Dept. of Botany,

Fac. of Science, Qena, Assiut Univ., Egypt.

Head of Dept. Prof. Dr. S. Mohawed.

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

R.A.M. BADRAN and A.E. YASSEN* (Received at 8/10/1994)

تأثير بعض مبيدات الرخويات على العائل الوسيط للبلمارسيا والفطريات المتواجده عليهم

ر مصنان بصران ، أجمط يـس

تم جمع ٣٥٠ عينه من قوقعى بيومفلاريا الكسندريناوبولينس ترانكاتس من أماكن مختلفه من نهر النيل وفروعه ثم اجرى اختبار فاعلية مبيدى 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 السابحة والارضية

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

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^{*:} Dept. Zoology, Fac. of Science, Qena, Assiut University, Egypt.

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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 aslo affected on the cell division of both tested snails.

Keywords: Effect, molluscicides, intermediate host, human bilharziasis, associated micoflora, 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 Rive 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 molluscides on the mycotic inhibitans 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 advant of efficient drugs and molluscicides. Several authors showed the dangers of pesticides on the damage of the heriditory 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 suphate and Bayluscide are the most effective molluscicides against the intermediate host of human Bilharziasis. Therefore our investigation aimed to test the inhibitive ablility of these molluscicides on the mycotic inhabitans as well as the cell divition 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 containg each concentration. The number of dead snails were recorded evert 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 containg 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 nethod of KLIGERMAN and BLOOM (1977) with some modification made by

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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 KCI 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 *Biomphalariea 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 Bictyuchus were recorded from treared snails. D. anomalus and S. sterile appeared on Biomphalaria alexandrina treated with Bayluscide but they reduced or missed on Bulinus trucatus. 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 suphate 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 suphate 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 Bayluside. 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 Bayluside respectivelty. 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 molluscidides 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 spicemens (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 molluscides. In fact their counts reduced on treated snails especially at 0.06 of copper suphate. 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 cladosporiodes appeared in all treated snails butits 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 althrough all treatments. It's 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 greately 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 althrough 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 althroungh 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 althrough the experiment.

Generally we can conclude that both of the tested mollusicides affects the mycotic inhabitans 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 snailes, 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 alexandina and Bulinus truncatus showed the diploid chromosome number of 2n = 18 and 2n = 36 respectively (Fig. la 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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Tieghem 118.6 44.0 161 87.0 2.0 93.0 54.0 14 16.0 9.0 3.0 13 9.0 3 17.0 13.0	circinelloides van	544.0	389.0							120.0	115.5	1.0	43	73.0	1.6	31.0	14.5	,				18.3	9.0			•	A.P.
184.6 44.0 151 87.0 3.0 35.0 54.0 14 16.0 9.0 1.0 13 9.0 1 17.0 13.0 - 14.0 13.0 - 17.0 13.0 - 17.0 13.0 - 18.0 - 17.0 13.0 14.0 14.0 14.0 14.0 14.0 14.0 14.0 14	Tieghen																										
HATTO 10915, 468 230-12 105-0 555-0 405-0 205-0 105-0 58-0 105-0 55-0 410 34-0 - 14-0 105-0 105-0 55-0 105-0 55-0 105-0 55-0 410-0 105-0 55-0	Lienalis wehner	310.6	44.0							16.0	9.3	3.0	13	9.0	•		13.0	,					0.81			•	RI
1427.0 1098.5 468 290.2 105.0 555.0 405.0 330 336.0 220.7 133.0 558 449.0 432 316.0 125.0 64 1135.0 970.0 711 155.0 103.0 52 955 915 677 Those 1125.0 954.5 315 212.3 13.0 448.0 312 51 0 200.0 120.0 487 456.0 411 256.0 111.0 44 1102.0 961.0 711 134.0 102.0 52 934 915 677 Jacrolx 83.0 54.0 61 55.5 14.0 73.0 57.0 19 43.0 17.0 17.0 54 12.0 13 51 0 14.0 . 13.0 9.0 . 15.0 . 27	racemosus Presentus	0.4.0	37.0				18.0			LS.C	4.3		•	4.0			0.40		14.0	3.0			0.51		2	1	L
1125.0 554.5 115 212.3 13.0 448.0 132.9 131 134.0 200.0 120.0 487 416.0 411 126.0 111.0 44 1102.0 961.0 711 134.0 102.0 62 934 915 677 93.0 54.0 61 55.5 14.0 71.0 57.0 19 43.0 17.0 17.0 54 12.0 19 53 0 14.0 . 33.0 5.0 . 15.0 . 27 4.0	icillium	1427.0	1095.5		-		- 57	-		336.0	228.7	139.0	554	449.0	***	70	25.0									-	H
49.0 \$6.0 61 \$5.5 14.0 72.0 \$7.0 19 43.0 17.0 54 \$2.0 19 \$3.0 14.0 5 17.0 57 0 17.0 57.0 17.0 54 \$2.0 1.0 19 5.0 1.0 19 5.0 1.0 19 5.0 1.0 19 5.0 1.0 19 5.0	chrysogenum Thom	1125.0	954.5				1		212	251 0	200.0	120.0	487	416.0	107	77	11.0				.,						Al
	corylophilium Dierckx	93.0	56.0	61	UP UP				13	43.0	17.0	17.0	3.6	22.0	179	OES	14.0		33.0	9.0		15.0				,	«Z
ASIS	duclauxi Delacroix	49.0	11.0	14		٠	4.0	٠		0.0	3.0		-	,	,	0	,	,		,		0.4	,		٠		1.
S																											AS1
																											S

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Table (1) continued

BADRAN & YASSEN

P. funiculosus Thos	18.0	4.0	0 16	4.4		2.0		,	3.0	,		,	,		1.0										3.
P. oxalicum Curzia & Thom	59.0	26.0	47	16.3	9.0	29.0	0.4		15.0	8.7	2.0	7.7	11.0	~	,										•
P. stackii Zaleski	83.0	44.0	15	3.0					12.0		,														,
Rhizopus stolonifer	616.0	411.0	221	185.0	73.0	99.0	87.0	15	195.0	114.0	33.0	72	\$3.0		28.0	14.0		17.0	12.0	3 29	25.0 13	13.0	us	*	N
(Ehrenb.) Lindt																									
Scopulariopsis brevicaulis	26.0	53.0			,										0.8							•			
(Sacc.) Bainer																									
Trichoderma harrianum Refea	16.0	12.0	12	9.0	2.0	13.0	0	64	0.	3.0	3.0		6.0	•	0.6	0.0	0	7.0	3.0	2 13	11.0 4	4.0 2.0	9.0	10	N
Ulocladium utrum Preuss	4.0		**			0.0		,													•			ı	
Verticillis lateritius	3.0		г			3.0	1		,		,				,						•		٠,		
	:	-	i	!	:	:	-	:	:	1	1	:		1	-	:		:	:	:	:	1	:	;	1
Total count of terrestrial		4966.2	3883	7.6705	6984.4 4966.2 2863.0 2702.5 2703 2831.6 2263.2 2831.5 2265.7 1865.3 3049 2766.0 2331 1952.0 986.7 238 2805.0 2034.0 1253 1260.5 618.5 239 18848 1804 1805	3910.0	3210.5	2703	811.5	2159.7	1655.3	3099	0.894	2311 1	22.0	86.7 2	8 2905	5.0 202	4.0 12	53 1260	. S 618	.8 239	1846	1504	1058
Lungi																									
	-	4	:	:	!	:	:		-	:			:	:			-				-	:	i	:	:
Gross total count	7043.9	7043.9 4998.7 3926	3926	3102.7	3402.7 2504.5 3544.0 3724.0 2730 2439.0 2379.2 3673.0 3236 1941.5 992.7 236 2356.5 3030.5 1281.5 628.5 238.5 3881 3804 1038	0.0166	0.422	2703 2	0.668	2179.2	1673.3	3000	0.877	316 11	41.5 9	2.7 23	. 2916	.5 202	8.5 12	1321 2361	.5 628	.5 239	3658	1504	1056
	:	-	:	i	:	:	:	1	-	:		;	:	-								!	:	1	;
Total No. of genera	20.0	36.0	16	13.0	11.0	17.0	15.0		12.0	10.0	11.0	2	12.0	7	13.0	12.0	4 11	11.0	10.0	11 9	11.0	9.0	11	•	w
		-	:	-			:	1	-		:			:	-	-			-			;	:	:	
Total No. of species	0.0	34.0	38	28.0	24.0	37.0	25.0	15	30.0	25.0	23.0	T	22.0	17	28.0	18.0	7 30	20.0	15.0	6	23.0 15.0	0	16	10	2
THE RESIDENCE															!			!							
the statement of the statement of																									
Bi . 0.001 g/L of Bayluscide					C1 = 0.02 g/L of Copper sulphate	2 9/1 0	Coppe	r sulph	ate.																
B2 . 0.002 g/L of Bayluscide					CZ = 0.04 g/L of Copper sulphate	4 9/2 0	Copper	dine a																	
B3 . 0.003 g/L of Bayluscide	1122				C3 = 0.06 g/l of Copper sulphane	6 9/1 0	Copper	ding a	# T. 8																
Section of the sectio																									

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Table (1) conclumed

Blouph. . Blomphalaria alexan. . slexandrins

Table (2) : The number of dead individuals of Biomphairia alexandrine and Bulinus truncatus.

		Stomphalatta alexandrina		*andrina	1		Bulinus truncatus	***************************************	J	Bulinus truncatus	uncatus			
	Copper sulphace	Iphace		Bayluscide	ide		Control	Copp	Copper sulphate	150	38	Bayluscide		Control
Mortality of enails in		S.L. L. H.L.	H.E.		S.L. L.	R.L.		13.0	S.L. L. K.L.	K. E.		S.L. L. H.L.	×	
	0.03	0.02 0.04	0.06	0.001		0.002 0.003		0.03	0.04	0.06	0.001	0.003	1	
2 nd day	20.0	22.0	28.0	19.0	18.0	27.0	1.0	10.0	10.0	12.0	8.0	10.0	14.0	1.0
e ch 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	23.0	1.0
e th day	6.0	3.0		5.0	0.8		0.4	0.0	18.0	15.0	13.0	20.0	14.0	3.0
s ch day	7.0			1.0			1.0	9.0	2.0		16.0	0	. •	
10 ch day							1.0	10.0	٠.		8.0	•		1.0
12 th day			4				2.0	2.0			1.0			1.0
Total Mo. of dead snails 50.0	\$6.0	0.02	50.0		80.08	50.0 14.0		50.08	50.08	80.08	50.08	50.0	80.08	7.0

S.L. . Sublethal concentration

N.L. - Mighlethal concentration

L. * Lathal concentration

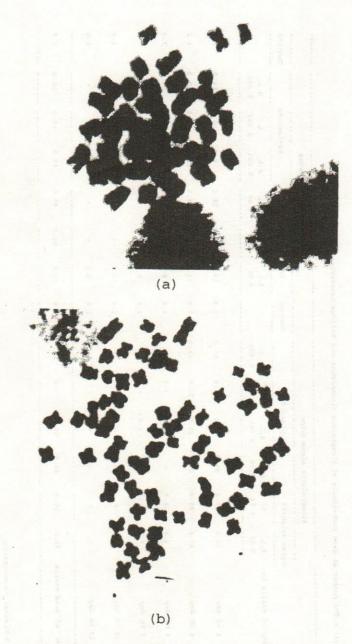


Fig. (1): Mitotic spread of the control, Biomphalara alexandrina
(a) and Bulinus truncatus (b), based on a colchicinetreated metaphase.

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