Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 56 Issue: 03: 2023
DOI10.17605/OSF.IO/V47FE

ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLE AGAINST RHIZOPUS ORYZAE ISOLATED FROM DIFFERENT CORN

NUHA M. ALHAZMI*

Department of Biology, College of Science, University of Jeddah, Jeddah 21589, Saudi Arabia. *Correspondence Email: nmalhazmi@uj.edu.sa

Abstract

Rhizopus oryzae is work as a model study for evaluation fungal evolution. It represents as a dangerous infection characterized by fast angioinvasive growth. Furthermore, Azole resistance R. oryzae isolates represent an economic problem. Green fabrication of silver nanoparticles is better than chemical methods, as it is environmental and cheap-cost. In the present study, azole resistance R. oryzae isolates were collected from maize grains fields that had been sprayed with azole fungicides and then isolated. Thenm applied silver nanoparticles were synthesized by biological methods, using Aspergillus niger as a reducer. Silver nanoparticles were characterized by X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM), the antifungal activity of silver nanoparticles against Azole resistance R. oryzae isolates were collected from maize grains soil that had been remedied with azole fungicides was evaluated using the disc diffusion method, minimum inhibitory concentration (MIC). The physiochemical results emphasized the fabrication of Ag-NPs in spherical shapes with a diameter of 14 nm without any aggregation. MIC of Ag-NPs against azole resistance R. oryzae isolates from white and yellow maize was 62.5, 31.25 mg/mL, respectively. Finally, the goal of the report is utilize the silver nanoparticles as antifungal agents against Azole resistance Rhizopus oryzae isolates.

Keywords: Rhizopus oryzae**,** silver nanoparticle, yellow and white corn, inhibition zone and minimum inhibition concentration (MIC).

INTRODUCTION

Rhizopus oryzae is belonging to the polyphyletic basal lineages which classify as the zygomycetes [1]. The zygomycetes is emerged spread filamentous which isolated from patients with mucormycosis, with a lethal infection in immunocompromised host [2]. More than 90% of rhinocerebral cases are caused by *R. oryzae* [3]. *Rhizopus* is ubiquitous in nature and many of their species enter in the food fermentation such as tempeh, ragi. Also, it enters in the fermentation to produce lactic acid and fumaric acid [4]. The analytic data of the *R. oryzae* genome refers to existence of multiple lines which demonstrated that the whole - -genome duplication (WGD), also, the result refer to the duplication of all protein complexes that including in the respiratory electron transport chain, the V-ATPase and the ubiquitin–proteasome system which confirmed that the genetic plasticity of *R. oryzae* may be lead rapid adaptation to adverse environmental conditions, including host immune [5].Maize is economic crop which enter in the nutrients for human and livestock. It cultivate in many tropical area such as Australia, Africa and Middle East countries. It divided into three types (red, yellow and white) [6]. Nanoscience is a multidisciplinary science which interest in the brilliant properties of metal nanoscale. It concerned as convert the bulk material into nanosize (1-100 nm). Interestingly, it is contributing in many different fields such as industry and agriculture [7]. Nowadays, researchers are focus on the benefits of nanomaterial such as large surface are, optical and chemical properties.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 56 Issue: 03: 2023
DOI10.17605/OSF.IO/V47FE

Nanomaterials consist of many branches such as noble metal and metal oxide metal [8]. Metal nanoparticles have a wide variety of physicochemical properties. Nanomaterials have excellent properties due to their high surface energy, large surface area. In medical science [9], nanomaterials play an important role in a variety of applications [10]. Silver nanoparticles are promising materials in the fields of antibacterial, antitumor, and antiviral therapy [11-12]. Interestingly, Ag-NPs have a broad spectrum of antibacterial activity [11]. The antibacterial mechanism of silver nanoparticles depends upon liberated silver ions [12]. In this study, we Isolate of *Rhizopus oryzae* from 100 samples from maize grains (50 samples from white maize and 50 samples from yellow maize) and then investigate the *Rhizopus oryzae* susceptibility of isolates against antifungal drugs, determine MIC of the collected isolates and finally, utilized silver nanoparticles as fungicide against Azole resistance *Rhizopus oryzae* isolates.

MATERIAL AND METHODS

Sampling, isolation and identification of *Rhizopus oryzae*

According to Alhazmi and Sharaf (2023), azole-resistant *R. oryzae* isolates were collected from corn grain soil that had been treated with azole antifungal [13].

Synthesis of silver nanoparticles using *Aspergillus niger*

Aspergillus niger was used to synthesize silver nanoparticles in an environmentally friendly manner. According to Alhazmi and Sharaf (2023) [13].

Characterization of silver nanoparticle

The silver nanoparticle was characterized using the following techniques: the Fourier transformed infrared (FT-IR) spectrum using the Nicolet 6700 apparatus (Thermo Scientific Inc., CA, USA), The crystalline nature and grain size were analyzed by XRD (D8 Advance X-ray Diffractometer, Bruker, Germany), and the morphology and visualization of zinc oxide nanoparticles were evaluated by transmission electron microscopy (TEM, JSM-2100F, JEOL Inc., Tokyo, Japan), and scanning electron microscopy (SEM, JSM-690, JEOL Inc., Tokyo, Japan).

The susceptibility of *Rhizopus oryzae* isolates

The antifungal susceptibility of all isolates investigated according to Clinical Laboratory Standard Institute M38-A2 reference method [14].

Table1: The antifungal agent with their concentration

Antifungal disc	Concentration
Terbinfine	100 ug/ml
Fluconazole	25 ug/ml
Ketoconazole	30 ug/ml
Voricazole	1 ug/ml
Amphotericin	100 ug/ml
Nystatin	100 ug/ml

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 56 Issue: 03: 2023
DOI10.17605/OSF.IO/V47FE

The anti-fungal activity of silver nanoparticle against Azole resistance *R. oryzae* isolates

The Susceptibility Tests were determined according to NCCLS recommendations (National Committee for clinical laboratory Standards, 1993). The inhibition zone assay was carried out by the well diffusion method [15].

Results

Isolation and identification of *R. oryzae*

As table 2 summarized the total number of *R. oryzae* isolates were 2 out of 50 samples of white maize while the number of *R. oryzae* isolates that isolate from yellow maize was 3 isolates. Figure 1 and 2 showed the *R. oryzae* from white and yellow maize respectively.

Figure 1: displayed the *Rhizopus oryzae* on sabouraud dextrose agar.

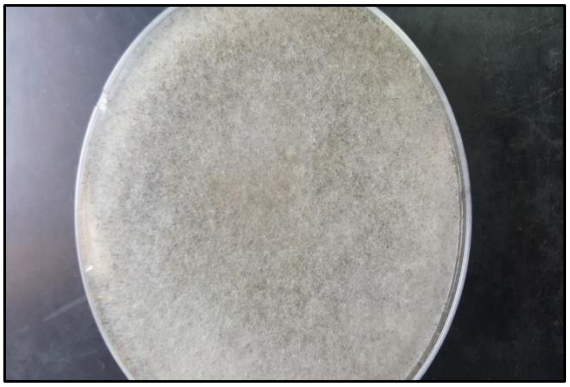

Table 2: Isolation and identification of *R. oryzae*

The source of Azole resistance *R. oryzae* isolates	No of isolate
White corn	2
Yellow corn	3

Characterization of silver nanoparticle

In this study, the silver nanoparticles were characterized by X-ray diffraction (XRD), SEM and TEM. The XRD patterns for Ag-NPs displayed that the presence of five characteristic peaks for Ag were observed at 2 Θ = 35.634, 43.415, and 65.264°, which are assigned to 111, 220, and 400, respectively. It was consistent with the standard silver diffraction pattern according to the Joint Committee on Powder Diffraction Standards (JCPDS-4-0783 Diffraction Card), as Figure 2 showed. The SEM images, as shown in Figure 3, highlight the presence of silver nanoparticles in a spherical shape with a smooth surface. The TEM image displayed the silver nanoparticle with particle sizes of 14 nm, as shown in Figure 4, emphasizing the synthesis of silver nanoparticles in a spherical shape.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 56 Issue: 03: 2023
DOI10.17605/OSF.IO/V47FE

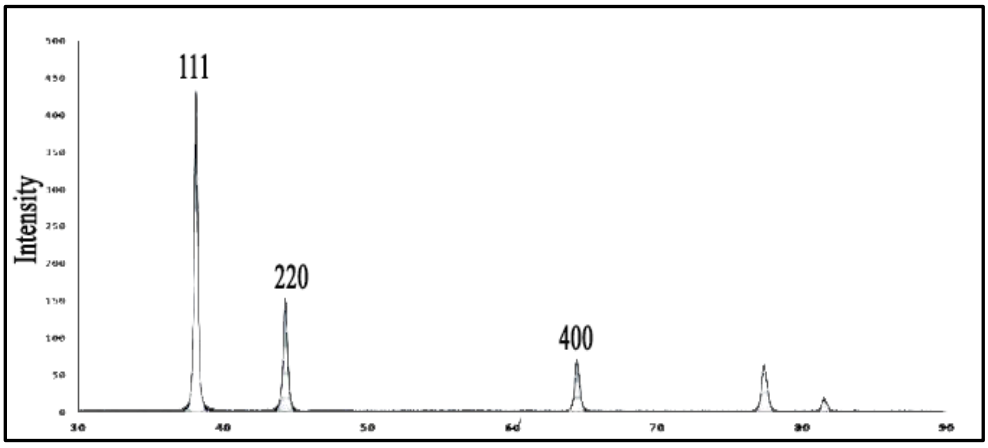

Figure 2. XRD of silver nanoparticles

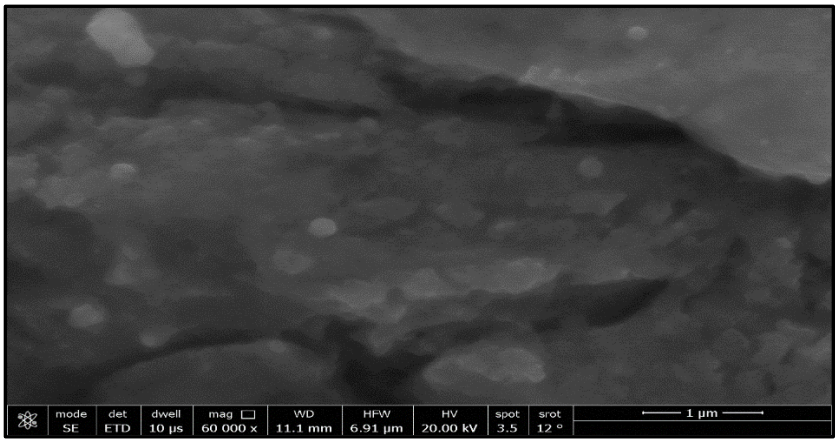

Figure 3: SEM images of silver nanoparticles

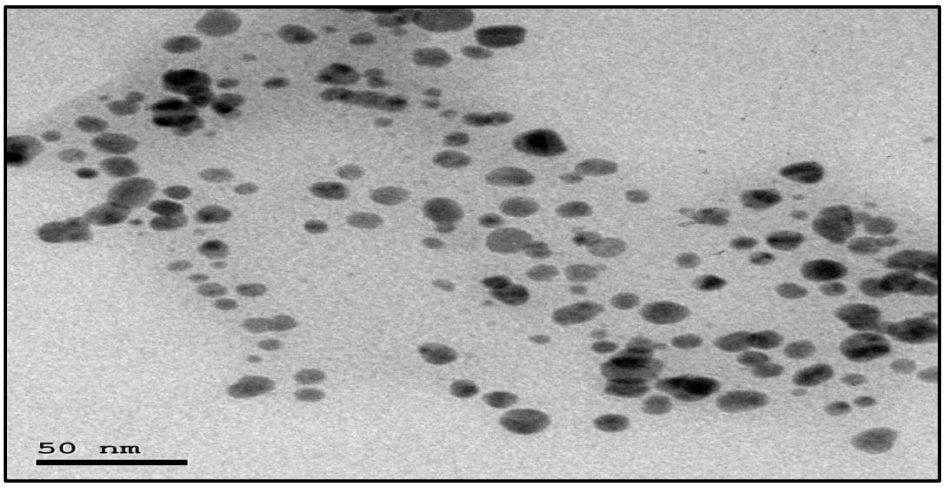

Figure 4: TEM images of silver nanoparticles

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 56 Issue: 03: 2023
DOI10.17605/OSF.IO/V47FE

The susceptibility of *Rhizopus oryzae* isolates against antifungal agents

The antifungal susceptibility of all isolates investigated against different antifungal agents as table 3 display, there is any significance effect of the common antifungals agents against *R. oryzae* that isolated from white and yellow maize except Terbinfine *against against R. oryzae* which isolated from white corn and Amphotericin against *R. oryzae* which isolated from yellow corn.

Table 3: The antifungals activity of common antifungals drugs against *R. oryzae* isolated from white and yellow corn.

Tested M.O.	Terbinfine	Fluconazole	Ketoconazole	Voricazole	Amphotericin	Nystatin
R. oryzae (white corn)	16	NA	NA	NA	NA	NA
R. oryzae (yellow corn)	NA	NA	NA	NA	13	NA

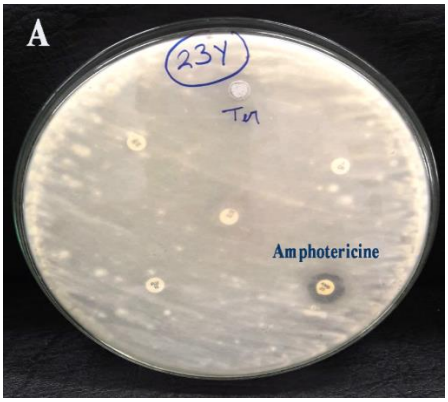

Figure 5A: The antifungals activity of common antifungals drugs against *R. oryzae* isolated from White corn,

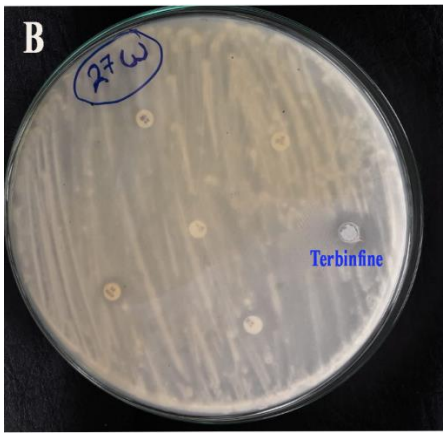

Figure 5B: The antifungals activity of common antifungals drugs against R. oryzae isolated from yellow corn

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 56 Issue: 03: 2023
DOI10.17605/OSF.IO/V47FE

The susceptibility of *Rhizopus oryzae* isolates against silver nanoparticles

Silver nanoparticle has a good anti-fungal activity against many funguses such as *Candida Albicans*, *Aspergillus niger* and *Pestalotiopsis maculans* [16]. As table 4 and figures 5 and 6 reveled. Silver nanoparticles which synthesized by reduction of silver ions by *Aspergillus niger* has a strong effect against both isolates of Azole resistance *Rhizopus oryzae* isolates. The inhibition zone of *R.oryzae* which isolated from white maize is 26 nm while, silver nanoparticle is less effect on *R. oryzae* isolated from yellow maize, the inhibition zone is 21 nm. Similarly, the minimal inhibition concentration of silver nanoparticles against *Azole resistance R. oryzae* isolated from yellow corn is better than white corn (62.5 and 31 mg) respectively.

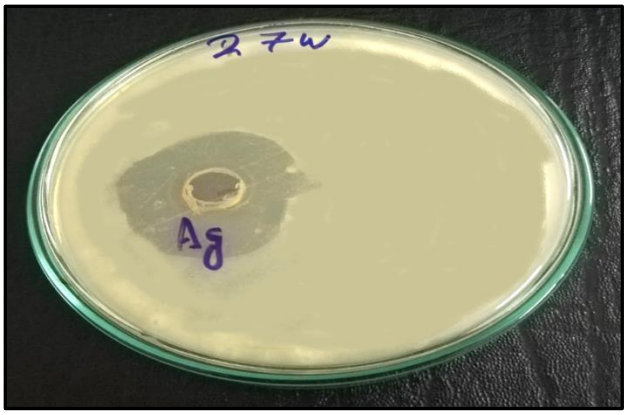

Figure 6: The antifungals activity of silver nanoparticles against *R. oryzae* isolated from white corn

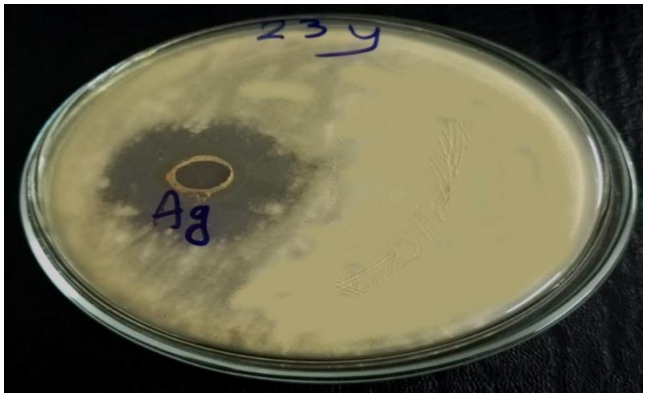

Figure 7: The antifungals activity of silver nanoparticles against *R. oryzae* isolated from yellow corn

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 56 Issue: 03: 2023
DOI10.17605/OSF.IO/V47FE

Table 4: The antifungals activity of silver nanoparticles against *R. oryzae* isolated from white and yellow corn

Tested microorganisms	Inhibition Zone (nm)	MIC (mg)
R.oryzae (white corn)	**26 nm**	**62.5 ± 2.1**
R.oryzae (yellow corn)	**21 nm**	**31.25 ± 1.2**

DISCUSSION

Rhizopus oryzae is related to the zygomycetes family. It causes a highly dangerous infection. The analysis data of the *R. oryzae* genome displayed the presence of the multiple lines which support the ancient whole-genome duplication (WGD). Interestingly, the fungal infection diseases still represent a great threat for individuals' health although novel antifungal have been developed [5]. The penetration and damage of endothelial cells lining blood vessels are occurred is the pathogenetic strategy of *R. oryzae's* due its attached to and phagocytosis via endothelial cells [14]. The angioinvasion is an indiaction of zygomycotic infections. Previous study reported that *R*. *oryzae* spores and hyphae can be penetrate and damage HUVECs [15]. Nanotehnology is the modern technology in fabrication and utilization of structures of material in nanosize scale, it can be utilize as a modern route to create a novel, drug [16-17]. Ten years ago, the nanoscience has magnificent role in medicine for example, cancer diagnosis and therapy. It can be categorized into different division such as noble metal or a metal oxide material. Also, it is typify into one dimension and two dimensions [18]. Silver nanoparticles are categorized as highly effective antimicrobial against a wide range of bacteria [19-20]. In this work, Azole resistance *R. oryzae* isolates were collected from maize grains soil that had been treated with azole fungicides and then isolated by pitt et.al (2009) method [12]. Followed by study the resistivity of the isolated against the common convention antifungal drugs. After that, the antifungal activity of silver nanoparticles, which synthesis by reduction method via *Aspergillus niger* as a reducer agent, against *Azole resistance R. oryzae* isolates from white and yellow by the inhibition zone and MIC method. The results showed that the total number of *R. oryzae* isolates were 2 out of 50 samples of white maize while the number of *R. oryzae* isolates that isolate from yellow maize was 3 isolates. The antifungal activity against *Azole resistance R. oryzae* isolates has no significance effect of the common antifungals agents against *R. oryzae* that isolated from white and yellow maize except Terbinfine *against against R. oryzae* which isolated from white corn and Amphotericin against *R. oryzae* which isolated from yellow corn as table 2 displayed. The physiochemical characterization of the silver nanoparticle with particle sizes of 14 nm. The inhibition zone of *R.oryzae* was 26 nm and 21 nm which isolated from white and yellow maize respectively. The mimium inhibition concentration (MIC) of *R.oryzae* was 62.5 mg and 31.25 mg which isolated from white and yellow maize respectively. The antimicrobial activity and surface –volume ratio increase as the size of the nanoparticle decreased [21]. The noble metal such as silver and gold has highly antibacterial activity with low toxicity for animal cells [22]. Currently, the scientists are interests in silver nanoparticles, especially in the field of treating skin infections including dermatomycosis [23] whatever, nanoparticle work alone or association with other drug which increase their

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 56 Issue: 03: 2023
DOI10.17605/OSF.IO/V47FE

impact against drug resistant microorganism (bacterial or fungus) [24]. The antimicrobial mechanism of the silver nanoparticle is release of silver ions throughout the accumulation of extracellular Ag-NPs which activate the penetration of Ag^{+} inside the cell [19]. Also, the attachment of Ag-NPs toward DNA and thiol groups of proteins with rectify with phosphorus- or sulphur-containing compounds inside DNA may lead to damage the yeasts by inhibiting DNA replication and protein inactivation [25]. Finally, silver nanoparticles are recommended as antifungal agents against Azole resistance *Rhizopus oryzae*.

CONCLUSION

In the present study, Azole resistance *R. oryzae* isolates were collected from maize grains fields that had been treated with azole antifungal agent and then isolated Followed by study the resistivity of the isolated against the common convention antifungal drugs. After that, the antifungal activity of silver nanoparticles, which synthesis by reduction method via *A. niger* as a reducer agent, against *Azole resistance R. oryzae* isolates from white and yellow by the inhibition zone and MIC method. The results showed that the total number of *R. oryzae* isolates were 2 out of 50 samples of white maize while the number of *R. oryzae* isolates that isolate from yellow maize was 3 isolates. The antifungal activity against *Azole resistance R. oryzae* isolates has no significance effect of the common antifungals agents against *R. oryzae* that isolated from white and yellow maize except Terbinfine *against against R. oryzae* which isolated from white corn and Amphotericin against *R. oryzae* which isolated from yellow corn as table 2 displayed. The physiochemical characterization of the silver nanoparticle with particle sizes of 14 nm. Also, the antifungal activities of silver nanoparticle against R. oryzae evidence those Ag-NPs. The inhibition zone of *R.oryzae* was 26 nm and 21 nm which isolated from white and yellow maize respectively. The minimum inhibition concentration (MIC) of *R.oryzae* was 62.5 mg and 31.25 mg which isolated from white and yellow maize respectively. Finally, evidence that silver nanoparticles is a promising agent against Azole resistance *R. oryzae* isolates.

References

1. James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, et al. (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443: 818–822.
2. Liu YJ, Hodson MC, Hall BD (2006) Loss of the flagellum happened only once in the fungal lineage: phylogenetic structure of kingdom Fungi inferred from RNA polymerase II subunit genes. BMC Evol Biol 6: 74.
3. Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, et al. (2007) A higher-level phylogenetic classification of the Fungi. Mycol Res 111: 509–547.
4. Abe A, Oda Y, Asano K, Sone T (2007) Rhizopus delemar is the proper name for Rhizopus oryzae fumaric-malic acid producers. Mycologia 99: 714–722.
5. Ma L-J, Ibrahim AS, Skory C, Grabherr MG, Burger G, et al. (2009) Genomic Analysis of the Basal Lineage Fungus Rhizopus oryzae reveals a Whole- Genome Duplication. PLoS Genet 5(7): e1000549. doi:10.1371/journal.pgen.1000549.

6. Fountain JC, Scully BT, Ni X, Kemerait RC, Lee RD, Chen Z-Y and Guo B(2014) Environmental influences on maize-Aspergillus flavus interactions and aflatoxin production. Front. Microbiol. 5:40. doi: 10.3389/fmicb.2014.00040
7. Ikonomova SP, Moghaddam-Taaheri P, Jabra-Rizk MA, Karlsson AJ.Engineering improved variants of the antifungal peptide histatin 5with reduced susceptibility to Candida albicans secreted aspartic proteases and enhanced antimicrobial potency. FEBS J. 2018;285:146–159. doi:10.1111/febs.14327
8. Yaqoob AA, Ahmad H, Parveen T, Ahmad A, Oves M, Ismail IMI,Qari HA, Umar K and MohamadIbrahim MN (2020) Recent Advancesin Metal Decorated Nanomaterials andTheir Various Biological Applications:A Review. Front. Chem. 8: 341.doi: 10.3389/fchem.2020.00341.
9. Oves, M., Rauf, M. A., Aslam, M., Qari, H. A., Sonbol, H., Ahmad, I., ... & Saeed, M. (2022). Green synthesis of silver nanoparticles by Conocarpus Lancifolius plant extract and their antimicrobial and anticancer activities. *Saudi journal of biological sciences*, *29*(1), 460-471.
10. Oves, M., Rauf, M. A., Warsi, M. K., Husain, F. M., Ansari, M. O., & Ismail, I. M. (2021). Aerogels as microbial disinfectant. In Advances in Aerogel Composites for Environmental Remediation (pp. 201-215). Elsevier.
11. H. Yu, L. Li, and Y. Zhang, “Silver nanoparticle-based thermal interface materials with ultra-low thermal resistance for power electronics applications,” Scripta Materialia, vol. 66, no. 11, pp. 931–934, 2012.
12. L. A. Austin, M. A.MacKey, E. C. Dreaden, and M. A. El-Sayed, “The optical, photothermal, and facile surface chemical properties of gold and silver nanoparticles in biodiagnostics, therapy, and drug delivery,” Archives of Toxicology, vol. 88, no. 7, pp. 1391– 1417, 2014.
13. Alhazmi, N.M.; Sharaf, E.M. Fungicidal Activity of Zinc Oxide Nanoparticles against Azole-Resistant Aspergillus flavus Isolated from Yellow and White Maize. Molecules 2023, 28, 711. https://doi.org/10.3390/ molecules28020711.
14. Ibrahim, A. S., J. E. J. Edwards, and S. G. Filler. 2003. Zygomycosis, p.241–251. In W. E. Dismukes, P. G. Pappas, and J. D. Sobel (ed.), Clinical mycology. Oxford University Press, New York, N.Y.
15. Ibrahim, Ashraf S., Brad Spellberg, Valentina Avanessian, Yue Fu, and John E. Edwards Jr. "Rhizopus oryzae adheres to, is phagocytosed by, and damages endothelial cells in vitro." Infection and immunity 73, no. 2 (2005): 778-783.
16. Hassan, A., Sorour, N. M., El-Baz, A., and Shetaia, Y. (2019). Simple synthesis of bacterial cellulose/magnetite nanoparticles composite for the removal of antimony from aqueous solution. Int. J. Environ. Sci. Technol. 16, 1433–1448. doi: 10.1007/s13762-018-1737-4
17. Hassan A, Elebeedy D, Matar ER,Fahmy Mohamed Elsayed A and Abd El Maksoud AI (2021) Investigation of Angiogenesis and Wound Healing Potential Mechanisms of Zinc Oxide Nanorods.Front. Pharmacol. 12:661217. doi: 10.3389/fphar.2021.661217
18. Hassan A, Al-Salmi FA, Abuamara TMM, Matar ER, Amer ME, Fayed EMM, Hablas MGA,Mohammed TS, Ali HE, Abd EL-fattah FM, Abd Elhay WM, Zoair MA, Mohamed AF, Sharaf EM,Dessoky ES, Alharthi F, Althagafi HAE and Abd El Maksoud AI (2022) Ultrastructural analysis of zinc oxide nanospheres enhances anti-tumor efficacy against Hepatoma. Front. Oncol. 12:933750. doi: 10.3389/fonc.2022.933750.
19. Sharaf EM, Hassan A, AL-Salmi FA, Albalwe FM, Albalawi HMR, Darwish DB and Fayad E (2022) Synergistic antibacterial activity of compact silver/magnetite core-shell nanoparticles core shell against Gram-negative foodborne pathogens. Front. Microbiol. 13:929491. doi: 10.3389/fmicb.2022.929491.
20. Alhazmi, N.M. Fungicidal Activity of Silver and Silica Nanoparticles against Aspergillus sydowii Isolated from the Soil in Western Saudi Arabia. Microorganisms 2023, 11, 86. https://doi.org/10.3390/ microorganisms11010086.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 56 Issue: 03: 2023
DOI10.17605/OSF.IO/V47FE

21. Ahmed S, Ahmad M, Swami BL, Ikram S (2016) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J Adv Res 7:17–28. https://doi.org/10.1016/j.jare.2015.02.007.
22. Lansdown ABG (2010) A pharmacological and toxicological profile of silver as an antimicrobial agent in medical devices. Adv Pharmacol Sci. https://doi. org/10.1155/2010/91068 6
23. Aljuffali I, Huang C-H, Fang J-Y (2015) Nanomedical strategies for targeting skin microbiomes. Curr Drug Metab 16:255–271. https://doi. org/10.2174/13892 00216 66615 08121 24923
24. Bera RK, Mandal SM, Raj CR (2014) Antimicrobial activity of fluorescent Ag nanoparticles. Lett Appl Microbiol 58:520–526. https ://doi.org/10.1111/ lam.12222
25. Vazquez-Munoz R, Avalos-Borja M, Castro-Longoria E (2014) Ultrastructural analysis of *Candida albicans* when exposed to silver nanoparticles. PLoS ONE 9:1–10. https ://doi.org/10.1371/journ al.pone.01088 76

Data availability statement

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

Funding

This work was fully funded by the author herself

Conflict of interest

The author declare that the research was conducted in the absence of any commercial or financial relationships that co

List of figures: