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## Synthesis and investigations on tellurium myconanoparticles

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### ABSTRACT

Tellurium has attracted the attention of many researchers and manufacturers due to its unique properties. Through the current work, six fungal isolates have been screened for their ability to reduce potassium tellurite ( $K_2TeO_3$ ) into elemental tellurium nanoparticles (TeNPs). The most promising fungal isolate was identified as *Aspergillus welwitschiae* and given the accession number (KY766958) based on molecular basis and has been used for biogenic (enzymatic) production of TeNPs. The produced TeNPs have been characterized using DLS, TEM and FTIR. Data showed that, the particle size is 60.80 d.nm with oval to spherical shape. The produced TeNPs have been evaluated for antimicrobial activity at 25 mg/ml. Data revealed antibacterial activity against *E. coli* and *Staphylococcus aureus* (MRSA). Evaluation of the effect of  $\gamma$ -irradiation on TeNPs production showed that, the productivity was improved at 1 kGy and suppressed gradually at higher doses.

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### 1. Introduction

Nanotechnology is a fast growing discipline of the high tech economy [1,41]. Products of nanoparticles or nanomaterials (substances with particle sizes less than 100 nm) became a part of everyday life and the focus of many industries including cosmetics, clothing, foods and drug products [18,43,44,46]. Although may cause adverse health effects on human, animal and environment, the different properties of nanoparticles compared to their respective bulk materials such as a high surface area to volume ratio, new mechanical, chemical, electrical, optical, magnetic, electro-optical, and magneto-optical [17] made them useful for many applications. Consequently, careful environmental, human and animal health safety assessment [10,12] should be considered during the speedy commercialization of nanotechnology.

Baerman et al. [5] indicated that, tellurium (Te) is a toxic metalloid present in the earth crust at very low levels (0.002 ppm) [5], in seawater at a range from 0.5 pmol/l at depth to 1 to 2 pmol/l in open surface waters and in coastal (up to 7 pmol/l) and fresh (26 pmol/l) rain waters [4].

Tellurium is a semiconductor usually added to copper, tin, gold or silver to produce a certain desired electrical characteristics. Tellurium is used as a secondary vulcanizing agent for rubber, in alloying agents for copper and stainless steel to make them easier to machine and mill

and in color glass and ceramics [21]. The relatively low melting points of tellurium (Te) nanostructures, along with catalytic activity in hydration and oxidation reactions, high thermoelectric along with piezoelectric responses, photoconductivity and nonlinear optical responses have attracted wide attention [19,25–27,31,45]. Nanostructures of tellurium may find many possible future uses including cancer drug development, antibiotics and in medicine [8,23].

The aim of the current work is to prepare stable-dispersed and pure tellurium nano-particles (TeNPs) in easy, fast and simple method, studying the effect of radiation on productivity and testing for antimicrobial activity.

### 2. Materials and methods

#### 2.1. Microorganisms and growth

Six Egyptian fungal isolates; coded F1G, F3k, F4Sew, F5k, F7k and F8k (Table 1) have been grown and preserved on Czapek Dox agar. Czapek Dox medium has the following composition [13] (g/l): 30, sucrose, 2, sodium nitrate, 1, dipotassium phosphate, 0.5, magnesium sulphate, 0.5, potassium chloride and 0.01, ferrous sulphate. The final pH was adjusted at  $7.3 \pm 0.2$ . Medium was autoclaved at 121 °C for 15 min before inoculation.

#### 2.2. Screening for tellurium nanoparticles-producing fungi

All fungal isolates have been grown on Czapek Dox broth medium at 30 °C for 7 days under 150 rpm shaking condition. At

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**Table 1**  
Sources of fungal isolation and growth on Czapek's Dox medium supplemented with sodium tellurite.

| Isolate code | Source of isolation                             | Growth on Czapek's Dox agar medium supplemented with sodium tellurite at <sup>a</sup> : |    |
|--------------|---|---|----|
|              |   | 3%  | 4% |
| F1G          | Spoiled garlic                                  | +++   | ++ |
| F3k          | Abo Zaabal waste composting plant (soil sample) | ++  | +  |
| F4Sew        | Agricultural sewage water                       | ++  | +  |
| F5k          | Kitchen drainage tube                           | +++   | ++ |
| F7k          | Kitchen drainage tube                           | +++   | ++ |
| F8k          | Kitchen drainage tube                           | +++   | ++ |

<sup>a</sup> Where: +: good growth, ++: very good growth, +++: excellent growth.

the end of the incubation period, media were filtered through microbial filter (0.45 nm pore size) to get a clear broth. For each organism, 1 ml of the broth medium was added to 1 ml of 4 mmol potassium tellurite ( $K_2TeO_3$ ) to get a final concentration of 2 mmol and incubated for 48 h at 30 °C. The presence of black suspended particles indicates elemental tellurium [3,42]. Equal volumes of fungal extracts and distilled water were used as blanks. The most promising tellurium nanoparticles-producing fungal isolate(s) was selected based on the intensity of blackening.

### 2.3. Molecular based identification of the most promising fungal isolate

The most promising fungal isolate was identified based on 18s-rRNA. Ribosomal RNA was extracted using GeneJet Plant genomic DNA purification Kit (Thermo) according to the manufacturer protocol. Primers ITS1-F (5'TCC GTA GGT GAA CCT TGC GG 3') & ITS4-B (5'TCC TCC GCT TAT TGA TAT GC 3') have been used for isolation of the 18s-rRNA. PCR product has been cleaned up using GeneJET™ PCR Purification Kit (Thermo). Sequencing of the PCR product was performed at GATC Company using ABI 3730xl DNA sequencer. The sequence results were processed by using the web-based blasting program, basic local alignment search tool (BLAST), at the NCBI site (<http://www.ncbi.nlm.nih.gov/BLAST>), and the data were compared with the NCBI/Genbank database [2].

### 2.4. Characterization of the produced elemental tellurium

The promising fungal isolate (F5k) selected based on production of black suspension have been tested for optical properties using Spectro UV–vis Double Beam UVD 3500, Labomed, Inc USA.

Tellurium nanoparticles produced by the most active fungal isolate, based on spectral analysis, has been analyzed for particle size, morphology and FT-IR. Particles nano-size was measured by dynamic light scattering (DLS) using Zetasizer NANO-ZS (Ver. 7.04, Serial Number: MAL 1074157, Malvern Instruments Ltd., United Kingdom) at the wavelength of 633 nm and a power of 4.0 mW as a light source collecting data at a fixed scattering angle of 173°. TeNPs morphology was determined using JEOL Transmission Electron Microscope (JEM-1230, Japan, with 500.000 x magnification power, 100 KV acceleration voltages and 0.5 nm resolving power)

For Fourier transform infrared (FT-IR) spectra, TeNPs were dried under reduced pressure and the dried sample was packed in KBr-form pellets and spectrum was recorded using FT-IR JASCO 6100, Japan in the range of 400–4000  $cm^{-1}$ .

### 2.5. Effect of $\gamma$ -irradiation of potassium tellurite/broth culture mixture

For studying the effect of  $\gamma$ -irradiation on the mixture of potassium tellurite/F5k broth culture, one ml of the broth medium was added to 1 ml of 4 mmol potassium tellurite ( $K_2TeO_3$ ) to get a final concentration of 2 mmol. The mixtures were subjected to

$\gamma$ -radiation ( $^{60}Co$ ) at dose rate of 3.2 KGy/h using Russian  $\gamma$ -radiation cell at Nuclear Research Center, Inshas, Egypt. The mixtures were subjected to nine doses; 1, 2, 5, 10, 15, 25, 30, 35 and 40 kGy and then incubated for 48 h at 30 °C. The irradiated mixtures were tested for optical properties and compared with non-irradiated positive control and radiated negative controls.

### 2.6. Evaluation of TeNPs for antimicrobial activity

Luria-Bertani (LB) agar medium was used in this respect. It was poured in Petri dishes and allowed to dry. Colonies of *E. coli*, *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Klebsiella*, *Candida albicans* and *Aspergillus niger* were spread on the surface of LB medium. Only 100  $\mu$ l of a suspension of – washed, dried and pre-weighed – TeNPs in distilled water (25 mg/ml) was added to a well of 1 cm diameter in plates cultured with the test strains under study. The plates were incubated for 48 h at 30 °C and then inspected for growth inhibition zones.

## 3. Results

### 3.1. Screening for tellurium nanoparticles-production

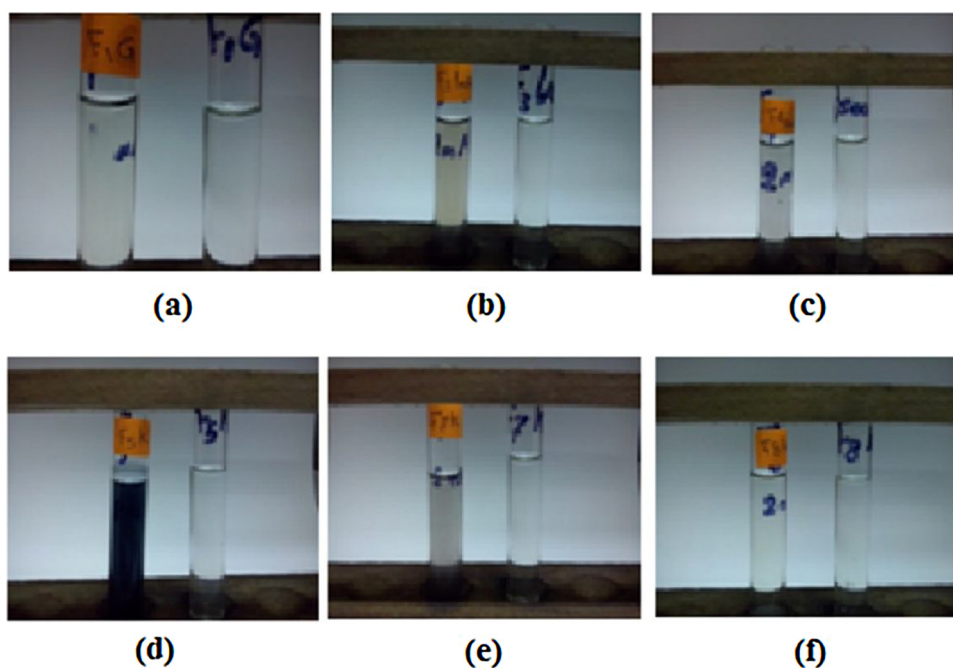
The extracts of the six fungal isolates showed different activities for production of elemental tellurium. Fig. 1(a–g) showed only three tubes with black turbidity; namely F3k, F5k and F7k, while the rest fungal isolates didn't give any blackening. The presence of black turbidity is an indicator of elemental tellurium.

Ultra violet/visible light spectral analysis of the tellurium nanoparticles produced from potassium tellurite/broth culture medium of the isolates F3 K, F5k and F7k are shown in Fig. 2. In addition to visual observation as provided in Figs. 1 and 2, shows that F5k is the most active fungus for production of tellurium nanoparticles (TeNPs) with maximum absorption at wavelength (~700 nm) and therefore it was the focus for further studies. The absorbance at 700 nm was 0.426, 0.529 and 0.311 for F3 K, F5k and F7k, respectively.

The most promising fungus (Fig. 3) was identified as *Aspergillus welwitschiae* based on 18s-rRNA and was given the accession number of (KY766958) by the Gene bank. Phylogenetic analysis of 18s-rRNA identification is presented in (Fig. 4).

Size distribution report by number using dynamic light scattering (DLS) and Transmission Electron Microscopy (TEM) analysis of tellurium nanoparticles (TeNP) produced by F5k are shown in Figs. 5 and 6, respectively. For size distribution (Fig. 5), a good quality results have been shown with particle size of 60.80 d. nm, Z-average of 114.8 d.nm and a polydispersity index (PDI) of 0.22. TEM (Fig. 6) showed oval to spherical-shaped TeNPs.

Fourier transform infrared (FTIR) spectroscopic analysis of the produced TeNPs (Table 2 and Fig. 7) indicates the presence of amino acid molecules which are the building blocks of the protein structure. Bonds represented by peaks number 4 (3428.81  $cm^{-1}$ ) and number 11 (1599.66  $cm^{-1}$ ) may correspond to the primary



**Fig. 1.** Screening for blackening by tellurium nanoparticles production; tubes on the right hand are controls and those on the left hand are tests; (a): FIG, (b): F3k, (c): F4 sew, (d): F5k, (e): F7k and (f): F8k.

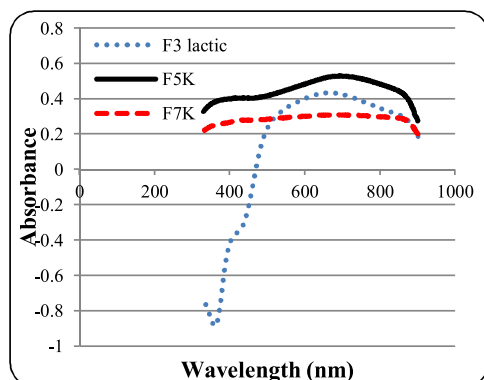
amine or amide owing to bond N—H and the bond —NH<sub>2</sub>, respectively. While bonds represented by peak number 9 at 1737.55 cm<sup>-1</sup> and peaks 5, 6 and 7 at 2925.48, 2856.06 and 2515.69 cm<sup>-1</sup>, respectively, may correspond to the C=O and O—H of the carboxylic acid. The peak number 17 (1078.01 cm<sup>-1</sup>) may correspond to the bond of C—O.

### 3.2. Effect of irradiated of potassium tellurite/broth culture medium mixture

The effect of different doses of  $\gamma$ -irradiation on productivity of TeNPs (Fig. 8) showed that the dose 1 kGy increased TeNPs production compared to the non-irradiated control. On the other hand, the increase in the dose of radiation resulted in gradual decrease in TeNPs production up to 30 kGy. Increase of  $\gamma$ -radiation over 30 kGy inhibits TeNPs production.

### 3.3. Evaluation of the antimicrobial effect of TeNPs

Screening for antimicrobial activity of TeNPs against different microbial isolates was provided in Table 3 and Fig. 9. Results



**Fig. 2.** UV/Vis Spectral analysis of the most active fungal isolates (diluted to 1:10). Molecular identification of F5 K based on 18s-rRNA.

revealed inhibition of *E. coli* and MRSA growth with 29 mm and 31 mm inhibition zone diameter, respectively. Alternatively, the growth of *Staph aureus*, *klebsiella*, *C. albicans* and *A. niger* were not inhibited.

## 4. Discussion

Microbial nano-biotechnology had attracted the attention of scientists and lead to progress and development in many fields including engineering, chemistry and biological sciences [24,35].

The biogenic production of nanoparticles became far superior over that of chemical methods. Despite chemical methods are able to, rapidly, produce large quantities of nanoparticles with a well-defined shapes and sizes; they are inefficient, costly and produce toxic and harmful wastes. Alternatively, the biogenic production of nanoparticles is a “green” and environmentally friendly process and requires less energy compared with chemical methods [7,38]. Microbial biosynthesis of nanoparticles may be intra- or extra-cellular process that is, highly, sensitive to changes in temperature, pH and nanoparticles concentration [24]. Tellurium nanoparticles (TeNPs) are often produced inside the microbial cells and hence the isolation of these particles from these cells requires additional downstream processing steps in order to release the metal, through ultrasound treatments and the use of detergents [11]. In the current study, TeNPs have been produced through cell-free enzymatic process because the enzymatic production of nanoparticles is more advantageous as it less sensitive to environmental changes compared to cellular processes and easier to obtain pure nanoparticles.

In the present investigation, screening for the most active fungal isolate(s) for biogenic production of TeNPs was done, using supernatant aliquots of six fungal isolates, in two steps; the formation of black suspension indicating reduction of potassium tellurite into elemental tellurium (Te<sup>0</sup>) [3,5,42] and spectral analysis at wavelength range (300–900 nm). The first step in screening resulted in three fungal isolates with high TeNPs yield, namely; F3 K, F5k and F7k which have been subjected to the second step. The isolate F5k exhibited the highest TeNPs production.

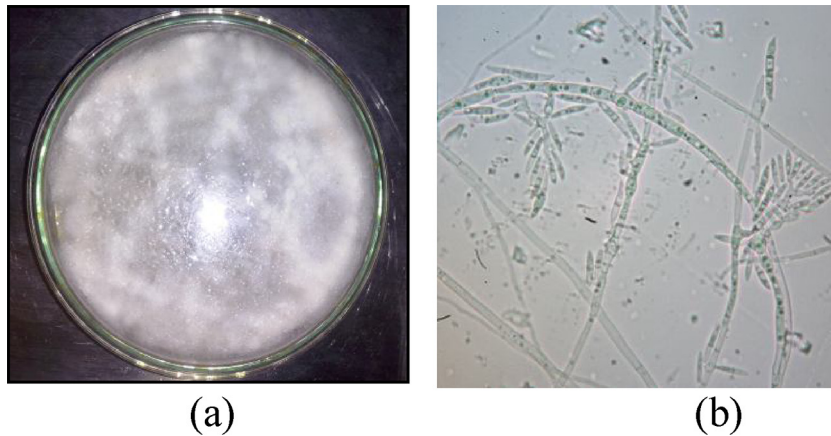


Fig. 3. Growth of *Aspergillus welwitschiae* KY766958 on Czapek's Dox agar medium (a) and under light microscope (b) at power 400×.

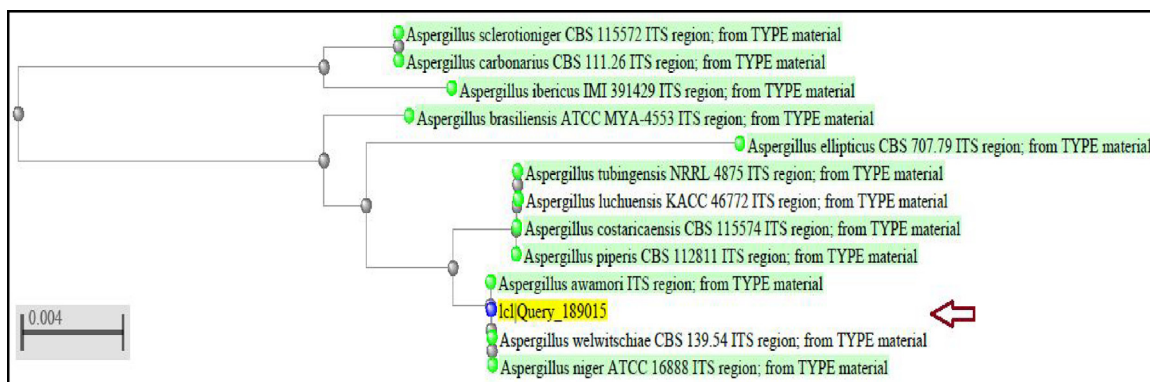


Fig. 4. Phylogenetic tree based on F5 K 18s-rRNA gene sequences.

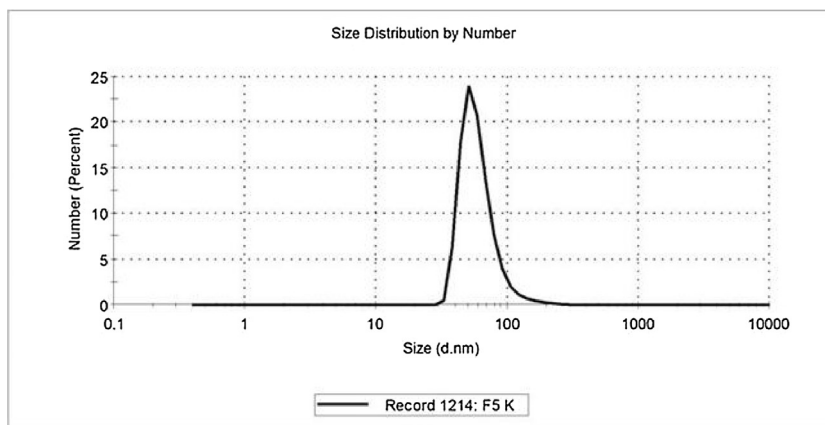


Fig. 5. Size distribution of tellurium nanoparticles produced by *Aspergillus welwitschiae* KY766958.

Spectral analysis of TeNPs suspensions showed that maximum absorption was at wavelength (~700 nm) and the absorbance was 0.426, 0.529 and 0.311 for F3 K, F5k and F7k, respectively, at this wavelength. Alternatively, Zare et al. [47] used wavelength of 500 nm to measure the turbidity produced by elemental tellurium. The wavelength in the present study, 700 nm, is the correct one as it measure in black range color, while wavelength 500 nm measures in green color range as it was reported by [9].

In the present study, F5k represented the most potent fungal isolate for TeNPs production, so it has been subjected to characterization using molecular techniques. The molecular

methods used for identification of fungi are highly sensitive and selective compared to morphological and physiological characterization that is sensitive to environmental conditions and hence turned out to be more difficult [48]. The phylogenetic tree in Fig. 4 shows phylogenetic analysis of the isolate F5k using 18 s rRNA sequence data with highest similarity of 100% to *Aspergillus welwitschiae* and was taken the accession number of (KY766958) by the Gene bank.

The shape and size of the produced TeNPs has been characterized using dynamic light scattering (DLS) and transmission electron microscopy (TEM). TeNPs was oval to spherical-



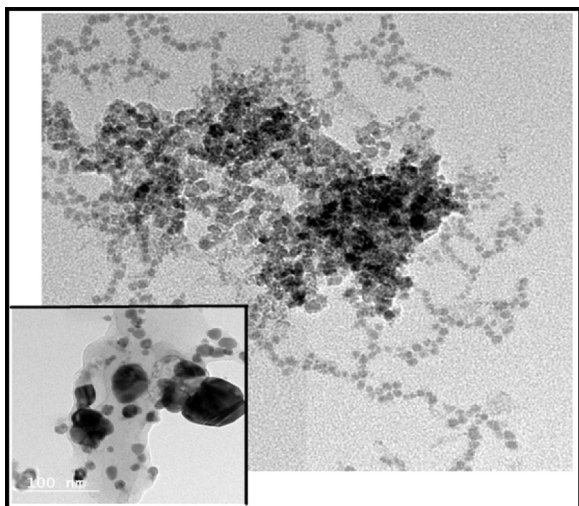


Fig. 6. TEM of tellurium nanoparticles produced by *A. welwitschiae* KY766958.

shaped particles with size distribution of 60.80 d.nm. The nanoparticles shape is similar to that demonstrated by [11]. On the other hand, Zare et al. [47] obtained rod-shaped tellurium nanoparticles by *Bacillus* sp. BZ. In addition, nano-size tellurium particles (68 nm) have been produced enzymatically as it was reported by Pugin et al. [35].

FT-IR analysis of the produced TeNPs indicated the presence of many peaks that may correspond to primary amine or amide and carboxylic acid. These bonds confirm the presence of amide or amine and carboxylic acid groups of the protein amino acids. Most likely, this protein is the enzyme(s) catalyzing the reduction of tellurite group into elemental tellurium ( $\text{Te}^0$ ).

Gamma rays are short wave, high-energy electromagnetic radiations capable of ionizing other atoms [15]. Gamma irradiation of potassium tellurite/broth culture mixture improved TeNPs production at 1 kGy. This increase in productivity may be

attributed to the activation of the enzyme(s) responsible for reduction of tellurite group. Gamma radiation has also been reported to increase the level of activity of many enzymes at low levels [16,29]. At higher levels of  $\gamma$ -radiation, the enzyme(s) responsible for reduction of potassium tellurite is disrupted and subsequently, reduction in TeNPs productivity. Maity et al. [28] and Kiong et al. [22] reported that,  $\gamma$ -radiation is considered to produce amino acids by breaking down of proteins. In addition, Stoeva and co-workers [40] reported the disruption of enzyme activity by high dose of  $\gamma$ -radiation.

The produced TeNPs have also been studied for their potential antimicrobial activity. Evaluation of antimicrobial activity of the produced TeNPs was performed at concentration of (25 mg/ml) on six microbial isolates including bacteria, yeast and fungi; *E. coli*, *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Klebsiella*, *Candida albicans* and *Aspergillus niger*. Of these microbial isolates, only *E. coli* and *Methicillin-resistant Staphylococcus aureus* (MRSA) have been inhibited with inhibition zone diameters of 29 mm and 31 mm, respectively.

The antimicrobial activity of nanoparticles has been studied and applied by many researchers [14,20,36,37] and showed promising results. Although, the mechanism responsible for the antimicrobial activity of TeNPs is not yet completely understood, there are possible modes of action that has been proposed to explain the toxicity of these nanoparticles. One possible mode of action is the production of reactive oxygen species (ROS) (Manke et al.). Other possible mechanisms include the functional damages of cell membrane or wall by disrupting their integrity [33] and the surface features of the nanoparticles may, also, be involved in conferring toxicity to NPs [6].

In a similar result to the current, it has been reported by Pérez et al. [32] that the damage of *E. coli* may be attributed to reactive oxygen species (ROS) released by TeNPs that is probably tellurite. This suggestion was confirmed when tellurium nano-rods were observed inside *E. coli* after being exposed to TeNPs. Alternatively, it has been reported by Pugin et al. [34] that *S. aureus* exhibits much more resistance to tellurite and generates lower amounts of ROS

Table 2

FTIR spectra of the biogenic TeNPs synthesized using *Aspergillus welwitschiae* KY766958.

| No | Position | Intensity | No | Position | Intensity | No | Position | Intensity | No | Position | Intensity |
|----|----------|-----------|----|----------|-----------|----|----------|-----------|----|----------|-----------|
| 1  | 3744.12  | 95.6689   | 2  | 3734.48  | 95.6053   | 3  | 3710.37  | 95.2484   | 4  | 3428.81  | 57.4692   |
| 5  | 2925.48  | 81.8676   | 6  | 2856.06  | 87.4928   | 7  | 2515.69  | 98.0415   | 8  | 2228.34  | 97.9764   |
| 9  | 1737.55  | 91.7427   | 10 | 1627.63  | 76.5812   | 11 | 1599.66  | 76.9506   | 12 | 1455.03  | 74.4186   |
| 13 | 1385.6   | 77.4897   | 14 | 1319.07  | 90.2641   | 15 | 1263.15  | 91.2372   | 16 | 1188.9   | 92.4491   |
| 17 | 1078.01  | 88.6119   | 18 | 1038.48  | 87.9958   | 19 | 872.631  | 94.2795   | 20 | 773.315  | 92.9797   |
| 21 | 624.823  | 86.0347   | 22 | 543.828  | 88.9867   | 23 | 450.297  | 95.5862   |    |          |           |

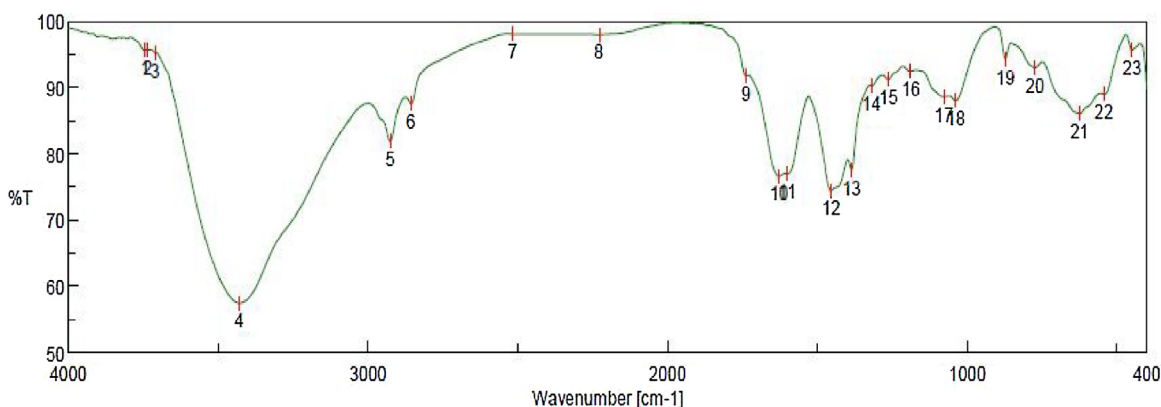


Fig. 7. FTIR spectra of the biogenic TeNPs synthesized using *Aspergillus welwitschiae* KY766958.

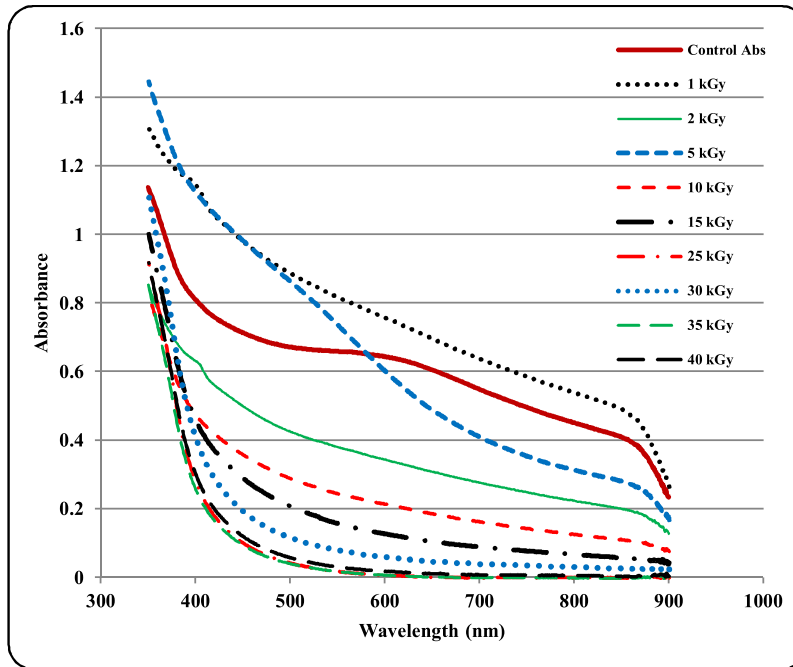


Fig. 8. UV/Vis Spectral analysis of irradiated potassium tellurite/broth culture medium mixture at different doses.

**Table 3**  
Evaluation of TeNPs antimicrobial activity at (25 mg/ml).

| Microbes             | <i>E. coli</i> | <i>S. aureus</i> | MRSA | <i>klebsiella</i> | <i>C. albicans</i> | <i>A. niger</i> |
|----------------------|----------------|------------------|------|-------------------|--------------------|-----------------|
| Inhibition zone (mm) | 29             | Nil              | 31   | Nil               | Nil                | Nil             |

which explains the resistance of *S. aureus* to TeNPs in the present study.

Although it may be surprising to obtain a resistant *Staph aureus* and sensitive MRSA, *S. aureus* resistance of methicillin has a different mechanism from that of ROS [39] and therefore the

resistance of *S. aureus* to methicillin does not influence or interfere with its sensitivity to TeNPs. Furthermore, Pugin et al. [34] showed no growth inhibition of *S. aureus* ATCC 6538 in presence of tellurium nano-particles.

In similar studies, tellurite NPs were reported to inhibit the growth of *S. aureus* by Gupta et al. [49] and had no inhibitory effect in a study by Molina-Quiroz et al. [30]. In addition, Zare et al. [47] demonstrated the antimicrobial activity of tellurium nanoparticles produced by *Bacillus* sp., isolated from the Caspian Sea against different clinical isolates (*S. aureus*, *P. aeruginosa*, *S. typhi* and *K. pneumonia*) between 125 and 500 mg/l. This conflict of the results may be attributed to that, TeNPs have a strain-specific antimicrobial activity. Conclusively, the use of TNPs as antimicrobial agents is

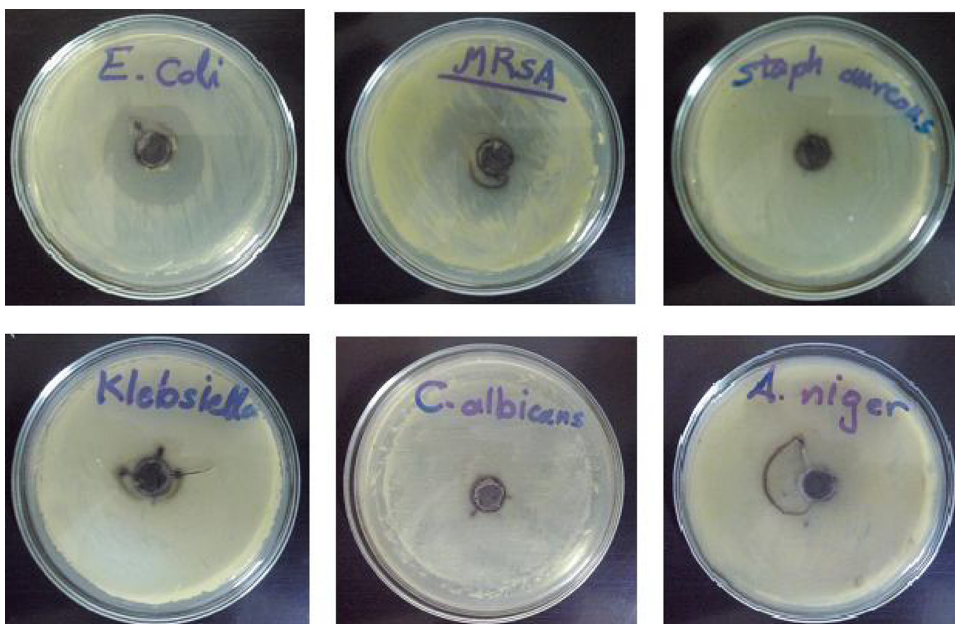


Fig. 9. Antimicrobial activity of TeNP against *E. coli*, MRSA, *Staphylococcus aureus*, *Klebsiella*, *C. albicans* and *A. niger*.

a novel and feasible strategy to face the antibacterial multi-resistance problem.

## 5. Conclusion

Tellurium nanoparticles have been synthesized using the fungal isolate *Aspergillus welwitschiae* KY766958. The produced TeNPs was oval to spherical shaped and have particle size of 60.80 d.nm. The use of  $\gamma$ -radiation showed that the used of 1 kGy increased TeNPs yield compared with the non-irradiated sample. The increase of the  $\gamma$ -radiation dose negatively affects the yield of TeNPs. Studying the antimicrobial effect of tellurium nanoparticles on bacterial and fungal strains revealed a good activity against *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA).

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This work was not funded by any organization.

## Conflict of interest

All authors declare that there is no conflict of interest and that this article does not contain any studies with human participants or animals performed by any of the authors.

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