CHAPTER-2

Materials and Methods
Materials and Methods

This chapter deals with the materials and methodology used in the present investigation. Experiments were conducted on the egg masses of *Lymnaea stagnalis* and *Gyraulus convexiusculus* and the details of the same are as follows:

1. **Selection of Pestiferous Snails**

For this present investigation the pestiferous snails were selected as follows –

(i) Common pond snail, *Lymnaea stagnalis* belonging to family-*Lymnaeidae*.

(ii) *Gyraulus convexiusculus* belonging to family-*Planorbidae*.

2. **Procurement and Rearing of Snail**

Sexually mature specimens of *Lymneae stagnalis* and *Gyraulus convexiusculus* of approximately same age, size and weight were procured from the Sagar lake by fishing nets or picking by hands and were kept into aerated freshwater glass containers (2 to 5 lit. capacity) provisioned with adequate aquatic vegetations e.g. Hydrilla to avoid the stress of starvation (as these species are herbivorous) and were acclimatized for 7 days on normal laboratory conditions according to method adopted after Subbarao (1989).

3. **Test Animals**

The young ones hatched from the freshly laid egg masses of *Lymnaea stagnalis* and *Gyraulus convexiusculus* were used for the
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experimental purposes. The egg masses laid by these snails were introduced to different concentrations of plants seed extracts used in the present investigation.

4. **Plants seed selected for extraction are as follows:**

   (i) *Cassia fistula* (Amaltas)

   (ii) *Delonix regia* (Gulmohar)

5. **Extraction of Plant seeds by Soxhlet Method:**

   Seeds of *Cassia fistula* and *Delonix regia* were procured locally. These seeds (*Cassia fistula* and *Delonix regia*) were washed, shadow dried, mechanically grind into coarse pieces, weighed 100 g. and then extracted with methanol (B.P. 60-80°C, procured from B.D.H.) by Soxhlet method adopted after Sharma, (1988) as shown in the photograph no. 1. After completion of 8 cycles in the Soxhlet, the extracts were filtered.

   After evaporation of solvent the extracted antifertility agents were weighed 3.9 g from *Cassia fistula* and 1.0 g from *Delonix regia*.

   The colour of the extracts are as followed:

   (i) Reddish colour semi-liquid was extracted from *Cassia fistula* seeds.

   (ii) Mustard colour semi-liquid was extracted from *Delonix regia* seeds.

   1% stock solution was prepared in distilled water and kept in dark coloured tightly closed glass bottles and stored in refrigerator at 4°C.
Soxhlet Apparatus

Photograph 1
The stock solution was further diluted and used at room temperature for the detection of LC values and after calculated the sublethal concentrations the experiments were done in triplicate.

General morphology of *Cassia fistula* and *Delonix regia* is described in chapter - 3.

6. **Experiments with different dosages of seed extracts**:

Fresh egg masses with subsequent developmental stages of *Lymnaea stagnalis* and *Gyraulus convexiusculus* of F₀ generation were introduced via media to different concentrations of plant seed extracts and the data was collected in triplicate and calculated the values of LC₁₀₀, LC₅₀ (method adapted after Finney, 1971) LC₀ and sublethal concentrations and data was summarized in Table No.1 to 5 and Graph No. 1, 2.

Fresh adult snails of *Lymnaea stagnalis* and *Gyraulus convexiusculus* of same age and same size were introduced via media to different concentrations of plant seed extracts in triplicate and calculated the values of LC₁₀₀, LC₅₀ (method adapted after Finney, 1971) LC₀ and sublethal concentrations and data was summarized in Table No. 6 to 9 and Graph No.3, 4.

7. **Studies on the behaviour, mortality and reproductive performance in Lymnaea stagnalis and Gyraulus convexiusculus under the influence of Cassia fistula and Delonix regia plant seed extracts**:

The data was recorded and summarized in Table No. 10 to 13.
8. Experiments were done on the different developmental stages of *Lymnaea stagnalis* and *Gyraulus convexiusculus* selected in the present investigation are as follows:

8.1 Cleavage
8.2 Blastula
8.3 Gastrula
8.4 Post gastrular changes e.g. Morphogenesis and Organogenesis.
8.5 Formation of trophophore larvae.
8.6 Number of trophophore transformed into veliger larvae.
8.7 Torsion in veliger larvae.
8.8 Metamorphosis of veliger into young snails.
8.9 Hatching of young snails from their respective egg capsules.

The data was recorded and summarized in Table No.14 to 17.

9. Residual analysis by Thin Layer Chromatography:

9.1 Preparation of TLC plates:

Rectangular glass plates of size 22.5 x 10 cm. were first thoroughly washed with chromic acid and then with detergent solution. The plates were finally rinsed with distilled water and dried. Thirty gms Silica gel “G” and 60 ml of distilled water were stirred and the plates were coated to a thickness of 250 μ with this adsorbent. The plates were then allowed to dry at room temperature. The direction of coating was noted and the coated plates were activated at 90°C for 24 hrs. in a chromatography oven.
9.2 Preparation of Samples:

The egg masses with subsequent different developmental stages of control and experimental groups were thoroughly washed and digested for 24 hrs. with chloroform : Ethanol : Acetone : Glacial acetic acid (60 : 40 : 15 : 06) mixture for residual analysis of Cassia fistula seed extract and Butanol : Acetic acid : Water (100 : 10 : 30) mixture for residual analysis of seeds extract of Delonix regia respectively.

The samples (egg masses containing 50 egg capsules) were homogenized in 5 ml of respective digestive solutions. All the content was then transferred to 12 ml centrifuge tubes.

The tubes were then shaken for 2 minutes and centrifuged at 3000 rpm for 15 min. The tubes along with materials were chilled in their respective digestive solutions and then decanted. After one hour this extract was used for residual analysis. In this method separation of residues of different seed extracts was done on Silica gel “G” coated plates.

9.3 Apparatus and Requirements:

The apparatus consist of a rectangular glass jar (30 x 15 x 12 cm) with glass lid, glass plates (size 22.5 x 10 cm), glass capillaries, sprayer or automizer, spraying regents, chromatograph oven, digestive solvents of analytical grades etc. Authentic pure samples of known quantity of seed extracts of Cassia fistula and Delonix regia were used for plotting the calibration curve.
9.4 Application of Samples on TLC Plate:

Samples extracted by centrifugation of control and experimental developmental stages of *Lymnaea stagnalis* and *Gyraulus convexiusculus* were applied on separate TLC plates by capillaries. The area of application was kept as small as possible because the smaller the area of application the sharper will be the resolution. Finally the samples were applied approximately 1.5 cm above the solvent system used in the residual analysis.

9.5 Solvent System:

The following solvent systems were used for the development of chromatograms:

(a) For residual analysis of *Cassia fistula* seed extract

Chloroform : Ethanol : Acetone : Glacial acetic acid

: 60 : 40 : 15 : 06

(b) For residual analysis of *Delonix regia* seed extract

Butanol : Acetic acid : Water

100 : 10 : 30

9.6 Development of TLC Plates:

After application of the samples the TLC plates were introduced into the appropriate solvents in developing tank and the residues were resolved into discrete spots when the solvent front approaches the top (usually 10 cms. above the distance from the point of application of the sample), which normally took 38-60 minutes. The TLC plates were dried and the position of the spots visualized by spraying with spraying reagents. Spraying was done with the help of glass automizer.
9.7 Spraying reagents:

(i) For residual analysis of *Cassia fistula* seed extracts:

(a) TLC plates developed in the appropriate solvent were kept on the vapours of concentrated HCL in a desiccator for sometimes.

(ii) For residual analysis of *Delonix regia* seed extracts:

(b) Using Van Urks reagent to develop spot on TLC plates.

9.8 Colour of the spots:

(i) In residual analysis of *Cassia fistula* seed extract:

a. Blue colour spot was developed.

(ii) In residual analysis of *Delonix regia* seed extract:

b. Brown colour spot was developed.

9.9 The Rf values of the spots were calculated by the formula as follows:

\[ Rf = \frac{\text{Distance of spot center from the start point}}{\text{Distance of solvent front the start point}} \]

Rf values vary with layer thickness hence in this study layer thickness was always kept constant i.e. 250 μ.

(i) Rf values for residual analysis of *Cassia fistula* seed extract:

The Rf value calculated in the present investigation was 0.16.

(ii) Rf values for residual analysis of *Delonix regia*:

The Rf value calculated in the present investigation was 0.36.
The data regarding the calibration of spot area in mm. sq. with respect to increase in concentration of seed extracts of Cassia fistula and Delonix regia Residual analysis of seed extracts in different developmental stages of control and experimental groups were recorded and summarized in Table No. 18 to 25 and in Graph No. 1 to 7.

(Note : Where 100 divisions = 0.1mm$^2$)

10 Method for Electrophoresis

10.1 Detection of Negatively Charged Protein and Lipoprotein Fractions in the Different Developmental Stages of Control and Experimental Groups under the Influence of Different Types of seed extracts in Lymnaea stagnalis and Gyraulus convexiusculus by Paper Electrophoresis :

Principle of Electrophoresis :

It is the technique for the separation of the charged molecules. If a mixture of the substance (applied as a sample on an adsorbent media) placed in buffer solution is introduced with electric current, the charged particles (or substance or molecules) moves towards the opposite pole i.e.

- Cation (+ve ion) moves towards cathode (-ve pole)
- Anion (-ve ion) moves towards anode (+ve pole)

The rate of migration will depend on the size, shape, molecular weight, total or net charge, pH of the buffer solution, current, voltage and other factors.
10.2 Requirement:

Egg masses containing different development stages of control and experimental groups of *Lymnaea stagnalis* and *Gyraulus convexiusculus*, Borate buffer (pH 8.6), centrifuge, digital power pack with vertical migration electrophoresis chamber, filter paper (Whatman No. 1), scissor, forceps, scale, pencil HB, centrifuge test tubes Mercuric Bromophenol Blue stain, 95% ethanol, Sudan black 'B' stain, 1% acetic acid, 55% ethanol, 40% ethanol, crystals of Bromophenol Blue, capillaries or micropipettes, etc.

10.3 Method:

(As manual supplied by Systronics Co. Ltd. Ahmedabad)

(i) Preparation of Borate Buffer (pH 8.6)

(a) 8.80 g of sodium tetraborate and

(b) 4.65 g of Boric acid, both were dissolved in 1 litre of distilled water.

(ii) Cutting the strip of paper (filter paper Whatman No.1) of appropriate size (39 x 1 cm). Six strips were used at a time.

(iii) Before sampling, paper strips were dipped in buffer and blotted lightly between the folds of filter papers and dried at room temperature.

(iv) Preparation of "Mercuric Bromophenol Blue" (Hg. B.B.) stain for staining the bands (Protein fractions) on the strips: 1 gm
Bromophenol blue in 95% ethanol saturated with Mercuric Chloride (Mercuric Chloride denatures the protein directly) was prepared.

(v) Preparation of ‘Sudan black ‘B’ stain : Saturated solution of Sudan black ‘B’ in 55% ethanol was prepared.

(vi) Preparation of 1% Acetic acid for removal of extra Hg B.B. stain from the strips.

(vii) Preparation of 40% ethanol for removal of extra Sudan black ‘B’ stain from the strips.

(viii) Preparation of samples for the detection of protein and lipoprotein fractions in control and experimental egg masses undergoing different developmental stages.

About 50 eggs capsules of control and experimental developmental stages of *Lymnaea stagnalis* and *Gyraulus convexiusculus* were cut into fine pieces and grind with Bloor's mixture into a mortar and pastle, then centrifuged at 3000 rpm for 20 minutes and the supernatant contained the lipoprotein fractions were separated (thrice with Bloor's mixture) and the residue was treated with 10% Trichloroacetic acid (TCA) and again centrifuged at 3000 rpm for 20 minutes. The supernatant was discarded and the residue was treated with 0.1 N NaOH (1 ml) (Boiled at 90°C, protein denatured and dissolved in this solvent) which contains protein fractions.
11. Applications of samples on the Whatman No.1 Filter paper strips:

The samples extracted from the egg masses of different developmental stages of control and experimental groups of *Lymnaea stagnalis* and *Gyraulus convexiusculus* were applied separately (in triplicate) on Whatman No.1 filter paper strips on cathode to separate the negatively charged protein and lipoprotein fractions of control and treated egg masses of *Lymnaea stagnalis* and *Gyraulus convexiusculus* by capillaries or micropipettes.

12. Preparation of Vertical Migration Chamber (V.M.C.) for paper Electrophoresis:

(a) Both the compartments (containing cathode and anode marked with black and red sockets for black and red lead connections with power pack) were filled with Borate buffer (pH 8.6) above the level of electrodes.

(b) Fixation of strips on triangular paper holding frame:

The paper strips presoaked in buffer and applied with samples were dropped over the upper cord. The lower cords hold the ends of the strips of papers, so they fall conveniently into the buffer. The frame was then placed into electrophoresis apparatus in such a way that the paper strips were dipped in the buffer solutions but samples have to be dipped into the buffer solution.

(c) Application of buffer solution on the strips by dropper or pipette so the continuity of electric current is maintained. Some crystal
of Bromophenol blue stain were added in the cathode side buffer compartment.

(d) Then replaced the transparent lid on the V.M.C. to minimize the evaporation of buffer solution.

(e) Fixed the three pin plug of the mains cord of power supply to mains supply sockets and switched the button to "ON" position.

(f) Constant voltage of about "250 V" was supplied for six paper strips.

(g) Left the V.M.C. undisturbed for about 3 to 3½ hrs.

(h) The migration or separation of corresponding fractions was visualized as the movement of blue colour on the strips towards opposite directions. The dye Bromophenol blue was fairly tightly bound to very low molecular weight fractions during the course of electrophoresis and did not alter the mobility of other fractions. The dye was not interfere with the subsequent staining of the strip. The progress of electrophoresis was visualized by the progress of blue colour on the strips. Excess of Bromophenol blue dye was moved as the purplish band and eventually move off.

(i) Electrophoresis was carried out at the room temperature.

(j) At the end of the run the current was switched off and the paper strips were dried in an oven at 100°C for 10 minutes to denature the protein fractions prior to staining.
Electrophoresis Instrument

Photograph 2
13. Techniques used for staining the paper strips for the Detection of Protein and Lipoprotein fractions:

13.1 Detection of Protein fractions on the strips:

Strips were immersed for 15 minutes in the Mercuric Bromophenol blue stain. The strips were then rinsed for several times in 1% Acetic acid solution until a relatively clear background was obtained.

13.2 Detection of Lipoprotein fractions on the Strips:

Strips were immersed for one hour in saturated solution of Sudan Black ‘B’ and then rinsed several times in 40% ethanol until a clear background was obtained.

The protein and lipoprotein fractions of different developmental stages of control and experimental egg masses of *Lymnaea stagnalis* and *Gyraulus convexiusculus* were detected out on paper strips and the data was analysed in Table No. 26 to 31 and summarised in Electrophoretograms No. 1 to 12.
Table - 1

Relevant Information about seed extracts opted for experimentation in *Lymnaea stagnalis* and *Gyraulus convexiusculus*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the plant used</th>
<th>Part of the plant used</th>
<th>Common Hindi name of plants</th>
<th>Seeds procured from</th>
<th>Colour of the extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Cassia fistula</em></td>
<td>Seed</td>
<td>Amalta</td>
<td>Locally collected</td>
<td>Reddish colour</td>
</tr>
<tr>
<td>2.</td>
<td><em>Delonix regia</em></td>
<td>Seed</td>
<td>Gulmohar</td>
<td>Locally collected</td>
<td>Mustard colour</td>
</tr>
<tr>
<td>S. No.</td>
<td>Name of the plant seed extract</td>
<td>Concentration of the plant seed extract</td>
<td>Duration (Hrs.)</td>
<td>Mortality</td>
<td>Lethal conc. value</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------------</td>
<td>----------------------------------------</td>
<td>----------------</td>
<td>-----------</td>
<td>-------------------</td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td>1.0%</td>
<td>96</td>
<td>100%</td>
<td>LC&lt;sub&gt;100&lt;/sub&gt;</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>0.5%</td>
<td>96</td>
<td>50%</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>3.</td>
<td><em>Cassia fistula</em></td>
<td>0.28%</td>
<td>96</td>
<td>Nil</td>
<td>LC&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>0.26%</td>
<td>96</td>
<td>Nil</td>
<td>Sublethal concentration</td>
</tr>
</tbody>
</table>

0.26% Concentration of seed extracts of *Cassia fistula* was considered as sublethal concentration throughout the experiments.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the plant seed extract</th>
<th>Concentration of the plant seed extract</th>
<th>Duration (Hrs.)</th>
<th>Mortality</th>
<th>Lethal conc. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td>0.26%</td>
<td>96</td>
<td>100%</td>
<td>LC&lt;sub&gt;100&lt;/sub&gt;</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>0.15%</td>
<td>96</td>
<td>50%</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>3.</td>
<td><em>Delonix regia</em></td>
<td>0.12%</td>
<td>96</td>
<td>Nil</td>
<td>LC&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>0.11%</td>
<td>96</td>
<td>Nil</td>
<td>Sublethal concentration</td>
</tr>
</tbody>
</table>

0.11% Concentration of seed extracts of *Delonix regia* was considered as sublethal concentration throughout the experiments.
Table - 4
Toxicity of Mehtanolic seed extract of *Cassia fistula* in *Gyraulus convexiusculus*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the plant seed extract</th>
<th>Concentration of the plant seed extract</th>
<th>Duration (Hrs.)</th>
<th>Mortality</th>
<th>Lethal conc. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td>1.0%</td>
<td>96</td>
<td>100%</td>
<td>LC(_{100})</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>0.5%</td>
<td>96</td>
<td>50%</td>
<td>LC(_{50})</td>
</tr>
<tr>
<td>3.</td>
<td><em>Cassia fistula</em></td>
<td>0.26%</td>
<td>96</td>
<td>Nil</td>
<td>LC(_{0})</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>0.25%</td>
<td>96</td>
<td>Nil</td>
<td>Sublethal concentration</td>
</tr>
</tbody>
</table>

0.25% Concentration of seed extracts of *Cassia fistula* was considered as sublethal concentration throughout the experiments.

Table - 5
Toxicity of Mehtanolic seed extract of *Delonix regia* in *Gyraulus convexiusculus*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the plant seed extract</th>
<th>Concentration of the plant seed extract</th>
<th>Duration (Hrs.)</th>
<th>Mortality</th>
<th>Lethal conc. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td>0.26%</td>
<td>96</td>
<td>100%</td>
<td>LC(_{100})</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>0.20%</td>
<td>96</td>
<td>50%</td>
<td>LC(_{50})</td>
</tr>
<tr>
<td>3.</td>
<td><em>Delonix regia</em></td>
<td>0.13%</td>
<td>96</td>
<td>Nil</td>
<td>LC(_{0})</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>0.12%</td>
<td>96</td>
<td>Nil</td>
<td>Sublethal concentration</td>
</tr>
</tbody>
</table>

0.12% Concentration of seed extracts of *Delonix regia* was considered as sublethal concentration throughout the experiments.
Table - 6

Data on toxicity of methanolic seed extract of *Cassia fistula* on the adult specimens of *Lymnaea stagnalis*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the plant seed extract</th>
<th>Concentration of the plant seed extract</th>
<th>Duration (Hrs.)</th>
<th>Mortality</th>
<th>Lethal conc. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td>1.5 ml/l</td>
<td>96</td>
<td>100%</td>
<td>LC$_{100}$</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>1.0 ml/l</td>
<td>96</td>
<td>50%</td>
<td>LC$_{30}$</td>
</tr>
<tr>
<td>3.</td>
<td><em>Cassia fistula</em></td>
<td>0.7 ml/l</td>
<td>96</td>
<td>Nil</td>
<td>LC$_{0}$</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>0.5 ml/l</td>
<td>96</td>
<td>Nil</td>
<td>Sublethal concentration</td>
</tr>
</tbody>
</table>

0.5ml/l Concentration of seed extracts of *Cassia fistula* was considered as sublethal concentration throughout the experiments.

Table - 7

Data on toxicity of methanolic seed extract of *Delonix regia* on the adult specimens of *Lymnaea stagnalis*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the plant seed extract</th>
<th>Concentration of the plant seed extract</th>
<th>Duration (Hrs.)</th>
<th>Mortality</th>
<th>Lethal conc. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td>1.0 ml/l</td>
<td>96</td>
<td>100%</td>
<td>LC$_{100}$</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>0.7 ml/l</td>
<td>96</td>
<td>50%</td>
<td>LC$_{30}$</td>
</tr>
<tr>
<td>3.</td>
<td><em>Delonix regia</em></td>
<td>0.5 ml/l</td>
<td>96</td>
<td>Nil</td>
<td>LC$_{0}$</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>0.4 ml/l</td>
<td>96</td>
<td>Nil</td>
<td>Sublethal concentration</td>
</tr>
</tbody>
</table>

0.4 ml/l Concentration of seed extracts of *Delonix regia* was considered as sublethal concentration throughout the experiments.
Table - 8
Data on toxicity of methanolic seed extract of *Cassia fistula* on the adult specimen of *Gyraulus convexiusculus*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the plant seed extract</th>
<th>Concentration of the plant seed extract</th>
<th>Duration (Hrs.)</th>
<th>Mortality</th>
<th>Lethal conc. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td>1.5 ml/l</td>
<td>96</td>
<td>100%</td>
<td>LC₁₀₀</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>1.0 ml/l</td>
<td>96</td>
<td>50%</td>
<td>LC₅₀</td>
</tr>
<tr>
<td>3.</td>
<td><em>Cassia fistula</em></td>
<td>0.7 ml/l</td>
<td>96</td>
<td>Nil</td>
<td>LC₀</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>0.5 ml/l</td>
<td>96</td>
<td>Nil</td>
<td>Sublethal concentration</td>
</tr>
</tbody>
</table>

0.5 ml/l Concentration of seed extracts of *Cassia fistula* was considered as sublethal concentration throughout the experiments.

Table - 9
Data on toxicity of methanolic seed extract of *Delonix regia* on the adult specimen of *Gyraulus convexiusculus*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the plant seed extract</th>
<th>Concentration of the plant seed extract</th>
<th>Duration (Hrs.)</th>
<th>Mortality</th>
<th>Lethal conc. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td>1.0 ml/l</td>
<td>96</td>
<td>100%</td>
<td>LC₁₀₀</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>0.7 ml/l</td>
<td>96</td>
<td>50%</td>
<td>LC₅₀</td>
</tr>
<tr>
<td>3.</td>
<td><em>Delonix regia</em></td>
<td>0.5 ml/l</td>
<td>96</td>
<td>Nil</td>
<td>LC₀</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>0.4 ml/l</td>
<td>96</td>
<td>Nil</td>
<td>Sublethal concentration</td>
</tr>
</tbody>
</table>

0.4 ml/l Concentration of seed extracts of *Delonix regia* was considered as sublethal concentration throughout the experiments.
Graph No. 1 Showing lethal concentration values, dosage and mortality % in *Lymnaea stagnalis* after treatment with methanolic seed extracts of *Cassia fistula* and *Delonix regia*.

Graph No. 2 Showing lethal concentration values dosage and mortality % in *Gyraulus convexusculus* after treatment with methanolic seed extracts of *Cassia fistula* and *Delonix regia*.
Graph No. 3 Showing lethal concentration values dosage and mortality % of methanolic seed extracts of *Cassia fistula* and *Delonix regia* on the adult specimens of *Lymnaea stagnalis*

![Graph](image)

Graph No. 4 Showing lethal concentration values dosage and mortality % of methanolic seed extracts of *Cassia fistula* and *Delonix regia* on the adult specimens of *Gyrulus convexiusculus*

![Graph](image)