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Growth-inhibiting effect of cerium oxide nanoparticles on some fungal isolates that may cause the football players' skin diseases

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ABSTRACT

Football sport is a public sport that is widely distributed around the world. Football players are subject to the same skin conditions as others, but participation in a sports environment characterized by unique stresses on the skin that are not normally seen in inactive individuals can cause skin issues. Nano-technology approach involving the synergistic interaction between microorganisms and nanoparticles offers an affordable, environmentally, and beneficial solution for fungal skin infection prevention. Herein, CeO_2NPs were properly characterized using different spectral analyses. Moreover, different fungal species were systematically isolated from the eleven volunteer football players' different skin parts, Port Said sporting city, Port Said, Egypt, and grown on a modified sabouraud dextrose agar medium. Eleven pure-culture fungal isolates were identified using spacer between 18S and 28S rRNA genes identification technique. The growth-inhibiting effect of different CeO_2NPs concentrations on the fungal isolates after ten days of incubation was studied. Furthermore, the reduction activity of used CeO_2NPs towards the isolated fungal species was inclusivity researched. The outcome data experimentally symbolized that the fungal isolates were remarkably stable under a pH of 5.5 and temperature of 25°C (optimized conditions). Besides, the growth-inhibiting of different fungal isolates was directly proportional to increasing the concentration of CeO_2NPs and completely stopped at the optimum dosage of 12 g/L compared to the control sample (non-treated CeO_2NPs specimen). Overall, the present study illustrates an ample perspective of cerium oxide nanoparticles as a futuristic platform for the decontamination of fungal skin diseases.

Key Words:

Cerium oxide, Football players, Fungi, Nanoparticles, Skin.

1. INTRODUCTION

Sport has become part of recent life, mainly among athletes. Although the health benefits of sports are unquestionable, participating in sports can be associated with several skin problems. Mechanical friction, contact with contaminated environment and different infectious agents, as well as exposure to various other factors and with the skin of other athletes increase the chances of getting a microbial skin infection consequently skin infections in sports players are extremely common [1].

Football players are subject to the same skin conditions as others, but participation in-sports environments characterized by unique stresses on the skin that are not normally seen in inactive individuals can cause skin issues. Combined with the close quarters shared by athletes and commonly poor hygiene practices, it isn't difficult to understand why microbial skin infections cause significant disturbance to individual and team activities [2,3].

Compounded by perspiration, friction commonly results in blisters that can be quite painful and diminishes the athlete's ability to perform [3]. Skin friction is governed by two main mechanisms: adhesion and deformation. At the interaction between skin and different materials, van der Waals bonds are formed between asperities, generating a sliding resistance due to adhesion. Skin's soft and elastic behavior means it will conform around the responding surface, requiring a force to deform the skin during relative movement. Both factors are influenced by an individual's skin properties, furthermore loading conditions, contact material, and environmental conditions [4].

A number of bacterial, viral, fungal, and parasitic infections occur in athletes. fungal infections are the most common type of microbial infections seen in athletes affecting skin, hair, and nail. These are caused by a group of closely related keratinophilic fungi, which are capable to invade keratinized tissues of the skin. These fungi are present in the environment all over the world and they are potentially pathogenic to humans. Fungal infections of the skin, hair, and nails are acquired through direct contact with other infected individuals [5,6].

Many environmental factors can be stimuli to the chance of athletes fungal infection, including pH, heat, and many other factors. These factors can create new skin disorders or enhance preexistent disorders [7].

Nanomaterials have demonstrated operative as unique materials for biotechnology applications because of their specific surface area and higher reactivity [8]. Nanoparticles (NPS) have been generally working as catalysts to improve numerous applications, according to their significantly high surface-to-volume ratio and other unique physic-chemical properties [9]. The nanoparticle's consequence on fungi has also been located into noteworthy consideration [10]. Some relevant studies have identified the NP's impact on the biological reaction rates as inhibiting the fungal growth rate [11]. The developed antifungal activity of NPS is usually signified by their exclusive characteristics and great accessible active surface areas and functionality [12].

The antimicrobial mechanisms of NPs are poorly understood [13]. The nanoparticles could reduce the viability of the fungal cell by permitting the binding of metal ions to sulfhydryl (thiol) and phosphate groups in the fungal cell wall, which delay the electron transport chain and energy generation. Moreover, nanoparticles prevent DNA replication and the respiratory chain in fungi, leading to fungal cell death by the generation of ROS [14].

The previous studies have demonstrated the significance of cerium oxide nanoparticles (CeO_2NPs) with their unique properties and biocompatibility to achieve various treatment actions, such as antimicrobial, anticancer, anti-diabetic, and antioxidant effects and drug delivery, by acquiring various mechanistic approaches at the molecular level [15]. However, there is a need to investigate the application of CeO_2NPs in the prevention of fungal infection in sports environments; it offers the potential of new

functional substances with unique activity toward the prevention of fungal contaminants in the sports environment.

Therefore, the present study aims the assessing the reduction activity of CeO_2NPs towards some fungal species that are isolated from the football players' different skin parts, in Port Said sporting city, Port Said, Egypt. Specifically, the CeO_2NPs can be introduced as an efficacious fungal growth-inhibiting agent applied to remediate the fungal-contaminated environment. A fungal group was set up to test the role of CeO_2NPs with different concentrations on fungal growth reduction. The fungal isolates were cultivated through a modified Sabouraud Dextrose agar medium and analyzed by spectrophotometer at 550.0(nm) after ten days of incubation. Eleven fungal isolates were isolated from football players' different skin parts.

2. MATERIALS AND METHODS

2.1. Chemicals

All reagents were employed as received without extra treatment (Sigma-Aldrich, Merck, Darmstadt, Germany).

2.2. Cerium oxide nanoparticles (CeO_2NPs)

A powder sample (5 g) of CeO_2 nanoparticles (size ≤25 nm) was obtained from Sigma-Aldrich® chemical company, Ontario, Canada, and used in the study.

2.3. Characterization of CeO_2NPs

The XRD analysis was applied to demonstrate the size and peaks of the CeO_2NPs to confirm their crystalline structure and pattern. The analysis was done using differential light scattering by the DLS particle size analyzer NanoBrook 90Plus, Brookhaven Devices Company، Holtsville, NY, USA. The transmission electron microscope (TEM-2,2100HR, JEOL Ltd., Tokyo, Japan) was the tool for the ultra-high-accuracy description of ferric nanoparticles that occurred in the aqueous solution formerly the full deposition on carbon lattices surfaces. A Nano Zeta Sizer (Nano-ZS Malvern Instruments Ltd., London, UK) was applied to count the zeta potential of the formed nanoparticles at neutral pH and room temperature-Ultraviolet-visible (UV-Vis) spectroscopy was used for the optical observation of the NP construction by monitoring the changes in the solution color over the incubation time. The UV–Vis analysis was carried out using JASCO NIR Spectrophotometer/ model: V-770. To determine the presence of specific surface functional groups of the studied nanoparticles, an FT-IR spectrophotometer was used. The analysis was performed using Infrared Spectrum Origin Jasco: model, FT-IR 6800typeA. All these analyses were carried out at Nano Science and Technology Institute, Kafr Elsheikh University, Egypt.

2.4. Samples collection

The fungal isolation sources were the football players' body as Foot, toes press, between toes, hand, fingers press, between fingers, underarm, posterior knee fold, and groin area of eleven volunteer football players (under 20 years old). Isolation was done immediately after training on the grass-land football playground. Sampling was performed at Port Said Sporting City, Port Said, Egypt. Mycological study was conducted in the faculty of science at Port Said University. The samples were obtained and stored in an icebox vessel. The trial was preserved in the fridge at 5 °C to evade any deviations in the fungal assemblages, and then transported to Environmental Science Department laboratory, Port Said University,

Egypt for examination. The fungal growth-effecting parameters including pH, temperature, and cerium oxide nanoparticles (CeO_2NPs) were examined.

2.5. Isolation and identification of fungal isolates via via spacer between 18S and 28S rRNA genes

To isolate the study fungal species, modified Sabouraud Dextrose agar media, pH 5.6 was used, and it contained [dextrose (40 g), a peptic digest of animal tissue (5 g), a pancreatic digest of casein (5 g) and agar (15 g) +1.0 L distilled water]. Chloramphenicol 500 mg (antibiotic) in 10 ml of water for bacteria growth inhibition was added to a molten medium and stirred, [16]. The swabbing technique was applied, in this technique; the cotton swab bud applied on a surface recover fungi spores, cotton swabs were then used to distribute fungi on the media in Petri-dishes [17]. The press culture technique was applied to isolate fungi from fingers and toes only by pressing them onto the medium. Pure fungal isolates were attained subsequently sub-culturing on a similar medium plate. The dishes of isolated fungi were incubated at room temperature approximately 25 °C, examined the agar plates daily for 14 days for growth [3]. For purification and identification of fungi, the growing fungi were purified in pure cultures on slants of the medium. Eleven pure culture fungal slants were identified. The spacer between 18S and 28S genes identification technique was completed and intended for the eleven isolated fungal species identification. The gene investigation was established by the Sigma company laboratory, in Egypt [18]. To get the maximum prolonged possible high-quality sequences, forward and reverse reads similar to each fungal strain were collected by consuming Phrap (version 0.990329) with evasion parameters. Then, the gathered structures were cut such that some basis with a valuable record of less than 30 (99.9% base call precision) was rejected. The PCR outcome sequence study was accomplished by the software of Finch TV which completed the spacer between 18S and 28S genetic material of the nucleotide building. BLAST (Basic Local Alignment Search Tool) operational record that refers to NCBI (National Center for Biotechnology Information) was used to define the achievements of the subject's sequences placed in the global nucleotide databases (Gen Bank), recognizing the utmost identical with the query sequence [19].

2.6. Experimental setup

2.6.1. Inoculum preparation

Fresh, mature (5-day-old) fungal cultures grown on modified Sabouraud Dextrose agar slants were used for inoculum suspension preparation. For each fungal isolate, the colonies were covered with 10 mL of distilled sterilized water. Tween 20 (5%) was added to help in the fungal inocula preparation. The inocula were done by gently rubbing the colonies with a sterile loop; the isolates were then shaken strongly for 15 seconds with a vortex mixer (Cole-Parmer Vortex Mixer, 0 to 3400 rpm, 115 VAC, China) and then transferred to a sterile tube [20]. Then, the inoculum hyphae were separated by filtering the spore suspension through the sterilized muslin cloth [21]. The suspension was filtered and collected in a sterile tube. This procedure removed the majority of the hyphae, producing an inoculum mainly composed of spores.

2.6.2. Influence of some environmental factors on the fungal growth

To examine the fungal growth under different environmental conditions, 1.0 mL of each pure fungal spore suspension was inoculated separately inside 100 ml sterilized modified Sabouraud Dextrose broth medium and incubated for ten days. The Influence of three medium pH values (e.g., 4.5, 5.5, 6.5, and 7.5), and three temperature degrees (e.g., 15.0, 25.0, 35.0, and 45.0 ^{0}C) were examined. The solution pH was adjusted before the sterilization step using HCl (0.5 M), and NaOH (0.5 M) to maintain pH values around 4.5, 5.5, 6.5, and 7.5 respectively. The mycelial mat was separated by filtering the spore suspension through the sterilized muslin cloth. The stock suspension was kept on Rotary Flask Shaker (MAC, MSW-301, India) for 30 min. The fungal spore count as an indicator of fungal growth was measured using a Jenway model 6800 spectrophotometer at a wavelength of 550 nm [21]. Tests had been carried out in triplicate and the averages had been recorded.

2.6.3. Influence of different concentrations of CeO_2NPs on the fungal isolates growth inhibition

To examine the influence of CeO_2NPs on fungal growth, different quantities of CeO_2NPs (e.g., 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 g/l) were dissolved separately in modified Sabouraud Dextrose broth media and autoclaved at 121.0 °C for 20.0 min [22]. Afterward, 1 mL of each pure fungal spore suspension was inoculated inside 100 mL media, then incubated under optimized conditions of pH 5.5, and a temperature of 25.0°C for ten days. CeO_2NPs-free media cultured under the same condition were used as controls; moreover, the absorbance of the culture-free medium was recorded. The fungal spore count as an indicator of fungal growth was measured as described earlier in section 2.6.2. Tests had been carried out in triplicate and the averages had been recorded.

2.7. Statistical investigations

The outcomes were stated with an average ± Standard Deviation, and whole experiments were performed in triplicate. Statistical Package for the Social Sciences (SPSS version 19. IBM Corporation) was employed to evaluate the obtained data. The statistic value of $P < 0.05$ was deemed substantial.

3. Results and discussion

3.1. Characterization of used CeO_2NPs

As documented previously [23], the XRD pattern diffraction peaks of CeO_2NPs planes showed peaks at 28.14°, 32.64°, 47.16°, 56.00°, 58.84°, 68.96°, 76.24° and 78.90° corresponding to [346], [112], [218], [166], [38], [50], [76] and [50] planes of cubic CeO_2 lattice Fig (1). This pattern showed that the particles have very sharp peaks with ultrafine structure and great crystalline cubic spinel construction, which confirms the purity and good formation [24]. TEM photomicrograph of CeO_2NPs Fig (2). Indicating that, the nanoparticles have an isotropic shape [25], within a range of 20–40 nm in size. The Zeta potential analysis value of used CeO_2NPs was 1.5 mV Fig (3). Indicating that, the particles have more colloidal stability with repulsive forces to prevent agglomeration. According to previous research articles, nanoparticles having a zeta potential larger than +25 mV or less than -25 mV have higher degrees of colloidal stability due to repulsive forces that prevent NPs from aggregating together [26]. Large positive or negative zeta potential magnetic particles repel each other, resulting in a non-aggregated solution with good particle stability [27]. The obtained result of studied CeO_2NPs indicated that the studied CeO_2NPs have a suitable dispersion capability in the aqueous medium. Ultraviolet–visible analysis of CeO_2 NPs spectra at wavelengths of 200–800 nm was used to explain the great absorption peak Fig (4), which related to superficial Plasmon excitation [28]. The sharp peak assumed by the UV–Vis spectrum at the absorption wavelength is 340 nm. Fourier transform infrared (FT-IR) analysis of CeO_2 NPs in terms of wavenumber vs transmittance (%) using FT-IR spectrometer in the wavelength range of 400–4000 Cm^{-1} at a resolution of 2 Cm^{-1} in KBr pellets [29], where the maximum transmittance is 38.75% and the minimum transmittance is 7.31% Fig (5).

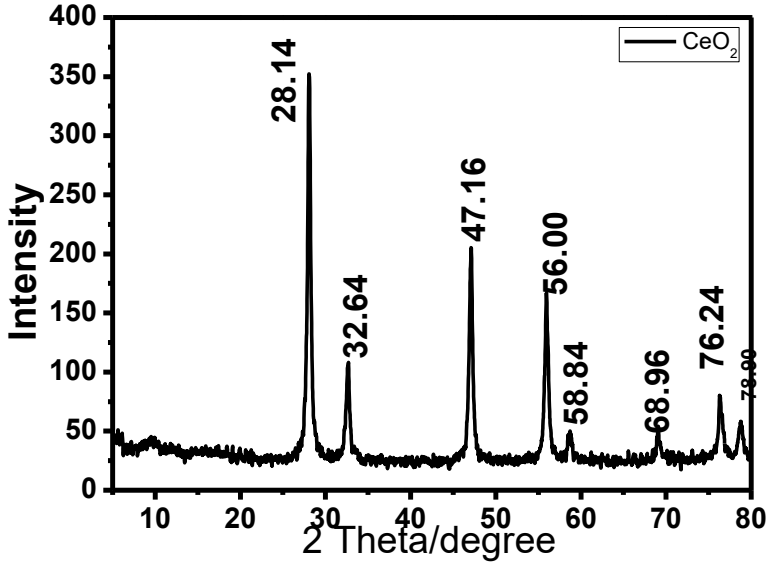

Fig (1) cerium oxide nanoparticles (XRD) analysis.

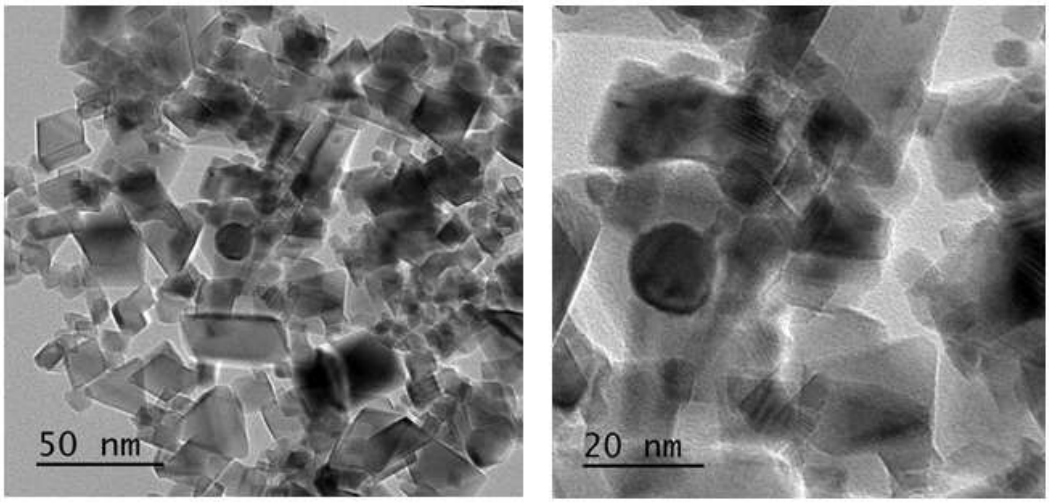

Fig (2) Cerium oxide nanoparticles TEM photomicrograph.

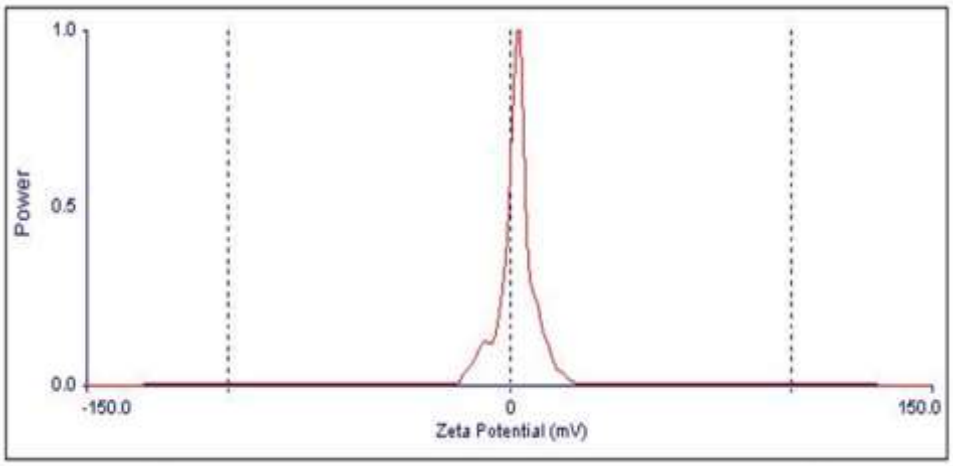

Fig (3) Cerium oxide nanoparticles zeta potential analysis, showing a peak at 1.5 mV.

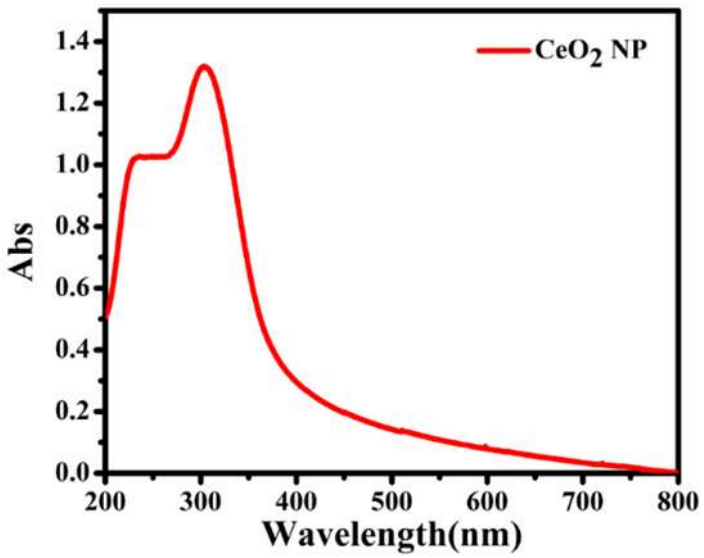

Fig (4) Cerium oxide nanoparticles (U-V) analysis.

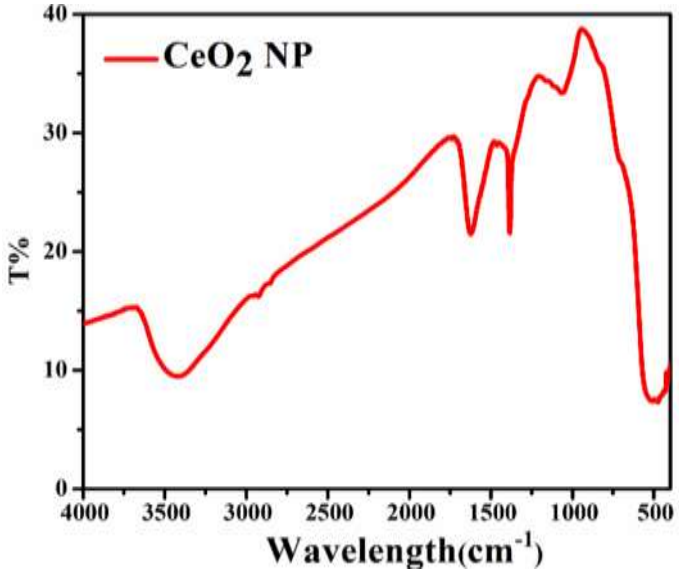

Fig (5) Cerium oxide nanoparticles (FTI) analysis.

3.2. Isolation and identification of fungal isolates

Isolated eleven fungal species from football players' different body parts with a total count of 634 is shown in Table 1. For football players' bodies, the maximum count was detected between the toes area, and the minimum count was detected with the posterior knee folds area. The phylogenetic analysis of eleven different fungal strains was successfully performed as displayed in Table 2. The results showed great identity, high query cover, max score, and a total score while zero for E-value with other world fungal species [19]. Referring to the literature, the isolated fungal species in the present study have been demonstrated to cause skin diseases for football players' as illustrated in Section 3.3.

3.3. Role of isolated fungal species in skin diseases for football players' from literature

To assess the inclusive effect of the isolated fungal species, a comprehensive survey was particularly researched to reveal the contribution of various species in the fungal skin disease studies. Satisfactory findings are listed in Table 3.

Table 1. Mean, ± SD, and total colonies count of eleven fungal species isolated from football players' different body parts.

Fungal Name		Isolation football players' different body parts									Total count (T)
		Foot	Toes press	Between toes	Hand	Fingers press	Between fingers	Underarm	Posterior knee folds	Genital area	
A. flavus	N	6	10	12	4	7	8	3	5	11	66
	Mean	0.55	0.91	1.09	0.36	0.64	0.73	0.27	0.45	1.00	-
	± SD	0.69	0.30	0.70	0.50	0.92	1.10	0.65	0.52	0.89	-
A. alternata	N	4	7	8	5	6	10	1	4	7	52
	Mean	0.36	0.64	0.73	0.45	0.55	0.91	0.09	0.36	0.64	-
	± SD	0.67	0.50	0.65	0.69	0.52	0.83	0.30	0.67	0.92	-
A. iranianum	N	6	9	10	5	7	11	3	2	9	62
	Mean	0.55	0.82	0.91	0.45	0.64	1.00	0.27	0.18	0.82	-
	± SD	0.52	0.60	0.94	0.69	0.92	0.63	0.65	0.40	0.87	-
P. chrysogenum	N	9	12	14	7	7	8	3	3	5	68
	Mean	0.82	1.09	1.27	0.64	0.64	0.73	0.27	0.27	0.45	-
	± SD	0.40	0.83	1.01	0.92	0.67	1.01	0.47	0.47	1.04	-

Table 1. Continued.

Fungal name		Isolation football players' different body parts									Total (T)
		Foot	Toes press	Between toes	Hand	Fingers press	Between fingers	Underarm	Posterior knee folds	Groin area	
A. nidulans	N	3	4	8	5	7	8	6	2	5	48
	Mean	0.27	0.36	0.73	0.45	0.64	0.73	0.55	0.18	0.45	-
	± SD	0.47	0.50	0.90	0.69	0.81	0.90	0.52	0.40	0.82	-
A. lentulus	N	7	9	11	6	9	12	3	0	4	61
	Mean	0.64	0.82	1.00	0.55	0.82	1.09	0.27	0.00	0.36	-
	± SD	0.67	0.87	1.00	0.52	0.98	1.30	0.47	0.00	0.81	-
C. cladosporioides	N	5	6	8	6	7	9	9	2	6	58
	Mean	0.45	0.55	0.73	0.55	0.64	0.82	0.82	0.18	0.55	-
	± SD	0.69	0.52	0.79	0.93	0.92	0.40	0.87	0.40	0.52	-
C. dominicanum	N	4	5	7	5	10	13	10	2	6	62
	Mean	0.36	0.45	0.64	0.45	0.91	1.18	0.91	0.18	0.55	-
	± SD	0.50	0.52	0.50	0.69	0.94	0.60	0.83	0.40	0.69	-

Table 1. Continued.

Fungal name		Isolation football players' different body parts									Total (T)
		Foot	Toes press	Between toes	Hand	Fingers press	Between fingers	Underarm	Posterior knee folds	Groin area	
C. sphaerospermum	N	9	10	13	7	7	7	0	4	3	60
	Mean	0.82	0.91	1.18	0.64	0.64	0.64	0.00	0.36	0.27	-
	± SD	0.75	0.94	0.87	0.67	0.50	0.67	0.00	0.67	0.47	-
C. oxysporum	N	5	6	8	6	8	12	5	3	5	58
	Mean	0.45	0.55	0.73	0.55	0.73	1.09	0.45	0.27	0.45	-
	± SD	0.52	0.82	0.65	0.69	0.65	0.54	0.52	0.47	0.69	-
C. halotolerans	N	5	5	6	5	3	5	4	2	4	39
	Mean	0.45	0.45	0.55	0.45	0.27	0.45	0.36	0.18	0.36	-
	± SD	0.52	0.69	0.52	0.69	0.47	0.52	0.50	0.40	0.50	-
Total (T)		63	83	105	61	78	103	47	29	65	634

Table 2. Fungal isolates spacer between 18S and 28S genes sequence information.

Sequence association description	Sequence association No.	Genome size (bp)	Identity	Associated NCBI tax ID
***Aspergillus flavus* clone SF 591 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**	MT529867.1	595	100%	5059
***Alternaria alternata* genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene**	OU989241.4	596	100%	5599
***Aureobasidium iranianum* isolate 4Y44 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence**	MT116788.1	515	99.75%	1226028
***Penicillium chrysogenum* isolate broom corn millets small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA**	MT229079.1	589	100%	5076

gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence				
***Aspergillus nidulans* strain HM22 large subunit ribosomal RNA gene, partial sequence**	MT609897.1	562	100%	162425
***Aspergillus lentulus* 28S ribosomal RNA (IFM58399_r29), rRNA,**	XR_004500616.1	3470	100%	293939
***Cladosporium cladosporioides* strain EECC-462 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**	KP942857.1	554	100%	29917
***Cladosporium dominicanum* strain SCAU014 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence,**	KY827344 .1	538	100%	1052092
***Cladosporium sphaerospermum* strain RCPF 90 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**	MT534178. 1	536	100%	92950
***Cladosporium oxysporum* strain EpF3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**	MZ578166.1	522	100%	29919

Table 2. Continued.

Sequence association description	Sequence association No.	Genome size (bp)	Identity	Associated NCBI tax ID
***Cladosporium halotolerans* strain AUMC 11387 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**	MN826823.1	554	100%	1052096

Table 3. Fungal name and pathogenicity of eleven fungal species isolated from football players' different body parts.

Fungal name	Pathogenicity
Aspergillus flavus	Common clinical syndromes associated with *A. flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections, and osteomyelitis following trauma and inoculation [30].
Alternaria alternate	Cause several different types of human infections, including paranasal sinusitis, ocular infections, onychomycosis, and cutaneous and subcutaneous infections [31].
Aureobasidium iranianum	*Aureobasidium* species are reported as the etiologic agent of pheohyphomycosis, keratomycosis, septicemia, peritoneal sepsis, and dermatological infections in humans [32].
Penicillium chrysogenum	A case of cutaneous penicilliosis in a young man [33].
Aspergillus nidulans	Causing cutaneous aspergillosis [34].
Aspergillus lentulus	Cause infections that range from superficial infections of the skin to life-threatening systemic infections [35].
Cladosporium cladosporioides	Reported as a cause of subcutaneous phaeohyphomycosis with cysts and nodules on his face and neck. Phaeohyphomycosis refers to infections of the skin, subcutaneous tissues, and internal organs of man [36,37].
Cladosporium dominicanum	These species have been associated with a number of human infections such as chromoblastomycosis, an opportunistic infection described as chronic skin and subcutaneous tissue fungal infection, and phaeohyphomycosis, a term generally used to describe infections caused by dematiaceous fungi [38].
Cladosporium sphaerospermum	Commonly distributed allergen. *Cladosporium* can cause several different types of infections, including skin, eye, sinus, and brain infections [39].
Cladosporium oxysporum	Reported as a cause of subcutaneous phaeohyphomycosis [40].
Cladosporium halotolerans	Reported as a cause of cutaneous infections in a wet environment [41].

3.4. Optimized conditions for the growth of isolated fungi

The growth of isolated fungal species under different environmental factors of medium pH (e.g., 4.5, 5.5, 6.5, and 7.5), and temperature (e.g., 15.0, 25.0, 35.0, and 45.0 ^{o}C) was regularly evaluated. A considerable (optimized) microbial consortium growth was achieved at a pH of 5.5 and a running

temperature of 25.0 ^{o}C. The absorbance pattern values of fungal growth media at wavelength 550.0 nm among different environmental factors and their statistical significance were illustrated in Tables S1-S4 and Figures S1 and S2 (see Supplementary information). Environmental conditions like pH, and temperature play an important role in fungal growth. Our experimental outcomes are in accordance with other studies from the literature, indicating that ranges pH 5.5-6 and a range temperature 25.0-30°C for fungal growth are the key factors that present the best experimental conditions enhancing the fungal growth [42-44] and the later study experiment performing. For superficial fungal infections, a normal stratum corneum in a fungal cell needs the continual synthesis of the lipid layer which includes numerous pH-dependent enzymes. Among these enzymes are β-glucocerebrosidase, acid sphingomyelinase, acid lipases, phosphatases, and phospholipases. The optimal pH condition for β-glucocerebrosidase in the synthesis of the important ceramides is 5.6. It is also recognized that the acidity in the extracellular environment of the stratum corneum may affect intracellular enzymatic activities [45]. Every fungus has an optimum growth temperature at which it shows the highest growth. Usually, most of the skin fungi grow at the temperature range of 25 ^{o}C to 30 ^{o}C, at this temperature range the fungal colony diameter was found to be the widest measure [46]. The high temperature might lead to fungal cell rupture and damage of membrane or loss of the cytoplasmic compounds and cell death. Because the skin fungi grow well in the culture at a temperature lower than 37 ^{o}C. Increasing or decreasing temperature than the optimum range may lead to the breakdown of enzymes. The optimum growth temperature enhances the enzyme activity which reaches the top and then they use the food source in the media to build macro-molecular and then build the fungal mass [47]. In the present study, the ranges of various environmental factors considered as limiting factors for skin fungal growth and can be employed in enhancing and controlling its growth.

3.5. Influence of CeO_2NPs on fungal isolate's growth

The growth of isolated fungal species under different cerium oxide NPs concentrations was regularly evaluated. Optimized nanoparticles doze for fungal isolates inhibition was achieved at a concentration 12 g/L. The absorbance pattern values of fungal growth media at wavelength 550.0 nm among different cerium nanoparticle concentrations and their statistical significance were illustrated in Tables 4 and 5. Many studies verified that NPs have important effects on microbial growth. Our experimental outcomes are in accordance with other studies from the literature, indicating that the antimicrobial activity of CeO_2 NPs was examined and the test results at a concentration of 10 -12 g/L showed higher activities than the other concentrations on the microbial growth inhibition [22]. The fungal cell may be destroyed by the effect of nanoparticles by different methods such as: accelerating lipid peroxidation, perforation, and the leakage of the fungal cell [48], blocked cell division in mycelia, and condensation of chromatin [49] and inhibiting enzyme catalytic_sites and caused the denaturation of enzymes, resulting in the arrest of translation, protein assemblage, and protein folding and the induction of chitin oxidation [50,15].

Table4. Average (±SD) of microbial growth media absorbance at (550 nm), using different concentrations of CeO_2NPs with different fungal isolates, after ten days of incubation.

Fungal Isolate	CeO_2NPs concentration (g/L)						
	Control	**2.0**	**4.0**	**6.0**	**8.0**	**10.0**	**12.0**
Aspergillus flavus	0.665 ± **0**.020	0.640±0.008	0.536± 0.005	0.514± 0.003	0.439± 0.018	0.250± 0.020	0.010±0.001
Alternaria alternate	0.557±0.009	0.520±0.009	0.442±0.017	0.353±0.033	0.315±0.013	0.288±0.011	0.010±0.001
Aureobasidium iranianum	0.340± 0.017	0.323± 0.012	0.229± 0.005	0.188± 0.013	0.146± 0.006	0.130± 0.007	0.010±0.001
Penicillium chrysogenum	0.409± 0.006	0.386± 0.013	0.342± 0.016	0.329± 0.008	0.294± 0.008	0.230± 0.007	0.010±0.001
Aspergillus nidulans	0.365±0.015	0.296±0.007	0.261±0.015	0.220±0.005	0.193±0.006	0.171±0.004	0.010±0.001
Aspergillus lentulus	0.255±0.013	0.211±0.010	0.184±0.004	0.162±0.005	0.126±0.002	0.090±0.010	0.010±0.001
Cladosporium delicatulum	0.744±0.014	0.670±0.006	0.507±0.007	0.414±0.014	0.394±0.023	0.274±0.015	0.010±0.001

Continued table 4.

Fungal Isolate	CeO_2NPs concentration (g/L)						
	Control	**2.0**	**4.0**	**6.0**	**8.0**	**10.0**	**12.0**
Cladosporium dominicanum	0.440±0.012	0.425±0.006	0.382± 0.017	0.305± 0.003	0.245± 0.011	0.176± 0.005	0.010±0.001
Cladosporium sphaerospermum	0.631±0.037	0.533±0.012	0.424±0.022	0.338±0.006	0.259±0.010	0.207±0.003	0.010±0.001
Cladosporium oxysporum	0.636± 0.017	0.576± 0.011	0.478± 0.016	0.417± 0.016	0.351± 0.013	0.236± 0.014	0.010±0.001
Cladosporium halotolerans	0.522± 0.010	0.418± 0.023	0.367± 0.011	0.307± 0.006	0.278± 0.010	0.130± 0.013	0.010±0.001

Note: The absorbance of culture-free medium is 0.010±0.001

Table 5. GLM test for variation in fungal growth media absorbance at (550.0 nm), using different concentrations of CeO_2NPs (g/L) with different fungal isolates, after ten days of incubation.

Growth media	Source	DF	Seq SS	Adj SS	Adj MS	F-value	P-value
Fungal growth media + different concentrations of CeO_2NPs	Conc. of NPs (g/L)	6	5.52269	5.52269	0.92045	299.79	0.000
	Fungal isolates	10	1.87167	1.87167	0.18717	60.96	0.000
	Error	214	0.65706	0.65706	0.00307	-	-
	Total	230	8.05142	-	-	-	-

GLM: General Linear Model; DF: Degree of freedom; Seq SS: Sequential sums of squares; Adj SS: Adjusted sums of squares; Adj MS: Adjusted mean squares.

4. Conclusions

In summary, the present study provides momentous insights into the introduction of cerium oxide nanoparticles (CeO_2NPs) as growth-inhibiting material for some fungal species isolated from football players' bodies as hand, fingers press, between fingers, knee, foot, toes press, between toes and groin area of eleven football players at Port Said sporting city, Port Said government, Egypt. Eleven fungal species which isolated from eleven football players' different body parts with a total count of 634. For football players' bodies, the maximum count was detected between the toes area, and the minimum count was detected with the posterior knee fold area. The progress in the fungal isolates was analyzed spectrophotometrically at wavelength 550.0 (nm) after ten days of incubation. Moreover, the designed mixtures of CeO_2NPs and grown fungal isolates have been extensively employed in the reduction of fungal growth. The findings revealed that the fungal isolates were remarkably enhanced under a pH of 5.5 and temperature of 25 °C. Moreover, the fungal growth markedly decreased with an increase in the CeO_2NPs concentration and stop completely at a concentration of 12 g/L. compared to the non-treated CeO_2NPs sample. To sum up, the present study proposed a momentous scenario for the potency of cerium oxide nanomaterials in football players' skin fungal infection control.

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SUPPLEMENTARY INFORMATION

Table S1. Average (±SD) of fungal growth media absorbance at (550.0 nm), using different pH values.

Fungal isolates	pH values			
	4.5	5.5	6.5	7.5
Aspergillus flavus	0.454±0.009	0.613±0.046	0.533±0.008	0.404±0.003
Alternaria alternata	0.356±0.011	0.530±0.007	0.402±0.007	0.313±0.007
Aureobasidium iranianum	0.268±0.003	0.353±0.013	0.307±0.006	0.219±0.002
0Penicillium chrysogenum	0.313±0.003	0.409±0.006	0.353±0.011	0.296±0.015
Aspergillus nidulans	0.209±0.002	0.339±0.006	0.291±0.007	0.181±0.007
Aspergillus lentulus	0.171±0.003	0.270±0.005	0.195±0.005	0.145±0.002
Cladosporium delicatulum	0.587±0.005	0.710±0.010	0.674±0.012	0.518±0.009
Cladosporium dominicanum	0.297±0.015	0.460±0.013	0.370±0.017	0.226±0.11
Cladosporium sphaerospermum	0.467±0.007	0.636±0.033	0.531±0.002	0.406±0.003
Cladosporium oxysporum	0.527±0.006	0.636±0.017	0.580±0.012	0.470±0.010
Cladosporium halotolerans	0.400±0.014	0.522±0.010	0.454±0.004	0.330±0.009

Table S2. GLM test for variation in fungal growth media absorbance at (550.0 nm), using different pH values after ten days of incubation.

Growth media	Source	DF	Seq SS	Adj SS	Adj MS	F-value	P-value
Fungal growth media using different pH values	pH values	3	0.58695	0.58695	0.19565	414.98	0.000
	Fungal isolates	10	2.13682	2.13682	0.21368	453.23	0.000
	Error	118	0.05563	0.05563	0.00047	-	-
	Total	131	2.77941	-	-	-	-

GLM: General Linear Model; DF: Degree of freedom; Seq SS: Sequential sums of squares; Adj SS: Adjusted sums of squares; Adj MS: Adjusted mean squares.

Table S3. Average (±SD) of fungal growth media absorbance at (550.0 nm), using different temperature degrees (^{0}C).

Fungal isolates	Temperature degrees (^{0}C)			
	15	25	35	45
Aspergillus flavus	0.462±0.005	0.607±0.004	0.567±0.005	0.389±0.013
Alternaria alternata	0.406±0.003	0.519±0.008	0.429±0.004	0.300±0.019
Aureobasidium iranianum	0.181±0.007	0.310±0.010	0.212±0.003	0.143±0.011
0Penicillium chrysogenum	0.256±0.011	0.387±0.014	0.312±0.006	0.211±0.001
Aspergillus nidulans	0.199±0.010	0.316±0.004	0.274±0.005	0.150±0.006
Aspergillus lentulus	0.109±0.006	0.235±0.006	0.177±0.003	0.086±0.010
Cladosporium delicatulum	0.482±0.006	0.667±0.015	0.545±0.007	0.397±0.016
Cladosporium dominicanum	0.298±0.008	0.410±0.006	0.357±0.009	0.250±0.010
Cladosporium sphaerospermum	0.364±0.021	0.589±0.005	0.424±0.022	0.274±0.007
Cladosporium oxysporum	0.477±0.005	0.603±0.002	0.568±0.008	0.395±0.019
Cladosporium halotolerans	0.310±0.003	0.498±0.008	0.397±0.009	0.274±0.006

Table S4. GLM test for variation in fungal growth media absorbance at (550.0 nm), using different temperature degrees (^{0}C) after ten days of incubation.

Growth media	Source	DF	Seq SS	Adj SS	Adj MS	F-value	P-value
Fungal growth media using temperature degrees (^{o}C)	Temperature degrees (^{o}C)	3	0.77774	0.77774	0.25925	433.50	0.000
	Fungal isolates	10	1.96424	1.96424	0.19642	328.45	0.000
	Error	118	0.07057	0.07057	0.00060	-	-
	Total	131	2.81255	-	-	-	-

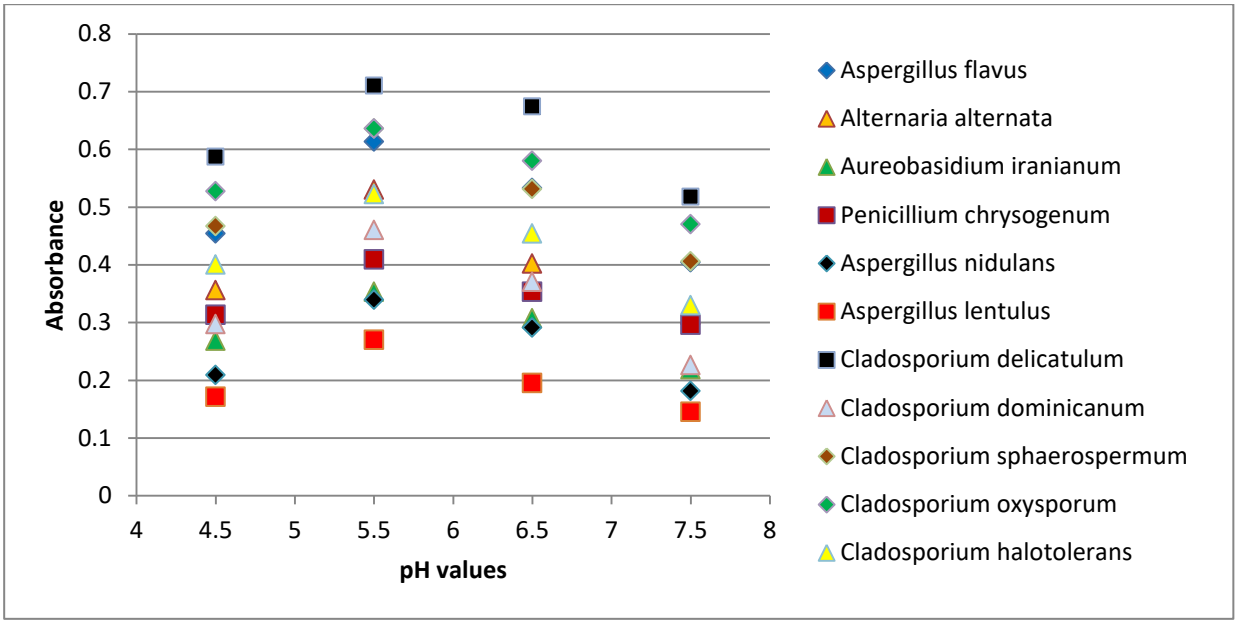

Figure S1. Fungal growth media absorbance at (550.0 nm), using different pH values.

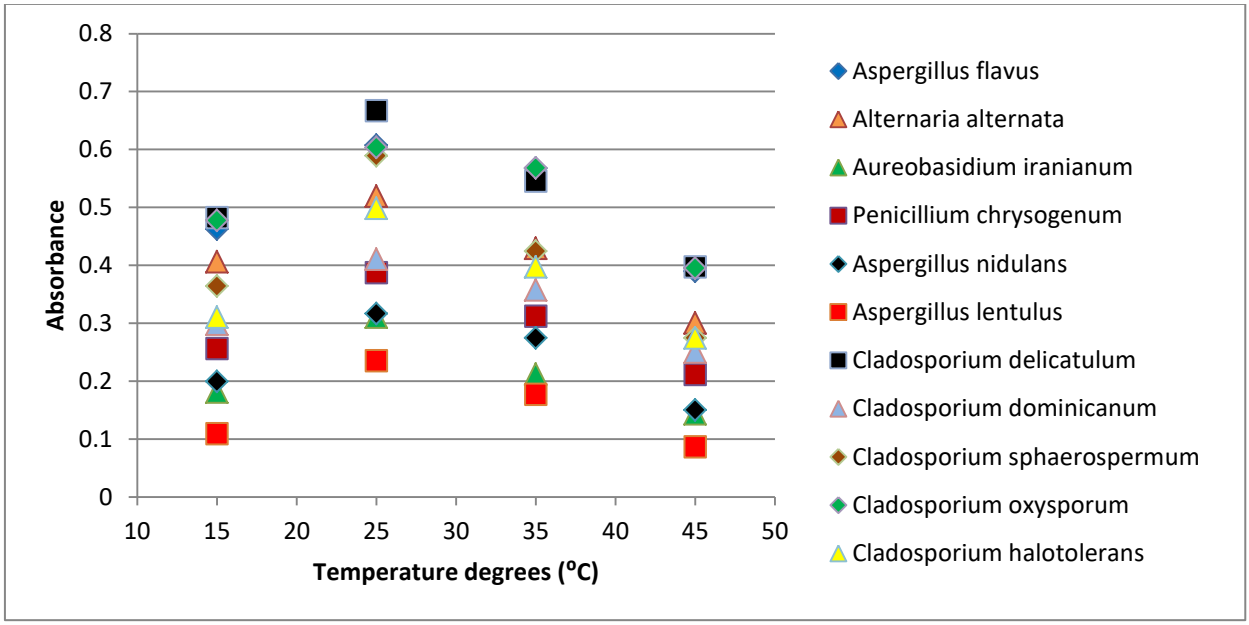

Figure S2. Fungal growth media absorbance at (550.0 nm), using different temperature degrees (°C).