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Deviation of negatively charged protein fractions in the trochophore and veliger larvae by the larvicidal action of baygon in freshwater pulmonate *Gyraulus convexiusculus* (Planorbidae)

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Abstract: *In the present investigation egg capsules of Gyraulus convexiusculus were treated with different concentrations of baygon. A dose and duration dependent deviations in the number of negatively charged protein fractions in the trochophore and veliger larval stages were observed. It resulted into anomalies in the morphogenesis and organogenesis of corresponding larval stages. Most of the protein bands showed the decrease in the protein positive intensities in comparison to control. This suggested that baygon causes larval toxicity in Gyraulus convexiusculus.*

Key words: *Baygon, Larvicidal action, Gyraulus convexiusculus.*

Introduction

The work on the larvicidal action of pesticides in freshwater snails is yet scanty and has been done by Bhide (1987) on *Pila globosa* after thiourea and DDT treatment, Bhide (1989) in *Lymnaea stagnalis* after thiourea and BHC application, Bhide (1991) in *Lymnaea stagnalis* after some organophosphorus pesticide treatment, Bhargava (1992) in *Lymnaea stagnalis* after thiourea and DDT application, Panigrahi (1997) on two fresh water snails of medical importance after rogor treatment, Bhide (1998) in *Lymnaea stagnalis* after nuvan, methyl parathion and thimet treatment, Panigrahi (1998) in *Lymnaea acuminata* and *Bellamya bengalensis* after molluscicides nuvan treatment, Gupta and Bhide (2001) in *Lymnaea stagnalis* after nuvan treatment and Bhide *et al.* (2004b) in *Gyraulus convexiusculus* after treatment of some pesticides.

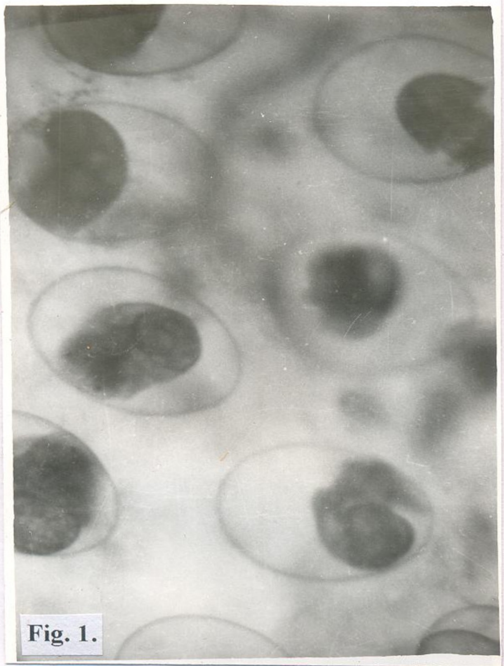
Though the literature concerning the arrest of development at any stage and malformation induced by intoxication of pesticides in gastropods is very scanty and has been reported only by Gupta and Bhide (2001), Bhide *et al.* (2004a) in some freshwater gastropods by residual analysis. This investigation is intended to know more about the toxicity of baygon in pestiferous freshwater snail *Gyraulus convexiusculus* and correlation of the data with the deviation in protein profiles by "Paper Electrophoresis" which is the integral part of the present investigation.

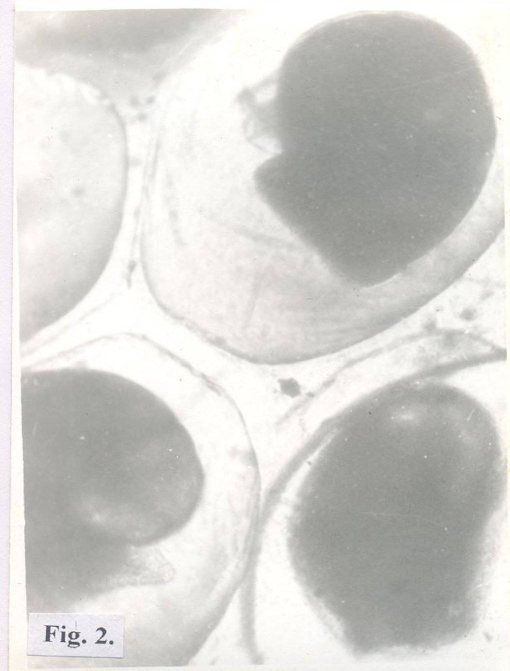
Materials and Methods

Procurement of specimens of snail *G. convexiusculus* and rearing them for obtaining the egg capsules for morphological and biochemical studies on different larval stages: Sexually mature adult specimens of *G. convexiusculus* of approximately same size and weight were collected from the botanical garden ponds of Sagar University and were acclimatized for 10 days under normal laboratory conditions in 5liter glass troughs. They were fed regularly with aquatic vegetation e.g. *Hydrilla* to avoid the stress of starvation and the egg masses laid by these snails were introduced to different concentrations of baygon (2-isopropoxyphenyl methyl carbamate, 0.5%, Bayer (India) Ltd, Kolshet Road, Thane, Maharashtra) separately in glass petri dishes (50 ml. capacity) in triplicate and calculated the percentage mortality and lethal concentration values for baygon (0.5% LC_{100}, 0.33% LC_{50}, 0.1% LC_0 and 0.05 % sub lethal concentration exhibited in Table 1) by probit analysis (Finney, 1971). Different lethal concentrations of baygon were used for the experimental purposes while untreated egg capsules (in triplicate) exhibiting different larval developmental stages were used as control and were developed in pond water. Egg masses containing about 50 egg capsules were used for each larval developmental stage of control and experimental groups. For morphological studies the egg masses of control and experimental groups were developed and preserved in commercial formalin (Fig. 1-8) and for the biochemical studies e.g. "detection of negatively charged protein fractions", the egg masses of control and experimental groups exhibiting different larval stages were processed as follows:-

Preparation of protein samples of different larval developmental stages of control and treated groups: The egg masses exhibiting different larval stages e.g. trochophore and veliger larval stage were processed for the extraction of protein samples by the method adapted after Jairaman (1985). The samples were applied in the form of streak on the 1cm. width x 39cm. long whatman no.1 chromatography paper strips presoaked in borate buffer (8.6 pH). Six samples were applied at a time.

Electrophoresis of extracted samples: Samples were applied at negative pole for the separation of negatively charged protein fractions by VMC (vertical migration chamber) Systronics model No. 604 V using digital power supply Systronics model EPA 610 by the method supplied in the manual of Systronics Co. Ltd. Ahmedabad.





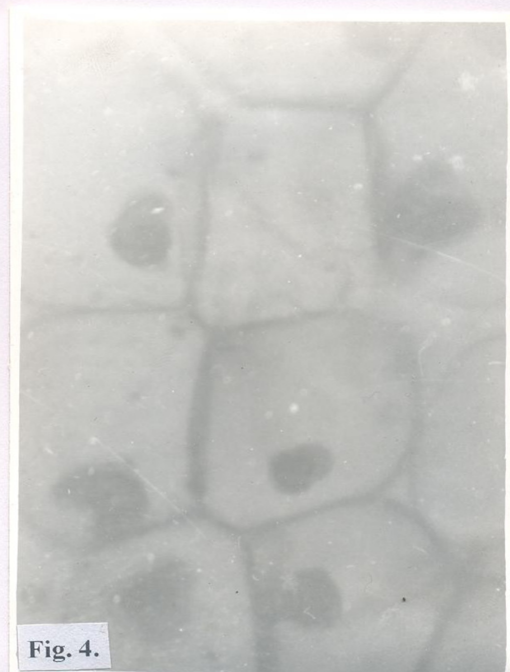
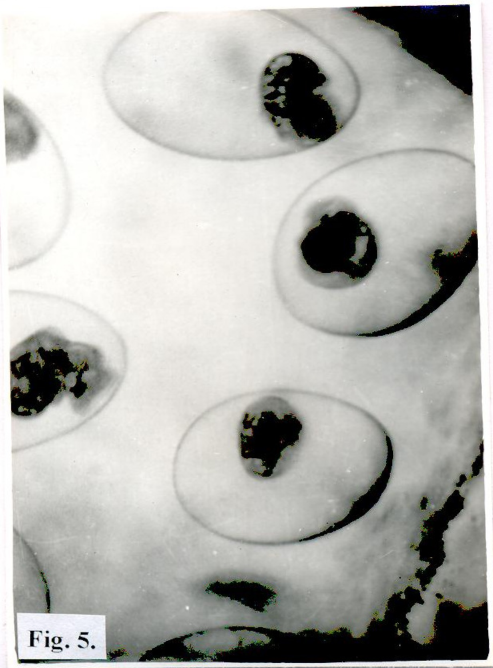


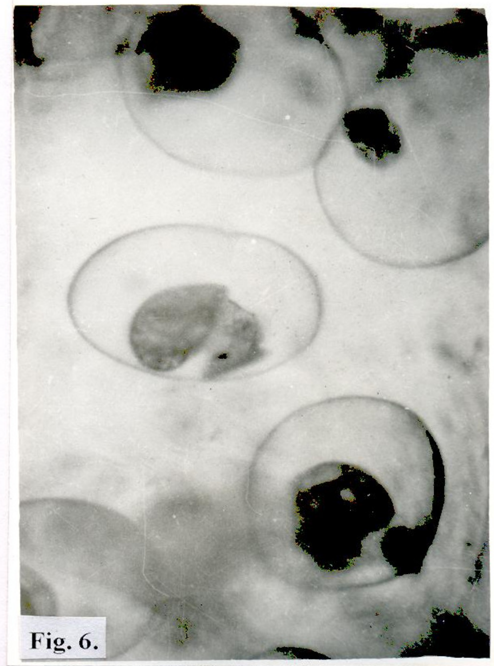
Fig. 1: Photograph of egg capsules of *G. convexiusculus* of control group showing trochophore larvae developed into veliger larvae (X 100).

Fig. 2: Photograph of egg capsules of *G. convexiusculus* of control group showing veliger larvae developed into young snails after completing the phenomenon of torsion (X 100).

Fig. 3: Photograph of two egg capsules of *G. convexiusculus* of control group showing well developed young snails (X 100).

Fig. 4: Photograph of egg capsules of *G. convexiusculus* treated with sub lethal concentration of baygon showing development arrest at trochophore larval stage (X 100).





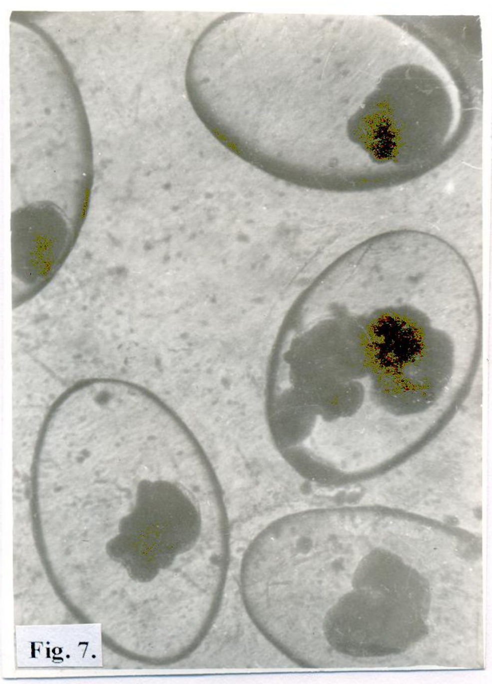


Fig 5: Photograph of egg capsules of *G. convexiusculus* treated with concentration LC_0 of baygon showing the development arrest at trochophore larval stage (X 100).

Fig 6: Photograph of egg capsules of *G. convexiusculus* treated with concentration LCo of baygon showing development arrest at veliger larval stage (X100).

Fig 7: Photograph of egg capsules of *G. convexiusculus* treated with concentration LC_{50} of baygon showing teratogenesis in morphogenesis of trochophore larval stage (X100).

Fig 8: Photograph of egg capsules of *G. convexiusculus* treated with concentration LC_{50} of baygon showing teratogenesis in morphogenesis of veliger larval stage (x100).

Table 1: Toxicity of baygon in *Gyraulus convexiusculus*

S. No.	Name of pesticide	Concentration of pesticide	Duration	Mortality	Lethal conc. value
1.		0.5 %	96 hr	100%	LC_{100}
2.	Baygon	0.33 %	96 hr.	50%	LC_{50}
3.		0.1 %	96 hr	Nil	LC_0
4.		0.05*%	13 days	Nil	Sub lethal conc.

* 0.05 % concentration of baygon was considered as sub lethal conc. throughout the experiment.

Table – 2: Effect of baygon on the developmental period of different larval stages, mortality and survival of young snails in *Gyraulus convexiusculus:*

S. No.	Concentration of baygon	No. of egg capsules	Percent mortality at the time of hatching of young snails	Trochophore larval period (in hr)	Veliger larval period (in hr)	Young snail period (in hr)	Percent survival of young snails after hatching
1.	No trace of any pesticide (Control group)	50	Nil %	52 ± 3	56 ± 4	47 ± 3	100 %
2.	0.05% (Sub lethal concentration)	50	Nil %	56 ± 4	58 ± 2	50 ± 1	100%
3.	0.1% (LC_0)	50	Nil %	58 ± 3.5	60 ± 2.5	53 ± 2	100%
4.	0.33 % (LC_{50})	50	50 %	61 ± 2.5	62 ± 2	56 ± 2.5	50 %

Staining of paper strips for the detection of protein fractions: Mercuric bromophenol blue staining method was used for the detection of protein bands on the already prepared electrophoresed strips after clearing the background with 1% acetic acid solution and fixing the bands in methanol.

The data on the number and intensities of protein fractions was detected out by densitometer (Systronics Co. Ltd. Ahmedabad) and summarized in the form of electrophoretograms 1- 2.

Results and Discussion

Morphological observations: The data on the morphological observations on different larval developmental periods, percentage mortality and hatchability of young snails was recorded and summarized in Table 2. Most of the egg capsules showed development arrest. Both the larval stages were more susceptible and also showed teratogenesis due to pesticide toxicity (Fig. 4-8). The young snail percent hatchability was very low with high percentage of mortality at LC_{50} dose in comparison to lethal and sub lethal concentration of baygon (Table 2). Control groups exhibited the normal larval development with no mortality and 100% hatchability in young snails (Fig.1-3).

Biochemical observations: In the present investigation, the egg capsules exhibiting different larval stages were introduced into different experimental concentrations of baygon e.g. 0.05% (sub lethal), 0.1% (LC_0) and 0.33% (LC_{50}). They exhibited various anomalies dependent upon dose and duration of treatment, not only in their organogenesis (as evident by Fig. 4 to 8) but also showed deviations in the number of negatively charged protein fractions as well as decline in protein positive intensities in the corresponding larval stages of experimental groups of *Gyraulus convexiusculus*. The data was recorded in electrophoretograms 1 and 2 and analyzed as follows:

Detection of negatively charged protein fractions in control and experimental trochophore larvae of *G. convexiusculus* treated with different concentration of baygon:

Note: PF = Protein fraction.

In control groups:

1. Total 8 PF were detected out in trochophore larval stage.
2. PF 4 was of very high intensity.
3. PF 7 was of high intensity.
4. PF 1, 3, 5, 6 and 7 were of variable density.
5. PF 2 was of very low density.

In experimental groups:

(a) In sub lethal concentration treated group:

1. Total 9 PF were detected out in trochophore larval stage.
2. PF 9 was of very high intensity.
3. PF 1 and 6 were of high intensity.
4. PF 2, 3, 4, 5, and 8 were of variable density.
5. PF 7 was of very low density.

(b) In LC_0 concentration treated group:

1. Total 8 PF were detected out in trochophore larval stage.
2. PF 1 was of very high intensity.
3. PF 3 and 4 were of equal density.
4. PF 5, 6, 7 and 8 were of variable density.
5. PF 2 was of very low density.

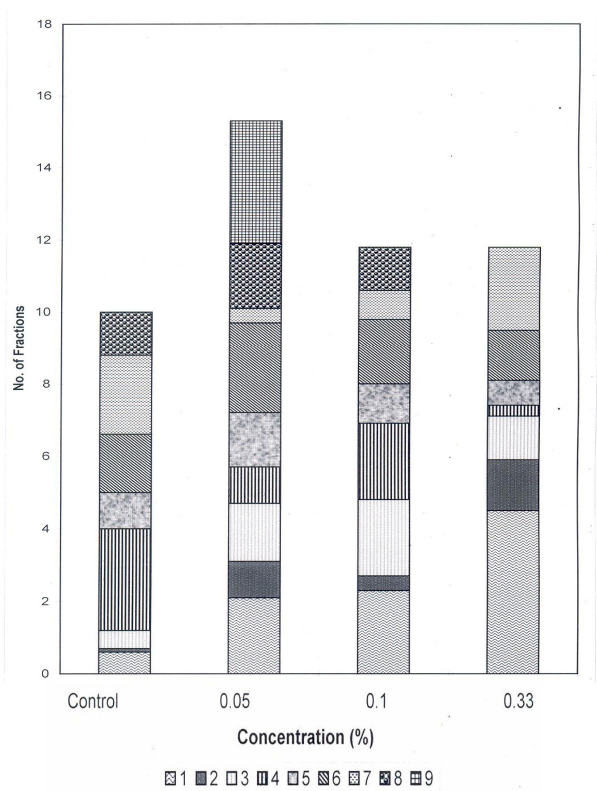
(c) In LC_{50} concentration treated group:

1. Total 7 PF were detected out in trochophore larval stage.
2. PF 1 was of very high intensity.
3. PF 2, 3, 5 and 6 were of equal density.
4. PF 7 was of high density.
5. PF 4 was of very low density.

Analysis:

1. The number of PF gradually decreases with respect to increase in the treated concentration.
2. PF 1 was of very high intensity in treated groups. The protein positive density gradually increases from sub lethal to LC_{50} concentration.
3. Gradual decrease in the protein positive intensity was observed from sub lethal to LC_{50} dose treatment.
4. Very low intensity band was variable (7th band in 0.05%, 2nd in 0.1% and 4th band in 0.33% concentration) showed the depletion of protein
5. Deviation as well as depletion in protein fractions resulted into development arrest, malformation or deformities in organogenesis in trochophore larvae suggesting the larvicidal action of baygon.

Detection of negatively charged protein fractions in control and experimental veliger larvae of *G. convexiusculus* treated with different concentration of baygon:



Electrophoretogram 1: Showing detection of negatively changed protein fractions in control and baygon treated egg masses undergoing trochophore larval stage in *Gyraulus convexiusculus*.

In control group:

1. Total 7 PF were detected out in veliger larval stage.
2. PF 5 was of very high intensity.
3. PF 1 was of high intensity.
4. PF 3, 6 and 7 were of variable density.
5. PF 6 was of very low density.
6. PF 2 and 4 were of equal density.

In experimental groups:

(a) In sub lethal concentration treated group:

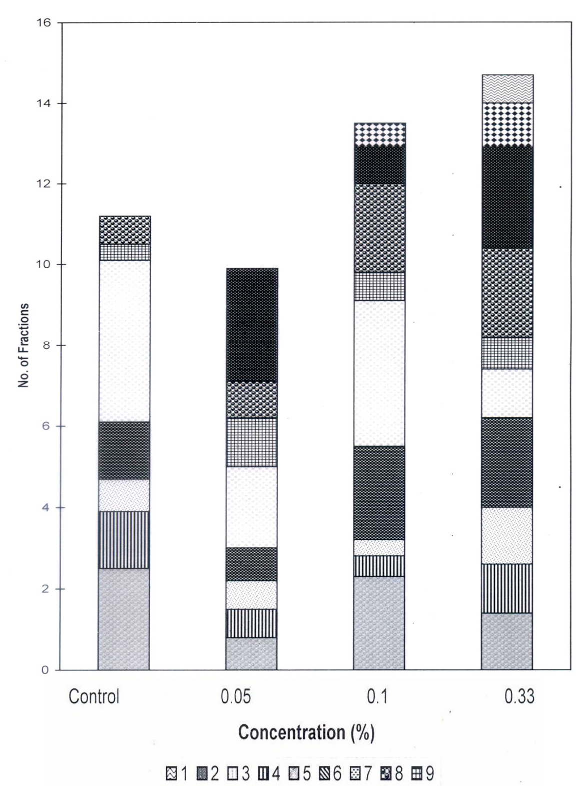
1. Total 8 PF were detected out in veliger larval stage.
2. PF 8 was of very high intensity.
3. PF 5 was of high intensity.
4. PF 1, 2, 3, and 4 were of equal density.
5. PF 6 and 7 were of moderate density.

(b) In LC_0 concentration treated group:

1. Total 9 PF were detected out in veliger larval stage.
2. PF 5 was of very high intensity.
3. PF 1, 4 and 7 were of high density.
4. PF 6, 8 and 9 were of equal density.
5. PF 2 and 3 were of equal in density but very low in protein positive intensity.

(c) In LC_{50} concentration treated group:

1. Total 10 PF were detected out in veliger larval stage.
2. PF 4, 7 and 8 was of very high intensity.



Electrophoretogram 2: Showing detection of negatively changed protein fractions in control and baygon treated egg masses undergoing veliger larval stage in *Gyraulus convexiusculus*.

3. PF 1, 2, 3, 5 and 9 were of equal and high density bands.
4. PF 6 and 10 was of high density.
5. PF 4 was of very low density.

Analysis:

1. The number of PF gradually increases with respect to increase in the treated concentration.
2. PF 1 was of very high intensity in treated groups. The protein positive density gradually increases from sub lethal to LC_{50} concentration.
3. Gradual decrease in the protein positive intensity was noticed from sub lethal to LC_{50} dose treatment.
4. Dose and duration of treatment dependent deviation and gradual increase in protein fractions in comparison to control resulted into malformation or deformities in organogenesis and teratogenesis in veliger larvae in *G. convexiusculus* suggesting the larvicidal action of baygon.

Biochemical study is an integral and important part of this investigation and there were practically no such studies on the detection of protein fractions in different larval developmental stages of *Gyraulus convexiusculus* by paper electrophoresis and the studies on the residual analysis of pesticides by TLC method in different developmental stages of *G. convexiusculus* and *Lymnaea stagnalis* by Gupta (2003) and Bhide *et al.* (2004a) suggested that the anomalies in morphogenesis and organogenesis of different larval stages were due to the accumulation of pesticides in the corresponding developmental stages.

The duration of larval stages was increased by 8 ± 1 hr. in comparison to the control. Organogenesis was also delayed. The number of protein fractions was decreased corresponding to the increase in concentration of baygon in trochophore larvae suggesting the increased rate of toxicity resulted into slow rate of development of larvae with some teratological symptoms in veliger larvae as evident by Fig. 4 to 8.

Toxicity data on mortality and development arrest with decline fecundity have been reported by Bhide (1987) in *Pila globosa* after thiourea and DDT treatment, Bhide (1989) in *Lymnaea stagnalis* after thiourea and BHC application, Bhide (1991) in *Lymnaea stagnalis* after some organophosphorus pesticide treatment, Bhargava (1992) in *Lymnaea stagnalis* after thiourea and DDT application, Panigrahi (1997) in two freshwater snails of medical importance after rogor treatment, Bhide (1998) in *Lymnaea stagnalis* after nuvan, methyl parathion and thimet treatment, Panigrahi (1998) in *Lymnaea acuminata* and *Bellamya bengalensis* after molluscicide nuvan treatment, Gupta and Bhide (2001) and Bhide *et al*. (2004b) in *Gyraulus convexiusculus* after treatment with some pesticides and reported the deformities in trochophore larvae which were correlated with the depletion in protein fractions while teratogenesis was the result of increase in the protein fractions in veliger larval stage as also observed in *G. convexiusculus* after treatment with different experimental concentration of baygon in the present investigation.

Protein is the main component which play major role in the progressive development as investigated by Holmes (1900), Ranjah (1942), Yonge *et al.* (1964), Hyman (1967), Barth and Broshears (1982) and Goel (1999) in some freshwater molluscs while in the present investigation pesticide intoxication exhibited adverse effect on the protein synthesis in different larval stages of *G. convexiusculus* which was proved by the significant reduction (in trochophore larvae) or increase (in veliger larvae) in the number of protein fractions in the treated groups in comparison to control larvae as observed by Gupta and Bhide (2001) in *Lymnaea stagnalis* after nuvan administration.

In treated groups deviation was not only found in the number of protein fractions but also in the protein positive intensities from trochophore to veliger larval stages in comparison to control larvae. In the present investigation in *G. convexiusculus* after treatment with baygon as also observed by Bhide *et al.* (2004b) in the different developmental stages of the same snail after treatment of some organophosphorus and carbamate pesticides.

In the present investigation it could be concluded that the adverse effect of pesticide baygon resulted into depletion in the number of protein fractions as well as decrease in protein positive intensities could be correlated with the abnormal morphogenesis and organogenesis during the larval period due to partial or total arrest of protein synthesis in trochophore larvae resulted into the development of reduced number of young snails due to high larval mortality rate or partial or total arrest of larval development due to larvicidal action of baygon or the young snails were unable to break down the wall of the corresponding egg capsules and remained trapped inside it and due to lack of nutrition they became starved and died within the egg capsules resulted into 100% mortality and zero percent hatchability and in this way pestiferous snail control programme would be done because *G. convexiusculus* is an intermediate host of number of trematode parasites which cause disorder and pathological changes in the liver of vertebrates.

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