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THE INTERACTIVE EFFECT OF SOME MOLLUSCICIDES ON THE INTERMEDIATE HOST OF HUMAN BILHARZIASIS AND THEIR ASSOCIATED MYCOFLORA IN THE RIVER NILE, EGYPT.

(With 2 Tables & One Fig.)

By

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تأثير بعض مبيدات الرخويات على العائل الوسيط للبلهارسيا والفطريات المتواجده عليهم

رمضان بدران ، أحمد ياسين

تم جمع ٣٥٠ عينه من قوقعة بيومفلاريا الكسندريناوبولينس ترانكاتس من أماكن مختلفه من
نهر النيل وفروعه ثم اجرى اختبار فاعلية مبيد البيلوسيد وكبريتات النحاس ضد القواقع
والفطريات المتواجده عليهم وتم تلخيص النتائج كالتالى :

تم تعريف ٤٣ و ٣٤ نوعاً من الفطريات تنتمى الى ٢٠ و ١٦ جنساً من الفطريات ذات الجراثيم
السابحه والارضيه

أظهر المبيدان تأثيراً قاتلاً على القواقع بيومفلاريا الكسندرينا مع ظهور التأثير الضار لكل
منهما على الانقسام الخلوى لكل من القوقعين .

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SUMMARY

The mycotic inhabitants of both control and treated snails were surveyed at the 7th and 15th day interval. The highest number of zoosporic species (16) were collected from control samples. The highest population of zoosporic fungi was collected from *Biomphalaria alexandrina*, the lowest from *Bulinus truncatus*. *Achlya*, *Dictyuchus* and *Saprolegnia* were the common genera. The Total colonies of zoosporic fungi were lowered after 15 days of treatment. Both of zoosporic and terrestrial fungi were affected markedly by copper sulphate than Bayluscide. On the other hand the collective counts of some terrestrial fungi were increased in the second period of (15 days) of snail treatment. *Aspergillus*, *Cladosporium* and *Penicillium* were the most prevalent genera. The two molluscicides affected more on the mortality of *Biomphalaria alexandrina* than *Bulinus truncatus*. They also affected on the cell division of both tested snails.

Keywords: Effect, molluscicides, intermediate host, human bilharziasis, associated microflora, River Nile, Egypt.

INTRODUCTION

During the last few decades there have been numerous reports on the role and organization of fungal community in the freshwater ecosystem (WICKLOW and CARROLL, 1981). Most of these reports were focused on fungi colonizing plant materials in rivers and lakes (WILLOUGHBY and ARCHER, 1973, CUMMINS, 1974, KHALLIL, 1990).

The occurrence of aquatic mycoflora in River Nile water and its tributaries have been extensively studied (KHALLIL, 1984, EL-NAGDY, 1985, EL-SHAROUNY, 1987 and EL-HISSY & KHALLIL, 1989) but we have no information concerning the effect of some molluscicides on the mycotic inhabitants of snails.

Shistosomiasis which has been known in Egypt since ancient times, is the most important parasitic disease, however suitable progress in the control was achieved only in the past decade with the advent of efficient drugs and molluscicides. Several authors showed the dangers of pesticides on the damage of the hereditary material. AMER and ALI (1980) reported the effect of a number of pesticides in inducing chromosomal

aberration on both meiotic and mitotic division, while BRUNERI (1971) obtained a complete inhibition of cell division where the mitotic index were zero. AXELSON and SUNDELL (197) reported the increasing of the frequency of cancer among people who have exposed to pesticides.

In Egypt recently, application of different molluscicides were widely used. Copper sulphate and Bayluscide are the most effective molluscicides against the intermediate host of human Bilharziasis. Therefore our investigation aimed to test the inhibitive ability of these molluscicides on the mycotic inhabitants as well as the cell division of snails.

MATERIALS AND METHODS

I - Collection of snail sample:

350 healthy specimens from each of *Biomphalaria alexandrina* and *Bulinus truncatus*, used in this study, were carefully collected from different localities near the shore of the Nile and its tributaries around Qena city.

II- Treatment of snail samples:

Three different concentration from each molluscicide were used, for CuSo₄ (S.L. (0.02), L (0.04) and H.L. (0.06) g per liter), while for Bayluscide (S.L. (0.001), L. (0.002) and H.L. (0.003) g per liter).

each 50 snails from each type were put in a sterilized pan containing each concentration. The number of dead snails were recorded every two days interval.

III- Determination of snail-inhabiting fungi:

A - Zoosporic fungi:

The baiting technique using sterilized sesame seeds was employed (KHALLIL, 1990).

B - Terrestrial fungi:

One ml. of wash water of control and treated snails, using sterile MENZIESS (1957) dipper, was transferred to 10 cm sterile Petri dish containing about 15 ml of glucose-Czapek's agar medium. 5 plates were used for each sample. Rose bengal was added as a bacteriostatic agent (SMITH and DAWSON, 1944). The plates were incubated at 28 °C for one during which the developing colonies were examined, identified and counted. This experiment carried out after 7 and 15 days of snail treatment.

IV- Chromosomal preparations:

The materials were prepared by the warm-dry method of KLIGERMAN and BLOOM (1977) with some modification made by

KAKAMURA (1986) as follows: The snails were kept in 0.005% colchicine solution for two hours before being sacrificed, then gonads and gills were removed and cut into small pieces and soaked in 0.075 M KCl hypotonic solution, these pieces were fixed in freshly mixed Carnoy's fixative (3 : 1 methyl alcohol: acetic acid). Tissues were then minced gently in 50% acetic acid to prepare a cell suspension. A drop of the cell suspension were pipetted and placed on heated clean glass slides and stained in 2% Giemsa solution. The prepared slides were examined under a research microscope and suitable metaphase plates were photographed.

RESULTS AND DISCUSSION

7043.9 and 4998.7 colonies related to 43; 34 species and 20; 16 genera of zoosporic and terrestrial fungi were isolated from control samples of *Biomphalaria alexandrina* and *Bulinus truncatus* respectively (Table, 1).

I - Fungi recorded on *Biomphalaria alexandrina* and *Bulinus truncatus* treated with molluscicides for 7 days.

a - Zoosporic fungi:

16 species of zoosporic fungi belonging to 6 genera were obtained from control snails. On the other hand *Leptolegnia caudata* and *Pythium ultimum* completely missed from treated snails. *Achlya* (5 species), *Dictyuchus* (3 species) and *Saprolegnia* (3 species) were the common genera. Two species of *Achlya* namely, *A. colorata* and *A. Klebsiana* appeared on the two snails and at the different concentrations of Bayluscide. Moreover, *A. Dubia* isolated from *Bulinus truncatus* and *A. racemosa* from *Biomphalaria alexandrina* at the different concentrations of Bayluscide.

Most of these *Achlya* species were previously recorded on the same snails in the River Nile water in Egypt (EL-SHAROUNY, 1988). *Achlya* was also common in river water in Ibadan, Nigeria (HLBAI, 1971), in Shatt Al-Arab (Iraq) (RATTAN et al., 1980) as well as in the Nile water (EL-HISSY et al., 1982). KHALLIL et al., (1991) reported that *Achlya* was among the common genera on Leeches of the Nile water.

Three species of *Dictyuchus* were recorded from treated snails. *D. anomalus* and *S. sterile* appeared on *Biomphalaria alexandrina* treated with Bayluscide but they reduced or missed on *Bulinus truncatus*. *D. monosporus* have been isolated from snails also in River Nile water (EL-SHAROUNY, 1988). *Dictyuchus* was also recorded on Nile fishes (BADRAN, 1986) and from freshwater plants (KHALLIL, 1990).

Pythium debaryanum was among the common species isolated from the two snails. It was missed at 0.06 concentration of copper sulphate in both *Biomphalaria alexandrina* and *Bulinus truncatus* but it appeared at the later snail treated with Bayluscide (Table, 1). EL SHAROUNY (1988) reported that *P. monospermum* and *P. undulatum* were among the common species on *Biomphalaria alexandrina*.

Saprolegnia represented by three species among which *S. diclina* appeared on both snails treated with 0.02 and 0.04 g/l of copper sulphate but disappeared at 0.06 concentration of the same molluscicide. Moreover it was isolated in a remarkable number (10 colonies / snail) on the two snails treated with Bayluscide (Table, 1). The rest of *saprolegnia* species were missed on both snails treated with copper sulphate but less encountered in the case of Bayluscide. *Saprolegnia* is well known fungi causing disease to freshwater fish (WILLOUGH by et al., 1983 and BADRAN, 1986). EL-SHAROUNY (1988) classified it among the common genera on Nile water snails. The rest of zoosporic fungal species namely, *Aphanomyces stellatus* and *Leptolegnia caudata* were disappeared on treated snails.

b - Terrestrial fungi.

20 and 26 fungal species were isolated from *Biomphalaria alexandrina* and 17 and 23 were isolated from *Bulinus truncatus* treated with copper sulphate and Bayluscide respectively. It has been noticed that the total counts of terrestrial fungi were markedly reduced on treated than control snails (Table, 1). Generally all of the isolated fungal species affected by both of tested molluscicides but that effect was more recognised in case of copper sulphate.

Alternaria alternata was among the main component of fungal species. It missed on the two snails treated with 0.06 of copper sulphate but it was encountered on all samples treated with Bayluscide but in low counts when compared with control specimens (Table, 1) EL-SHAROUNY (1988) proved that *A. alternate* was less encountered on *Biomphalaria alexandrina* and *Bulinus truncatus*.

Aspergillus was also common on the two snails. It was represented by five species and one species variety among of them *A. flavus*, *A. fumigatus* and *A. niger* were the most dominant. They were recorded on both snails treated with both molluscicides. In fact their counts reduced on treated snails especially at 0.06 of copper sulphate. The least count (14 colonies of *A. flavus* / snail) was yielded by *Biomphalaria alexandrina* treated with 0.06 copper sulphate and the highest (716 colonies of *A. fumigatus* / snail) was yielded by the same snail treated with 0.001 of Bayluscide. Numerous investigations

showed that *Aspergillus* species were dominant in different habitates (EL-NAGY, 1981, ABDEL-HAFEZ and BAGY, 1985 and EL-NAGDY and ABDEL-HAFEZ, 1990).

Cladosporium cladosporioides appeared in all treated snails but its count reduced (8 colonies) when *Bulinus truncatus* treated with 0.06 of copper sulphate. KHALLIL *et al.* (1991) isolated that species from leeches and freshwater samples of Nile River.

The most prevalent genus was *Penicillium*. It was represented by 6 species. *P. chrysogenum* was the most frequent although all treatments. Its highest count (315 colonies) recorded on *Biomphalaria alexandrina* at 0.001 of Bayluscide but the lowest (44 colonies) appeared on the same snail at 0.06 of copper sulphate. The rest of *Penicillium* species were affected greatly by copper sulphate than Bayluscide. Some of *Penicillium* species have been isolated from aquatic habitates (DICK, 1971 and ABDEL-HAFEZ and BAGY, 1985).

Mucor circinelloides, *M. hiemalis* and *Rhizopus stolonifer* were among the basic component of fungal species. They emerged from the two snails treated with Bayluscide but they missed at 0.06 of copper sulphate (Table, 1).

Treatments of snails with copper sulphate affected also *Fusarium oxysporum* and *F. solani* and that effect was observed at 0.04 and 0.06 concentrations. The same species were isolated also although snail treatments with Bayluscide but with variable counts.

Trichoderma harzianum was recorded on both treated snail in variable counts also.

The remaining fungal species namely *Cochlibobolus lunatus*, *Drechslera halodes*, *Scopuloriopsis brevicaulis*, *Ulocladium utrum* and *Verticillium lateritium* were the least records in this investigation.

II - Fungi recorded on *Biomphalaria alexandrina* and *Bulinus truncatus* treated with molluscicides for 15 days:

The results of this part were basically similar to those previously mentioned except for the following observations.

1 - The number of collective colonies of all zoosporic fungi were lowered than those collected at 7 days.

2 - *Aphanomyces stellatus* appeared in low count (1 colony) each at 0.001 and 0.002 on *Biomphalaria alexandrina* only treated with Bayluscide.

3 - The collective counts of terrestrial fungi were markedly increased on the two snails although the treated and that increase attributed to the accidental increase in counts of some species like *Alternaria alternata*, *Aspergillus flavus*, *A.*

fumigatus, *A. niger*, *Cladosporium cladosporioides* and *Penicillium chrysogenum*. That increase may due to the viable spores which resist the molluscicides at first week reproduce rapidly thereafter utilizing the dead snails (Table 1).

4 - *Fusarium oxysporum*, *F. solani*, *Mucor hiemalis*, *M. circinelloides*, *Penicillium duclauxi*, *P. funiculosum* and *P. oxalicum* were missed on the two snails treated with copper sulphate. On the other hand *P. steckii* and *Scopulariopsis brevicaulis* disappeared although the experiment.

Generally we can conclude that both of the tested molluscicides affects the mycotic inhabitants of snails spicemens especially in the first week but copper sulphate was more effective than Bayluscide.

Concerning the effect of molluscicides on the mortality of the two types of snails, we found a varying degree of mortality in both of *Biomphalaria alexandrina* and *Bulinus truncatus*. These variations were depend on the type of pesticide used and its concentration. Both of tested molluscicides was more toxic for *Biomphalaria alexandrina* than *Bulinus truncatus* as shown in Table (2).

Prepared slides from gills and gonads of control specimens of both *Biomphalaria alexandrina* and *Bulinus truncatus* showed the diploid chromosome number of $2n = 18$ and $2n = 36$ respectively (Fig. 1a and b).

The two molluscicides have the ability to reduce the cell division in both two species which ranged from 95% to complete inhibition where the mitotic index were zero. Reduction of mitotic activity may be due to the interference of pesticides which caused the inhibition of D.N.A. synthesis and consequently prevent the cell to inter prophase stage. There is no clear chromosomal aberration in the few spreads of chromosomes obtained during the treatment with molluscicides.

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Table (1) : Total counts (per small) of small-inhabiting fungi recovered on glucose - Czapek's agar at 25 C after 7 and 15 days of molluscicide treatment.

Genus and species	Control samples						Eryluicide						Treated samples									
	Slooph.		Bul.		Strophalaria alexandrina		Bryluicide		Bulinus truncatus		Strophalaria alexandrina		Bulinus truncatus		Strophalaria alexandrina		Bulinus truncatus					
	At the end of 7 days	At the end of 15 days	At the end of 7 days	At the end of 15 days	At the end of 7 days	At the end of 15 days	At the end of 7 days	At the end of 15 days	At the end of 7 days	At the end of 15 days	At the end of 7 days	At the end of 15 days	At the end of 7 days	At the end of 15 days	At the end of 7 days	At the end of 15 days	At the end of 7 days	At the end of 15 days				
Achlya	12.0	5.5	10.0	8.5	7.0	7.0	2.0	-	5.5	5.5	4.0	3.0	1.0	-	5.0	-	3.0	-	5.0	-	1.0	-
A. colorata Fringhala	3.5	2.5	2.0	3.0	2.0	1.0	1.0	-	2.5	2.5	1.0	1.0	0.5	-	2.0	-	2.0	-	2.0	-	1.0	-
A. dubia Coker	1.0	1.0	-	-	1.0	-	-	-	1.0	1.0	1.0	1.0	0.5	-	1.0	-	-	-	1.0	-	-	-
A. Plebsiana Plechers	2.0	2.0	2.0	2.0	2.0	2.0	-	-	2.0	2.0	2.0	1.0	-	-	2.0	-	-	-	2.0	-	2.0	-
A. otion Coker & ouch	2.5	-	1.0	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. excesosa Hildrethend	3.0	-	2.0	3.0	2.0	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aphanomyces scallatus	1.0	-	-	-	1.0	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
De Bary																						
Dictyuchus	9.5	5.0	7.0	5.0	5.0	4.0	1.5	-	2.0	-	-	-	-	-	5.5	-	3.5	1.0	-	-	2.0	-
D. anomalis	5.0	3.0	4.0	2.0	2.0	2.0	1.5	-	2.0	-	-	-	-	-	3.0	-	2.0	1.0	-	-	-	-
Barnson																						
D. monosporus Leitzgeb	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5	-	1.0	-	-	-	-	-
D. acervia Coker	3.0	2.0	3.0	3.0	3.0	2.0	-	-	-	-	-	-	-	-	1.0	-	0.5	-	-	-	2.0	-
Lepidogonia caudata	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
De Bary																						
Pythium	18.0	10.0	12.0	-	-	10.0	3.0	-	8.0	1.0	1.0	1.0	2.0	2.0	7.5	5.0	3.0	2.0	-	6.0	8.0	5.0
P. debaryanum Kesse	11.5	6.0	8.0	-	-	7.5	3.0	-	8.0	1.0	1.0	7.0	2.0	2.0	7.5	5.0	3.0	2.0	-	8.0	8.0	5.0
P. ultimum Trow	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. Ghadlatus Petersen	4.0	2.0	4.0	-	-	3.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saprolegnia	18.0	12.0	14.0	10.0	10.0	8.0	5.0	-	11.0	12.0	9.0	5.0	3.0	2.0	1.0	-	2.0	1.0	-	2.0	2.0	2.0
S. diclina Humphrey	12	10.0	10.0	10.0	10.0	5.0	5.0	-	10.0	10.0	10.0	7.0	5.0	3.0	2.0	1.0	-	2.0	1.0	-	2.0	2.0
S. fema (Gruth)	4.0	2.0	2.0	-	-	1.0	-	-	2.0	2.0	2.0	2.0	-	-	-	-	-	-	-	-	-	-

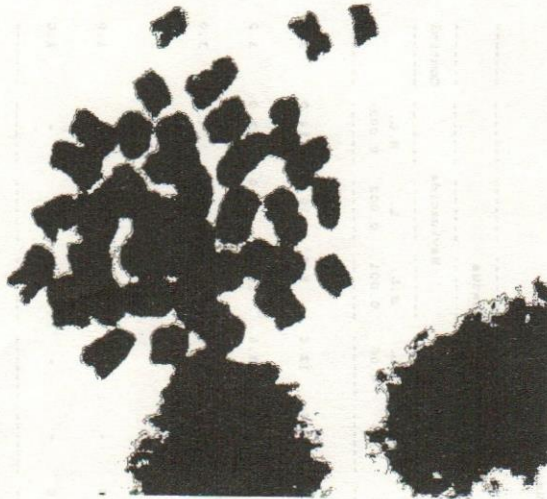
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EFFECT OF SOME MOLLUSCICIDES & HUMAN BILHARZIASIS

Table (2) : The number of dead individuals of *Biomphalaria alexandrina* and *Bulinus truncatus*.

Mortality of snails in	<i>Biomphalaria alexandrina</i>				<i>Bulinus truncatus</i>									
	Copper sulphate		Bayluscide		Control		Copper sulphate		Bayluscide					
	S.L.	H.L.	S.L.	L.	S.L.	H.L.	S.L.	L.	S.L.	L.				
2 nd day	20.0	22.0	28.0	19.0	25.0	27.0	2.0	10.0	10.0	12.0	5.0	10.0	14.0	1.0
4 th day	17.0	25.0	22.0	25.0	30.0	23.0	3.0	11.0	20.0	23.0	10.0	17.0	22.0	1.0
6 th day	6.0	3.0	-	5.0	5.0	-	4.0	8.0	18.0	15.0	13.0	20.0	14.0	3.0
8 th day	7.0	-	-	1.0	-	-	2.0	9.0	2.0	-	16.0	3.0	-	-
10 th day	-	-	-	-	-	-	1.0	10.0	-	-	5.0	-	-	1.0
12 th day	-	-	-	-	-	-	2.0	2.0	-	-	1.0	-	-	1.0
Total No. of dead snails	50.0	50.0	50.0	50.0	50.0	50.0	14.0	50.0	50.0	50.0	50.0	50.0	50.0	7.0

S.L. = Sublethal concentration
 L. = Lethal concentration
 H.L. = Highlethal concentration



(a)



(b)

Fig. (1) : Mitotic spread of the control, *Biomphalaria alexandrina* (a) and *Bulinus truncatus* (b), based on a colchicine-treated metaphase.