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

SOLANUM NIGRUM SEED POWDERED EXTRACTS ARE USED AS POTENT MOLLUSCICIDES TO CONTROL HARMFUL SNAIL *LYMNAEA ACUMINATA*

***Pradeep Kumar**
Department of Zoology, Government Degree College
Sukrauli, Kushinagar, (U.P.) India, 274207
**Author for Correspondence: pkumar_gpu@yahoo.co.in*

ABSTRACT
One of the major carriers of *Fasciola hepatica* and *F. gigantica* is the freshwater host snail *Lymnaea acuminata.* These cause liver fluke among cattle's and humans. One important strategy to lower the liver fluke is to manage the host snail population. The effectiveness of synthetic molluscicides is higher, but they have adverse ecological and non-target organism impacts. The aim of the present study was to assess *S. nigrum* molluscicidal efficiency against the host snail *L. acuminata*. The molluscicidal efficacy of *S. nigrum* was found to be both concentration and time dependent. For 96 hours, the toxicity of *S. nigrum* dry seed powder and its various organic extracts and column purified seed powder against *L. acuminata* was continually observed at varying concentrations. Toxic effects were observed for 24, 48, 72 and 96h. Host snail mortality was measured every 24h for 96h, and lethal concentration (LC_{50}) values were calculated. The 24h LC_{50} of dried seed powder of *S. nigrum* were 175.21mg/l and at 96h 165.41 mg/l. The 24h and 96h LC_{50} of column purified fraction of dried seed powder of *S. nigrum* were 95.25 and 73.25 mg/l, respectively. The present study found that *S. nigrum* formulations could be effective molluscicides for controlling host snails.

Keywords: *Solanum nigrum; Molluscicides; Lymnaea acuminata; Liver fluke*

INTRODUCTION

Fasciola hepatica and *F. gigantica* are the causative agents of fascioliasis, a significant trematode parasite disease in tropical and subtropical regions (Hacariz *et al.,* 2014). *Fasciola* infections have been reported in 81 countries worldwide (Furst *et al.,* 2012). In general, the distribution of fascioliasis is worldwide zoonotic disease in ruminant animals and humans (Mas-Coma *et al.,* 2014; Cwiklinski *et al.,* 2016; Ram and Kumar, 2025). However, these parasitic diseases in India are mainly caused by *F. gigantica* (Dalton, 1999). *Fasciola* has a complex lifecycle among intermediate host snails and definitive mammals hosted, including humans (Carvedo and Cabad, 2020). Their presence in the liver of cattle reduces the productivity of ruminants, especially livestock with significant economic value (Kuchai *et al.,* 2011; Eshetu *et al.,* 2017; Kumar, 2020; Kumar, 2021). In the northern part of Uttar Pradesh (India), freshwater snail *Lymnaea acuminata* is an intermediate host of *F. gigantica*, which is responsible for endemic fasciolosis (Singh and Agarwal, 1981; Kumar and Singh, 2006; Kumar *et al.,* 2011; Kumar *et al.,* 2012; Kumar *et al.,* 2013a; 2013b; Kumar and Singh, 2014; Kumar *et al.,* 2016; Kumar *et al.,* 2018; Kumar *et al.,* 2020). Certain slug and snail species are also harming crops, which results in higher financial losses (Kumar, 2020). Therefore, the control of the harmful snail population, thereby breaking the life cycle of *Fasciola* (Kumar *et al.,* 2009; Kumar *et al.,* 2018; Kumar, 2021; Ram and Kumar, 2025) and reducing the fascioliasis and economic loss. Synthetic molluscicides that are widely recognized can be used to control the number of harmful snails. The use of synthetic molluscicides has been advocated because; it is not safer for the environment (Agarwal and Singh, 1988). Due to their increased acceptability, safety for non-target organisms, biodegradability, and friendly to the environment, plant products are increasingly being

used as alternative for synthetic molluscicides (Harston and Hostettmann, 1985; Kumar, 2021; Kumar, 2024).

The herbaceous weed plant *Solanum nigrum* is commonly known as garden nightshade or black nightshade in English, Munatakali in Tamil, Gudakami in Bengali, Kachchipandu in Telugu, and Makoya in Hindi (Mani *et al.,* 2022). It is an annual plant that can reach a height of 25 to 100 cm. Simple subescent hairs that are coarsely pubescent and angular cover the entire plant (Mani *et al.,* 2022). *S. nigrum* contains a variety of phytochemicals compounds which have various pharmacological activities. Polyphenolic components such as gallic acid, catechin, and naringenin, as well as polysaccharides, glycoalkaloids, and glycoproteins, are active ingredients of *S. nigrum* (Ravi *et al.,* 2009). *S. nigrum* leaves are frequently used to treat skin diseases, gouty joints, joint pain, and anti-tuberculosis medications (Chopra *et al.,* 1956). The gastric mucosa is protected against ulceration by the methanolic extracts of *S. nigrum* (Jainu and Devi, 2004). The active ingredients of ethyl acetic acid, which is used as a mosquito larvicidal agent, are extracted from *S. nigrum* (Bhatia *et al.,* 2011). The aim of the present study is to evaluate the molluscicidal efficacy of *S. nigrum* seeds against freshwater host snail *L. acuminata.*

MATERIALS AND METHODS

Collection of Lymnaea acuminata

Adult snail *L. acuminata* were collected from low-lying submerged fields and ponds from Sukrauli, Kushinagar (U.P.) India. The same size (2.63±0.34 cm in length) snails were acclimatized for 72 hours in dechlorinated tap water at 28±4^{0}C in lab conditions. The pH of the water was 7.2-7.3 and DO (dissolved oxygen), free CO_2, and bicarbonate alkalinity were 6.1-7.2 mg/l, 5.3-6.5 mg/l, and 105.0-106.0 mg/l, respectively.

Preparation of plant crude products

The fresh seed of *S. nigrum* was isolated from mature ripe fruits and washed with fresh water and dried in sunlight for 10 to 15 days and pulverized in the electric grinder for crude powders thus obtained, which were then sieved with the help of fine mesh cloth. This fine crude powder was then used for experiments against the host snail *L. acuminata*.

Extraction of seed powder in organic solvent

Two gram seed powders of *S. nigrum* were separately extracted with 250 ml of 98% ether, 99.7% chloroform, 98% methanol, 98% acetone, and 95% ethanol at lab conditions for 72h. Each preparation was separately filtered through sterilized Whatman No-1 filter paper and filtered extracts were subsequently evaporated under a vacuum machine (Jaiswal and Singh, 2008). The seed powder of *S. nigrum* yielded 112 mg ethanol, 106 mg chloroform, 112 mg ether, and 120 mg acetone extracts. The residues, thus obtained, were used for the determination of lethal concentration (LC_{50}) values against *L. acuminata*.

Column extracts purification

Two litter ethanol organic extract fraction of dried crude seed powder of *S. nigrum* were subjected to silica gel (60-120 mesh, Qualigens Glass, Precious Electrochemidus Private Limited, Bombay, India) chromatography through a 5 × 45 cm column. Fifty-milliliter fractions eluted with ethanol (95%) were collected. Ethanol was evaporated under a vacuum machine and the remaining solids column extract obtained were used for the determination of LC_{50} value of each fraction.

Determination of toxicity (LC_{50}) value

Toxicity of crude powder, different organic extracts and column purified of *S. nigrum* seed was performed by the method of Kumar and Singh, (2006). In each experiment ten host snails were kept in a clean glass aquarium containing 3 liters dechlorinated tap water. These experimental snails were exposed continuously for 96h to different concentrations and preparation of *S. nigrum* seed powder and snail mortality was observed for 24, 48, 72, and 96h. Six experimental aquariums were set up for each concentration. Snail mortality was recorded at intervals of 24h each up to 96h. In the control groups of experiment the snails were kept in an equal volume of water under similar laboratory conditions without

treatment. Snail's mortality was established by the contraction of the body within the shell, no response to the touch by needle probe was declaring evidence of snail death. The values of LC_{50}, slope values, t-ratio, 'g' value, and heterogeneity factor were calculated using the POLO computer program (Robertson *et al.,* 2007).

RESULTS

The dried seed powder of *S. nigrum*, and their different organic extracts, and column fractions against snail *L. acuminata* were time and concentration-dependent. The 24h LC_{50} of dried seed powder of *S. nigrum* were 175.21 mg/l and at 96h 165.41 mg/l (Table-1). Among all the organic extract the ethanol extract of dried seed powder of *S. nigrum* were more effective against *L. acuminata*. The 24h, 48h, 72h, and 96h LC_{50} were 155.21, 150.23, 149.12 and 145.40 mg/l, respectively (Table-1) (Fig-1). Whereas, the ethanolic column purified fractions of all the organic extract were highly toxic against the host snail *L. acuminata*. The 24h LC_{50} of the column purified fractions of dried seed powder of *S. nigrum* were 95.25 mg/l. The 96h LC_{50} of column purified fraction of dried seed powder of *S. nigrum* were 73.26 mg/l (Table-1) (Fig-1).

The slope values given in Table-1 were steep and the separate estimates of LC based on each of the six replicates were found to be within the 95% confidence limits of LC_{50}. The t- ratio was greater than 1.96 and the heterogeneity factor was less than 1.0. The g- value was less than 0.5 at all probability levels (90, 95, and 99) (Table-1).

DISCUSSION

According to a phytochemical investigation, the whole *Solanum nigrum* plant contains proteins, carbohydrates, flavonoids, tannins, alkaloids, glycosides, saponins, phytosterols, and coumarins (Nyeem *et al.,* 2017). The organic extracts ether, chloroform, acetone, ethanol, and column purified fraction of *S. nigrum* seed powder have molluscicidal activity at 24, 48, 72, and 96h exposure the LC_{50} value against fresh water snail *Lymnaea acuminata* were 95.25, 90.36, 84.37, and 73.26 mg/l, respectively. Ravi *et al.,* (2009) has been reported that *S. nigrum* contain a variety of compounds that have pharmacological properties. Active ingredients include polysaccharides, glycoproteins, and glycoalkaloids; polyphenolic

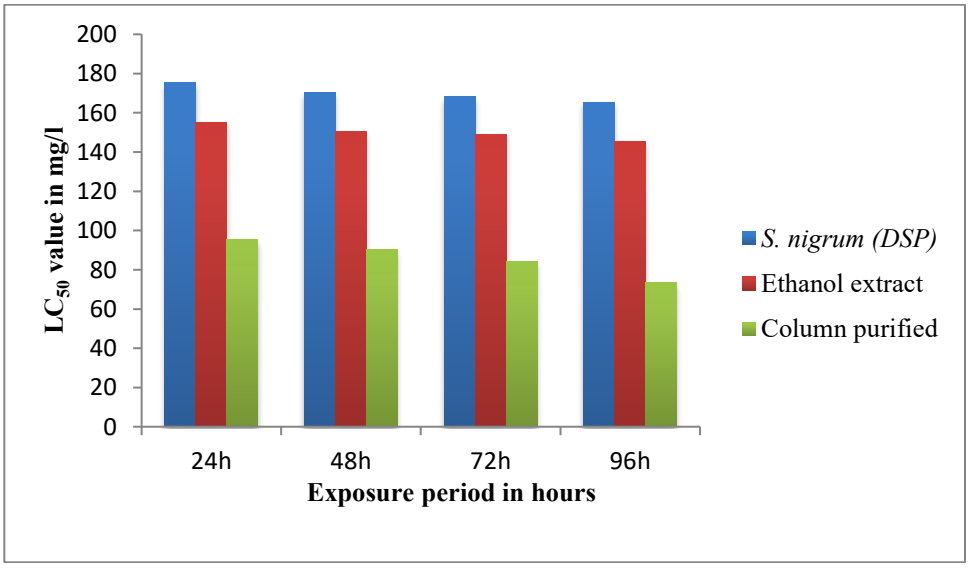

Figure 1: Histogram shows the toxic effect of ethanol extract, and column purified fractions of *S. nigrum* dried seed powder (DSP) against host snail *L. acuminata.*

Table 1: Toxic effect of dried seed powder, different organic extract, and column purified fractions of *S. nigrum* against the snail *L. acuminata.*

Prepared formulations (mg/l)	Exposure period in hours (h)	LC_{50} value in mg/l (w/v)	LCL value	UCL value	Slope-value	t-ratio	g-value	Heterogeneity
S. nigrum (DSP)		175.21	158.92	193.26	0.16±0.32	2.45	0.32	0.14
Ether extract		166.50	151.50	170.72	0.31±0.55	2.62	0.31	0.13
Chloroform extract		163.32	156.82	173.25	0.50±0.10	2.23	0.44	0.14
Methanol extract	**24h**	166.90	158.23	175.31	0.46±0.61	2.25	0.11	0.12
Acetone extract		161.31	154.05	176.62	0.52±0.10	2.41	0.23	0.16
Ethanol extract		155.21	148.47	163.96	0.44±0.36	2.63	0.30	0.14
Column purified		95.25	88.30	99.96	0.58±0.40	2.82	0.31	0.12
S. nigrum (DSP)		170.23	157.60	182.31	0.16±0.34	2.26	0.31	0.15
Ether extract		163.42	152.50	170.16	0.33±0.30	2.57	0.32	0.14
Chloroform extract		162.32	155.72	173.42	0.32±0.21	2.46	0.45	0.18
Methanol extract	**48h**	165.32	152.62	172.70	0.42±0.44	2.31	0.23	0.12
Acetone extract		159.34	150.64	172.51	0.49±0.46	2.25	0.23	0.12
Ethanol extract		150.23	138.25	163.26	0.53±0.32	2.23	0.43	0.15
Column purified		90.36	88.17	99.72	0.47±0.42	2.16	0.35	0.11
S. nigrum (DSP)		168.21	157.22	172.25	0.54±0.21	2.17	0.26	0.22
Ether extract		160.23	154.40	178.93	0.31±0.52	2.21	0.26	0.27
Chloroform extract		157.30	149.32	172.64	0.43±0.51	2.16	0.35	0.14
Methanol extract	**72h**	162.37	152.55	170.91	0.30±0.75	2.26	0.34	0.22
Acetone extract		156.40	147.25	165.61	0.12±0.18	2.32	0.35	0.21
Ethanol extract		149.12	138.34	155.80	0.30±0.61	2.81	0.33	0.28
Column purified		84.37	73.45	89.91	0.32±0.22	2.25	0.43	0.16
S. nigrum (DSP)		165.41	152.81	177.60	0.44±0.15	2.33	0.32	0.20
Ether extract		159.25	145.75	180.51	0.34±0.81	2.63	0.72	0.15
Chloroform extract		155.18	146.41	170.33	0.26±0.71	2.34	0.38	0.26
Methanol extract	**96h**	158.35	144.52	174.42	0.25±0.15	2.32	0.18	0.46
Acetone extract		152.84	148.31	162.50	0.26±0.18	2.45	0.23	0.42
Ethanol extract		145.40	136.90	153.36	0.55±0.36	2.72	0.26	0.18
Column purified		73.26	52.81	84.22	0.42±0.25	2.22	0.62	0.44

Abbreviation: DSP- Dried Seed Powder, LCL- Lower Confidence Limits, UCL-Upper Confidence Limits. Six batches of 10 snails were exposed in different concentrations of the above preparations. Mortality of snails was recorded in every 24h. Significant negative regression ($p<0.05$) was observed between exposure period and LC_{50} of treatments.

chemicals, such as gallic acid, epicatechin, gallic acid, catechin, protocatechuic acid, rutin, caffeic acid, and naringenin are found in *S. nigrum*. Solamargine, solasonine, solanine, and solasodine solanidine are glycoalkaloids found in unripe fruits of *S. nigrum* plants that are harmful to cattle and humans when consumed. According to An Lei *et al.,* (2006), *S. nigrum* have anticancer potential stems from its ability to disrupt the structure and function of tumor cell membranes, disrupt RNA and DNA synthesis, alter cell cycle distribution, block the NF-Kappa B anti-apoptotic pathway, activate caspase cascades reaction, and increase nitric oxide production. *S. nigrum* can either withstand high nitrate nitrogen (NO3-N) concentrations or be harmful to animals. According to Albouchi *et al.,* (2018), acute nitrate toxicity can result in death, while chronic toxicity causes a reduction in milk production, abortion, muscle tremors, a

staggering gait, a rapid heartbeat, frequent urination, labored breathing, collapse, and coma, with or without convulsions. However, it is unclear to what extent nitrate poisoning can harm human health and animal production, and the effects that are actually seen following nightshade plant administration may be caused by solanine, NO3-N, or both (Weller and Phipps, 1978). The crude extracts of *S. nigrum* fruit have anti-diarrheal properties. The fruit extract significantly reduced the frequency of stools and increased the mean latent duration in rats with castor oil-induced diarrhea at concentrations of 250 and 500 mg/kg body weight (Bhatia *et al.,* 2011). As reported by Ahmed *et al.,* (2001), an ethanolic leaf extract of *S. nigrum* has both larvicidal and molluscicidal properties. The concentration demonstrated the most notable larvicidal movement against hatching of two mosquito species, *Aedes caspius* and *Culex pipiens* (LC_{50}-51.29 and 125.89 mg/l in approximately 24 hours and 21.38 and 38.11 mg/l in 48 hours or less, respectively), as well as molluscicidal action (LC_{50}-3.37 mg/l in approximately 24 hours). The ethyl acetic acid derivations concentrate of *S. nigrum* could be utilized as a mosquito larvicidal agent (Rawani *et al.,* 2010). Significant anthelmintic activity was demonstrated by the water and ethanol extracts of *S. nigrum* leaves (Elias *et al.,* 2013).

The ethanolic columns purified of dried seed powder of *S. nigrum* have a potent molluscicidal activity against *Lymnaea acuminata*. The active ingredients in *S. nigrum* seed may dissolve easily in the organic solvent ethanol. The ethanol extract of dried seed powder of *S. nigrum* were more effective against *L. acuminata*. The 24h, 48h, 72h, and 96h LC_{50} were 155.21, 150.23, 149.12 and 145.40 mg/l, respectively. This result indicates that the active substance of *S. nigrum* is easily soluble in ethanol and it also dissolves slowly in water which causes the death of the host snail, *L. acuminata*. Fascioliasis can be prevented by reducing the host snail population using the active phytochemicals produced by *S. nigrum*. This fact has prompted further research to understand how the phytochemicals which are found in *S. nigrum* seeds exert their effects at the enzyme level inside host snails.

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