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Inhibition effect of cascutachinasis silver nanoparticles on oral candida species

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Abstract:

The study aimed to use AgNps (green synthesis) in inhibition growth of Candida spp In this study 95 samples were collected from saliva patients samples were positive (32 for the presence of fungal elements. Yeasts that isolated were included Candida parapsilosis, C. albicans, C. glabrata, and C.Krusie. While the filamentous fungi includedAspergillusniger, Penicilliumchrysogenum, Geotrichumcandidium Antifungal activity of AgNps revealed that each species of Candida had been affected more than others, as follow C. glabrata was highest inhibition in diameter (35 mm) at the concentration of 10 %, while the C. albicans was (34 mm), C. Parapsilosis (mm) and C. krusie growth (15 mm) .

Key word: Cuscutachinesis, silver Nanoparticles, Candida spp., candidiasis

Introduction :

Nano silver is one of the most thoroughly investigated nano-materials and its antimicrobial activity is associated with the characteristic structure of nanoparticles. Theyare characterized by a high fraction of surface atoms so that nanoparticles have a greateraffinity for interactions with thiol groups; it exhibits a high antifungal activity, forinstance against Candida albicans as well as against some others pathogenic strains (1).

Oral fungal diseases affect a significant, then rising, proportion of the population (2)

Oral candidiasis was the most usual human fungal infection. Even though C. albicans

was the most common pathogen responsible for candidiasis, other Candida species

.causing oral infections have also been identified including C. glabrata, C. krusei, C

.(parapsilosis, C. dublieniesis, C. tropicalis, C. kefyr and C. guilliermondii. (3, 4, 5

The most usual Candida species encountered is Candida albicans and accountsfurther than 90% of oral space isolates. (6, 7)

Material and Methods :

Collection of fungal samples from cases .

This experimental study was taken on 95 donors from Babylon University in Hila

city, Iraq, during 2018 (September to December). The samples were taken by using2

sterile swab sticks which were labeled indicating the source, date; age and sex of patients

then put in translate media, and sent to the fungal laboratory in Babylon University

college of science, department of biology .

Diagnostic tests for yeasts .

The isolates were cultured on Sabouraud dextrose agar and the petri dishes

incubated at a temperature 28-35 0C for 24-48 hours. Diagnosed isolates initially

substantially the smooth colonies cream color isolated (8), keeping the principals coloniesin the Sabouraud dextrose agar medium (SDA) for the purpose of conducting subsequenttests. It was the perpetuation of the assets of the isolates through re culturing isolates onthe medium (SDA) every 30 days to avoid depletion of nutrients, and underwent thefollowing diagnostic tests.

CHROM agar Candida medium :

This medium used to differentiate between the species of Candida, depending on the

color and characteristics of the surface of the colony, where the yeasts cultured on the

CHROM agar medium at 37oc for 24-48 hours, after incubation examined the petri dishesand was noted the presence of colonies with different colors, each color refers to aspecific species of Candida (9).

Formation of germ tube test :

It was taken 0.5 ml of human serum after separating blood components in centrifuge

and blend well with a colony of yeast isolates from the medium and incubated at 37oc forhours and then took a drop of the mixture and place on slide with a drop of dye(3-4) lacto phenol cotton blue and examined under a microscope to detect the species of yeast that component the germ tube, this is a rapid diagnostic test for the C. albicans from other yeasts species (10).

Haemolysis Test :

Yeast isolates was culture blood agar plate and incubated at 28 oc for three days the

colony was observed which surround haemolysis aria with hyaline colour that referee

type alpha haemolysis refers to a specific species of C. albcanus and other species

.appeared as colony surround green area represent beta type

Prepare various concentrations of silver nanoparticles :

Followed this method for AgNps this was done in the following order, 5 g dry matter

was taken for the AgNps on was completed to 100 ml of distilled water and the stock

solution was obtained Concentration of 5% or 50% mg / ml was obtained from which

concentrates (1, 5 and 10) were prepared As a reminder (12,13) .

4-Preparation of the 0.5 McFarland standards

ml of 0.048 M from BaCl2 (1.17% w/v BaCl2.2H2O) was added to 99.5 ml of0.5

M H2SO4 (1% v/v) with constant stirring. Distribute the standard into screw cap 0.18

tubes of the same size and with the same volume as those used in growing the broth

cultures. Seal the tubes tightly to prevent loss by evaporation. Store protected from light3at room temperature. Vigorously agitate the turbidity standard on a vortex mixer beforeuse (14)

Tested effect silver nanoparticles on yeasts in vitro

Determine the antifungal activity of AgNps was followed by agar disc diffusion

method was done by method (15). Silver concentrations were prepared (1%, 5%, and

mg/ml. placed discs of filtration paper diameter of 5 millimeters, which were (%10

previously sterilized by the autoclave. Suspended isolated colonies from Sabouraud dextrose agar was added to 5 ml from 0.85% sterile normal saline to achieve 0.5 McFarland turbidity to form a yeast stock suspension of 1.5×106 cells/mL, which shouldproduce semi confluent growth with most Candida spp. isolates (16). Then move it 0.1mlto the surface SDA And publish using swabbed (sterile cotton swabs) Then left the dishesto dry the suspension, and Transfer the filtration papers using sterile forceps and placedon the surface of the cultivated dishes with a rate of three Tablets in each dish and withthree replicates per concentration and incubated dishes at 37 ° C for a period of 48 h. afterwhich the inhibition diameter for each concentration .was measured

Statistical analysis -

Data were analyzed statistically using SPSS program through an ANOVA. Means were compared by (5%) LSD test to determine the significant difference between the different treatments assayed. (17) .

Results and Discussion.

1. Isolate and diagnose the pathogenic fungi isolated from the oral

In this study, 95 samples were collected from saliva patients, positive (32 infection) for the presence of fungal elements. Yeasts that isolated were included *Candida parapsilosis, C. albicans, C. glabrata,* and *C. Krusie*. While the filamentous fungi included *Aspergillusniger, Penicilliumchrysogenum*, *Geotrichumcandidium.*

Table (1) types of fungi, number of sample and Percentage (%) of fungiisolates.

Type of fungi	Number of isolates	Percentage(%)of isolates
C. krusie	**5**	**15.6**
C. glabrata	**7**	**21.9**
C. albicans	**12**	**37.5**
C.parapsilosis	**4**	**12.5**
P. chrysogenum	**1**	**3.1**
G. candidium	**1**	**3.1**
A. *niger*	**2**	**6.3**
Total number	**32**	**100%**

2: CHROM agar

The yeast cells were identified according to morphology and color of colonies on CHROM agar as appear in table (4-6) and plate (4-1), according to the color key that putted by (18) and (19). The differentiation in colors belong to the containing this agar a chromosomal material interacts with an enzyme hexosaminidase that secreted by the same species, this leads to a quick diagnosis, depending on the color and appearance of the colony (20).

The advantages of CHROM agar are easy to prepare boiling and dispensing in Petri plates, facilitates the rapid isolation and identification of yeast species. CHROM agar facilitates identification between *candida* spp. from specimens containing mixture of *candida spp.* in the present study and do not affect the viability on sub-sequent subcultures. CHROM agar has the advantage of rapid identification of *Candida species*, technically simple, rapid and cost effective compared to technically demanding time consuming and expensive conventional method (21).

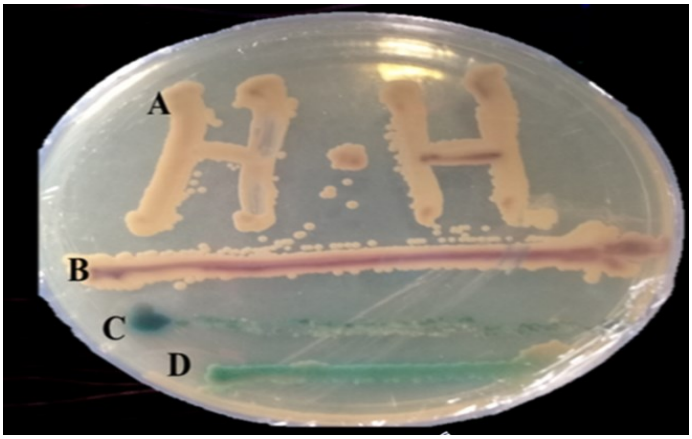


Plate (1): *Candida* colors on CHROM agar; A :*C. parapsilosis*

B:C. *krusie* C: *C. glabrata* D: *C. albican*

3: Test of germ tube formation

It has been evaluating the efficiency of the isolates that showed the green color on the CROM agar to form a germ tube. The test showed that the results of all isolates with green color related to the type *C. albicans*. This study is agreed with (22)

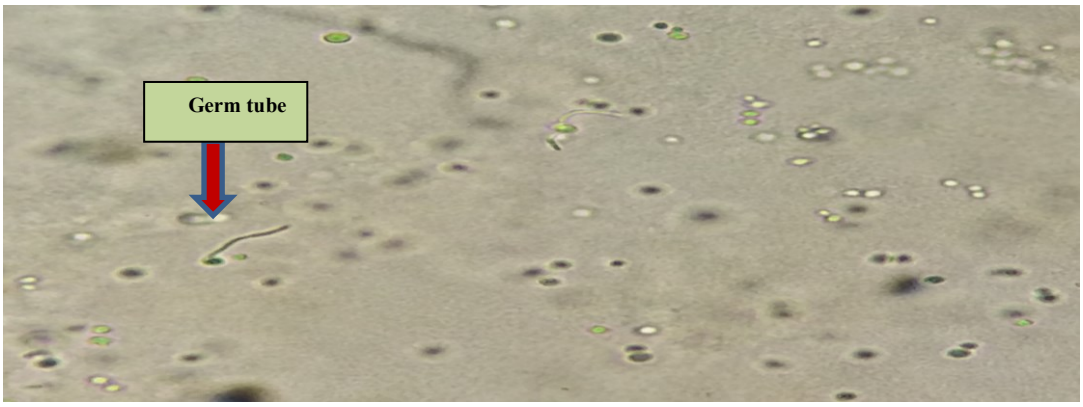


Figure (1): germ tube of *C. albicans* under light microscope (40x)

4: Hemolysis test

*C. albicans*it demonstrated its ability to analyze blood compared to other types of *Candida* where the character of blood analysis characterizes it from other types of *Candida*the result which agreed with previous studies as(23) *C. albicans* exhibits hemolytic activity when grown on glucose-enriched blood agar. This activity is present on intact organisms, and it is secreted into the culture medium. Hemoglobin released from analysis erythrocytes can restore the transferrin-inhibited growth of *C. albicans* Concluded that *C. albicans* expresses a hemolytic factor whichallows it to acquire iron from host erythrocytes.

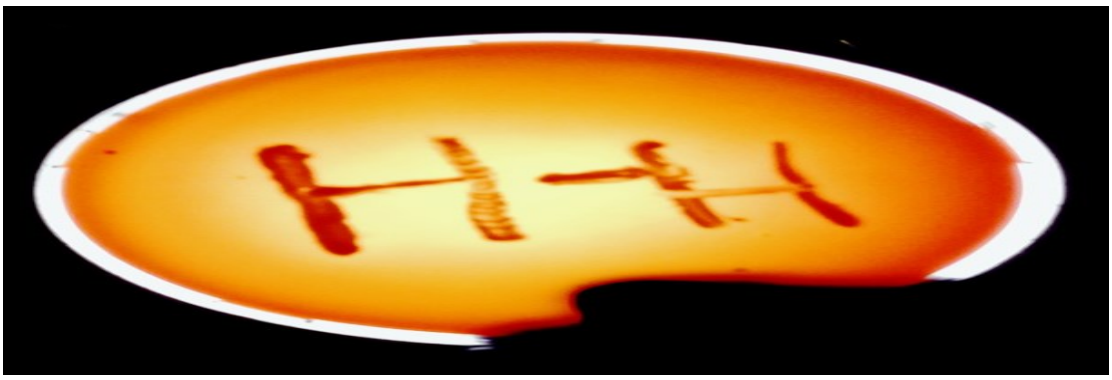


Plate (2): *C. albicans* on blood agar

5: Agar discdiffusion method:

A- Effects AgNps on *C. albicanus*growth:
The results of the present study showed that there were significant differences between concentration of silver nanoparticles the results showing that 10% concentration was the most concentrated inhibition The rate of 34 mm, while the concentration was 5% the result of inhibition 28 mm, while concentration 1% was the lowest concentration inhibited 20.3 mm diameter of the inhibition zone.

as shown in figure (2)and plate (3) which appear the diameter of inhibition zone increase with increase concentration, Results agreed with the study of (25),The nanoparticle particles act as effective antifungal agents against two types of yeast (*Candida albicans*, *Saccharomyces cerevisiae*) Observed through the scan by

electron microscopy Changes in the structure of the membrane of both yeast in the interaction with the particles of silver Nanoparticles, as they are observed holes on the surface of the membrane leading to the death of the cell as a result of these holes and noted the inhibition of the process of buds resulting from the crash of the membrane and thus inhibit the growth of yeast, and withfounded(26)

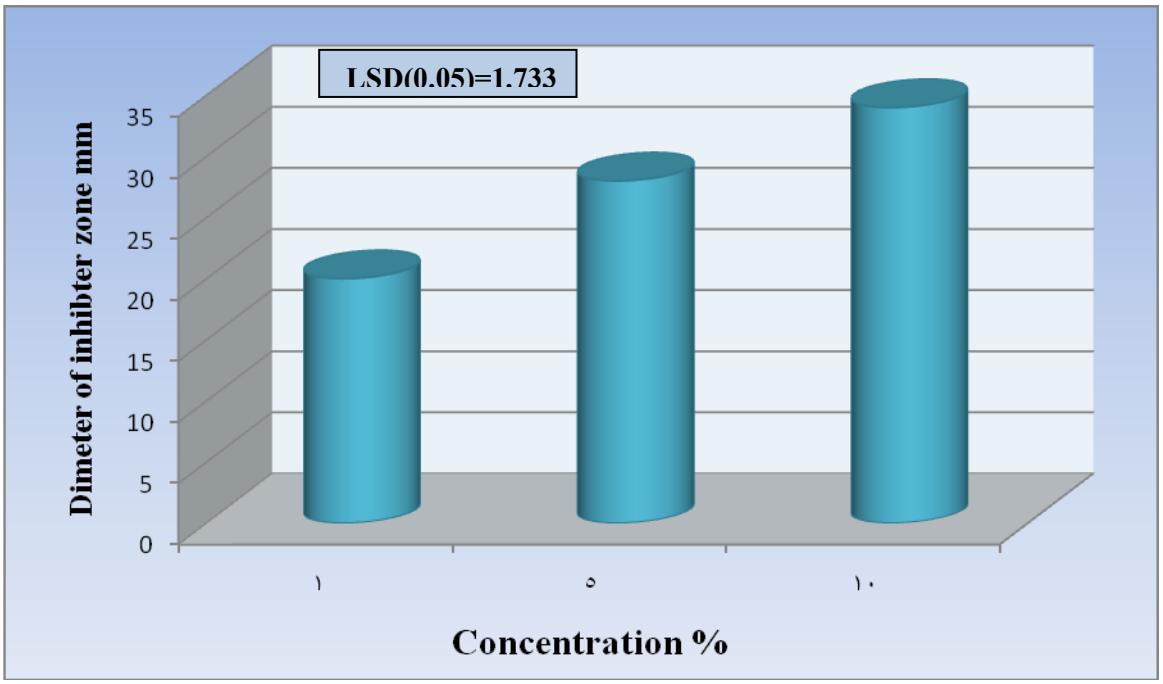


Figure (2): affect different concentration AgNps on *C. albicanus*

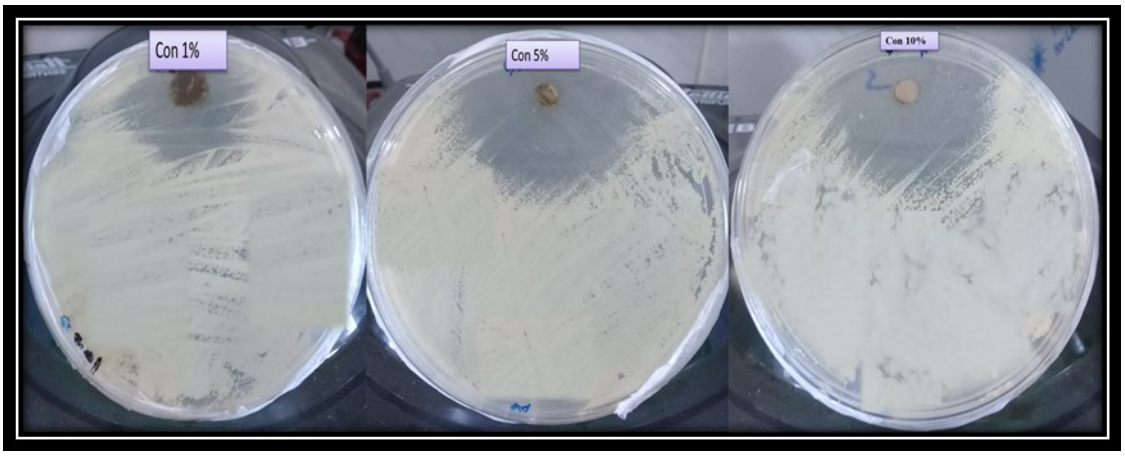


Plate (3):Effects AgNps on *C. albicanus*growth:

B- Effects AgNps on *candida krusie* growth

The results of the present study showed that there were significant differences between concentration of silver nanoparticles the results showing that 10% concentration was the most concentrated inhibition The rate of 15 mm, while the concentration was 5% the result of inhibition 12 mm, while concentration 1% was the lowest concentration inhibited 9.3 mm diameter of the inhibition zone as shown in

figure (3) and plate (4)The results agree with study of (27; 28; 29; 30; 31) Action of AgNPs against yeasts of Candida genera (*C. glabrata* and *C. kruse*).

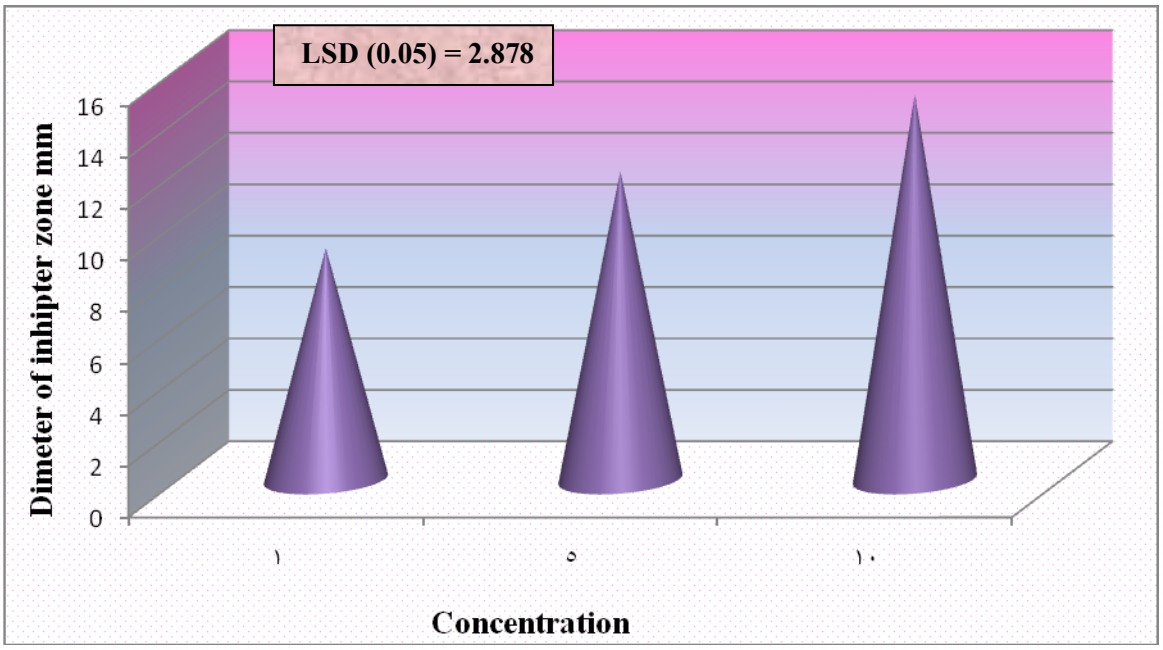
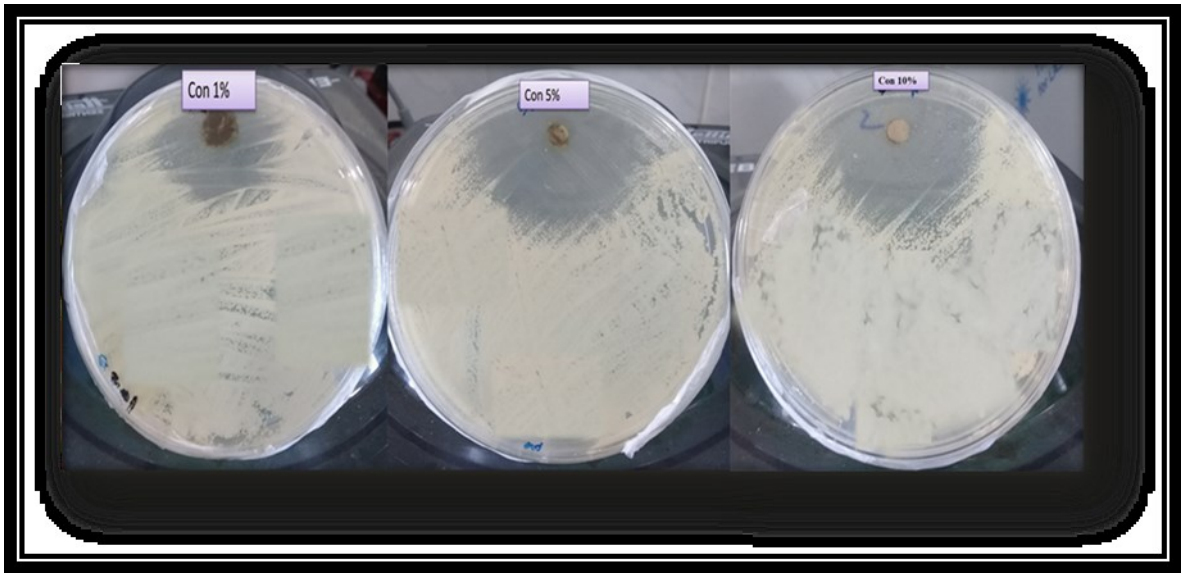


Figure (3): affect different concentration AgNps on *C. krusie*



Plate(4): Effects AgNps on *candida krusie* growth

C- Effects AgNps on*C.glabrata* growth:

The results of the present study showed that there were significant differences between concentration of silver nanoparticles the results showing that 10% concentration was the most concentrated inhibition The rate of 35 mm, while the concentration was 5% the result of inhibition 23 mm, while concentration 1% was the lowest concentration inhibited 15 mm diameter of the inhibition zone.

The figure (4) and plate (5) papered by increase con. Inhibition zone increased the result approve with study (32) AgNPs could be considered as an alternative potential in the development of new antifungal agents with minimum cytotoxicity in

fibroblasts and lethal action on *Candida* species with resistance to conventional antifungal compounds.

And founded that *candida* spp. this product agree with (33) biosynthesized Ag nanoparticles from *G. corticata* have an effective antifungal activity against *C. albicans* and *C. glabrata* (34) Ag-Nps has been shown to inhibit the growth of bacteria and yeast

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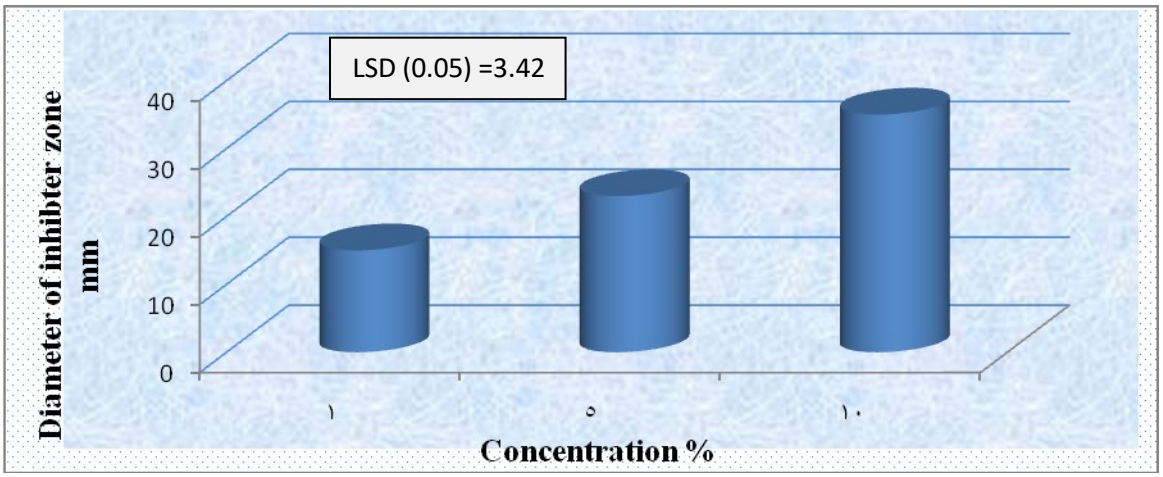

Figure (4): Effects AgNps on *C. glabrata*growth

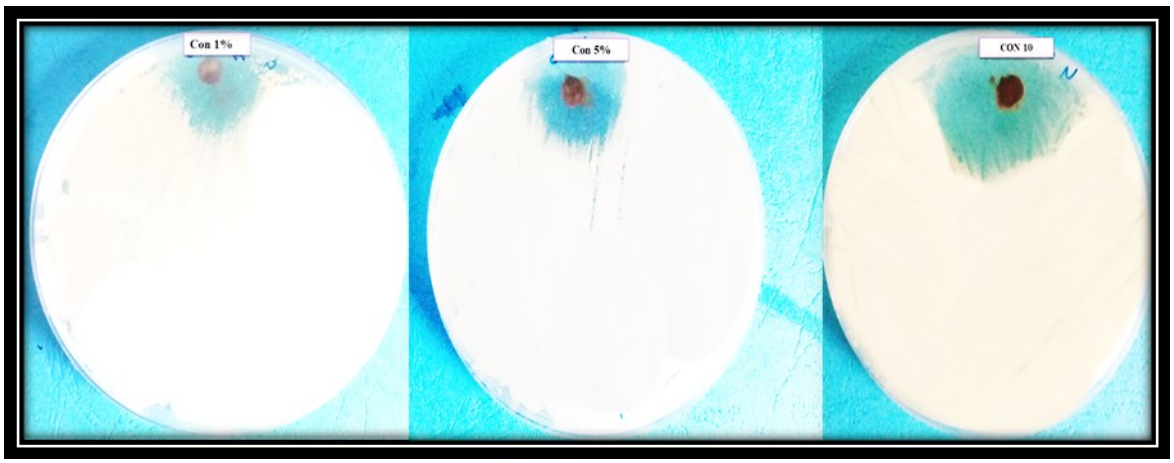

Plate (5) Effects AgNps on C.glabrata growth:

D- Effects AgNps on *C.parapsilosis* growth:

The results of the present study showed that there were significant differences between con. (1%, 5%, 10%) mg /ml of AgNps on the evolution of C. *parapsilosis* (according to the diameter of inhibition zone) appear as follow (10, 12, and 25) mm, showed in figure (4-18).

The resulted of study agree with study (35) included the strong AgNPs activity against *C. parapsilosis* and *X. axonopodis*pv. citri (Xac) that was morphologically characterized,pointing to strong AgNPs effects on microorganisms' membranes.

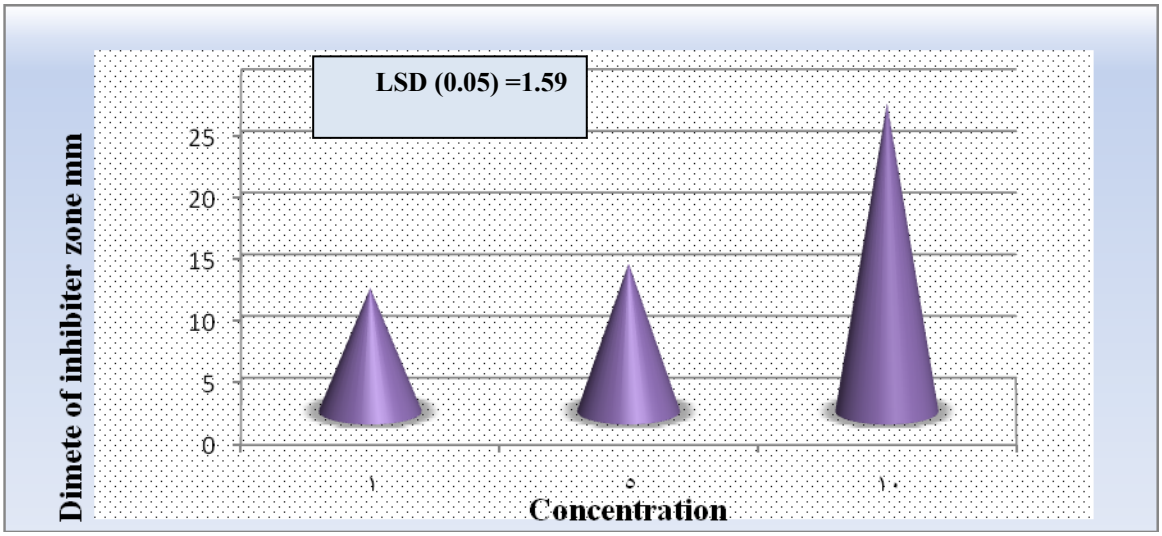

Figure (5): Effect silver nanoparticles on *C. parapsilosis*growth.

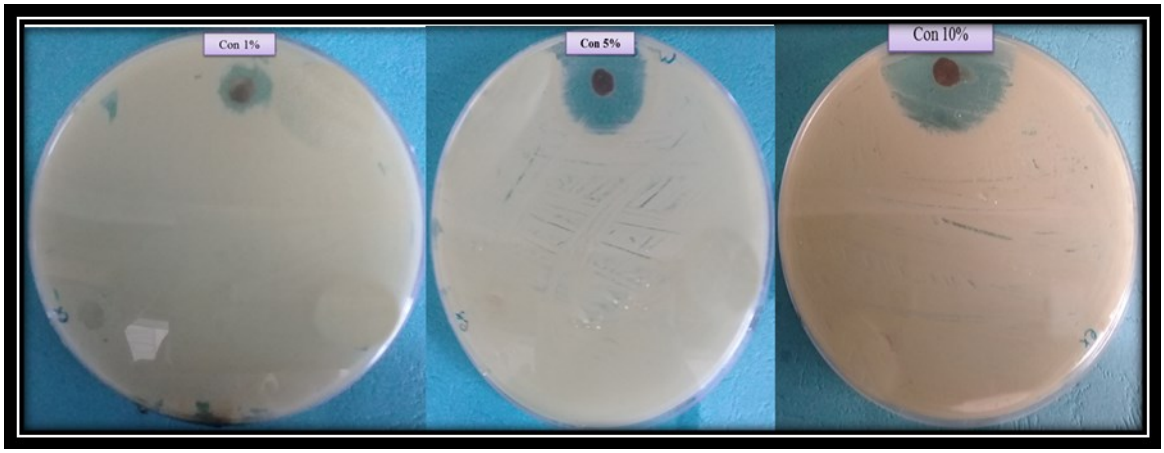

Plate (6): Effects AgNps on *C. parapsilosis*growth

Silver nanoparticles appear the heist effect at concentration 10% especially on *C. glabrata*growth which gave 35 mm in the diameter of inhibition zone followed by *C. albicans* which gave 34 mm in the diameter of inhibition zone, then *C. parapsilosis*, and *C. krusie* which gave 25, 15 mm in the diameter of inhibition zone respectively (plates 3,4,6).

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Conflict of Interest

The authors have declared no conflict of interest .

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