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Control of black fungus associated with COVID-19 by some safety methods

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***Abstract*---**Antibacterial, antifungal, and antiviral activities are all present in silver nanoparticles. Silver nanoparticles have the capacity to penetrate bacterial cell walls, altering cell membrane structure and even causing cell death. The efficiency of silver nanoparticles on the fungi that cause the black fungus linked with Covid was investigated in this study,The poisoned food technique was utilized with concentrations of 5%, 10%, and 20%, and while all of the concentrations were efficient, the effect was larger when the greatest concentration was employed.The efficacy of mushrooms was also tested against these fungi that cause mucormycosis,The antifungal activity of ethanol extracts of Agaricus bisporus against Rhizopus arrhizus was determined in vitro using the poison food approach and different concentrations of the extract (8, 10, 16 mg/ml). The maximum effect of ethanolic extract against Rhizopus arrhizus growth was achieved at 8 mg/ml, and the maximum effect of ethanolic extract was achieved at 16 mg/ml, with a growth rate of 57.5mm.

***Keywords*---**black fungus, mucormycosis, silver nanoparticle, mushroom, agaricus bisporus, COVID-19.

Introduction

Mucormycosis is a rare fungal infection with a high death and morbidity rate that has interest of health experts and physicians worldwide, particularly in India (1,2). Excessive drug usage and advanced age are also risk factors for pharmaceutical side effects. Many older persons have many chronic ailments, which requires them to take multiple drugs, increasing their risk of contracting opportunistic epidemic infections (3). Mucormycosis has resurfaced in the news in 2020, in part due of its link to severe COVID-19 infection which Its symptoms are fever, shortness of breath, phlegm, brain pain, coughing, muscle pain or fatigue.

Immunosuppressive medications, and uncontrolled diabetic mellitus (4,5) Mucorales fungi invade various kinds of damp, organic materials and are an inextricable aspect of the human environment. They are economically important as soybean product fermenting agents and enzyme makers, as well as plant parasites and spoiling organisms. Several taxa can cause life-threatening infections, especially in those with weakened immune systems. The order Mucorales, which includes 261 species in 55 genera, has been classified to the phylum Mucoromycota infections in humans have been observed in 38 of the recognized species, resulting in a clinical condition known as mucormycosis (6*) Rhizopus, Mucor, Absidia, and Cunninghamella* belong to the Mucorales, The Mucorales fungus is responsible for the majority of human fungal infections, with *Rhizopus oryzae* being the most dangerous of these diseases(7).These fungi have unrestricted cottony mycelia that ranges in color from white to grey to black(8).

Many antifungal drugs are resistant to them, and the types of antifungal agents available for treatment are extremely limited. Because fungi are eukaryotic organisms with a structure and metabolism comparable to their eukaryotic hosts, this is the case. As a result, antibiotics with novel antibacterial mechanisms are unavoidably and urgently required in medicine(9,10,11).Because of scientific advances in metal nanoparticle research, Ag-Nps have gotten a lot of attention as a probable antibacterial agent and for therapy,Nanoparticles are also designed to target drug delivery and release drug continuously or delayed in fungal infections, this is owing to the mechanical, thermal, and catalytic capabilities of nanoparticles with a significant surface to volume ratio, which influence the nanoparticles' mechanical, thermal, and catalytic nature(12,13,14,15,16).Recent progress in metal nanoparticle research appears to be promising. Ag-Nps made using various synthetic processes have been demonstrated to have effective antibacterial action(17) Nanotechnology, which deals with nanometer-sized things, has sparked a lot of attention in recent years, nanoparticles are made via physical, chemical, and biological processes(18).

A mushroom is a macro fungus with a characteristic fruiting body that can be epigeous (above ground) or hypogenous (below ground) and large enough to be seen and collected by hand,the most common mushroom is an umbrella-shaped mushroom with a pileus (cap) and stipe (stem), with some species having an annulus (ring), such as *Agaricus bisporus* (19,20).Higher fungi are Macromycetes that belong to the phylum Basidiomycota and some that belong to the phylum Ascomycota(21).,The fruit bodies of mushrooms contain a number of essential minerals such as copper, zinc, iron, potassium, selenium, sodium, phosphorous, manganese and magnesium.(22), This mushroom possesses a number of medicinal and therapeutic properties. It is known worldwide for its anti-cancer, anti-tumor, pharmacological, cholesterol-lowering, immunostimulating, antimicrobial, anti-inflammatory, and antioxidant activities (23,24)

Materials and Methods

Materials

Silver nanoparticles/origin USA, ketocanazole (antifungal), dimethyl sulfoxide (DMSO) solution /origin India, mushroom (*Agaricus bisporus*)

Isolation of the fungi causing black fungus from Covid patients

- A -Specimens were obtained by nasal swab method from the patients that infected with Covid 19 virus. Specimens were collected in a sterile way, cultured, and then transported to the lab for incubation and testing
- B - The isolates were phenotypically and molecularly characterized (*Rhizopus arrhizus*). *(*25)

Preparation of silver nanoparticles concentration

The different concentrations of silver nanoparticles were prepared using sterile distilled water and DSMO, 20, 10 and 5 mg of silver nanoparticles were dissolved in 5ml of DSMO and 95 ml of distilled water to prepare concentrations (20%, 10%, 5%) respectively by used Hot plate with magnetic stirrer.(26)

Prepation of *Agaricus bisporus* extract

Mushroom (*Agaricus bisporus*) was obtained from Al-Wadaq farm for the production of mushrooms located in Baghdad near Basmaya residential complex in December 2021, which Diagnosed phenotypically and molecularly. The mushrooms were washed with plain water, then with distilled water, and cut into small pieces with a clean knife and dried by air at a temperature of 50 degrees Celsius and then grinded for the purpose of obtaining on powder. The alcoholic extract was made using 400 ml of (80%) methanol alcohol as a solvent for 100 gm of dry powder using a Continuous Soxhlet Extraction device (27)

Preparation of ketocanazole soulution

5 ml of Dimethylesulphoxide (DMSO) was placed at a concentration of 100% in an airtight tube, add 50 mg of antifungal to it, then shake the solution vigorously using the Vortex, and consider the solution at a concentration of 10,000 µg/ml.(as negative control)

Screening silver nanoparticls for antifungal activity

The experiment was carried out using the poisoned food approach:

- Measurement of colony growth rate on solid medium**,** involved pouring SDA medium at 50 °C over 1 ml of each concentration (5 %,10 %, and 20%)from Ag-Nps solution in Petri dishes and making a circular motion for it to homogenize with the medium.Following the above step of solidifying the medium, the inoculum was transferred using a 5 mm diameter cork borer from new isolates that were 4-5 days old and placed in the middle of the dish with three replicates of each concentration for each isolate, then the dishes were incubated at 37 °C for four days and the measurements were taken. The colony's diameter was measured and compared to a positive control plate and a negative control plate. (antifungal dish is made by pouring 1 ml of the antifungal solution ketaconazole into a Petri dish, then pouring the SDA medium and allowing it to harden, as well as inoculating

with a cork piercing 5 mm in diameter and transferring part of the fungal culture.)

- On a liquid media, the dry weight of fungus growth is measured. Using the poisoned food approach, the experiment was carried out by pouring SDB medium at 50 °C over 1 ml of the concentrate in a 20 ml tube. Inoculum with a diameter of 5 mm was transferred from fresh 4-5 day old isolates and deposited in the tubes, which were subsequently cultured at 37°C for 4 days. The growth is filtered using filter paper after incubation, then dried in an oven at 50°C for 24 hours before the filter papers are weighed. The negative and positive control tubes were compared.

Testing *Agaricus bisporus'* antifungal activity

Using the poisoned food technique, its effect on mucorales was investigated (28):

- On solid media, the rate of colony growth is measured.
  3 ml of each concentration of alcoholic extract (8,10,16 mg/ml) was placed in the center of a Petri dish, then SDA medium was poured over it at 50-45°C and the medium was poured in a circular motion, then the medium was let to solidify with three replicates for each concentration (29). It was incubated for 5 days at 37°C, and colony growth in the poisoned and non-poisoned plates was compared, and the colony growth rate was calculated.
- On a liquid media, the dry weight of fungus growth is measured.
  At 50 °C, 3 mL of each alcoholic extract was placed in glass vials (20 mL capacity) containing SDB medium in concentrations of 8, 10, and 16 mg/mL. 5 days at 37°C incubation After incubation, the growth is filtered using filter paper and dried in a 50 °C oven for 24 hours before being weighed and compared to the negative and positive control tubes.

Result

***Rhizopus arrhizus* identification**

Rhizopus arrhizus was identified according to agricultural and microscopic features, and identified by using the identification keys (fig 1,2),as well as a molecular diagnosis

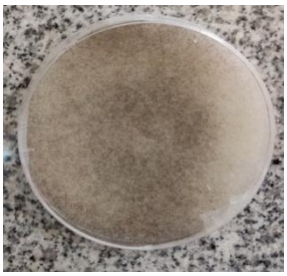

Fig. 1. *Rhizopus arrhizus* isolate on SDA Media

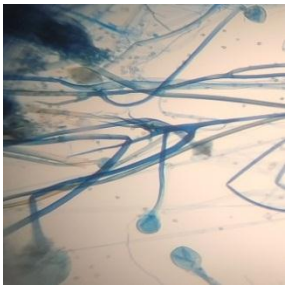

Fig 2. *Rhizopus arrhizus* isolate under microscope

Antifungal activity

- A - The results of the antifungal response shown by silver nanoparticles solutionare summarized in tables (1-a),(1-b) figure (3-B)
- The results of the antifungal response shown by alcoholic extract of *Agaricus bisporus* are summarized in tables (2-a),(2-b) figure(3-C)

Table 1-a
Effect of silver nanoparticles on the radial growth of isolated *Rhizopus arrhizus*

Con. / The specimen	5%		10%		20%		Antifungal		Positive control
	Fungal growth (mm)	%	Fungal growth (mm)	%	Fungal growth (mm)	%	Fungal growth (mm)	%	
1	25.22±3.45	71.97± 3.84	22±2.82	75.55± 3.14	15.88±3.1	82.35± 3.44	5.72±0.44	93.64±0.18	90±0
2	26± 3.09	71.11± 3.44	22±3.09	75.55± 3.44	18.16±2.85	79.82± 3.17	5.4±0.35	94±0.08	90±0
LSD (P<0.05)	3.681								

Table 1-b
Effect of silver nanoparticles on the dry weight of isolated *Rhizopus arrhizus*

Con. / The specimen	5%		10%		20%		Antifungal		Positive control
	The weight (mm)	%	The weight (mm)	%	The weight (mm)	%	The weight (mm)	%	
1	0.135±0. 034	67.31± 8.8	0.102±0 .006	75.33±4.45	0.074±0.009	82.08±4.08	0.041±0.001	90.07±0.14	0.413±0. 015
2	0.146±0. 04	65.23± 10.2	0.100±0 .005	76.19±2.26	0.071±0.009	83.09±1.29	0.043±0.005	89.76±0.06	0.420±0. 005
LSD (P<0.05)	0.087								

Table 2-a
The effect of alcoholic extract of *Agaricus bisporus* on the redial growth of *Rhizopus arrhizus*

Con. / The specimen	8%		10%		16%		Antifungal		Positive control
	Fungal growth (mm)	%	Fungal growth (mm)	%	Fungal growth (mm)	%	Fungal growth (mm)	%	
1	51.33±3.46	41.88± 3.84	43.33± 4.47	50.9 4±4.96	38.66±1	56.23±1.11	5.44±0.37	93.84±0.08	88.33±2.5
2	50± 1.26	43.92± 1.4	41.66± 3.66	53.27± 4.07	36.33± 3.44	59.25±2.18	5.41±0.37	93.93±0.06	89.16±2.04
LSD (P<0.05)	4.118								

Table 2-b
Effect of alcoholic extract of *Agaricus bisporus* on the dry weight of isolated *Rhizopus arrhizus*

Con. / The specimen	8%		10%		16%		Antifungal		Positive control
	The weight (mm)	%	The weight (mm)	%	The weight (mm)	%	The weight (mm)	%	
1	0.390±0.006	7.58±1.08	0.371±0.007	12.08±1. 6	0.334± 0.015	20.85±0.78	0.043±0.005	89.81±0.02	0.422±0.005
2	0.401±0.01	6.96±0.45	0.373±0.007	13.45±1. 02	0.341± 0.012	20.88±1.23	0.043±0.005	90.02±0.02	0.431±0.005
LSD (P<0.05)	0.014								

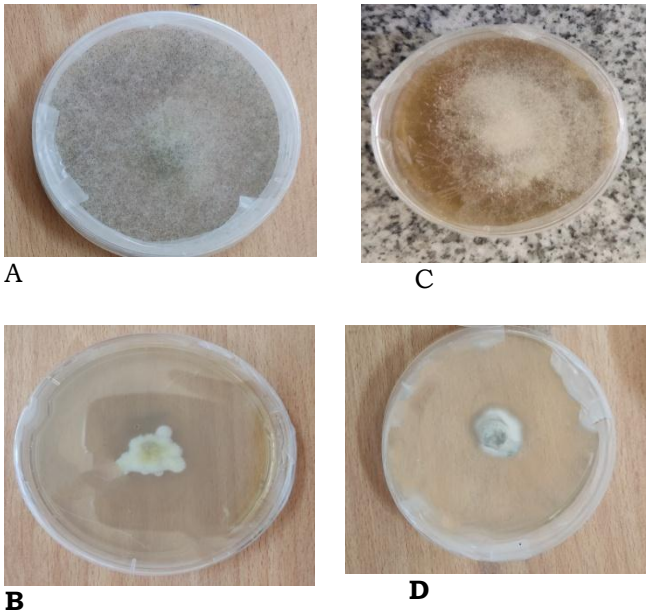

Fig 3. A-*Rhizopus arrhizus* under the same conditions and without any treatment, B-Effect of silver nanoparticles on the growth of *Rhizopus arrhizus*, C-Effect of alcoholic extract of *Agaricus bisporus* on the growth of *Rhizopus arrhizus*, D-Effect of antifungal ketocanazole on the growth of *Rhizopus arrhizus*

Discussion

- The effect of silver nanoparticles solution on *Rhizopus arrhizus* growth was extremely evident; the maximum effect was archived at a concentration of 20%, resulting in a growth rate of 16.5 mm .According to (30, 9): Because of their high affinity for fungal cell membranes, Ag-Nps connect to them and penetrate them, causing cell lysis by generating structural changes in the fungus' core, resulting in a low molecular weight site, and finally Ag-Nps bind to the respiratory chain, causing cell death. Silver ions are released by Ag-Nps in fungal cells, boosting antifungal activity. Nano-Ag has been proven to exhibit antibacterial properties in numerous studies (11, 31,10).
  Kim and other in 2008 (17) noted that Nano-Ag can inhibit the growth of dermatophytes, which cause superficial fungal infections.
- There was a clear effect of alcoholic extract on the growth of *Rhizopus arrhizus* ; the maximum effect of alcoholic extract of *Agaricus bisporus* was archived at a concentration of 16 mg/ml, resulting in a growth rate of 57.5 mm .Mushrooms contain a variety of proteins and chemicals that have antifungal, antibacterial, and antiviral properties; polypeptide alveolarin and peptide eryngin, for example, have strong antifungal properties (32 ,33 ,34,35,36 ,37). The ethanolic extract of *Agaricus bisporus* displayed antifungal efficacy against some fungi according to Kumar and Yadav (38).Many research have been conducted on the antimicrobial property of mushroom extracts against a variety of microorganisms (39). And the ethanolic extract of *Agaricus bisporus* displayed antifungal efficacy against some fungi according to Kumar and Yadav (38).In 2020, Sultan and other found that mushrooms can inhibit the growth of *Aspergillus flavus*(29) .And in 2020,Karnwell and others (40) showed in their study the effectiveness of an alcoholic extract against several species of bacterial pathogens.

Conclusion

This research focuses on the use of Nano-Ag on pathogenic fungal strains. Nano-Ag is thought to be a possible anti-infective agent for human fungal illnesses**.**We have seen this with effectiveness against the *Rhizopus arrhizus* fungus.Many scientists feel that silver nanoparticles are beneficial can discharge silver ions in a continuous stream to kill pathogens. According to the findings of this study, the ethanol extracts of *Agaricus bisporus* showed good activity against *Rhizopus arrhizus* , indicating that *Agaricus bisporus* is beneficial to human health and could be used as a natural source of antifungal agent in the development of a new drug for fungal infections instead of commercial antifungal drugs, which can lead to drug resistance.

Competing interest's statement

The author(s) declare(s) that there is no conflict of interest.

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