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Manufacturing and characterization of an effective and eco-friendly nano insecticide with the aid of green alga *Chlorella vulgaris* in contrast to traditional insecticide

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ABSTRACT There is a growing demand for the manufacture of eco-friendly insecticide. This study aimed to establish an aqueous extract of *Chlorella vulgaris* as a green factory to manufacture nano-insecticide of titanium nanoparticles to control house flies (*Musca domestica*) by describing the basic properties of TiO_2 solution before and after manufacturing. The absorbance was raised to 0.58, while transmission decreased to 38 under UV-Visible spectra. Regarding to XRD analysis, seven sharp diffraction peaks appeared for a bulk solution while only three sharp peaks were noticed after phyco-based synthesis. The crystal size of the prepared titanium nanoparticles was determined to be 27.39 nm. Furthermore, the observed size for bulk particles ranged from 92.33 to 249.6 nm through SEM, while for nanocrystalline the size ranged from 9.395 to 206 nm. Various phytochemicals were detected within the algal extract, including phenols, tannins, alkaloids, flavonoids, resins, and saponins. All of these active compounds participated in nano-synthesis by acting as reducing and stabilizing agents. Finally, titanium nanoparticles were used as a controlling agent against house flies *Musca domestica*. In this study, this nanoparticles application also has been compared with traditional insecticide Imidacloprid. The high mortality percentages reached 100% against the first larval stage, 70% against the third larval stage, and 93.3% in adult flies. These mortalities were higher after using Imidacloprid for all tested stages. Many phenotypic distortions were also observed in house flies treated with TiO_2 NPs prepared by *Chlorella*, including failure in pupal emergence and maturity, incomplete development in the head, legs, and wings, and disappearance of the genital organs. The study demonstrated that *C. vulgaris* is a good candidate for nanomanufacturing and a rich naturally derived nanopesticide.

KEYWORDS Alkaloids; Chlorophytes; Dextran; Emergence; House fly; Stabilizing agents

1. Introduction

Musca domestica is the most popular flying insect in the world. Since the presence of *M. domestica* closely with domestic animals, areas of human activities like cafés, hospitals, the food industry, and marketing centers, these insects may transmit pathogens that may cause serious diseases to humans and animals. These pathogens include: eggs of helminths, cysts and Trophozoites of protozoa, viruses, bacteria, and fungi via mechanical transmission process through their secretions such as vomits. House flies can transmit pathogens through many parts within their bodies. The number of pathogens investigated in the fly gut is totally higher than the amount observed on the body surfaces. This true suggesting that the secretions of this insect (vomits and feces) may also serve as a chief pathogenic transmission route (Guerrero et al. 2014). In general, biopesticides provide a significant donation to sustainable agriculture as well as give assistance to eliminate the reliance on detrimental chemical pesticides (Abdel-Rahim and Mohamed 2013). Biopesticides are termed to biologically derived agents that are usually used for pests' management. These agents are mostly extracted from many organisms, such as fungi, bacteria, nematodes, algae, or natural products like pesticides derived from plants that are termed botanical (Guerrero et al. 2014). Biopesticides participate in many applications; thus, they are considered an important requirement to continuously discovering novel constructions (Saber et al. 2018). In the last decade, nanotechnology is a tool that has expressed numerous applications; including the Nanoparticulated development systems concerned in agro-industrial areas, such as fertilizer industry, production of pesticides, and herbicides (Paramo et al. 2020). Titanium dioxide (TiO_2) is the second metal oxide that is widely used in the production of consumer products such as food additives. Brun et al. (2014) reported that in 2006-2010, TiO_2 was produced at 5,000 tons/year and reached 10,000 tons/year in 2011-2014. Microalgae consists of diverse unicellular algae that found in the aquatic ecosystem, both fresh and sea water, as well as terrestrial ecosystems. Depending on the obtainable nature of the carbon source, these algae are classified as either heterotrophic or photoautotrophic. Thus, their cultivation is considerably non-expensive and simple (Rosales-Mendoza et al. 2020). Algae are authorized rich sources of constituents that are novel in their structure and authenticated as biologically active metabolites. Primary or secondary metabolic products produced by these organisms may exhibit potential bioactivity in the pharmaceutical industry (Usharani et al. 2015). Among the fields of nanotechnology, phyconanotechnology has been regarded as a recent nanoscience branch that depends on the production of nanoparticles based on algal-derived compounds as they are quite easy to handle and are less toxic in nature (Sharma et al. 2016). Several authors have surveyed the biological impacts of phyco-synthesized nanoparticles that are characterized by different sizes and shapes under changed conditions. It was noticed that the algal-derived bio-molecules act as a reducing and capping agent that is capable of producing stabilized nanoparticles without using any toxic compounds (Negi and Singh 2018). Nanoparticles of TiO_2 have been used as catalyst agents in various reactions, based on their benign properties, minimal toxicity, elevated stability, and significant photocatalytic and optical properties. In addition, the toxicity and cellular responses of TiO_2 NPs are determined by several parameters, such as the surface/mass ratio, surface reactivity, crystallinity, purity, solubility, adsorbed fractions, coating materials, shape and size (Shah et al. 2017). However, the intense use of TiO_2 NPs in many industrial fields poses a danger to the ecosystem (Sugeçti et al. 2021). In this study, the authors attempted to prepare a novel insecticidal agent by using phyco-synthesized titanium oxide nanoparticles using the dried biomass of green alga *Chlorella*, which was isolated from local fresh water. As it is a relatively eco-friendly, non-toxic, naturally based reducing and capping agent. In addition, these nanoparticles have been experimented with as an insecticide to manage the spread of house flies within the human surrounded environment, as compared with traditional insecticide.

2. Materials and Methods

2.1. Preparation of algal material

A fresh isolate of *Chlorella vulgaris* was gained from the laboratory of higher graduates in the biology department, Mustansiriyah University. The green alga was cultured in flasks using Chu-10 medium (HIMEDIA, USA). In an illuminated incubator (Han Yang, Korea) with about (200 μE/m²/s) light intensity and 26±2 °C. After obtaining heavy biomass, algal cells were washed three times using distilled water to get rid of the components of culture media, then harvested through centrifugation and dried in a glass plate at 55 °C for 24 h. Finally, the dried algal biomass was removed from the plates and powdered using a mortar. And used in the second step to prepare nanomaterials.

2.2. Phyco-based manufacturing of titanium dioxide nanoparticles (TiO_2NPs)

The manufacturing process was carried out according to the previous studies (Hameed et al. 2019) with several modifications. In order to prepare water extract from green alga *C. vulgaris*, a weight of (2 g) from the algal powder was mixed with 200 mL of deionized distilled water (DW). Then heated at 70 °C for 1 h. And filtered using Whatman No.1 to obtain a homogenous solution which will be use during nanoparticle preparation. The bulk of titanium dioxide was purchased from Sigma Aldrich (China). The properties of this oxide were; (79.87 g/mol), density of (4.2 g/cm^3), and average size of (550 nm). A concentration of 10 mg/mL was prepared by dissolving TiO_2 in deionized distilled water (DW), in order to the preparation of nanoparticles, a volume of (50 mL) of this solution was added drop-wise to 10 mL of algal extract solution, after that, a weight of (1 g) of dextran was added and pH adjusted up to 9. Using a magnetic stirrer at 300 rpm for 30 min duration and the mixing process was done on a hot plate at a temperature of 70 °C till the color change was observed.

2.3. Characterization of TiO_2 nanoparticles

Several analyses have been carried out for the characterization of prepared nanoparticles as explained in Hameed et al. (2019). The first one was UV–visible Spectroscopy and achieved with three samples including algal extract, titanium oxide solution, and prepared TiO_2NPs using Shimadzu 1601 spectrophotometer (Shimadzu, Japan). Another important analysis was X-Ray diffraction by using the XRD diffractometer (Shimadzu 6000, Japan) with the size and configuration of particles for both bulk and NPs were determined through this analysis. Finally, scanning electron microscope (SEM) analysis for NPs has been carried out using electron microscope AIS2300C (Oxford,India) to allow visualization of morphology and sizes of the particles.

2.4. Exploration of some active phytochemicals in algal extract

The presence of active compounds, such as (alkaloids, tannins, flavonoids, phenolic, and saponins), in this study was estimated by adopting standard protocols by Shaikh and Patil (2020).

2.5. Rearing of insects and insecticidal bioassay

The house fly, (*Musca domestica*), was chosen as an experimental model for insecticidal bioassay. The collection of their colony was done in the animal house belonging to the Biology Department, College of Science, Mustansiriyah University. The insect individuals were free from insecticides and pathogens. The colonies were kept under 28±2 °C as well as in a healthy environment. The experiment was carried out in three larval stages till it reached the adult form, via feeding with nutritional media containing (10 g of yeast extract, 200 g of fish food, and 100 mL of D.W). Two mL from each concentration (200, 400, 800 µg/mL) of experimented solutions including algal extract, TiO_2, TiO_2NPs, and Imidacloprid, was mixed with the feeding media. On the other hand, adults were sprayed with 2 mL of each applied solution. The whole experiment was carried out in cups; all cups were covered with muslin cloth and maintained at room temperature. After 24 h, the motility of insects was observed to determine their status. The percentage of mortality was calculated by applying Abbott's formula (Hameed et al. 2019). In addition, the obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6. The values were presented as the mean with standard deviation (SD).

3. Results and Discussion

3.1. Description of prepared nanoparticles

The successful performance of NPs was identified through multiple considerations. First of all, the visual color change was observed during the preparation reaction by converting the constancy from homogenous aqueous into colloidal as shown in (Figure 1). The colloidal constancy of manufactured NPs resulted from the addition of dextran into the reaction mixture. Since dextran coated the core particles, this actually increases the stabilization of prepared NPs during dispersion by steric repulsion, the content of dextran also eliminates degradation temperature (De Oliveira et al. 2019).

Regarding the UV-Visible spectra analysis, as displayed in (Figure 2), the differences between the UV-Visible spectra were detected between the three examined solutions; (algal extract, TiO_2 and TiNPs). Both TiO_2 and algal extract have got a peak of 259 nm, while TiNPs has got a peak of262 nm. On the other hand, absorbance values for the three samples were 0.213, 1.02 and 0.58 respectively. These results agree with (Bagheri et al. 2013).

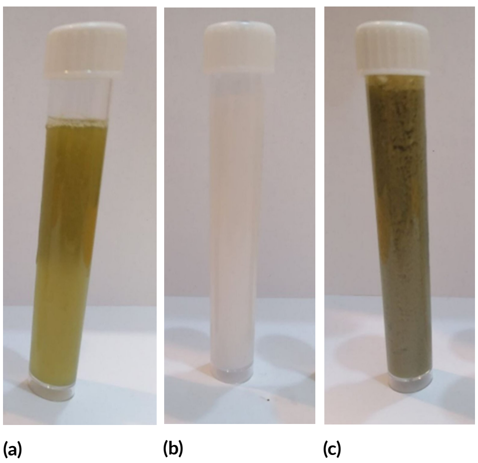


FIGURE 1 Color change and appearance of colloidal constancy during the preparation procedure, (a) water extract of *Chlorella vulgaris*, (b) TiO_2 bulk solution, (c) TiO_2 NPs.

The previous study explained the differences between UV-spectra of samples in which a decrease in crystalline size resulted in an increase in absorbance values. Furthermore, peaks become sharper. In Figure 3a and 3b, it showed that the transmission intensity for TiO_2 is decreasing in particle size of (61 at 232 nm and, the core TiO_2 solution of 38 at 310 nm. In phyco-synthesized TiO_2 NPs) the plasmon peak appearance was detected at 28 T% in 233 nm. due to the quantum size effect.

In the (Figure 4), it showed the presence of seven sharp diffraction peaks (2-theta 27.39°, 31.74°, 41.19°, 54.24°, 56.59°, 68.94°, 69.04°) in the current study. The XRD experimental pattern agrees with the JCPDS card no. 21-1272 (Shah et al. 2017). After phyco-based synthesis of TiNPs, only three strong diffraction peaks appeared (2-theta 27.39°, 41.9°, and 54.42°). Since the average crystallite size of prepared TiNPs was 27.39 nm according to Scherer's equation.

Through SEM analysis, the total morphology and sizes were observed for bulk and prepared TiNPs. As shown in (Figure 5), clusters of crystalline nanoparticles were formed using the green alga. The size observed for bulk particles ranged from 92.33 to 249.6 nm, while for nanocrystalline, the size ranged from 9.395 to 206 nm. These values confirmed the successful synthesis of TiNPs resulting in the current study (Antić et al. 2012).

3.2. Exploration of some active phytochemicals in the algal extract

The presence or absence of the active phyto-components that has been displayed in Table 1 have many active phytochemicals in the extract of *C. vulgaris*; these phytochemicals support the creation process of NPs. The enhancement activity discussed by (Sinha et al. 2015), by which these phytochemicals act as reducing and capping factors for metal oxide as well as stabilize the created nanoparticles by avoiding agglomeration. Within the cell components of *C. vulgaris*, lipids are made up of different cellular ingredients, mainly glycolipids, and hydrocarbons. Besides lipids, proteins occupy half of the dry weight within the fully grown algal cells. Furthermore, carbohydrates such as cellulose and starch exist as crucial structural components in *C. vulgaris*. All these components are suggested to play an essential role in both reduction and stabilization along with AgNPs preparation (Torabfam and Yüce 2020).

3.3. Insecticidal and distortions bioassay

As compared to other tested solutions, the phyco-based synthesized TiO_2 NPs exhibited significant insecticidal impacts on all tested larval stages as well as on adult individuals of house flies. These findings consider the best evidence of improvement of insecticidal impact of *Chlorella aqueous* extract through the use of it as an agent to bottom down the bulk of TiO_2 into nanoscale particles. Also, the TiO_2 NPs solution exhibited the ability to overcome the effect of traditional insecticide (imidacloprid). The most susceptible stage was in first stage; with 100% and 96.6% for 200 µg/mL prepared TiO2 NPs and Imidacloprid, re-

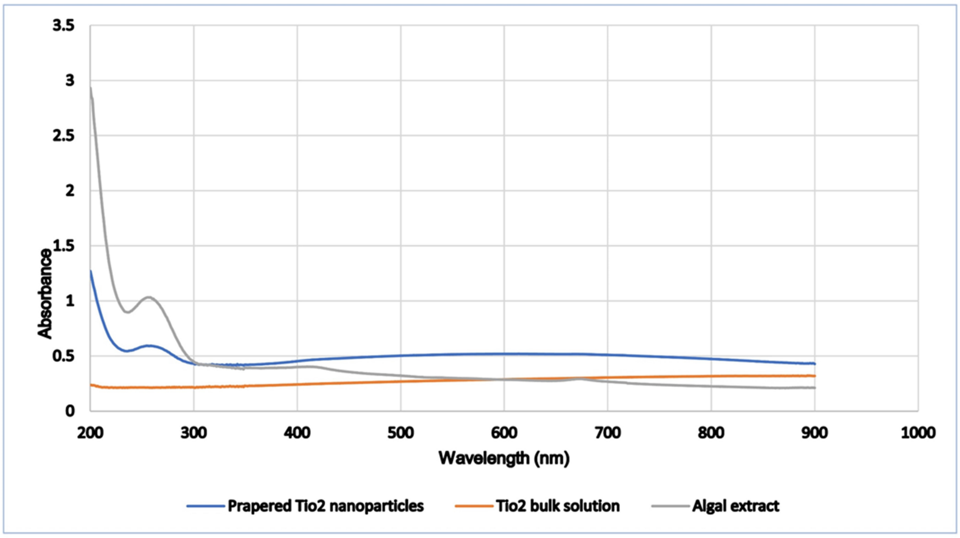


FIGURE 2 Absorbance spectra of the solutions (water extract of *Chlorella vulgaris*, TiO_2 bulk, manufactured TiO_2 NPs) under spectrum from 200 to 900 nm.

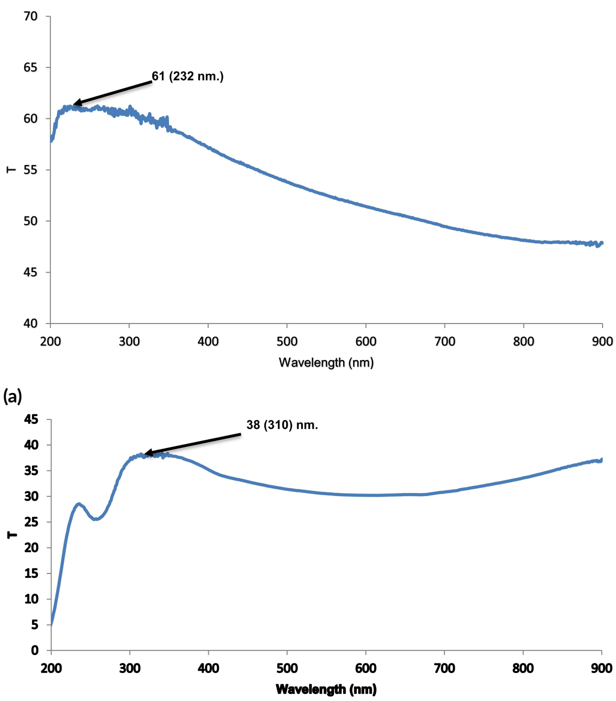


FIGURE 3 Transmission spectrum of A: TiO_2 bulk solution, B: manufactured TiO_2 NPs using *Chlorella vulgaris*. Under spectrum from 200 to 900 nm.

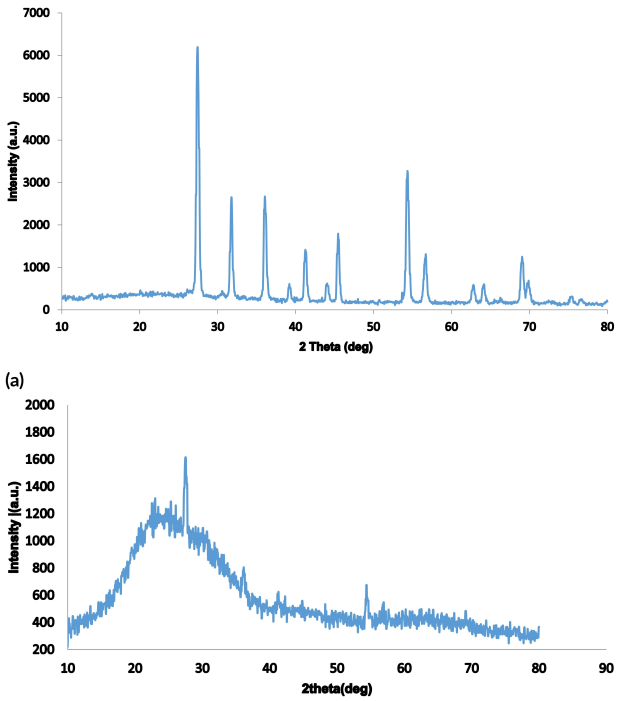


FIGURE 4 Shows the X-Ray Diffractometer pattern of A: TiO_2 bulk solution, B: manufactured TiO_2NPs using *Chlorella vulgaris*.

spectively, while in third stage, 70% and 63.3% for 200 µg/ml prepared TiO_2 NPs and imidacloprid, respectively. For instance, in adults, 93.3% mortality has gotten from 200 µg/ml prepared TiO2 NPs as compared to 53.3% mortality after adults feed with Imidacloprid. Furthermore, no

TABLE 1 Presence or absence of active compounds in *Chlorella vulgaris*.

Chemical compound	Phenols	Tannins	Alkaloids	Flavonoids	Resins	Saponins
Presence	+	+(condensed)	+	+	+	+

Note: +: presence

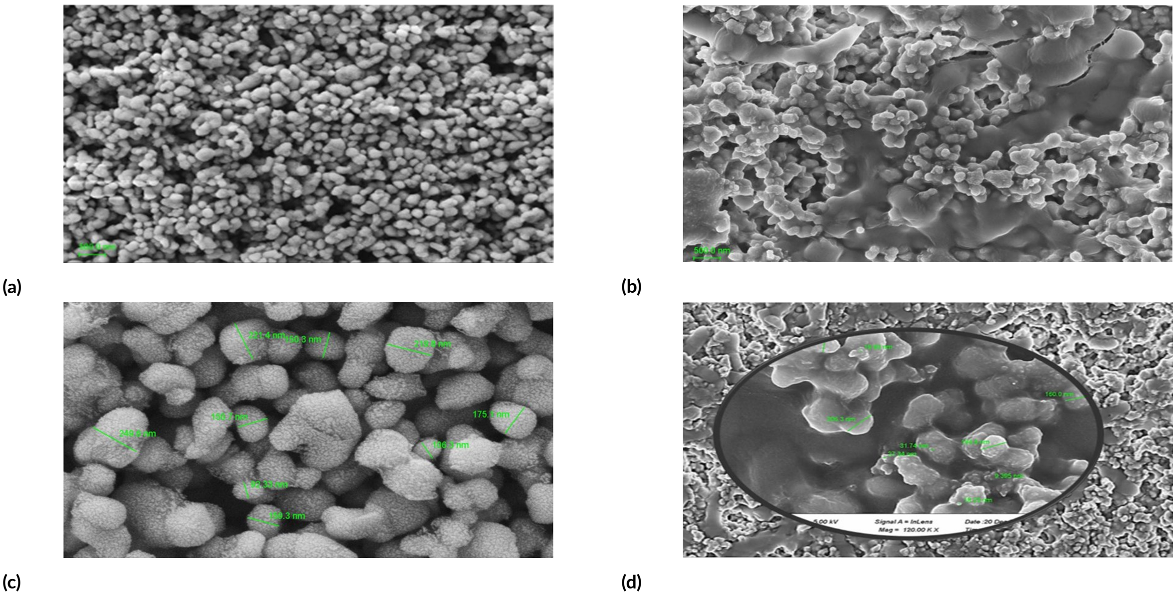


FIGURE 5 SEM images of bulk and manufactured TiO2 NPs: a, b: for bulk and manufactured TiO_2 NPs under 30.00 K × magnification respectively, the scale bar 1 µm.; c, d: for bulk and manufactured TiO_2 NPs under 120.00 K × magnification respectively. The scale bar is 100 µm.

difference in mortality percentages has been observed after feeding of the second larval stage with both previous solutions at 73.3%. In addition, a significant difference was statistically detected between the applied solutions with their different concentrations on the larvae and the adults. However, no significance different was detected for the concentrations of prepared TiO2 NPs in adults and first larval stage (Figure 6). This confirms the high impact of

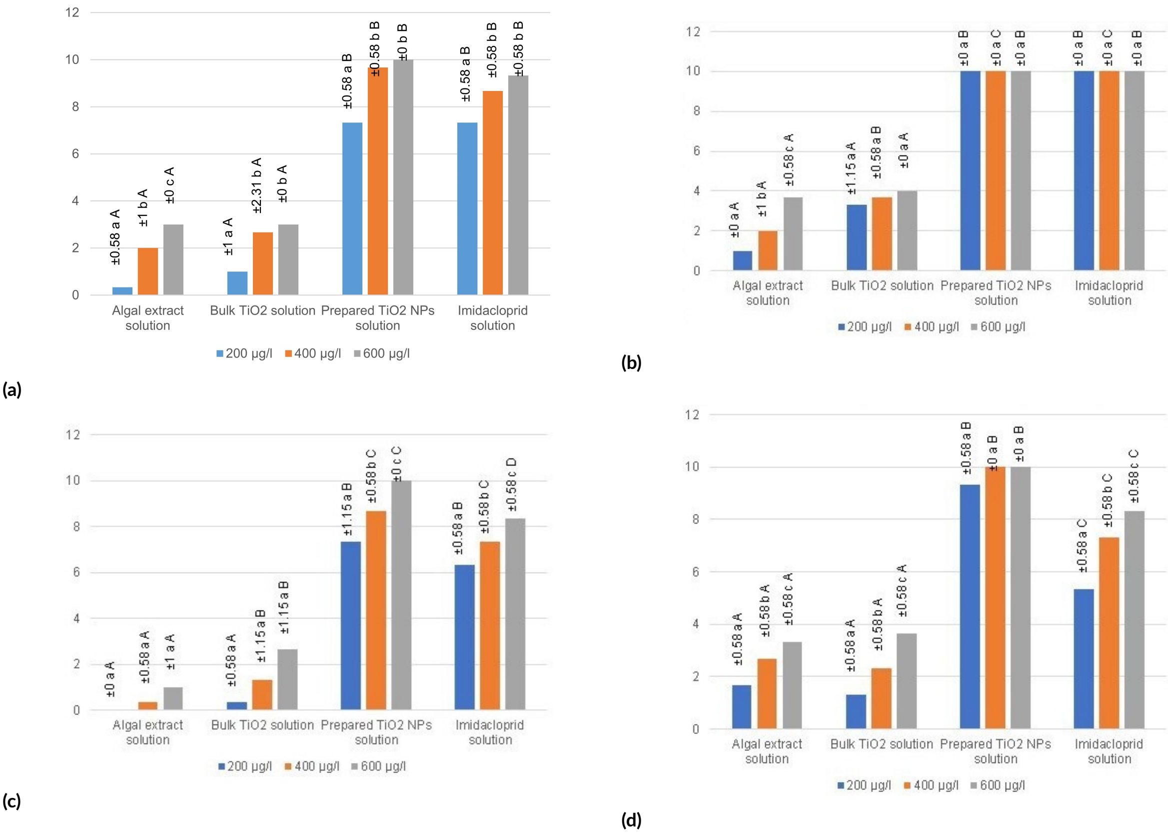
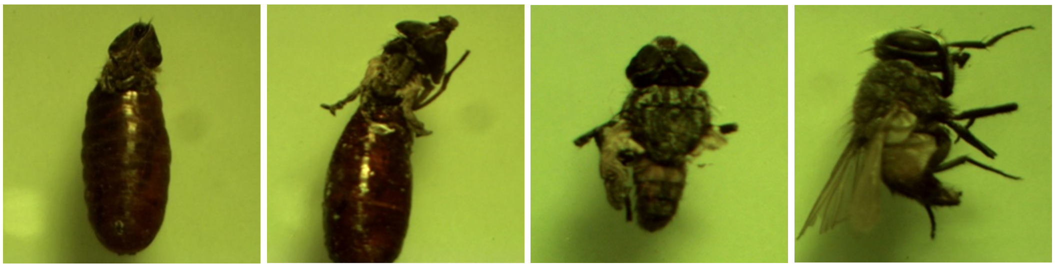


FIGURE 6 Mean and SD of the dead individuals of house fly calculated after treating with different concentrations of examined solutions: a.1st larval stage; b.2nd larval stage; c.3rd larval stage and d. adults.



(a) (b) (c) (d)

FIGURE 7 Phenotypic distortions detected in house fly treated with TiO_2 NPs manufactured by *Chlorella*.: a. Complete failure in emergence; b. Partial failure in the maturity of pupal into adults, in complete development in both head and wings; c. Malformed adult, disappearance in genital organs, and d. Distortion in legs, wings fragility.

the prepared NPs as an insecticide.

In the current study, different phenotypic malformations were observed after exposure of the second and forth larval instars and adults to TiO_2 NPs produced from *Chlorella*. All these abnormalities are displayed in (Figure 7) with their descriptions.

In general, the insecticidal activity of nanomaterials has been examined using the oral route via mixing these materials with certain concentrations of food for the insects. The larvae feed on treated food after hatching from the egg. Once the food passes through the larval gut, nanoparticle interference has occurred, leading to the appearance of unusual phenotypes ending with neuronal defects and then larval death (Sabat et al. 2016). One of the probable mechanisms behind the fatality of house flies has been explained by (Hameed et al. 2019). The latter study suggested that when metal nanoparticles have dispersed orally or even through rupturing of the cuticle membrane, by these routes the Ti NPs reached the cavity of the insect. This leads to generating the particles of Reactive Oxygen Species (ROS). Due to the toxic tension of ROS, egg damage happens before proceeding to the next larval stage.

There are many biochemical parameters that were eliminated after insect exposure to biogenerated TiO_2 NPs. These parameters include (elimination in levels of total lipids and proteins, a decrease in some enzymes such as; lactate dehydrogenase, alkaline phosphatase, and acid phosphatase). These events lead to decrease in growth and development, and finally death (Nadeem et al. 2018). In their diets. TiO_2 NPs caused dose-dependent cellular damage in the *Gellaria mellonella* larvae hemolymph and maximized the levels of the non-enzymatic uric acid and bilirubin antioxidants. Furthermore, the total protein content in hemolymph remarkably decreased at the highest concentration of TiO_2 NPs. Whereas the glucose amounts were not affected. These findings established that TiO_2 NPs caused dose-dependent toxic effects on *G. mellonella* larvae (Sugeçti et al. 2021). Further, studies demonstrated that when the second and fourth instar larvae of *S. littoralis* were exposed to TiO_2 nanoparticles, several biological aspects were affected, such as larval period, pupation, the emergence of adults, fertility rate, longevity, and hatching of the egg, and also caused malformations in all insect stages (Shaker et al. 2017). At concentrations related to oral exposure of humans, TiO_2 nanoparticles exhibited low insecticidal activity on the fruit fly, *Drosophila melanogaster*, but significantly increased the period needed for pupation. In addition, the gene expression for catalase was significantly downregulated after treatment (Gutiérrez-Ramírez et al. 2021).

4. Conclusions

Increased demand is needed to improve eco-friendly, cheap, nontoxic approaches to get NPs that are suitable for getting an environmental application, especially insect control. In this study, coordination was achieved between a local algal isolate and a titanium oxide solution. This coordination results in the formation of nanoparticles with accepted properties. This nano-solution was approved as an active controlling agent against house flies with lower concentrations as compared with applied traditional chemical insecticide. Further studies should be suggested in the future to examine the efficiency of the prepared titanium nanoparticles against other insects and focus on those transferring serious human pathogens as well as those attacking agricultural crops.

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Authors' contributions

RSHA, RJF, RSN, and MAAS proposed the idea of this study. All authors contributed equally in designing the experimental work, making the measurements, analyzing and interpreting the data, and writing the manuscript. RJF performed the calculations and statistical analysis. All authors participated in the revision of the manuscript.

Competing interests

The authors declare no conflict of interest.

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