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***Research Article** (Open Access)*

EFFECT OF THE EXTRACT OF *MORUS ALBA* BARK AND *ABRUS PRECATORIUS* SEEDS AND THEIR ACTIVE COMPONENTS ON THE REPRODUCTIVE PATTERN IN FRESH WATER SNAIL *LYMNAEA ACUMINATA*

Km Suman and Vinay Kumar Singh*
Malacology Laboratory
Department of Zoology, Deen Dayal Upadhyaya Gorakhpur University,
Gorakhpur- 273009 (U.P.), Bharat
**Author for Correspondence: vinaygkpuniv@gmail.com*

ABSTRACT
Effect of sub lethal treatment (20% and 60% of 24h and 96h LC_{50}) of the crude extract and active molluscicidal component of plants *Morus alba* (apigenin, morusin) and *Abrus precatorius* (abrin) on the reproduction and biochemical changes of the snail *Lymnaea acuminata* is studied. Earlier it has been reported in our laboratory that apigenin, morusin and abrin are active molluscicide. In the present study, it has been noted that crude extract as well as active molluscicidal component caused a significant reduction in the fecundity, hatchability and survival of young snails. There was a significant negative correlation in between the fecundity and exposure time. Withdrawal of the snails to fresh water after 96h exposure period caused a significant recovery in the fecundity of the snail *Lymnaea acuminata*. Treatment with 60% of 96h LC_{50} apigenin, morusin and abrin caused significant decrease in protein, (19.75 of control), Total amino acid (48.93 of control), DNA (16.92 of control) and RNA (19.60 of control) in gonadal tissue of snail *L. acuminata* after 96h exposure period. Biochemical changes in the gonadal tissue of *L. acuminata* are the cause of decrease in fecundity, hatchability and survival of young snails. This effect is reversible in nature so that their use at sub lethal concentration is eco-friendly.

***Keywords*:** *Morus alba, Abrus precatorius, Fecundity, Protein, Nucleic acid, Lymnaea acuminata*

INTRODUCTION
Fasciolosis is caused by *Fasciola hepatica* and *F. gigantica* (Arjona *et al*., 1995). Fresh water snail *Lymnaea acuminata* act as the intermediate host of *Fasciola* sp. These flukes complete their life cycle within the mollusc an intermediate host snail and a final mammalian host (Singh *et al*., 2021). Effective control of fasciolosis transmission, involves reducing the population of vector snails. The snail *L. acuminata* reproduces round the year and lays eggs on the underside of aquatic vegetation leaves. Although various synthetic molluscicides have been utilized to control vector snail population, yet its prolonged use caused many toxic effects. Some time it also caused on resurgence of mollusc populations (Garba *et al* 2024). Treating *Fasciola* in mammalian hosts requires multiple doses of anti-helmintic drugs, which caused side effects (Abdul-Samie *et al*., 2010). It is recommended in snail management programs that if molluscicides cans reduce their reproductive capacity, even at sublethal concentrations, it will be more effective in controlling of fasciolosis (Singh *et al*., 2021).
The present study aims to evaluate the anti-reproductive activity of *Abrus precatorius* L. (Fabaceae) seed and *Morus alba* L. (Moraceae) and their active component against the snail *L. acuminata*. *A. precatorius* is potential herbal medicine. It is known as Indian licorice, Crab's eye, Jequirity and Rosary pea. It is also called Gunja in Sanskrit and Ratti in Hindi. It is found in tropical and subtropical regions worldwide (Aswin *et al*., 2022). *A. precatorius* seeds can treat skin diseases, ulcers, and nervous system disorders. When processed into a paste, the seeds can be applied to the skin for shoulder joint stiffness, sciatica,

bruises, and paralysis (Aswin *et al*., 2022). The seeds can also serve as a laxative, but in large doses, they are toxic and can cause cholera-like symptoms. Additionally, *A. precatorius* seeds can be used as a natural contraceptive (Bhakta *et al*., 2019, Akbar, 2020). Earlier Singh and Singh (1999) reported that the natural molluscicidal activity of *A. precatorius* seed and its active component abrin against snail *L. acuminata. Morus alba* L. (Moraceae), from the genus *Morus,* is found in Africa, South America, and Asia. *M. alba* has a wide range of medicinal applications, either as a single drug or in compound formulations for various ailments (Mahmood *et al*., 2013). *M. alba* used in Unani medicine as an anti-tissue, diuretic, expectorant and hypotensive. The phenolic compounds in *M. alba* exhibit antioxidant and antibacterial activities (Suriyaprom *et al.,* 2021). The bark of *M. alba* has anti-helminthic properties, and its extracts show antibacterial and fungicidal activities (Rao *et.al*. 2012). Earlier Hanif *et al.* (2013) have reported the molluscicidal activity of the bark and leaf of *Morus alba* against the target snail *L. acuminata* investigates further its potential and managing fasciolosis.

MATERIALS AND METHODS

Test animal

The adult *L. acuminata* (measuring 2.60 ± 0.30 cm in length) were collected from nearby lakes, ponds, and pools and kept in a glass tank with dechlorinated tap water for 72h in order to acclimatize them to laboratory condition. *L. acuminata* are hermaphrodite although self-fertilization uncommon. These snails deposit their eggs on the underside of aquatic vegetation's leaves in the form of gelatinous capsules holding 5-300 eggs. According to Singh and Singh (2004) groups of twenty snails in 5 l of dechlorinated water were subjected to sublethal doses (20 and 60% of 24 h LC_{50} and 96h LC_{50}) of *M. alba* bark and *A. precatorius* seed extract and their active component. Every 24 up to 96 h, the total number of eggs laid by the treated and control groups of snails was counted. Capsules containing eggs from each treated group were incubated at 30°C±1°C in covered Petri-dishes holding the same concentration as supplied to adult snails, since it is challenging to identify the mother snail for a specific spawn. Up to hatching, the development of the embryos was monitored under a binocular microscope at regular intervals. In this study only the percentage of eggs that hatched after being exposed for 24 h were counted. The removal of dead embryos was done to prevent contamination. Young snail's survival was monitored up to 72 h after hatching. The withdrawal trials involved withdrawal of the snails to the aforesaid 96 h after treatments for 96 h, following which they were moved to fresh water (Singh *et al.* 1999; Singh and Singh, 2004) and fecundity of snail was noted for next 72 h.

Pure Compounds

Pure active molluscicidal component of *Morus alba* (Apigenin, Morusin) and *Abrus precatorius* (Abrin) were procured from sigma chemical Co (USA).

Biochemical analysis

Snails were subjected to sublethal concentrations of apigenin, morusin and abrin (20 and 60% of 24h LC_{50} and 96h LC_{50}) (Table 1). Six batches were made for each concentration. The sole treatment used in the control aquarium was dechlorinatedtap water. The treated snails were taken out of the aquaria and given a water rinse after a 24h period. The gonadal tissue was promptly taken out, weighed, and then put on filter paper to get rid of any clinging water. Various biochemical tests were conducted on both the treatment and control groups.

Estimation of Protein

Levels of protein (µg/mg) in gonadal tissue estimated according to the method of Lowry *et al*. (1951) with a standard of bovine serum albumin. Tissue homogenates (1 mg/ml, w/v) were prepared using 10 percent w/v trichloroacetic acid (TCA). Values have been expressed as µg protein/mg tissue.

Estimation of total free Amino Acids

Estimates of total free amino acid in the gonadal tissue of snail (µg/mg) were done using the method of Spies's (1957). Free amino acids have been expressed as µg/mg tissue.

Table 1. Sublethal concentrations (20% and 60% of the 24h LC_{50} and 96h LC_{50}) of *M. alba* crude bark and of *A. precatorius* seed extract and their active component against *L. acuminata*.

Molluscicides	**24h LC_{50} (mg/l)**	**20% of 24h LC_{50} (mg/l)**	**60% of 24h LC_{50} (mg/l)**	**96h LC_{50} (mg/l)**	**20% of 24h LC_{50} (mg/l)**	**60% of 24h LC_{50} (mg/l)**
Crude bark extract of *Morus alba**	325.19	65.03	195.11	173.17	34.63	103.90
Apigenin*	12.57	2.51	7.54	1.92	0.38	1.15
Morusin*	13.40	2.68	8.04	2.12	0.42	1.27
Crude seed extract of *Abrus precatorius***	196.10	39.22	117.66	93.83	18.76	56.29
Abrin**	0.36	0.07	0.2	0.04	0.008	0.02

**Hanif and Singh, 2012 **Singh and Singh, 1999.*

Table 2. Effect of sublethal exposure (20% and 60% of 24h LC_{50}) to crude bark of *M. alba* and *A. precatorius* seed and their active component molluscicide on the fecundity of the *Lymnaea acuminata.*

Molluscicide(s)	Sublethal Concentration (mg/l)	Fecundity (eggs/20 snails)			
		24h	**48h**	**72h**	**96h**
Control		260.0±3.15	255.1±3.21	252.5±2.53	246.0±2.75
Crude bark extract of *M. Alba*	20% of LC_{50} (65.03)	205.1±0.69*†	196.6±0.91*	190.9±0.74*	180.1±0.74*
	60% of LC_{50} (195.11)	185.3±0.74*†	145.9±0.70*	122.9±0.76*	102.7±0.74*
Apigenin	20% of LC_{50}(2.51)	195.9±0.80*†	180.9±0.75*	165.0±0.73*	110.0±0.77*
	60% of LC_{50} (7.54)	165.8±0.76*†	160.8±0.75*	112.9±0.68*	95.0±0.78*
Morusin	20% of LC_{50} (2.68)	190.9±0.77*†	145.2±0.81*	130.2±0.76*	110.1±0.74*
	60% of LC_{50} (8.04)	115.2±0.74*†	100.2±0.78*	85.0±0.76*	83.9±0.78*
Crude Seed extract of *A. precatorius*	20% of LC_{50} (39.22)	175.9±0.82*†	186.8±0.75*	175.7±0.79*	165.8±0.74*
	60% of LC_{50}(117.66)	139.6±0.90*†	130.8±0.65*	120.2±0.76*	110.8±0.79*
Abrin	20% of LC_{50} (0.07)	132.1±0.79*†	125.9±0.77*	115.0±0.77*	100.9±0.73*
	60% of LC_{50} (0.2)	95.8±0.81*†	86.8±0.76*	80.8±0.80*	70.9±0.75*

***Note:** Each value is the mean ± SE of six replicates; each replicate represents the eggs laid by the group of 20 snails.*
**Significant ($p<0.05$) by student's 't' test applied to treated and control groups.*
† Product-moment correlation coefficient showed that there was a significant ($p<0.05$) negative correlation between exposure period and fecundity of the L. acuminata.

Estimation of Nucleic Acids

DNA and RNA in the gonadal tissue of *L. acuminata* were estimated according to the method of Schneider (1957) using diphenylamine and orcinol reagents, respectively (µg/mg). Gonadal tissue homogenates (1 mg/ml, w/v) were preparedin 5% TCA and centrifuged at 5000g for 20 min. and supernatant used for the estimation. For DNA standard curves were drawn using different concentration ofcalf thymus, DNA, whereas RNA standard curveswere drawn using yeast RNA as standard. Both DNA and RNA have been expressed as µg/mg tissue.

Statistical Analysis

The data have been expressed of as mean ± SE at least six times. The student's t-test was applied between controls and treated groups to analyses significant changes ($P < 0.05$), if any. A product-moment correlation coefficient was used to determine the relationship between snail fecundity/ hatchability/ survival and exposure time (Sokal and Rohlf, 1973).

Table 3. Effect of sub lethal exposure (20% and 60% of 24h LC_{50}) to crude bark of *M. alba* and seed extract of *A. precatorius* and their active component of molluscicide on the hatchability and survival of eggs of the snail *Lymnaea acuminata*.

Molluscicide(s)	Fecundity after 24h(eggs/20 snails)	Hatchability percentage	Percent survival		
			24h	**48h**	**72h**
Control	260±3.15	100(7-10)	100	100	100
Crude Bark Extract of *M. alba*	105.1±0.69	95.0±0.4 (9-11)	93.0±0.47*†	90.0±0.45*	88.0±0.44*
	185.7±0.74	83.0±0.42 (9-12)	75.0±0.38*†	70.0±0.35*	64.0±0.32*
Apigenin	195.9±0.80	96.0±0.76 (8-10)	84.0±0.66*†	72.0±0.57*	68.0±0.53*
	165.8±0.76	94.0±0.74 (9-12)	79.0±0.76*†	69.0±0.54*	60.0±0.47*
Morusin	190.9±0.77	92.0±0.72 (8-13)	85.0±0.67*†	79.0±0.62*	75.0±0.59*
	115.2±0.74	90.0±0.71 (10-13)	80.0±0.63*†	65.0±0.51*	58.0±0.45*
Crude Seed Extract of *A. precatorius*	175.9±0.82	90.0±0.45 (10-11)	84.0±0.42*†	74.0±0.39*	73.0±0.37*
	139.63±0.90	85.0±0.43 (9-12)	80.0±0.40*†	75.0±0.38*	68.0±0.34*
Abrin	132.1±0.79	65.0±0.51 (8-13)	60.0±0.47*†	56.0±0.44*	51.0±0.40*
	95.8±0.81	58.0±0.47 (8-13)	52.0±0.41*†	39.0±0.30*	30.0±0.23*

***Note:** Each value is the mean ± SE of six replicates; each replicate represents the eggs laid by the group of 20 snails. Values given in parenthesis indicate the hatching periods.*
**Significant ($p<0.05$) by student's t' test applied to treated and control groups.*
† Product-moment correlation coefficient showed that there was a significant ($p <0.05$) negative correlation between exposure time and survival of the miniature snail.

RESULTS AND DISCUSSION

Effect on Fecundity

A group of twenty snails laid 246-260 eggs/day in control groups. There was a significant ($P < 0.05$) reduction in the fecundity after *Lymnaea acuminata* treated with 20% and 60% of 24h LC_{50} and 96h LC_{50} of two plants namely crude bark extract of *M. alba* and its active component (apigenin and morusin) and seed extract of *A. precatorius* (abrin) the active component, respectively (Table-2). Maximum decrease in fecundity (70.9 eggs/20 snails) within 24h was observed in snail expressed to 60% of 24h LC_{50} of abrin (Table-2). The maximum reduction in fecundity (83.9 eggs/20 snails) was observed in 60% of 96h LC_{50} (Table-4). There was a significant negative correlation between the exposure period and fecundity. Withdrawal of snails after exposure to 20% and 60% of 96h LC_{50} of *M. alba* and *A. precatorius* plant extract and their active components apigenin, morusin and abrin to fresh water indicates that there was a positive correlation between the withdrawal period (24h, 48h and 72h) and fecundity of withdrawn snails (Table-6). The order of reduction in fecundity of snails exposed to higher concentration of both plants and their active component.

Hatchability and Survival

Percentage hatchability and hatching period was observed in the eggs laid after 24h treatment of different compounds.In control groups all the eggs hatched into young ones at temperature 30°C±1°C for 7-10 days. Percent hatching of eggs of snails exposed to 20% and 60% of 24h LC_{50} and 96h LC_{50} of *M. alba* bark extract and its active component (apigenin and morusin) and seed extract of *A. precatorius* and its active component (abrin). Maximum reduction in hatching (58% of control) was observed in 60% of 24h LC_{50} treated with abrin the active component of *A. precatorius* (Table-3). Same trend was found, when abrin 60% of 96h LC_{50} treated to snails. The maximum reduction was found (47.0% of control) Table (3 and 5). Hatching period was prolonging to 9-18 days with respect to control groups (7-10 days) (Table 3 and 5). The newly hatched snails were mostly found attached to the wall of the container. They have very thin shell in comparison to control groups. Movement of newly hatched snails in treated groups was slow with smaller tentacle in comparison to control groups. Survival time and survival of young snails hatched from the eggs laid by snails exposed to crude bark extract of *M. alba* and their active component (apigenin, morusin) and *A. precatorius* seed and their active component (abrin) caused maximum mortality (30.0 % of control) in offspring's after 72h hatching period of 24h LC_{50} and maximum mortality (35.0 % of control) in offspring's after 72h hatching period of 96h LC_{50} (Table 3 and 5). In withdrawal experiments snails were withdrawn from treatments of 96h LC_{50} of crude bark extract of *M. alba* (apigenin, morusin) and crude seed extract of *A. precatorius* (abrin) for 96h and then for next 72h a significant recovery in fecundity of snail *L. acuminata* was noted (Table 6). There was a significant positive correlation between withdrawn period and the fecundity of withdrawn snails. Maximum recovery (240.2 eggs/20 snails) in fecundity was observed in snail withdrawal after 96h treatment of 20% of crude extract of *M. alba* (Table 6).

Biochemical changes

The biochemical changes in gonadal tissue of *L. acuminata* exposed to different treatments of 20% and 60% of 96h LC_{50} of plant molluscicides *M. alba* and *A. precatorius* and their active component (apigenin, morusin and abrin). After 96h of exposure changes in the level of protein, total amino acids, DNA and RNA in gonadal tissue of snails were measured. The gonadal tissue of *L. acuminata* had a protein level of (91.6 µg/mg) at 96h LC_{50}. Snails subjected to 60% of the 96h LC_{50} of abrin showed the significant decrease in the protein content (19.75% of control) in gonadal tissue of *L. acuminata* (Table 7). In control group's the levels of free amino acids in the gonadal tissue of *L. acuminata* was (31.9 µg/mg). The active component of *M. alba* (apigenin and morusin) caused significant decrease in the levels of amino acid (37.93% and 46.70 of control) exposed to 60% of 96h LC_{50}, respectively (Table 7). In the control groups, the levels of DNA in the gonadal tissue of *L. acuminata* were (76.8 µg/mg). The gonadal tissue of *L. acuminata* treated with the 60% 96h LC_{50} of abrin showed the maximum reduction in DNA (16.92% of

control). In the control groups, RNA levels was (65.8 µg/mg).There was a significant reduction in the gonadal tissue of *L. acuminata* treated to the 60% 96h LC_{50} of abrin showed the maximum decrease RNA (19.60% of control).

Table 4. Effect of sublethal exposure *(*20% and 60% of 96hLC_{50}) to crude bark of *M. alba* and *A. precatorius* seed extract and their active component on the fecundity of the *Lymnaea acuminata*.

Molluscicide(s)	Sublethal dose concentration (mg/l)	Fecundity (eggs/20 snails)			
		24h	**48h**	**72h**	**96h**
Control		**260.0±3.15**	**255.1±3.21**	**252.5±2.53**	**246.0±2.75**
Crude Bark extract of *M. alba*	20% of LC_{50} (34.63)	213.1±0.51*†	209.1±0.69*	204.9±0.89*	201.7±1.17*
	60% of LC_{50} (103.90)	190.0±0.77*†	150.9±0.69*	130.9±0.76*	105.0±0.77*
Apigenin	20%of LC_{50}(0.38)	148.9±0.79*†	122.0±0.87*	116.9±0.75*	100.1±0.70 *
	60%of LC_{50} (1.15)	114.1±0.75*†	106.0±0.72*	99.2±0.82 *	95.0±0.76 *
Morusin	20% of LC_{50} (0.42)	171.0±0.74 *†	156.9±0.78*	147.0±0.74*	144.0±0.75*
	60% of LC_{50} (1.27)	113.9±0.77 *†	105.8±0.74*	92.7±0.78*	83.9±0.69*
Seed extract of *A. precatorius*	20% of LC_{50}(18.76)	200.1±0.78*†	195.0±0.72*	178.0±0.73*	157.0±0.77*
	60% of LC_{50} (56.29)	146.9±0.77*†	119.8±0.68*	109.7±0.71*	101.8±0.71*
Abrin	20% of LC_{50} (0.008)	141.1±0.70*†	128.1±0.73*	122.8±1.90*	112.6±1.83*
	60% of LC_{50}(0.02)	109.1±0.78*†	101.0±0.65*	96.1±0.80*	83.9±0.81*

***Note:** Each value is the mean ± SE of six replicates; each replicate represents the eggs laid by the group of 20 snails.*
**Significant ($p<0.05$) when student's 't' test was applied in between treated and control groups.*
† Product-moment correlation coefficient showed that there was a significant ($p<0.05$) negative correlation between exposure period and fecundity of the L. acuminata.

It is clear from the results section that the 20% and 60% of 24h LC_{50} and 96h LC_{50} of *M. alba* bark extract and their active component (apigenin and morusin) and *A. precatorius* and their active component (abrin) significantly reduced the reproductive capacity of snail *L. acuminata.* The time dependent reduction in fecundity is evidenced by the negative correlation between fecundity and exposure time. There are a number of pharmacological effects of *M. alba* and *A. precatorius* have been established (Timalsina *et al*., 2021). Yet, its reports on the snail reproduction are not well explored. Apigenin is a naturally occurring substance from plants and the aglycon of numerous naturally occurring glycosides belong to the flavones class (Kashyap *et al*., 2018). The enzyme CYP2C9, which is a charge of the body's metabolism of numerous pharmacological medications, is strongly inhibited by apigenin (Si *et al.*, 2009). The flavonoid morusin exhibit strong cytotoxicity against the murin leukemia cell P-388, with an IC_{50} of 3.1 Sg/ml

(Suhartati *et al.*, 2009). Antifertility activity was one of the stimulating outcomes of *Abrus* seeds (Nwodo, 1991).

Table 5. Effect of sublethal exposure (20% and 60% of 96h LC_{50}) crude bark extract of *M. Alba* and crude seed extract of *A. precatorius* plant and their active component molluscicide on the hatchability and survival of eggs of the snail *Lymnaea acuminata.*

Molluscicide (s)	Fecundity after 24h(eggs/20 snails)	Hatchability percentage	Percent survival		
			24 h	**48 h**	**72 h**
Control	**260±3.15**	**100 (7-10)**	**100**	**100**	**100**
Crude Bark Extract of *M. alba*	213.0±0.51	96.0±0.48 (9-12)	95.0±0.48*†	93.0±0.47*	89.0±0.45*
	190.9±0.77	85.0±0.65 (9-12)	83.0±0.42*†	82.0±0.41*	78.0±1.39*
Apigenin	148.9±0.79	98.0±0.77 (9-12)	87.0±0.68*†	75.0±0.59*	70.0±0.55*
	114.1±0.75	97.0±0.76 (10-13)	80.0±0.63*†	70.0±0.55*	62.0±1.95*
Morusin	171.0±2.14	96.0±0.76 (9-12)	90.0±0.71*†	80.0±0.63*	72.0±0.51*
	113.9±0.77	94.0±1.74 (11-14)	81.0±0.64 *†	68.0±0.53 *	60.0±0.47 *
Crude Seed Extract of *A. precatorius*	200.1±0.78	92.0±0.46 (9-13)	86.0±0.43*†	80.0±0.40*	78.0±0.39*
	146.9±0.77	85.0±0.43 (9-14)	76.0±0.38*†	70.0±0.39*	65.0±0.33*
Abrin	141.0±0.70	69.0±0.54 (9-13)	65.0±0.51 *†	60.0±0.47 *	55.0±0.43 *
	109.1±0.78	70.0±0.55 (9-14)	60.0±0.47 *†	42.0±0.33 *	35.0±0.27 *

Note: Each value is the mean ± SE of six replicates; each replicate represents the eggs laid by the group of 20 snails. Values given in parentheses indicate the hatching periods.
**Significant ($p<0.05$) by student's 't' test applied to treated and control groups*
† Product-moment correlation coefficient showed that there was a significant ($p<0.05$) negative correlation between exposure time and survival of the miniature snail L. acuminata.

It has been reported earlier that active molluscicidal component tigogenin, hecogenin and acetogenin reduced the fecundity of the snail *L. acuminata* by affective the CDC cells (Caudo-dorsal cells) (Singh *et al.*, 1999; Singh and Singh, 2004). CDC cells in the brain of the snail *L. acuminata* release ovulation hormone (Roubos e*t el.*, 1981). It seems that seeds of *A. precatorius* and active component (abrin) and (apigenin, morusin) an active component *M. alba* and bark extract may cause snails to absorb less oxygen, which may impact their metabolism and result in decreased fertility due to this they reduces the release of ovulation hormone, which resulted a decrease in the fecundity of treated snails. It is possible that the interference with the snail's embryonic growth and development is the cause of the decrease

hatchability of *L. acuminata* exposed to active component of apigenin, morusin, which is found in *M. alba* and abrin in *A. precatorius* seed. Because there is less surface tension between the water and the respiratory surface, freshly born snails may consume less oxygen, which could lead to a decrease in survival rate (Lamba, 1970). In treated groups some young larvae were weak; they are unable to break the egg capsule and died due to starvation. Young snails hatched from the treated egg masses showed much delay in attaining maturity in comparison to control groups. They were mostly found attached to the wall

Table 6. Effect of withdrawal after 96h exposure to crude bark of *M. alba* (apigenin, morusin) and crude seed extract of *A. precatorius* (abrin) molluscicides on fecundity of the snail *Lymnaea acuminata*.

Molluscicide(s)	**Fecundity after 96h(eggs/20 snails)**		
	24h	**48h**	**72h**
Control	268.1±0.70	263.1±0.68	258.0±0.64
Crude Bark Extract of *M. alba*	225.0±0.85*†	237.1±0.82*	240.2±0.79*
	218.3±0.88*†	230.0±0.71*	235.1±0.73*
Apigenin	190.1±0.76*†	195.9±0.72*	200.2±0.72*
	170.1±0.75*†	177.0±0.69*	186.0±0.81*
Morusin	180±0.83*†	187.9±0.79*	192.8±0.81*
	162.0±0.72*†	169.1±0.79*	182.9±0.70*
Crude Seed Extract *A. precatorius*	205.8±0.72*†	207.9±0.75*	216.8±0.78*
	195.1±0.77*†	209.8±0.80*	223.8±0.86*
Abrin	150.1±0.79*†	160.8±0.86*	175.8±0.78*
	130.1±0.83*†	142.1±0.83*	163.0±0.80*

***Note:** Each value is the mean ± SE of six replicates; each replicate represents the eggs laid by the group of 20 snails.*
**Significant ($p<0.05$) by student's 't' test applied to treated and control groups.*
† Product-moment correlation coefficient showed that there was a significant positive correlation between withdrawal time and fecundity of the snail L. acuminata after withdrawal.

of container and were apathetic towards feed. High percentage of mortality and low percentage of fecundity was found by the treatment of plant products and their active components (apigenin, morusin and abrin) suggests that there are able to control to population density of this snail by inhibiting their developmental at any stage. Significant decrease in the different biochemical parameter viz. Protein, total free amino acids, DNA and RNA in the gonadal tissue of *L. acuminata* indicate that the biochemical changes in the gonadal tissue of *L. acuminata* is the cause of reproduction in fecundity, hatchability and survival of young snails. Reduction in protein levels may be due to the direct interference of these plants

and their active component with the protein synthesis. Total free amino acids level in the gonadal tissue of the snail is lower than control. It indicates that they also interfere with the biosynthesis of amino acids in

Table 7. Effect of sub lethal concentration (20% and 60% of 96h LC_{50}) of active component (apigenin, morusin) of *M. alba* and (abrin) of *A. precatorius* seed on the (µg/mg) of protein, total free amino acid, DNA, and RNA in the gonadal tissue of the snail *Lymnaea acuminata* at 96h exposure period.

Molluscicide(s)		**Protein (µg/mg)**	**Total free Amino acid (µg/mg)**	**DNA (µg/mg)**	**RNA (µg/mg)**
Control		91.6±0.59* (100)	31.9±0.35* (100)	76.8±0.43* (100)	65.8±0.60* (100)
Apigenin	20% of LC_{50}	56.0±0.78* (61.13)	20.0±0.75* (62.69)	50.7±0.73* (66.01)	44.8±0.79* (68.08)
	60% of LC_{50}	39.9±0.78* (43.55)	12.1±0.38* (37.93)	43.8±0.80* (57.03)	35.0±0.74* (53.19)
Morusin	20% of LC_{50}	68.7±0.46* (75.0)	24.7±0.78* (77.42)	54.2±0.83* (70.57)	49.8±0.92* (75.68)
	60% of LC_{50}	42.1±0.25* (45.96)	14.9±0.77* (46.70)	47.6±0.32* (61.97)	39.2±0.57* (59.57)
Abrin	20% of LC_{50}	26.4±0.28* (28.82)	23.8±0.84* (74.60)	20.8±0.80* (26.17)	18.4±0.59* (28.06)
	60% of LC_{50}	18.1±0.55* (19.75)	15.61±0.32* (48.93)	13.0±0.97* (16.92)	12.9±0.88* (19.60)

***Note:** Each value is the mean ± SE of six replicates.*
**Significant ($p<0.05$) by student's 't' test applied to treated and control groups.*
Values given in parentheses indicate the percent level, with control taken as 100%.

the cells (Singh *et al.*, 2010). The present result indicates that the following treatment with 20% and 60% of 96h LC_{50} of active component of these plant. The level of DNA was reduced in a time and dose dependent manner. DNA is an index of cell number and decreases in DNA content indicates of cell death, and it may be caused by release of toxic substances. There is change in the level of RNA is noted with reduction in RNA level, there is bound to be a fall in protein levels because of reduction in the synthesis of new protein (Singh *et al*., 2008).

CONCLUSION

The present study clearly indicates that sub lethal treatment of plant *Morus alba* and A. *precatorius* and their active components apigenin, morusin and abrin significantly change the reproductive pattern and biochemical parameters in freshwater snail *L. acuminata*. This effect is reversible in nature so that their use at sublethal concentration is ecofriendly. Since these plant molluscicides are the safest and cost-effective method for control of freshwater snail as compared to other synthetic counterparts that plant molluscicides harmless to other non-target aquatic biotas.

Authors' Contributions

VKS suggested the concept, design, and the topic and provided the technical guide. Km Suman performs the experiment and writing the first hand manuscript. Both the authors read and approved the final manuscript.

***Conflict of Interest:** No*

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