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Leaf Power Unleashed: Exploring the Molluscicidal Potential of *Antigonon leptopus* against *Bellamya bengalensis* and *Lamilidens marginalis* Snails

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Abstract

The use of plant-based molluscicides for snail control is gaining interest due to their eco-friendliness, affordability, availability, and ease of application compared to synthetic molluscicides. In this study, we investigated the molluscicidal potential of the aqueous leaf extract of *Antigonon leptopus* Hook. & Arn. against two snail species, *Bellamya bengalensis* Lamarck. 1882 *and Lamilidens marginalis* Lamarck. 1819. The adult snails were exposed to various concentrations of the plant extract for different time intervals in laboratory conditions. Mortality rates were observed, and the median lethal concentration (LC_{50}) was calculated using probit regression analysis. Water quality parameters were recorded using standard screening methods. The 96-hour LC_{50} and LC_{84} values against *B. bengalensis* were determined as 0.46g/L and 3g/L respectively. For *L. marginalis*, the 120-hour LC_{50} and LC_{84} values were found to be 0.7g/L and 1.12g/L respectively. Phytochemical screening tests revealed the presence of saponins, flavonoids, alkaloids, terpenoids, cardiac glycosides, and tannins in the leaf extract create a synergistic effect of test snails. The observed toxic effect of *A. leptopus* leaf extract on the snails suggests its ability to interfere with their physiological metabolism, ultimately leading to their death and proofs its potentiality as an effective herbal molluscicide and stupefactant for Pisciculture advancement.

Keywords: *Antigonon leptopus*, phyto-molluscicide, *Bellamya bengalensis, Lamilidens marginalis*

Introduction

Invertebrate snails belonging to the Gastropoda class play a crucial role in aquatic ecosystems and have both ecological and economic significance. However, certain snail species can pose significant challenges. They act as intermediate hosts for widespread diseases like schistosomiasis and can also spread diseases among fish populations. Moreover, some snail species are considered agricultural pests, causing damage to crops, and their overproduction in nursery ponds can disrupt fish food resources and hinder effective fish culture management.

To address these challenges, the use of molluscicides is essential for aquaculture development and disease control, particularly when molluscs act as disease vectors. However, the use of synthetic molluscicides is becoming less favored due to their high cost and potential environmental hazards (Coelho and Caldeira, 2016). As a result, molluscicides of plant origin are gaining interest as an alternative due to their desirable properties, including eco-friendliness, affordability, biodegradability, and target specificity. Extensive research is underway to screen various botanical sources for their molluscicidal potential (Mourad AA, 2014; De et al. 2021; Sing et al., 2010).

In this context, the present study focuses on evaluating the snailicidal effect of *Antigonon leptopus* on two locally available and edible snail species found in the Paschim Medinipur district. While previous studies have examined plant-based molluscicides, *A. leptopus* has been identified as a new organic molluscicide. Moreover, the molluscicidal effect of *A. leptopus* on *Bellamya bengalensis* and *Lamilidens marginalis* snails has not been reported before. Additionally, it has been observed that *A. leptopus* exhibits piscicidal effects at certain concentrations and exposure durations (De et al. 2022a; De et al. 2022b; De et al. 2022c).

The findings of this study have significant implications for the development of environmentally friendly molluscicides and the effective control of snail populations. By exploring the molluscicidal potential of *A. leptopus,* researchers can contribute to the advancement of sustainable aquaculture practices and disease management strategies. However, further research is needed to better understand the specific mechanisms of action of *A. leptopus* on snails and fish, as well as to assess its long-term effects on non-target organisms and the surrounding environment

Materials

2.1 Plant material collection and method of extraction

The selection of *A. leptopus* as a potential molluscicide was based on a preliminary screening test. Mature green leaves of the plant were collected from a location in Rangamati, Paschim Medinipur, which is situated near Midnapore town in West Bengal, India. The identification of the plant was conducted by the first author of the study, taking into account relevant literature. A voucher specimen (SCANT-10) was preserved in the herbarium of the Department of Botany at Midnapore College, Paschim Medinipur, West Bengal, for future reference and verification.

2.1.1 Ecological Status:

A. leptopus Hook. and Arn., commonly known as coral vine or chain of love, is a fast-growing, evergreen plant native to Mexico (Fig. 1). The plant is characterized by its dark green, heart-shaped to arrowhead-shaped leaves, which typically measure around 5 inches in length. The unique shape of the leaves, resembling hearts, adds to the plant's aesthetic appeal.

One of the distinguishing features of *A. leptopus* is its delicate pink flowers, which contribute to its common name "Chain of love" in Mexico. The inflorescence axis of the plant is modified into tendrils, allowing it to climb and reach heights of up to 40 feet. This climbing ability makes it an excellent choice for covering walls, fences, or trellises, providing a vibrant and eye-catching display.

The flowers of *A. leptopus* are small, with elongated sepals that provide a brilliant burst of color. The color of the flowers can range from pure white to various shades of rose pink, and some varieties even display deep coral-colored flowers. This diversity in flower color adds to the visual charm and versatility of the plant, making it a popular choice among gardeners and plant enthusiasts.

The combination of heart-shaped leaves, delicate pink flowers, and climbing tendrils make *A. leptopus* a captivating addition to gardens and landscapes. Its fast growth rate, evergreen nature, and attractive floral display make it a sought-after plant for adding beauty and charm to outdoor spaces.

The plant coral vine is listed as category II invasive, exotic by Florida's Pest Plant Council (Florida Exotic Pest Plant Council.2019)

2.1.2 Aqueous extract and stock solution preparation:

The aqueous fresh leaf extract of *A. leptopus* was prepared by following a series of steps. Mature green leaves (10g) were carefully collected, ensuring that only healthy leaves without visible damage or disease were chosen. The collected leaves were then thoroughly washed to remove any dirt or impurities. After washing, the leaves were ground using a mortar and pestle or a blender to break down the plant material and release the active compounds. The resulting ground material was soaked in 50mlwater with constant stirring for 24h at room temperature and filtered using Whatman filter paper No.1. This filtrate represented the aqueous fresh leaf extract. To prepare a concentrated stock solution, a specific amount of the filtered extract was dissolved in a known volume (100ml) of distilled water. The concentration of the stock solution depended on the desired concentration range for the experiment. To obtain test solutions with different concentrations (100, 200, 400, 600 & 800 mg/L), appropriate dilutions of the stock solution were made by measuring a specific volume and diluting it with a known volume of distilled water. The dilution ratios were calculated accurately to ensure consistent and reproducible results. These steps followed guidelines from the APHA standard methods (APHA, 2012) or other relevant protocols specific to the study, allowing for the availability of different concentrations of the *A. leptopus* leaf extract for conducting experiments and evaluating its molluscicidal effects

2.2 Snail collection:

The adult snails of *Bellamya bengalensis* and *Lamilidens marginalis* were collected from a nearby stream, ensuring that the collection process did not harm or damage the snails or their natural habitat. The snails were carefully placed in a clean bucket filled with water taken from the same stream. This step was taken to maintain the natural water conditions and minimize stress during transportation to the laboratory (Fig. 2).

Upon arrival at the laboratory, the snails were acclimatized for a period of 3 days. The acclimatization process involved keeping the snails in a controlled environment with room temperatures set at 27°C ± 3°C. The snails were placed in dechlorinated tap water, ensuring that the water quality was suitable for their survival and well-being.

During the acclimatization period, the snails were provided with food to meet their nutritional requirements. They were fed boiled dried shreds of lettuce (*Lactuca sativa* L.) leaves, which were prepared in a way that ensured their suitability as a food source for the snails. This feeding regimen aimed to mimic their natural diet and provide them with essential nutrients.

Throughout the course of the experiment, the snails were handled in accordance with ethical guidelines and principles of animal welfare in scientific experiments, as outlined in the APHA (American Public Health Association) guidelines of 2012 (APHA, 2012). This means that the well-being and welfare of the snails were taken into consideration, and steps were taken to minimize any potential discomfort or harm to the snails during the experimental procedures.

By following these detailed protocols and ethical considerations, the study ensured the appropriate care, acclimatization, and handling of the snails, promoting their welfare while conducting the necessary experiments to evaluate the molluscicidal effects of *A. leptopus* leaf extract on *Bellamya bengalensis* and *Lamilidens marginalis* snails.

Methods

This experimental study has been designed to investigate the snailicidal effect of *A. leptopus* aqueous leaf extract on *Bellamya bengalensis and Lamilidens marginalis* in laboratory condition.

3.1 Experimental set up

In this experiment, rectangular plastic containers measuring 20cm x 20cm x 10cm were utilized. Each container was filled with 5 liters of non-chlorinated tap water, which was pre-aerated for 20 minutes to ensure full oxygen saturation before introducing the different volumes of the *A. leptopus* leaf extract.

For the experiment, five different concentrations of the plant extract were prepared, each with three replicates. These concentrations were 100, 200, 400, 600, and 800 mg/l. A control group was also maintained, where no extract was added. Adult snails of two species, *Bellamya bengalensis* and *Lamilidens marginalis*, were exposed to the various concentrations prepared from fresh *A. leptopus* leaves for durations of 24, 48, 72, 96, and 120 hours. Each container contained 30 snails. The mortality of the snails was observed and recorded at 24-hour intervals.

To analyze the mortality data, a probit regression model was employed. Additionally, the phytochemical content of the plant was determined using standard screening methods. The experiment followed standard static bioassay procedures based on the guidelines provided by (APHA-AWWA- WPCF, 1971; APHA- AWWA- WPCF, 1980).

3.2 Phytochemical analysis:

The aqueous extract of *A. leptopus* leaf underwent qualitative phytochemical analysis using established protocols recommended by Odebiyi and Sofowara (1990), Trease and Evans (1989), and Harborne (1998) to identify its constituent compounds.

Table 1. Phytochemical analysis of *A. leptopus*

Plant	Parts used	Flavonoids	Alkaloids	Terpenoids	Cardiac glycoside	Tannins	Saponin
A. leptopus	Leaves	+	+	+	+	+	+

(+) = Present ; (-) = Absent

3.3 Water quality parameters:

Throughout the entire research period, the water quality of each glass aquarium in the three trials was monitored for phytochemical parameters before and after the application of the plant extract. The parameters considered, following APHA 2012 guidelines (APHA 2012), included temperature, pH, dissolved oxygen, alkalinity, hardness, and total dissolved solids (TDS).

3.4 Statistical analysis:

The LC_{50} value, representing the effective dose for 50% mortality, was determined using probit regression analysis (Finney, 1971). This statistical analysis method is commonly employed to estimate the concentration or dosage at which 50% of the test subjects exhibit mortality.

By fitting a probit regression model to the observed mortality data, the LC_{50} value can be estimated, providing valuable information about the toxicity or effectiveness of a particular treatment or substance. The LC_{50} value serves as a crucial parameter in assessing the potency or lethal dose of a compound and is widely used in toxicological studies.

Using the probit regression analysis, researchers can gain insights into the concentration or dosage required to achieve a specific level of mortality, allowing for further understanding of the potential effects and risks associated with the tested substance.

Result and Discussion

4.1 Phytochemical

The phytochemical screening of the aqueous leaf extract of *A. leptopus* indicated the presence of various important compounds. Saponins, which are glycosides known for their soap-like properties, were detected in the extract. Saponins have been extensively studied for their diverse biological activities and are often associated with beneficial effects on human health.

The extract also contained cardiac-glycosides; a class of compounds known for their ability to affect heart contractility. These compounds have been widely investigated for their potential cardiovascular benefits.

Tannins, a group of polyphenolic compounds, were found in the extract as well. Tannins are recognized for their astringent properties and have been reported to possess antioxidant and antimicrobial activities.

Flavonoids, a large and diverse class of plant secondary metabolites, were identified in the extract. Flavonoids have attracted significant attention due to their potential health benefits, including their antioxidant, anti-inflammatory, and anticancer properties.

Alkaloids, another class of bioactive compounds, were also present in the aqueous leaf extract of *A. leptopus*. Alkaloids are known for their diverse pharmacological activities and are often associated with effects on the central nervous system.

The presence of these phytochemical constituents in the extract suggests that *A. leptopus* leaf extract contains a range of bioactive compounds that may contribute to its potential therapeutic properties. However, further research is necessary to determine the specific effects and potential applications of these compounds within the context of *A. leptopus* leaf extract

4.2 Water quality

Fig. 3 illustrates the changes in TDS levels in different experimental sets from the initial to the final stages. The results indicate that the TDS values increased over time and with increasing concentration of the plant extract. The experimental set with the highest concentration exhibited the most significant changes in TDS value compared to the control group.

Fig. 4 shows the changes in pH levels in different experimental sets from the initial to the final stages. The results indicate that the pH of the different experimental sets increased over time. Initially, the solutions with different concentrations were slightly acidic, but at the end of the experiment, they became neutral or slightly alkaline.

These findings demonstrate that the application of the plant extract had an impact on the water quality parameters, particularly TDS and pH, in the experimental aquaria. The increase in TDS values suggests the presence of additional dissolved solids resulting from the application of the plant extract, while the increase in pH levels indicates a shift towards a more neutral or alkaline environment. Monitoring water quality parameters, as per APHA 2012 guidelines, is essential for assessing the potential effects of the plant extract on the aquatic environment during the experimental period

Fig. 5 displays the levels of salinity in different experimental sets compared from the initial to the final stages. The results of the experiment indicate that the salinity of the different experimental sets remained unchanged over time and with respect to the concentration of the plant extract.

No significant changes in salinity were observed throughout the experiment, indicating that the application of the plant extract did not have an impact on the salinity levels in the experimental aquaria. The salinity levels remained constant, suggesting that the plant extract did not contribute to an increase or decrease in the salt content of the water.

These findings indicate that the salinity parameter was not influenced by the application of the plant extract, and the water maintained its original salinity levels throughout the research period

Fig. 6 illustrates the changes in dissolved oxygen (D.O.) levels in different experimental sets compared from the initial to the final stages. The results of the experiment indicate that the D.O. levels in the different experimental sets decreased over time and with increasing concentration of the plant extract.

The last two experimental sets, with the highest concentrations, exhibited a rapid decrease in D.O. levels compared to the control group. This suggests that higher concentrations of the plant extract had a more pronounced effect on reducing the dissolved oxygen content in the water.

Furthermore, the control set showed a slower and gradual decrease in D.O. levels compared to the experimental sets, indicating a relatively stable oxygen concentration over time.

These findings demonstrate that the application of the plant extract, particularly at higher concentrations, resulted in a significant decrease in dissolved oxygen levels in the experimental aquaria. Monitoring dissolved oxygen is crucial as it directly affects the aquatic organisms' respiratory processes and can have implications for their survival and overall ecosystem health.

4.3 Snailicidal effect:

The molluscicidal effect of *A. leptopus* was assessed against *Bellamya bengalensis* and *Lamilidens marginalis* in the study. To confirm mortality, the snails in each container were carefully examined, and the number of deceased snails was recorded. A mechanical stimulus was applied to the footsole of the snails, and their inability to retract their foot back inside the shell and failure to close the trapdoor indicated their death.

The presence of active plant extracts from *A. leptopus* resulted in notable changes in the snails' physical appearance showing in Fig. 7 and Fig. 8. The cephalopodal mass of each snail became severely swollen and turgid, indicating a detrimental effect on the snail's internal tissues. Additionally, the observation of mucous secretion covering most of the foot suggested a physiological response to the plant extract.

One significant characteristic of the affected snails was the pungent and rotting smell emanating from their foot. This smell is likely attributed to the disruption of normal physiological processes caused by the molluscicidal activity of the *A. leptopus* extract. Furthermore, the color of the snail shells underwent a transformation to a fossy white appearance, which can be indicative of shell damage or degradation.

These findings align with previous studies conducted by Ndarnukong et al. (2006) and Kiros et al. (2014), who also observed similar effects of *A. leptopus* extracts on mollusks. The swelling, turgidity, mucous secretion, foul smell, and shell discoloration collectively suggest the potent molluscicidal activity of *A. leptopus* against *Bellamya bengalensis* and *Lamilidens marginalis*.

These results contribute to the understanding of the potential of *A. leptopus* as a molluscicidal agent, providing insights into the specific changes and symptoms exhibited by the affected snails. Further investigations are warranted to elucidate the underlying mechanisms responsible for the observed molluscicidal effects and to explore the potential applications of *A. leptopus* in controlling mollusk populations in various settings.

The statistical analysis conducted in this study revealed the molluscicidal efficacy of *A. leptopus* leaf extract against *Lamilidens marginalis*. The LC_{16}, LC_{50}, and LC_{84} values at 96 hours of exposure were determined to be 0.07g/L, 0.46g/L, and 3g/L, respectively. Probit analysis of the experimental data provided a lower limit of 444.76 mg/L and an upper limit of 490.35 mg/L. Similarly, at 120 hours of exposure, the LC_{16}, LC_{50}, and LC_{84} values were found to be 0.4g/L, 0.7g/L, and 1.12g/L, respectively. The probit analysis yielded a lower limit of 134.9 mg/L and an upper limit of 3.9 g/L.

These findings highlight the potency of *A. leptopus* leaf extract as a molluscicidal agent against *Lamilidens marginalis*. The obtained LC values indicate the concentration required to achieve specific levels of mortality in the target organism. The availability of such information is valuable for future applications of *A. leptopus* extract in controlling mollusk populations.

Moreover, the use of *A. leptopus* as an alternative plant product offers several advantages over synthetic chemicals. It is environmentally friendly, readily accessible, and cost-effective. Utilizing *A. leptopus* extract for industrial development and socio-economic benefits can contribute to the well-being of local communities.

To gain a deeper understanding of the mode of action of *A. leptopus* on these organisms, ongoing molecular-level investigations are being conducted in the laboratory. Exploring the specific mechanisms by which *A. leptopus* extract affects the target mollusks will provide insights into its potential applications and broaden its scope for controlling different classes of fishes and mollusks. However, further studies are necessary to ensure the safety and efficacy of *A. leptopus* extract on non-target species.

In conclusion, this study demonstrates the molluscicidal efficacy of *A. leptopus* extract and highlights its potential as an environmentally friendly alternative to synthetic chemicals. The ongoing molecular investigations and the need for further research emphasize the importance of understanding the broader applicability and potential toxic effects on non-target organisms.

Conclusion

In conclusion, the aqueous leaf extract of *Antigonon leptopus* demonstrated significant molluscicidal potential against *Bellamya bengalensis* and *Lamilidens marginalis* snail species. The extract exhibited dose-dependent toxicity, as indicated by the calculated LC_{50} and LC_{84} values. Phytochemical screening of the plant extract revealed the presence of various bioactive compounds such as saponins, flavonoids, alkaloids, terpenoids, cardiac glycosides, and tannins. These compounds may contribute to the observed molluscicidal effect by interfering with the snails' physiological metabolism. The findings suggest that *A. leptopus* leaf extract holds promise for the development of eco-friendly herbal molluscicides for snail control or as a means of stupefying snails for harvesting purposes. However, further research is necessary to identify the specific compounds responsible for the molluscicidal activity and to assess the potential impacts on non-target organisms and the environment. These investigations will provide a better understanding of the extract's mechanism of action and its safety profile, contributing to its potential application in snail management strategies.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest regarding the publication of this article.

Credit Author Statements

Dulal Kumar De: Conceptualization, funding acquisition, project administration, resources, original draft writing and methodology development

Bratati Bhanja: Experiment, observation and Data collection and recording/ reporting of data, Software use, statistical analysis.

Tapan Seal: Data curation, validation, review and editing.

Rama Prasad Bhattacharya: Visualization, supervision and revision.

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Fig. 1. Flowering twig of *Antigonon leptopus* Hook.and Arn

Fig. 2. Experimental Snail

Fig. 3. Effect of *Antigonon leptopus* in TDS during exposure period

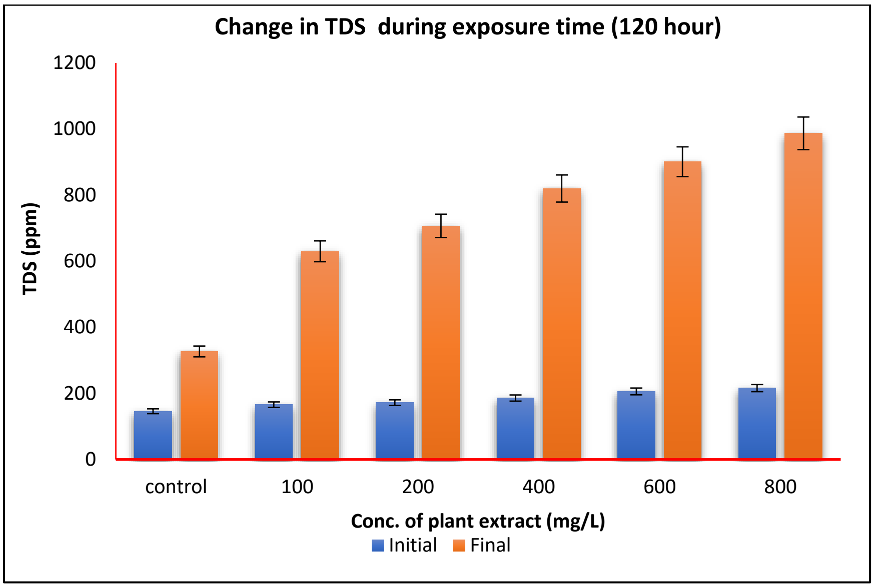

Fig. 4. Effect of *Antigonon leptopus* in pH during exposure time

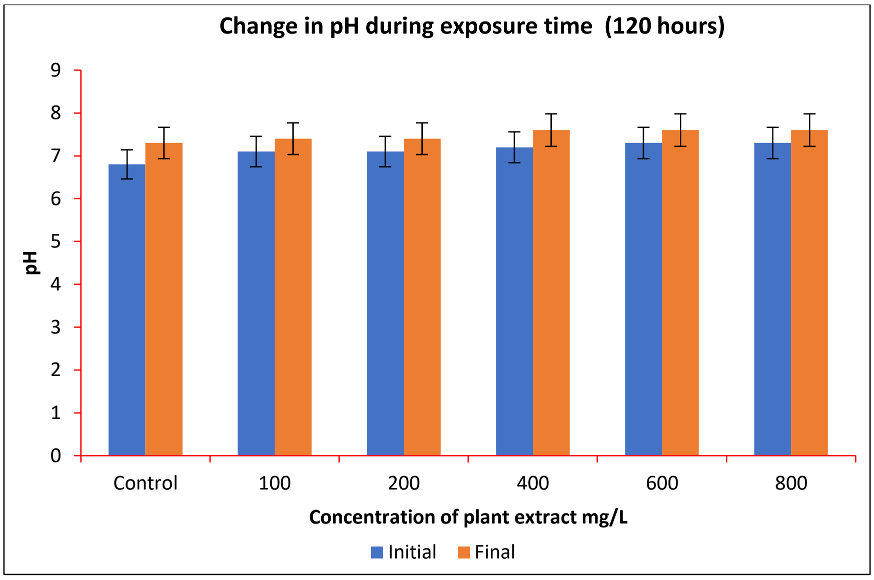

Fig. 5. Effect of *Antigonon leptopus* in salinity during exposure time

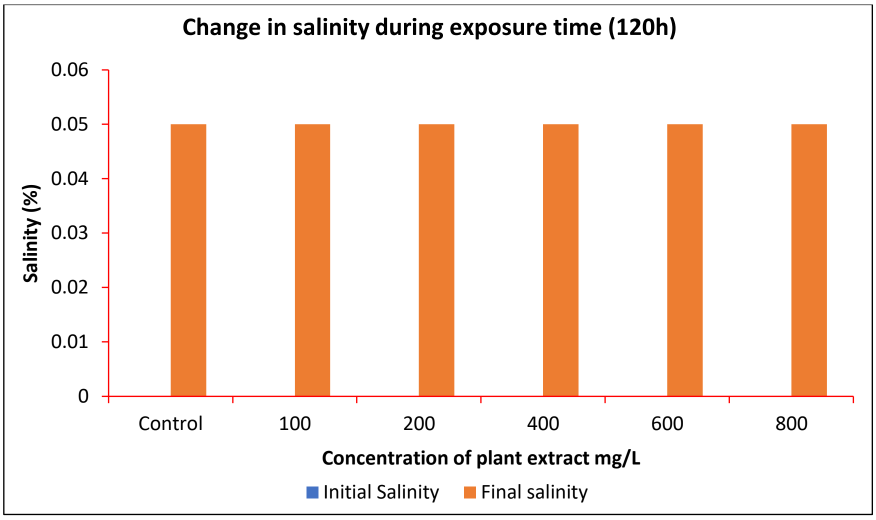

Fig. 6. Effect of *Antigonon leptopus* in dissolved oxygen during exposure time

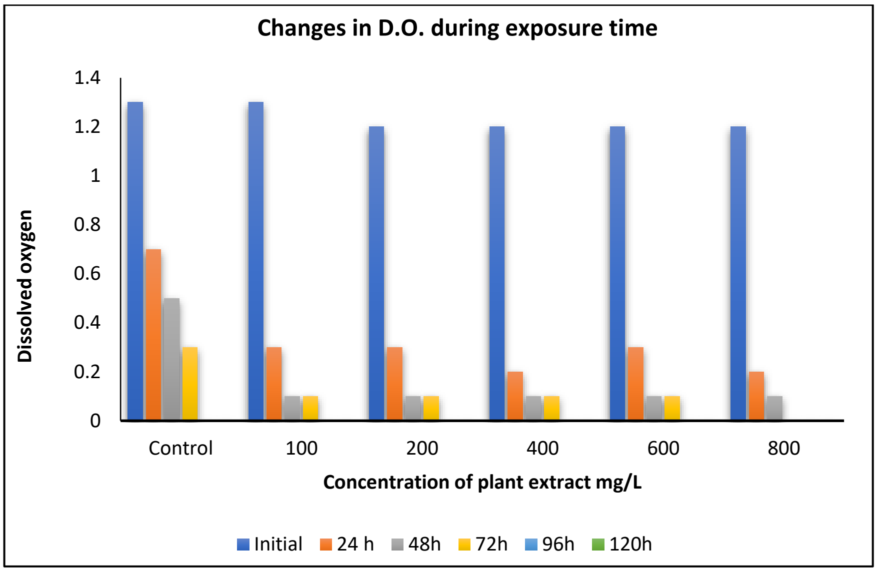

Fig. 7. Effect of *Antigonon leptopus* on *B. bengalensis*

Fig. 8. Effect of *Antigonon leptopus* extract on L. marginalis