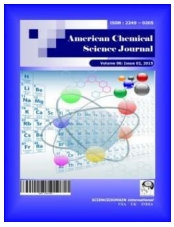

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Corrosion Resistance and Antimicrobial Activity of Extra- and Intracellular Fe(II) Nanoparticles Biosynthesized Via *Aspergillus foetidus ATCC 14916*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study aims to synthesizing Fe (II) nanoparticles with green synthesis method. For this purpose fungal isolate was chosen from the most potent four isolates and its metallotolerance ability towards Fe (II) was studied. Extracellular and intracellular biosynthesis of the nanoparticles was achieved. The nanoparticles were characterized using atomic absorption spectrophotometer (AAS), dynamic light scattering (DLS) and transmission electron microscope (TEM). The shape of metal nanoparticles mostly was spherical. The size of the particles ranges from 80 nm to 370 nm & from 31.53 nm to 61.94 nm for extra- and intracellular particles respectively. The isolate was identified based on morphological and physiological and genetic characteristics and it was found to be closely related to *Aspergillus foetidus* strain ATCC 14916 with 99% similarity. The antimicrobial activity of the produced intracellular and extracellular nanoparticles was done. The samples have shown antimicrobial activity against some of the used test organisms with different results and different diameter of the inhibition zones among each other. In addition, the corrosion inhibition

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efficiency of the extracellular nanoparticles was studied. The inhibition efficiency of the inhibitor by polarization curves exhibited the maximum inhibition efficiency 93.877% at the highest concentration of 907.2 ppm.

Keywords: Metallotolerant; metal nanoparticles; iron; green synthesis; TEM; DLS; AAS; andantimicrobial activity.

1. INTRODUCTION

There is an enormous interest in the synthesis of nanomaterials due to their unusual optical [1], chemical [2], photoelectrochemical [3], and electronic properties [4]. There are various physical and chemical methods employed for the synthesis of metal nanoparticles [5,6]. However, these methods have certain disadvantages due to involvement of toxic chemicals and radiation. Therefore, research is shifting towards biological methods of synthesis of metal nanoparticles, as these are rapid, cost effective, and eco-friendly. Thus, microorganisms have been applied in metal nanoparticle production [7]. The use of fungi in the synthesis of nanoparticles is a relatively recent addition to the list of microorganisms. The use of fungi is potentially exciting since they secrete large amounts of enzymes and are simpler to deal with in the laboratory. However, the genetic manipulation of eukaryotic organisms as a means of overexpressing specific enzymes identified in nanomaterial synthesis would be much more difficult than that in prokaryotes [8]. Due to the outbreak of infectious diseases caused by different pathogenic bacteria and fungi and the development of antibiotic or metal resistant strains, there is increasing need to find new antibacterial products [9]. The broad spectrum antimicrobial properties of metallic NPs encourage their use as disinfectants in purification processes (medicine, water and air), food production, cosmetics, clothing, and numerous household products [10]. The objective of our study was to synthesize iron nanoparticles by metallotolerant microorganisms as benign technique in chance to produce metal nanoparticles with unique properties. Also, characterization of the produced metal nanoparticles was essential. In addition, the produced metal nanoparticles were used as antimicrobial agents as required important application and as corrosion inhibitor.

2. MATERIALS AND METHODS

2.1 Isolation

The Supplemented-metal-nutrient (SMN) agar medium which has the same composition as nutrient agar medium with the addition of different concentration of Fe^{+2}*viz* (50, 100, 200 and 500 ppm). The medium was poured under aseptic conditions in sterile plates. The plates were inoculated with either 0.1 ml of soil suspension or 0.1 ml of waste water came from detergents processing wastes from (Savo factory) in Alamereia, Cairo. The plates were incubated at 30℃ for 2 days in case of bacterial isolates and 7 days for fungal isolates.

2.2 Metallotolerance Ability Examination of the Isolates

The isolates obtained were purified and then, tested for their ability to grow on SMN agar medium containing higher gradient concentrations of the same metal from which it was previously isolated. The maximum concentration was achieved after which no growth was determined.

2.3 Biosynthesis of Fe^{+2} Nanoparticles

Supplemented-Metal-Nutrient (SMN) broth medium was prepared and the selected metallotolerant isolates were allowed to grow on sub-lethal concentration. Incubation was carried out at 30℃ for 2 days in case of bacterial isolates and 7 days for fungal isolates.

2.4 Separation of Intracellular and Extracellular Nanoparticles Samples

At the end of the incubation period for bacterial and fungal isolates the extracellular filtrate was separated by centrifugation and filtration, respectively and cells were washed with distilled water.

The intracellular contents of the bacteria and fungi were obtained by ultrasonic disruption of cells with an ultrasonic processor (Cole Parmer Ultrasonic Homogenizer CPX 400) over three 15 s periods, and with an interval of 45 s between periods. The sonicated samples were centrifuged at 15,000 rpm for 30 min at 4℃ to remove cell-debris. The supernatants were then used for the characterization of the intracellular nanoparticles [11].

2.5 Characterization of Fe^{+2} Nanoparticles

The intracellular and extracellular nanoparticles were characterized and examined using atomic absorption spectrophotometer (AAS), dynamic light scattering (DLS). According to the comparison of all the results of metallotolerance ability and DLS measurements, the TEM examination was carried out for the sample giving the most relevant results [12,13].

2.6 Characterization of the Fungal Isolate

The morphological and physiological characteristics of the isolate were studied. The isolate was identified also based on 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, and 28S ribosomal RNA gene sequences.

2.7 Studying the Antimicrobial Activity of the Nanoparticles Samples

The antimicrobial activity was carried out for the selected sample which previously subjected to TEM examination using agar well diffusion method against six test organisms and compared to control. In addition, the antimicrobial activities of the selected nanoparticles were confirmed by measuring MIC against another set of test organisms.

2.8 Application of $F_{2\ Fe/S}$ Extracellular Nanoparticles in Corrosion Resistance

The extracellular nanoparticles were obtained from *Aspergillus foetidus*, $F_{2\ Fe/S}$ grown on a medium containing 3000 ppm of Fe^{+2}. Different concentrations were prepared from the extracellular nanoparticles sample *viz*. (0, 226.8, 680.4 and 907.2 ppm). The corrosion resistance efficiency of samples was calculated using polarization technique [14].

Carbon steel working electrode of the following chemical composition was used as the metal to be protected: 0.07% C, 0.24% Si, 1.35% Mn, 0.017% P, 0.005% S, 0.16% Cr, 0.18% Ni, 0.12% Mo, 0.01% Cu and the remainder is Fe. A pre-treatment was carried out prior to each experiment, in which specimen surface was mechanically ground with 400, 600, 800 and 1000 emery paper, washed with acetone and bi-distilled water then dried and put into the cell [15]. The electrochemical measurements were carried out using Volta lab 40 (Tacussel-Radiometer PGZ301) potentiostate and controlled by Tacussel corrosion analysis software model (Voltamaster 4) under static condition. The corrosion cell used had three electrodes. The reference electrode was a saturated calomel electrode (SCE). A platinum electrode was used as auxiliary electrode. All potentials given in this study were referred to this reference electrode. After measuring the Eocp, the electrochemical measurements were performed. The polarization curves were obtained in the potential range from -800 to -300 mV (SCE) with a 2 mVs-1 scan rate.

The corrosion inhibition efficiency (η) was calculated using the following equation [16,17].

$$\eta = (i_{corr} - i^{o}_{corr}) / i_{corr} * 100$$

where $i^{o}corr$ and icorr are the corrosion current density values with and without inhibitor, respectively, which determined by extrapolation of the cathodic and anodic Tafel lines to the respective free corrosion potential.

3. RESULTS AND DISCUSSION

Isolation on SMN agar medium lead to selection of one bacterial and three fungal Fe^{+2} isolates.

A metallotolerance ability examination for the selected isolates was performed by increasing metal concentration up to lethal concentration. The most resistant microbial isolates were selected and grow at sub- lethal concentration.

Selection was made according to metallotolerance ability of the microbial isolates and DLS measurements. In addition, the easier isolates to manipulate were preferred in selection.

In this connection, for the metallotolerance of Fe^{+2} it was revealed that, when a novel cyanobacterium isolated from an iron-depositing hot spring its iron requirements and tolerance were estimated by measuring cell yield in media supplemented with a range of $FeCl_3 \cdot 6H2O$ concentrations *viz.* 0–1000 μM. The growth of the isolate was suppressed in culture media supplemented with $FeCl_3 \cdot 6H2O$ concentrations less than 40 μM and greater than 1000 μM but, iron concentrations between 40 and 400 μM stimulated its growth which suggestd that, exponential growth of the isolate required elevated concentrations of soluble iron (>40 μM) [18].

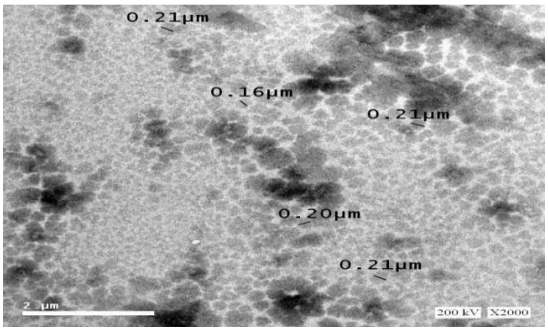
it was revealed in an earlier study that, when *E. coli* has been cultured in a nutrient broth which supplemented with Fe^{+2} and incubated at 37℃ for 5 h, bacterial growth reduced at presence of 0.5 mM/l Fe^{+2} in comparison with control. Growth of the bacteria was completely inhibited by 1 mM/l concentration of ferrous ion [19].

Also, the growth of *Aspergillus fumigatus, A. wentii, Curvularia lunata*and *Chaetomium globosum*on was tested on ferric oxide (Fe_2O_3) and both the bacteria *Alcaligenes faecalis*and *Bacillus coagulans* were tested on ferric oxide and ferrous sulphate ($FeSO_4$). The salts were tested at 1000 and 10000 ppm. It was found that, the growth of all microorganisms tested was very poor at 10000 ppm [20].

The isolate (**F**$_{2\ Fe/S}$) was allowed to grow at 3000 ppm of Fe^{+2}. It was chosen for nanoparticles production extracellularly and intracellularly.

Studying the extracellular and intracellular nanoparticles samples with AAS has shown that, the isolate **F**$_{2\ Fe/S}$ is capable of uptake about 5.42% of the used concentration of the metal (2398 ppm) as indicated in Table 1.

Studying the extracellular and intracellular nanoparticles samples with DLS has shown the size distribution of the nanoparticles in the extracellular sample from (34.03-477.7 r.nm) with different mean number for each size, the maximum mean number 26.4% was that of the particles with size 52.85 r.nm. The size



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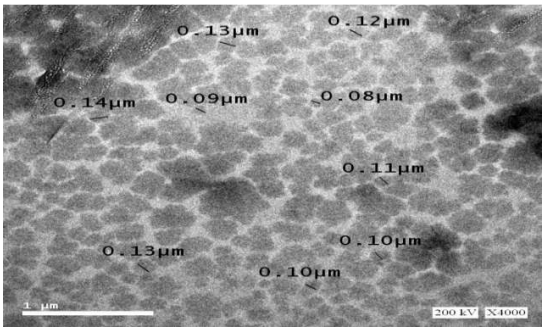

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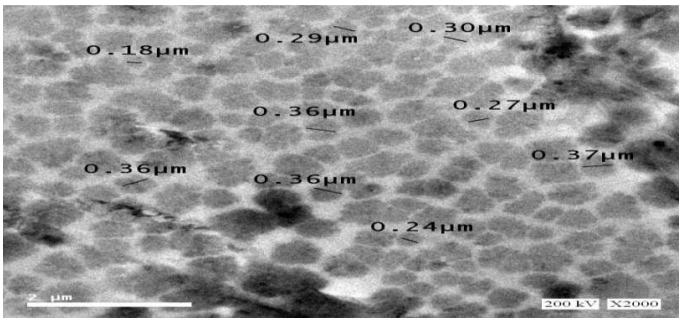

(c)

Fig. 1. TEM image of the extracellular Fe^{+2} nanoparticles of the isolate $F_{2\ Fe/S}$ (a, b, c, d) showing the spherical form and accurate size of the nanoparticles

Table 1. Distribution of the metal ion inside and outside the selected metallotolerant $F_{2\ Fe/S}$ isolate (Atomic absorption measurements)

Selected isolate	Type of the metal	The isolated microorganism NPs producer	Type of nanoparticles	Uptake percentage of the metal by the isolated microorganism (%)
F$_{2\ Fe/S}$	Fe^{+2}	*Aspergillus foetidus*, **F**$_{2\ Fe/S}$	Extracellular & intracellular	5.42

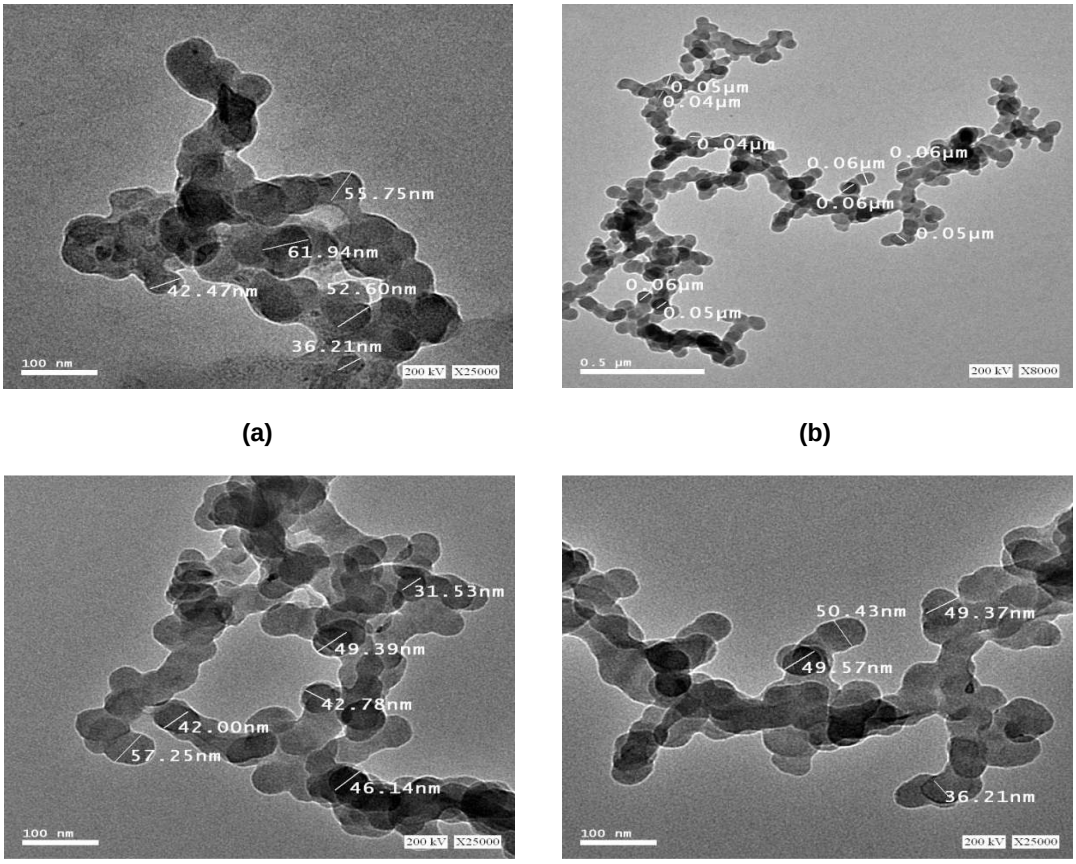

Fig. 2. TEM image of the intracellular Fe^{+2} nanoparticles of the isolate $F_{2\ Fe/S}$ (a, b, c, d) showing the spherical form and accurate size of the nanoparticles

distribution of the metal nanoparticles in the intracellular sample from (52.85-82.09 r.nm) with different mean number for each size, the maximum mean number 45.3% was that of the particles with size 70.89 r.nm.

The nanoparticles samples were studied with TEM and it was found that, the shape of the extracellular Fe^{+2} nanoparticles of the isolate $\mathbf{F_{2}}_{Fe/S}$ is spherical. The size of the particles with respect to the spherical form ranges from 80 nm to 370 nm. The shape of the intracellular Fe^{+2} nanoparticles of the isolate $\mathbf{F}_{2Fe/S}$ were spherical. The size of the particles with respect to the spherical form ranges from 31.53 nm to 61.94 nm.

Concerning iron, Magnetic Fe sulfide nanoparticles are synthesized by using sulfate-reducing bacteria where particles having a size of a few nanometers are formed on the surface [21].

Another researcher reported the synthesis of magnetic nanoparticles by using *Fusarium oxysporum*and *Verticillium* sp. at room temperature. Both fungi secreted proteins which were capable of hydrolyzing iron precursors extracellularly to form iron oxides nanoparticles [22]. In addition, it was revealed that, *Curvularia lunata*, *Chaetomium globosum*, *A. fumigatus*, *A. wentii*and the bacteria *Alcaligenes faecalis* and *Bacillus coagulans*were tested to produce nanoparticles in ferric oxide (Fe_2O_3) while the two bacteria tested in two salts ferric oxide and ferrous sulphate ($FeSO_4$) for nanoparticles production. Growing fungal suspension of *A. fumigatus, A. wentii, C. globosum*and *C. lunata*with Fe_2O_3 (1000 ppm) yielded different sizes of nano-particles. *A. fumigatus* produced the nanoparticles with the average size of 42.4 nm causing 28.7% scattering of light. *C. lunata* produced the nanoparticles with the average size of 20.7 nm resulting in 20% light scattering. *C. globosum* showed 67% peak of light

scattering due to the nanoparticles with the average size of 25 nm while in case of *A. wantii,* the production of 46 nm nanoparticles was recorded causing 6% light scattering. *Bacillus coagulans*failed to give any response to nanoparticle production while *A. faecalis* produced nanoparticles in both the salts giving the particles with the average size of 12 nm with 27.4% scattering of light in Fe_2O_3 while giving nanoparticles of the average size of 43 nm with a light scattering of 27.5% in $FeSO_4$ [20]. In addition, it was reported that, iron nanoparticles of average size have been synthesized using dried leaves of plant *Spinancia oleracea* [23].

The isolate identification was based on using Compendium of soil fungi [24], Atlas of clinical fungi [25] &using an Image Analysis System. It was identified also based on genetic characteristics on comparison of 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence and it was found to be more closely related to *Aspergillus foetidus* strain ATCC 14916 18S with 99% similarity according to the collected data Fig. 4.

The antimicrobial activity study of the nanoparticles samples has shown that , both samples has antimicrobial activity against some of the used test organisms indicated by the inhibition of their growth, Tables (2 and 3).

It was reported that, iron oxide nanoparticles have antibacterial activity and it was approved that the efficiency of the activity can be altered by changing the surface potential and accessible surface functional groups. The changes cause change in interaction pattern at the nano-bio interface [26].

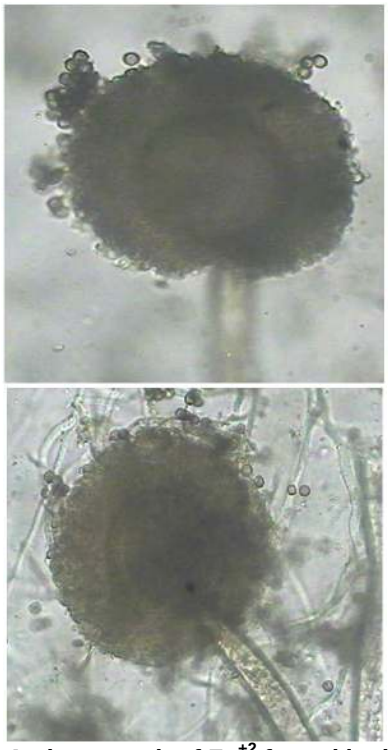

Fig. 3. A photograph of Fe^{+2} fungal isolate on microscopical examination (magnification X40)

Also, magnetic iron oxide (α-Fe_2O_3) nanoparticles were synthesized by pulsed laser ablation. Antibacterial activities of iron oxide nanoparticles were tested against Gram-positive; *Staphylococcus aureus* and Gram-negative; *Escherichia coli, Pseudomonas aeruginosa* and *Serratia marcescens* and it was found to have good antibacterial activity against both used organisms [27]. In addition, recent

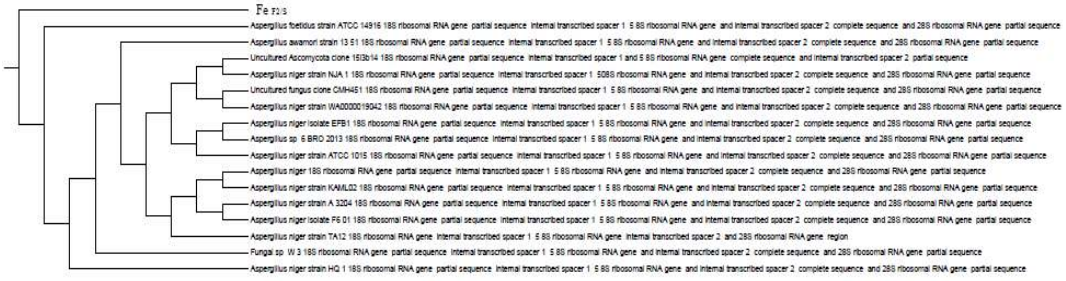

Fig. 4. A dendrogram showing the sequence relationships between *Aspergillus foetidus* ATCC14916 and several other strains based on 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

study reported the synthesis of iron nanoparticles by *Alternaria alternari* and it was found to haave antibacterial activity against gam positive and gram negative bacteria used in its study [28].

The extracellular nanoparticles sample of isolate *Aspergillus foetidus*, **$F_{2\ Fe/S}$** was selected for determining its corrosion resistance efficiency at different concentrations *viz*.(0, 226.8, 680.4 and 907.2 ppm) as recorded in Table 4 and Fig. 5.

It was concluded that, there is slight increase in inhibition efficiency with increasing concentrations of nanoparticles which may be attributed to an increase in the surface coverage of inhibitor on steel surface. In addition, the inhibition efficiency of the inhibitor by polarization curves exhibited the maximum inhibition efficiency (93.877%) at the highest concentration of 907.2 ppm. It can be concluded that, the presence of extracellular nanoparticles of isolate *Aspergillus foetidus*, **$F_{2\ Fe/S}$** prevented the attack of acid medium on steel surface.

In a recent study, it was reported that, magnetite-RK/amidoxime nanoparticles act as good corrosion inhibitor for mild steel and inhibition efficiency increased with inhibitor concentration. The inhibition efficiency of the inhibitor by polarization technique exhibited the maximum inhibition efficiency 96.6% at the highest concentration 150 ppm [14].

In addition, it was approved that, iron oxide nanoparticles (IONPs) found to be highly active as corrosion inhibitor. Its corrosion inhibition efficiency was measured using Weight Loss Method. It was found that, olive oil stabilized IONPs can be applied as anticorrosive additive for epoxy paint coated on mild steel with corrosion inhibition efficiency 80.88% [29].

Table 2. Antimicrobial activity results of the *Aspergillus foetidus*, $F_{2\ Fe/S}$nanoparticles

Nanoparticles sample	NPs conc. (ppm)	Diameter of inhibition zone (mm)					
		S. aureus	***B. subtilis***	***E. coli***	***P.* auriogenosa**	***C. albicans***	***A. flavus***
Extracellular NPs of *Aspergillus foetidus*, $F_{2\ Fe/S}$	2268	- ve	24.5	- ve	21.5	24	- ve
Intracellular NPs of *Aspergillus foetidus*, $F_{2\ Fe/S}$	130	- ve	- ve	17.5	18.5	21.5	- ve

Table 3. Minimum inhibitory concentration measurements of the produced *Aspergillus foetidus*, $F_{2Fe/S}$ nanoparticles

Tested microorganisms	Nanoparticle sample		Standard (ppm)
	Extracellular NPs of *Aspergillus foetidus*, $F_{2\ Fe/S}$	Intracellular NPs of *Aspergillus foetidus*, $F_{2\ Fe/S}$	
	Minimum inhibitory concentration (ppm)		
Fungi			amphotericin
Aspergillus fumigatus (RCMB 02564)	7.81	31.25	0.98
Candida albicans (RCMB 05035)	31.25	62.5	3.9
Gram positive bacteria			Ampicillin
Staphylococcus aureus (RCMB 010027)	62.5	62.5	0.015
Streptococcus pyogenes (RCMB 010015)	62.5	125	0.06
Gram negative bacteria			Gentamycin
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	62.5
Eschericia coli (RCMB 010056)	15.63	62.5	0.12

Table 4. Inhibition efficiency values for steel in HCl with different concentrations of extracellular nanoparticles of isolate *Aspergillus foetidus*, $F_{2\ Fe/S}$ calculated by polarization technique

Inhibitor	Concentration (ppm)	$-E_{corr}$, mV vs. SCE	I_{corr}, mA cm^{-2}	β_a, mV dec^{-1}	$-\beta_c$, mV dec^{-1}	η_p, %
Extracellular NPs of isolate *Aspergillusfoetidus*, $F_{2\ Fe/S}$	0	-529.8	0.731	158.9	-164.9	0
	226.8	-576.1	0.049	130	-155	93.025
	680.4	-582.2	0.042	173	-151	93.592
	907.2	-593.5	0.043	126	-139	93.877

Where: (E_{corr}): The corrosion potential in volts
(I_{corr}): The corrosion current in amps
(β_a): The anodic Beta Tafel Constant in volts/decade
(β_c): The cathodic Beta Tafel Constant in volts/decade
(η_p): Corrosion inhibition efficiency

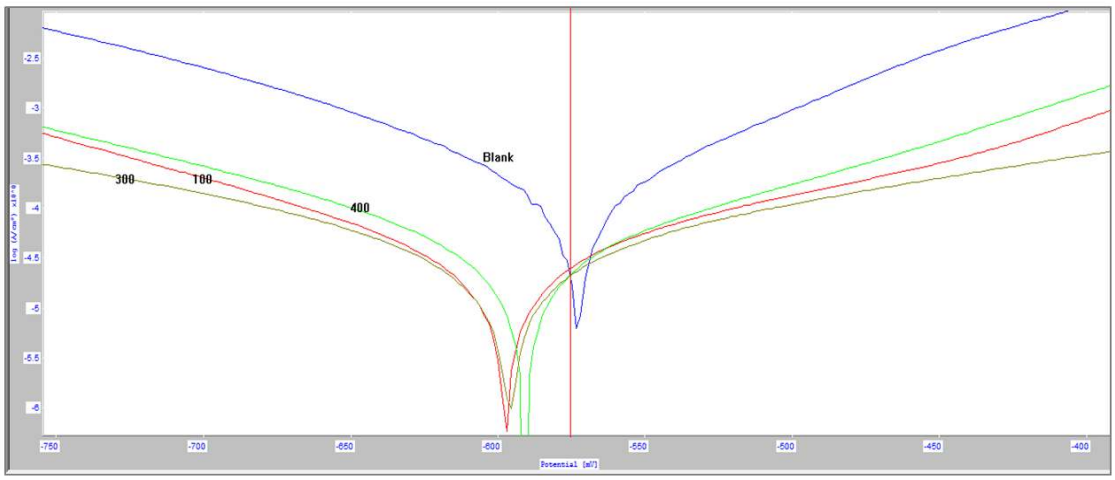

Fig. 5. Polarization curve of Carbon steel in HCl solution containing nanoparticles of isolate *Aspergillus foetidus*, $F_{2\ Fe/S}$ at different concentrations

4. CONCLUSION

Iron (II) nanoparticles have been synthesized by *Aspergillus foetidus* metallotorent fungi and it was found that, it can produce nanoparticles intracellularly and extracelullarly and both nanoparticles samples have antimicrobial activities. Also, extracellular nanoparticles of isolate *Aspergillus foetidus*, $F_{2\ Fe/S}$ were found to have corrosion inhibitory effect with exceptional results which indicate the promising properties of these nanoparticles offering them to be used in further applications with the advantage green synthesis of the metal nanoparticles.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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