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EFFECT OF PROLONGED SUBLETHAL CONCENTRATIONS OF BAYLUACIDE AND TRIFENMORPH ON GLYCOGEN AND PROTEIN CONTENT OF *Biomphalaria alexandrina*

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ABSTRACT

The Effect of chronic application of sublethal concentration of molluscicides, bayluscide (0.05 –0.025 ppm) and trifenmorph (0.25 – 0.125 ppm) on the glycogen and protein content of *Biomphalaria alexandrina* was determined. Bayluscide and trifenmorph produced significant reduction in the glycogen content of *Biomphalaria alexandrina,* but a significant decrease in the protein content of the snails was not apparent until after 4 weeks of continuous exposure. The results indicate that the molluscicides may exert their primary molluscicidal action on the carbohydrate metabolism of the snails.

The both molluscicides significantly reduced the gain in shell diameter of the snails.

INTRODUCTION

The intermediate host of *Schistosoma spp.* and other trematodes are different species of freshwater snails. Therefore, the control of those snails is the most important and effective method to interrupt the transmission cycle and thus protect the final hosts from being infected. Although powerful molluscicides are available to this purpose, their use is very problematic due to their high toxicity towards other aquatic organisms. To overcome this disadvantage, the use of sublethal doses for a long time application is thought to be a more environmental accebtable method.

The total protein concentration was reduced by application of sublethal doses of molluscicides in *Australorbis glabratus* (Gilberston *et al.*, 1967). During starvation the reserves first utilized seam to be glycogen in *Planorbis cornaus* (Emerson, 1967) or glycogen and lipids in *Helisoma duryi* (Bedding, 1979). El-Emam and Madsen (1982) found that *Biomphalaria alexandrina* juviniles were dead after 7 days of application. The molluscicidal activity of bayluscide and trifenmorph had been recorded against mollusces by (Hagen *et al.*, 1978; Cardarelli, 1979). Although a number of slow-release molluscicide formulations have been developed for aquasystem for control the water snails, the intermediate host of Schistosomiasis (Mc-Cormick and Fooladi, 1977; Cardarelli, 1979; Kronstein, 1979; Bahadir and Pfister, 1985; Bahadir and Pfister, 1990 and El-Naggar *et al.*, 1991). The present study reports the results of the effect of low doses of molluscicides, bayluscide and trifenmorph on the glycogen and protein content of *Biomphalaria alexandrina* over a period of 8 weeks.

MATERIALS AND METHODS

Source of snails

Biomphalaria alexandrina (8 –12 mm) were collected from irrigation canals in Kafr El-Sheikh Governorate. They were kept in laboratory 25 °C ± 2 °C, dechlorinated water, pH 7 ± 0.4 for one month before being used (El-Emam and Ebeid, 1989). Snails were fed with artificial food ad-libidum.

Molluscicides

Bayluscide: (the ethanolamine salt of niclosamide) 5,2'- dichloro – 4-nitro-salycylanilids, common name, Bayer 73 (Bayer A.G., Leverkusen Germany), analytic standard (99%)

Trifenmorph: (N- tritylmorpholine) or N-Triphenylmethyl-morpholine, Shell Chemical Company, analytical standard (99%)

Application of molluscicides:

Sublethal concentration of 0.05, 0.025 and 0.25, 0.125 ppm were used for bayluscide and trifenmorph, respectiviely. Snails were changed both in the control and treated groups twice weekly.

The first group of snails was exposed for 2 weeks, the second group for 4 weeks, the third for 6 weeks and the fourth group for 8 weeks. A 3-4 days were chosen as a period for the replacement of snail water and the molluscicides in snail water, because molluscicidal concentrations of bayluscide and trifenmorph have been shown to produce 100% mortality to the snails 5 days after exposure to trifenmorph in snail water and even after 7 days for bayluscide in experiments to determine the half-life of the molluscicides (Adewunmi *et al.*, 1987b).

This biological assay is used for the estimation of the effective concentrations of molluscicides using the snail itself as the animal model (WHO,1965).

Preparation of snail cephalopedal and hepatobancreas tissues

The tissues were dissected by taking away every bit of the shell, the tunica propria, the gasrrointestinal sling runing through the hepatobancreas, the stomach, the whole parts of the elimentary canal and the reproductive gland. The hepatobancreas and cephalopedal tissues were the removed for analysis.

The preparation of the homogenized tissues was a modification of the method of Keppler and Decker (1983) as described by Ratka (1985). Briefly, the cephalopedal and hepatobancreas tissues were frozen in liquid air for a short time, Each tissue was ground with a pestle and mortar previously cooled to –70 °C. The ground powder was the transferred into an Eppendorf tube which had been previously cooled to -70 °C and weighed in order to know the weigh of the tissue. The tissue was then boiled for 5 min. in a bath after the addition of o.5 ml of boiling distilled water. If the tissue was more

than 100 mg the amount of boiling water added was adjusted accordingly. The tissue was then homogenized with a potter- Elevehjem homogenizer after which the volume of the material was adjusted to 1.3 ml in a graduated reagent glass with distilled water and mixed throughly.

This material was then transferred into another previously weighed Eppendorf tube which had also been cooled at –70 °C. The prepared tissue was left at the same temperature until required for glycogen and protein analysis.

Glycogen and protein analysis

The glycogen content of the sanails was determined by the method of Keppler and Decker (1983) using diazyme (Boeringer, Manheim) which contains 1,4 and 1,6- x amyloglucosidase, which catalyse the degredation of glycogen to glucose. The amount of glucose formed was then determined by hexokinase and glucose-6- phosphate dehydroginase. This was assayed by spectroscopy at 340 nm using spectrophotometer.

The protein content was determined by the method of Lowry *et al.*, 1951) with using bovine serum as standard. This was measured at 650 nm using spectrophotometer.

The statistical evaluation of the results for comparison of the means values was carried out by the Mann Whitney U-test (Siegel, 1956) and the student's t- test. Significant values are quoted at indicated levels in the text.

RESULTS

The effect of trifenmorph and Bayluscide on glycogen content

Results in table (1) showed that the mean glycogen content of snails in control check (untreated groups) vary between 9.3 and 17.87 mg/g of snail tissue. In absolute values, the results were about the same as those reported by Ratica (1985). Also, in terms of percentages, they are about the same as those reported by Chiang (1977) for *Biomphalaria glabrata* and by Calvin (1981) for mussel tissue.

It is interesting that trifenmorph (Table 1) appeared to produce concentration-related significant effects on the glycogen content of *Biomphalaria alexandrina* after contiuous expossure of the snails to the molluscicide for 2 weeks. The effect of bayluscide did not appear to be dose-related at the two concentrations tested.

This concentration- related effect of trifenmorph on the glycogen content of the snails can also as the same as snails under contiuous exposure for 4,6 and 8 weeks. It is quit clear from the results that the longer of exposure time, the more deleterious the effect that trifenmorph had on the level of glycogen of the snails. Snails exposed for 2, 4, 6, 8 weeks at a concentration of 0.25 ppm. had 43.01% , 43.31%, 10.52%, 6.22% of the glycogen content of control snails, respectively.

The effect of bayluscide on glycogen content of the snails exposed for 2 weeks at a concentration of 0.05 ppm had 37.48% of the glycogen content of

control snails, while snails exposed for 4 weeks had 23.24%. Snails exposed for 6 and 8 weeks had 22.05% and 25.91%, respectively.

This means that both mollusciciodes, trifenmorph and bayluscide significantly reduced glycogen content, specially when the snails exposed long time.

Table (1): Effect of continuous application of trifenmorph and bayluscide on the glycogen content of the snail, *Biomphalaria alexandrina* (N per test group = 6)

Treatments	Concentration (ppm.)	Exposure time (week)	Mean ± S.D.of Glycogen content (mg/g)	% glycogen	U-test (P)
Control	--	2	10.30 ± 2.83	-----	--
Trifenmorph	0.125	2	8.35 ± 2.75	81.07	>0.05
Trifenmorph	0.25	2	4.43 ± 1.95	43.01	<0.002
Bayluscide	0.025	2	1.85 ± 1.23	17.10	<0.002
Bayluscide	0.05	2	3.86 ± 1.60	37.48	<0.002
Control		4	11.06 ± 2.71	-----	--
Trifenmorph	0.125	4	7.76 ± 2.37	70.16	<0.01
Trifenmorph	0.25	4	4.79 ± 1.39	43.31	<0.002
Bayluscide	0.025	4	5.04 ±1.70	45.57	<0.002
Bayluscide	0.05	4	2.57 ± 1.28	23.24	<0.002
Control		6	17.87 ± 3.18	------	--
Trifenmprph	0.125	6	3.43 ± 1.74	19.19	<0.002
Trifenmorph	0.25	6	1.88 ± 1.04	10.52	<0.002
Bayluscide	0.025	6	2.88 ± 1.95	16.12	<0.002
Bayluscide	0.05	6	3.94 ± 1.13	22.05	<0.002
Control		8	9.30 ± 2.23	-----	--
Trifenmorph	0.125	8	1.36 ± 0.70	14.62	<0.002
Trifenmorph	0.25	8	0.56 ± 0.24	6.22	<0.002
Bayluscide	0.025	8	3.93 ± 1.02	42.26	<0.002
Bayluscide	0.05	8	2.41 ± 0.59	25.91	<0.002

Each value represent the mean of 6 replicates. S.D. = standard deviation

$$\% \text{ Glycogen} = \frac{\text{Glycogen content in treatment}}{\text{Glycogen content in control}} \times 100$$

The effect of the molluscicides on the protein content of the snails

The effect of trifenmorph and bayluscide was not as dramatic as the effect on glycogen. It can be seen from Table 2 that both molluscicides appear to be no difference between the treated and control snails exposed to sublethal concentration of the molluscicides for 2 and 4 weeks ($P > 0.05$, Student's *t*- test). However, from the sixth week onwards, the molluscicides produced significant effects on the protein content of the snails. The effect of the molluscicides on the protein content did not appear to be concentration-related.

Table (2): Comparison of the effect of trifenmorph and bayluscide on the protein content of *Biomphalaria alexandrina* to molluscicides(N per test group = 6)

Treatments	Concentration (ppm.)	Exposure time (week)	Mean ± S.D. of protein content (mg/g)	U-test (P)
Control	--	2	2.92 ± 0.53	--
Trifenmorph	0.125	2	2.32 ± 0.61	>0.05
Trifenmorph	0.25	2	2.20 ± 0.76	<0.002
Bayluscide	0.025	2	2.23 ± 0.54	<0.002
Bayluscide	0.05	2	2.23 ± 0.75	<0.002
Control		4	5.13 ± 0.81	--
Trifenmorph	0.125	4	4.52 ± 0.41	<0.01
Trifenmorph	0.25	4	4.67 ± 0.52	<0.002
Bayluscide	0.025	4	4.98 ±0.48	<0.002
Bayluscide	0.05	6	4.91 ± 62	<0.002
Control		6	6.47 ± 0.98	--
Trifenmprph	0.125	6	4.48 ± 0.49	<0.002
Trifenmorph	0.25	6	4.48 ± 0.81	<0.002
Bayluscide	0.025	6	4.69 ± 0.06	<0.002
Bayluscide	0.05	6	4.39 ± 0.46	<0.002
Control		8	6.99 ± 1.36	--
Trifenmorph	0.125	8	3.99 ± 1.30	<0.002
Trifenmorph	0.25	8	4.51 ± 1.07	<0.002
Bayluscide	0.025	8	3.30 ± 1.39	<0.002
Bayluscide	0.05	8	4.66 ± 0.41	<0.002

Each value represent the mean of 6 replicates. S.D. = standard deviation

The effect of the molluscicides on the gain in shell diameter of *Biomphalaria alexandrina*.

The data in table (3) showed that both molluscicides, trifenmorph and bayluscide significantly reduced the gain in shell diameter of the snails.

Table (3): Comparison of the effects of trifenmorph and bayluscide on the gain in shell diameter of *Biomphalaria alexandrina* exposure fior 8 weeks (N per test group = 6)

Treatments	Concentration (ppm.)	Mean ± S.D.of shell diameter (mm)	U-test (P)
Control	--	12.13 ± 1.21	--
Trifenmorph	0.125	8.23 ± 1.17	>0.05
Trifenmorph	0.25	9.93 ± 0.68	<0.002
Bayluscide	0.025	8.25 ± 2.27	<0.003
Bayluscide	0.05	9.27 ± 1.27	<0.005

Each value represent the mean of 6 replicates. S.D. = standard diviation

DISCUSSION

Our results clearly demonstrated the significant deleterious effects of prolonged administration of low concentrations of the molluscicides, trifenmorph and bayluscide on the glycogen and protein content of the snails, *Biomphalaria alexandrina*. The reasoning behind using sublethal concentrations rather than lethal concentrations is that this should reduce the hazard for non- target organisms and thereby possible ecological damage.

Biomphalaria alexandrina is an intermediate host of Schistosomiasis. After penetrating the cephalopedal region of the snail, the miracidia become mother sporosysts by polyembriony and migrate to the hepatopancreas via the mantel region. Whithin the hepatobancreas, the mother sporosysts mature (Chiang, 1977) and produce cercariae which are then shed from the snails to seek their human hosts. The availability of more glycogen in the hepatobancreas- ovotestis complex has been suggested partly as the reason why this tissue is preferred for the development of cercariae from daughter sporosysts (Ching, 1977).

A deleterious effect on glycogen stores would be expected to hinder the development of cercariae as has been partly shown for bayluscide.

Bayluscide at a concentration of 0.06 ppm. has been shown to lengthen the prepartenly period of *Schistosoma mansoni* in *Biomphalaria sudanica* (Sturrock, 1966). But the exposure peripod used by this author was short. Longer exposure periods (as in this study would be required to bring about a reliable effect. This suggests the suggesion raised by Andrews *et al.*, (1983) that a longer exposure periods is necessary to bring about a reliable molluscicidal effect.

Gastrobods, in general or reproductive factors (von Brand, 1931; Meenakshi, 1956; Emerson, 1965; Goddard and Martin, 1966). It can be inferred from the results of this study and those of Adewunmi *et al.* (1987b) that the significant reduction in the glycogen and protein content could be held responsible for the reduction in egg production and growth rate of *Biomphalaria glabrata* and *L. columella* (Adewunmi *et al.*, 1987b). The significant reduction of the gain in sheil diameter confirms an earlier study and also supports our proposal that the effect on the growth and reproductive capacity of the snails is primarily a result of a deleterious effect of those molluscicides on the metabolism of carbohydrates and to a lesser extend on the protein content of the snails. The results are in agreement with previous findings on bayluscide (even thought the exposure period was much shorter) that this agent could be producing most of its effects on carbohydrate metabolism (Ishak *et al.*, 1972; El-Gindy and Mohamed, 1978; El-Emam and Ebeid, 1989); Mohamed *et al.*, 1981) claimed that niclosamide showed no significant effect on the protein content of *Biomphalaria alexandrina* exposed for a maximum period of 24 days. This is an indication that the time of exposure used by these authors was probably not long enough for a significant effect to be produced on the protein content of the snails. The present work is a positive complementary addition to the literature on the efficacy of bayluscide and trifenmorph in particular.

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تأثير التركيزات تحت المميتة والمستمرة لمبيدات القواقع البيلوسيد والترفينمورف على المحتوى الكلى للجليكوجين والبروتين فى قواقع البيومفلاريا ألكسندرينا

محمد محمد فتحى النجار

قسم المبيدات – كلية الزراعة بكفر الشيخ – جامعة طنطا – ج م ع

فى هذا البحث تم دراسة التأثير المزمن للتركيزات تحت المميتة) لمبيدات القواقع البيلوسيد (٠,٠٥ . ٠,٠٢٥جزء فى المليون) والتريفنمورف (٠,٢٥ & ٠,١٢٥ جزء فى المليون) وذلك على المحتوى الكلى لكل من الجليكوجين والبروتين فى قواقع البيومفلاريا الكساندرينا العائل الوسيط للبلهارسيا. ولقد أوضحت نتائج التجارب أن كلا المبيدين أدى الى نقص كبير فى المحتوى الكلى للجليكوجين . كما أدى المبيدين الى نقص كبير فى المحتوى الكلى للبروتين وخصوصا من الأسبوع الرابع فى حالة مبيد البيلوسيد والتعرض المستمر للتركيزات المنخفضة (تحت المميتة). وأرجعت المراجع هذا الى التأثير على هضم الكربوهيدرات فى القواقع.

كما كان للمبيدين تأثيرا معنويا على صدفة القواقع وأدا الى نقص كبير فى محيط محارة القوقع.