International Journal of Science, Environment and Technology, Vol. 3, No 3, 2014, 1090 – 1104

ISSN 2278-3687 (O)

BEHAVIOURAL CHANGES IN FRESHWATER SNAIL *BELLAMYA BENGALENSIS* DUE TO ACUTE TOXICITY OF COPPER SULPHATE AND *ACACIA SINUATA*

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Abstract: Behavioural changes were recorded in the freshwater snail *B. bengalensis* when subjected to mean LC_{50} concentrations of copper sulphate (0.56 ppm) and *A. sinuata* (2.32 ppm) for different exposure periods. Different physiological and morphologically changes were observed in experimental animals it includes protective response, tentacular movements, foot movements and its secretion, response to external stimuli, mucus secretion of gills and courtship behaviour. In this concept to improve that any toxicants or any molluscicide were responsible for the alterations in behavioural of animal body. From obtained results, we concluded the toxicity of copper sulphate and *A. sinuata* was responsible for the behavioural changes in freshwater snail *B. bengalensis.*
Keywords: *Bellamya bengalensis*, copper sulphate, *Acacia sinuata* and behaviour.

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INTRODUCTION

The toxicology not only protect human and the environment from the deleterious effects of toxicants, but also to facilitate the development of more selective toxicants with clinical drugs and pesticides. Toxicant is a quantitative concept, where substance become harmful at some higher doses but, at lower become less 422 effective to organisms. In between these limits there is a range of possible effects, from long-term chronic toxicity to immediate lethality. The importance of dose is well illustrated by metals that are essential in the diet but proved toxic at higher doses. Thus iron, copper, magnesium, cobalt, manganese and zinc can be present in the diet at too low level (deficiency), at an appropriate level (maintenance) or at too higher level (toxic) (Ernest Hodgson, 2004). According to Rostein (1959), heavy metals directly affected to the tissue and may interact with cell membrane. Higher concentration of toxic metals, in aquatic environment can cause adverse effects on aquatic organism at cellular or molecular level and ultimately leads to disorder in biochemical composition of organism.

Molluscs are therefore of interest not only to farmers and the pesticide industry but also to ecotoxicologists as monitor species for environmental pollution. They encounter toxic

*Received May 08, 2014 * Published June 2, 2014 * www.ijset.net*

materials either by contact or during feeding. If they crawl over surfaces recently sprayed with pesticides, on toxic molluscicide baits or in heavily polluted substrates, the skin is the first point of contact (Triebkorn *et al*., 1996). Generally, the animal behaviour depends on the fluctuations of environmental conditions and their capacity of animal body. The behaviour of an organism is defined as an act or conduct in particular way or exact way an organism responses to stimulation of environment especially those responses that can be observed (Webster's, 2002). Some biotic as well as abiotic factors play very important role to change activities and behaviour of the animals. The toxicological nature of surrounding environment was assessed with the help of behaviour and metabolic changes in animals. The behavioural modification in animals can be taken as the most sensitive indicators of environmental stress (Eisler, 1979). Nimgnare (1992), Shaikh, (1999) and Sarojini *et al*., (1990), studied the effect of detergents on *Kasbora daniconium* and correlated the behavioural changes in relation to metabolic rates. The activities like opening of operculum, extension of foot and other body parts, crawling movement were minimized when animals were exposed to folithion and lebycide (Muley and Mane, 1988). Aluminium and salicylic acid in the environment have changed the behaviour of *Lymnaea stagnalis* (Compbell *et al*., 2000). The pesticides nuvan, methyl parathion and thimet have changed the normal behaviour of *Lymnaea stagnalis* (Bhide, 1998) and *pila globosa* (Bhide, 1987).

Gokhale and Mane (1990), studied the toxicity of molluscicide in bivalves *Lamellidens marginalis*, where they documented that, after intoxication, bivalve closed their valves, immediately after exposure to molluscicide and showed diapedesis in all exposed animals after 12 hrs. with white coagulated matter of mucus. These effects can potentially gives out structural and functional changes in freshwater ecosystems (Camargo, 2003).

Present study is designed to investigate toxicity of copper sulphate and pod extract *Acacia sinuata* on behavioural responses of freshwater snail *B. bengalensis*.

MATERIALS AND METHODS

Experimental animal:

1) Collection Site:

For the present investigation, experimental animal freshwater snail *Bellamya bengalensis* (Lamark) (Prosobranch) were collected from Rajaram tank, near Shivaji University, Kolhapur, Maharashtra, India.

Experimental Design:

For behaviour study 50 healthy experimental animals (23-26 mm shell height and 2.8-3.5 gm weight) were used. Animals were divided into 5 set. As control set-I, set-II, set-III, set-IV and set-V 10 in each trough (5 litters of capacity). For the intoxication study, pre-determination LC_{50} concentration of heavy metal copper sulphate 0.56 ppm was induced in each of experimental set. Set-I was (control), Set-II (exposed upto 24 hrs.), Set-III (exposed upto 48 hrs.), Set- IV (exposed upto 72 hrs.) and Set-V (exposed-96 hrs.). At the same time controlled group run upto 96 hrs by providing natural food and aeration. For the confirmation, the whole or total experiment repeated thrice. The above procedure was followed for intoxication of pre-determined LC_{50} concentration of *A. sinuata* 2.32 ppm and for the confirmation repeated thrice.

The differentiation of behavioural changes was recorded as per the intoxication and time of exposure by considering protective behaviour, foot movement, mucus secretion and tentacular movement.

RESULTS

Behavioural changes were recorded in the freshwater snail *B. bengalensis* when subjected to mean LC_{50} concentrations of copper sulphate (0.56 ppm) and *A. sinuata* (2.32 ppm) for different exposure periods at 24, 48, 72 and 96 hrs. Behavioural changes occurred due to intoxication was compared with control animals. The results were as follows-

1) Control group:

Control group of snails were found completely immersed in the water, initially retracted their body inside the shells and operculum was closed. Immediately after 6-8 minutes snails has opened the univalve shell. Control snails were very quick in their protective response. Animals protruded their body outside the shell and showed regular movements, including radular movements for feeding. Tentacular movement was quick and fast. Snails showed free movement of foot with firm attachment to the trough. Ample amount of mucus was secreted by foot. All the animals were grouped attached to the floor and wall of trough. Their courtship behaviour was seen normal (Fig. No. 1). Normally young whitish or colourless embryos were seen in the trough (Table No.1).

2) Behavioural changes due to intoxication:

a) Intoxication of copper sulphate:

When the snails were exposed to toxicity of copper sulphate, body parts were expanded after 24 hrs. of exposure. The snails released excreta in the trough. The tentacular movements were side to side. The foot of the snails was highly stretched. In stressful condition the mucus secretion was more in trough. The snails lowered response to the external stimuli sometimes they became immovable and added white gelatinous mucus. Few snails were in groups or paired. They remain attached.

After 48 hrs. of intoxication, snails discharged more amounts of excreta into the trough. Snails showed very poor side to side tentacular movement, with less response to external stimuli. Size and shape of the foot was reduced. Mucus secretion of foot was increased. The snail does not gave response to pin touch or vibrations. White gelatinous mucus mass was secreted over the buccal mass and shell. They did not form pair or groups. The snails dragged their body inside the shells.

After 72 hrs. of exposure the snails lost the protective behaviour. They closed their opening of the shells by operculum and remained steady. The tentacular movements were not observed in the snails. As all the body parts were retracted into the shell, the foot movement was not observed but large amount of mucus was found in the trough. The snails did not show any response to any mechanical stimuli.

After 96 hrs of copper sulphate intoxication, snails remained in same position under toxic chemical stress. They tightly closed their shell opening by operculum. Tentacular movements and foot movements were not seen. White, thick gelatinous mucus secretion observed into the trough. The courtship behaviour was not observed and snails were remained alone (Fig. No. 2).

Behavioural changes in freshwater snail *B. bengalensis* at pre-determined mean LC_{50} 0.56 ppm of copper sulphate at 24, 48, 72 and 96 hrs. exposures have recorded in Table No. 2.

b) Intoxication of *Acacia sinuata*:

When snails were exposed to pod extract of *A. sinuata,* after 24 hrs. of exposure some body parts were protruded out through the shell. Quick responses to pin touch or any stimulus was initially seen. The tentacles showed slow side to side movements. The foot movement was not fast. Snails have initiated mucus secretion against toxicity. After 48 hrs. of intoxication, body parts were dragged inside the shell showing excess amount of excreta in trough. A

tentacular movement was not regular. Few snails were in paired, but courtship behaviour was not seen.

After 72 and 96 hrs of exposure tentacular movement and foot movements were reduced. Response to external stimuli was not found. Quantified mucus secretion was observed in trough. In courtship behaviour snails tried to escape from each other. Snails showed loss of firm grip of operculum due to stress of toxic solution. Tentacular and foot movements was not seen. No response to external stimuli. Mucus secretion was much more in trough. No courtship behaviour was observed. At the end experimental snails were became immotile and were lost their normal movement in trough (Fig. No.3). Altered behaviour in freshwater snail *B. bengalensis* at pre-determined mean LC_{50} 2.32 ppm of pod extract of *A. sinuata* at 24, 48, 72 and 96hrs. exposure has been recorded in Table No. 3.

DISCUSSION

In control groups of our experiment, snails were showed normal behaviour with ample mucus secretion. But intoxication of metal and molluscicide showed increased quantity of mucus. Animals lost the response to external stimuli. Operculum was tightly closed after 72 and 96 hrs. Similarly, hypersecretion of mucus by the mucus glands of mantle was found in snails *Lymnaea stagnalis* due to toxicity of molluscicide and in *Pila globosa* due to intoxication of DDT (Bhide, 1987). The Mucus secretion was found higher due to mercury intoxication as that of cadmium and zinc (Devi, 1996). Nanaware and Awati (2004), recorded, increased mucus secretion in gills of snail *V. Bengalensis* due to fruit extract of *Sapindus laurifolius* and synthetic pesticides Thimet 10-G and Sodium pentachlorophenate.

Intoxication of toxicants altered the normal behaviour of animal including, food intake, normal locomotion, reproductive behaviour, etc. (Cooke, 1971). Kamble (2007) documented that, animal behaviour provides information about type of contamination and its concentration in the aquatic bodies. Muley and Mane (1988), observed that the behavioural changes were influenced by the toxic compounds of mercury salts in *Viviparus bengalensis*. Similar behavioural changes were observed by Gokhale and Mane (1990), in bivalve molluscs, *Lamellidens marginalis* due to fluoride and endosulfan toxicity and due to metasystox toxicity (Muley *et al*., 1987) in clams. Devi (1996) found, very similar results when intertidal gastropod *Morulas granulate* (Duclos) exposed to mercury, cadmium and zinc. Escaping behaviour was recorded in *Biomphalaria glabrata* after intoxication (Nolan *et al*., 1953); the distress syndrome (Harry and Aldrich, 1963); withdrawing into the shell

(Cheng and Sullivan, 1973) and the ability of the snail to avoid high doses of the product when exposed to a concentration gradient (Etges, 1963).

The risk of chronic exposure to toxicants under natural condition showed higher sensitivity to several toxic compounds (Oehlmann and Schulte-Oehlmann, 2002; Valenti *et al*., 2006; Duft *et al*., 2007; Wang *et al.,* 2007). In the last few years the, New Zealand mud snail *Potamopyrgus antipodarum* (Hydrobiidae mollusca) has used in ecotoxicology (Duft *et al*., 2003; Mazurova *et al*., 2008; Pedersen *et al*., 2009) because of its partheogenetic reproduction (Mazurova *et al.,* 2008). Water flea found most widely used aquatic invertebrates to test fluoride toxicity (LeBlanc 1980; Fieser *et al*., 1986; Metcalfe-Smith *et al*., 2003), as it showed relatively high tolerance to acute toxicity of fluoride. Effects of fluoride on animal behaviour have been found in aquatic animals as apathetic behaviour, loss of equilibrium and altered migratory movements (Damkaer and Dey, 1989). However, to assess long-term consequences of fluoride on the behaviour of a freshwater macro-invertebrate, showed that, the activity of snail was impaired by intoxication of higher fluoride concentrations.

The relative importance of morphological and behavioural characteristics for controlling body temperature was estimated by using a mechanistic heat-budget model (Porter and Gates, 1969; Gates, 1980; O, Connar and Spotila, 1992; Porter and Kearney, 2009). In most of rocky intertidal gastropods they keep foot attached to the substratum to maintain position on the shore, but it comes at the expense of increasing conductive heat flux between the foot and a potentially hot substratum (Denny and Harley, 2006). Withdrawing the foot into shell has added benefit for allowing snails to close there operculum, thereby reducing water loss (Mc Mahon and Britton, 1985). Nagarajah *et al*., (1985) noticed the behavioural changes in same intertidal molluscs after exposure to water soluble fraction of diesel. The mercury and mercurial salts changed the behaviour in *M. articulata* (Saliba and Vella, 1977) and in *V. Bengalensis* (Muley and Mane, 1988) respectively. Akarte and Mane (1988), reported that, the bivalve molluscan when exposed to different test concentration of folithion in different seasons they showed reduced shell valve movement with mucus secretion and maximum excreta.

Literature survey indicated that, the behaviour of the molluscan animals were changed due to toxic compounds including pesticides, metals, phenolic compounds and oils in the surrounding medium. *Helix aspersa* was changed due to application of some organophosphrous compounds (Rorke *et al*., 1974). Muley and Mane (1988), observed such

behavioural changes in snail due to toxicity of mercury salts. Thickness and quantity of voluminous mucus secretions near mouth cause the death of snails at 240 ppm from 72 hrs. in *V. bengalensis* due to intoxication by folithion and lebaycid (Mulley and Mane, 1988).

In our study we found that, in control snails behavioural responses to external stimuli were quick and sharp. But, concentration and exposure time has changed the response in animals. In courtship behaviour, chemical intoxication has impact on release of young once in trough. Thick and whitish mucus was more in trough. We observed that, behaviour changes due to toxicity of copper sulphate and *A. sinuata* 24hrs, 48hrs, 72hrs and 96 hrs were more or less similar but comparatively the behavioural alterations recorded due to cooper sulphate intoxication were more acute and earlier than intoxication of *A. sinuata* proving copper sulphate has more toxicity than pod extract of *A. sinuata* in freshwater snail *Bellamya bengalensis.*

ACKNOWLEDGEMENT

Authors are very much thankful to the UGC and Head Dept. of Zoology for providing infrastructural and laboratory facilities for carried out present research work.

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TABLES AND FIGURES

Sr. No.	Type of Response	Behaviour of Normal group of snails
1	Protective Response	Control group of snails showed quick response.
2	Tentacular movements	Fast and regular movements.
3	Foot movements and its secretion	Foot movement was fast. It tightly attached to the surface with the help of foot. Secretion by foot was ample as per locomotory activity.
4	Response to external stimuli	Generally, any movement in water or wave of water stimulated snail and stoped all the movement and again snail retain the normal motility.
5	Mucus secretion of gills	In control group of snails mucus secretion by gill was not seen.
6	Courtship behaviour	In control group, snails were remained, attached with each other in group.

Table 1: Normal behaviour of control group of freshwater snail *Bellamya bengalensis.*

Sr. No.	Type of response	Behaviour of Copper sulphate intoxication group			
		24hrs.	48hrs.	72hrs.	96hrs.
1	Protective behaviour	Tolerate toxicity with the help of operculum.	Operculum slowly closed.	Tightly closed.	Tightly closed.
2	Tentacular movements	Slowed down.	Movement was reduced.	Remained steady.	No free movement.
3	Foot movements	Food extended initially.	Movement was slowed down.	Foot retracted.	Foot movement not seen.
4	Response to external stimuli	Initially quick response.	Response to stimuli was reduced.	Very poor response.	No response.
5	Mucus secretion of gills	Mucus secretion initiated in trough.	Secretion increased in trough.	Mucus secretion quantitatively increased.	Thick white mucus seen in trough.
6	Courtship behaviour	Normal courtship behaviour showing pairing among the snail.	Only few snails were paired.	Snails detached form each other.	No courtship behaviour was observed.

Table 2: Behaviour changes in freshwater snail *B. bengalensis* against Copper sulphates intoxication at different exposure periods.

Sr. No.	Type of response	Behaviour of *Acacia sinuata* intoxication group			
		24hrs.	**48hrs.**	**72hrs.**	**96hrs.**
1	Protective behaviour	Body parts were protruded showing maximum response.	Body part dragged inside the shell.	Protective behaviour was reduced to any stimuli.	Snails tightly closed opening of shell by operculum due to stress of toxic solution.
2	Tentacular movements	Normal side to side movement was seen.	Side to side movement was reduced.	Remained at one position.	Movement was not seen.
3	Foot movements	Normally extended foot movement.	Reduced movement.	Remained contracted.	No movements.
4	Response to external stimuli	Response was quick to external stimuli.	Reduced response.	Remained in the same position.	No response.
5	Mucus secretion of gills	Initiated mucus secretion.	Mucus secretion was fast.	Mucus secretion increased.	Increased thick whitish mucus.
6	Courtship behaviour	Normal courtship behaviour showing pairing among snail.	Snails detached.	Remain steady.	No courtship behaviour was observed.

Table 3: Behaviour changes in freshwater snail *B. bengalensis* against pod extract of *Acacia sinuata* intoxication for different exposure periods.

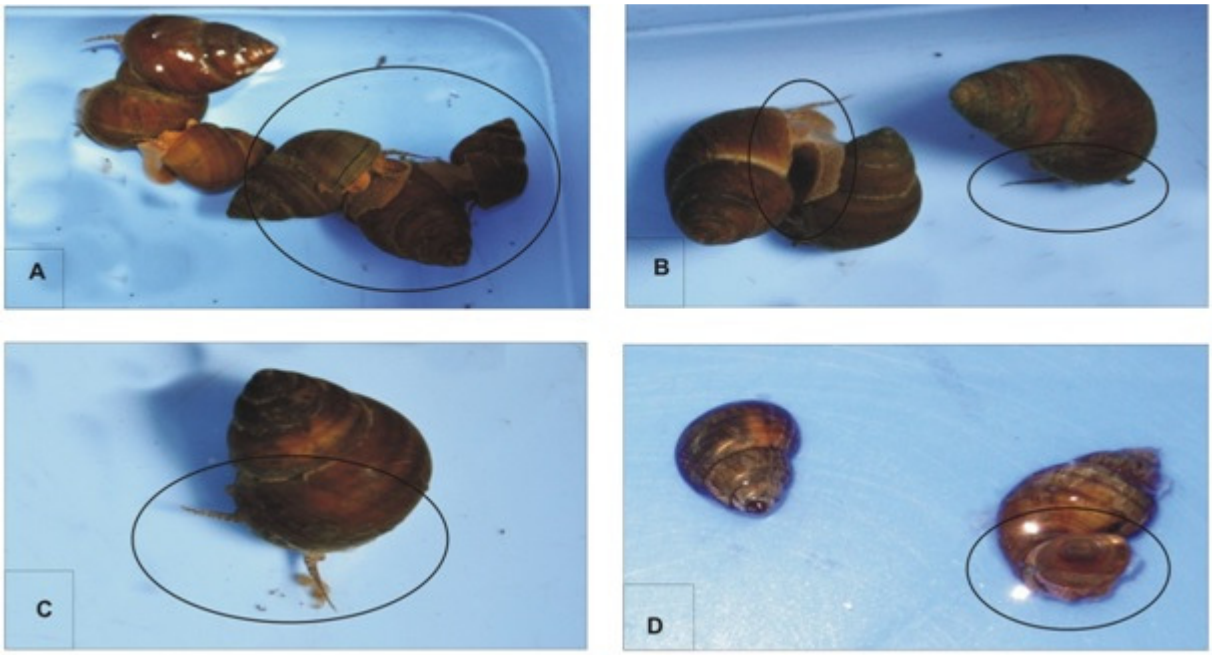

Fig-1- Behaviour of *B. bengalensis* from control group. A- Courtship behaviour of Control group of freshwater snail *B. bengalensis.* **B-** Foot movement in Control group of freshwater snail *B. bengalensis.* **C-** Tentacular movement in Control group of freshwater snail *B. bengalensis.* **D-** Mucus secretion in Control group of freshwater snail *B. bengalensis.*

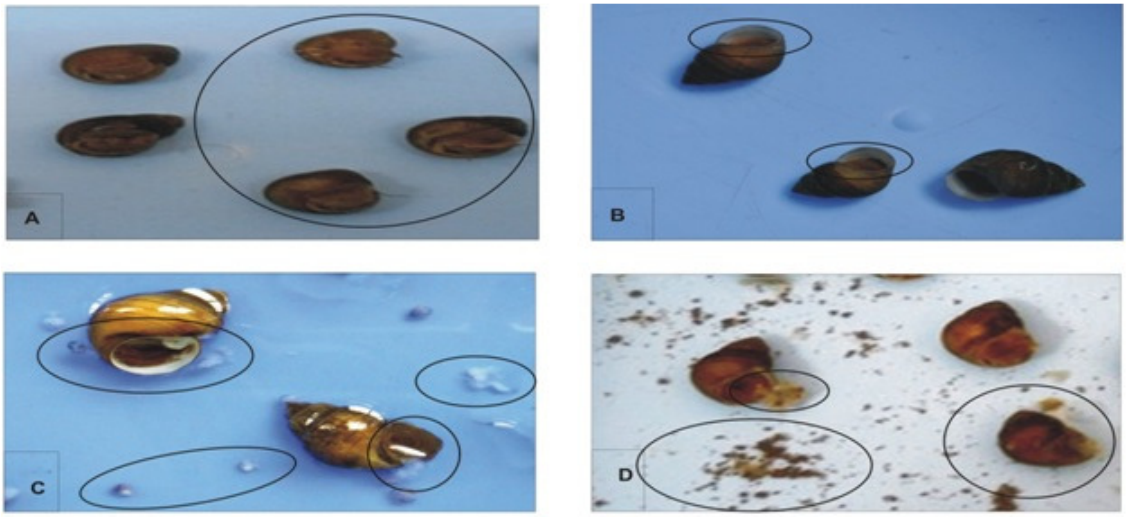

Fig-2- Behaviour of *B. bengalensis* from intoxication of copper sulphate. A- After intoxication of copper sulphate changes in courtship behaviour of freshwater snail *B. bengalensis.* **B-** After intoxication of copper sulphate changes in foot movements of freshwater snail *B. bengalensis.* **C-** After intoxication of copper sulphate changes in tentacular movement of freshwater snail *B. bengalensis.* **D-** After intoxication of copper sulphate Mucus secretion increased in freshwater snail *B. bengalensis.*

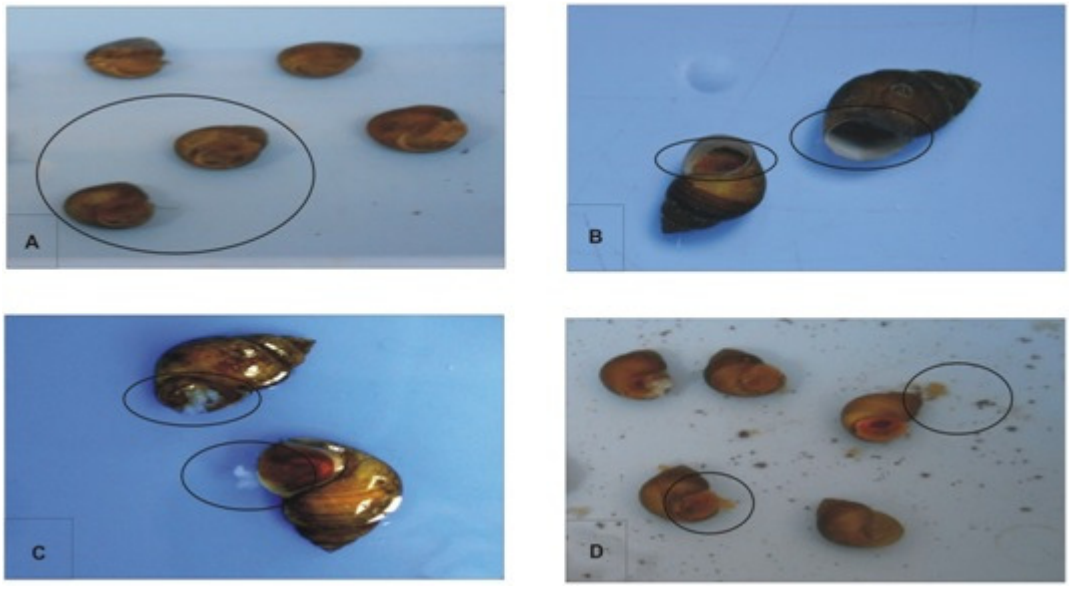

Fig-3- Behaviour of *B. bengalensis* from intoxication of *Acacia sinuate.* A- After intoxication of *Acacia sinuata* changes in courtship behaviour of freshwater snail *B. bengalensis.* B- After intoxication of *Acacia sinuata* changes in foot movements of freshwater snail *B. bengalensis.* C- After intoxication of *Acacia sinuata* changes in tentacular movement of freshwater snail *B. bengalensis.* D- After intoxication of *Acacia sinuata* Mucus secretion increased in freshwater snail *B. bengalensis.*