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THE USE OF SOLID AND LIQUID MEDIUM TO STUDY THE EFFECTS OF HEAVY METALS ON THE GROWTH OF CANDIDA ALBICANS .

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ABSTRACT

Sabouraud's dextrose (solid and liquid) medium has been used to examine the response of *Candida albicans* to Cobalt , Nickel , Silver , Copper and Lead in three doses per each (10 , 25 and 50) mg/l in different exposure periods . In the solid medium the number of colonies was not effected by all metals ions , except Ag ions (25 and 50) mg/l which completely inhibited the colonies numbers . In the liquid medium the effect of these metals on the growth was in the following sequence Co , Ni , Ag , Cu and Pb respectively.

Most elements studied showed increase in the inhibition effect on the growth with increasing the ions concentration in the medium , while the inhibition effect decreased with the increasing periods of exposure.

INTRODUCTION

A number of fungal activities including respiration , mycelial growth , spore production and germination are known to be inhibited by heavy metals pollutants (1).Yeasts are known to possess the ability to accumulate metals from aqueous solution by physico-chemical interaction like adsorption and absorption or by metabolism (2,3,4). Sorption processes depend on the nature of the metal ion and on disposable functional groups on the cell surface (5). The concentration ligand charge and cavity size influence the selectivity of the metal uptake (6,7) . On the other hand , the composition of the solution to which the microorganisms is exposed affects the amount of the metal taken up (8) . The substrate composition influences the cell wall structure and the metabolic state of the cell(9,10). There is only a few studies about the effect and removal of the heavy metal on the pathogenic isolates of *Candida albicans*. Therefore , the present study was designed to assess the effective element and concentration of heavy metals on the growth of this microorganism in vitro , and to determine the effect of exposure period on the growth .ate.

MATERIALS AND METHODS

In this work *Candida albicans* isolated from case of women vaginal candidiasis (11) was used . The species studied was identified following the schemes of (12,13). Two loopfuls of isolated colony to be tested from 1-2 day on Sabouraud's dextrose agar old cultures were inoculated into tubes containing 5ml of sterilized distilled water . Then diluted by 1:100 with sterilized distilled water , shacked vigorously .

The suspension was adjusted to 10^6 cell / ml . by using improved Neubauer chamber (14). Nine ml of Sabouraud's dextrose broth were dispensed into each tube . After autoclaving heavy metals ions (cobalt as $Co(NO_3)_2.6H_2O$, nickel as $Ni(NO_3)_2.6H_2O$, silver as $AgNO_3$, copper as $Cu(NO_3)_2.3H_2O$ and lead as $Pb(NO_3)_2$ were added at three doses 10 , 25 and 50 mg/l. under aseptic condition . One ml of cell suspension (10^6 cell / ml) was introduced into each tube , also one ml of cell suspension was added to medium free from heavy metal (control). By L-shaped spreader , 0.02 ml of cell suspension (10^6 cell / ml) was spreaded on each Sabouraud's dextrose agar (15 ml / Petridissh) incorporated with heavy metal ions. For each treatment triplicate tubes and plates were used.

Optical density (O.D.) at 590 nm was detected for all treatment after exposure periods (1,2,3 and 4 days) by using spectrophotometer (Milton Roy Spectronic 601) . After 1,2,3 and 4 days of incubation number and diameters of colonies were compared , also mean rates of colonies number and diameter were calculated.

Data were analyzed statistically by using the analysis of variance (ANOVA) and Revised Least Significant Differences (RLSD) test to compare between the means.

RESULTS

The results of the statistical analysis (ANOVA test) showed that the effect of study metals ions was significant ($P< 0.01$) on the growth of *Candida albicans* compared with control treatment .

By RLSD test (RLSD = 0.03995) revealed no significant differences between the effect of lead and copper on the growth rate . The effect of the study metals ions was in the following sequence :

Co , Ni , Ag , Cu and Pb respectively .

Fig. (1) : *the effect of cobalt ions on the growth of C. albicans.*

In broth medium the effect of cobalt ions was highest on the lst day of the experiment , whereas the lowest effect was on the 4th day (except 50 mg/l treatment) . The effect of cobalt ions was increased with the increase of cobalt concentration in the medium .

In the solid medium the effect of cobalt ions on the colonies numbers on the 1st day at 10 mg/l was not effective but 25 mg/l treatment decreased the numbers , while no growth was revealed in 50 mg/l treatment . On the other hand , there were no significant differences on the colonies numbers in all treatments on the different periods used compared with control treatment Table(1).

Colonies diameter were significant decreased at 25 mg/l treatment , and least diameter was recorded at 50 mg/ltreatment (1.25 mm) . Table (2)

Fig : (2) Effect of nickel ions on the growth of C.albicans

The growth rate of *C.albicans* was significant decreased with the increase of ions concentration in the broth medium , the highest effect was seen on the 1st day of exposure periods comparable with the 4th day in different concentration used.

At 10 mg/l treatment the growth rate did not show any differences on the 1st or 2nd day , also on 3rd or 4th day . At 25 mg/l treatment effect was found on the growth rate at 3rd and 4th day . On the other hand , no effect was found between 1st and 2nd day . In 50 mg/l treatment on significant effect was seen at different exposure periods .

In the solid medium the colonies numbers were not effect at 10 and 25 mg/l treatments whereas 50 mg/l treatment act to elongation the lag phase period. The effect of 10 mg/l ions on the colonies diameters was not significantly different in comparison with the control treatment , while 25 and 50 mg/l treatments were decreased significantly the diameters of colonies . (Table 1 and 2).

Fig. (3) the effect of silver ions on the growth of C. albicans

In the broth medium the highest effect was recorded in the 25 and 50 mg/l treatments . On the other hand , 10 mg/l treatment did not show differences comparable with the control treatment (except at 2nd day), and the growth rate significantly increased with the exposure period , while the similar effect was seen in the 3rd and 4th day . At 25 and 50 mg/l treatments the growth rate was not affected with the exposure periods .

In the solid medium at 10 mg/l treatment the colonies number was not different but colonies diameter was decreased compared with control treatment ,

while the colonies growth disappeared in 25 and 50 mg/l treatments . (Table 1 and 2).

Fig. (4) the effect of copper ions on the growth of C. albicans

Broth medium showed that copper ions increase the growth rate with the exposure periods (except at the 4th day which did not differ from the 3rd day). The

growth rate at 10 and 25 mg/l treatments were decreased , but the 50 mg/l treatment incrased the growth rate compared with the control treatment.

In the solid medium no clear effect on colonies numbers was seen. Whereas colony diameter was increased at 10 mg/l and decreased at 25 and 50 mg/l treatments. (Table 1 and 2) .

Fig. (5) effect of lead ions on the growth of C. albicans

Ten mg/l treatment decreased the growth rate on 1st and 2nd days , also a similar effect was seen on the 3rd and 4th days compared with the control . At 25 mg/l treatment the decreases in growth occurred only in the 2nd day , while at 50 mg/l treatment the decreases in the growth rate was occurred in 2nd and 3rd day.

No clear effect was seen with lead ions on the colonies number in the solid medium , but a clear effect (decrease) was seen in colonies diameter in different treatments . (Table 1 and 2)

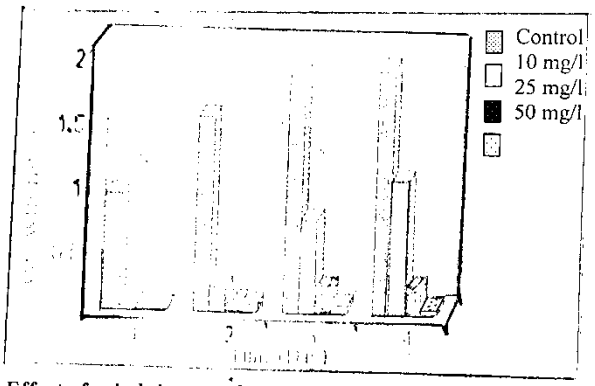

Fig. (1): Effect of cobalt ions on the growth rate of *C.albicans* in liquid medium.

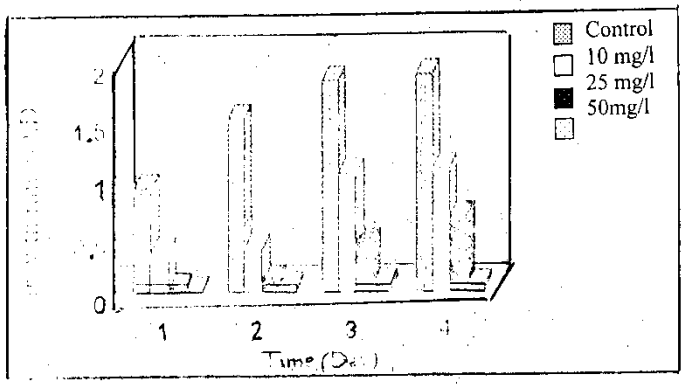

Fig. (2): Effect of Nickel ions on the growth rate of *C.albicans* in liquid medium.

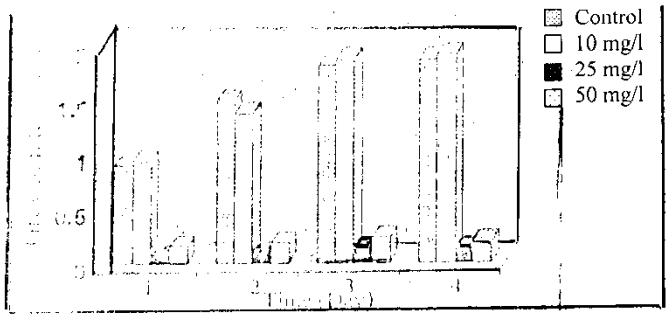

Fig. (3): Effect of Silver ions on the growth rate of *C.albicans* in liquid medium.

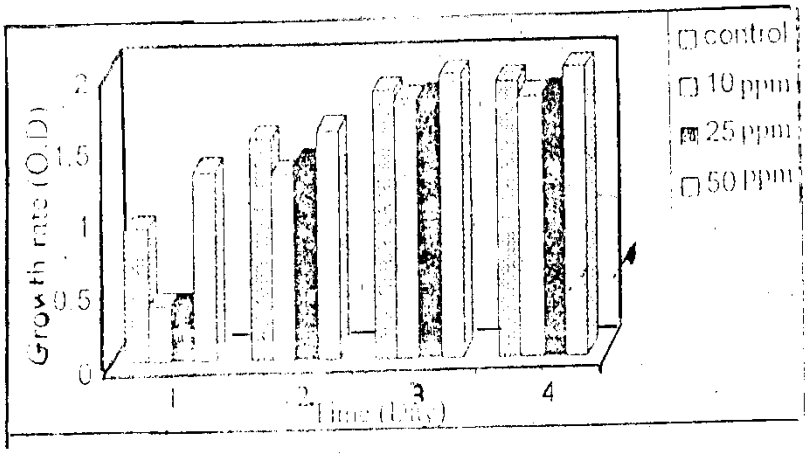

Fig. (4): Effect of Copper ions on the growth rate of *C.albicans* in liquid medium.

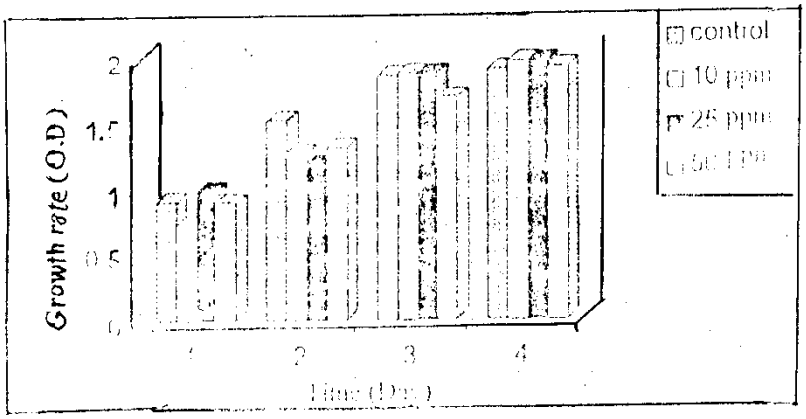

Fig. (5): Effect of Lead ions on the growth rate of *C.albicans* in liquid medium.

Treatments	Ions Concentrations (mg/l)	Exposure periods (days)			
		1	2	3	4
Control	0	> 200	>200	> 200	>200
Co	10	> 200	>200	> 200	>200
	25	100 - 150	>200	> 200	>200
	50	No growth	>200	> 200	>200
Ni	10	> 200	>200	> 200	>200
	25	> 200	>200	> 200	>200
	50	2 – 5	>200	> 200	>200
Ag	10	> 200	>200	> 200	>200
	25	No growth	No growth	No growth	No growth
	50	No growth	No growth	No growth	No growth
Cu	10	> 200	>200	> 200	>200
	25	> 200	>200	> 200	>200
	50	> 200	>200	> 200	>200
pb	10	> 200	>200	> 200	>200
	25	100 – 150	>200	> 200	>200
	50	> 200	>200	> 200	>200

Table (1) : Effect of heavy metals ions on the colonies numbers of *Candida albicans* on solid medium

Treatments	Ions Concentrations (mg/l)	Exposure periods (days)			
		1	2	3	4
Control	0	0.5 – 2	0.75 – 4	0.75 – 5.5	0.75 – 6.5
Co	10	0.5 – 1.5	0.5 – 4.5	0.5 – 5.5	0.5 -6.5
	25	0.1 – 1.25	0.2 – 2.25	0.2 - 3.5	0.2 – 4.5
	50	No growth	0.1 – 1	0.1 – 1	0.1 – 1.25
Ni	10	0.5 – 2	0.5 – 3.5	0.5 – 4	0.5 - 4.5
	25	0.1 – 0.5	0.1 – 1.75	0.1 – 2	0.2 – 2.5
	50	0.1 – 0.5	0.1 – 0.6	0.1 – 0.8	0.1 – 1
Ag	10	0.1 – 0.75	0.5 – 2.5	0.5 – 3.5	0.5 – 4.5
	25	No growth	No growth	No growth	No growth
	50	No growth	No growth	No growth	No growth
Cu	10	0.5 – 2	0.7 – 3.5	0.7 – 6	0.7 – 7.5
	25	0.5 – 2	0.5 – 3.5	1 – 4	1 – 4.75
	50	0.5 – 2	0.5 – 3.7	0.5 -4	0.5 – 5.7
pb	10	0.5 – 2	0.5 – 3	0.5 – 3.2	0.5 – 3.5
	25	0.5 – 1.5	0.5 – 3.7	0.5 – 4	0.5 – 4
	50	0.5 – 1.7	0.5 – 3	0.5 – 3.2	0.5 – 3.5

Table (2) : Effect of heavy metals ions on the colonies diameters (mm) of *Candida albicans* on solid medium

DISCUSSION

The effect of heavy metals ions on the organisms was varied according to the metals type , concentration and exposured periods (15) . In the present study we found that cobalt ions was the toxic ions on the growth rate of *C.albicans* in liquid medium , this is in line with finding of (16) who found that cobalt ions were most toxic for basidiomycetes , also (17) found the same result in other 21 species of basidiomycetes . The toxicity of these ions may be due to the ability of these strains to take up most ions from the culture medium . Norris (9) reported two step process for *saccharomyces cerevisiae* accumulating cobalt , the first step was a rapid binding to negatively charged groups on the cell surface , following by metabolism dependent uptake .

Nickel ions had the nearest effect to that cobalt ions on the growth rate in liquid medium , this may be due to the similar mechanism in which the strain take up these ions . Our result was in contrast with finding of (18) who found that mycelial dry weight of *Myrothecium verrucaria* and *Phoma humicola* was not affected by any dose (10 , 50 and 100 mg/l) of nickel ions , but the growth of *Fusarium solani* was significant inhibited by the high dose (100 mg/l) only and was not affected by the other doses (10 and 50 mg/l) . these varied in results perhaps related to the type species which used in the present study. The effect of copper ions in our results increases the growth rate with the exposure periods except on the 4^{th} day . Our results show a disagreement with the results obtained by (19) , they found that the toxicity was Cu > Co > Zn . And in contrast with the results of (20) who found out that copper was the most toxic metal to *Aueobasidium pululIans* .

El – Sharouny , *et al* (18) showed that *Aspergillus* were not affected by any dose (10 , 50 and 100 mg/l) of copper sulphate . This element make colony colouration (brown colony) in our opinion this may lead to decrease the effect of copper ions due to the change in the composition from effective form to another form useful for the growth . This result was in agreement with finding of (21) who found that the ability of more tolerant isolates to change the colour of agar containing $CuCl_2.2H_2O$ from green to

brown and suggestes that a change in the state of the copper ion complex , possibly involves a change in oxidation state and solubility . Copper tolerated isolates of *Saccharomyces cerevisiae* deposite copper as the sulphate at the cell periphery or within the cytoplasm , and the production of cysteine – sulpher and thiol compounds as chelating agents has been proposed as a mechanism to reduce the availability and toxicity of the metal . In other fungi , resistance to copper depends on deposition of copper oxalate with the cytoplasm.'

The lead ions had a less effect compared with other elements . Our result was in agreement with (18) they found that *Phoma humicola* was not affected at any dose (10 , 50

and 100 mg/l) of lead nitrate . Also they found that *Aspergillus niger* was not inhibited by 1000 mg/l pb^{+2} . Al – Rikabi (1) showed that pb^{+2} at concentration of 0.15 ppm significantly increased the vegetative growth of *Achlya racemosa* while depreesing effect was found at higher concentration . It is apparent that the phenomenon of microbial resistance to toxicity of heavy metals ions allows no simple explanation . This is undoubtedly due to the multiplicity of interactions that can occur between microbial cells , heavy metal ions and other environmental constituents (18).

Cobalt ions affected significantly the colonies numbers and diameters at 25 and 50 mg/l . Nickel ions revealed the same result but the effect on colonies numbers restricted at 50 mg/l . Also the same effect on colonies diameters by silver ions was recorded .

No clear effect on colonies numbers occurred by copper and lead ions while a clear effect on diameter was seen especially in higher concentration of ions (at 25 and 50 mg/l) . Al – Rikabi (1) showed that Pb^{+2} at low concentration significant increased the vegetative growth of *Achlya racemosa* , whereas depressing effect was found at higher concentration .

Generally, in our study we found that the inhibition effect on the growth was decreased with the increase of the exposure periods in most concentration this may be due to the ability of *C.albicans* to adaptation by several mechanisms.

CONCLUSION AND RECOMMENDATION

1. This study showed that Ag , Co and Ni ions were more effective as heavy metals elements than others (Cu and Pb) in liquid medium.
2. Most elements used revealed increases in the inhibition effect on the growth rate of the studied microorganism with increased ion concentration in the medium .
3. The inhibition effect decreased with the increase of periods of exposure to the elements in general .
4. We recommend for further study to investigate the activity of these effective metals (Ag ,Co and Ni) and ion concentration in the treatment of infection caused by *Candida albicans* in the future .

أستخدام الوسط الزرعي الصلب والسائل لدراسة تأثير العناصر الثقيلة على نمو الخميرة *Candida albicans*

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الخلاصة

أستخدم الوسط الزرعي Sabouraud's dextrose الصلب والسائل لدراسة استجابة الخميرة *Candida albicans* لتأثير العناصر الثقيلة (الكوبلت ، النيكل ، الفضة ، النحاس والرصاص) بثلاثة تراكيز هي (50,25,10) ملغم / لتر وعلى فترات تعريض مختلفة .

أظهرت النتائج عدم تأثر المستعمرات في الوسط الزرعي الصلب عند أضافة تراكيز تلك العناصر في الوسط عدا عنصر الفضة الذي أثر بصورة كاملة على عدد المستعمرات وبالأخص عند التركيزين (50,25) ملغم / لتر ، أما في الوسط السائل فكان تأثير تلك العناصر حسب ترتيبها الرصاص < النحاس < الفضة < النيكل < الكوبلت .

من هذه الدراسة نستنتج ان معظم العناصر ازداد تأثيرها التثبيطي بزيادة تركيزها في الوسط الزرعي وانخفض ذلك التاثير بزيادة فترات التعريض .

REFERENCES

1- **Al – Rikabi** , S. A. W. (1997) A study on the effect of Zn^{+2} , Pb^{+2} and Mn^{+2} on the vegetative and sporogenesis of *Achlya racemosa* .Al – Mustansiriya . J. Sci , 8(3): 109 – 115 .

2-Gadd, G. M. (1983a). Heavy metal accumulation by bacteria and other microorganisms. Experientia , 46, 834 – 849.

3-Gadd, G. M. (1986) . The uptake of heavy metals by fungi and yeasts: the chemistry and physiology of the process and applications for Biotechnololgy. In immobilisation of inos by Biosorption, eds H. Eccles and S. Hunt. Ellis Horwood Ltd. Chichester. UK. 135 – 157 pp.

4- Brady , D. and Duncan , J. R. (1994) . Bioaccumulation of metal cations by *Saccharomyces cerevisiae* . Appi. Microbiol. Biotechnol , 41 , 149 – 154.

5- Beveridge , T. J. (1989). Role of cellular design in bacterial metal accumulation and mineralization . Annu. Rev – Micro. 43 , 147 – 171.

6-Williams , R. J. P. (1981). Physico – chemical aspects of inorganic elements transfer through membranes. Phil. Trans. R. Soc. Lond. B. 294, 57 – 74 .

7- **Fransto** da Silva, J. J. R. and Williams, R. J. P. (1996). The uptake of elements by biological systems. In Structure and Bonding, eds. J. D. Dunitz, P. Hemmerich. J. A. Ibers, C. K. Forgensen , J. B. Neilands, D. Reinen and R. J. P. Williams. Springer – verlag, Berlin , Heidelberg, New York. 67 – 122 pp.

8- **Grupa,** U. Kunz, B. and Bauer, W. (1992). Intrazellulare Akkumulation von cadimium durch *E. coli* zur Reinigung belaster wasser. Bioengineering , 3, 34 – 37 . Cited by Engl, A. and Kunz, B. (1995). Biosorption of heavy metals by *Saccharomyces cerevisiae* : Effects of Nutrient Conditions. J. Chem. Tech. Biotechno. 63, 257 – 261.

9- **Norris**, P. R. (1977). Accumulation of cadmium and cobalt by *Saccharomyces cerevisiae* J. Gen. Microbiology. 99, 317 – 324.

10- **Norris**, P. R. and Kelly, D. P. (1979). Accumulation of metals by bacteria and yeasts . Developments in industerial microbiology, 20, 299 – 308.

11- **Khudor** , M. H. (1998) . A study on vaginal candidiasis in Basrah women and effect of Five antifungal drugs on some clinical isolates. M. Sc. Thesis, College of Science, University of Basrah.

12- **Ellis** , D. H. (1994) . Clinical mycology: the human opportunistic mycosis. Pfzor , New York 166 pp.

13- **Hoog,** G. S. de., and Guaro, J. (1995). Atlas of clinical fungi universital Rovira I virgilli, Reus, Spain and centraalbureau voor schmmelcultures, Baarn Delft, the Netherlands . 720 pp.

14-.**Anonymous** (1995) . Antifungal susceptibility testing . Faculity of Medicine , University of Rovira . I virgilli , Spain . 39 pp.

15-. **Khudor**, M. H. (2001). Effect of cadmium and zinc ions on the growth of *Candida albicans* and *Saccharomyces cerevisiae*.Accepted for publication in Al – Qadisiya the scientific journal of vet. Medicine.

16-. **Baldrian** , P. and Gabriel , J. (1997) . Effect of heavy metals on the growth of selected wood – rotting basidiomycetes . Folia microbiol , 42 (5) , 521 – 523 .

17-. **Sanglimsuwan** S., Yoshida N., Morinaga T. and Murooka, Y. (1993) . Resistance to and uptake of heavy metals in Mushrooms. J. Ferment Bioeng. 75, 112 – 114.

18-. **El – Sharouny** , H. M. M., Bagy , M. M. K. and El – sharawany A. A. (1989). Effect of six heavy metals ions on the mycelial growth of some Egyptian soil fungi. Bull. Fac. Sci, Assiyt Unive., 18 (1-D) 1 – 13.

19-**Hooley**, P. and Shaw, D. S. (1985). Inheritance of sensitivity to heavy metals in ***Phytophthora drechsleri.*** **Trans. Br. Mycol. Soc. 85 (4). 677 – 681.**

20-. **Gadd**, G. M. (1983b) . The use of solid medium to study effects of cadmium , copper and zinc on yeasts and yeast – like fungi : applicability and limitations . J. of Applied Bacteriology . 54, 57 – 62.

21- **Ross**, I. S. (1975). Some effects of heavy metals on fungal cells. Transactor of the British Mycological Society . 64, 175 – 193.