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Comparative karyological studies on the two Egyptian *schistosome* vectors, *Biomphalaria glabrata* and *B. alexandrina*, with reference to chromosomal aberrations due to Za'ater plant

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ABSTRACT: *Karyological character variations, included G-banded patterns, in the two Egyptian vectors of schistosomiasis, Biomphalaria glabrata and B. alexandrina (Gastropoda, Planorbidae) had studied. Chromosomes of the present snails that treated with Za'ater-plant extract (Origanum syriacum, Labiatae) produced three types of chromosomal aberrations; chromosomal addition in the two species, chromosome gab in B. glabrata and chromatid break in B. alexandrina. The results had been analyzed using suitable statistical methods. The present study estimated the effective dose of Za'ater that led to revealing these chromosomal aberration-types.*

Key Words: *Karyotype, Biomphalaria sp., Botanical molluscicides, Origanum, chromosomal aberrations.*

I. INTRODUCTION

Trematodes of the genus *Schistosoma* are responsible for chronic schistosomiasis in over 200 million people worldwide. Debilitating hepatic, intestinal, and urinary pathology make schistosomiasis a parasitic disease of global socioeconomic and public health concern. In this respect, schistosomiasis represents an increasing health problem in many developing countries and in non-endemic regions, due to the increasing number of immigrants [1]. The fresh-water snails *Biomphalaria glabrata*, *B. alexandrina* and *Bulinus natalensis*, that lead to schistosomiasis, and *Lymnaea stagnalis*, that leads to fascioliasis, are the most important intermediate hosts of the human blood-fluke helminth parasite and becoming serious pests in Egypt [2]. Several extracts of plant origin had been studied as biological control-agents against these snails[3]; of which the study of Mostafa *et al.* (2005) on *Anagalis arvensis* [4] and the study of Singab *et al.* (2006) on *Iris germanica alba* against *B. alexandrina* [5].

Cytogenetically, chromosomal studies of gastropods have greatly increased in the recent years, but many questions related to the susceptibility of the intermediate hosts to infection by respective trematode remain unanswered. Thus, several recent works studied DNA amplification, using RAPD-PCR technique, to show genetic variations between susceptible and resistant strains to parasite infection within intermediate hosts. From these works the study of Amera *et al.* (2007) [6] on the target snails *B. glabrata* and *B. alexandrina*. Furthermore, Silva *et al.* (2007) [7] studied Ki-67-protein, of infected *B. glabrata* with *Schistosoma mansoni*, that expressed in the nucleus during cell division, being absent during the G0 resting phase of the cellular cycle. They found this protein in the parasite while the snail tissues were completely negative.

Thereby, the present work includes significant karyological data, supported by G-banding patterns, about the Egyptian fresh-water snails, *B. glabrata* and *B. alexandrina* as well as the chromosomal aberrations that revealed under the effect of Za'ater-extract, as a botanical control agent the present snails.

II. MATERIALS AND METHODS

II.1. Tested snails:

Biomphalaria glabrata and *B. alexandrina* obtained from the laboratory colonies of Theodor Bilharz Institute, Imbaba, Giza. Egypt.

The stock colonies of these snails were reared under suitable laboratory conditions in glass aquaria (50X30X20 cm^3) filled with continuously aerated dechlorinated tap water. The target snails fed on fresh lettuce leaves. Only fully-grown and non-infected snails used in the present studies.

II.2. Fixation:

The healthy-adult snails and the treated ones subjected to LC_{50} and LC_{90} of Za'ater plant extract injected with colchicine solution (0.1%, 0.5 µl/mg of soft body weight) into the abdominal cavities (after poring the shell) for 8 hours (Webb, 1976) [8]. Then, incisions were made in the snails to obtain genetalia that immersed in a fixative (1:3 glacial acetic acid: absolute ethyl alcohol) for at least 6 hours at 4°C.

II.3. Preparation of plant extracts:

The dried seeds of Za'ater's plant (*O. syriacum*) obtained from a folk market in Cairo, Egypt. They dried in an electric oven at 50°C for three days, homogenated, stirred for 10 minutes in ethanol and the obtained ethanolic-extracts filtered and left to evaporate in the oven at 70°C until a crude gum-shaped substance formed.

II.3.1. Treatment:

The obtained residue of Za'ater-extract dissolved in distilled water (1:5 by volume) and the obtained solution used to determine LC_{50} and LC_{90}.

II.4. Cytogenetical preparations:

II.4.1. Chromosomal Staining:

The fixed genetalia cut into small pieces, 2-3 pieces were stained with 10% Giemsa stain for 5 minutes, the specimens squashed on clean glass slides and immediately photographed.

II.4.2. Chromosomal Measurement:

Measurement of the chromosomes of metaphase cells (100 cells/species) accurately accomplished using light microscope special scaled slide and eyepiece. The mean chromosomal lengths calculated per microns.

II.4.3. Karyotype preparation:

After chromosomal measurement, the karyotypes of the present snails were prepared by arranging the chromosomes, according to their relative lengths and photographed.

II.4.4. G–banding pattern preparation:

G-banding pattern preparation were made according to the technique recommended by Shay (1982) [9]. The colchicine-treated-metaphase cells immersed in trypsin solution, washed in phosphate-buffered-saline solution followed by immersion in 4% Giemsa stain.

II.4.5. Chromosomal aberrations:

The resulted types of chromosomal aberrations in the target snails photographed and scored (100 cells/species, three times). The obtained data were represented by histograms of Bailey (1976) [10].

III. RESULTS AND DISCUSSION

Until now, the snails are controlled using chemical molluscicides that unfortunately leave toxic residues and cause chemical environmental contamination (Ghamry *et al.,* 1993) [11]. The appealing aspects with plant extracts as botanic control-agents are that: they are highly effective, rapidly biodegradable, inexpensive, readily available and easily applicable with simple techniques (El-Kassas, 2001) [3].

The present work was carried out to estimate the LC_{50} and LC_{90} of Za'ater plant against *B. glabrata* and *B.alexandrina* snails. Table (1) shows LC_{50} and LC_{90} of this plant after 48 hours, where, *B. alexandrina* snails recorded higher concentrations (226.8 & 422.9 ppm) than *B. glabrata* (215.2 &415.7 ppm) of LC_{50} and LC_{90} respectively.

Several extracts of plant origin had been studied as biological control-agents against the snails; of which the study of both Mostafa *et al.* (2005) [4] on *Anagalis arvensis* and Singab *et al.* (2006) [5] on *Iris germanica alba*

against *B. alexandrina*, as well as Ndamukong *et al.* (2006) [12] on *Nicotiana tabacum*, *Aframomum citratum*, *A. melegueta*, *Curcuma domestica* and *Solanum scabrum* against *Bulinus camerunensis* and *B. truncates*.

Singab *et al.* (2006) [5] declared that LC_{90} of *Iris germanica alba*-extract against *B. alexandrina* was 1.26 mg/l. In addition, they chromatographically isolated two compounds, flavone and flavanone derivatives, that had molluscicidal activities:.

Cytogenetically, ovotestes of the present snails provided a good opportunity to study the karyotype and chromosomal aberrations due to Za'ater-extract. The present results revealed that the diploid chromosome number (2N) of the two species is 36. The whole chromosomes of the two species arranged according to their relative lengths (Figs. 3&4). The mean chromosomal lengths in *B. glabrata* are slightly higher than those of *B. alexandrina* snails, except 3, 4 and 5 autosomes (table 2 & Fig.1). There are four types of chromosomes in *B. glabrata* and *B. alexandrina*; 10 metacentric, 4 submetacentric, 2 acrocentric and 2 telocentric members. Nevertheless, the present work revealed differences in the centromeric position in 12 pairs of autosomes of *B. glabrata* and *B. alexandrina*, as displaying in table (2).

The G-banding bands revealed that chromosome 8 of *B. glabrata* exhibited secondary constriction, that represents the nucleolar organizer regions, and this autosome can be used as a marker in the this species (Fig. 3).

Several studies carried out on the karyotypes of the fresh-water snails. From which, Goldman *et al.* (1980 [13]&1983 [14]) and Brown *et al.* (1996) [15] studied the karyotype of *Bulinus natalensis* and *B. tropicus* (Planorbidae). However, Brown *et al.* (1996) [15] found differences in the centromeric position in four pairs of chromosomes than those of Goldman *et al.* (1983) [14] as well as appearance of a secondary constriction in one chromosome of a third genus (*B. fatalness*). The results of Raghunathan (1976) [16] supported the present work who studied karyotype of one of the target snails *B. glabrata* and declared that diploid chromosome number is 2N=36 and its chromosome pairs are classified into 4 groups; 10 pairs of metacentric, 4 pairs of submetacentric, 2 pairs of acrocentric and 2 pairs of telocentric chromosomes. Moreover, he showed that 8-autosome was with secondary constriction that can be used as a marker in genetic experiments. The present result reinforced this work in the same species. Nevertheless, Goldman *et al.* (1983) [14] declared that autosomes of *B. glabrata* classified into 3 groups; 15 pairs of metacentric, 2 pairs of submetacentric and 2 pairs of acrocentric chromosomes and the secondary constriction is absent in all autosomes. The work of Sidky *et al.* (1982) on chromosomes of *B. alexandrina* reinforced the present karyotype on the same species.

In general, G-banded-chromosomes revealed more detailed information about chromosomal aberrations than classical chromosomal morphology (karyotype). The present results revealed four types of structural chromosomal aberrations after LC_{90}-treatment; chromosomal addition in the two species, chromosome gab in *B. glabrata* and chromatid break in *B. alexandrina* (Fig. 5).

Concerning polyploidy**,** Goldman *et al.* (1980) [13] studied the hybrid origin of polyploidy in *Bulinus tropicus* and *B. natalensis* and stated that the same two subgenera of *Bulinus* revealed polyploidy and showed ability to transmit the parasite *Schistosoma haematobium*. In this respect, Brown and Shaw (1989) [18] declared that chromosome number in *Bulinus truncatus* and *B. permembranaceus* was tetraploid. However, Mukaratirwa *et al.* (1998) [19] stated that *Bulinus truncatus/tropicus* complex, collected from Zimbabwe, vary widely in chromosome number.

Amera *et al.* (2007) extracted DNA from *B. glabrata* and *B. alexandrina* snails and found that RAPD profiles of DNA genome of the same strain were relatively homogenous, whereas the profiles of snails from different strains were quite distinct. They also declared that the resistant character was ascendant in contrast to a decline in the susceptibility of snails from one generation to the next [6].

Silva *et al.* (2007) studied Ki-67-protein (which expressed in the nucleus during cell division and being absent during G-resting phase of the cellular cycle) in *B. glabrata* infected with *Schistosoma mansoni*, found this protein in the parasite while the snail tissues were completely negative. These data are worth registering to

complement general data on Ki-67 protein and to help future studies on the relationship of the parasite and of its intermediate host [7].

The present results offer a candidly recommendation to the Theodor Bilharz Institute concerning the effective dose of the botanical control agent (*Origanum syriacum*) to be applied on *B. alexandrina* and *B. Glabrata* snails, for controlling these pests or at least minimizing their numbers.

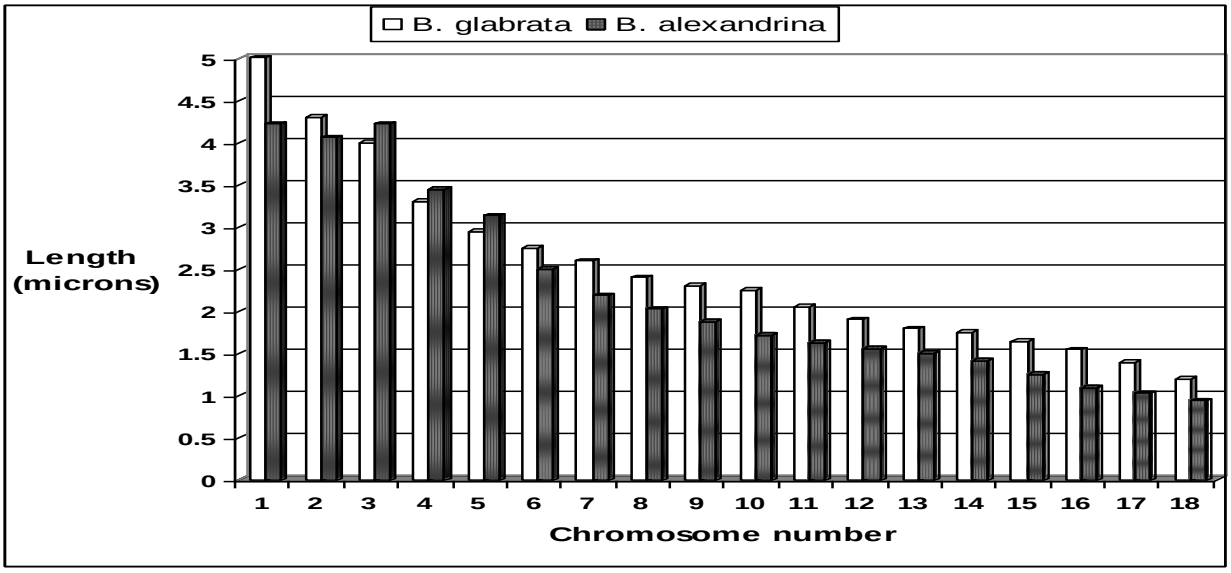

Fig. (1): Diagrammatic representation of chromosome lengths of the two species of the snails.

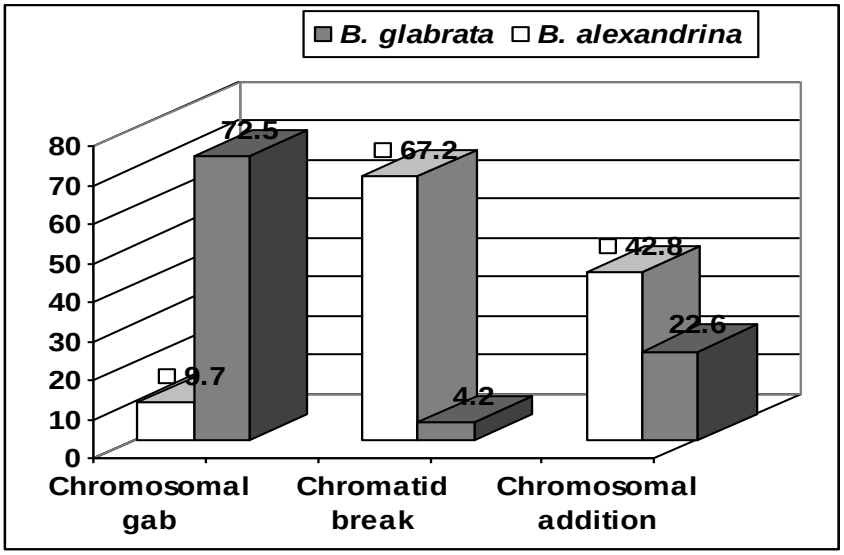

Fig. (2): Diagrammatic representation of chromosome aberration-types of the two species of the snails.

Table (1): LC_{50}, LC_{90} of the plants *Origanum syriacum* to the snails *B. glabrata* and *B. alexandrina*.

Concentrations	Mean Concentrations (ppm) ± SD	
	B. glabrata	*B. alexandrina*
LC_{50}	↓ 215.2 ± 0.65	226.8 ± 0.66
LC_{90}	↓ 415.7 ± 0.33	422.9 ± 1.00

Table (2): Measurements of the mitotic chromosomes of the freshwater snails, *B.glabrata* and *B.alexandrina* (in micrmeters).

Chromo. number	*B. glabrata*				*B. alexandrina*			
	Mean chromosomal length				Mean chromosomal length			
	Chromo. type	1st arm	2nd arm	Total length	Chromo. type	1st arm	2nd arm	Total length
1	submeta.	1.50	3.00	4.50	submeta.	1.25	2.97	4.22
2	meta.	2.10	2.20	4.30	meta.	1.88	2.19	4.06
3	meta.	1.90	2.10	4.00	meta.	1.88	2.34	4.22
4	submeta.	1.30	2.10	3.40	submeta.	1.25	2.19	3.44
5	submeta.	1.30	1.90	3.20	acro.	0.63	2.50	3.13
6	meta.	1.50	1.60	3.10	meta.	1.09	1.41	2.50
7	meta.	1.40	1.50	2.90	meta.	0.94	1.25	2.19
8	acro.	0.50	2.20	2.70	submeta.	0.63	1.41	2.03
9	meta.	1.25	1.35	2.60	meta.	0.94	0.94	1.88
10	telo.	-	2.30	2.30	telo.	-	1.72	1.72
11	meta	1.00	1.20	2.20	meta.	0.78	0.84	1.63
12	acro.	0.40	1.60	2.00	acro.	0.21	1.35	1.56
13	meta.	0.90	1.00	1.90	meta.	0.63	0.88	1.50
14	meta.	0.85	0.95	1.80	submeta.	0.47	0.94	1.41
15	meta.	0.80	1.90	1.70	meta.	0.47	0.78	1.25
16	meta.	0.80	0.80	1.60	meta.	0.47	0.63	1.09
17	meta.	0.70	0.80	1.50	meta.	0.47	0.56	1.03
18	telo.	-	1.30	1.30	telo.	-	0.94	0.94

Chromo. = chromosomal, meta. = metacentric, submeta. = submetacentricacro. = acrocentric, telo. = telocentric.

Table (3):Chromosomal aberration-types obtained in the snails *B. glabrata* and *B. alexandrina*.

Chromosomal aberration-types	Mean Score ± SD	
	B. glabrata	*B. alexandrina*
Chromosomal addition	22.6 ± 0.67	42.8 ± 1.33
Chromatid break	↓ 04.2 ± 0.33	67.2 ± 1.67
Chromosomal gab	72.5± 1.33	↓ 09.7± 0.67

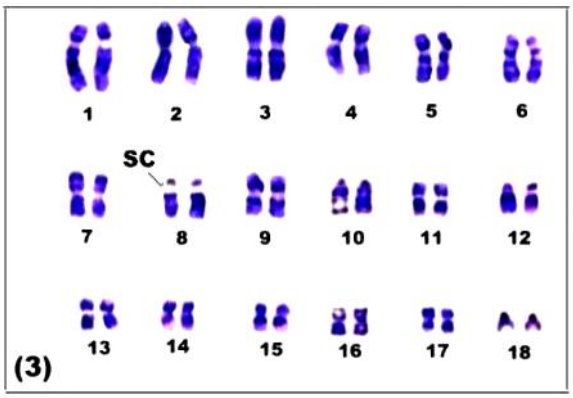

Fig. (3): G-banding patterns of untreated snail *B. glabrata* (SC=secondary constriction, X=2000).

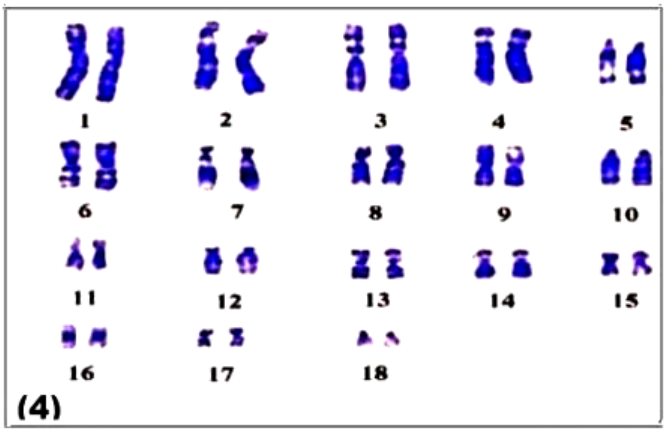

Fig. (4): G-banding patterns of untreated snail *B. alexandrina* (X=2000).

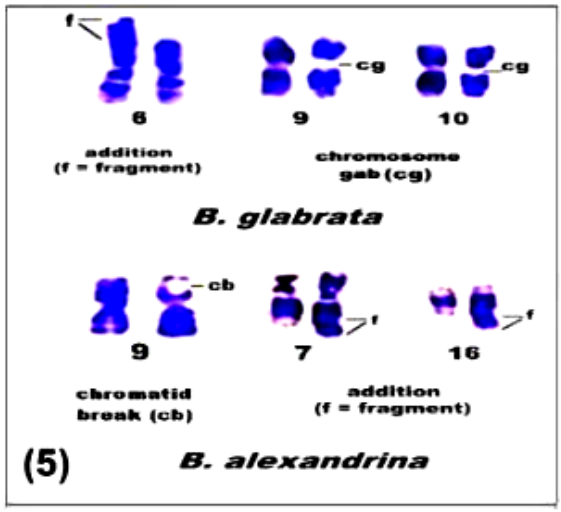

Fig. (5): G-banding patterns of structural chromosomal aberration-types in both species (X=2000).

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