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Morphological effects of alcoholic extract of mushroom and silver nanoparticles on black fungus

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***Abstract*---**The present study aimed to know the extension effects of alcoholic extract of *Agaricus bisporus* in concentrations (16)mg\ml and silver nanoparticles (20)% on morphology (macro and micro-features) of *Rhizopus arrhizus* that causes co infection (mucormycosis)in covid-19 patients .Results show ,decrease in the growth rate of the tested strains ,some parts hyphae was atrophied and increased lateral branching points and swelling of the cell wall and condensing the material cytoplasm and other changes.

***Keywords*---**black fungus, morphological effect, agaricus bisporus, silver nanoparticles.

Introduction

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.(Walther *et al* 2019). Mucorales have coenocytic hyphae and a polysaccharide chitosan-containing cell wall (an N-deacetylated version of chitin) Asexual resting spores that come into contact with skin or mucosa cause mucormycosis. The innate immune system's physical barriers prevent tissue invasion to a significant extent, Many people who were suffering from dangerous diseases have survived as a consequence of recent advancements in medicine and

the use of contemporary technologies in therapy, yet antibiotic resistance has increased at the same time.(Stevanovic *et al* 2012)This spurred scientists to look for anti-fungal alternatives that are effective against pathogenic fungus, are low-cost, non-toxic, and function in ways that are unknown to fungi in order to overcome the high resistance to fungi.(Kanhed *et al* 2014) although mucormycosis might develop from a malfunction of the immune cell effectors or damage to the skin or mucosa. So it is an opportunistic fungus (Nicolas *et al* 2020)

Material and Methods

Isolation of the fungi causing black fungus from Covid patients

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

Preparation of silver nanoparticles concentration

The concentration of silver nanoparticles were prepared using sterile distilled water and DSMO, 20, mg of silver nanoparticles were dissolved in 5ml of DSMO and 95 ml of distilled water to prepare concentration by used Hot plate with magnetic stirrer.(Dixit *et al* 1976)

Preparation of the alcoholic extraction of mushroom

Mushroom(*Agaricus bisporus*) was obtained from Al-Wadaq farm for the production of mushrooms located in Baghdad .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. And prepared 16mg\ml concentration

Cultivation

One ml of each concentration of mushroom alcoholic extract at concentrations (16 mg/ml) and solution of silver nanoparticle (20%) was placed in the center of a Petri dish and then SDA medium was poured over it and a circular motion was made for the medium and then left to solidify with three replicates for each concentration .Then the inoculum was transferred by a 5 mm diameter cork borer from isolates 4-5 days old. and incubation for seven days.

Study the effect of plant extract on the morphology of fungal cells

Followed method (Benson, 2002), a Slide Culture Technique , as follows:

1. Sterility petri dish shower glaze on wet filter paper and glass rod in the letter V attic and put a clean glass slide and sterile.
2. Cut amid agar containing mushroom extract alcohol concentration of 16 mg / ml and 20% silver nanoparticle in the form of small cubes and dimensions of no more than (1 cm) cubes and transfer and placed on the surface of the glass slide and covered with a sterile cover slide.

3. inoculted corner of the agar cube by part of species fungal and wet the surface of filter paper 3 ml of distilled water and put the lid sterile petri dish and incubated at 28 c for a period of 7 days.
4. Slide the cover was lifted with forceps and placed on a glass slide another container on a drop of Lactophenol-cotton blue stain.
5. For the purpose of evaluated the morphological changes that occurred as a result of the treatment with nanoparticles and the alcoholic extract of mushrooms, an electron microscope (SEM)was used to show those effects and the changes that occurred.

Results and discussion

Macro-features

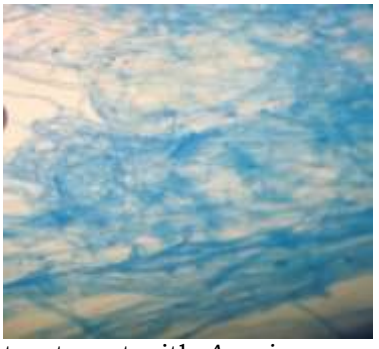
The decrease in the growth rate of the tested strain due to the effect of the treatments is usually accompanied by morphological changes of the developing mycelium .We noticed this from the fungal growth on the plate**.** Fig (1) appear A decrease in the formation of spores and a change in the color of the colony in addition to the lower of the growth compared with control treatment (Widana et al., 2021) .

16mg/ml 20% Control

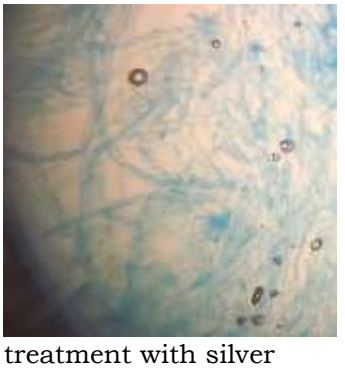
Fig .1. Effect of treated on macro-features of *Rhizopus arrhizus*

Micro-features

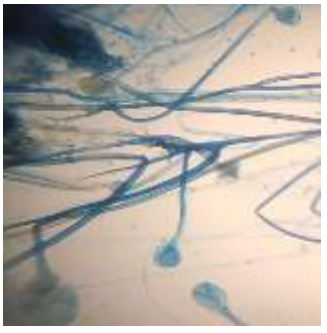
Fig. (2) explain effect of treated with mushroom extract and silver nanoparticle on shape of hypha and mycelium as well as fungal cell of *Rhizopus arrhizu*s. It shows abnormal growth,excessive branching and swelling of the cell wall. And absent of sporangiospores.



treatment with *Agaricus bisporus* extract



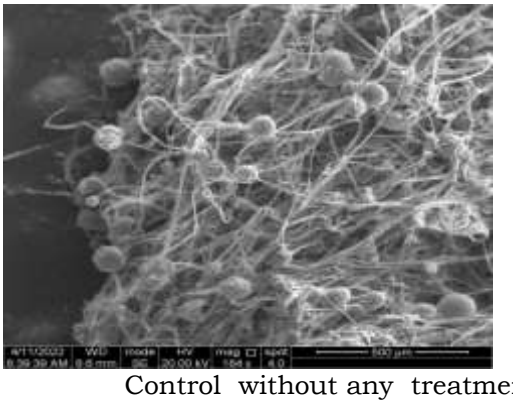
treatment with silver nanoparticles

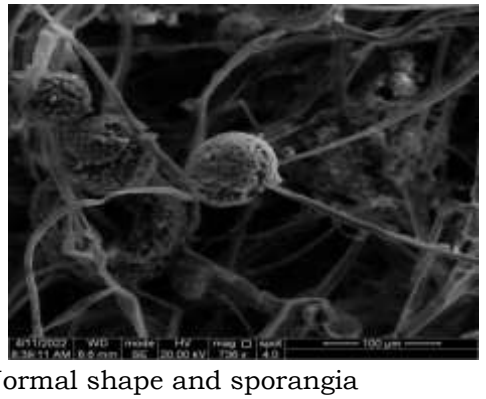


Control

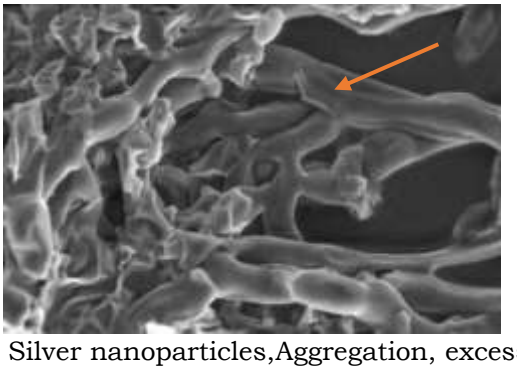
Fig.2.morphological change in mycelium of *Rhizopus arrhizus*under a light microscope

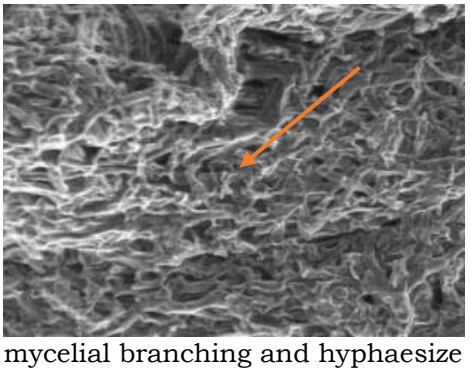
While fig. (3) appear morphological change of mycelium under an electron microscope(SEM). It is show,aggregation, excessive mycelial branching and hyphae size sporulation reduction, it was discovered that abnormal growth ,excessive branching and swelling of the cell wall .



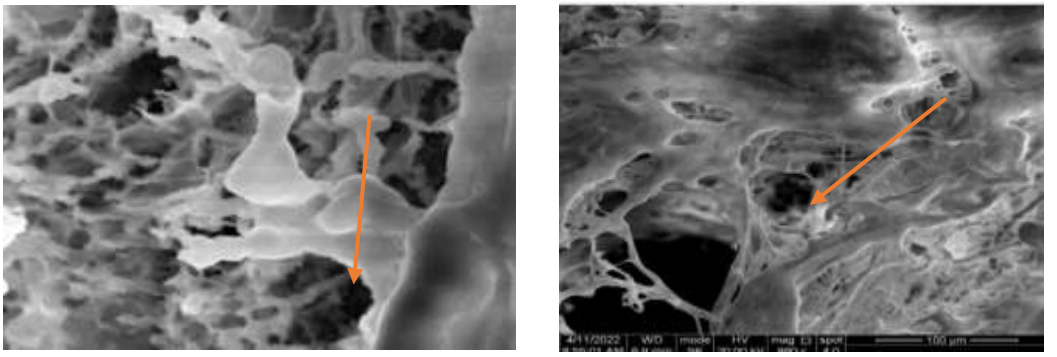


Control without any treatmentNormal shape and sporangia





Silver nanoparticles,Aggregation, excessive mycelial branching and hyphaesize sporulation reduction



Mushroom treatment, Abnormal growth ,excessive branching and swelling of the cell wall

Fig.(3)morphological change after treatment with mushroom extract (16 mg/ml) and silver nanoparticles(20 %)under SEM

Many research coordinated with our results such as (Jasim, et.*al*,2019 ;Jasim,*et.al*,2018; Radhi, and Jasim) .Radhakrishnan *et al* (2018) noticed that there were morphological changes that occurred on the genus Candida after treating it with silver nanoparticles ,they noticed alterations in the cell walls of the cells, their transformation into a rough shape .Also Nasrollahi *et al* (2011) saw fungal cells with a pore in their cell membrane being destroyed. Scanning Electron Microscopy (SEM) results on two model yeast (*Saccharomyces cerevisiae and Candida albicans*) reveal a reciprocal relationship between Ag-NPs and membrane structure, resulting in fungal cell damage and death. The capacity of Ag-NPs to damage the fungus's surface membrane structure was tested using SEM.

Conclusion

From the results that were presented above, it can be concluded that both the alcoholic extract of mushroom and silver nanoparticles have an inhibitory effect on the fungus that causes black fungus disease associated with Covid, and they can be promising treatments to reduce the secondary infection of Covid-19 disease.

References

Benson, H. j. (2002).Microbiology application (Laboratory manual in general microbiology). Gth ed. published by McGra -Hill, New York. U.S.A : 202-203.

Dixit, S. N.; Tripathy, S. C. and upadyyey, R.R. (1976). The antifungal substasnce of rose flower (Rose indica) Economic Botany. 30:371-373

Jasim, N.O., Hasan, T.K., Flieh, H. Characterization of silver nano particles synthesized by leaves green tea extract. Journal of Global Pharma Technology, 2018, 10(10), pp. 423–427.

Jasim, N.O., Nabel, B., Taleb, A. Characterization and biological activity of green synthesis silver nanoparticles. International Journal of Drug Delivery Technologythis link is disabled, 2019, 9(3), pp. 94–97.

Kanhed, P., Birla, S., Gaikwad, S., Gade, A., Seabra, A. B., Rubilar, O., ... & Rai, M. (2014). In vitro antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. *Materials Letters*, *115*, 13-17.

Nasrollahi, A., Pourshamsian, K. H., & Mansourkiaee, P. (2011). Antifungal activity of silver nanoparticles on some of fungi.

Nicolás, F. E., Murcia, L., Navarro, E., Navarro-Mendoza, M. I., Pérez-Arques, C., & Garre, V. (2020). Mucorales species and macrophages. *Journal of Fungi*, *6*(2), 94. oryzae adheres to, is phagocytosed by, and damages endothelial cells in

Radhakrishnan, V. S., Mudiam, M. K. R., Kumar, M., Dwivedi, S. P., Singh, S. P., & Prasad, T. (2018). Silver nanoparticles induced alterations in multiple cellular targets, which are critical for drug susceptibilities and pathogenicity in fungal pathogen (Candida albicans). *International journal of nanomedicine*, *13*, 2647.

Radhi, Z.E., Jasim, N.O. Synthesis, characterization and antimicrobial activity of MgO nanoparticles. IOP Conference Series: Earth and Environmental Sciencethis link is disabled, 2021, 790(1), 012089.

Stevanović, M. M., Škapin, S. D., Bračko, I., Milenković, M., Petković, J., Filipič, M., & Uskoković, D. P. (2012). Poly (lactide-co-glycolide)/silver nanoparticles: Synthesis, characterization, antimicrobial activity, cytotoxicity assessment and ROS-inducing potential. *Polymer*, *53*(14), 2818-2828

Walther, G., Wagner, L., & Kurzai, O. (2019). Updates on the taxonomy of Mucorales with an emphasis on clinically important taxa. *Journal of fungi*, *5*(4), 106.

Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, *29*(3), 550–561. https://doi.org/10.1002/cae.22202