Biological synthesis of Titanium Dioxide nanoparticles by *Curcuma longa* plant extract and study its biological properties

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**ABSTRACT**

The objective of this study was biosynthesis of titanium dioxide nanoparticles (TiO\(_2\) NPs) using *Curcuma longa* aqueous extract and characterize them. Study their effect on the growth, sporulation, pathogenicity of Fusarium graminearum and some wheat plants parameters compared with standards industrials nanoparticles. *C. longa* aquatic extract was used to biosynthesis TiO\(_2\) NPs by two methods. At first method, TiO\(_2\) was found in both colloidal solutions (CS) and nanopawder while it found in jest nanopawder in second method. All biosynthetic nanoparticles were in nano size. It was: 91.37 nm, 76.36 nm of (CS) and nanopawder for first methods respectively while it was 92.6 nm of nanopawder in second method. All nanoparticles have good optical properties. Crystal’s shape of nanopawder were in three form: anatase, rutilr and brookite and it was anatase in colloidal solutionat first method while it was pure anatase innanopawder when at second method. The average crystallite size of was calculate by Scherer's equation, it was 43.088 nm and 22.881 nm for nanopawder and colloidal solution respectively at first method. It was 45.808 nm for nanopawder at second method. All concentrations of nanoparticles were reduced fungal and spores. These decreasing were more effective using biosynthetic compare with industrial synthetic nanoparticles. There were reductions in damping-off caused by *F. graminearum* by biosynthetic NBs in both varieties of plant (Al-Rasheed and Tamuze-2). These was better than the effect of industrial synthetic nanoparticles. The resistance to damping off and the growth of plant in Al-Rasheed variety was more sensitive compared with Tamuze-2 variety especially at higher concentrations. There were decrease in all plant's parameters at most concentrations of TiO\(_2\) biological synthetic compare with industrial synthetic nanoparticles in Al-Rasheed variety, while there were inductions in some plant's parameters by biosynthetic nanoparticles.
compared with industrial synthetic in Tamuze-2 variety. Finally, *C. longa* can be used to biosynthesis TiO$_2$ NPs with good biological properties.

**Keyword:** biosynthesis; *Curcuma longa*; damping-off; nanoparticles; plant; TiO$_2$

1. INTRODUCTION

Nanotechnology is the term used to cover the design, construction and utilization of functional structures with at least one characteristic dimension measured in nanometers, (1). Such materials can be designed to exhibit novel and significantly improved physical, chemical and biological properties, phenomena and processes as a result of the limited size of their constituent particles or molecules, (1). Generally metal nanoparticles can be prepared and stabilized by physical, chemical and biological methods with little modifications for different metals, (2), (3), (4), (5), (6). The properties of nanoparticles and its applications are different and dependent on their size, distribution and morphology, (7), or even on synthesis methods. The main disadvantages of physical methods are the quality of the product, which is less as compared to nanoparticles produced by chemical methods. Usually these methods require costly vacuum systems or equipment to prepare nanoparticles,(8).

Increasing sensibility towards green chemistry and biological processes has led to develop an environment-friendly process for the synthesis of non-toxic nanoparticles. A vast array of biological resources available in nature including living plants, (9), plant products, plant crude extracts, algae, fungi, yeast, (10), bacteria, (11) and viruses could all be employed for synthesis of nanoparticles. Biological methods are regarded as safe, cost-effective, biocompatible, non-toxic sustainable and environment friendly processes, (12). In addition, most bioprocesses occur under normal air pressure and temperature, resulting in vast energy savings, high-yield and low cost, (13).

Recent reports of plants towards production of nanoparticles is said to have advantages such as easily available, safe to handle and broad range of biomolecules which mediate synthesis of nanoparticles (Salam *et al.*, 2012) (4). Some mineral nanoparticles have been synthesized inside live specimens of the plants *Brassica juncea*, *Medicago sativa* and *Heliannus annus*, (9). Ag-NPs were successfully synthesized under room temperature at different volumes of C. longa extract with an average size of 10.46, 8.18, and 4.90 nm for 5, 10, and 20 mL of aqueous C. longa tuber powder extract, with spherical-shaped structures (14). Other plant mediated process to developed its extract for phytosynthesis of titanium dioxide nanoparticles: *Nyctanthes*, *Annona squamosapeel* extract, (15), *Catharanthus roseus* leaf aqueous extract (16) and *Ecliptaprostrata* (17), *Azadirachta indica* (18).

TiO$_2$ nanoparticles become a new generation of advanced materials due to their brilliant and interesting optical, dielectric, and photo-catalytic characteristics from size quantization. It is one of the most widely used nanostructures in various Areas, (19). Some researches were focused on its effect on bacteria, algae, plankton, fish, mice, and rats, (20), (21) but its effect on fungi or plants' growth was unclear. Some studies suggest that interactions of TiO$_2$ NPs with other chemicals or physical factors may result in an increase in toxicity or adverse effects, (22), such as photo-catalytic activity of titanium dioxide nanoparticles that prevents the fungal colonization of wood samples over long time when compared to untreated ones, (23). TiO$_2$ nanoparticles have some effect on plant growth.
The results of (24) approve that TiO$_2$ nanoparticles (NPs) (50 nm of anatase shape) were increased promoter indicator of Latyfia variety of wheat but it has negative effect on germination percentage, germination rate and Shoot and root length. Additionally, exposed wheatgrass seeds to high concentrations of nano TiO$_2$ particles (80 ppm) led to diminished germination rate, (25). In the same time, (26) showed that the use of TiO$_2$ NPs on the germination of wheat at the proper and lower concentrations have additive effect and in high-concentration have reduction effect.

The objective of this study was biosynthesis of TiO$_2$ nanoparticles by *C. longa* plant extract and characterized the products Moreover, we study its biological properties and if it is different compared with the same size of industrial synthesis nanoparticles including their effect on fungal growth and germination of two varieties of wheat (*T. aestivum*).

2. MATERIALS AND METHODS

2.1. Biological synthesis of Titanium Dioxide nanoparticles by *C. longa* plant extract

- **Preparation**

  **Plant extraction:** Fifteen gram of powdered material of *C. longa* was extracted by a Soxhlet extractor with 300 ml of distal water at 40 °C for 3-4 h. The extract was filtered by Whatman number 4 filter paper. The residue was removed. The filtrate was used for biological synthesis of nanoparticles directly as soon as possible.

  **Titanium Dioxide bulk particles (TiO$_2$ BPs):** It procured from Sigma Aldrich, China. Its molar mass, density and size were: 79.87 g/mol, 4.2 g/cm$^3$ and (400-800 nm), respectively. Distil water was used to prepare solutions with different concentrations.

- **Synthesis:** This was done by two methods. In first method, 50 ml of filtrate was mixed with 2.5 ml of titanium dioxide bulk particles (50 mg/ml), in flask. So, the final concentrations of it was 2.38 mg/ml. It placed in magnetic steer hot plate with 50 °C and 1000 rpm /second for 5 hr. The same above method had done in second method but the filtrate was mixed with 5 ml of TiO$_2$ bulk particles (TiO$_2$ BPs), (50 mg/ml). The final concentrations of it was 4.55 mg/ml. The period time was 8 hr. The solutions allowed to cool at room temperature. The solution was repeated centrifugation at 15,000 rpm for 10 min. The supernatant (colloidal solutions) kept for Characterization. The precipitate formed was washed with double distilled water and then centrifuged at 1500 rpm for 10 min. This was repeated three time. The obtained precipitate (nanopawder) was dried at room temperature for 24 h. and characterized as described falling.

**Characterization**

The exact configuration of the fabricated particles, phase purity, structure, average particle size, morphology of crystals and distribution were measured using the falling technique. Standard industrials NPs were, also, analyzed.

- **Atomic force microscopy (AFM):** size, surface topography and granularity volume distribution of biosynthesized nanoparticles characterized using Atomic Absorption Spectroscopy (AA-680, Shimadzu-Japan), (characterized by Dr. Abdul Kareem Al-Samarai Lab. Baghdad/Iraq,(27).
UV–visible analysis: nanoparticles were recorded by a Schimadzu 1601 spectrophotometer in 200-800 nm range operated at a resolution of 1 nm (28). The absorption coefficient (α) calculated from the optical spectrum using the formula, \( \alpha = 2.3026 \frac{A}{t} \), where: (A) and (t) are the measured absorbance and thickness of the sample, respectively. The optical band gap energy (Eg) evaluated from the absorption spectrum, and the optical absorption coefficient (α) near the absorption edge gave according to (31):

\[
\alpha h\nu = B(h\nu-E_g)^n
\]

where: h, ν, B, and Eg are Planck's constant, frequency of incident photons, constant, and optical bandgap energy respectively. Energy gap of TiO\(_2\) NPs (Eg) was estimated by plotting hv versus \((\alpha h\nu)^{1/2}\) according Tauc plot, (32).

X-ray diffraction. X-ray diffraction (XRD) was used to confirm the crystal structure (crystal phases and crystallite size of each Phase) of TiO\(_2\) nanoparticles. XRD analysis was performed using an X-ray diffractometer with Cu-K\(\alpha\) crystal radiation (\(\lambda = 1.54 \text{ Å}\)) scanning at a rate of \((5^\circ/\text{min})\) for \((2\theta)\) range of \((20^\circ-70^\circ)\). The diffraction peaks were identified by comparison with (JCPDS-84-1286), (JCPDS 21-1272), according 2θ. The full width at half maximum (FWHM) in the XRD was used to determine the crystallite size using Scherer's equation (33).

The strain value \(\eta\) and the dislocation density \(\delta\) value can be evaluated by using the relations in the following equations:

\[
\eta = \frac{\beta \cos \theta}{4} (34) \quad \text{and} \quad \delta = \frac{1}{G_s^2} (35)
\]

SEM analysis: TiO\(_2\) biosynthetic nanoparticles were analysis by scanning electron microscope (SEM), Vega Tescan (USA), in the Center of Nanotechnology and Advanced Materials/ University of Technology/ Iraq. Biological synthesis nano-powder (which synthesis according above first method) used to compare its effect, in all following experiments, with the effect of industrial synthetic NPs, which procured from Sigma Aldrich (USA). These nanoparticles were white color, anatase shape with size 50 nanometer, assay 99.0%. Sterilized distilled water was used to prepare different concentrations of industrial and biological synthesis NPs.

Antifungal and anti-pathogenic activity of nanoparticles

A pure isolate of F. graminearum fungi were get from Department of Biology, College of Science, AL-Mustainsiriyah University, Baghdad, Iraq. The antifungal activity of all nanoparticles were evaluated against F. graminearum. PDA medium prepared with different concentrations of nanoparticles. There was negative control of distill water in all experiment. All experiment has run in three replicate. Petri dishes were inoculated in the center with 4 mm of fungal plugs. Incubated at 28 ±2 °C for 8-10 days. The radials growth of the colonies measured. The percentage of inhibition of mycelial growth was calculated. The spore
suspending (10 ml of sterile distilled water per petri old dishes), was collected, centrifuged then calculated by hemocytometer. The percentage of spores’ inhibition rate was calculated.

**Anti-pathogenic activity:** two variety of dry wheats’ seeds (*T. aestivum*) (Al Rasheed variety and Tamuze-2 variety) were taken from Ministry of Science and Technology-Seed Technology Center. Germination percentage of them were 100% for both varieties. Mycelium agar plug technique was used for pathogenicity test, (36). Seeds immersed in 1% sodium hypochlorite solution for three minute then washed with sterilized distilled water three times. Washed seeds were soaked with various concentrations of each of industrial and biological synthetic nanoparticles for 72 hours. Five seeds per petri dishes, were inoculated with (0.8 cm) diameter of old cultures mycelium on the center of petri dishes. Untreated seeds exposed to fungi and untreated seeds without exposed to fungi were used as control negative and positive respectively. Five replicate were get for each treatment. Each replicate contains ten seeds. The seeds were germinated in 28 °C for ten days. The symptoms were: seedlings fail to emerge (pre-emergence damping off) or seedlings collapse, submerged in a mass of whitish fungal growth. The number of dead seeds or dead seedling were determined after seven days to calculate total percentage of damping off as followings: 

\[
\text{Damping-off \%} = \left(\frac{S - s}{S}\right) \times 100
\]

Where is: 
S = average of germinated seed in control plates, 
s = average of germinated seed in plates treated with NPs.

### 2. 2. The effect of nanoparticles on germination parameters of wheat.

The seeds of two variety of wheat (*T. aestivum*) (Al Rasheed variety and Tamuze-2 variety) were soaked in different concentrations of nanoparticles suspensions for 72 hours. There was negative control (distill water) for all experiment. All seeds were incubating at (27 ±1 °C, 12 h. light: 12 h. dark). All experiment has run in three replicate. The number of new germinated seeds was recorded daily. A seed was considered germinated when the radicle showed at least 2 mm in length. The following parameters were calculating: germination percentage, (37); germination rate, (38) and mean germination time, (37).

### 3. RESULTS

#### 3. 1. Biological synthesis of TiO₂ NPs by *C. longa* plant extract

**First method:** In the first method, TiO₂ was found in both supernatant and precipitate. We called them colloidal solutions and nanopowder respectively. Size range were (80 - 110) and (50 - 110) nm with average diameter: 91.37 nm, 76.36 nm of colloidal solutions and in nanopowder (precipitate) respectively, (Figure 4). Root mean square (RMS) and roughness average (RA) of colloidal were: 0.872 nm and 0.745 nm. While root mean square (RMS) and roughness average (RA) of nanopowder were: 0.773 nm and 0.674 nm, respectively. Figure (1) showed AFM topographic images of biosynthesis TiO₂ nanoparticles by first method.

The XRD pattern of this biosynthesis nanopowder showed the presence of seven peaks. Strong diffraction peaks were: (25.3201° (101), 48.0516° (200) and 37.818° (004) indicating that nanoparticles structure of these peaks were anatase crystalline. Other peaks were: 27.5° (110) and 54.4° (211) corresponded to anatase form and one brookite form (crystallographic plane = 121) with 2 theta = 30.8°. The average crystallite size of TiO₂ nanoparticles was calculate by Scherer’s equation, it was 43.088 nm. The XRD patterns of biosynthesis colloidal
solutions shown in Figure (3) indicated that it was in the form of anatase TiO$_2$. Optimum peaks at 25.37° corresponded to the 101 planes of anatase form. Table (1) showed a summary of X-ray characterization of TiO$_2$ biosynthesis nanoparticles. Scherer's equation was used to calculate the average crystallite size of TiO$_2$ nanoparticles. It was 22.881 nm. The XRD patterns of pure stander industrial synthetic nanoparticles were shown in Figure (3). The crystal shape of them was pure anatase form. Strong diffraction peaks were: 25.3425°, 48.0686°, 37.8455°, 53.86°, 62.78°, 68.78° and 75.2° corresponded to: (101), (200), (004), (111), (002) (116) and (215) crystallographic planes. The average crystallite size of TiO2 nanoparticles (according to Scherer's equation) was 16.473 nm. Figure (4) showed SEM image of TiO$_2$ biosynthesis nanoparticles (nanopawder) using first method. Particle size (>50 nm).

**Fig. 1.** (A) Granularity volume distribution chart of TiO$_2$ NPs synthesis by *C. longa* plant extract using first method. (B) and (C): AFM topographic images of colloidal solutions and nanopawder respectively.
Industrial synthetic NPs

Biosynthesis NPs (nanopowder)
Fig. 3. X-ray pattern of TiO$_2$ biosynthesis NPs (nanopowder) using first method. Identified phases of anatase (A), rutile (R) and brookite (B).

Table 1. Summary of X-ray characterization of TiO$_2$ biosynthesis NPs (nanopowder and colloidal solutions) compared with industrial synthetic NPs.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Sample</th>
<th>(hkℓ) planes</th>
<th>Crystal shape</th>
<th>2 theta (deg)</th>
<th>FWHM (deg)</th>
<th>D (nm)</th>
<th>$\eta \times 10^{-4}$ (lines$^2$mm$^{-4}$)</th>
<th>$\delta \times 10^{14}$ (lines/m$^2$)</th>
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<tbody>
<tr>
<td>First biosynthesis method</td>
<td>nanopowder</td>
<td>101 anatase</td>
<td>25.320</td>
<td>0.198</td>
<td>40.926</td>
<td>33.866</td>
<td>5.971</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>200 anatase</td>
<td>48.052</td>
<td>0.175</td>
<td>49.474</td>
<td>28.015</td>
<td>4.086</td>
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<tr>
<td></td>
<td></td>
<td>004 anatase</td>
<td>37.818</td>
<td>0.215</td>
<td>38.864</td>
<td>35.663</td>
<td>6.6207</td>
<td></td>
</tr>
<tr>
<td></td>
<td>colloidal</td>
<td>101 anatase</td>
<td>25.37</td>
<td>0.354</td>
<td>22.881</td>
<td>60.574</td>
<td>19.1</td>
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<td>second biosynthesis method</td>
<td>nanopowder</td>
<td>101 Anatase</td>
<td>25.330</td>
<td>0.179</td>
<td>45.222</td>
<td>30.649</td>
<td>4.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 Anatase</td>
<td>48.075</td>
<td>0.168</td>
<td>51.602</td>
<td>26.86</td>
<td>3.756</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>004 Anatase</td>
<td>37.814</td>
<td>0.206</td>
<td>40.601</td>
<td>34.137</td>
<td>6.066</td>
<td></td>
</tr>
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<td>industrial synthetic</td>
<td>nanopowder</td>
<td>101 anatase</td>
<td>25.343</td>
<td>0.4957</td>
<td>16.34</td>
<td>84.825</td>
<td>37.456</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 anatase</td>
<td>48.067</td>
<td>0.529</td>
<td>16.368</td>
<td>84.678</td>
<td>37.327</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>004 anatase</td>
<td>37.846</td>
<td>0.5</td>
<td>16.713</td>
<td>82.93</td>
<td>35.801</td>
<td></td>
</tr>
</tbody>
</table>

(hkℓ) planes: crystallographic plane; FWHM: Full width at half maximum; D: dimension of crystal in nm; $\eta \times 10^{-4}$: strain value; $\delta \times 10^{14}$: dislocation density.
**Fig. 4.** SEM image of TiO$_2$ biosynthesis NPs (nanopowder) using first method. Particle size (>50 nm).

**Second method:** TiO$_2$ was found in precipitate only as nanopowder and absent in supernatant (colloidal solution). Size of nanoparticles were range between (10 - 140) nm with average diameter 92.6 nm of nanopowder. The percentage volume of particles with size 30 nm was 29.145. Figure 5 (B), showed AFM topographic images of TiO$_2$ nanoparticles synthesis by *C. longa* plant extract (supernatant) using second method.
Fig. 5. (A) Granularity volume distribution chart of TiO$_2$ NPs powder synthesis by C. longa plant extract using second method. (B): AFM topographic images.

The XRD patterns shown in Figure (7) indicated that the crystal shape of biosynthesis nanopowder was pure anatase form. Optimum peaks were: 25.3302°, 48.0745 °, 37.8144°, 53.86° and 62.82° corresponded to: (101), (200), (004), (105) and (002) crystallographic planes, Figure (7). Table (1) showed a summary of X-ray characterization of TiO$_2$ biosynthesis nanoparticles. The average crystallite size of TiO$_2$ nanoparticles was calculated by Scherer's equation, it was 45.808 nm.

Fig. 7. X-ray pattern of TiO$_2$ biosynthesis NPs (nanopowder) using second method. Identified phases is pure anatase (A).
SEM analysis: Figure (8) showed SEM image of TiO$_2$ biosynthesis nanoparticles (nanopowder) using second method. Particle size (<90 nm).

**Fig. 8.** SEM image of TiO$_2$ biosynthesis nanoparticles (nanopowder) using first method. Particle size (<90 nm).

There are some advantages of using plants for the synthesis of nanoparticle such as: they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction. A number of plants are being currently investigated for their role in the synthesis of nanoparticle, (39). According to (40) plant extracts are believed to act as reducing and stabilizing agents in the nanoparticle synthesis. The nature of plant extract affects the kind of nanoparticles synthesized in a highly critical manner with the source of plant extract being the most vital factor and different concentrations of biochemical reducing agents affecting the morphology of synthesized nanoparticles, (41).

In current study *C. longa* successful to synthesis pure anatase nano-size TiO$_2$ NPs. This ability of this plant may be due to presence of terpenoids, flavonoids and proteins was considered to be responsible for the formation and stabilization of titanium nanoparticles, (42). The different concentrations of *C. longa* plant extract may be responsible for different sizes nanoparticles that synthesis in current result. This result similar to (14) who reported that Ag-NPs were synthesized with an average size of $10.46 \pm 5.58$, $8.18 \pm 3.53$, and $4.90 \pm 1.42$ nm for 5, 10, and 20 mL of different concentrations of plant aqueous extract, with spherical-shaped structures. There are no reports about biosynthesis of TiO$_2$ NPs by *C. longa* but there were some reports of other plants which able to synthesis TiO$_2$ NPs, for example: Nyctanthes success to synthesis titanium dioxide nanoparticles with size of nanoparticles ranging from 100 to 150nm, (43). TiO$_2$ NPs were synthesized from Annona squamosapeel extract (Roopan et al., 2012) (16), *Catharanthus roseus* leaf aqueous extract (16), *Ecliptaprostrata* extract (44),
and Azadirachta indica extract.(45). (42) reported that TiO$_2$ NPs biologically synthesis using leaf extract of Azadirachta indica, with mixture of rutile and anatase phases, size ranged from 15 to 42 nm. (46) reported that Euphorbia prostrata extract can synthesis TiO$_2$ NPs with spherical shape and an average size 83.22 nm. (47) successes to biosynthesis of tetragonal TiO$_2$ NPs with (32 nm) average in particles size from Aloe vera extract.

3. 2. Antifungal and anti-pathogenic activity of nanoparticles

Table (2) showed that the highest fungal and spore Inhibition rate found in (200 mg/ml) of industrial synthetic nanoparticles, while the lowest fungal Inhibition rate found in (20, 0.2) mg/ml. The highest fungal and spore Inhibition rate were found at (20 mg/ml) of biological synthetic nanoparticles and the lowest of them were found at (0.2 mg/ml). There were decrease in fungal and spore Inhibition rate at all concentrations of biological synthetic compare with industrial synthetic nanoparticles except (20 mg/ml) concentration, it has the same spores’ Inhibition rate in each treatment (40.816 %). In both varieties, Rasheed and Tamuze-2, there were decrease in percentage of damping-off at all concentrations of biological synthetic compare with industrial synthetic nanoparticles. The response to nanoparticles of Al-Rasheed variety was more sensitive to resistance damping off compared with Tamuze-2 variety.

**Table 2.** Comparison between the effect of industrial synthetic NPs and biological synthetic NPs on Inhibition rate, Spores inhibition rate and pathogenicity of F. graminearum

<table>
<thead>
<tr>
<th>Con. (mg/ml)</th>
<th>% F.IR IS</th>
<th>% S.IR BS</th>
<th>% F.IR IS</th>
<th>% S.IR BS</th>
<th>Al-Rasheed variety IS BS</th>
<th>Tamuze-2 variety IS BS</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>39.583</td>
<td>53.061</td>
<td>29.166</td>
<td>34.693</td>
<td>50 33.333 50 40</td>
<td>50 33.333 50 40</td>
</tr>
<tr>
<td>20</td>
<td>12.5</td>
<td>40.816</td>
<td>31.25</td>
<td>40.816</td>
<td>46.667 40 56.667 46.667</td>
<td>46.667 40 56.667 46.667</td>
</tr>
<tr>
<td>2</td>
<td>14.583</td>
<td>46.938</td>
<td>12.5</td>
<td>20.408</td>
<td>53.333 46.667 56.667 50</td>
<td>53.333 46.667 56.667 50</td>
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<tr>
<td>0.2</td>
<td>12.5</td>
<td>40.816</td>
<td>8.333</td>
<td>6.122</td>
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<td>53.333 56.667 53.333 63.333</td>
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<tr>
<td>CT-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100 100 100 100</td>
<td>100 100 100 100</td>
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<tr>
<td>CT-2</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>0 96.667 0 96.667</td>
<td>0 96.667 0 96.667</td>
</tr>
</tbody>
</table>

Con.: concentrations; F.IR: fungal inhibition rate; S.IR: spores’ inhibition rate; Path.: pathogenicity; IS: industrial synthetic NPs; BS: biological synthetic NPs; CT-: untreated fungi or untreated seeds exposed to fungi; CT-2: untreated seeds without exposed to fungi.

3. 3. Effect of nanoparticles on germination parameters of T. aestivum

In Al-Rasheed variety, there were decrease in all plant's parameters at all concentrations of biological synthetic NPs compare with industrial synthetic NPs except (20-2 mg/ml)
concentrations. These concentrations of biological synthetic were induction mean germination time compare with industrial synthetic. In compared with control, there were:

a. **Germination percentages**: There were reduction in seed germination using different concentrations of industrial and biosynthetic nanoparticles.

b. **Germination rate**: The higher concentration of industrial synthesis nanoparticles reduced these parameters significantly. The same reduction found using (2-200) mg/ml of biosynthesis nanoparticles, (P < 0.05). All other treatments were not affected.

c. **Mean germination time**: it was not affected by all concentrations of industrial or biosynthesis nanoparticles, (P < 0.05), except the induction in these parameter at 2 mg/ml of biosynthesis nanoparticles, significantly. All other treatments were not affected.

d. **Mean daily germination**: each concentrations of: (20-200) mg/ml of industrial synthesis nanoparticles and (2-200) mg/ml of biosynthesis nanoparticles were reduced MDG.

e. **Germination value**: the reduction in these parameters were significance at all treatment of biosynthesis nanoparticles while these significant reduction was found at (20-200) mg/ml concentrations of industrial synthesis.

f. **Promoter Indicator**: the reduction in these parameters were significance at all treatment of industrial synthesis while these significant reduction was found only at higher concentrations, (Table 3).

In Tamuze-2 variety: the comparison between the effect of industrial and biosynthetic nanoparticles were different in action. The decreasing was found in different concentrations of only promoter indicator. While there were inductions by biosynthetic nanoparticles compared with industrial synthetic in: germination percentage (2-20) mg/ml, germination rate (2-200) mg/ml, mean germination time (all concentrations), mean daily germination (2-20) mg/ml and germination value (2-20) mg/ml. All other concentrations were reduced above parameters.

a. **Germination percentages**: There were reduction in seed germination using different concentrations of industrial and biosynthetic nanoparticles.

b. **Germination rate**: The changing in these parameters were not significant in all treatment except the induction on it at (200 mg/ml) of biological synthetic nanoparticles.

c. **Mean germination time**: There were reduction in it using different concentrations of industrial and biosynthetic nanoparticles at (P < 0.05).

d. **Mean daily germination**: The same feature found in MDG.

e. **Germination value**: the significant reduction found at (20 mg/ml) of industrial synthesis and (20-200) mg/ml of biosynthetic nanoparticles. All other treatments were not significant.

f. **Promoter Indicator**: the reduction in these parameters were significance at all treatment of biosynthesis nanoparticles while these significant reduction was found at (20-200) mg/ml concentrations of industrial synthesis.

Overall, the result found that Al-Rasheed variety was more sensitive to nanoparticles compared with Tamuze-2 especially at the higher concentrations.
Table 3. Comparison between the effect of industrial synthetic NPs and biological synthetic NPs on Germination percentage, Germination rate, Mean germination time, mean daily germination, Germination Value and Promoter Indicator of Al-Rasheed variety of T. aestivum.

<table>
<thead>
<tr>
<th>V.</th>
<th>Con. (mg/ml)</th>
<th>Industrial synthetic nanoparticles</th>
<th>Biological synthetic nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% GP</td>
<td>GR</td>
<td>MGT</td>
</tr>
<tr>
<td>Al-Rasheed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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Data shows: means; V: variety of plant; Con.: concentration; CT - : untreated; GP: Germination percentage; GR: Germination rate; MGT: Mean germination time; MDG: mean daily germination; GV: Germination Value; PI: Promoter Indicator; Similar letters are not significance at (P < 0.05) vertically.

This study agrees with the positive and negative effect results of (24) who approve that TiO2 industrials nanoparticles (NPs) (50 nm of anatase shape) were increased promoter indicator of Latyfia variety of wheat but it has negative effect on germination percentage, germination rate and Shoot and root length. It, also, increased total number of chemical compounds that identified in leaves plant compared with control. Additionally, exposed wheatgrass seeds to high concentrations of nano TiO2 particles (80 ppm) led to diminished germination rate, (25). In the same time, (26) showed that the use of TiO2 NPs on the germination of wheat at the proper and lower concentrations have additive effect and in high-concentration have reduction effect. This positive and negative effects of NPs on plant growth may depends on the concentration, size, particles shape and physical and chemical properties of NPs, plant species, (48) as well as the methods of NPs synthesis. Some studies found that physiological and chemical methods for NPs synthesis were more toxic to living cells and vice versa. In current studies, there were decrease in all plant's parameters at most concentrations of TiO2 biological synthetic compare with industrial synthetic nanoparticles in Al-Rasheed variety, while there were inductions in some plant's parameters by biosynthetic nanoparticles compared with industrial synthetic in Tamuze-2 variety. (49) studied the effect of biologically synthesized Ag NPs on hydroponically grown Bacopa monnieri growth metabolism, and found that biosynthesized AgNPs showed a significant effect on seed germination and induced the synthesis of protein and carbohydrate and decreased the total phenol contents and catalase and peroxidase activities.

4. CONCLUSIONS

*C. longa* can be used to biosynthesis TiO2 NPs by two methods. All biosynthetic particles were in nano size with good optical properties. Crystals shape were in three form anatase, rutile and brookite when first method was used while it was pure anatase when second methods was used. This Nanoparticles were reduced fungal growth, spores and pathogenicity of *F. graminearum*. Al-Rasheed variety of wheat plant was more sensitive to resistance damping off compared with Tamuze-2 variety. But its growth was more sensitive to industrial and biological synthetic NPs compared with Tamuze-2 especially at higher concentrations. More study of the stability of TiO2 NPs produced by *C. longa* and if it
agglomerates to be in micro-size are benefit. Study its activity against other organism compared with bulk and industrial nanoparticles will gave good knowledge.

References


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