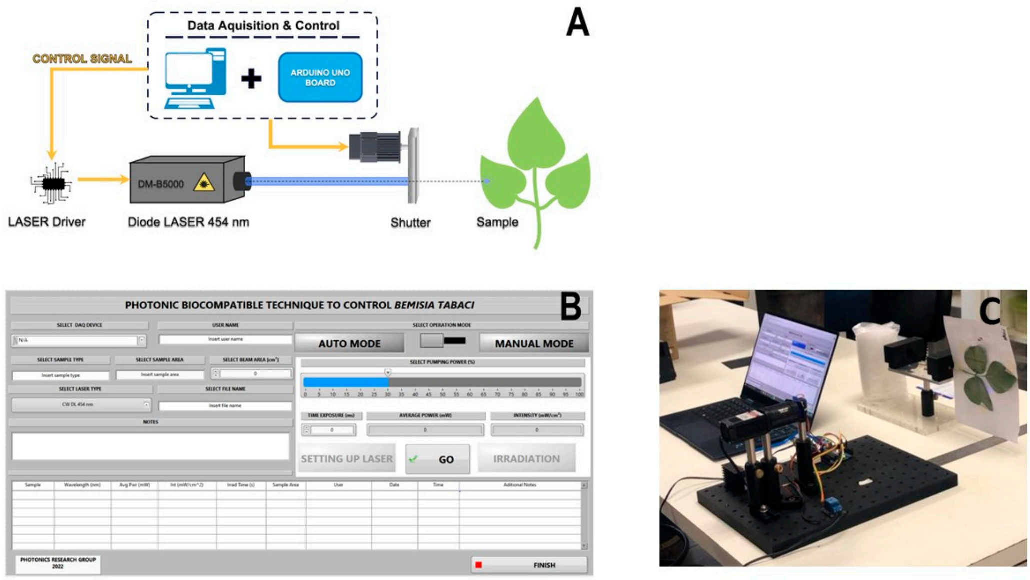


**Figure 3.** An automated assay was developed to study the effect of laser irradiation on whitefly nymphs. (**A**) Simplified block diagram illustrating the homemade automated setup used to obtain accurate exposure irradiation times. In addition, (**B**) presents the developed GUI's print screen. (**C**) Incidence of the CW-DL DB-B5000 (454 nm) on common bean detached leaves containing 3rd instar whitefly nymphs, controlling the irradiation exposure time.

## 3. Results

In the preliminary tests, i.e., the manual irradiation of the physical system plant–insect using the CW-DL models Inferno (640 nm), Krypton (527 nm), and Artic (444 nm) manually, revealed that only 527 nm and 444 nm wavelengths caused 100% insect mortality (Table 2). The best irradiation performance belongs to the wavelength 444 nm, which only required about 1 s to eliminate all irradiated whiteflies. On the other hand, the 527 nm wavelength needed 60 s to eliminate all the irradiated insects, causing damage to the plant's development. The lowest recorded performance is from the lowest energy wavelength (640 nm), in which the irradiation achieved only 4% of mortality with an exposure time of 60 s. We want to emphasize that the results obtained for the 3rd instar nymphs represent what was observed for the earlier stages, which are usually more susceptible to stress and adverse conditions. The mortality of younger nymphs (1st and 2nd instar) and eggs was also significant (data not shown).

**Table 2.** Insect mortality and laser parameters used in the preliminary assays for insect stage of 3rd instar nymphs. The uncertainty associated with the intensity is about 12%, as reported in Section 2.2, while the uncertainty associated with the manually controlled time exposure varies from 5% for longer times (60 s), while for shorter times (5 s and 1 s) it drastically increases to around 50%.

| | **Continuous-Wave Diode Lasers** | | |
|---|---|---|---|
| | **Artic 444 [nm]** | **Krypton 527 [nm]** | **Inferno 640 [nm]** |
| Intensity [$Wcm^{-2}$] | 6.0 | 6.0 | 6.0 |
| Exposure time [s] | 1 | 60 | 60 |
| Total number of insects evaluated | 24 | 24 | 24 |
| Number of dead insects after laser irradiation | 24 | 24 | 1 |
| Insect mortality [(i)] [%] | 100 | 100 | 4 |
| Damage to plant development | NO | YES | YES |

[(i)] The proportion of dead insects in relation to the total number of insects evaluated.

It is pointless to show a photonic technique to eliminate pests from plants if such a technique causes permanent harm to the plant, i.e., that will damage the plant's development. That being said, we carefully analyzed how any possible damage from the laser would influence the plant's growth. The most critical data is shown in Table 3, where we show that plants treated with laser beams did not differ from the controls (not treated) regarding plant development (plant yield and seed viability). As one can check, the most effective irradiation within the least amount of time possible that eliminates the insects (444 nm, $I = 6.0$ $Wcm^{-2}$, $t = 1$ s) does not cause any harm to the plant development (Tables 2 and 3). The 527 nm wavelength did not harm the plants when applied on the leaves for only 1 s (Table 3). However, at a longer exposure time (60 s), damage to plant development was observed for 527 nm and 640 nm (Table 2).

**Table 3.** Average number of pods and grains per plant and seed germination rate after exposing the common bean plants to irradiation three times using different laser wavelengths and beams. The uncertainty associated with the intensity is about 12%, as reported in Section 2.2, while the uncertainty associated with the manually controlled time exposure varies from 5% for longer times (60 s), while for shorter times (5 s and 1 s) it drastically increases to around 50%.

| | **CW-DL Artic (444 nm) and Krypton (527 nm)** | | |
|---|---|---|---|
| | **444 [nm]** | **527 [nm]** | **Control Experiment** |
| | **$I = 6$ $[Wcm^{-2}]$ $t = 1$ [s]** | **$I = 6$ $[Wcm^{-2}]$ $t = 1$ [s]** | **No Irradiation** |
| Average number of pods per plant | 6.00 (a) | 5.00 (a) | 5.85 (a) |
| Average number of grains per plant | 22.33 (a) | 18.75 (a) | 25.14 (a) |
| Seed germination rate [%] | 97.60 (a) | 99.15 (a) | 96.90 (a) |

(a) means followed by the same letters in the rows do not differ by Tukey's test ($p < 0.05$).

Thermal images showed that the leaf temperature, initially at 26 °C (room temperature) (Figure 4A), rapidly increased during 1 s of laser irradiation, reaching 36 °C on average (Figure 4B) in about 3 s. However, the temperature dropped quickly, returning to room temperature about 10 s after laser irradiation (Figure 4C). We emphasize that the analyzed damage concerns plant development, which was not negatively impacted. However, that does not mean that the laser irradiation is innocuous to plants: a temporary, isolated burn, with no side effects on the plant's development, was observed on some of the leaves irradiated in the various experiments conducted. This effect, which was previously reported in the literature [16], was not observed in plants presenting a normal state of turgidity. That is, it is possible that the plants that presented the small burns were dehydrated, or were not in their normal state of turgidity. In most irradiated plants, the only effect observed was a slight discoloration of the leaf at the site of application. This observation is important, as it leads us to think about technology application recommendations considering satisfactory irrigation levels before laser application for future studies.

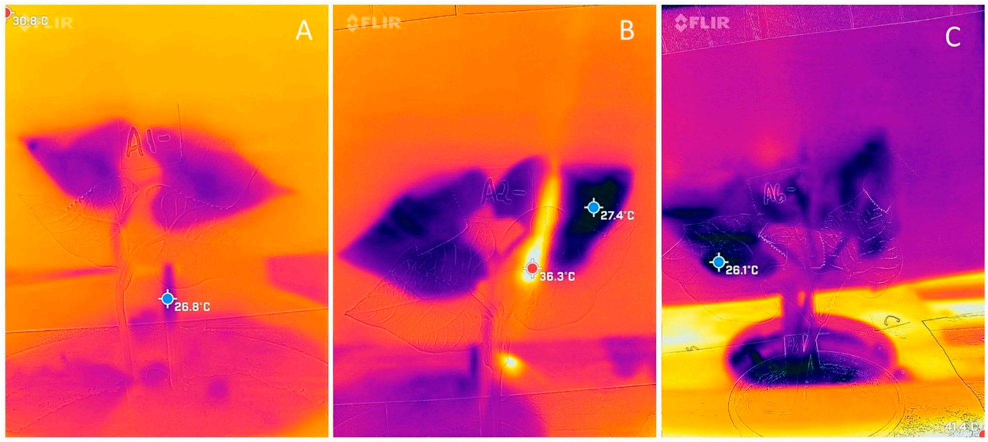


**Figure 4.** Thermal images of the common bean leaf before (**A**), during (**B**), and after (**C**) laser irradiance.

With the previously presented results, it was clear that a wavelength close to 444 nm was suitable for testing in a more controlled environment from the data and acquisition control point of view. Therefore, we have achieved reliability values regarding optical intensity and time exposure using a homemade automated system with a laser source at 454 nm wavelength. The results are presented in Table 4 for three different optical intensities with a maximum of 4 s of irradiation time. Even with the lowest optical intensity applied ($\sim 2.0$ Wcm$^{-2}$), the full insect mortality was achieved, but it took 4 s. The second lowest optical intensity ($\sim 4.0$ Wcm$^{-2}$) reached the total mortality within 3 s. As expected, the total mortality with an optical intensity above 6.0 Wcm$^{-2}$ would get the complete mortality. In this case, we tested an optical intensity of approximately 10.0 Wcm$^{-2}$. Regarding plant damage, a punctual burn in the irradiation site was observed on the leaves. However, this is considered temporary damage without an effect on plant development, as explained before.

**Table 4.** Data corresponding to 100% insect mortality achieved with laser wavelength 454 nm in the automated assays for insect stage of 3rd instar nymphs. The uncertainty associated with the intensity is about 12%, as reported in Section 2.2, while the uncertainty associated with the automated controlled time exposure is negligible.

| CW-DL Model DM-B5000 (454 nm) | | | |
|---|---|---|---|
| Intensity [Wcm$^{-2}$] | $I_1 = 10$ | $I_2 = 4$ | $I_3 = 2$ |
| Exposure time [s] | $t_1 = 1$ | $t_3 = 3$ | $t_4 = 4$ |
| Total number of insects evaluated | 56 | 51 | 52 |
| Number of dead insects after laser irradiation | 56 | 51 | 52 |
| Insect mortality [(i)] [%] | 100 | 100 | 100 |

[(i)] The proportion of dead insects in relation to the total number of insects evaluated.

## 4. Discussion

Recent advances in physical methods for insect management, mainly using electromagnetic (EM) radiation, have been reported for several insect species, including ants, stored grain beetles, aphids, leaf miners, spider mites, mosquitoes, and whiteflies [12–22,28]. All of these previous works reported the usage of different laser systems, ranging from VIS to IR (infrared), using both continuous-wave and pulsed beams. However, two of those works have attracted our attention, particularly [13,21], which used low-cost and compact laser systems with wavelength emission at 405 nm and 445 nm, respectively. The low-cost and reduced dimension factors are crucial for our work proposal, which presents a potential alternative method to the chemical and biological processes for pest control by small farmers. In Ref. [13], the authors reported an exposure time of 30 min to achieve 100% mortality in adults of *Tribolium castaneum* species. However, they did not mention what optical intensity they used. Such exposure time is a considerable drawback to presenting a practical alternative pest control method.

On the other hand, Ref. [21] has shown that a 445 nm laser with an average optical power of approximately 670 mW focused was able to cause mortality rates depending on the emitted pulse energy. With all this in mind, we have decided to choose the VIS spectral region (445 nm, 520 nm, and 635 nm) to check which wavelength and dose will be efficient to achieve 100% mortality of the whitefly (*B. tabaci*), without causing permanent damage to the plant (*Phaseolus vulgaris* L.). Moreover, to the best of our knowledge, our report is the first one showing the use of a laser to control the whitefly *B. tabaci*.

Our results show that the whitefly *B. tabaci* can be controlled, i.e., the mortality can be fully achieved, with the following doses: $D_1 = I_1\, t_1 \approx 10$ J cm$^{-2}$, $D_2 = I_2\, t_3 \approx 12$ J cm$^{-2}$, and $D_3 = I_3\, t_4 \approx 8$ J cm$^{-2}$. One can compare our results with previously reported ones in terms of dose evaluation. One of the closest examples in terms of wavelength and type of laser that we have found in the literature for comparison purposes is the work reported by Gaetani et al. [16]. In their work, the authors reported they used a Diode Pump Solid State (DPPS) CW laser, with an emission centered at 532 nm, delivering a dose of about 54 J cm$^{-2}$ to control aphids. The dose used to eliminate the aphids is around five times higher when compared to the doses we used to eliminate the whitefly *B. tabaci*, which is reasonable, considering that aphids are usually at least three times larger than whiteflies. Still, the amount of energy required to cause significant mortality of insects also varies with the insect species [29].

There are few reports on the effect of EM radiation within the visible (VIS) spectral window on insect mortality. Ultraviolet (UV) light is the best-known EM radiation to have lethal effects on most organisms, being highly damaging to biological systems because it can induce a variety of mutagenic and cytotoxic DNA alterations. For example, a significant reduction of the number of eggs and nymphs of the whitefly *B. tabaci* was reported in plants treated with UV (315–400 nm) irradiation [28]. These authors discuss that UV-B radiation might have lethal effects on plants, so they decided to work with UV-A, which is not absorbed by native DNA [30], but can still damage cell constituents, such as lipids, proteins, and even DNA, by increasing the synthesis of reactive oxygen species (ROS) [31]. One might consider that using UV radiation is not safe to non-target organisms. Therefore, in addition to its potential to be harmful to DNA and other cellular components, UV lasers are highly expensive, so they would not serve our future goals of providing an affordable technology for small farmers.

Moreover, it has been suggested that VIS EM radiation around 444 nm can damage insect cells by increasing the synthesis of ROS [29], which may be one of the possible causes of the mortality rate presented in our work. As an alternative to the toxic effect of ROS, insect mortality could be caused by a rapid increase in body temperature. In the present work, insect mortality does not seem to be related to a thermal effect, considering that thermal images showed a rapid increase in the leaf temperature, from ~26 °C to ~36 °C during laser irradiation, and then dropping down to ~26 °C about 10 s after the irradiation. The observed temperature gradient did not influence plant survival and development, although some tissue damage was observed.

Other types of laser beams should be considered for pest management, such as pulsed laser systems. In the interaction of a laser beam with a biological tissue, which in our context is a physical system plant–insect, the main advantage of using a pulsed laser system, with the same wavelength 454 nm, would be related to the reduction of thermal effects on the plant [32,33], which represents an unquestionable advantage. However, in the context of this work, which is to present a photonic technique that small farmers could use, the considerable drawback of a pulsed laser system is the cost factor compared to a CW DL system. From a practical point of view, a CW DL system is a robust, small-sized, and affordable laser system emitting a 454 nm wavelength that will achieve 100% mortality of whiteflies without permanently damaging the plant.

## 5. Conclusions

In the present study, we reported for the first time a mortality of 100% of whitefly *Bemisia tabaci* 3rd instar nymphs on common bean (*Phaseolus vulgaris* L.), using a continuous-wave, low-cost, diode laser emitting a 454 nm beam to deliver lower doses ($\leq$ 12 Jcm$^{-2}$) to the plants. In addition, preliminary irradiations with 444 nm, 527 nm, and 640 nm laser wavelengths have been conducted to understand the effect of impaired plant development. Although temporary damage appeared after the laser irradiation, it was not enough to impair the plant development, suggesting that 454 nm laser wavelength irradiation could be used on a large scale to control the 3rd instar nymphs of *B. tabaci*. In order to record a reliable dose value, we have developed an automated homemade system that controls, with precision, the exposure time. The low-cost automated system associated with the low-cost laser source is an excellent indicator that this system could be, in the near future, an alternative for small and medium farm producers to have a biocompatible photonic technique available.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/photonics10060636/s1, Figure S1-A: Inferno's spectral emission used in the preliminary tests, where the emission peak is centered at c.a. 640 nm; Figure S1-B: Inferno's spatial profile was acquired with an automated knife-edge home system (step of 50 µm), revealing an elliptical-like spatial profile. No additional lens was used besides the set of lenses coupled to the output facet by the manufacturer. The cross-section was acquired at ~40 cm from the laser. The inset is a laser beam photograph in a black target to give the reader a qualitative aspect ratio of the beam; Figure S2-A: Krypton's spectral emission used in the preliminary tests, where the emission peak is centered at c.a. 527 nm; Figure S2-B: Krypton's spatial profile was acquired with an automated knife-edge home system (step of 50 µm), revealing an elliptical-like spatial profile. No additional lens was used besides the set of lenses coupled to the output facet by the manufacturer. The cross-section was acquired at ~40 cm from the laser. The inset is a laser beam photograph in a black target to give the reader a qualitative aspect ratio of the beam; Figure S3-A: Artic's spectral emission used in the preliminary tests, where the emission peak is centered at c.a. 444 nm; Figure S3-B: Artic's spatial profile was acquired with an automated knife-edge home system (step of 50 µm), revealing an elliptical-like spatial profile. No additional lens was used besides the set of lenses coupled to the output facet by the manufacturer. The cross-section was acquired at ~40 cm from the laser. The inset is a laser beam photograph in a black target to give the reader a qualitative aspect ratio of the beam; Figure S4-A: DM-B5000's spectral emission used in the preliminary tests, where the emission peak is centered at c.a. 454 nm; Figure S4-B: DM-B5000's spatial profile was acquired with an automated knife-edge home system (step of 50 µm), revealing an elliptical-like spatial profile. A 13.5 cm bi-convex lens was used in addition to the set of lenses coupled to the output facet by the manufacturer. The vertical and horizontal cross-sections were acquired at ~20 cm from the focal point. The inset is a laser beam photograph in a black target to give the reader a qualitative aspect ratio of the beam; Figure S5: Calibration procedures of the thermal camera used in this work; Table S1: Some of the technical specifications provided by the manufacturer regarding the Inferno laser (635 nm) model from Wicked Lasers (China) used in the preliminary tests conducted in this work. Wavelength emission, laser power, and beam shape were tested in the laboratory using an optical spectrometer (Avantes, AvaSpec-HERO, Netherlands), power meter (Coherent, PowerMax, USA), and automated homemade knife-edge system with a 50 µm step, respectively; Table S2: Some of the technical specifications provided by the manufacturer regarding the Krypton laser (520 nm) model from Wicked Lasers (China) used in the preliminary tests conducted in this work.

Wavelength emission, laser power, and beam shape were tested in the laboratory using an optical spectrometer (Avantes, AvaSpec-HERO, Netherlands), power meter (Coherent, PowerMax, USA), and automated homemade knife-edge system with a 50 µm step, respectively; Table S3: Some of the technical specifications provided by the manufacturer regarding the Artic laser (445 nm) model from Wicked Lasers (China) used in the preliminary tests conducted in this work. Wavelength emission, laser power, and beam shape were tested in the laboratory using an optical spectrometer (Avantes, AvaSpec-HERO, Netherlands), power meter (Coherent, PowerMax, USA), and automated homemade knife-edge system with a 50 µm step, respectively; Table S4: Some of the technical specifications provided by the manufacturer regarding the DM-B5000 laser (445 nm) model from Sunshine Eletronics (China) used in the automated tests conducted in this work. Wavelength emission, laser power, and beam shape were tested in the laboratory using an optical spectrometer (Avantes, AvaSpec-HERO, Netherlands), power meter (Coherent, PowerMax, USA), and automated homemade knife-edge system with a 50 µm step, respectively.

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## References

1. De Barro, P.J.; Liu, S.-S.; Boykin, L.M.; Dinsdale, A.B. *Bemisia tabaci*: A Statement of Species Status. *Annu. Rev. Entomol.* **2011**, *56*, 1–19. [CrossRef] [PubMed]
2. Brigide, P.; Ataide, T.D.R.; Canniatti-Brazaca, S.G.; Baptista, A.S.; Abdalla, A.L.; Filho, V.F.N.; Piedade, S.M.S.; Bueno, N.B.; Sant'ana, A.E.G. Iron Bioavailability of Common Beans (*Phaseolus vulgaris* L.) Intrinsically Labeled with 59Fe. *J. Trace Elem. Med. Biol.* **2014**, *28*, 260–265. [CrossRef] [PubMed]
3. Martins, S.M.; Melo, P.G.S.; Faria, L.C.; Souza, T.L.P.O.; Melo, L.C.; Pereira, H.S. Genetic Parameters and Breeding Strategies for High Levels of Iron and Zinc in *Phaseolus vulgaris* L. *Genet. Mol. Res.* **2016**, *15*, 28011. [CrossRef] [PubMed]
4. Nasar, S.; Shaheen, H.; Murtaza, G.; Tinghong, T.; Arfan, M.; Idrees, M. Socioeconomic Evaluation of Common Bean (*Phaseolus vulgaris* L.) Cultivation in Providing Sustainable Livelihood to the Mountain Populations of Kashmir Himalayas. *Plants* **2023**, *12*, 213. [CrossRef]
5. Souza, T.L.P.O.; Faria, J.C.; Aragao, F.J.L.; Del Peloso, M.J.; Faria, L.C.; Aguiar, M.S.; Wendland, A.; Quintela, E.D.; Diaz, J.L.C.; Magaldi, M.; et al. BRS FC401 RMD: Cultivar de Feijão Carioca Geneticamente Modificada Com Resistência Ao Mosaico-Dourado. ISSN 1678-961X. 2016. Available online: http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1055465 (accessed on 2 April 2023).
6. Lal, R. Carbon Emission from Farm Operations. *Environ. Int.* **2004**, *30*, 981–990. [CrossRef] [PubMed]
7. Dusenbury, M.P.; Engel, R.E.; Miller, P.R.; Lemke, R.L.; Wallander, R. Nitrous Oxide Emissions from a Northern Great Plains Soil as Influenced by Nitrogen Management and Cropping Systems. *J. Environ. Qual.* **2008**, *37*, 542–550. [CrossRef] [PubMed]
8. Gan, Y.; Liang, C.; Hamel, C.; Cutforth, H.; Wang, H. Strategies for Reducing the Carbon Footprint of Field Crops for Semiarid Areas. A Review. *Agron. Sustain. Dev.* **2011**, *31*, 643–656. [CrossRef]
9. Liu, C.; Cutforth, H.; Chai, Q.; Gan, Y. Farming Tactics to Reduce the Carbon Footprint of Crop Cultivation in Semiarid Areas. A Review. *Agron. Sustain. Dev.* **2016**, *36*, 69. [CrossRef]
10. Zhang, D.; Shen, J.; Zhang, F.; Li, Y.; Zhang, W. Carbon Footprint of Grain Production in China. *Sci. Rep.* **2017**, *7*, 4126. [CrossRef]

11. Jaiswal, B.; Agrawal, M. Carbon Footprints of Agriculture Sector. In *Carbon Footprints: Case Studies from the Building, Household, and Agricultural Sectors*; Muthu, S.S., Ed.; Environmental Footprints and Eco-design of Products and Processes; Springer: Singapore, 2020; pp. 81–99, ISBN 9789811379161.
12. Rashid, S.N. Effect Of Nd:YAG Laser On Flour Beetle. *Tikrit J. Pure Sci.* **2018**, *23*, 83–89. [CrossRef]
13. Ubaid, J.M. Using Laser Energy for Controlling Some Stored Product Insects. *Al-Kufa Univ. J. Biol.* **2016**, *2016*, 37–41.
14. Rashid, S.N.; Mahdi, E.M.; Jasim, A.S. Effect of Diode Laser on Ants (Camponotus Consobrinus). *Mater. Today Proc.* **2021**, *42*, 1980–1985. [CrossRef]
15. Abdel-Kader, M.H.; El-Nozahy, A.M.; Ahmed, S.M.S.; Khalifa, I.A. Biochemical Studies of the Effect of Two Laser Radiation Wavelengths on the Khapra Beetle Trogoderma Granarium Everts (Coleoptera: Dermestidae). *AIP Conf. Proc.* **2007**, *888*, 225. [CrossRef]
16. Gaetani, R.; Lacotte, V.; Dufour, V.; Clavel, A.; Duport, G.; Gaget, K.; Calevro, F.; Da Silva, P.; Heddi, A.; Vincent, D.; et al. Sustainable Laser-Based Technology for Insect Pest Control. *Sci. Rep.* **2021**, *11*, 11068. [CrossRef]
17. Hamzah, A.K.; Al-Hamzah, F.A. Effect of Laser Radiation on the Phenotypic Mutations of Drosophila Melanogaster (Diptera:Drosophilidae). *J. Adv. Zool.* **2022**, *43*, 104–110.
18. Ibrahim, R.; Abomaaty, S.; Ammar, M. Effect of Non-Conventional Methods to Control Liriomyza Trifolii, Aphis Gossypii and Tetranychus Urticae. *Egypt. J. Agric. Res.* **2017**, *95*, 1359–1368. [CrossRef]
19. Ikeshoji, T. Mortality, Inherited Sterility and Phototactic Responses of Mosquitoes (Diptera:Culicidae) to Laser Beams of Different Spectra. *Appl. Entomol. Zool.* **1992**, *27*, 277–284. [CrossRef]
20. Marx, C.; Kiesow, T.; Hustedt, M.; Kaierle, S.; Poehling, H.; Rath, T. Application of NIR-Lasers for the Control of Aphids and Whiteflies. In Proceedings of the DGG-Proceedings, Bonn, Germany, 29 April–3 May December 2013.
21. Mullen, E.R.; Rutschman, P.; Pegram, N.; Patt, J.M.; Adamczyk, J.J. Johanson, 3ric Laser System for Identification, Tracking, and Control of Flying Insects. *Opt. Express OE* **2016**, *24*, 11828–11838. [CrossRef]
22. Turner, W.K.; Callahan, P.S.; Lee, F.L. Lack of Response of Cabbage Looper, Corn Earworm and Fall Armyworm Moths1 to 28, 118 and 337 Mm Laser Radiation. *Ann. Entomol. Soc. Am.* **1977**, *70*, 234–236. [CrossRef]
23. Ferreira, A.L.; FARIA, J.; Moura, M.C.; Zaidem, A.L.M.; Pizetta, C.S.R.; Freitas, E.; Coelho, G.R.; Silva, J.F.A.; Barrigossi, J.A.F.; Hoffmann, L.V.; et al. Whitefly-Tolerant Transgenic Common Bean (*Phaseolus vulgaris*) Line. *Front. Plant Sci.* **2022**, *13*, 984804. [CrossRef]
24. Pizetta, C.S.R.; Ribeiro, W.R.; Ferreira, A.L.; da Costa Moura, M.; Bonfim, K.; Pinheiro, P.V.; Cabral, G.B.; Aragão, F.J.L. RNA Interference-Mediated Tolerance to Whitefly (*Bemisia tabaci*) in Genetically Engineered Tomato. *Plant Cell Tiss. Organ. Cult.* **2021**, *148*, 281–291. [CrossRef]
25. Laporta, P.; Brussard, M. Design Criteria for Mode Size Optimization in Diode-Pumped Solid-State Lasers. *IEEE J. Quantum Electron.* **1991**, *27*, 2319–2326. [CrossRef]
26. Kogelnik, H.; Li, T. Laser Beams and Resonators. *Appl. Opt.* **1966**, *5*, 1550–1567. [CrossRef] [PubMed]
27. d'Errico, F.; Abegão, L.; Souza, S.O.; Chierici, A.; Lazzeri, L.; Puccini, M.; Vitolo, S.; Miyamoto, Y.; Nanto, H.; Yamamoto, T. Entrance Surface Dosimetry with Radiophotoluminescent Films. *Radiat. Meas.* **2020**, *137*, 106423. [CrossRef]
28. Dáder, B.; Gwynn-Jones, D.; Moreno, A.; Winters, A.; Fereres, A. Impact of UV-A Radiation on the Performance of Aphids and Whiteflies and on the Leaf Chemistry of Their Host Plants. *J. Photochem. Photobiol. Biol.* **2014**, *138*, 307–316. [CrossRef] [PubMed]
29. Hori, M.; Shibuya, K.; Sato, M.; Saito, Y. Lethal Effects of Short-Wavelength Visible Light on Insects. *Sci. Rep.* **2014**, *4*, 7383. [CrossRef] [PubMed]
30. Sinha, R.P.; Häder, D.-P. UV-Induced DNA Damage and Repair: A Review. *Photochem. Photobiol. Sci* **2002**, *1*, 225–236. [CrossRef]
31. Rastogi, R.P.; Richa; Kumar, A.; Tyagi, M.B.; Sinha, R.P. Molecular Mechanisms of Ultraviolet Radiation-Induced DNA Damage and Repair. *J. Nucleic Acids* **2010**, *2010*, 592980. [CrossRef]
32. Vogel, A.; Venugopalan, V. Mechanisms of Pulsed Laser Ablation of Biological Tissues. *Chem. Rev.* **2003**, *103*, 577–644. [CrossRef]
33. Taha, S.; Mohamed, W.R.; Elhemely, M.A.; El-Gendy, A.O.; Mohamed, T. Tunable Femtosecond Laser Suppresses the Proliferation of Breast Cancer in Vitro. *J. Photochem. Photobiol. Biol.* **2023**, *240*, 112665. [CrossRef]