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MOLLUSCICIDAL POTENCY OF *PITTOSPORUM TOBIRA VARIGATUM* AND *HEDERA CANARIENSIS* PLANTS AGAINST JUVENILE AND ADULT *BIOMPHALARIA ALEXANDRINA* SNAILS

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ABSTRACT

In the search for new molluscicidal plants for controlling the snail vector of schistosomiasis, laboratory evaluation was made to assess the molluscicidal activity of *Pittosporum tobira varigatum* and *Hedera canariensis* plants against *Biomphalaria alexandrina* snails. Results indicated that *P. tobira* dried powder was more potent than *H. canariensis* as LC_{90} values were 110 and 160 ppm for the two plants respectively. The effect of three sublethal concentrations (LC_0, LC_{10} and LC_{25}) of each of the molluscicidal plants was investigated against both juvenile and adult *B. alexandrina* snails. The studied parameters included the survival rate, fecundity, reproductive rate and the growth of the experimental and control groups. The sublethal concentrations of both plants markedly suppressed the survival rate and increased the mortality with increasing the concentration and the observation period. The results also revealed that juvenile snails could resist the cumulative toxicity of these plants more than adult snails, as the survival rates of juvenile were higher during all the observation weeks. Juvenile snails exposed to 36 ppm of *H. canariensis* survived the whole experimental periods, while the corresponding adult snails died by the 14^{th} week. On the other hand, juvenile snails treated with *H. canariensis* and the control ones started to lay eggs at the third week of the experiment, while the onset of oviposition of juvenile snails treated with *P. tobira* was retarded. This delay was proportional to the concentration used. The reduction in reproductive rates of all experimental groups treated with 60 ppm of *P.*

tobira was also remarkable as it reached 99.51% of that of the control snails. The histological studies of the hermaphrodite gland of snails exposed to 27 or 48ppm of *P. tobira* and 36 or 60 ppm of *H. canariensis* for 4 weeks was also carried out. The hermaphorodite gland of treated snails showed degeneration in the male and female gamatogenic cells, intertubular connective tissue and Ancle's layer. These degenerations were more prominent in snails exposed to higher plant concentrations. Numerous hermaphrodite tubules were totally void of any gametogenic cells.

INTRODUCTION

Schistosomiasis is long known to be endemic throughout Africa, Far East and South America, affecting more than 250 millions people in over than 76 countries (Sturrock, 2001). One way to attack the problem of schistosomiasis is to destroy the vector snails and thus remove a vital link in its life cycle. This may be achieved by means of synthetic products such as Bayluscide or alternatively with molluscicides from plant sources (Brackenbury, 1999; Rug and Ruppel. 2000). The use of molluscicidal plants growing abundantly in areas where schistosomiasis is endemic is a simple, inexpensive and appropriate technology for local control of the snail vector and may become a useful future complement for the control of this disease (Schall *et al.*, 2001).

Meanwhile, molluscicidal potency has been observed in numerous plant families (Liu *et al.*, 1997) and attributed to several major classes of natural products including saponins,other terpenes and alkaloids (Hostettmann *et al.*, 1997). However, no plant molluscicides have so far gained wide application. Only a few plants have been extensively studied on their long term effects on snails such as *Euphorbia splendens* (Giovanelli *et al.*, 2001; Schall *et al.*, 2001).

In preliminary laboratory tests, *Hedera canariensis* plant showed molluscicidal activity against *Biomphalaria alexandrina* (El-Emam *et al.*, 1990), though it still needs further detailed studies. Therefore, it is resonable to study the effect of sublethal concentrations of *Pittosporum tobira varigatum* and *Hedera canariensis* plants on both juvenile and adult *B. alexandrina* snails. The tested parameters include the survival, fecundity, reproductive rate and growth rates of treated and control snails. Besides, a histological study was also carried out to investigate the changes noticed in the hermaphrodite gland after molluscicidal application.

MATERIALS AND METHODS

Plants:

The plants under investigation are *Pittosporum tobira varigatum* (Family Pittosporaceae) and *Hedera canariensis* (Family Araliaceae). They were collected during summer months, 2005 from a private garden near Banha and from El-Zohryia Garden, Cairo respectively. The plants were kindly identified by Dr Wafaa M. Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University. Voucher specimens of the plants were deposited at the Department of Medicinal Chemistry, Theodor Bilharz Research Institute. The plants were shade dried and powdered by an electric mill.

Snails:

Wild *Biomphalaria alexandrina* snails, were collected from irrigation canals in Giza Governorate. They were kept in plastic aquaria with dechlorinated water under optimum laboratory conditions of pH (7.5-7.7) and Temp.(25± 2°) and fed on lettuce leaves for 3 weeks before being used in tests in order to accommodate to laboratory conditions. During this period, snails were examined for trematode infection and positive snails were excluded.

Snail's toxicity tests:

The LC_{50}, LC_{90} and the slope function for each plant were determined from the mortality curves according to the method of Litchfield and Wilcoxon (1949).Three replicates of gradual concentrations were prepared from the aqueous suspension of the dry powder of each tested plant on basis of weight/volume. Ten snails (8-11 mm diameter) were used in 1 L- capacity jar, for each replicate. The exposure periods were 24 hrs followed by another 24 hrs as a recovery period. The molluscicidal concentrations were expressed in terms of ppm.

Evaluation of survival, fecundity and growth:

This study was carried out to test the effect of sublethal concentrations of *P. tobira varigatum* and *H. canariensis* on the survival, reproductive and growth rates of both juvenile and adult snails. Three sublethal concentrations were chosen from each molluscicidal plants; LC_0; LC_{10} and LC_{25}. These concentrations are equivalent to 27, 48 and 60 ppm of *P.tobira varigatum* and 36, 60 and 80 ppm of *H. canariensis*. Two snail groups were used in this experiment; 210 juvenile snails (3.3-3.6 mm diameter) and 210 adult snails (5.8-6.3 mm). Each group was subdivided into 21 subgroups each of 10 snails. Three replicates of each of the tested concentrations and the control were prepared in 1.5 L

capacity plastic aquaria. They were filled with 1L of the experimental solution and each was supplied with 10 juvenile or adult snails. The water temperature was kept at 25 ± 2° and the aquaria were supplied with dried lettuce leaves and tetramine [Ingredients: fish meat, aquatic plants, shrimp meat, vitamins and oat flour]. A piece of chalk was added as a calcium source. The aquaria were also supplied with white plastic foams as a favorite place for snail's egg deposition. Dead snails were removed daily and the molluscicidal solutions were changed once weekly. The experiment was planned to last for 20 weeks unless the experimental snails died. Egg masses were collected once weekly and the eggs were counted using a stereomicroscope. The shell diameter of each snail was measured using a caliper at the beginning of the experiment and thereafter once weekly.

Calculations:

The parameters measured in this study are the ratio of surviving snails in each week (L_x), the mean No. of eggs per snail per week (M_x). Also the reproductive rate in each week was represented by the term L_xM_x. The growth rate in any week was expressed as the mean shell diameter of snails in any week. The total egg production and the total reproduction rate were calculated by the end of the experiment. Also, the percent reductions in these two parameters were estimated.

Histological study:

This was carried out to record the possible histological changes in the hermaphrodite gland and the digestive gland of treated snails compared to the control snails. Two sublethal concentrations of each plant were chosen: 27 and 48 ppm for *P. tobira varigatum* and 36, 60 ppm for *H. canariensis*. Snails were exposed to these concentrations for 4 successive weeks. Thereafter, both treated and control snails were washed with water then dried. The shells were gently crushed between two glass slides and the soft parts of the snails were carefully removed out of the shell.

The hermaphrodite gland of each snail was gently separated then fixed in Bouin's solution. The glands were dehydrated using ascending grades of ethanol then cleared in terpineol, embedded in paraffin wax and finally sectioned at 6 μ using a microtome. Sections were stained with haematoxylin and eosin stain (H-E), dried then microscopically examined and photographed by a Zeiss video camera, Germany.

RESULTS

Results concerning the molluscicidal potency of the dry powders of *Pittosporum tobira varigatum* and *Hedera canariensis* plants were listed in Table (1). Data showed that both plants possess marked activity as their LC_{90} values were 110 and 160 ppm for the two plants respectively after 24 hrs exposure. Meanwhile, the LC_{50} values recorded 74 and 98 ppm for the two plants respectively. The slope functions of the regression lines representing the toxicity of the two plants were 1.36 and 1.41 respectively (i.e. < 1.5).

Data concerning the effect of sublethal concentrations of the two plants on juvenile *B.alexandrina* snails are listed in Tables (2 and 3). The parameters studied were the survival rate, the onset of oviposition, fecundity, reproductive and growth rates. Results in Table (2) proved that the survivorship of snails treated with *P. tobira varigatum* showed variable patterns of response. Snails exposed to 60 ppm suffered a sharp depression in survivorship as all snails (except one) died by the end of the first week (L_x=0.03). Also snails exposed to 48 ppm showed a sharp decline at the first week (L_x=0.20), thereafter they decreased gradually. However, snails exposed to 27 ppm decreased gradually and steadily and then died at the 18th and 19th week for the two groups respectively. The onset of oviposition of snails exposed to 48 and 60 ppm of the plant was delayed when compared with that of the control group. Control snails started egg laying at the 3rd week of the experiment, while in, the 48 ppm and 60 ppm exposed groups egg laying was delayed to the 4th and the 5th week respectively. Meanwhile, the onset of oviposition in snails exposed to lower concentration (27 ppm) was not affected.

Comparing the fecundity (M_x) and reproductive rates (L_xM_x) of all experimental groups with that of the control group revealed that those two parameters were markedly affected by exposure to the plant and this effect was proportional to the concentration used. The M_x values were lower than that of the control during all the observation weeks. They recorded peaks values of 33.46, 28.25 and 19 for the treated groups respectively, while the maximum value of the control was 128.17. The three experimental groups showed a reduction in the total reproductive rate which reached 85.51%, 96.49% and 99.51% of that of the control group by the end of the experiment. Also the rate of growth of exposed snails was moderately lowered and the net growth values were 5.74, 7.38, and 7.38 for the treated groups, compared with 8.3 for the control group.

Regarding the effect of *H.canariensis* on juvenile snails, results (Table 3) revealed that 80 ppm of the plant had a strong cumulative effect as snails exposed to this concentration did not survive long and died by the 7^{th} week with a sudden collapse at the first week (L_x=0.27).However, snails treated with lower concentrations of 36 and 60 ppm could survive the whole observation period and their survivorship values at the 20^{th} week were 0.27 and 0.17 for the two groups respectively. Also it can be noticed that they showed many periods of stability in their L_x values such as from the (4^{th}-6^{th} weeks), (10^{th}-12^{th} week) in the 36 ppm-group and during the (3^{rd}-5^{th} week), (10^{th}-12^{th} week) and (13^{th}-17^{th} week) in the 60-ppm group .The onset of oviposition in all experimental snails was not affected by exposure to different concentrations of the plant as all the tested and the control groups started to lay eggs at the 3^{rd} week of the observation period. The egg laying capacity of all experimental groups showed fluctuations as their L_x values were variable from week to another. The maximum-recorded L_x values were 46.47, 29.68 and 23.80 for the groups exposed to 36, 60 and 80 ppm respectively. However, the L_x values were always lower than that of the control during all the observation weeks.

The total reproductive rates (L_xM_x) for the treated groups were 203.51, 136.36 and 6.88 compared with 851.46 for the control group. Therefore, the reduction in L_xM_x values from that of the control were 76.1%, 83.99% and 99.19% by the end of the experiment. The net growth, was inversely proportional to the plant concentration used, as it was 5.58, 5.53 and 4.58 mm for the three exposed groups at the end of the observation period, while it was 8.47 for the control group at the same period.

Regarding the effect of *P.tobira varigatum* on adult snails, results in Table (4) showed that almost half the numbers of snails exposed to 48 or 6o ppm were dead after the first week of exposure, while no mortality was recorded at the same week in the group exposed to 27 ppm of the plant. Thereafter, live snails continued to decrease gradually till the 3 experimental groups died in 3 successive weeks: the 13^{th}, 12^{th} and 11^{th} week. The egg laying capacity values (M_x) of the 3 groups followed a fluctuated pattern with some peaks. The peak recorded for the 27 ppm-group was 36 in the first week, while those recorded for the last two groups were 28.53 and 23.29 in the first two weeks. This means that the suppressive effect of the plant did not appear during the first weeks. Thereafter, the fecundity and the reproductive rates of treated snails were

lower than those of the control snails in all the observation weeks. Moreover, all experimental groups stopped egg laying completely in some weeks. The percentages of reduction in fecundity were 85.44%, 87.16% and 92.07% , while the reduction in reproductive rates were 87.66%,93.52% and 96.23% for the three exposed groups respectively. The mean shell diameter of the treated snails was lower than that of the control during the whole observation period. Therefore, the net growth values of snails by the end of the experiment were 2.26 mm, 2.0 mm and 2.88 mm for the experimental groups compared to 5.18 mm for the control group.

Concerning the survivorship of adult snails exposed to *H. canariensis*, data in Table (5) showed that it declined gradually starting from the first week without any sudden collapse. The experimental groups were dead by the 14^{th}, 11^{th} and 8^{th} week for the 3 groups respectively. The fecundity and the reproductive rates were greatly affected by exposure to the plant and their values were relatively higher in the first weeks and markedly lower in the last weeks. The group treated with 80 ppm stopped egg laying at the last week of survival (M_x=0.00). The reduction in reproductive rates of the 3 exposed groups were 81.27%, 86.88% and 94.80% from that of the control group. The growth of the experimental groups was lower than that of the control in all weeks. The net increases in shell diameter were 3.58 mm, 3.88 mm and 2.48 mm for the experimental groups and 5.38 mm for the control group.

The histological study was conducted on the hermaphrodite gland of adult snails exposed to 27 or 48 ppm of *P. tobira* and 36 or 60 ppm of *H. canariensis* for 4 weeks as well as on control snails. The control specimens showed that the normal hermaphrodite gland consists of huge number of identical tubules that are connected together with a connective tissue. These tubules are covered with a thin layer (Ancle's layer). The tubules are lined with layers of epithelial cells that differentiate into male and female gametogenic cells (spermatogenesis and oogenesis). These gametogenic cells appeared in normal snails arranged in clusters at different development stages. Inside the lumen of the tubules, the fully mature ova and sperms are aggregated in large numbers (Fig. 1, A).

Comparing the treated snails showed various histology alterations in most tubules. The Ancle's layer was severely damaged and ruptured at many positions, while in the connective tissue between the tubules; numerous areas of degenerations could be noticed. The numbers of mature ova and sperms were reduced and their outlines were shrinked.

Also all premature stages suffered from great damage in their shape and were vacuolated and therefore their cytoplasm was weakly stained.

Histological examination of the hermaphrodite gland of snails exposed to higher concentrations of both plants showed more prominent changes with greater decline in the number of mature ova or sperms. Moreover, in many tubules, the ova and sperms were mostly lacking and the tubules appeared almost empty. Besides the connective tissue between the tubules disappeared completely (Figs. 1, B and C).

DISCUSSION

In this study the dry powder of both *P. tobira varigatum* (Pittosporaceae) and *H.canariensis* (Araliaceae) plants showed marked molluscicidal potency as their LC_{90} values were 110 and 160 ppm for the two plants respectively. Moreover, the recorded median lethal concentration (LC_{50}) for both plants ranged from 74 -98 ppm, which falls well below the threshold of 100 microg/ml (ppm) set for a potential molluscicidal plant by the World Health Organization (WHO, 1993 and Silva *et al.*, 2005). However, these results indicate that *P. tobira varigatum* is a more potent molluscicide than *H. canariensis*. The minor difference in molluscicidal potency between the two plants could be attributed to the difference in the nature or amount of their active chemical constituents. *H. canariensis* was previously reported to contain triterpene saponins (Ahmed, 1997). The reported potency of the latter plant supports the previously obtained data on the molluscicidal effect of *H. canariensis* (El-Emam *et al.*, 1990).

Meanwhile, to the best of our knowledge, this is yet the first report of the molluscicidal effect of *P. tobira varigatum*. However, an other subspecies of *P. tobira* was previously reported to possess a marked molluscicidal effect (Hamed, 1999) and this similarity verifies the chemotaxonomic relationship between the two subspecies. This relationship is also supported by various studies on many plant species from family Araliaceae that showed molluscicidal activity such as *Dizygotheca elegantissima and Didymopanax morototoni* (El-Nahas, 1999; Melendez and Capriles, 2002).

Results concerning the cumulative effect of the sublethal concentrations (low and high) of both plants on the survival of snails proved that lower concentrations of the two plants (LC_0 and LC_{10}) almost have the same destructive effects on snails. However, on using higher

concentrations, *H. canariensis* became more destructive on both juvenile and adult snails than *P. tobira varigatum.*

It was also noticed that in all groups exposed to relatively low concentrations, the reduction in survival rate was gradual and slow on the contrary to snails exposed to LC_{25} of the plants as the decline in survival was sharp and sudden. This means that snails could acclimatize to low concentrations of the molluscicide and tolerate its effects at the beginning of the experiment. This result agrees with that previously recorded by Abdel Hafez *et al.* (1997) that low concentrations of *Azolla pinnata* almost have no effect on the survival of *B. alexandrina.* On the other hand, Tantawy (2002) concluded that low concentrations of *Atrilplex halimus* caused a sharp decline in the survival of the same snail species. The present investigation also revealed that juvenile snails could resist the destructive effect of the plants more than adult snails as they survived for longer experimental periods. This may be explained by the assumption that juvenile snails being exposed to the plants at their younger age could acclimatize to their toxic effect. Similarly, Dos Santos and Sant Ana (2001) reported that *Annona sp.* plant is more active against adult forms of *Biomphalaria* sp. However, Anto *et al.* (2005) and Singh *et al.* (2005a) noticed that some plant derived molluscicides caused a significant reduction in the fecundity and survival of young snails than adult snails.

Regarding the egg laying capacity (M_x), the reproductive rate (L_xM_x), the total fecundity ($\sum M_x$) and the total reproductive rates ($\sum L_xM_x$) by the end of the experiment, data obtained revealed that all these values were greatly reduced by exposure to the two plants for 20 successive weeks. The reduction in total fecundity and reproduction ranged from 87.23%-99.51 % in snails exposed to LC_{25} of the two plants. This enormous reduction means that the snail's reproductive ability was almost completely destroyed due to a complex process involving more than one factor. This is in full agreement with results of Rao and Singh (2001) that the sublethal exposure of plant derived molluscicides on snail reproduction can be attributed to their effect on many metabolic elements of exposed snails such as ascorbic acid, total protein, albumin, GOT, GPT and other enzymes such as AAT and AChE (Farag and Risk, 1992; El-Hawary, 1996; El-Ansary *et al.*, 2001; El-Nahas and El-Deeb, 2002; Tiwari *et al.*, 2004). Later on, Singh and Singh (2004) and Singh *et al.* (2005b) stated that sublethal treatment with herbal molluscicides caused a great reduction in the protein, amino acid, DNA and RNA in the ovotestis of treated snails that are very important for several metabolic processes

including gametogenesis. The metabolic disorders are also reflected on snail survival (Abdel Kader *et al.*, 2005). On the basis of the present and other previous findings on other molluscicidal plants, this depression in fecundity can be matched with the histological damage noticed in the hermaphrodite gland, including the reproductive suppression of exposed snails (El-Deeb and El-Nahas, 2005). This explanation was further confirmed by some authors who reported that exposing snails to certain bacteria and virus spp. affected both the number and structure of ova and sperms which finally suppress the snails, egg laying (El-Deeb, 2000). Another explanation was that these molluscicides may greatly suppress the secretion of steroidal sex hormones responsible for maturation of the hermaphrodite gland and stimulation of gametogenesis (El-Nahas, 1998).

It can also be concluded that *P. tobira varigatum* has more destructive effect on snail fecundity than *H. canariensis*. Also juvenile snails exposed to *P. tobira varigatum* showed retarded onset of oviposition that was proportional to the concentration used. Also, adult snails treated with *P. tobira varigatum* stopped egg laying completely through some of the experimental weeks, while this was not noticed in case of using *H.canariensis*. It was also noticed that the depressive effect on egg laying capacity and reproductive rate is a molluscicidal-dose dependent as it was more pronounced in case of using higher concentration. This result is in agreement with other reported data (Bakry and Sharf El-Din, 2000 and Rao; Singh, 2000).

The great inhibition in growth rates of snails exposed to the molluscicidal plants may be attributed to the disturbance in metabolism or physiology of snails (Abdel-Kader *et al.*, 2005). The results also showed that reduction in growth rate was highly significant when high concentration was applied. This result is in agreement with previous investigations on other synthetic or plant molluscicides on either juvenile or adult snails (Mahmoud ,1993; El-Deeb, 1996; Mostafa, 1996).

Regarding the histological effect of the two plants on the hermaphrodite gland of exposed snails, it was noticed that the depression recorded in the reproductive ability of snails exposed to these two plants was confirmed by the enormous histological changes in this gland. The reduction noticed in all gametogenic stages as well as in the mature ova and sperms and in the other histological damage recorded in the Ancle's layer and the connective tissue can explain the great reduction noticed in fecundity. Also, higher plant concentrations caused more prominent histological alterations than low concentrations. These findings agree with

the suppressive effect of the saponin-containing *Tetrapleura tetraptera* on the gonadotrophic hormone (Bode *et al.*, 1996). Similarly, Mossalem (2003) reported a great histological damage in hermaphrodite gland of *B. alexandrina* exposed to *Panicum repens*, *Solanum nigrum* and *Dyzygotheca kerchoveana*. While El-Deeb and El-Nahas (2005) reported similar histological changes in hermaphrodite gland of snails exposed to *S. sesban*. Moreover, in the present work, the depression in reproductive rates of exposed snails to the tested plants was accompanied by increase in the number of empty eggs that contained no embryos. This is in accordance with Bakry *et al.* (2002) about the effect of molluscicidal treatment in producing abnormal eggs by *B. alexandrina*.

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EXPLANATION OF FIGURE

Fig (1): Section in hermaphrodite gland of *B alexandrine* (Bouin-Hx&E):

A) (1) Control snails 100 X.
(2) 200 X.

B) Snails exposed to *P. tobira varigatum* plant:
(1) 27 ppm 200 X.
(2) 48 ppm 200 X.

C) Snails exposed to *H. canariensis* plant:
(1) 36 ppm 200 X.
(2) 60 ppm 200 X.

A: Ancyl's layer.
c: connective tissue.
g: gametogenesis.
h: hermaphrodite tubules.

Table (1): Comparative susceptibility of adult *Biomphalaria alexandrina* snails exposed to the dry powder of *Pittosporum tobira varigatum* and *Hedera canariensis* plant for 24 hours.

Plants	*Pittosporum tobira varigatum* (ppm)	*Hedera canariensis* (ppm)
LC_{50}	74 (63.25-86.58)	100 (84.03-119.2)
LC_{90}	110	160
Slope	1.36	1.41
Sublethal concentrations		
LC_0	27	36
LC_{10}	48	60
LC_{25}	60	80

Table (2): Survivorship (L_x), fecundity (M_x), reproductive rate (L_xM_x) and mean shell diameter of juvenile *Biomphalaria alexandrina* snails exposed to sublethal concentrations of *Pittosporum tobira varigatum* plant.

Observation period (weeks)	*Biomphalaria alexandrina* snails exposed to:															
	27 ppm				48 ppm				60 ppm				Control			
	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)
0	1.00	-	-	3.42 ± 0.53	1.00	-	-	3.42 ± 0.53	1.00	-	-	3.42 ± 0.53	1.00	-	-	3.42 ± 0.53
1	0.83	0.00	0.00	3.86 ± 0.67	0.20	0.00	0.00	3.93 ± 0.64	0.03	0.00	0.00	3.35 ± 0.00	1.00	0.00	0.00	3.93 ± 0.51
2	0.80	0.00	0.00	4.18 ± 0.87	0.20	0.00	0.00	4.28 ± 1.06	0.03	0.00	0.00	4.20 ± 0.00	1.00	0.00	0.00	5.21 ± 0.53
3	0.77	7.60	5.85	5.67 ± 1.02	0.20	0.00	0.00	4.77 ± 0.70	0.03	0.00	0.00	4.35 ± 0.00	0.97	21.50	20.86	5.92 ± 0.77
4	0.77	24.91	19.18	6.32 ± 0.76	0.17	24.33	4.14	5.15 ± 1.57	0.03	0.00	0.00	5.20 ± 0.00	0.93	49.70	46.22	6.50 ± 0.63
5	0.43	27.30	11.74	7.18 ± 0.86	0.17	18.40	3.13	6.10 ± 1.24	0..03	16	0.48	6.50 ± 0.00	0.90	43.00	38.70	6.77 ± 0.54
6	0.43	33.46	14.39	7.42 ± 0.77	0.13	26.20	4.45	6.62 ± 0.71	0.03	11	0.33	7.00 ± 0.00	0.87	40.39	35.14	7.17 ± 0.65
7	0.43	32.85	14.13	7.61 ± 0.83	0.13	18.75	2.44	7.53 ± 1.29	0.03	19	0.57	7.50 ± 0.00	0.87	45.40	39.50	7.33 ± 0.65
8	0.43	30.69	13.20	8.01 ± 0.97	0.13	28.25	3.67	8.20 ± 0.91	0.03	14	0.42	8.50 ± 0.00	0.80	77.56	62.05	7.58 ± .0.58
9	0.40	23.00	9.20	8.09 ± 0.81	0.13	11.25	1.46	8.43 ± 1.09	0.03	8	0.24	8.60 ± 0.00	0.80	90.20	72.16	8.14 ± 0.69
10	0.40	13.42	5.37	8.47 ± 0.85	0.13	9.75	1.27	8.73 ± 1.99	0.03	7	0.21	8.80 ± 0.00	0.80	101.53	81.22	8.94 ± 0.79
11	0.37	21.42	7.93	8.60 ± 0.81	0.13	3.75	0.49	8.75 ± 0.88	0.03	4	0.12	9.10 ± 0.00	0.77	128.17	98.69	9.14 ± 0.65
12	0.33	18.45	6.09	8.62 ± 0.81	0.10	10.50	1.05	8.88 ± 1.13	0.03	8	0.24	9.40 ± 0.00	0.77	95.41	74.27	9.75 ± 0.73
13	0.33	15.40	5.08	8.74 ± 0.94	0.07	16.00	1.12	9.37 ± 0.47	0.03	9	0.27	9.90 ± 0.00	0.73	72.72	53.09	10.22 ± 0.52
14	0.33	2.80	0.92	8.83 ± 0.80	0.07	27.50	1.93	9.85 ± 0.92	0.03	11	0.33	10.2 ± 0.00	0.73	69.52	50.75	10.53 ± 0.49
15	0.33	1.18	0.39	8.99 ± 0.95	0.07	19.00	1.33	10.25 ± 0.35	0.03	15	0.45	10.5 ± 0.00	0.73	24.50	17.89	10.82 ± 0.62
16	0.30	15.20	4.56	9.02 ± 0.93	0.07	16.50	1.16	10.40 ± 0.57	0.03	14	0.42	10.5 ± 0.00	0.70	29.34	20.54	10.93 ± 0.73
17	0.27	5.33	1.44	9.12 ± 1.02	0.07	34.50	2.42	10.80 ± 0.57	0.03	0.00	0.00	10.8 ± 0.00	0.67	57.29	38.38	11.25 ± 0.65
18	0.03	7.00	0.21	9.16 ± 0.00	0.00	0.00	0.00	-	0.00	0.00	0.00	-	0.67	64.32	43.09	11.43 ± 0.54
19	0..00	0.00	0.00	-									0.63	54.28	34.20	11.72 ± 0.51
Total		280.01	119.68			264.68	30.06			136	4.08			1064.83	826.77	
% Reduction		73.70	85.52			75.14	96.36			87.23	99.51					
Net growth				5.74				7.38				7.38				8.3

Table (3): Survivorship (L_x), fecundity (M_x), reproductive rate (L_xM_x) and mean shell diameter of juvenile *Biomphalaria alexandrina* snails exposed to sublethal concentrations of *Hedera canariensis* plant.

Observation period (weeks)	*Biomphalaria alexandrina* snails exposed to:															
	36 ppm				60 ppm				80 ppm				Control			
	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)
0	1.00	-	-	3.42 ± 0.53	1.00	-	-	3.42 ± 0.53	1.00	-	-	3.42 ± 0.53	1.00	-	-	3.42 ± 0.53
1	0.97	0.00	0.00	3.97 ± 0.67	0.87	0.00	0.00	3.63 ± 0.61	0.27	0.00	0.00	3.70 ± 0.58	1.00	0.00	0.00	3.93 ± 0.51
2	0.97	0.00	0.00	4.74 ± 0.87	0.87	0.00	0.00	5.21 ± 0.72	0.23	0.00	0.00	4.44 ± 0.86	1.00	0.00	0.00	5.21 ± 0.53
3	0.90	9.45	8.51	5.61 ± 1.02	0.83	19.42	16.12	6.30 ± 0.83	0.17	13.14	2.23	6.20 ± 0.92	0.97	21.50	20.86	5.92 ± 0.77
4	0.87	46.67	40.60	6.62 ± 0.76	0.83	19.92	16.53	6.98 ± 1.06	0.13	23.80	3.09	7.50 ± 0.42	0.93	49.70	46.22	6.50 ± 0.63
5	0.87	33.31	28.98	7.04 ± 0.86	0.83	29.68	24.63	7.09 ± 0.84	0.07	8.25	0.58	7.76± 0.56	0.90	43.00	38.70	6.77 ± 0.54
6	0.87	27.15	23.62	7.11 ± 0.77	0.77	23.72	18.26	7.29 ± 0.90	0.07	14.00	0.98	8.00 ± 0.63	0.87	40.39	35.14	7.17 ± 0.65
7	0.77	19.35	14.90	7.25 ± 0.83	0.73	13.17	9.61	7.53 ± 0.89	0.00	0.00	0.00	-	0.87	45.40	39.50	7.63 ± 0.65
8	0.67	13.83	9.27	7.40 ± 0.97	0.67	13.82	9.26	7.56 ± 1.08					0.80	77.56	62.05	7.88 ± .0.58
9	0.67	4.05	2.71	7.46 ± 0.81	0.60	4.45	2.67	7.72 ± 1.11					0.80	90.20	72.16	8.14 ± 0.69
10	0.57	6.55	3.73	7.73 ± 0.85	0.50	1.66	0.83	7.91 ± 1.28					0.80	101.53	81.22	8.94 ± 0.79
11	0.57	8.00	4.56	7.99 ± 0.81	0.50	13.65	6.83	8.10 ± 1.19					0.77	128.17	98.69	9.14 ± 0.65
12	0.57	20.93	11.93	8.08 ± 0.81	0.50	12.94	6.47	8.12 ± 1.19					0.77	95.41	74.27	9.75 ± 0.73
13	0.53	11.00	5.83	8.23 ± 0.94	0.33	6.65	2.19	8.12 ± 1.23					0.73	72.72	53.09	10.22 ± 0.52
14	0.50	6.50	3.25	8.43 ± 0.80	0.33	1.33	0.44	8.18 ± 1.32					0.73	69.52	50.75	10.53 ± 0.49
15	0.47	23.50	11.05	8.61 ± 0.95	0.33	7.53	2.48	8.41 ± 1.22					0.73	24.50	17.89	10.82 ± 0.62
16	0.47	15.40	7.24	8.64 ± 0.93	0.33	26.71	8.81	8.45 ± 1.23					0.70	29.34	20.54	10.93 ± 0.73
17	0.43	23.00	9.89	8.79 ± 1.02	0.33	16.07	5.30	8.45 ± 1.32					0.67	57.29	38.38	11.25 ± 0.65
18	0.43	29.10	12.51	8.85 ± 0.90	0.30	13.00	3.9	8.67 ± 1.19					0.67	64.32	43.09	11.43 ± 0.54
19	0.37	9.78	3.62	8.93 ± 0.94	0.23	6.0	1.38	8.82 ± 1.27					0.63	54.28	34.20	11.72 ± 0.51
20	0.27	4.86	1.31	9.00 ± 1.28	0.17	3.83	0.65	8.95 ± 1.38					0.60	42.51	25.51	11.89 ± 0.45
Total		312.43	203.51			233.55	136.36			59.19	6.88			1107.34	852.28	
% Reduction		71.79	64.51			78.91	83			94.65	92.75					
Net growth				5.58				5.53				4.58				8.47

Table (4): Survivorship (L_x), fecundity (M_x), reproductive rate (L_xM_x) and mean shell diameter of adult *Biomphalaria alexandrina* snails exposed to sublethal concentrations of *Pittosporum tobira varigatum* plant.

Observation period (weeks)	*Biomphalaria alexandrina* snails exposed to:															
	27 ppm				48 ppm				60 ppm				Control			
	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Meanshell diameter ± S.D. (mm)
0	1.00	-	-	6.12 ± 0.76	1.00	-	-	6.12 ± 0.76	1.00	-	-	6.12 ± 0.76	1.00	-	-	6.12 ± 0.76
1	1.00	36.0	36.0	7.03 ± 0.79	0.57	12.82	7.31	6.81 ± 0.63	0.57	23.29	13.28	6.79 ± 0.85	1.00	20.15	20.15	6.96 ± 0.81
2	0.93	24.96	23.21	7.14 ± 0.84	0.57	28.53	16.26	7.21 ± 0.60	0.50	12.06	6.03	6.81 ± 0.91	0.97	53.44	51.84	7.47 ± 0.87
3	0.93	11.85	11.02	7.40 ± 0.08	0.53	16.18	8.58	7.32 ± 0.59	0.33	6.40	2.11	6.91 ± 0.79	0.97	54.28	52.65	7.70 ± 0.93
4	0.80	3.60	2.88	7.65 ± 0.81	0.47	7.07	3.32	7.36 ± 0.45	0.33	12.10	3.99	7.13 ± 0.58	0.93	48.61	45.21	7.89 ± 0.91
5	0.77	0.00	0.00	7.75 ± 0.93	0.37	1.55	0.57	7.40 ± 0.50	0.27	1.13	0.31	7.15 ± 0.86	0.90	80.43	72.39	8.24 ± 0.79
6	0.60	3.61	2.17	7.97 ± 0.99	0.37	19.72	7.30	7.50 ± 0.73	0.20	0.00	0.00	7.30 ± 0.89	0.87	90.27	78.53	8.51 ± 0.89
7	0.47	1.92	0.90	8.17 ± 0.78	0.33	0.80	0.26	7.63 ± 0.52	0.10	1.66	0.17	7.54 ± 0.91	0.87	123.38	107.34	8.41 ± 1.18
8	0.43	0.69	0.30	8.18 ± 1.06	0.27	1.37	0.37	7.69 ± 0.65	0.07	2.00	0.14	7.67 ± 1.20	0.87	98.20	85.43	8.63 ± 0.95
9	0.40	12.66	5.06	8.20 ± 0.83	0.10	8.33	0.83	7.83 ± 0.75	0.03	1.00	0.03	8.25 ± 0.00	0.83	110.50	91.72	9.60 ± 0.89
10	0.23	13.14	3.02	8.22 ± 1.05	0.03	5.00	0.15	8.00 ± 0.00	0.03	3.00	0.09	9.00 ± 0.00	0.83	40.50	33.62	10.60 ± 0.28
11	0.17	5.0	0.85	8.34 ± 1.25	0.03	0.00	0.00	8.10 ± 0.00	0.00	0.00	0.00	-	0.80	33.00	26.40	10.85 ± 0.35
12	0.07	1.5	0.11	8.38 ± 1.41	0.03	0.00	0.00	8.20 ± 0.00					0.76	11.00	8.36	11.00 ± 0.82
13	0.00	0.00	0.00	-	0.00	0.00	0.00	-					0.76	25.7	19.53	11.30 ± 0.75
Total		114.93	85.52			101.37	44.92			62.64	26.15			789.46	693.17	
% Reduction		85.44	87.66			87.16	93.52			92.07	96.23					
Net growth				2.26				2.08				2.88				5.18

Table (5): Survivorship (L_x), fecundity (M_x), reproductive rate (L_xM_x) and mean shell diameter of adult *Biomphalaria alexandrina* snails exposed to sublethal concentrations of *Hedera canariensis* plant.

Observation period (weeks)	*Biomphalaria alexandrina* snails exposed to:															
	36 ppm				60 ppm				80 ppm				Control			
	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)	L_x	M_x	L_xM_x	Mean shell diameter ± S.D. (mm)
0	1.00	-	-	6.12 ± 0.76	1.00	-	-	6.12 ± 0.76	1.00	-	-	6.12 ± 0.76	1.00	-	-	6.12 ± 0.76
1	0.97	19.93	19.33	6.93 ± 0.59	0.97	24.63	23.89	6.80 ± 0.57	0.77	20.30	15.63	6.39 ± 0.65	1.00	20.15	20.15	6.96 ± 0.81
2	0.97	29.78	28.89	6.92 ± 0.67	0.93	22.90	21.30	7.07 ± 0.68	0.67	13.29	8.90	7.09 ± 0.56	0.97	53.44	51.84	7.47 ± 0.87
3	0.90	27.63	24.87	7.47 ± 0.62	0.80	21.63	17.30	7.54 ± 0.68	0.47	17.07	8.02	7.63 ± 0.64	0.97	54.28	52.65	7.70 ± 0.93
4	0.87	35.77	31.12	7.65 ± 0.69	0.73	16.71	12.20	7.58 ± 1.24	0.33	8.00	2.64	7.71 ± 0.62	0.93	48.61	45.21	7.89 ± 0.91
5	0.67	23.80	15.95	8.13 ± 0.79	0.57	27.23	15.52	7.67 ± 0.66	0.17	7.60	1.29	8.30 ± 0.27	0.90	80.43	72.39	8.24 ± 0.79
6	0.43	13.83	5.95	8.50 ± 0.70	0.37	17.24	6.38	8.00 ± 0.92	0.10	9.67	0.97	8.50 ± 0.75	0.87	90.27	78.53	8.51 ± 0.89
7	0.20	13.80	2.76	8.55 ± 0.80	0.20	22.27	4.45	8.21 ± 0.97	0.03	0.00	0.00	8.60 ± 0.00	0.87	123.38	107.34	8.41 ± 1.18
8	0.10	9.33	0.93	8.75 ± 0.35	0.10	3.83	0.38	8.68 ± 1.03	0.00	0.00	0.00	-	0.87	98.20	85.43	8.63 ± 0.95
9	0.07	36.00	2.52	9.10 ± 0.47	0.07	6.00	0.42	9.67 ± 0.76					0.83	110.50	91.72	9.60 ± 0.89
10	0.07	7.00	0.49	9.17 ± 0.76	0.03	5.00	0.15	10.0 ± 0.00					0.83	40.50	33.62	10.60 ± 0.28
11	0.07	11.00	0.77	9.30 ± 0.28	0.00	0.00	0.00	-					0.80	33.00	26.40	10.85 ± 0.35
12	0.03	19.00	0.57	9.50 ± 0.00									0.76	11.00	8.36	11.00 ± 0.82
13	0.03	22.00	0.66	9.70 ± 0.00									0.76	25.70	19.53	11.30 ± 0.75
14	0.00	0.00	0.00	-									0.76	34.80	26.45	11.50 ± 0.83
Total		268.87	134.81			167.44	101.99			75.93	37.45			824.26	719.62	
% Reduction		67.38	81.27			79.69	86.88			90.79	94.80					
Net growth				3.58				3.88				2.48				5.38

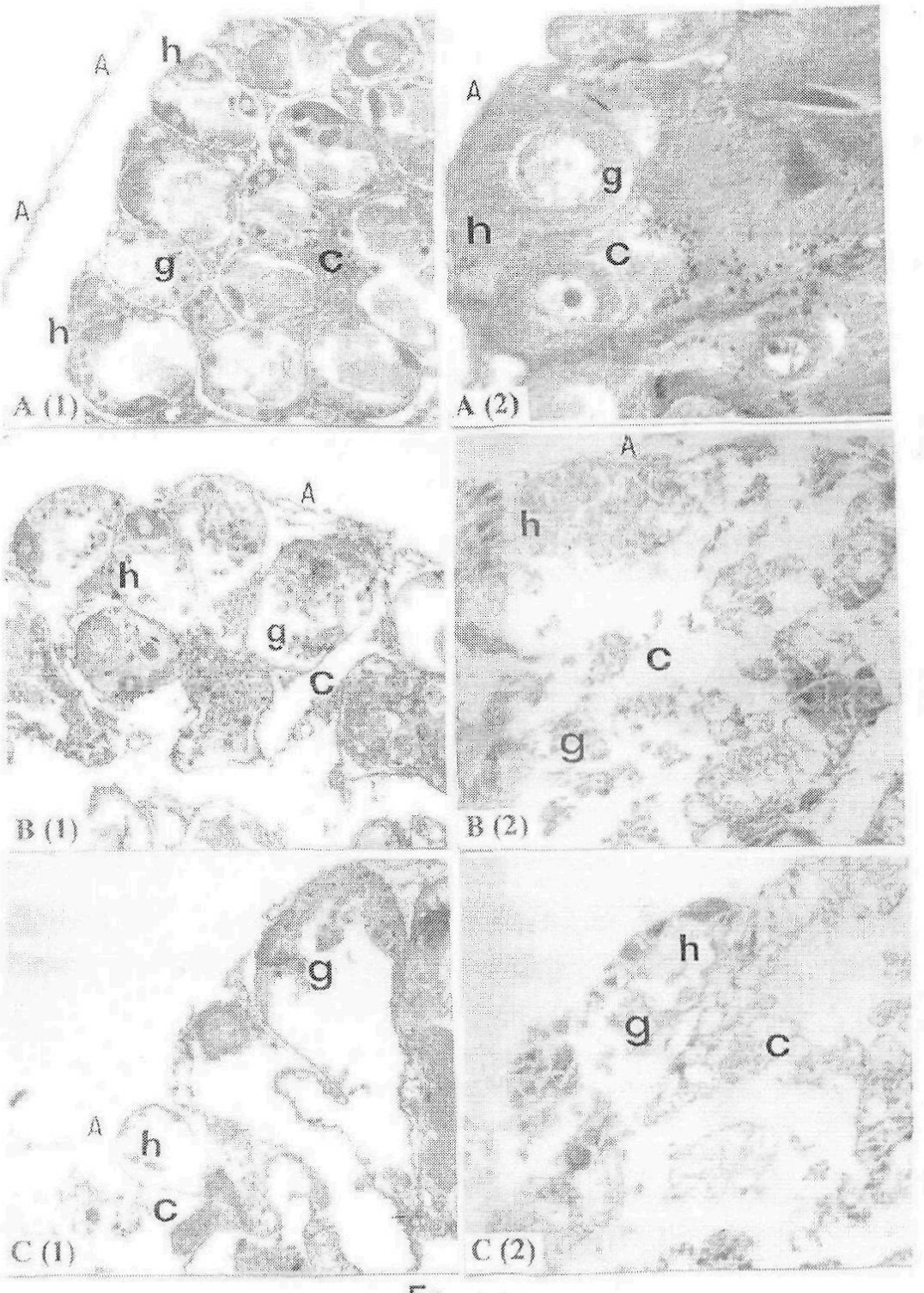

Fig (1)