Effect of Silver and Cobalt Ions on the Growth of the fungus *Fusarium chlamydosporum* (Wollenweber & Reinking)

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Abstract

An isolate of the fungus *Fusarium chlamydosporum* was exposed to different concentrations (10, 20, 40, 60, 80, and 100ppm) either of silver Ag^{+1} or of cobalt Co^{+2} ions, singly and a mixture *in vitro* and the growth rate (colony diameter) of the fungus was measured. The results showed that the toxic effect of Ag^{+1} ions was more than those of Co^{+2} ions and at concentration 100ppm Ag^{+1} ions gave the highest effect on the fungus growth. The results also showed that the mixture of Ag^{+1} and Co^{+2} ions has a synergistic effect on the growth rate of the fungus. In general, the effect depened on the heavy metal ions concentration and the exposure periods.

Key words : *Fusarium chlamydosporum* , Heavy metals effect, Silver, Cobalt.

Introduction:

The management of fungal disease on human, food crops and ornamental plants is economically important. Recently more efforts have been given to develop safe management methods that pose less danger to humans, animals and plants (1;2).

Investigations showed that some heavy metals ions such as (Silver ions) having broad spectrum of antimicrobial activity and also very effective for reducing human and plant disease caused by pathogenic fungi (1).

However, the extrapolation of these findings to more general cases is limited due to the fact that most studies are based on *in vitro* Petri dish and in plants growth chamber evaluation, still those researches provide valuable preliminary efficacy data of heavy metal compounds for controlling microbial diseases (3;4). Recently, some heavy metal compounds have been widely used for developing of many biological and pharmaceutical processes products as well as appliances such as coating material for medical devices, orthopedic and dental graft materials, topical aids for wound repair, water sanitization and even washing machines (1;5). In spite of these numerous applications of these compounds and the numerous reports of their antimicrobial activities, the antifungal properties of different forms of these compounds have not been investigated fully therefore, the aim of this study was to determine the inhibitory property of both cobalt (Co^{+1}) and silver ions (Ag^{+1}) singly versus the mixture on the growth rate of the fungus *Fusarium chlamydosporum* which is a well-known fungus taken from natural environments as having the ability to cause sever disease to human animals and plants.

Materials and Methods:

An isolate of the fungus *Fusarium chlamydosporum* was isolated from a patient with tenia pedis infection and identified in the fungal laboratory of the college of science depending on (6).This isolate was grown on the malt extract agar (MEA) medium for 7 days, then an inoculum (6 mm diam.) was taken by a cork borer and placed on to new plates which have 15 ml from of the culture medium submitted with one of the concentrations (10, 20, 40, 60, 80, or 100ppm) of silver ions as $AgNO_3$ salt or cobalt ions as $(CoNO_3)_2.6H_2O$ salt singly or as mixed. Duplicates were used for each treatment in addition to the control. Cultures were incubated under 25C° in dark for 9 days.

After inoculation the fungal growth at each treatment was measured every day until the 9^{th} day of exposure period.

Analysis of variance (ANOVA test) and Revised least significant differences (RLSD test)

were used among means to calculate significant differences ($P<0.05$).

Results:

The analysis of variance (ANOVA test) showed significant differences effects of the heavy metals ions on the growth rate (colonies diameter) of the fungus *Fusarium chlamydosporum* ($P<0.05$) as compared with the control treatment when these ions were added whether singly or mixed.

The Effect of Silver Ag^{+1} Ions Singly:

The growth rate of the fungus was decreased significantly ($P<0.05$) at all treatments, except at 10 and 20ppm concentrations as compared with the free metal treatment (control) as shown in (fig.1 plate 1). The results showed that the highest effect of Ag^{+1} ions on the growth rate of the fungus appeared at 100ppm, in fact the growth of the fungus at 40, 60, 80 and 100ppm treatments became not only slow but also similar to each other ($P>0.05$) but differs from the control, 10 and 20ppm treatments.

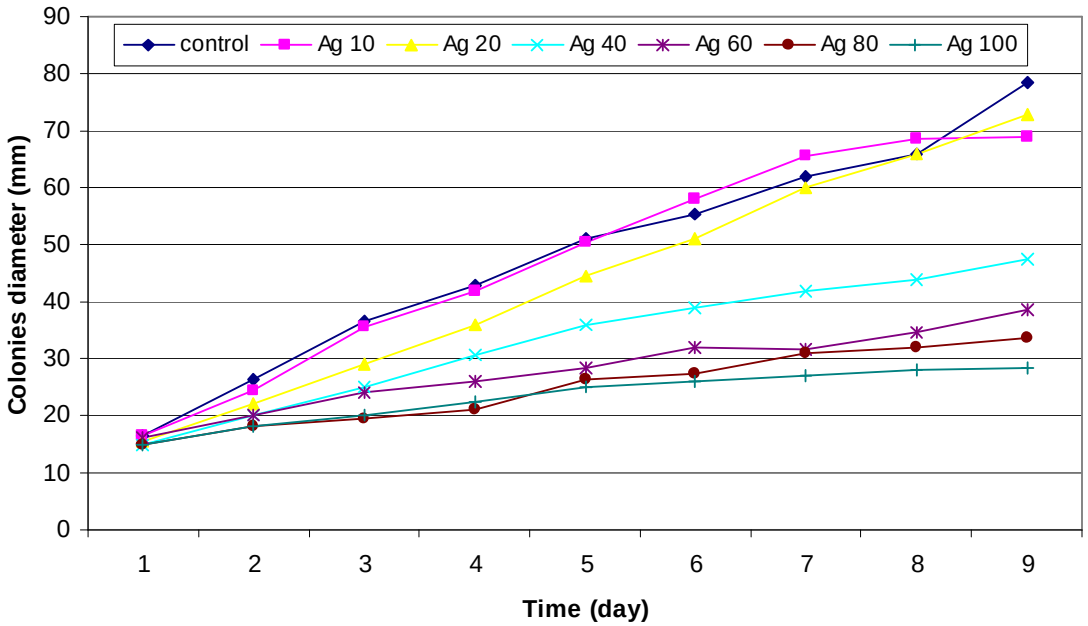

Fig. 1: Effect of Silver ion on the Colonies diameter of *Fusarium chlamydosporum at* different incubation periods.

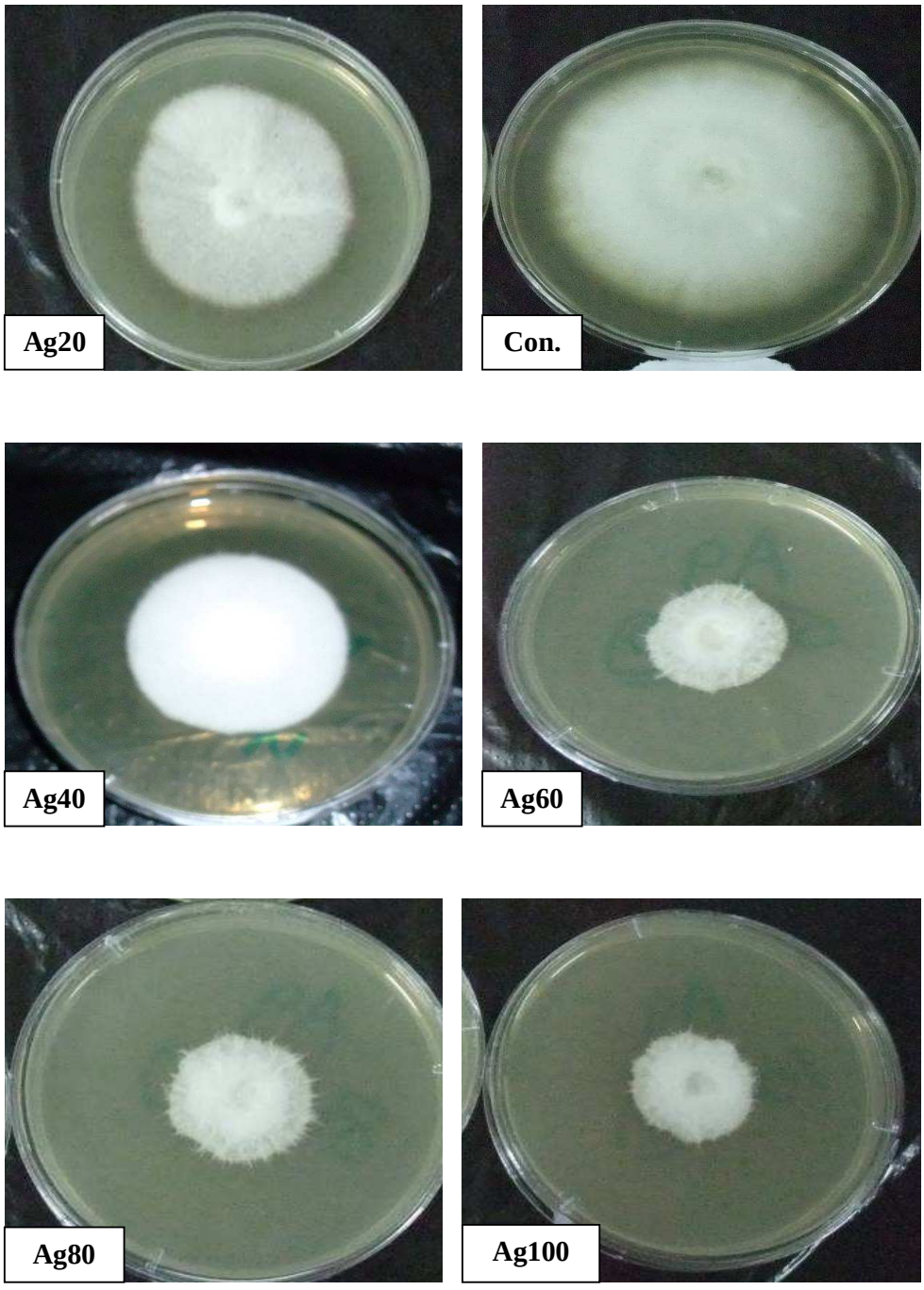

Plate 1: Effect of Silver ion concentrations (ppm) on the colonies diameter of *Fusarium chlamydosporum* after (9) days of incubation.

The Effect of Cobalt Co^{+2} Ions Singly :

There were no significant differences ($P<0.05$) in the growth rate of the fungus colonies when they are exposed to different concentrations of Co^{+2} ions as compared with the control treatment, but these ions mad the growth slow in fact some of the Co^{+2} ions

concentration stimulate the growth of the fungus as shown in (fig.2 plate 2)

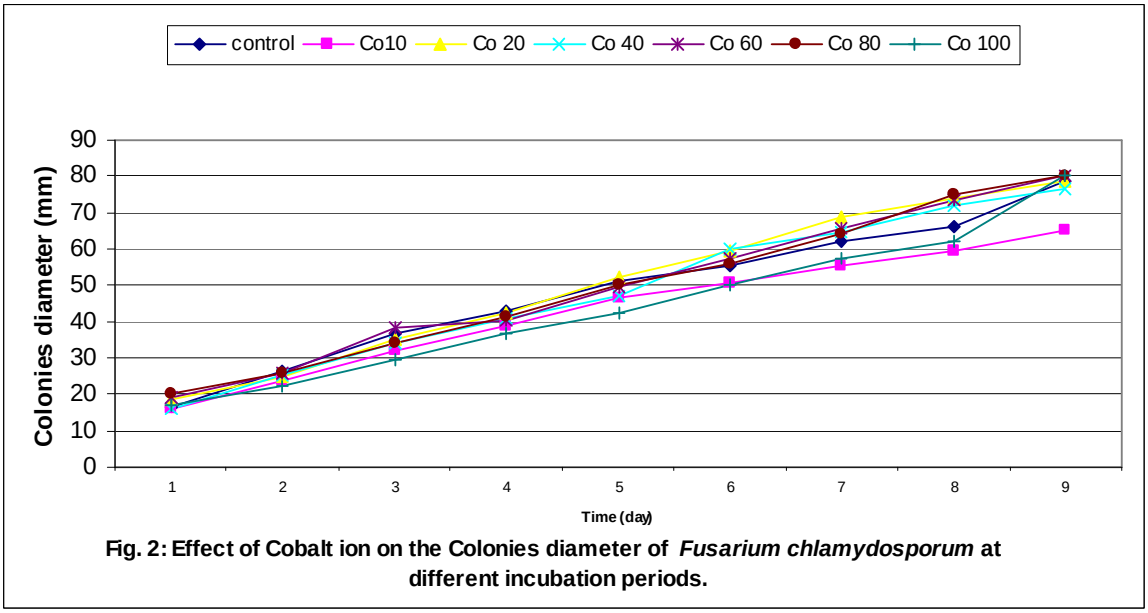

Fig. 2: Effect of Cobalt ion on the Colonies diameter of *Fusarium chlamydosporum* at different incubation periods.

The Effect of Ag^{+1} and Co^{+2} Ions Mixture:

When cobalt ions was added at different concentrations 10, 20 or 40ppm to the treatments which contained Silver ions at 10 or 20 ppm, there no significant differences ($P>0.05$) were found on the growth rate of fungus as compared with the control or with the treatments that contain 10 or 20ppm silver as singly whereas significant differences ($P<0.05$) were found when different concentrations of Co^{+2} ions were added to 40, 60, 80 and 100ppm Ag^{+1} treatments as compared with the control treatment. However, they have no differences from each other or from the treatments which have Ag^{+1} ions at 40, 60, 80 or 100ppm. Also here the growth rate of the fungus is stimulated when some concentrations of the Co^{+2} ions were added to the Ag^{+1} ions as shown in (fig. 3).

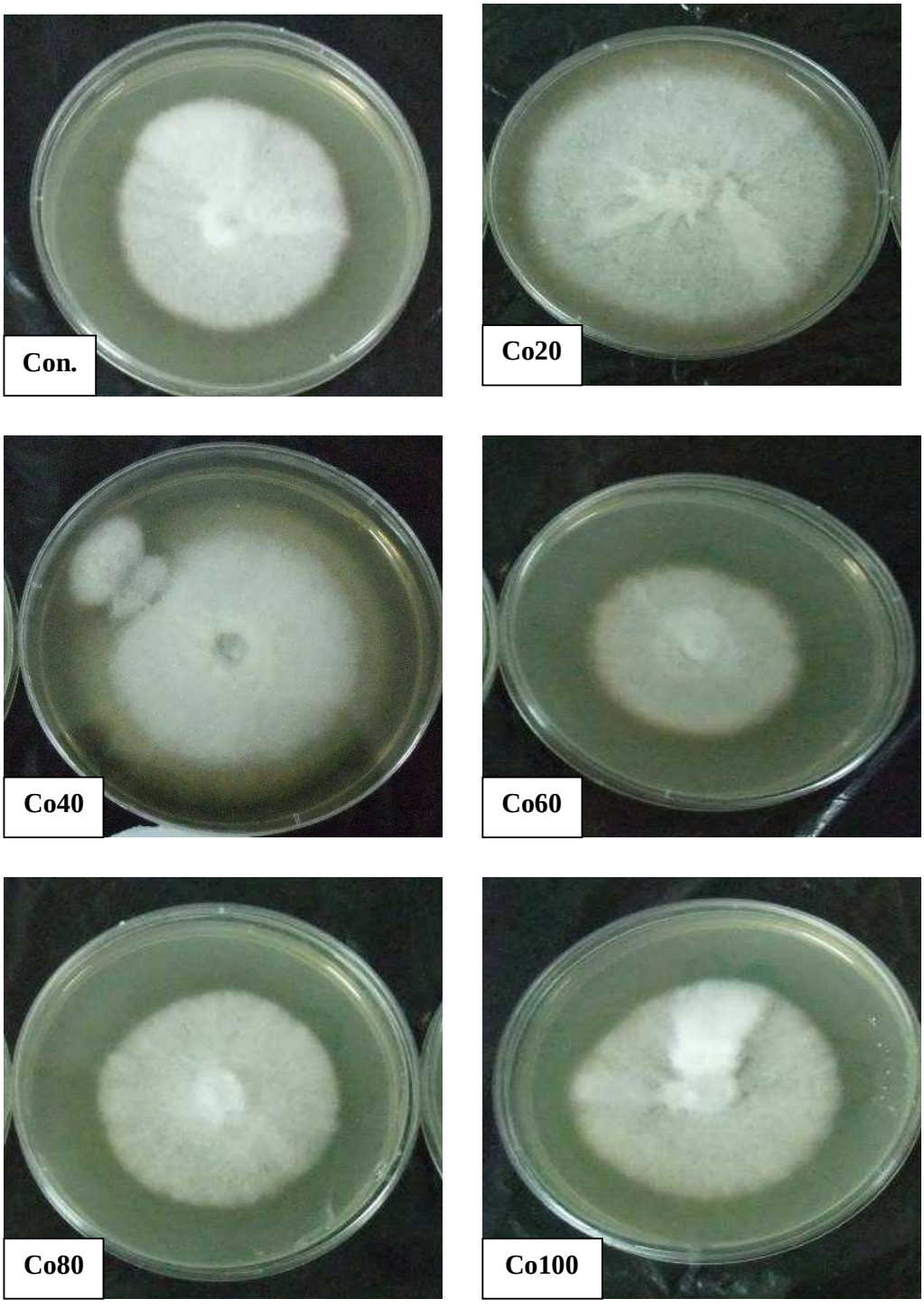

Plate 2: Effect of cobalt ion concentrations (ppm) on the colonies diameter of *Fusarium chlamydosporum* after (9) days of incubation.

This result indicates that the toxic effect of the Silver ions was greater than that of the cobalt ions on the growth rate of the fungus. Yet, the toxic effect of cobalt ions become more when mixed with silver ions together as compared with its toxicity as singly. Finally, high level from the chlamydospores

were produced as compared with the control. It has been noticed that a pink color would be visible in the medium when the fungus is exposed to high concentrations of Ag^{+1} ions whether alone or when mixed with Co^{+2} ions.

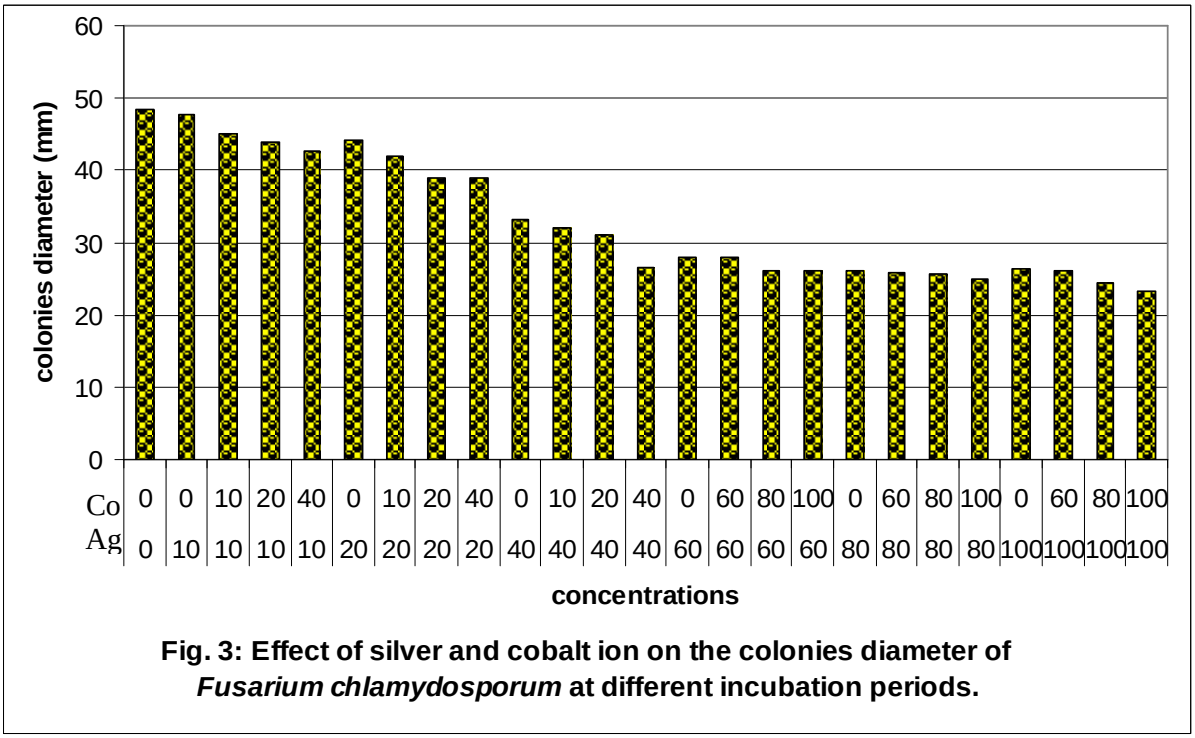

Fig. 3: Effect of silver and cobalt ion on the colonies diameter of *Fusarium chlamydosporum* at different incubation periods.

Discussion:

In fact, there have been a few studies focusing on the effect of heavy metals on the fungus *Fusarium chlamydosporum* which was a wide-speared fungus in the environment, causing sever disease to human, plants and animals (1;6;7;8;9).

In this study, it has been found that the Silver ion concentrations have various levels of inhibition on the fungus growth rate (colonies diameter).The degree of inhibition increased as Silver ion concentrations increased in the medium, these results go in agreement with (2;10;11).

The mechanism of the inhibitory effects of Ag^{+1} ion on Microorganisms is partially known, some studies suggest that the inhibitory effect of Ag^{+1} ion may be due to the directly attach to and penetrate the cell membrane of the spores and killed them (2;12), other studies has reported that antimicrobial activity of Ag^{+1} ion on microorganisms was dependent on the concentration of Ag^{+1} ion and was closely associated with the formation of pits in the cell wall of the microorganisms then Ag^{+1} ion accumulated in the membrane caused the permeability, resulting in cell death (13), and recently Kim *et al.* (14) found that the antimicrobial mechanism of Ag^{+1} ion is related to the formation of free radicals and subsequent this free radical–induced cell membrane damage, also Jung et *al.*(15) found that the interaction between Ag^{+1} ion and the constituents of the microbial cell membrane caused structural changes and damage to the membranes and intracellular metabolic activity which might be the cause of cell death.

The pink color which appeared at the culture medium and the formation of high level of chlamydospores in the presence of high concentration of the Ag^{+1} ions alone or when mix with the Co^{+2} ions may be due to the stress imposed by the ions present at the medium on the fungus growth (16;17).

In spite of the toxicity of silver ions, the low concentrations 10 and 20ppm hadn't affect the growth of the fungus because of some heavy metal ions connected at low concentrations with the organic and non-organic components of the growth medium this may reduce the toxicity of these ions. In general the degree of metals toxicity varied

depending on the fungus, the metal which is use and the growth medium (2;18;19). The results showed that the fungus tolerated a considerable amount of Cobalt ions while at certain concentrations of this ion stimulate the growth of the fungus. These phenomena may be due to that this fungus can translocating this heavy metal into intracellular structure up to particular amounts by binding and thereby detoxify it. Some fungi also offer a wide range of metal-binding functional groups which can detoxify the ions effect, also the cobalt ions can be used by some fungi at particular amount as Co enzyme which stimulate the fungus growth (20;21). Moreover the concentrations of cobalt ions used at these experiments may be lower than those affect the growth rate of this fungus.

When the fungus is exposed to mixed ions of silver and cobalt every metal may affect the growth independing from the other metal, this case may be due to different mechanisms for each metal to affect the growth or this mean that there no competition occurred between these two metals on the binding sites (13;16;17).

In brief, antifungal activity of silver ions was much greater than cobalt ions, when we put this finding with the fact that silver ions was less toxic to human and animal than synthetic fungicides and having multiple modes of action targeting abroad range of biological bath ways of microbes so its important to focus on more research to extend applicability of silver for controlling different pathogenic fungi.

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تاثير ايونات الفضة والكوبلت على نمو الفطر *Fusarium chlamydosporum* (Wollenweber & Reinking)

مصطفى عبد الوهاب نجم و مكية مهلهل الحجاج و زينب فاضل منصور
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الخلاصة:

عرضت عزلة من الفطر *Fusarium chlamydosporum* لتراكيز مختلفة (10، 20، 40، 60، 80 و100 جزء بالمليون) من ايونات الفضة او الكوبلت، بصورة منفردة او بشكل خليط وتحت الظروف المختبرية وتم قياس قطر المستعمرة للفطر.

اظهرت النتائج ان التاثير السمي لايونات الفضة كان اكبر من تاثير ايونات الكوبلت، كذلك كان التاثير المشترك لايونات الفضة والكوبلت بشكل تعاوني على معدل النمو للفطر، وقد اعطى تركيز 100 جزء بالمليون من ايونات الفضة اعلى تاثير على نمو الفطر، وعموماً كان تاثير الايونات معتمداً على تراكيز الايونات وفترة التعريض.