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EFFECTS OF *LAURUS NOBILIS* (LAURACEAE) ON *BIOMPHALARIA GLABRATA* (SAY, 1818)

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Experiments were carried out using aqueous extracts from leaves and flowers of Laurus nobilis *on* Biomphalaria glabrata. *Treatments were performed on blastula stage (± 15h after first cleavage) and on adult snails (11-18 mm). In both instances they were exposed for 24 h to different concentrations of the extracts on snails (200 to 2500 ppm) and embryos (20 to 300 ppm) at 25 ± 1 °C. The embryos were observed for a period of 20 days after treatment and the snails for 10 days.*

Results obtained with leaf aqueous extracts have shown a degree of toxicity on embryos starting at a concentration of 125 ppm, the flower extract being effective at 35 ppm. The malformation obtained with the different concentrations falls into the unespecific type category, however some cephalic and shell malformations were found in embryos treated with concentrations over 50 ppm (leaves) and 25 ppm (flowers).

The LD90 on adult snails obtained by treatments with flower and leaf extract was observed at concentrations of 340 ppm and 1900 ppm respectively.

Biomphalaria glabrata is a mollusk of medical interest because it is one of the main intermediate hosts of *Schistosoma mansoni*, the organism causing schistosomiasis. The study of *B. glabrata* is very important, since one of the means controlling schistosomiasis is by the preventive elimination of the host snails with molluscicides.

Interest in the search for plant extracts with molluscicidal activity arose as a function of environmental factors, since synthetic molluscicides are highly toxic to animals and plants, and of economic factors, in view of the increasing costs of chemical products (WHO, 1983). Several authors have investigated plants for molluscicidal activity. Lemma (1970) studied *Phytolacca dodecandra*, Sousa et al. (1970, 1974), Silva et al. (1971) and Rouquayrol et al. (1972, 1973, 1980), investigated plants of the Brazilian northeast, Pereira et al. (1974, 1978) studied *Anacardium occidentale* and *Euphorbia cotinifolia,* Medina & Ritchie (1980) worked with *Solanum nodiflorum*, Adewunmi & Sofowora (1980) with plant species from Nigeria, Ahmed et al. (1984) with plants native to Sudan, Shoeb & El-Sayed (1984) worked with Euphorbiaceae and Agavaceae and Mendes et al. (1984, 1986) with plants of the Brazilian flora.

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Extending the search for substances with possible molluscicidal activity, we tested several plant species. The plant that yielded the best results, *Laurus nobilis*, Linn (1753), was selected for the present study. Popularly known as laurel or Apollo's laurel, *Laurus nobilis* is used in folk medicine for its abortifacient (Farnsworth, 1975), stomach treatment, emmenagogue, diuretic (Font-Quer, 1978), antirheumatic and parasiticidal (Garner et al., 1961) activities. Its leaves are used in cooking (Barriga, 1974) and its volatile compounds have shown activity as cockroach repellents in the laboratory (Verna & Meloam, 1981).

The present study is a preliminary evaluation of aqueous extracts of flowers and leaves of *L. nobilis* tested in the laboratory on *B. glabrata* embryos and adult snails. The effect of the extracts on embryo development was studied more in depth because embryos usually show low susceptibility to plant extracts in relation to adult snails.

The choice of aqueous extract rather than extract using other solvents was due to the fact that the former is more easily biodegradable with reduced development of toxic residues in the environment, as well as to the fact that it consist in a more economic method (WHO, 1983).

MATERIAL AND METHODS

Aqueous extracts of *Laurus nobilis* leaves and flowers were prepared with five extractions of 10 minutes each in boiling water. A Clevenger attached to a condenser and coupled to a volumetric balloon was used in the extractions in order to recover part of the substances taken away by the steam.

The planorbid used in the tests was *Biomphalaria glabrata* originally from Belo Horizonte (State of Minas Gerais) and adapted to laboratory conditions for several years. The snails are maintained in aquaria with continuous aeration and fed lettuce and a balanced ration.

The tests were performed according to the methodology recommended by the World Health Organization (WHO, 1965, 1983). Snails with 11 to 18 mm shell diameters and embryos at the blastula stage (15 hours after the first egg cleavage) were used in the bioassays (Camey & Verdonk, 1970). The assays were repeated 5 to 7 times at different concentrations on an average number of 8 to 10 snails and 168 to 328 embryos per experiment. Snails and egg masses used as controls were maintained under the same experimental conditions, except that they were only left in filtered water.

At the end of exposure, animals and egg masses were washed and observed daily. The snails were analysed for 10 days by recording the death rate, and the embryos were examined with a stereomicroscope up to the 20th day after treatment. The following parameters were used to evaluate the effect of the extracts on the embryos: mortality, hatching, malformation, and apparently normal or teratomorphic embryos that did not hatch. The malformations were classified by the methods of Raven (1949), Verdonk (1965) and Geilenkirchen (1966). The lethal concentrations were determined according to the method of Cox, 1970.

RESULTS

The results of the tests conducted on embryos at the blastula stage treated with *L. nobilis* extracts at concentrations of 20 to 300 ppm are summarized in Table I and Fig. 1. Mortality was more than 90% at concentrations of 50 and 200 ppm of flower and leaf extracts, respectively.

The molluscicidal activity on adult sinails was significant starting at dilutions of 340 and 1900 ppm of flower and leaf extracts, respectively (Table II and Fig. 2). Snails that did not survive sublethal concentrations showed intense hemorrhage at the end of treatment.

Table III and Figs. 3 and 4 show the effect of *L. nobilis* on the hatching of embryos that resisted treatment. Control embryos exposed to a temperature of 25 ± 1 °C started hatching between the sixth and seventh day of development.

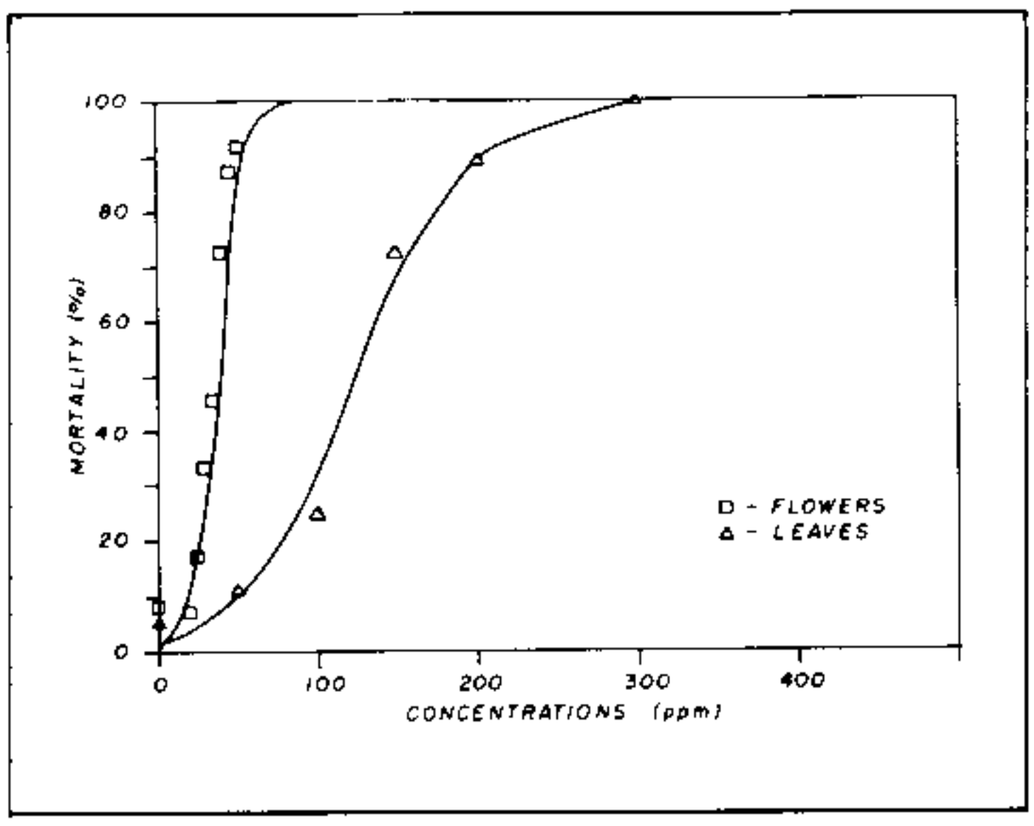

Fig. 1: mortality of *Biomphalaria glabrata* embryos exposed to aqueous extracts of *Laurus nobilis* flowers and leaves, on the 20th day after treatment.

	LD_{50}	LD_{90}
Flowers	34.3	50.1
Leaves	124.4	198.9

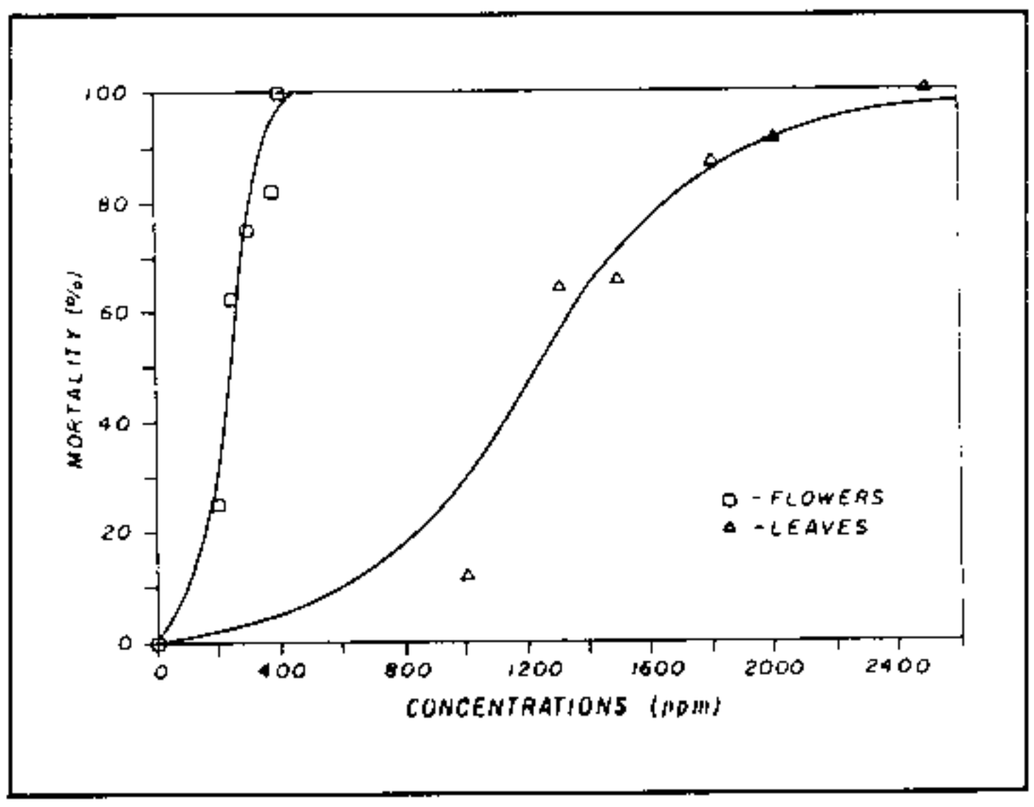

Fig. 2: mortality of *Biomphalaria glabrata* adult snails exposed to aqueous extracts of *Laurus nobilis* flowers and leaves, on the 10th day after treatment.

	LD_{50}	LD_{90}
Flowers	242	340
Leaves	1219	1900

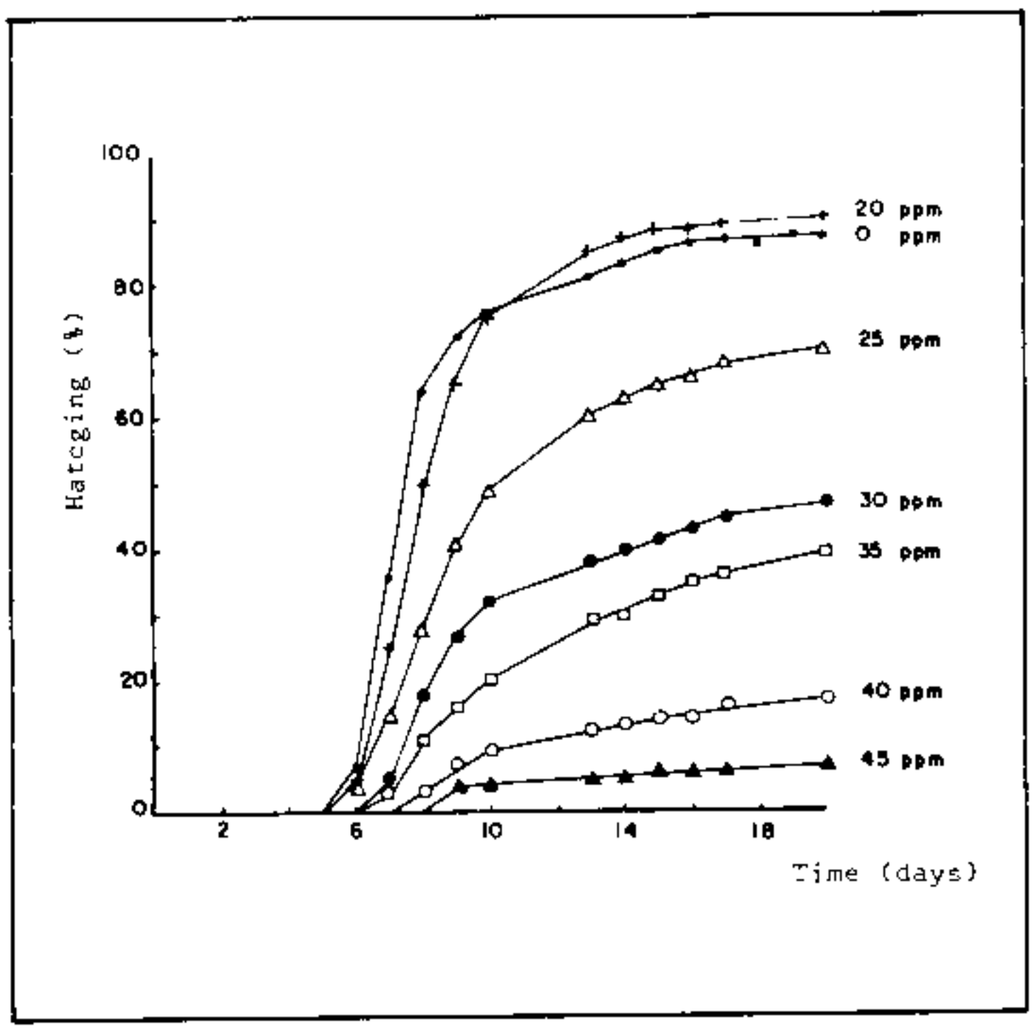

Fig. 3: hatching rate of embryos exposed to aqueous extracts of *Laurus nobilis* flowers at different concentrations during the 20th days observation.

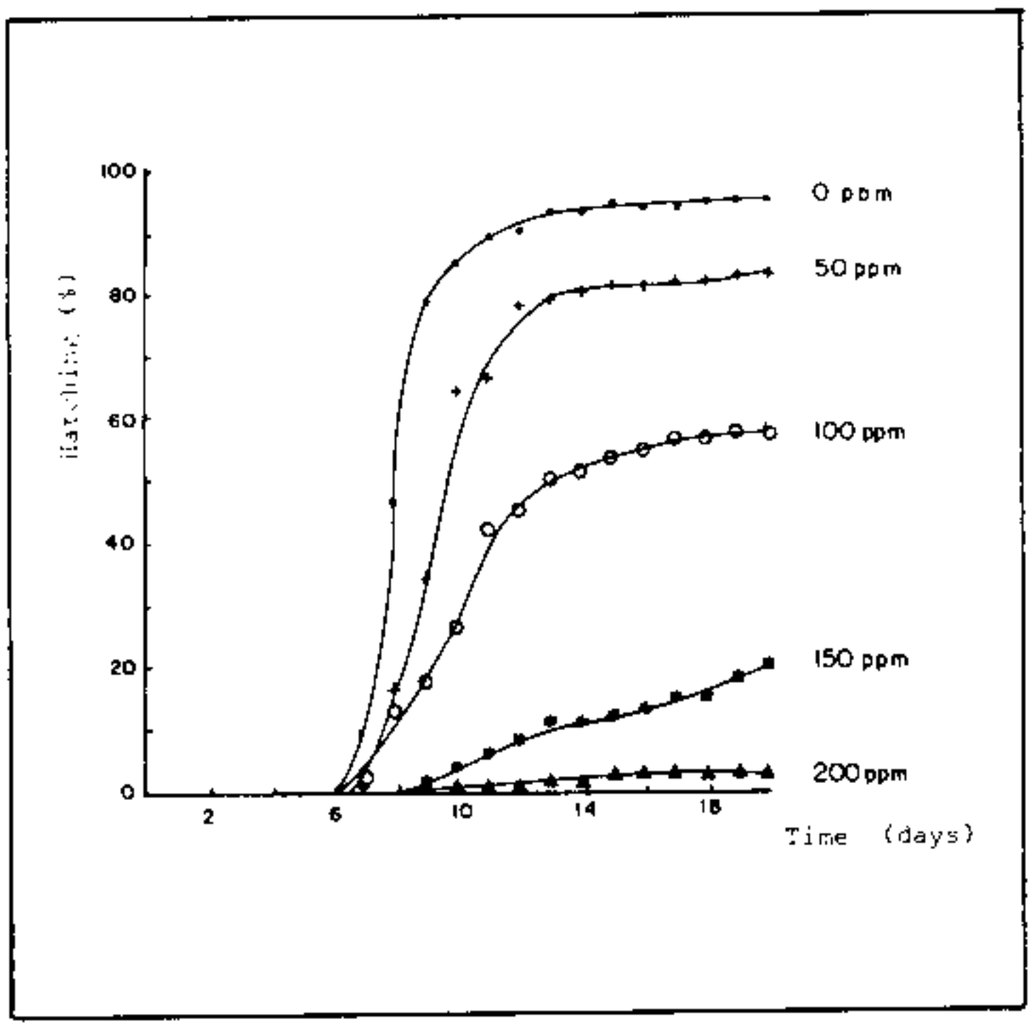

Fig. 4: hatching rate of embryos exposed to aqueous extracts of *Laurus nobilis* leaves at different concentrations during the 20th days of observation.

TABLE I

Mortality of *Biomphalaria glabrata* embryos exposed to aqueous extracts of *Laurus nobilis* flowers and leaves, on the 20th day after treatment. A total of 1000 to 2363 embryos were analyzed per concentration

	0	20	25	30	35	40	45	50	100	150	200	300
Flowers	8.11	7.02	17.32	33.30	45.63	72.98	87.54	91.90	100	100	100	100
Leaves	4.82	–	–	–	–	–	–	9.33	25.99	69.02	91.18	100

TABLE II

Mortality of *Biomphalaria glabrata* adult snails exposed to aqueous extracts of *Laurus nobilis* flowers and leaves, on the 20th day after treatment. A total of 50 to 58 snails were analysed per concentration

	0	200	250	300	350	400	1000	1300	1500	1800	2000	2500
Flowers	0	26.0	60.0	78.18	82.0	100	100	100	100	100	100	100
Leaves	5.95	–	–	–	–	–	12.07	64.44	65.96	87.72	90.57	100

TABLE III

Hatching rate of *Biomphalaria glabrata* embryos observed on the 20th day after treatment with aqueous extracts of *Laurus nobilis* flowers and leaves. A total of 1000 to 2363 embryos were analyzed per concentration

	0	20	25	30	35	40	45	50	100	150	200	300
Flowers	86.94	89.73	70.52	47.40	39.01	17.51	6.78	6.49	0	0	0	0
Leaves	95.01	–	–	–	–	–	–	82.86	57.57	20.28	3.44	0

TABLE IV

Percentage of malformed embryos observed during treatment with aqueous extracts of *Laurus nobilis* flowers and leaves

Extracts	Concentrations (ppm)	No. embryos	Unespecific malformations (%)	Cephalic malformations (%)	Shell malformations (%)
Flowers	0	1110	3.96	0.36	0.09
	20	1168	2.99	0.42	0
	25	1143	17.50	1.57	0
	30	1000	35.50	2.20	0.20
	35	1133	45.63	1.68	0
	40	1125	76.18	0.89	0
	45	1092	77.47	0.18	0
	50	1680	62.20	0.06	0
Leaves	0	2363	1.96	0.13	0
	50	1092	2.32	0.18	0.09
	100	1508	16.49	2.59	0.20
	150	1460	69.31	1.09	0.21
	200	1363	61.16	0.88	0.07
	300	1516	70.67	0	0

Teratomorphic embryos were observed after treatment with the various concentrations of flower and leaf extracts of *L. nobilis* (Table IV), with a predominance of unespecific malformations. Cephalic malformations reached maximum rates of 2.20% and 2.59% after treatment with flower and leaf extracts, respectively, the most frequent being right and left ocular reduplication. Right and left monoophthalmy and tetraophthalmy also occurred. Embryos with shell malformations, which occurred at low rates, showed reduced or elongated shells. (Fig. 5).

DISCUSSION

L. nobilis flowers showed greater molluscicidal activity than leaves against *B. glabrata,* with snails and embryos at the blastula stage showing 4- to 5- fold greater sensitivity to flower than to leaf extracts.

Embryos at the blastula stage were 8.5 times more susceptible to the extracts than the snails. Studies conducted on *Arthemisia verlotorum, Cassia rugosa, Euphorbia pulcherrima, Rumex crispus* (Mendes et al., 1984) and *Stevia rebaudiana* (Kawano & Simões, 1986) reported greater snail resistance in relation to the embryos. The low susceptibility of snail eggs to plant extracts had also been previously detected by Lemma (1970) working with *Phytolacca dodecandra,* by Rouquayrol et al. (1973) with *Pithecelobium multiflorum,* Pereira et al. (1974, 1978) with *Anacardium occidentale* and *Euphorbia cotinifolia,* and by Mendes et al. (1984, 1986) with several plant species.

Embryos exposed to extract concentrations close to, or higher than, LD_{50} which survived treatment showed a 24 to 48 hour delay in hatching in relation to the controls.

Unespecific malformations caused by treatment with flower and leaf extracts of *L. nobilis* predominated at all concentrations tested. At highly toxic concentrations, unespecific malformations were followed by embryo death during the first few days after treatment. Cephalic malformations involving eye number and position in embryos treated with *L. nobilis* extracts suggest that there was an alteration in the cephalic plate region (future region of eyes and tentacles) since these plates are not yet formed during the blastula stage (Camey & Verdonk, 1970). The occurrence of embryos with cephalic malformations has been previously noted in *B. glabrata* treated with lithium carbonate (Camey & Paulini, 1963), hycanthone (Souza & Katz, 1973), giberellic acid and 2-chloroethyl trimethyl ammonium chloride (Kawano et al., 1982), caffeine (Simões & Kawano, 1982), and with infusions of *Stevia rebaudiana* (Kawano & Simões, 1986).

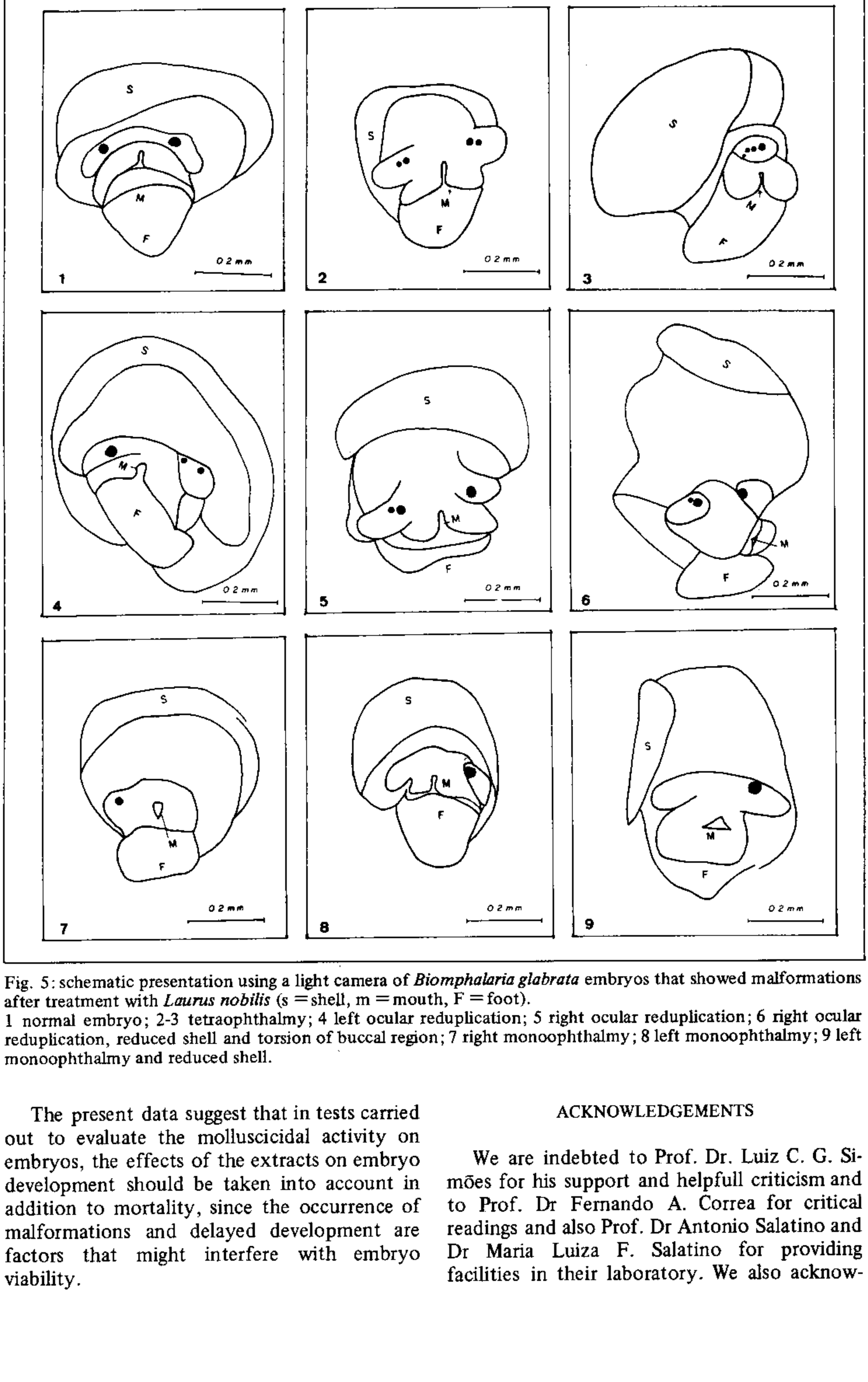

Fig. 5: schematic presentation using a light camera of *Biomphalaria glabrata* embryos that showed malformations after treatment with *Laurus nobilis* (s = shell, m = mouth, F = foot).
1 normal embryo; 2-3 tetraophthalmy; 4 left ocular reduplication; 5 right ocular reduplication; 6 right ocular reduplication, reduced shell and torsion of buccal region; 7 right monoophthalmy; 8 left monoophthalmy; 9 left monoophthalmy and reduced shell.

The present data suggest that in tests carried out to evaluate the molluscicidal activity on embryos, the effects of the extracts on embryo development should be taken into account in addition to mortality, since the occurrence of malformations and delayed development are factors that might interfere with embryo viability.

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