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Toxicity Study of Plant Extract of Cerbera thevetia on Egg Masses of Lymnaea stagnalis

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Abstract:-

In the present study, spawn of *Lymnaea stagnalis* was exposed to different concentration of plant extracted of *Cerbera thevetia* i.e. 1.0%, 0.5%, 0.26% and 0.25 % solution, they showed hyper-irritability manifested in climbing behavior at the surface of the water avoid contact with treated medium and to take in fresh water. The egg masses were swollen and were sticky then in controls. Snails are pest and prolific. So it is very essential to control the population from glycocides of plant extract of *Cerbera thevetia.*

Introduction:-

Lymnaea stagnalis snail is the most harmful pest of paddy crops and aquatic vegetation. Most of workers have investigated the toxicity of pesticides for land snails. Most of the study about snail's development was around or after hatching. In the present investigation has also been taken to study the effect of glycosides extracted of *Cerbera thevetia* on the egg masses of *Lymnaea stagnalis.* The embryos of *Lymnaea stagnalis* are well visible inside the egg capsules and therefore this snail is very easy ideal material to study the embryonic development.

The work on the glycoside toxicity in *Lymnaea stagnalis* is yet scanty, so this is attempt was performed to evaluate of glycoside on the toxicity on the different developmental stages of *Lymnaea stagnalis.*

Material and Methods:-

Adult snails of *Lymnaea stagnalis* were collected from their natural habitats in Sagar pond. The snails were kept in glass container filled of water. The snails were fed on fresh aquatic vegetation. The collected snails were acclimatized for 7 days under laboratory conditions according to method adopted by Subbarao (1989). The water was replaced with fresh water 3 times in a week.

They the number of eggs sacs and the eggs per sacs were counted for each snail. The egg masses laid by these snails were introduced via to different concentration concentrations of plants seed extracts and the data was collected in triplicate and calculated the values of LC_{100}, LC_{50}, LC_0 and sub-lethal concentration were detected out for each group separately and data was summarized in table no.1(Probit analysis method adopted after Finney, 1971). Each egg masses contain about 30 egg capsules. Different developmental stages of Lymnaea stagnalis selected in the present investigation as follows:

1. Cleavage
2. Blastula
3. Gastrula
4. Post gastrular changes e.g. Morphogenesis and Organogenesis.
5. Formation of trochophore larvae.
6. Number of trochophore transformed into veliger larvae.
7. Torsion in veliger larvae.
8. Metamorphosis of veliger into young snails.
9. Hatching of young snails from their respective egg capsules.

The seeds of *Cerbera thevetia* have been analysed to find out the glycosides.

The defatted seed powder (100 gm) of each plant was extracted with 95% ethanol for 20 hrs separately in a Soxhlet apparatus. The ethanolic extract on concentration gave a brownish syrupy mass. It was then successively extracted with acetone. The process of dissolving and precipitation was repeated several times and finally through a bed of activated charcoal. On removal of the solvent a brownish mass of glycoside was obtained in a yield of 6.83%.

Results- Cleavage is spiral, begins about $2^{1/2}$ to 3 hours after the eggs masses were laid in the control group of *Lymnaea stagnalis* but it started after 6±2 hours after treatment with glycoside in *Lymnaea stagnalis*. The dose and duration of treatment dependent increase in the duration of cleavage has been observed in the experimental snails of *Lymnaea stagnalis.*

In the present investigation in *Lymnaea stagnalis* it was observed that blastula period was increased in paclitaxel treatment. However, mortality during blastula stage ranges from 0.5 to 1.0 percent. The increase in the duration of blastula was more or less same in the experimental snails of *Lymnaea stagnalis*.

In the present investigation in *Lymnaea stagnalis* the gastrulation period increased by 5±2 hours in treatment. This stage was found to be more susceptible as mortality occurred in the later gastrula stages. After gastrulation three germinal layers were formed and organogenesis and morphogenesis were started in control as well as in experimental snails but the duration was somewhat more prolonged in experimental snails and was found to be dependent on dose and duration of treatment in comparison to the control groups.

The young snail hatchability percentage was very low with high percentage of mortality after high concentration of glycoside treatment in comparison to control. Data on the morphological observation on different survivality percentage, mortality percentage and hatchability of young snails was recorded and summarized in table No-2.

Discussion- In the present investigation egg lying was observed throughout the year in *Lymnaea stagnalis* and it was observed that egg masses were laid in large number from July to October as also reported by Gupta (2003), Nema (2005) Jain (2007) and Mahobiya et al (2012).

In the present investigation after treatment with glycocide as also observed by Grasveld (1949) in *Lymnaea stagnalis.* Isolated blastomeres generally give rise to the same sequence of cells as in normal development, while in the present investigation blastomeres were not isolated. In further development the micromeres spread over the macromeres. This is followed by slight invagination of the macromeres resulting in a small depression representing a rudimentary archentric cavity and blastopore as also observed in the present investigation in *Lymnaea stagnalis*. The endodermis cells originated from the macromeres and fill the whole of the interior of the embryos. The gastrula is therefore a sterogastrula.

Two larval stages e.g. trochophore and veliger larval stages were found in the development of *Lymnaea stagnalis*. as also observed by Gupta (2003) in *Lymnaea spp*. and *Gyraulus* spp., Nema (2005) in *Lymnaea* spp., Jain (2007) in pulmonates and Mahobiya et al (2012) in Lymnaea spp. while in the present investigation larval development arrest has been observed in large number of egg capsules in *Lymnaea stagnalis due* to the intoxication of alkaloid and the effect was more pronounced in paclitaxel treatment.

In the present investigation in *Lymnaea stagnalis* was observed that the snails developed from the treated egg masses had thin and transparent shell due to decalcification as also observed by Bhide (1991) in *Lymnaea stagnalis* after nuvan and methyl parathion exposure, Gupta (2003) in *Lymnaea stagnalis* and *Gyraulus convexiusculus* after some pesticides and dye treatment, Nema (2005)

in *Lymnaea* spp. and Jain (2007) in *Lymnaea* spp. and *Gyraulus* spp. after treatment with some pesticides respectively.

It could be concluded from the present investigation that the glycocide used for the treatment, were able to arrest the development at any stage, in which larval stages were most susceptible suggested the larvicidal nature of the glycocide which induces teratogenicity.

Table 1: Data on Toxicity of Plant Extract of *Cerbera thevetia* on Egg Masses of *Lymnaea stagnalis*.

S.No.	Name of the Glycocide compound	Concentration of the Glycocide Compound	Duration (hrs.)	Mortality (%)	Lethal conc. value
1. 2. 3. 4.	Plant Extract of *Cerbera thevetia*	1.0% 0.5% 0.26% 0.25%	72 72 72 72	100% 50% Nil Nil	LC_{100} LC_{50} LC_0 Sublethal concentration

Result: 0.25 % concentration of colchicine was considered as sublethal concentration value.

Table No. 2DEVELOPMENTAL DATA OF Lymnaea stagnalis UNDER THE INFLUENCE OF CERBERIN (METHANOLIC EXTRACT)

Kind of the Glycoside	Conc. of the Glycoside	Total No. of Egg Capsules	No. of eggs completed cleavage	No. of eggs completed blastula	No. of eggs completed gastrula	No. of trochophore formed	No. of veliger formed	No. of veliger completed torsion	Total No. of young snails hatched	No. of young snails survived upto adulthood
Control	No. of trace of any Glycoside	50	49 ± 1	49 ± 1	49 ± 1	48 ± 1	48 ± 1	47 ± 1	48 ± 2	47 ± 1
Plant Extract of *Cerbera thevetia*	1.0%	50	48 ± 1	47 ± 2	38 ± 1	32 ± 1	25 ± 2	24 ± 1	22 ± 2	19 ± 2
	0.5%	50	48 ± 1	47 ± 2	25 ± 2	24 ± 1	22 ± 2	19 ± 2	15 ± 2	15 ± 1
	0.26%	50	48 ± 1	47 ± 2	24 ± 1	19 ± 2	18 ± 2	16 ± 2	14 ± 1	11 ± 2
	0.25%	50	48 ± 1	47 ± 2	20 ± 2	15 ± 2	13 ± 2	11± 2	11 ± 1	9 ± 1

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