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Detection the Antifungal Effect of Zirconium Oxide Nanoparticles on Mold which Isolated from Domestic's

Dina Y. Mohammed
Department of Biology, College of Science, Mustansiriyah University, IRAQ.
**Author email: dr.dinayousif@uomustansiriyah.edu.iq*

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Abstract

The aim of this study is to detection the antifungal effect of Zirconium oxide nanoparticles on mold which isolated from domestic's bathroom during April 2017 in Baghdad City. Twenty species were isolated from one hundred samples which were *Aspergillus niger, Aspergillus. flavus,, Aspergillus duricaulis, Aspergillus nidulans Aspergillus. parasiticus, Aspergillus fumigatus, Aspergillus. brasiliensis, Aspergillus heteromorphus, Curvularia lunata, Penicillium sp., Fusarium oxysporum,, Alternaria alternate, Cladosporium sp. Trichoderma sp., Mucor, Rhizopus sp., Rhodotorula mucilaginosa,, Stachybotrys and yeast. Among the isolated species Aspergillus niger,* was the most abundant (14.92%) frequency, followed by *Aspergillus flavus* (10.14%), while less abundant (0.95 & 0.63 %) *Cladosporium sp. &, Mucor* respectively. The higher inhibition zone of fungal growth was recorded at 8mg/ml concentration of Zirconium oxide nanoparticles which was (3.8cm) in molds *Aspergillus niger, Aspergillus. flavus,, & Aspergillus. brasiliensis*.

Keywords: Nanoparticles, Nano-ZrO2 and Mold

الخلاصة

هدفت الدراسة الحالية التحري عن فعالية التضادية لدقائق الزركونيوم النانوية ضد الاعفان المعزولة من الحمامات المنزلية، حيث تم عزل وتشخيص الاعفان من الحمامات المنزلية لمدينة بغداد خلال شهر نيسان 2017.تم عزل عشرون نوع من الاعفان من مئة مسحة وهم *Aspergillus niger* و*Aspergillus. flavus* و *Aspergillus. duricaulis* و *Aspergillus. nidulans* و *Aspergillus. parasiticus* و *Aspergillus. fumigatus* و*Aspergillus. heteromorphus* و*Aspergillus. brasiliensis* و *Curvularia lunata* و *Penicillium sp* و *Fusarium oxysporum* و *Alternaria sp.*, و *Cladosporium sp* و *Trichoderma sp* و *Mucor sp.*, و *Rhizopus sp* و *Stachybotrys*و*mucilaginosa*, والخمائر . ومن بين هذة العزلات ، أظهرت النتائج أن اعلى نسبة تكرار للاعفان كانت للفطر *A.niger* بلغت (14.92%) تبعها الفطر *Aspergillus. flavus* بنسبة (10,14%) في حين كانت اقل نسبة تواجد للفطريات هو *Cladosporium sp* و*Mucor sp.*, حيث بلغت نسبة تواجدهما 0,95 % و0,63 % على التوالي). وقد بلغت اعلى نسبة تثبيط لدقائق الزركونيوم النانوية ضد الاعفان المعزولة عند تركيز 8 ملغم /مل حيث بلغت قطر التثبيط(3,8سم) عند الفطريات *Aspergillus niger* و*Aspergillus. flavus* و *A. brasiliens s*.

Introduction

Nanotechnology has got important application for enhancing agricultural productivity, along with other emerging technologies such as biotechnology including breeding of plant, controlling different diseases, genetics, fertilizer technology, agriculture roles, and with other relation fields [1,2,3,4]. In recent years, nanoparticle (NP) materials have received increasing attention because their special unique physical and chemical properties, which differs significantly from their conventional counterparts [5]. Many types of nanomaterials such as copper, zinc, silver titanium [13] magnesium, gold [6] alginate [10] and have been study. Although, little work has been done on the antibacterial activity of zirconium dioxide. However, zirconium NPs has been shown antibacterial and antifungal activity [7, 8].

Zirconium oxide NPS can consider one of the most intensively studied materials owing to its technologically of applications in O_2 sensors, cell electrolytes fuel, catalysts and chemical stability etc. [9, 10, 11, 12].

The current study has two aims which are: isolation, identification of fungi (mold and yeast isolated from domestic's bathroom. The second aim is to test the ability of (ZrO2 NPs) in inhibition of fungal.

Materials and Methodologies

Collection samples

During April 2017, one hindered samples were collect from random domestic's bathrooms in Baghdad city by used sterile cotton swabs .After collection they were to return the samples as soon as possible to the laboratory.

Isolation, and Identification of fungi

In the laboratory, all samples were inoculated on to Sabouraud Dextrose Agar (SDA) and then incubated at 28 ±2 °C for 7 days. When hypha growth was observed, purification was carried out by cutting a tiny piece of media with hypha at the edge of a colony and then transplanted onto new medium plates. The colonies of mold were identified macro and microscope for comparison of fungal morphology with descriptions given by [13, 14, 15, 16, 17, 18]. Routine culture approach, for the identification of *Aspergillus* in the level of species, used four differential media including: czapek dox agar (CZ), czapek yeast agar (CYA) malt extract agar (MEA) and Potato Dextrose Agar (PDA). The texture, the surface and reverse color of colony were noted in addition to any dye that diffused in to the media[24]. The purified fungal isolates were stored at freeze in 20% glycerol broth until antifungal susceptibility tests were performed.

Zirconium oxide nanoparticles (ZrO2NPs)

Dry synthetic industrial Zirconium oxide nanoparticles were procured from Eprui nanoparticles & microspheres company-china

Effect of different concentrations of ZrO2NPs on vegetative growth of fungi

Suspension of spores for different fungal isolates is collecting after spreading in SDA medium. The filter paper discs (0.5cm) in diameter were sterilized by autoclave and soaked in different concentration of ZrO2NPs (2, 4, 8, 16 and 32 mg/ml) were placed on the agar plates which was inoculated with the tested microorganisms at 28 ± 2 o C for 72 hrs. .Triple plates were used for each concentration. After incubation the growth inhibition zone were estimated by measuring the diameter of the zone of inhibition in millimeters [19].

Results and Discussions

Frequency of Mold Contamination in domestic's bathroom

Growth of mold a bear in different shape and vary color ranging from cream to radish and green to chocolate and dark black. Molds can sometimes be detected by its damp smell [20, 21]. The result observed that twenty different kinds of mold, isolate based on micro and maro characteristic morphology namely *Aspergillus sp. Curvularia sp., Penicillium sp., Fusarium oxysporum, Alternaria sp., Cladosporium sp. Trichoderma sp., Mucor sp., Rhizopus sp., Rhodotorula mucilaginosa, Stachybotrys*, and Yeast Table 1. The frequency of fungal species percentage which are 73.3%, 3.8%, 4.76%, 4.12%, 1.26%, 0.95%, 1.58%, 0.63%, 1.58%, 1.26%, 1.26%, 2.22% and 3.17% respectively as seen in Figure 1 . *Apergillus* sp. was predominant with a recovery of 73.33%. As indicated in Figure 2 Frequency growth of *Aspergillus* species are found the gravelly out all the bathrooms sample especially *A.niger* (14.92%), followed by *A. flavus* (10.47%) *A. duricaulis* (9.20%) *A. nidulans* (8.88%) *A. parasiticus* (8.25%) *A. fumigatus* (7.93%), *A. brasiliensis* (*6.98%*), and *A. heteromorphus* (6.66%).

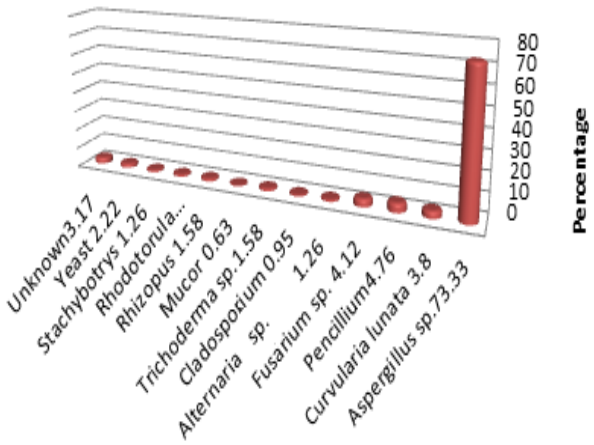

Figure 1 : Percentage growth of mold which isolated from domestic's bathroom.

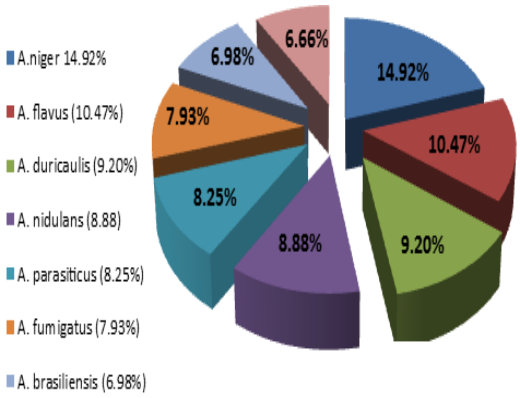

Figure 2: Growth Percentage of *Aspergillus* spp.

The study found that *Aspergillus niger* was the most common mold isolated from all the samples .Generally, *Aspergillus* species are saprophytic and ubiquitously distributed which found every wear and their conidia have been isolated at extreme environment. *Aspergillus* spores are tiny, typically ranging between 2 – 3µ and are thus easily dispersed the gravelly wind [26]. *Aspergillus* refer to Deutromycetes [21, 22] is ubiquitous, most species can survive in any environment, but certain species are more prevalent at certain niches and environments depending on its ecology and adaptation.

Cultural and microscopic features of isolated mold species

One of the primary tools to identified *Aspergillus* spp is the colonies color with slow to rapid growers; the colonies were colorless at first and turned to blue, green, yellowish-green, dark or cinnamon-chocolate. The surface was velvety, cottony, granular or fluffy. Beside that present and absent of scleirotia,, slushy dyes and other metabolic product secret in to the media could be useful to identified *Aspergillus* spp. Figure 3 and Table 1.

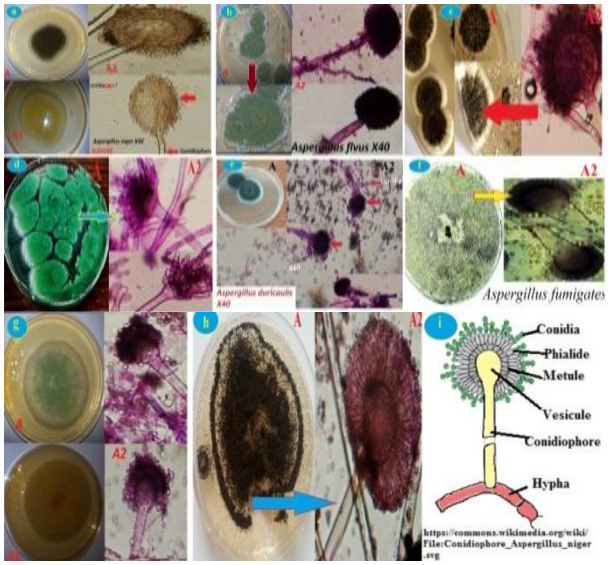

Figure 3: Cultural and microscopic features of Aspergillus species which isolated from bathroom. A= colonies on PDA A1= underside A2=under microscope X40. (a) A.niger (b) A. flavus (c) A. brasiliensis (d) A. nidulans (e)A. duricaulis (f) A. fumigatus (g)A. parasiticus (h) A. heteromorphus (i) Diagram of Aspergillus specie.

Table 1: General characteristics of the *Aspergillus* species that isolated from domestic's bathroom.

	Mould	dimeter (mm) on PDA	Stipe Texture	Serration	Blister Shape	Blister Diameter (µm)	Conidia Size (µm)	Color, Texture/Shape	Conidia Head/Shape
1	*A. niger*	49 - 59	So	D	S/ Or	36 - 52	3-7	b/r/ Or	Or
2	*A. flavus*	45 - 50	Gr	M	R	20 - 35	3 – 5.5	yg / Ro/ G	R
3	*A. fumigatus*	25 - 38	So	M	Sp/ C	22 - 34	2.5-4	g/r/ Or	S/C
4	*A. duricaulis*	32 -58	So	M	P to Clab	12 - 25	2.5-4	g/r/ Or	S/C
5	*A. brasiliensis*	35 - 49	So	D	P	32 - 38	3 - 4.5	b/r	Or
6	*A. heteromorphus*	32-57	Gr	D	S/ Or	11 - 14	3-4	b/r/ Or	Or S/ Or to Or
7	*A. parasiticus*	31 - 64	Gr	M	P/ Or	22 - 32	4 -6	g/r/ Or	R
8	*A. nidulans*	43 - 58	So	D	Sp /P	10- 14	3.5-4	g/s/S	L/C

The size of the colonies is after week of incubation; Seriation; M= Monoseriate, D= Diseriate; Blister Shape ; Sp= Spathulate, C= Clavate P = Pear form, Or = Orbicular, R = Radiate, S/ Or = Sub Orbicular. Conidia Color, Texture Shape; yg = Yellow Green, g = Green, So= Soft, Gr=Gravelly Or= Orbicular, S/C = Short Columnar, L/C = Long Columnar.

The results observed other different kinds of mold, isolate based on micro and maro characteristic morphology [22], [23], [24], [25], [26], [27], [28] namely *Curvularia sp., Penicillium sp., Fusarium oxysporum, Alternaria sp., Cladosporium sp. Trichoderma sp., Mucor sp., Rhizopus sp.,Rhodotorula mucilaginosa, Stachybotrys*, and Yeast, as see in Figure 4.

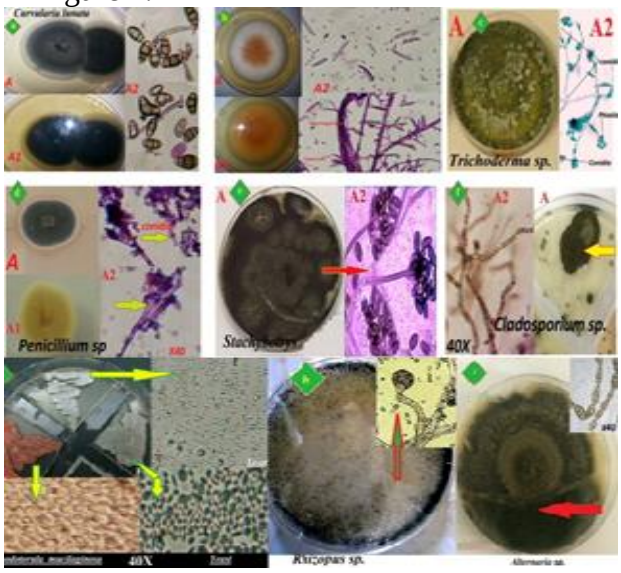

Figure 4: Cultural and microscopic features of some mold which isolated from bathroom 40X. A= colonies on PDA A1= underside A2=under microscope X40. (a) Curvularia lunata, (b) Fusarium oxysporum (c) Trichoderma (d) Penicillium sp. (e)Stachybotry sp. (f) Cladosporium sp.(g) Rhodotorula mucilaginosa (h) Rhizopus sp. (i) Alternaria sp.

Determination Antifungal activity by Paper Disk Diffusion Assay

The characteristic of Zirconium oxide nanoparticles (ZrO2 Nps) as the fallowing: colorless, with shape tetragonal and particle size: 20-30 nm, assay 99.0%.

Good reduction on all growth of molds which not depend on concentrations. In most cases [32], higher inhibition of fungal growth was recorded at a concentration of 8mg/ml. In spite of not always all fungi showed growth inhibition with the increment of concentrations of ZrO2Nps, The highest inhibition diameter (3.8 cm) was observed on PDA medium treated with 8 mg/ml concentration of ZrO2NPs against *A. niger*, *A. flavus* and *brasiliensis* .while the lowest inhibition diameter (0.5cm) was observed on PDA medium treated with both 2 and 32 mg/ml concentration of ZrO2NPs against *A. brasiliensis* and *A. fumigatus, A. heteromorphus, Penicillium sp, Curvularia lunata F. oxysporum. Trichoderma, Mucor sp, Stachybotrys sp*, Yesat1, Yesat2 respectively. As showed in Table 2 and Figure 6 there were significant different between different concentrations of ZrO2Nps on growth of mold while there was no growth inhibition in the negative control.

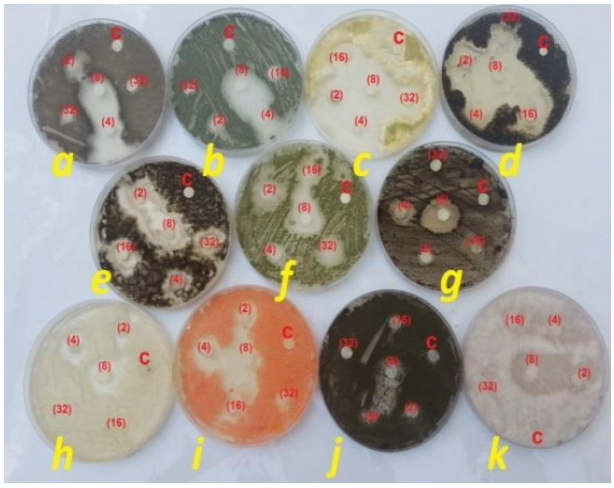

Figure 6: Inhibition zone of some mold which isolated from bathroom at different concentration. a= A. fumigatus b= Penicillium sp c= A. flavus d= A. niger e=A. brasiliensis f=A. nidulans g= A. heteromorphus h= Yeast1 i= Rh. mucilaginosa j=Curvularia lunata k=F. oxyspo.

Antifungal ability for the ZrO2 NPs may due to their huge surface was and lower size [29]. It can destroy, ergosterol in fungal cells membrane (making different gradients among the cytoplasmic membranes that keep membrane potential ability) and it causes cell death, [30,31,32].Current results of antifungal effect of Zirconium oxide NPs which proved inhibition effect of the ZrO2 NPs in treated cotton for hindering the growth of *Candida, albicans* and *A. niger* fungal strains,[33].

The current study agrees with the positive results of Abdul Jalill and others [34] they found that zirconium oxide NPs can reduce

the fungal growth and germination and decrease pathogenicity of it but they have some negative, effect on different parameters of plant growth [35]. These were compatible with other studies which proved antifungal against many pathogens, can inhibit *C. albicans* biofilm formation at the less concentration [38], and other studies [36].

ZrO2, NPs have become the most, commonly used NPs as anti- microorganisms[33], especially as antifungal[35]. Petra and others focused on an evaluation of the influence of ZrO2 NPs on the respiration and viability of microorganisms in activated sludge [38, 39]. It is good antimicrobial, agents such as Staphylococcus *aureus* and *Escherichia coli* bacterial pathogens and antifungal activity against *C.,albicans* and *A.,niger* in terms of safety, durability and heat resistance in comparison with conventional organic antibacterial agents [33, 36, 37, 38, 39].

Table 2: Effect of ZrO2NPS on the mold isolates at different concentration by Paper Disk Diffusion Assay.

Types of molds	Means(cm)different concentration mg/ml±S.D						L.S.D
	2	4	8	16	32	Cont	0.05
Aspergillus niger	**3.1±0.7**	**3.4±0.4**	**3.8±0.2**	**3.4±0.4**	**3.6±0.5**	**0.00**	**0.523 ***
Aspergillus. flavus	**2.4±0.5**	**1.4±0.5**	**3.8±0.2**	**1.3±0.2**	**2.1±0.2**	**0.00**	**0.624 ***
Aspergillus. fumigatus	**1±0.5**	**1.4±0.4**	**2.6±0.3**	**0.6±0.0**	**0.5±0.0**	**0.00**	**0.226 ***
Aspergillus duricaulis	**2.9±0.3**	**3.4±0.2**	**3.5±0.2**	**3.7±0.3**	**3.6±0.3**	**0.00**	**0.058 ***
Aspergillus parasiticus	**0.5±0.0**	**1.6±0.2**	**3.7±0.2**	**3.4±0.5**	**1.4±0.4**	**0.00**	**0.50 ***
Aspergillus heteromorphus	**0.6±0.00**	**1.5±0.1**	**3.6±0.4**	**3.3±0.3**	**0.5±0.0**	**0.00**	**0.334 ***
Aspergillus brasiliensis	**0.7±0.01**	**1.7±0.3**	**3.8±0.4**	**3.6±0.5**	**3.4±0.7**	**0.00**	**0.017 ***
A.6spergillus nidulans	**0.8±0.1**	***1.6*±0.3**	***3.6*±0**	**3.3±0.6**	**1.6±0.3**	**0.00**	**0.405 ***
Curvularia lunata	**0.6±0.1**	**0.7±0.4**	**3.8±0.4**	**0.6±0.1**	**0.5±0.0**	**0.00**	**0.213 ***
Penicillium sp	**0.7±0.1**	**1.8±0.6**	**3.8±0.6**	**1.7±0.2**	**0.5±0.0**	**0.00**	**0.929 ***
Fusarium oxysporum	**0.5±0.0**	**1.5±0.7**	**3.8±0.7**	**1.6±0.2**	**0.5±0.0**	**0.00**	**0.336 ***
Alternaria alternate	**0.7±0.0**	**1.8±0.4**	**3.8±0.4**	**1.5±0.2**	**0.7±0.1**	**0.00**	**0.912 ***
Cladosporium sp.	**0.8±0.2**	**1.6±0.3**	**3.8±0.3**	**1.1±0.2**	**0.9±0.1**	**0.00**	**0.506 ***
Trichoderma sp	**0.6±0.0**	**1.8±0.2**	**3.8±0.2**	**1.2±0.3**	**0.5±0.0**	**0.00**	**0.963 ***
Mucor sp.	**0.6±0.0**	**1.5±0.4**	**3.8±0.4**	**1.1±0.2**	**0.5±0.0**	**0.00**	**0.784 ***
Rhizopus sp.	**0.6±0.0**	**1.3±0.3**	**3.8±0.3**	**1.7±0.2**	**0.5±0.0**	**0.00**	**0.612 ***
Stachybotrys sp.	**0.6±0.0**	**1.4±0.4**	**3.8±0.4**	**1.1±0.4**	**0.7±0.1**	**0.00**	**0.841 ***
Rh.mucilaginosa	**1.4±0.2**	**2.8±0.5**	**3.8±0.5**	**1.1±0.2**	**0.6±0.1**	**0.00**	**0.339***
Yesat1	**0.6±0.0**	**0.6±0.4**	**2.2±0.4**	**0.8±0.2**	**0.5±0.0**	**0.00**	**0.305***
Yeast 2	**0.7±0.1**	**2.1±0.4**	**2.1±0.4**	**1.1±0.2**	**0.5±0.0**	**0.00**	**0.175***
L.S.D 0.05	**0.114 ***	**1.644 ***	**1.172 ***	**0.312***	**0.81***	**0.00 NS**	**---**
*** (P<0.05), NS: Non-0Significant.**							

Conclusions

The present study was designed to show the role of Zirconium oxide nanoparticles against mold It was concluded that the Zirconium oxide nanoparticles has been antifungal activity against mold and the higher inhibition zone of fungal growth was recorded against *A. niger, A. flavus,, & A. brasiliensis.*

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