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THE INTERACTIVE EFFECT OF SOME MOLLUSCICIDES ON THE INERMEDIATE HOST OF HUMAN BILHARZIASIS AND THEIR ASSOCIATED ZOOSPORIC FUNGI IN THE RIVER NILE, EGYPT

By R. A. M. BADRAN (1) (2)

Effet respectif de quelques molluscicides sur les hôtes intermédiaires de la schistosomose humaine et sur les champignons aquatiques associés, dans le Nil, Égypte.

Résumé : Biomphalaria alexandrina *et* Bulinus truncatus *sont les principaux hôtes intermédiaires de la schistosomose humaine en Égypte. Trois cent cinquante spécimens de chaque espèce ont été récoltés sur les bords du Nil et de ses affluents près de la ville de Qena et répartis en 7 groupes, 6 traités et 1 témoin. Deux molluscicides, sulfate de cuivre et Bayluscide® ont été utilisés à 3 concentrations différentes.*
Les champignons hébergés par les mollusques — traités ou témoins — ont été contrôlés à intervalles de 7 à 15 jours après le traitement. Les 2 molluscicides ont eu un effet létal plus important sur B. alexandrina *que sur* B. truncatus. *C'est également dans le cas de* B. alexandrina *que les isolements fongiques ont été les plus nombreux. Le sulfate de cuivre est plus efficace que le Bayluscide® sur les champignons hébergés par les 2 espèces. Les genres les plus communs étaient* Achlya, Dictyuchus *et* Saprolegnia, *tandis que* Leptolegnia caudata *et* Pythium ultimum *étaient complètement absents des mollusques traités. Une réduction du nombre des colonies fongiques a été constatée 15 jours après le traitement. Les 2 molluscicides inhibent la division cellulaire des 2 espèces de mollusques.*

Summary: Biomphalaria alexandrina *and* Bulinus truncatus *are the main vectors of human Bilharziasis in Egypt. The mycotic inhabitants of both control and treated snails were surveyed at 7 and 15 days intervals. The two molluscicides affected more on the mortality of* Biomphalaria alexandrina *than* Bulinus truncatus. *The highest population of zoosporic fungi was collected from* Biomphalaria alexandrina, *the lowest from* Bulinus truncatus. *Copper sulphate was more effective than Bayluscide® on the fungal species of both snails.* Achlya, Dictyuchus *and* Saprolegnia *were the common genera while* Leptolegnia caudata *and* Pythium ultimum *completely missed from treated snails. The total colonies of zoosporic fungi were lowered after 15 days of treatment. The two molluscicides inhibited the cell division of both tested snails.*

INTRODUCTION

Schistosomiasis 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. *Biomphalaria alexandrina* and *Bulinus truncatus* were the main intermediate hosts of Bilharziasis in Egypt.

The occurrence of aquatic fungi in the River Nile water and its tributaries have been extensively studied (6, 8-10) but we have no information concerning the effect of some molluscicides on the mycotic inhabitants of snails.

Recently, in Egypt, Bayluscide® and coper sulphate are widely used against the intermediate hosts of Bilharziasis. Several authors showed the dangers of pesticides on the damage of the heriditory material. AMER and ALI (2) reported the effect of a number of pesticides in inducing chromosomal aberration on both meiotic and mitotic division, while BRUNERI (5) obtained a complete inhibition of cell division where the mitotic index were zero. AXELSON and SUNDELL (3) reported the increasing of the frequency of cancer cells among people who have exposed to pesticides.

MATERIALS AND METHODS

Collection of snail sample

Three hundred and fifty 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.

Treatment of snail samples

Three different concentrations from each molluscicide were used, for $CuSO_4$ [S. L. 0.02, L. 0.04, and

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H. L. 0.06] g/l, while for Bayluscide® [S. L. 0.001, L. 0.002 and H. L. 0.003] g/l.

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

Determination of snail-inhabiting fungi

The baiting technique using sterilized sesame seeds were employed (11). In this procedure 3-4 sterilized seeds of sesame were placed in sterilized Petri dish containing about 10 ml of sterilized water. One snail, either dead or living, according to the effect of treatment, from treated or control samples was put in each plate and incubated at 20° C. Five plates were used for each sample. After the 7th day of incubation, the colonized seeds were examined, counted and identified. The same methods were repeated after the end of the experiment (15 days).

Chromosomal preparation

The materials were prepared by the warm-dry method of KLIGERMAN and BLOOM (13) with some modification made by NAKAMURA (14) as follows: the snails were kept in 0.005 % colchicine solution for two hours before being sacrified, 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 was 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

In control samples, 16 species (belonging to 6 genera) and 9 species (4 genera) of zoosporic fungi were collected from *Biomphalaria alexandrina* and *Bulinus truncatus* respectively, whereas 59.5 and 32.5 fungal colonies/snail were isolated from the former and the later molluscs respectively (table I). This observation reveals that *Biomphalaria alexandrina* was more hospitable than *Bulinus truncatus*. That hospitability could be due to its large surface or to its shell weakness more than to *Bulinus truncatus*. The more common genera were: *Achlya* (5 species), *Dictyuchus* (3 species) and *Saprolegnia* (3 species).

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

Data of Table I showed that *Leptolegnia caudata* and *Phythium ultimum* completely missed from treated snails. Two species of *Achlya*, namely *A. colorata* and *A. klebsiana* appeared on the two snails at the different concentrations of Bayluscide®. Moreover, *A. dubia* isolated from *Bulinus truncatus* and *A. racemosa* from *Biomphalaria alexandrina* treated with different concentrations of Bayluscide®. On the other hand, all of *Achlya* species were missed on 0.04 and 0.06 concentrations of copper sulphate. Most of these *Achlya* species were previously recorded on the same snails in the River Nile water in Egypt (9). *Achlya* was also common in river water in Ibadan, Nigeria (1), in Shat Al-Arab (Iraq) (15) as well as in the Nile water (12). Khallil *et al.* (12) reported that *Achlya* was among the common genera on Leaches of the Nile water.

Three species of *Dictyuchus* were recorded from treated snails. *D. anomalus* and *D. sterile* appeared on *Biomphalaria alexandrina* treated with Bayluscide® but they reduced or missed on *Bulinus truncatus*. Copper sulphate has a more inhibitive effect towards *Dictyuchus* species than Bayluscide®. *D. sterile* and *D. monosporus* have been isolated from snails also in River Nile water (9). *Dictyuchus* species were also recorded on Nile fishes (4) and from fresh water plants (11).

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 in the latter snail treated with Bayluscide® (Table I). Besides, EL-SHAROUNY (9) 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 g/l of the same molluscicide. Moreover, it was isolated in a remarkable number (10 colonies/snail) on the test snails treated with Bayluscide® (Table I). The rest of *Saprolegnia* species were missed on both snails treated with copper sulphate but less encountered on snails treated with Bayluscide®. *Saprolegnia* is a well known fungus causing disease to fresh water fish (4, 16). EL-SHAROUNY (9) 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.

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

The results after 15 days were basically similar to those previously mentioned except the following observations:

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

Tab. I. — Total counts (per snail) of snail-inhabiting fungi recovered by baiting-seed technique at 25° C after 7 and 15 days of molluscicides treatment. (two substances applied at various concentrations).

Tab. I. — Nombre total de champignons récoltés par mollusque à l'aide de la méthode des « graines pièges » à 25° C, 7 jours ou 15 jours après le traitement molluscicide (deux substances appliquées à différentes concentrations).

genera and species	control samples		treated samples																							
			Bayluscide ®												Copper sulphate											
	Biomphalaria alexandrina	*Bulinus truncatus*	*Biomphalaria alexandrina*						*Bulinus truncatus*						*Biomphalaria 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		
			B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
Achlya	**12.0**	**5.5**	**10.0**	**8.0**	**7.0**	**7.0**	**3.0**	**-**	**5.5**	**5.5**	**4.0**	**3.0**	**1.0**	**-**	**5.0**	**-**	**-**	**3.0**	**-**	**-**	**5.0**	**-**	**-**	**1.0**	**-**	**-**
A. colorata Pringsheim	3.5	2.5	3.0	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	1.0	0.5	-	1.0	-	-	-	-	-	1.0	-	-	-	-	-
A. klebsiana Pieters	2.0	2.0	2.0	2.0	2.0	2.0	-	-	2.0	2.0	2.0	1.0	-	-	2.0	-	-	-	-	-	2.0	-	-	-	-	-
A. orion Coker & Couch	2.5	-	1.0	-	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. racemosa Hilderbrand	3.0	-	3.0	3.0	3.0	2.0	2.0	-	-	-	-	-	-	-	-	-	-	1.0	-	-	-	-	-	-	-	-
Aphanomyces stellatus De Bary	1.0	-	-	-	-	1.0	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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. anomalus Emerson	5.0	3.0	4.0	2.0	2.0	2.0	1.5	-	2.0	-	-	-	-	-	3.0	-	-	2.0	1.0	-	-	-	-	-	-	-
D. monosporus Leitgeb	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5	-	-	1.0	-	-	-	-	-	-	-	-
D. sterile Coker	3.0	2.0	3.0	3.0	3.0	2.0	-	-	-	-	-	-	-	-	1.0	-	-	0.5	-	-	-	-	-	2.0	-	-
Leptolegnia caudata De Bary	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
Pythium	**18.0**	**10.0**	**12.0**	**-**	**-**	**10.5**	**3.0**	**-**	**8.0**	**2.0**	**2.0**	**7.0**	**2.0**	**2.0**	**7.5**	**5.0**	**-**	**3.0**	**2.0**	**-**	**8.0**	**8.0**	**-**	**5.0**	**-**	**-**
P. debaryanum Hesse	11.5	8.0	8.0	-	-	7.5	3.0	-	8.0	2.0	2.0	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. undulatum Petersen	4.0	2.0	4.0	-	-	3.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saprolegnia	**18.0**	**12.0**	**14.0**	**10.0**	**10.0**	**8.0**	**5.0**	**-**	**12.0**	**12.0**	**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	12.0	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	-	2.0	-	-
S. ferax (Gruith) Thuret	4.0	2.0	2.0	-	-	1.0	-	-	2.0	2.0	2.0	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. parasita Coker	2.0	-	2.0	-	-	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
total count	**59.5**	**32.5**	**43.0**	**23.0**	**22.0**	**30.5**	**13.5**	**-**	**27.5**	**19.5**	**18.0**	**19.0**	**8.0**	**5.0**	**20.0**	**6.0**	**-**	**11.5**	**4.0**	**-**	**15.0**	**10.0**	**-**	**10.0**	**-**	**-**
total number of species	16.0	9.0	12.0	6.0	6.0	13.0	6.0	-	7.0	6.0	6.0	6.0	4.0	2.0	8.0	2.0	-	7.0	3.0	-	5.0	2.0	-	4.0	-	-
total number of genera	6.0	4.0	4.0	3.0	3.0	5.0	5.0	-	4.0	3.0	3.0	3.0	3.0	2.0	4.0	2.0	-	4.0	3.0	-	3.0	2.0	-	4.0	-	-

B1 = 0.001 g/l of Bayluscide
B2 = 0.002 g/l of Bayluscide
B3 = 0.003 g/l of Bayluscide

C1 = 0,02 g/l of Copper sulphate
C2 = 0,04 g/l of Copper sulphate
C3 = 0,06 g/l of Copper sulphate

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

Generally we can conclude that both of the tested molluscicides affect the mycotic inhabitants of snails specimens but copper sulphate was more effective than Bayluscide®, especially at 0.02 and 0.04 g/l. Also, we noticed that the total colonies of fungi were lowered, after 15 days of snail treatment, and that may attribute to the biostability of that molluscicides or the dead snails may release some toxic metabolites which reduce fungal population.

Concerning the effect of molluscicide on the mortality of the two types of snails, we found a varying degree of mortality between the tested snails. These variations were depending on the type of molluscicide used and its concentration. *Biomphalaria alexandrina* was more sensitive towards the two molluscicides than *Bulinus truncatus* (Table II).

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

The two molluscicides have the ability to reduce the cell division in both the test snails, which ranged from 95 % to complete inhibition where the mitotic index were zero. Reduction of mitotic activity may be due to the interference of molluscicide which caused the inhibition of DNA synthesis and consequently prevent the cell to inter prophase stage. There is no clear chromosomal aberration in the few spreads of chromosomes obtained from treated snails.

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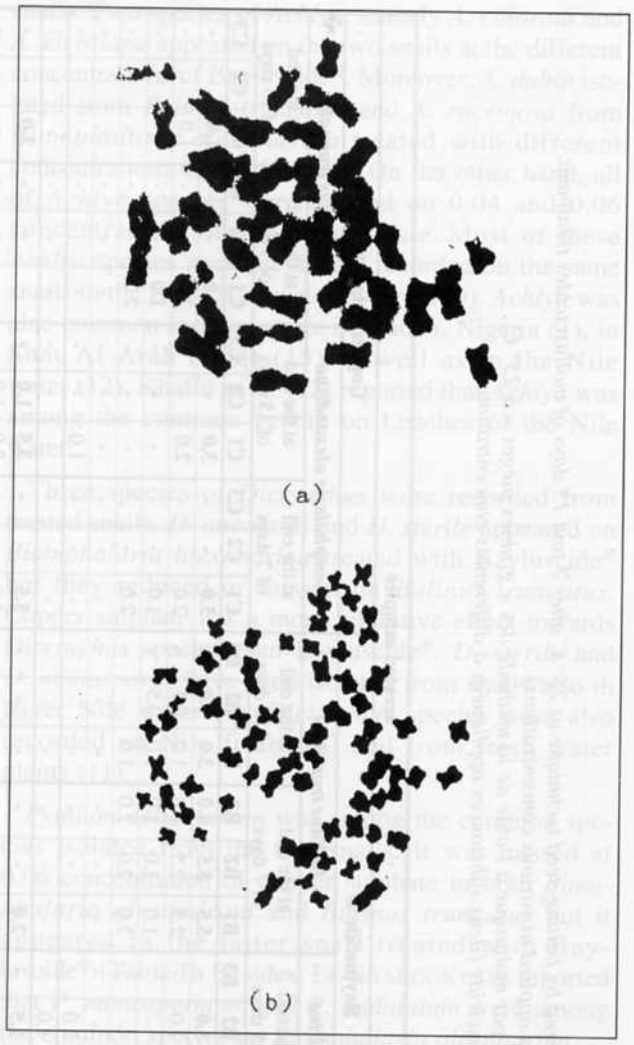

Fig. 1. — Mitotic spread of the control, Biomphalaria alexandrina (a) *and* Bulinus truncatus (b)*, based on a colchicine-treated metaphase.*

Fig. 1. — Préparation caryocinétique des témoins chez Biomphalaria alexandrina (a) *et* Bulinus truncatus (b) *(après traitement à la colchicine).*

Tab. II. — The number of dead individuals of Biomphalaria alexandrina *and* Bulinus truncatus. *(two substances applied at various concentrations).*

Tab. II. — Nombre de Biomphalaria alexandrina *et de* Bulinus truncatus *trouvés morts à différents intervalles après le traitement molluscicide (deux substances appliquées à différentes concentrations).*

	Biomphalaria alexandrina							***Bulinus truncatus***						
	Copper sulphate			**Bayluscide**			**control**	**Copper sulphate**			**Bayluscide**			**control**
mortality of snails in	**S. L. 0.02**	**L. 0.04**	**H. L. 0.06**	**S. L. 0.001**	**L. 0.002**	**H. L. 0.003**		**S. L. 0.02**	**L. 0.04**	**H. L. 0.06**	**S. L. 0.001**	**L. 0.002**	**H. L. 0.003**	
2nd day	20	22	28	19	25	27	2	10	10	12	5	10	14	1
4th day	17	25	22	25	30	23	3	11	20	23	10	17	22	1
6th day	6	3	-	5	5	-	4	8	18	15	13	20	14	3
8th day	7	-	-	1	-	-	2	9	2	-	16	3	-	-
10th day	-	-	-	-	-	-	1	10	-	-	5	-	-	1
12th day	-	-	-	-	-	-	2	2	-	-	1	-	-	1
total number of dead snails	**50**	**50**	**50**	**50**	**60**	**50**	**14**	**50**	**50**	**50**	**50**	**50**	**50**	**7**

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