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Fasciolosis: Causes, Challenges and Controls

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ISBN 978-981-16-0258-0

ISBN 978-981-16-0259-7 (eBook)

<https://doi.org/10.1007/978-981-16-0259-7>

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The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

*We dedicate this book to our research mentor
and pathfinder of our academic journey
Lt. Prof. R. A. Agarwal, Ex-Head, Department
of Zoology; Ex-Dean, Faculty of Science;
Ex-Pro Vice-Chancellor, D.D.U. Gorakhpur
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Preface

Fasciolosis is a waterborne disease caused by *Fasciola hepatica* and *F. gigantica*. Both species are important helminthiases and have a great impact on human development. The importance of fasciolosis infection and control measures against them is emphasized by the World Health Organization (WHO). The definitive host range of *Fasciola* is broad and includes many mammals and humans. The presence of *Fasciola* infection in cattle or human population basically depends upon the presence of substantial reservoir, intermediate host, opportunity for water resource contamination by human/nonhuman hosts, and dietary practices that include consumption of raw aquatic vegetation. The ability of *Fasciola* species to spread is related to the large capacities of fasciolids to colonize and their ability to adopt new environments. Various lymnaeid snails are intermediate hosts of *Fasciola*, which helps in spreading the disease. The present book is focused toward the current status of fasciolosis, their economic impact, evolutionary origin and genetic diversity, distribution of disease, and vector snails. Effective fasciolosis control requires proper diagnosis, vaccination, and treatment at various stages of infection. Snail control is one of the effective methods to reduce *Fasciola* infection. It is recommended in all the epidemic areas as the snail represents the weakest link in the life cycle of *Fasciola*. The population of snail can be maintained below a threshold level by various molluscicides. With the advancement of technology, various snail control methods such as use of plants products, cow urine, drug combinations, use of spectral color of the sunlight, and phytotherapy of snails/their larvae inside/outside the body are now explored to control fasciolosis.

Gorakhpur, Uttar Pradesh, India
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Acknowledgments

We, with profound sense of gratitude, are thankful to Prof. Santiago Mas-Coma, Chairman, Department of Parasitology, Faculty of Pharmacia, University of Valencia, Spain, Director of the WHO Collaborating Centre on Fasciolosis and Its Snail Vectors, and President of the International Federation for Tropical Medicine (IFTM). Prof. Mas-Coma is a world renowned scientist, and he has given his valuable suggestions on Fasciolosis Control Program time to time. We are associated with Prof. Mas-Coma since 2008. In his association, our Malacology Laboratory is recognized as a member of International Fasciolosis Control team of the WHO. He has also given valuable tips on DNA nucleotide sequencing of *Fasciola gigantica* larva. Our research team is also thankful to Prof. Syed Akhtar Husain, Department of Biosciences, Jamia Millia Islamia University, New Delhi, India, for genomic studies of *Fasciola* larva.

We take this opportunity to acknowledge all the members of Malacology Laboratory, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur—273009, U.P., India.

The authors are grateful to Mr. Ajay Pati Tripathi for his expert editorial help. Last but not least, we wish to thank our families and friends for their continuous support.

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About the Book

Fasciolosis is a waterborne disease which causes a huge impact on human health and economy. There is no consolidated literature on the causes, challenges, and controls of fasciolosis. This book attempts to provide a comprehensive overview of fasciolosis constrain and their control. The first chapter of the book provides a brief history, status, economy, life cycle, evolutionary origin, and genetic diversity of *Fasciola*. The second chapter contains the constrain of fasciolosis status in different states of India, whereas the chapter third focuses on the distribution and ecology of vector snail belonging to the Lymnaeidae/Planorbidae family in India. The fourth chapter is on fasciolosis control, which deals with the diagnosis, vaccination, and treatment of *Fasciola*. The fifth chapter deals with vector snail control and its future probability in controlling fasciolosis. Control methods are focused on the toxicity of synthetic as well as natural products against vector snails. The use of cow urine and cow dung as molluscicide for the effective control of vector snails is discussed in this book. The use of different visible monochromatic lights of seven colors with chemoattraction in trapping the snails for killing them by various combinations of molluscicides is described. Snail control by bait formulations as well as synergistic treatments of various combinations is also discussed. Phytotherapy of snails without killing the vector snail is one of the new approaches to control fasciolosis. One of the promising methods to control fasciolosis with the help of the photosensitive plant product chlorophyllin against host as well as parasite larvae is also discussed. A new biological tool, i.e., phytotherapy of snail by chlorophyllin, can efficiently manage fasciolosis infection, without killing the vector snail. The authors are confident that this book is a step toward a new approach in the field of fasciolosis control in India as well as other parts of the world.

Contents

1	Fasciolosis	1
1.1	Fasciolosis Status	2
1.2	Economic Importance	4
1.3	Human Fasciolosis	5
1.4	Animal Fasciolosis	7
1.5	Life Cycle of <i>Fasciola</i>	10
1.5.1	Miracidium	11
1.5.2	Sporocyst	11
1.5.3	Redia	11
1.5.4	Cercaria	12
1.5.5	Metacercaria	12
1.6	Genetic Characterization of <i>Fasciola</i>	13
1.7	Evolutionary Origin	18
	References	19
2	Fasciolosis Constrain in India	27
2.1	Humid Subtropical Climate with Dry Winter	32
2.1.1	Uttar Pradesh	32
2.1.2	Uttarakhand	33
2.1.3	Haryana	33
2.1.4	Delhi	34
2.1.5	Punjab	34
2.1.6	West Bengal	34
2.1.7	Odisha	35
2.1.8	Bihar	35
2.1.9	Jharkhand	35
2.2	Tropical Rain Forest Region	35
2.2.1	Meghalaya	36
2.2.2	Assam	36
2.2.3	Andaman and Nicobar Islands	36
2.2.4	Arunachal Pradesh	36
2.2.5	Sikkim/Manipur/Nagaland/Mizoram	37
2.3	Tropical Steppe	37

2.3.1	Gujarat	37
2.3.2	Rajasthan	38
2.4	Tropical Semi-Eric Steppe	38
2.4.1	Maharashtra	38
2.4.2	Tamil Nadu	39
2.4.3	Karnataka	39
2.4.4	Andhra Pradesh	39
2.4.5	Kerala	39
2.5	Tropical Savanna Region	40
2.5.1	Madhya Pradesh	40
2.5.2	Chhattisgarh	40
2.6	Mountain Climate	40
2.6.1	Himachal Pradesh	40
2.7	Temperate/Subtemperate Region	41
2.7.1	Jammu and Kashmir	41
	References	43
3	Distribution and Ecology of Lymnaeidae/Planorbidae Snails in India	49
3.1	Family: <i>Lymnaeidae</i>	50
3.1.1	Subgenus <i>Pseudosuccinea</i>	50
3.1.2	<i>Lymnaea (Pseudosuccinea) biacuminata</i> : (Mitra et al. 2005)	53
3.1.3	<i>Lymnaea luteola</i> Lamark, 1822	53
3.1.4	<i>Lymnaea ovalior</i> , Annandale and Prashad 1921	55
3.1.5	<i>Lymnaea horae</i> Annandale and Rao 1925	55
3.1.6	<i>Lymnaea gedrosiana</i> (Annandale and Prashad 1921)	56
3.1.7	<i>Lymnaea mimetica</i> Annandale, 1918	56
3.1.8	<i>Lymnaea shanensis</i> Annandale, 1918	56
3.2	Subgenus <i>Galba</i>	57
3.3	Subgenus <i>Radix</i> Montfort	58
3.3.1	Subgenus <i>Lymnaea</i>	59
3.3.2	Subgenus <i>Stagnicola</i>	59
3.3.3	Family <i>Planorbidae</i>	60
3.3.4	Genus <i>Indoplanorbis</i> Annandale and Prashad 1921	60
	References	63
4	Fasciolosis Control	65
4.1	Diagnosis	65
4.2	Clinical Diagnosis	66
4.3	Blood Parameters and Enzyme Diagnosis	66
4.4	Parasitological Diagnosis	67
4.5	Immunodiagnosis	67
4.6	Vaccination	68
4.7	Fatty Acid-Binding Proteins	69

4.8	Cysteine Peptidase	69
4.9	Leucine Aminopeptidase	69
4.10	Glutathione S-Transferase	70
4.11	Treatment	70
	References	71
5	Snail Control	75
5.1	Biological Control	76
5.2	Mechanical Control	77
5.3	Chemical Control	78
	5.3.1 Metaldehyde	78
	5.3.2 Niclosamide	78
	5.3.3 Carbamate	78
	5.3.4 Organophosphorous	79
	5.3.5 Synthetic Pyrethroids	79
5.4	Plant-Derived Molluscicides	80
5.5	Cow Urine as Molluscicides	89
	5.5.1 Cow Urine	89
	5.5.2 Pesticidal Use of Cow Dung/Urine	89
5.6	Combined Action of Molluscicides	91
5.7	Bait Formulations in Snail Control	97
5.8	Phytotherapy of Snails to Control Fasciolosis	104
5.9	Photosensitivity of Host/Parasite and Molluscicide in Control of Fasciolosis	105
	References	107

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Abstract

First chapter of the present book deals with the introduction of fasciolosis disease, their history, overall status, economic impact on livestock and milk production. Life cycle of *Fasciola* is briefly discussed to understand their mode of parasitism in cattle as well as human beings. Evolutionary origin and genetic characterization of *Fasciola hepatica*/*Fasciola gigantica* in different parts of world with respect to their distribution is also described to understand their global prevalence.

Fasciolosis is considered to be waterborne disease of historic period. Several prehistoric human coprolite studies indicate the presence of *Fasciola* eggs in that time (Bouchet 1994, 1995, 2003; Aspöck et al. 1999; Dittmar and Teegen 2003). According to Bouchet et al. (2003), *Fasciola* has been found in prehistoric human population 5000–5100 years ago at the end of Mesolithic period and Neolithic period of animal domestication and the development of agriculture. *Fasciola hepatica* has been reported in Bronze Age as well as middle age (Bouchet et al. 2003). Carvalho et al. (2002) have opinion that eggs of liver fluke found in European coprolites but never in the new world, suggesting that *fasciolosis* in America is a relatively recent introduction.

One of the best cattle breeders in France, Jean de Brie for the first time written a treatise in the year 1379 and gave an accurate description of *Fasciola hepatica* and its damage in the liver of sheep. Later, an English Judge, Anthony Fitzherbert (1523) emphasized the importance of the ecology of *Fasciola hepatica*. In 1684, Francesco Redi had published monumental tome, which is one of the earliest and best work on parasitology. He describes the *Fasciola hepatica* in rabbit. In 1881, F.R. Leuckart published a series of articles on the life cycle of *F. hepatica*. Algemon P.W.T. in 1881 described the life cycle of *F. hepatica* independently. Perhaps, this was the first

complete description of the life cycle of digenetic trematode. Further in 1882, Algemon P.W.T. in his second report of experiment on the development of liver fluke found that this fluke is transmitted by the intermediate host molluscs, particularly the snails *Linaeus pereger* and *L. truncatulus*. In spite of the extensive studies of Algemon and Leuckart, many gaps in the complete description of the life cycle of *F. hepatica* remained. Later, in 1892, Adolf Lutz has done the detailed study on its life cycle and explained that those animals acquire *F. hepatica* by swallowing the encysted stage. Dmitry F. Ssinitzin (1914) reported the new details of the life history of *Fasciola*. He demonstrated that young flukes migrate through the intestinal wall into the abdominal cavity and then penetrate the liver of rabbit.

Fasciolosis is an important helminth disease caused by two trematodes *Fasciola hepatica* and *Fasciola gigantica* of subfamily Fasciolinae (Mas-Coma et al. 2005; Tolan 2011). The subfamily fasciolinae includes three genera: *Fascioloides*, *Tenuifasciola*, and *Fasciola*. *Fascioloides* are represented by *F. magna*, the large American liver fluke, reported in the liver of North American and European bovinds. Genus *Tenuifasciola* is represented by *F. tragelaphi* found in the bile duct of *Tragelaphus spekei* (Pike and Condry 1966) and also in cattle in Zimbabwe (Mukaratirwa and Brand 1999). Third genus *Fasciola* includes four species (Yamaguti 1971). *F. hepatica* is found in the liver of domestic ruminants viz. sheep, goat, cattle, equines, camelids, pigs and many wild animals deer, rabbits, hares. *F. hepatica* is distributed in temperate and subtropical areas of the Americas, Europe, Asia, and Africa (Mas-Coma and Bargues 1997). Tropical liver fluke *F. gigantica* is a common parasite of liver of domestic animals sheep, goats, cattle, buffaloes, camels, pigs, horses and donkeys and wild animals—large antelopes, deer, giraffes, and zebras. *F. gigantica* is distributed in tropical and subtropical areas of Asia and Africa. *F. nyanzae* and *F. jacksoni* are found in liver of *Hippopotamus amphibious* and Asian elephants, respectively.

Many species of freshwater snail belonging to the family Lymnaeidae and Planorbidae are intermediate host of highly infective fluke larva of the genus *Fasciola*. These snails act as intermediate hosts found in natural ponds, lakes, canals, and paddy fields there by more chances of natural infection. Buffaloes are more exposed to the *Fasciola* larvae as they dwell in these water bodies infested with snails, while other cattle come in contact of infection by grazing near water submerge area and drinking water in the snail infested water bodies. Fasciolosis is reported in all the five continents of the world. Now, fasciolosis is recognized as an emerging-reemerging human disease (Mas-Coma et al. 2005). The World Health Organization has estimated that 2.4 million people are infected with *Fasciola*, and a further 180 million are at risk of infection (WHO 2007).

1.1 Fasciolosis Status

Fasciolosis is an important disease among the entire zoonotic helminthes worldwide (Haridy and Ibrahim 1999). The two liver fluke species infect a wide range of mammals especially cattle and sheep, while humans are regarded as accidental

hosts (Yamaguti 1958; Froyed 1975; Hillyer and Apt 1997; Daniel and Mitchell 2002; Mas-Coma et al. 2005). Fasciolosis is an up-and-coming threat among the list of parasitic infections in a large number of countries as a result of environmental changes and man-made modifications, occurring in areas, where conditions are suitable for the survival of its intermediate hosts. It is mostly prevalent in countries having well-developed cattle and sheep production farming. Furthermore, human fasciolosis has also been reported in developing countries, except Western Europe (Mas-Coma et al. 1999a, b). In some advanced countries, fasciolosis may persist up to 75% (Spithill and Dalton 1998), whereas, in humid regions which are underdeveloped, fasciolosis is considered to be the most prevalent infection in large ruminants prevailing up to 90% in Africa and Indonesia, ranging to an alarming 100% in India (Spithill et al. 1999). Infection in humans occurs, when they consume uncooked aquatic vegetables or drink freshwater contaminated with metacercariae/cercariae (Mas-Coma et al. 1999a, b, 2019).

In India, fasciolosis is widespread and primarily caused by *F. gigantica* although *F. hepatica* is reported in the temperate Himalayan region. Fasciolosis caused by *Fasciola gigantica* is one of the commonest parasitic problems of cattle and buffalo prevalent in all parts of India. Building of dams and the establishment of new irrigation systems have further widened the distribution of *Fasciola* by creating more water-covered areas suitable for propagation of its intermediate hosts, the lymnaeid snail vectors. Thus, fasciolosis has started to appear in the semi-arid and arid regions of western India, where it was hitherto non-existing. The onset and advancement of monsoon rains have a profound effect on the incidence and seasonality of fasciolosis in India. Most of the available information on the prevalence of *F. gigantica* come from abattoir surveys and coprological studies on animals visiting clinics, and is thus biased. It is, however, apparent that the prevalence of fasciolosis in a tropical country like India is largely determined by rainfall and production system. A review of some of the recently conducted survey indicates a high level of incidence in the endemic areas throughout India but in the northern plains, a high 10–39% infection was recorded in cattle and buffalo (Jairajpuri 1991). A nationwide survey in dairy animals organized by the National Dairy Development Board (NDDB) indicated two critical period in the year; July–September and February–March (Sanyal 1995). Incidence of endemic fasciolosis is very common in the eastern region of the state of Uttar Pradesh (Shukla et al. 2006). In eastern Uttar Pradesh, infection of *Fasciola gigantica* is very high. Ninety four percent buffaloes slaughtered in Gorakhpur District are infected with *F. gigantica* (Singh and Agarwal 1981). This situation of infection still exists in the cattle of this region, which may cause human fasciolosis accidentally or due to unhygienic condition of human population living around water logged areas (Ramachandran et al. 2012).

In the recent years, emergence of fasciolosis has been noted in pigs, horses, wild animals—hares (Walker et al. 2010), and even in human in different parts of the world (Mas-Coma et al. 2005). Now it has been mentioned as an emerging zoonosis (Mas-Coma et al. 2005). This re-emergence is due to climatic change, greater stock movement, and longer parasite host exposition resistance to anthelmintic drugs. Estimation of economic losses due to re-emergence of fasciolosis is difficult. Usually

direct economic losses are calculated on the basis of death of animals or liver condemnations. It is harder to calculate the economic losses due to frequency of animal treatments in clinics.

1.2 Economic Importance

The economic importance of fasciolosis in veterinary is well established and considered as a significant limiting factor that affects the production potential in small and large ruminants. Fasciolosis results economic losses in livestock, appearing in the form of mortalities, reduced fertility, abortions, slow growth and reduction of milk and meat production, infected livers, and withered carcasses. Fasciolosis in general causes colossal economic losses of 200 million US\$ annually to the agriculture budget. Apart from this it affects productivity of large ruminants worldwide by causing up to 20% loss in body weight, decreased milk/beef production, and decreased fertility in cattle (Torgerson and Claxton 1999). Furthermore, detrimental effects of fasciolosis in sheep consist of reduction of weight gain and wool production as well as sudden deaths of animals (Sinclair 1962; Roseby 1970). Foreyt and Todd (1982) and Berhanu et al. (2018) considered fasciolosis as a major factor of liver condemnations and reduced feed intake, resulting in reduced livestock productive efficiency.

In the United States, cattle and sheep producers bear annual economic losses of \$30,000,000 as a result of direct and indirect effects of fasciolosis. Direct losses are attributed to infected livers at slaughter (Malone 1986). In England, the direct losses of 600,000 bovine livers represented approximately £1 million/\$1.7 million U.S (Haseeb et al. 2002), while indirect losses include reductions in average daily weight gain and reduced milk yield (Rose 1970; Hope-Cowerdy et al. 1977; Schweitzer et al. 2005a), production in dairy cattle (Randell and Bradley 1980), and reduced herd performance in cow-calf operations (Dargie 1986). In France, *Fasciola* treated/infected dairy cows enhanced conception rates by 23% (Mage et al. 1989). An infection of even 30 adult *Fasciola* affects the quality and quantity of wool growth in sheep (Dargie 1986; Clarkson 1989). Animal productivity worldwide was affected by fasciolosis with an estimated loss ranging up to approximately US\$3.2 billion per annum (Spithill et al. 1999; Mehmood et al. 2017) (Table 1.1).

Suleiman and Nma (2017) in their extensive survey on fasciolosis noted that in north central Nigeria total economic losses in between year 2005 and 2015 was about 776,832 US\$. Earlier, Ardo et al. (2013) reported total economic loss of 1921 US\$ in Adamawa state of Nigeria. Similarly, Schweitzer et al. (2005b) and Regassa et al. (2012) reported loss of 42.8 million and 13,364.72 US\$ in Switzerland and central Ethiopia, respectively.

In India, cattle and buffaloes are the major source of milk and milk-based products for human. Besides this, they are also main source of meat for a section of Indian community. Fasciolosis is one of the major causes of productive loss in these animals. Among the livestock population, cattle (190–199 million) plays a

Table 1.1 Cattle population and milk production in NER (North Eastern Region) states and whole India

NER State of India	Area (sq. km)	Cattle population	Milk production 2011–2012 (Tonnes)
Arunachal	83,743	503	3.2
Assam	78,438	10,041	752
Manipur	22,327	342	78
Meghalaya	22,429	887	77
Mizoram	21,081	35	17
Nagaland	16,579	470	45
Sikkim	7096	135	42
Tripura	10,486	954	91
NER total	262,179	13,368	1134
All India	3,287,263	190,909	107,934

Source: Basic statistics of NER (2015): Livestock census (2012)

major role in India's economy, accounting 37.28% of total livestock population (Livestock Census 2012).

Economic losses due to fasciolosis in domestic animals under Indian condition are very high with the prevalence rate of infection in between 30 and 94% (Singh and Agarwal 1981; Bhatia et al. 1989; Rai et al. 1996). Fasciolosis caused by *F. gigantica* is one of the most economically important helminthic infections of cattle and buffalo in India. According to Copeman and Copland (2008), there are 283.2 thousand large ruminants in India, in which prevalence is in between 10 and 25%. About 99.12 thousands are infected with *Fasciola*, which caused economic loss of about 81.28 millions Australian dollars.

The annual milk loss in the state of Uttarakhand, India due to fasciolosis has been estimated to be Rs. 90.41 Crores. The milk loss has been found highest in buffaloes since the adult female buffaloes constitute 66% of the total bovine population in the state (Bardhan et al. 2014).

1.3 Human Fasciolosis

Human fasciolosis, which has been traditionally viewed as a secondary disease, is becoming more prevalent in the world (Chen and Mott 1990). The incidence of human cases has been reported in 51 countries of the five continents (Mas-Coma et al. 1999a, b; WHO 2007). Human infection of *Fasciola hepatica* is determined by the presence of the intermediate host snail, domestic herbivorous animal, climatic condition, and the dietary habit of man (Chen and Mott 1990). Sheep, goat, and cattle are considered to be predominant animal reservoirs. Humans are infected by ingestion of aquatic plants that contain the infected metacercaria (Markell and Voge 1999) by drinking unboiled contaminated water containing viable metacercaria (Chen and Mott 1990). Human consuming raw liver dishes from fresh liver infected with juvenile flukes could also be one of the resources for infection of *fasciolosis* (Taira

et al. 1997; Esteban et al. 1999). Adult flukes in the bile ducts cause inflammation and hyperplasia of the epithelium (Mas-Coma et al. 1999a).

Bouchet et al. (2003) reported that *Fasciola* existed in prehistoric human populations of the Stone Age, an era of agricultural development that led to domestic utility of animals. The first record of human fasciolosis infection was found in a female patient during an autopsy performed by Pallas in 1760 in Berlin (Dittmar and Teegen 2003). Before 1992, reported human fasciolosis was nearly 3000 cases (Hopkins 1992; Rin et al. 1994). Since 1950, human fasciolosis has been reported from 51 countries, where approximately 180 million people are at risk of infection and 2.4 million are estimated to be infected (Curtale et al. 2005; WHO 2007; Mas-Coma et al. 2009a). *F. hepatica* is considered to be more adapted to the human host than *F. gigantica*. The report also states that human disease caused by *F. gigantica* is reported in comparatively few geographical areas. Human coprolites have been examined for *Fasciola* eggs and their presence suggests that fasciolosis was common in prehistoric humans (Aspöck et al. 1999; Dittmar and Teegen 2003). The geographical distribution pattern shows that human fasciolosis is highest in South America (Bolivia, Peru, and Chile), followed by Europe, Africa, and Asia, with the fewest cases being reported in Oceania (New Zealand, Australia, and Philippines) (Mas-Coma 2005; Mas-Coma et al. 2005). Several researchers have shown human fasciolosis as a major health hazard in several areas globally including the Nile Delta in Egypt and central Vietnam (Lotfy and Hillyer 2003; Lotfy et al. 2008). The most prevalent (72–100%) human fasciolosis is reported in the Bolivian Altiplano. More than 300,000 humans are infected with *Fasciola* (Hillyer 1999; Mas-Coma et al. 1999b). Human fasciolosis is found mainly in indigenous peoples, who regularly share the same water sources with their animals. Infection is more prevalent in children but noted in all age groups of both sexes (Mas-Coma et al. 2005). Humans play a significant transmitter of disease, as 5000 eggs per gram feces in Bolivian children are reported (Mas-Coma et al. 1999b, 2009b). However, in other countries fasciolosis is more common in animals (Chen and Mott 1990; Esteban et al. 1998). The infection was limited in the past to specific and typical geographical areas, where animal to human transmission was common, but a few human outbreaks have been recorded due to human-to-human transmission in South America, which was attributed to watercress consumption (WHO 2007). Studies carried out in recent years have shown human fascioliasis to be an important public health problem (Chen and Mott 1990). In Europe, *Nasturtium officinale*, *Nasturtium sylvestris*, *Rorippa amphibia*, *Taraxacum dens leonis*, *Valerianella olitoria*, and *Mentha viridis* were reported as a source of human infections (Mas-Coma et al. 1999a, b). Because *F. hepatica* cercariae also encyst on water surface, humans can be infected by drinking of fresh untreated water containing cercariae (Chen and Mott 1990). In addition, an experimental study suggested that humans consuming raw liver dishes from fresh livers infected with juvenile flukes could become infected (Taira et al. 1997).

Ten thousand human fasciolosis is noted in Guilan and Mazandaran provinces of Northern Iran (Rokni et al. 2002; Moghaddam et al. 2004). Nineteen percent prevalence of human fasciolosis was noted in between the Neel delta region of

Cairo and Alexandria (Esteban et al. 2003). It was estimated that about 830,000 persons were infected with *Fasciola*. Human fasciolosis in Europe, particularly in France, Spain, and Portugal, there is increase of 50–100 cases per year (Mas-Coma et al. 2005).

In India, human fasciolosis has been reported in state of Assam, Bihar, Maharashtra, Uttar Pradesh, Arunachal Pradesh, and West Bengal (Narain et al. 1997; Elhence et al. 2001; Vatsal et al. 2006; Ramachandran et al. 2012). *F. gigantica* is mostly prevalent in cattle in north eastern region. Narain et al. (1997) from upper Assam reported *F. hepatica* infection in a 7-year-old girl having history of eating water cresses regularly. Ultrasonography revealed the presence of adult fluke in gallbladder. Ghildiyal et al. (2014) reported the infection of *F. hepatica* in a 27-year-old female living in Lucknow, U.P., India, belongs to lower socioeconomic family in outpatient department of Era Medical College, Lucknow. Liver fluke has been also reported previously from different parts of India (Narain et al. 1997; Elhence et al. 2001; Vatsal et al. 2006). Though, in India infection of *F. gigantica* is rare so far, but association of rural people with animals and poor hygienic conditions, lack of health services in remote village indicates existence of higher probability of human infection. It needs extensive survey of human health particularly related with liver problems (Gupta 2014).

1.4 Animal Fasciolosis

Animal fasciolosis occurs worldwide (Torgerson and Claxton 1999). According to Boray (1969) at least 46 species of domestic mammals, including cattle and sheep are infected either naturally or experimentally. The common liver fluke *Fasciola hepatica* is a parasitic flatworm that can be collected in large number from bile duct of infected cattle, sheep, buffaloes, goats, horses, ovine, swines, and other mammals. In tropical regions, fasciolosis is considered the single most important helminthes infection of cattle with prevalence rate of 30–90% in Africa, 25–100% in India, and 25–90% in Indonesia (Hansen et al. 1999). In Iran, the average prevalence of fasciolosis was in between 11.5% and 34.6% in different animals (Khosravi and Babaahmady 2012; Amiazare et al. 2018). In Northern part of India, heavy infection of *F. gigantica* is noted by Singh and Agarwal (1981). Ninety four percent buffaloes slaughtered in local houses of Gorakhpur district U.P., India are infected with *F. gigantica* (Singh and Agarwal 1981).

Fasciolosis belongs to the plant-borne trematode **zoonoses**. In Europe, the Americas, and Oceania, only *F. hepatica* is a concern, but the distributions of both species overlap in many areas of Africa and Asia (Mas-Coma et al. 2005). The definitive host range is very broad and includes many **herbivorous mammals**, including humans. The **life cycle** includes **freshwater snails** as an **intermediate host** of the parasite (Torgerson and Claxton 1999).

Adult flukes of both species (*F. hepatica* and *F. gigantica*) are localized in the **bile ducts** of the **liver** or **gallbladder**. *F. hepatica* measures 2–3 cm and has a **cosmopolitan distribution**. *F. gigantica* measures 4–10 cm in length and the

distribution of the species is limited to the [tropics](#) and has been recorded in Africa, the Middle East, Eastern Europe, and south and eastern Asia (Torgerson and Claxton 1999). Direct losses are those that indicate the condemnation of infected livers at slaughter house (Malone 1986), while indirect losses are those that includes reduction in average daily gain and reduced milk production in dairy cattle (Randell and Bradley 1980; Mage et al. 1989). In domestic livestock in Japan, [diploid](#) ($2n = 20$), [triploid](#) ($3n = 30$), and [chimeric](#) flukes ($2n/3n$) have been described, many of which reproduce [parthenogenetically](#) (Sakaguchi 1980). In India, a species called *F. jacksoni* was described in [elephants](#) (Singh et al. 1994).

The life cycle of *Fasciola* is complex. It involves a final host (where the adult worm lives), an intermediate host (where the larval stages of the worm develop), and a carrier (entailing suitable aquatic plants). The process starts when infected animals (cattle, sheep, buffaloes, donkeys and pigs but also horses, goats, dromedaries, camels, llamas, and other herbivores) defecate in freshwater sources. Since the worm lives in the bile ducts of such animals, its eggs are evacuated in feces and hatch into larvae that lodge in a particular type of water snail (the intermediate host). Once in the snail, the larvae reproduce and eventually release more larvae into the water. These larvae swim to nearby aquatic or semi-aquatic plants, where they attach to the leaves and stems and form small cysts (metacercariae). When the plants with the small cysts attached are ingested, they act as carriers of the infection. Watercress and water-mint are good plants for transmitting fasciolosis, but encysted larvae may also be found on many other salad vegetables. Ingestion of free metacercariae floating on water (possibly detached from carrier plants) may also be a possible mode of transmission.

Gupta and Singh (2002) extensively reviewed the several reports of epidemiology of fasciolosis from different endemic areas of India. Yadav et al. (2007) reported the epidemiological studies on the prevalence of *F. gigantica* infection in sheep and goats of western U.P., from January, 2001 to December, 2004. According to them, peak prevalence 2.61% of the infection in sheep and goat was noted in rainy season. Highest peak prevalence in sheep (5.1%) and goats (13.3%) was noted in month of July and March, respectively. The prevalence of infection in snails was highest (4.18%) during July to October, whereas it was lowest (0.76%) during summer (March to June).

There are many ecological factors affecting snail populations including temperature, light, [hydrogen ion](#) concentration ([pH](#)), vegetation, depth of water, current of the water, chemical composition of the soil, and snail population [competition](#) (Mas-Coma et al. 2005). It has been reported that the most important [intermediate host](#) for *F. gigantica* is [Radix auricularia](#) (Mas-Coma et al. 2005). However, *Lymnaea rufescens* and *L. acuminata* are the host snails in the [Indian subcontinent](#); *Radix rubiginosa* and *R. natalensis* are the hosts in [Malaysia](#) and in Africa, respectively, and the synonymous *L. cailliaudi* is the intermediate host in east Africa (Mas-Coma et al. 2005). *F. gigantica* is a causative agent (together with *F. hepatica*) of fasciolosis in [ruminants](#) and in humans worldwide (Mas-Coma et al. 2005).

Further, Singh et al. (2012, 2013) and Kumari et al. (2012) noted that abiotic factors such as temperature, pH, dissolved oxygen, carbon dioxide, and electrical conductivity in surrounding water and population density of snails are crucial factor, which can significantly affect the larval infection of *F. gigantica* in snails. Characteristics of *Fasciola* species is greatly influenced by local conditions and ethology of intermediate hosts molluscs and metrological factors. Strategies used to control fasciolosis depend on the extent and seasonality of disease transmission, the intermediate host's ability to survive climatic conditions. They have suggested that the treatment of water bodies with molluscicides for the control of snails and ultimately fasciolosis between July and October is not only more potent and cost effective, during these months than spending more by using higher concentration of the molluscicides during the remaining months of the year.

To complete its life cycle, *F. hepatica* requires a freshwater snail as an intermediate host, in which the parasite can reproduce asexually. *Galba truncatula* is the most common intermediate host of *F. hepatica* in Europe and South America. Intermediate hosts of *F. hepatica* are freshwater snails of family Lymnaeidae (Torgerson and Claxton 1999; Graczyk and Fried 1999). Snails of family Planorbidae also act as an intermediate host of *F. hepatica* very occasionally (Mas-Coma et al. 2005). However, throughout the years an increasing number of other molluscan intermediate hosts of *F. gigantica* have been reported (Mas-Coma et al. 2005).

The development of infection in definitive host is divided into two phases: the parenchymal (migratory) phase and the biliary phase (Dubinsky 1993). The parenchymal phase begins when excysted juvenile flukes penetrate the intestinal wall. After the penetration of the intestine, flukes migrate within the abdominal cavity and penetrate the liver or other organs. *F. hepatica* has a strong predilection for the tissues of the liver (Behm and Sangster 1999). The second phase (the biliary phase) begins when parasites enter the biliary ducts of the liver. In biliary ducts, flukes mature, feed on blood, and produce eggs. Hypertrophy of biliary ducts associated with obstruction of the lumen occurs as a result of tissue damage.

Clinical signs of fasciolosis are always closely associated with infectious dose (amount of ingested metacercariae). In sheep, as the most common definitive host, clinical presentation is divided into four types (Dubinsky 1993; Behm and Sangster 1999):

Acute Type I Fasciolosis: Infectious dose is more than 5000 ingested metacercariae.

Sheep suddenly die without any previous clinical signs. Ascites, abdominal hemorrhage, icterus, pallor of membranes, and weakness may be observed in sheep.

Acute Type II Fasciolosis: Infectious dose is 1000–5000 ingested metacercariae. As above, sheep die but briefly show pallor, loss of condition, and ascites.

Subacute Fasciolosis: Infectious dose is 800–1000 ingested metacercariae. Sheep are lethargic, anemic and may die. Weight loss is dominant feature.

Chronic Fasciolosis: Infectious dose is 200–800 ingested metacercariae. Asymptomatic or gradual development of bottle jaw and ascites (ventral edema), emaciation, weight loss.

Acute fasciolosis is not common but occurs in sheep. Acute forms of fasciolosis develop in 2–3 weeks after massive infection of a large number of metacercariae in short span of time, which ultimately resulted in massive invasion of the liver parenchyma, hemorrhaging, anorexia, abdominal pain, weight loss, and sudden death. Subacute fasciolosis occurs after 6–10 weeks of the ingestion of lower number of metacercariae over a longer period of time and reflects liver damage, followed by cholangitis. It is not so fatal as acute condition. It shows clinical signs during 1–2 weeks before death.

The chronic fasciolosis is seen after 4–5 months of ingesting a low number of metacercariae nearly throughout the whole year. The pathogenic effects are anemia, paleness of mucus membrane, submandibular edema, and ascites. Lack of appetite is related with the entry of flukes in the bile ducts in first phases of severe acute infection (Dargie 1986). Decrease in appetite was noted when hepatic deterioration is higher (Ferre et al. 1995).

1.5 Life Cycle of *Fasciola*

The adult mature and gravid fluke is flat having leaf shaped body. The adult parasitizes the liver/or gallbladder of the final hosts (Despommier and Karapelou 1987; Andrews 1999). The fluke has an elongated anterior end known as a cephalic cone having an oral and ventral sucker. The intestines are highly branched throughout the body. The male and female reproductive organs are present near the posterior sucker in the center of the body. The female reproductive tract is a dense ovary and is located just above the testes and is linked to a short convoluted uterus that opens into a genital pore above the ventral sucker. The vitellaria are highly dispersed and divided in the lateral and posterior region of the body. *F. gigantica* and *F. hepatica* are very similar to each other, varying in length and width. In addition, the cephalic cone of *F. hepatica* is shorter than *F. gigantica*. The shape of the eggs of the two flukes is also very similar (Soulsby 1982) with the measurements of *F. hepatica* and *F. gigantica* being approximately $150\ \mu\text{m} \times 90\ \mu\text{m}$ and $200\ \mu\text{m} \times 100\ \mu\text{m}$, respectively (Dunn 1978).

The life cycle of *Fasciola* species is a typical of digenetic trematodes. Eggs laid by the adult parasite in the bile ducts of their hosts pass into the duodenum with the bile. The eggs then leave the host through the feces. At this stage, eggs are still not embryonated; further development to maturation takes approximately 2 weeks. Eggs need adequate temperature and humidity (Harris and Charleston 1976). Ciliated larva miracidium develops inside the egg when light weakens the photoactive joint operculum of the cell, hypertonicity inside the egg and miracidium activity opens the operculum, and miracidium comes outside the egg. Usually this process takes

12 days at 26 °C in laboratory conditions, whereas in field conditions it may require 2 months and 10–12 °C.

1.5.1 Miracidium

The eggs then hatch to release motile miracidium, which rapidly swims in aimless direction to locate and penetrate the intermediate snail host (Aksy et al. 2005; Yesildag et al. 2009). Mollusc intermediate snail is located with the help of chemotactic stimuli. Factors such as temperature, pH, oxygen, ionic composition, and turbidity of water are major decisive factors in locating the intermediate host. Miracidia failing to locate suitable host generally die within 24 h (Dalton 1999).

1.5.2 Sporocyst

After penetrating the snail, the miracidium then differentiates into mother sporocyst by losing its cilia. The sporocyst then divides and form redia (forum with sucker and primitive gut), and a fully mature redia.

1.5.3 Redia

Redia is elongated, has cylindrical body wall, and consists of the usual layers, viz. cuticle, musculature, and the mesenchyme. It bears a mouth at its anterior end surrounded by sucker. Mouth leads into a short muscular pharynx followed by an elongated sac like intestine. Numerous unicellular pharyngeal glands open into the pharynx. Redia feeds on the hosts digestive juices. A birth pore is present at the anterior end, a little behind the collar through which the next generation larvae escape out. The protonephridia branches further and forms a much elaborate system. All the flame cells of each side open out through a common excretory duct. At the posterior end ventrolateral lappets or procruscula are present, which is the conspicuous feature of redia. The body of the larva is packed with germ balls and germ cells. The redia moves about in the host tissue. The movement is brought about by the muscular contractions of the body. Moving redia enters into various organs of snail but prefers to migrate to the digestive gland. The next larvae, i.e., cercaria, develop within the redia. These emerge out through the birth pore. During the summer months, when sufficient nourishment is available, instead of cercaria a second generation of redia is formed. However, during the winter months cercaria are formed. Each redia forms 14–20 cercarias (Radomyos et al. 2004; Garcia 2007).

1.5.4 Cercaria

The cercaria of *Fasciola* species have a rounded body measuring between 0.25 and 0.35 mm long, with a long thin unbranched tail measuring approximately 0.5 mm long. The mobile cercaria generally leaves the snail 4–7 weeks after infection by migrating through the tissues of snails, in moist conditions, when a critical temperature of 10 °C is exceeded. Thus, in winter when average day and night temperature is below 10 °C the development of miracidia in eggs and larval stages in snail is arrested. Above 10–28 °C these developments are faster in proportion to rise in temperature, higher temperatures result in heavy mortality of snails, negatively compensating the faster development of larval stages. Other influencing factors are drought and rains, the former resulting in estimation of snails and the later stimulating their multiplication as well as emergence of cercaria. The combination of these influencing factors marks out two critical periods in a year in India, when high population of metacercaria occurs in nature and animals are exposed to heavy doses of infection. The primary critical period is from July to September and secondary from February to March (Narsapur and Gatne 1993).

Number of cercariae formed inside the snail body is highly variable and it depends on the number of miracidia that infect host snails, ranging from 10 to 4000 cercariae. Intramolluscan development of *fasciola* larval stage lasts 8 weeks. Snail mortality prevalence of snail infection and number of cercariae produced are variable according to location. Periodicity of cercarial shedding is about 6 days. The number of cercariae shedding wave peaks at the second wave and subsequently decreases up to fifth wave (Rojo-Vazquez and Ferre-Perez 1999).

1.5.5 Metacercaria

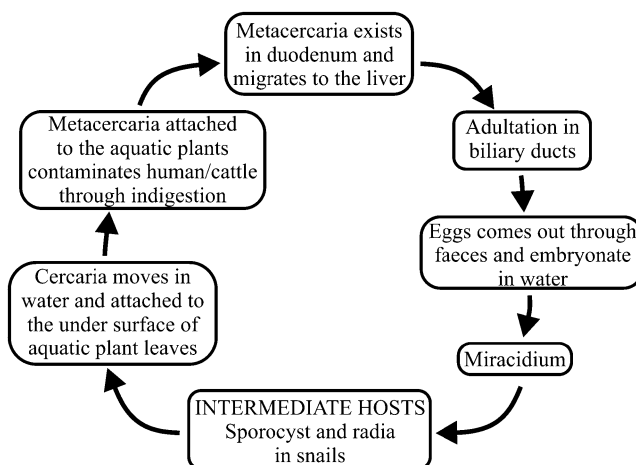
On emerging from the snail the cercaria attaches to submerged blades of grass or other vegetation like watercress; the tail falls away and the cercaria body secretes a four-layered cyst covering from cystogenous glands located on the lateral regions of the body. The formation of the cyst wall may take up to 2 days. The metacercaria encysted on water plants (Radomyos et al. 2004; Garcia 2007) is the infective form to the definitive host. Generally, metacercaria are infective to ruminants such as cattle and sheep, but also to other mammals including human beings. One miracidium hatching from a fluke egg can produce up to 4000 infective cysts (metacercaria) due to the vegetative multiplication at the sporocyst and redia stages. The metacercarial cyst is only moderately resistant, not being able to survive dry conditions; it is resistant to the acidic juices of the stomach (Dias et al. 1996; Haseeb et al. 2002; Mas-Coma 2005; Goral et al. 2011a, b). They may survive up to a year maintained in conditions of high humidity and cool temperatures (Andrews 1999; Soulsby 1982; Dunn 1978). The metacercarial cysts, when ingested along with the contaminated vegetation by the definitive host enter into the small intestine, releasing the young parasite, which penetrates the gut wall, entering the peritoneal cavity. From there, it migrates over a period of approximately seven days, directly to

the liver. The juvenile fluke (also referred to as *adelscoaria*) then penetrates the liver tissues, through which it migrates, feeding mainly on blood, for about 6 weeks. After this period, the fluke enters the bile ducts, maturing into a fully adult parasite after about 3 months from initial infection. Egg production then commences and completes the life cycle. Sometimes, in rare case, the immature flukes can cross the visceral peritoneum or through blood vessels reach the other organs such as lung, the lymph nodes under the skin, and uterus (Rojo-Vazquez and Ferre-Perez 1999). Adult flukes can survive for many years in the livers of infected hosts and lay between 20,000 and 50,000 eggs/day. The rate of egg production is responsible for the degree of pasture contamination and thus greatly influences the epidemiology of the disease. The epidemiology of the disease is also influenced by the grazing habits of the animals. Animals grazing in wet marshy areas, favored by the intermediate host, are more likely to become infected. Typically, long and wet seasons are associated with a higher rate of infection. However, sheep are more likely to ingest large numbers of cysts during dry periods following a wet season. This is due to a reduction in available pasture, forcing the animals to graze in swampy areas or in areas where the water has receded, thus exposing them to vegetation heavily infected with metacercariae. In the past, human fasciolosis was limited to populations within well-defined watershed boundaries; however, recent environmental changes and modifications in human behavior are defining new geographical limits and increasing the populations at risk (WHO 1998).

Now, it has been established fact that in the last five to six decades there is a marked increase in the temperature (about 1 °C) of the planet earth, if these conditions prolong, temperature may increase up to 4 °C by the end of twenty-first century (Barnett et al. 2006). Kenyon et al. (2009) is of the opinion that occurrence and timing of fasciolosis outbreaks, particularly the epidemiology in future will be directly affected by climatic change. Temperature has a direct effect on larval production of *Fasciola*, thus increasing the number of the cercarial production and transmission of infection in synergy with other factors (Poulin 2006) (Fig. 1.1).

1.6 Genetic Characterization of *Fasciola*

Molecular techniques appear to be very useful for the development of both diagnostic and epidemiological tools. These techniques are very useful in studying intraspecific variability of the *Fasciola*. According to Cwikilinski et al. (2015), *F. hepatica* genome is one of the largest known pathogen genomics at 1.3 GB. Polymorphism in genome may be an evolutionary potential for adaptation to host availability, climate change, drug/vaccine treatments (Cwikilinski et al. 2015; Mas-Coma et al. 2019). A variety of molecular methods have been applied for genetic structuring of the liver fluke. Different molecular methods have been extensively used for genetic characterization of *Fasciola*. These methods are PCR-RFLP of nucleotide genes (ribosomal, mitochondrial, etc.), RAPD variability, and microsatellite markers. These methods are expensive and time consuming. Single nucleotide polymorphism (SNP) assays after direct sequencing is the most reliable method used in genetic diversity



LIFE CYCLE OF *FASCIOLA* SPECIES

Fig. 1.1 Life cycle of *Fasciola* species

studies of *F. hepatica*. This method could solve a substantial problem to find the correlation between the species origin and genetic diversity of geography isolates.

Numbers of fasciolid genotyping studies have been done by various workers (Blair and McManus 1989; Barker et al. 1993; Hashimoto et al. 1997; Itagaki et al. 1998, 2005; Lee and McManus 2001; Semyenova et al. 2003; Huang et al. 2004; Hurtrez-Bousses et al. 2004; Mas-Coma et al. 2003, 2005, 2009) in different countries. In invertebrate, the nuclear ribosomal DNA (rDNA) ITS spacers are the most adequate markers for species differentiation of *F. hepatica* (Mas-Coma and Bargues 2009). Extensive studies on pure samples of various countries have demonstrated, only two haplotypes of ITS-2 differing in only one mutation, whereas the sequence of the other spacer ITS-1 is always identical in pure *F. hepatica*. In pure sample of *F. gigantica*, only one haplotype in ITS-2 and ITS-1 were noted (Mas-Coma et al. 2009).

Polymerase Chain Reaction (PCR) based molecular methods for genetic detection and identification of Fasciola species: PCR based molecular genomic analysis is used in taxonomy and evolution. PCR methods are random amplified polymorphic DNA (RAPD), simple sequence repeat polymorphism (SSR), restriction fragment length polymorphism (RFLP), the novel sequence-related amplified polymorphism (SRAP), etc.

Random Amplified Polymorphic DNA (RAPD): RAPD markers are random segments of genomic DNA (Semyenova et al. 2003), which are used for PCR amplification with primer of arbitrary nucleotide sequence technique in identification of genetic diversity of *F. hepatica* in cattle of Ukraine and Armenia. They found higher variation in examined liver flukes indicating multiple genetically

different parasites, but no relevant information in genetic diversity and population structure of liver flukes. Later, McGarry et al. (2007) studied differentiation of fasciolid species in the UK, Peru, Ghana, and Sudan.

Sequence-Related Amplified Polymorphism (SRAP): SRAP is an effective molecular method for studying genetic variation by the amplification of genomes from related organism on two primers (Li and Quiros 2001). Alassad et al. (2008) and Li et al. (2009) used SRAP method for studying genetic variability and diversity in different geographical locations in Spain and mainland China, respectively. They have noted four coding regions of genome cluster. These regions are not related to both species of *Fasciola*.

Simple Sequence Repeat Polymorphism (SSR): SSR is also known as microsatellites markers, in which repeated sequence is found in noncoding DNA. These microsatellites have high level of mutations. Study on variation in microsatellites in population can explain the population structure and diversity, genetic drift, etc. Hurtrez-Bousses et al. (2004) isolated and characterize six microsatellite markers in *F. hepatica*.

PCR-Restriction Fragment Length Polymorphism (PCR-RFLP): PCR-RFLP method is performed on amplification of particular DNA region that contains polymorphic sites and digestion of particular target amplicon by restriction enzyme. It depends on the comparison of band profiles generated after restriction enzyme digestion of target DNA in species. This technique is used in identifying genetic drift, whole genome and comparative mapping. In Spain, Marcilla et al. (2002) have used this method targeting 28S ribosomal DNA (rDNA) of *F. hepatica* and *F. gigantica*. Huang et al. (2004) and Ichikawa and Itagaki (2010) used ITS-2 (internal transcribed spacer-2) and ITS-1 (internal transcribed spacer-1) for differentiation between *F. hepatica* and *F. gigantica*. Later, Walker et al. (2007) noted 3 mitochondrial DNA (mtDNA) regions with high polymorphism and defined 52 complex halotypes specific for hosts and geographical location.

Single Strand Conformation Polymorphism (PCR-SSCP): PCR-SSCP is a sensitive and convenient method for detection of genetic variation (Sunnucks et al. 2000), in which single strand nucleic acids are electrophoretically separated. Alasaad et al. (2011) have developed PCR-SSCP assays for identification of *Fasciola* species. Identification of *Fasciola* species was done on the sequence of ITS-2 of the rDNA and fluorescence dyes labeled specific primers. Three specific SSCP profiles were developed for the identification of *F. hepatica*, *F. gigantica* and their intermediate in Spain, China, Egypt, and Nigeria.

Loop-mediated Isothermal Amplification (LAMP): LAMP method is used to amplify the target nucleic acid under same temperature conditions with help of two to three sets of primer. This method is accurate and fast and products are detected by photometry (Notomi et al. 2000). Intergenic spacer (IGS) region of rDNA for PCR amplification was in development of LAMP assay to differentiate *F. hepatica* and *F. gigantica* (Ai et al. 2010).

Single Nucleotide Polymorphism (SNP): SNP method is based on the analysis of particular single base pair variation at specific places inside/outside the coding DNA fragments. This method provides a fingerprint in identification of species,

molecular taxonomy, and evolution of genetic diversity investigations. Various methods are used for SNP genotyping, i.e., Reverse Dot Blots; HPLC genotyping, TaqMan assay, fluorescence polarization, microchips, pyrosequencing, SNaP shot, direct sequencing, etc.

SNP genotyping through direct sequencing is the analysis of sequencing region of desired fragment and comparison is made between suspected fragment frequency with wild type fragment or other sequenced fragment with the help of various software. This method is very reliable method used in genotype diversity studies of *F. hepatica*. SNP analyses are done with multiple genes: ribosomal, mitochondrial, and protein coding. Multiple gene studies are utilized in species identification, differentiation and diversity in intra- as well as interspecific relationship.

Ribosomal DNA regions used for SNP assay provide the exact information for systematic and phylogene of the species (Hillis and Dixon 1991). Commonly variable regions are ITS-1, ITS-2, and D-domains (divergent regions in 28S and 18S genes). Various workers have used these rDNA regions in genotype study of *F. hepatica* (Marcilla et al. 2002; Olson et al. 2003; Lee et al. 2007; Vara-Del Rio et al. 2007; Lotfy et al. 2008; Mas-Coma and Bargues 2009). Lotfy et al. (2008) used 28S rDNA, ITS-1 and ITS-2 spacers to describe the evolution of *Fasciolid* species. 28S rDNA fragment was used to explore genetic heterogeneity of *F. hepatica* in Spain (Vara-Del Rio et al. 2007). Teofanova et al. (2011) studied the genetic diversity of liver flukes from Eastern Europe.

Similar to rDNA mitochondrial DNA is also used commonly in molecular taxonomy and genetic diversity study. Mitochondrial DNA provides the maternal inheritance to identify the genealogy to make relationship and origin (Le et al. 2001, 2002). Full nucleotide sequence of *F. hepatica* mitochondrion is described by Le et al. (2001). Liver fluke mtDNA potentially polymorphic three genes viz. FhmtDNA COX3/ND4, FhmtDNA ATP6/ND1, and FhmtDNA COX1/1-rRNA are used in most studies (Walker et al. 2007). Commonly variation analysis of NAD1 and COX1 is used to study the variations in fasciolid isolates (Semyenova et al. 2006; Lee et al. 2007; Amer et al. 2010). Thirteen nad1 and 10 cox1 haplotypes have been identified, viz. Semyenova et al. (2006) studied the differentiation between Eastern European and Western Asian liver flukes *F. hepatica* population. The liver flukes dispersed in two main lineages (Ist and IInd) with 1.07% nucleotide difference and distributed unequally in the studied area. It is presumed that lineage I has Asian origin, but both of them present in European population. Teofanova et al. (2011) and Walker et al. (2011) have studied the population dynamics of *F. hepatica* and genetic diversity in Eastern Europe and Netherland, respectively. Kantozoura et al. (2011) have established the correlation in between south-eastern European genetic variants with the help of mathematical models.

Protein coding genes used for SNP assay have been studied for genetic population structuring. Commonly used proteins are cathepsin L-like enzyme cathepsin L protease family β -tubulin 3 (Rabinson et al. 2002; Irving et al. 2003; Rayan et al. 2008). Irving et al. (2003) reported 18 cathepsin L-like enzymes for evolution study

of fasciolides. They used fasciolide enzymes to study the divergence time of *F. hepatica* and *F. gigantica* species. Teofanova et al. (Alasaad et al. 2011) noted that although β -tubulin 3 gene is used for genetic diversity study, yet it is very suitable as marker of molecule, as it has multiple polymorphism.

In India, no extensive study on fasciolid genotyping has been done. Gunasekar et al. (2008) with the help of RAPD technique noted low level of genetic variation among *F. gigantica* isolates in different host animals. Sankar et al. (2010) has done sequence and phylogenetic analysis of internal transcribed spacer (ITS-1) ribosomal DNA sequence of *F. gigantica*. Sequencing of recombinant clone revealed that the length of the ITS-1 was 433 bp comprising 422 bp of complete ITS-1 gene. 98.8% homology was noted with published sequence of various countries and 99% homology with Meghalaya strain of *F. gigantica*. They suggested that enzyme *RsaI* could be used for restriction fragment length polymorphism (RELP) analysis. Hayashi et al. (2016) phylogenetically analyze *Fasciola* in Delhi, India and identified them as *F. gigantica* by using nucleotide analysis of the nuclear phosphoenolpyruvate carboxykinase (PEPCK) and DNA polymerase delta (POLD) genes. Based on the nucleotide sequence of mitochondrial NADH dehydrogenase subunit (nad 1) gene, the fluke had 18 haplotypes, classified them haplogroup A. This group predominates in South Asia. Delhi population of liver fluke *F. gigantica* showed higher π value than eastern India population. It is suggested that *F. gigantica* of haplogroup A might have spread from west to east India along with the artificial migration of the domestic Zebu cattle, *Bos indicus*.

Tripathi et al. (2018) for the first time in India reported the draft genome of *F. gigantica*. According to them assembled draft genome has a size of ~1.04 Gb with N 50 of 129 kb, with a total number of 20,858 genes. They have noted de nova repeats in the draft genome were 46.85%. They found that in pathway analysis genes of glycolysis, Krebs cycle and fatty acid metabolism were present, but the key genes for fatty acid production in fatty acid biosynthesis were absent, which indicates that fatty acid required for the survival of the fluke may be acquired from the host bile.

Rajanna et al. (2018) studied the genetic diversity of *F. gigantica* in the regions (Deccan plateau, Western Ghats and Coastal region) of Karnataka, India. Infection status of liver fluke in snails was established through cercaria shedding and nested polymerase chain reaction (PCR) based technique second internal transcribed spacers (ITS-2) of nuclear ribosomal DNA. They noted that the sensitivity of PCR (8.2%) for detection of *F. gigantica* infection within snail is significantly higher than cercarial shedding (4.3%) with an overall prevalence of 5.1%. According to them, prevalence of infection was higher in winter than in the rainy and summer seasons (6.2% instead of 4.6% and 4.3%, respectively). Deccan plateau (5.8%) showed a higher prevalence of infection compared to Western Ghats (5.2%) and Coastal region (3.6%). The sequencing IES-2 region permitted the identification of parasite *F. gigantica* population genetic structure of the parasite in the country.

1.7 Evolutionary Origin

Evolution and origin of *Fasciola* species depends on coevolution of definitive (mammals and intermediate freshwater snails) host during the Neozoan age. According to Lotfy et al. (2008), in Eocene Epoch 15 million ago fasciolids originated in Africa and then dispersed in Eurasia. He has also accepted the hypothesis that evolution of fasciolid as well as their host occurs simultaneously, as definitive host of ancient fasciolid were predecessors of the elephants. Intermediate hosts of fasciolids were freshwater planorbidae/lymnaeidae snails. According to Irving et al. (2003), the divergence between *F. hepatica* and *F. gigantica* has occurred in between 28 to 16 million years ago during the Miocene Epoch. It has been also hypothesized that evolution of the *Fasciola* is connected with the evolution of sheep, goats, and cows (Hiendlender et al. 2002; Semyenova et al. 2006), but there are no relevant evidence. Semyenova et al. (2006) have suggested fasciolid origin lineage with African origin and their subsequent dispersion in Europe; however, time of recolonization is not defined, it may be associated with the human migration from Asia to Europe at the end of Pleistocene Epoch.

As per investigation of Teofanova et al. (2011), additional hypothesis is that fasciolid has originated in Australia and Tanzania. Australian haplogroup (AF216697) (Le et al. 2000) and Tanzanian sequence (EU282862) (Walker et al. 2008) to CtCmt1 and CtCmt2.2.2, was determined respectively. These haplogroups have been specifically found in liver fluke of *F. hepatica* population of Australia and Northern Europe; it may be due to colonization from Northern Europe liver fluke populations from the United Kingdom. Second theory is that during continental drift in Miocene about 19 million ago Africa collided with Eurasia (Seyfert and Sirkin 1979). It may be possible that in that period Italy and Greece have not been connected to Eurasian continent so that differences between the liver fluke population in Central Europe (Poland) and South-Eastern Europe (Greece) were noted. Divergence and colonization may occur from either Eurasian liver fluke populations or populations from newly joint to Eurasia Greek.

Third hypothesis, which was given on the basis of Teofanova et al. (2011), investigation is also associated with recolonization of *F. hepatica* during last ice age of Pleistocene Epoch. According to Seyfert and Sirkin (1979), in that time glacier sheets did not cover the Bulgaria and Greece and found only on Northern Carpathian Mountain. Recolonization of Northern areas of Europe after ice age occurred not from far south (Greece) but from boundary of the glacial sheets, it supports the presence of β -tubulin 3 gene and mitochondrial profile of Bulgarian fasciolid. It may be possible that parallel genetic migration could occur from Asia to Europe, concerned with migration of human along with domesticated animals. The evidence was noted in the Asian origin mitochondrial lineage 1 (Semyenova et al. 2006) and identical CtCmt1 lineage (Teofanova et al. 2011) of Europe.

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Abstract

India is a large country having three seasons that is winter, summer, and monsoon, and seven distinct climatic zones. In the present chapter, constrain of fasciolosis caused by *Fasciola hepatica* and *Fasciola gigantica* in different climatic zones is discussed as per earlier studies of different workers in India. *Fasciola hepatica* is only found in temperate/subtemperate region and mountain climate of India, whereas *Fasciola gigantica* is found in all the climatic zones. Percent fasciolosis infection rate is given in all the states of India. Infection of fasciolosis in cattle across the India is between 1.69% and 94%.

Fasciolosis, a food-borne infection by the trematodes *Fasciola hepatica* and *F. gigantica* causes economic losses to the livestock sector estimated at US \$3.2 billion per annum (Spithill et al. 1999) and is considered as a re-emerging zoonosis in several countries (Mas-Coma et al. 2005; El-Rahimy et al. 2012). The spread and increased incidence of fasciolosis due to climate change, drug resistance, and intensified agriculture is noted with *F. hepatica* in Europe (Mas-Coma et al. 2009). Climate change, intensified agricultural practices, and urbanization have changed the epidemiology of *F. gigantica* in the domestic ruminants of the tropical countries.

Fasciola has two species *F. hepatica* and *F. gigantica*. *F. hepatica*, a natural parasite of sheep is smaller in size and abundantly reported in different parts of the world with temperate climate, whereas *F. gigantica*, a natural parasite of buffalo is found around the northern and southern region of equator with tropical or subtropical climate. *F. gigantica* is mostly found in India, whereas *F. hepatica* is reported in northern hilly region of India.

India is federal union of 29 states and 7 union territories having 1.25 billion populations. India covers an area of approximately 3.2 million km². It has a wide

range of weather conditions across a large geographic scale and varied topography. Basically, India has three seasons in all areas, i.e., winter, summer, and monsoon, and large dependency on agriculture-based economy. India has seven distinct climatic zones (Sanyal 1995, 2001).

1. *Humid subtropical climate with dry winter*—extending from Punjab to Assam covering Northern plains.
2. *Tropical rain forest region*—West coast, Goa and parts of Assam and Meghalaya.
3. *Tropical steppe*—comprising crescent between Saurashtra and Kutch in the South, Punjab in the North, and Rajasthan in East.
4. *Tropical semi-arid steppe*—Rain shadow region from Maharashtra to Tamil Nadu covering parts of Andhra Pradesh and Karnataka.
5. *Tropical savanna region*—comprising peninsular parts excluding semi-arid region.
6. *Mountain climate*—comprising Northern Mountains.
7. *Tropical desert*—comprising western parts of Rajasthan and Kutch area of Gujarat.

Most of the studies on prevalence of *F. gigantica* infection have been made on abattoir surveys and fecal detection of fluke eggs of animals visiting clinics or laboratories. A study based on surveys conducted in past indicates the high prevalence of fasciolosis. In India, *Fasciola* infection in Indian elephant *Elephas indicus* first time recorded by Cobbold (1869). Later, Bhalerao (1932) documented *Fasciola* infection in Indian elephant of Assam. Half a dozen *Fasciola* specimens were collected by Mr. F. Ware and H. Cooper from Assam.

Chowdhary (1994) reviewed the prevalence of fasciolosis in domestic livestock in India and reported 14 provincial states to be affected by this disease, covering north, south, east, and west regions. The infection has been found to occur in sheep of 11 states. Now, the constrain of fasciolosis infection has spread nearly all over the India (Table 2.1, Fig. 2.1).

F. gigantica has been reported to be the only species responsible for causing animal fasciolosis in the plains of India with the snail intermediate host identified as *Lymnaea auricularia rufescens* (Thapar and Tandon 1952). However, snails of the variety *L. auricularia* sensu stricto are reported as widely prevalent in the Kashmir valley (temperate agroclimatic zone of India) (Dhar et al. 1985). But, no reports on the transmission of *F. gigantica* or *F. hepatica* by *L. auricularia* sensu stricto in the field conditions in Kashmir valley are available till date, except for a single report on the laboratory transmission of *F. gigantica* by this variety of the snail (Sharma et al. 1989). Prevalence of *F. hepatica* was reported from the highland mountains of Gulmarg (Kashmir valley) in the local sheep breed (Sharma et al. 1989) but no further reports are available on the prevalence of *F. hepatica* in animals of the Kashmir valley or from other regions of the country.

The agroclimatic conditions in the Indian subcontinent differ considerably, consequently infection with *F. gigantica* varies with each geographical region of the

Table 2.1 Different climatic zone-wise prevalence of *Fasciola* infection in India

Name of States	Vector species		Percent infection/reference
	<i>Fasciola gigantica</i>	<i>Fasciola hepatica</i>	
Humid and subtropical climate			
Uttar Pradesh	+		Buffalo (32.8–94%)/Anon (1972), Verma and Subramanin (1977), Singh and Agarwal (1981), Singh et al. (2012a), Dwivedi et al. (1985) Sheep (1.69%)/Yadav et al. (2007) Goat (2.02–3.6%)/Bano and Sultana (2003)
Uttarakhand	+		Sheep (2.53–4.53%), Goat (1.2–3.24%)/Kumar et al. (2007) Buffalo (57.4–78.0%) Saxena et al. (2006)
Haryana			Buffalo (1.3%)/Gupta et al. (1986)
Delhi	+		Cattle, buffalo, sheep and goat (0.8–12.09%)/Yadav et al. (2008)
Punjab	+		Buffalo (21%), Cattle (18%)/Bannerjee and Agarwal (1992) Sheep (72.9%), Goat (56.8%)/Dhand et al. (2004) Dairy Animals (0.5%)/Haque et al. (2011) Cattle (1.88%)/Singh et al. (2012b)
West Bengal	+		Cattle (0.89%)/Samanta and Santna (2009), Paul and Dasgupta (2007)
Bihar	+		Goats (12.87%), Sheep (8.98%)/Kumari et al. (2010)
Jharkhand	+		Snails (11–14%), Tigga et al. (2014)
Odisha	+		Cattle (4.3%)/Mahalik (2015)
Tropical rain forest region			
Meghalaya	+		Cattle (52.25%)/Roy and Tandon (1989), Roy and Tondon (1992)
Assam	+		Cattle (34%)/Devi et al. (2012) Elephant (18–62%)/Islam (1997)
Andaman and Nicobar island	+		Buffalo (12.1–70%)/Rai et al. (1996) Cattle (5.5–16.5%)/Kundu et al. (2010)
Arunachal Pradesh	+		Mithun (4.08–28.9%)/Chamuah et al. (2014), Biswas et al. (2014), Joken et al. (2012)
Sikkim, Manipur, Nagaland, Mizoram	+		Cattle and goat (19.3–83%)/Katiyar et al. (1983, 1991) Cattle (10.16%) Laha et al. (2016)
Tropical steppe			
Gujarat	+		Cattle and buffalo (12–18%)/Pethkar and Hiregaudar (1972) Buffaloes (25–33%)/Pandya et al. (2015a, b) Cattle and buffaloes (36–42%)/Maharana et al. (2016)
Rajasthan	+		Cattle (2.42%)/Godara et al. (2003)

(continued)

Table 2.1 (continued)

Name of States	Vector species		Percent infection/reference
	<i>Fasciola gigantica</i>	<i>Fasciola hepatica</i>	
Tropical semi-arid steppe			
Maharashtra	+		Sheep (31.8%), Cattle (17.5%), Buffalo (23.8%), Goats (19.4%)/Bedarkar et al. (2000a, b) Buffalo, Cattle, Sheep, Goat (5–29%)/Ratnaparkhi (1991)
Tamil Nadu	+		Sheep (50%)/Soundararajan and Iyne (2005), Jeyathilakan et al. (2010)
Karnataka	+	–	Buffalo (1.5%), Cattle (2.4%)/Mamatha and D'souza (2006) Sheep (ND)/Muraleedharan (2005) Buffalo (22.5%), Cattle (26.6%)/Krishnamurthy and D'souza (2015)
Andhra Pradesh	+		Sheep/Rao and Madhubala (1998) Cattle, buffalo (3.8%)/Sreedhar et al. (2009)
Kerala	+		–
Tropical Savanna region			
Madhya Pradesh	+		Buffalo (3.51%), Cattle (6.02%), Goat (3.24%)/Agarwal et al. (2004) Goat (13.86–25.3%)/Solanki et al. (2016)
Chhattisgarh	+		Buffalo (0.6%), Cattle (0.3%)/Pal et al. (2001)
Temperate/subtemperate region			
Jammu and Kashmir	+	+	Cattle (4.46–19.4%)/Pandit et al. (2004) Sheep (15%)/Sharma et al. (2007) Calves (5.1%)/Khajuria and Kapoor (2003) Goat and Sheep (2.0–4.16%)/Yadav et al. (2004) Cow and Buffalo (2.6–6.9%)/Yadav et al. (2006) Cattle (18.7–55.6%)/Dhar et al. (1988) Cattle (85%)/Sharma et al. (1989)
Mountain climate			
Himachal Pradesh	+	+	Cattle and buffalo (36–48.7%)/Jithendran and Bhat (1999) Sheep and Goat (8.8–9.6%)/Jithendran (1998, 2000), Mathur (1986)

country. In India, fasciolosis of cattle and buffaloes is of more serious concern because these animals are the main source of milk and milk products for humans. Besides, buffaloes also constitute the main source of meat for a section of the Indian community and are important for beef export and a source of livelihood for the farmers engaged in dairy industry. Fasciolosis is a major cause of productivity loss in these animals but comprehensive studies on the disease prevalence including all the epizootiological factors are lacking. Most of the reports are primarily based on the data obtained from animals slaughtered at local abattoirs, fecal examination of

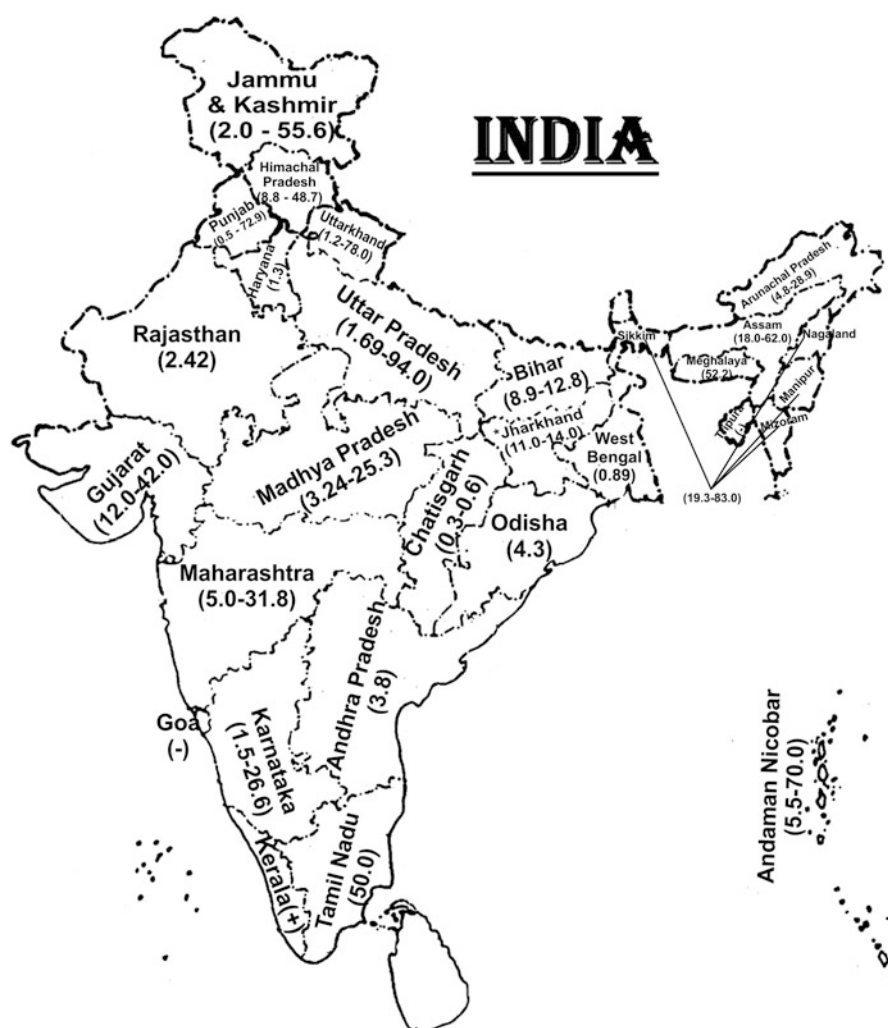


Fig. 2.1 Prevalence of fasciolosis constrain in different States of India. Values in parenthesis indicate percent infection of *Fasciola*. (–) no report

the domestic ruminants in the field conditions or limited experimental work that does not provide a comprehensive picture on the prevalence and economic losses caused by the helminthes. Also, studies on the epidemiology, dynamics of the snail transmission of the pathogen, and forecasting systems for the outbreaks of this snail-borne trematode disease have been scanty till date.

The infection rates in animals reported from various parts of the country vary from 30 to 80% (Katiyar et al. 1983 in Sikkim; Roy and Tandon 1989 in Meghalaya; Singh 1988 in Madhya Pradesh; Kathiria et al. 1990 in Gujarat; Ratnaparkhi et al.

1993 in Maharashtra; Rai et al. 1996 in Andaman and Nicobar Islands; Garg et al. 2009 in north India). Outbreaks of fasciolosis have been reported in sheep in Garhwal hills, Uttarakhand (Katiyar and Tewari 1962), in buffaloes in Great Nicobar Island and Bareilly region (Rao et al. 1985; Gupta and Paul 1987; Chandra et al. 2011). Dhar et al. (1988) and Sharma et al. (1989) reported more than 55% prevalence of fasciolosis in cattle in the Kashmir valley. The available data on the prevalence of fasciolosis in domestic ruminants in different climatic zones of the India are reviewed here.

2.1 Humid Subtropical Climate with Dry Winter

2.1.1 Uttar Pradesh

Uttar Pradesh is one of the largest states of India having mountain climate and Northern plains. It has humid subtropical climate with dry winter. About 40% of cattle and 50% of buffaloes examined by fecal examination were found positive for *F. gigantica* in Uttar Pradesh (Anon 1972). Verma and Subramanian (1977), in an abattoir survey in Uttar Pradesh during May 1970 to April 1971, noted 32.8% *F. gigantica* in buffaloes slaughtered in the Tarai area of U.P. Singh and Agarwal (1981) found heavy infection of *F. gigantica* (94%) in the buffaloes slaughtered in different slaughter houses of Gorakhpur District. Dwivedi et al. (1985) reported that in adjoining area of Izatnagar, Bareilly among 87 buffaloes 20 were positive to infection of *F. gigantica*. In 1995, Pachauri noted 24.4% fasciolosis in cattle of Uttar Pradesh.

Prevalence of fasciolosis caused by *F. gigantica* in goats of Kanpur, U.P. was recorded by Bano and Sultana (2003). They noted that peak infestation was observed during the rainy season 6.19% in 2001, 5.61% in 2002, and 6.25% in 2003. Velusamy et al. (2004) noted the prevalence of *F. gigantica* in buffaloes of Bareilly district of U.P. The highest prevalence of infection was recorded in month of October to December (20.66–23.97%). However, the prevalence was low during the months of January and February (17.9–19.6%). Prevalence of *F. gigantica* infection in intermediate host snail *Lymnaea* was maximum in the month of September (5.88%). Moderate infection was noted in the month of January (1.42%) and February (1.51%). Yadav et al. (2007) conducted epidemiological studies to observe prevalence of *F. gigantica* infection in sheep and goats of western Uttar Pradesh from January 2001 to December 2004. Overall, prevalence in sheep and goats was 1.85%, whereas peak prevalence (2.61%) was noted in rainy season. Animals of Bareilly district were most infected (4.75%), followed by Moradabad (3.50%), Meerut (0.79%), and Agra (0.35%) divisions. Prevalence of the infection in snails was highest (4.18%) during rainy season, whereas lowest (0.76%) in summer.

A recent study on *F. gigantica* infection in the snail intermediate host *L. auricularia* was carried out in some parts of north and central India using ribosomal markers ITS-2 and 28S rDNA and by screening of *L. auricularia* ($n = 3928$) for *F. gigantica* cercarial shedding for the period from 2012 to 2014

(Bauri et al. 2015) in three seasons of each year, viz. summer (March–June), rainy (July–Oct), and winter (Nov–Feb). *F. gigantica* infection in these snails had a significant ($P < 0.05$) occurrence in winter for both the years with 2.9% and 3.7% snails, respectively, infected with the parasite, followed by 1.9% and 0.9% in the rainy season. *F. gigantica* infected *L. auricularia* were prevalent throughout the year with a prevalence of 1.9% and 0.9% recorded for these 2 years (Bauri et al. 2015). A significant ($P < 0.05$) seasonal correlation with the snail density in the water bodies was observed, as in hot summers a heavy mortality of the snails occurred while snail density/m² was higher after rains. Climatic factors including rainfall and water temperature had a direct influence on the transmission of the *F. gigantica* infection by these snails. Climatic characteristics allow for fasciolosis transmission post-monsoon through to winter.

Kumari et al. (2012) noted that *F. gigantica* infection in host snail *L. acuminata* was highest in the month of October (55%), November (70%) followed by June–August (45–50%) in different natural ponds of Gorakhpur. Whereas, in further study Singh et al. (2012a) also found that another snail host *Indoplanorbis exustus* was highly infected (40%) in the month of October–November. These reports indicate that infestation in host snails as well as buffaloes in Gorakhpur is very high. Kumari et al. (2016) while, doing molecular identification of the cercaria larva and adult of *F. gigantica* in different ponds of Gorakhpur and adjoining area found in buffaloes liver belongs to *F. gigantica* because nucleotide sequencing were 99% similar. These findings were submitted to national center for biotechnology information. A gene bank accession number KT49356 and KT49357 were allotted (online) for cercaria larva and *F. gigantica*, respectively.

2.1.2 Uttarakhand

Literatures on prevalence of fasciolosis in Uttarakhand are insufficient. Saxena et al. (2006) identified the presence of *F. gigantica* in liver of buffaloes of Nainital district of Uttarakhand by Elisa method. They found high prevalence of fasciolosis (up to 78%), which was higher than bile sediment examination (57.45%). Kumar et al. (2007) studied the infection of fasciolosis in two geo-climatic conditions of Uttarakhand state, i.e., tarai (up to 1000 m elevation) and hills (above 1000 m elevation). In tarai area, prevalence of 4.53% and 3.49% fasciolosis was recorded in sheep and goats, whereas in hills 2.53% and 1.20% goats were harboring *F. gigantica* infection.

2.1.3 Haryana

In 1986, 1.3% incidence of fasciolosis has been noted in nonendemic area of Haryana (Gupta et al. 1986). Chaudhuri et al. (1993) examined fecal samples of cattle and buffalo at monthly intervals over a period of 6 years in Haryana for

F. gigantica infection and found a high prevalence of infection in both the hosts with greater prevalence in buffaloes. Most of the workers reported the occurrence of infection in cattle/buffaloes throughout the year, with a higher prevalence in winter months. Higher percentage of *Lymnaea auricularia* shedding *F. gigantica* cercariae was noted during August to November (post-rains).

2.1.4 Delhi

During January 2002 to December 2004, a seasonal prevalence of *F. gigantica* infection in cattle, buffaloes, sheep, and goats were carried out in NCR, Delhi. Coprological examination of cattle, buffaloes, sheep, and goats revealed an overall prevalence of 3.12, 4.27, 0.82, and 1.73%, respectively. Besides this, prevalence of *F. gigantica* infection in slaughtered buffaloes, sheep, and goats was 12.09, 2.97, and 2.07%, respectively (Yadav et al. 2008).

2.1.5 Punjab

Dhand et al. (2004) noted that overall 65.2% animals in Saunda village of Sirhand in Punjab were infected with fasciolosis morbidity being significantly less in goats (56.81%) than sheep (72.91%). Cumulative mortality was higher in sheep (0.41%) as compared to goats (0.057%). According to them, it is due to more resistance of goats to fasciolosis. Haque et al. (2011) reported that in western plains of Punjab, which includes Moga, Sangrur and Baramulla districts is among the hot and dry zones. In these districts, 37.97% prevalence of gastrointestinal parasitic infections in adult animals were noted, yet the infection of *F. gigantica* is low (0.51%). Singh et al. (2012b) reported 1.88% infection of *F. gigantica* in coprological examination of cattle in Ludhiana district.

2.1.6 West Bengal

Samanta and Santna (2009) carried out a survey to find out the prevalence of gastrointestinal helminthes in cattle in 52 villages of West Bengal during 2002–2005. They reported that overall parasitic helminthes infection was 76.17%. Infection was significantly lower in stall fed animals (70.3%). However, *F. gigantica* infection was only 0.89% of total helminthes infection (Pal and Dasgupta 2007) while preparing the cross-reactive antigens of *F. gigantica* found that only *F. gigantica* was found in buffalo at Tangea Kalkate.

2.1.7 Odisha

Prevalence of fasciolosis was noted in Bhubaneswar and adjoining area in August 2011 to January 2012. The overall prevalence of *F. gigantica* infection was 4.32% in cattle (Mahalik 2015). High incidence rate of infection was found in rainy season and in older animals.

2.1.8 Bihar

The geographical agro-ecological conditions of Bihar are endowed with hot and humid climate, multiple river banks, floods, less urbanization, and large agricultural land. Kumari et al. (2010) found that the incidence of helminthic infection was quite high. She noted high incidence of helminthic infection in gastrointestinal of sheep (89–90.9%) and goat (79.7–91.1%). Out of all helminthic infection, *F. gigantica* infection was 8.90% and 12.87% in sheep and goats, respectively.

2.1.9 Jharkhand

Fasciola cercaria infection in different host snails around Ranchi was reported by Tigga et al. (2014). According to them, *Indoplanorbis*, *Lymnaea*, and *Gyraulus* species of snails act as an intermediate host of *F. gigantica*. *Fasciola* infection in snails was highest in the month of September (11%) and October (14%). Bauri et al. (2015) identified the prevalence of *F. gigantica* cercaria infection in 28 randomly selected *L. auricularia* and nucleotide sequence analysis matched its adult *F. gigantica*. None of the sequence matched to *F. hepatica*. They concluded that in Ranchi *F. gigantica* was the species prevalent in host snails. *F. gigantica* infection in *L. auricularia* was prevalent throughout the year, maximum in winter (2.9–3.7%). There was a significant correlation of the season with density of the snail population (Bauri et al. 2015).

2.2 Tropical Rain Forest Region

Rao et al. (1985) reported that out of 452 buffaloes died in great Nicobar Island between April 1975 and December 1976, 35.3% were infected with *F. gigantica*, whereas in Meghalaya 52.25% cattle were infected with *F. gigantica* (Roy and Tandon 1989). Rai et al. (1996) noted 12.1–70.0% incidence of bovine fasciolosis in Andaman and Nicobar Island.

2.2.1 Meghalaya

Roy and Tandon (1989) conducted a survey on the prevalence of fasciolosis in Meghalaya between years 1985 and 1987. According to them in four districts viz. Shillong, Nongstom, Jowai, and Tura of Meghalaya, there was high (52.25%) prevalence of *F. gigantica* in beef cattle. Rate of infection among male host was higher. Prevalence rate was higher in winter than summer. Further, in 1992, Roy and Tandon noted 53.02% of *Fasciola* infection in cattle population of Meghalaya. Beef cattle are an important livestock of North-East India having more cattle per hundred of population than the rest of the country. *F. gigantica* was found to be one of the major parasites among slaughtered cattle and snail *L. auricularia* is the intermediate host of this fluke (Rajkhwa 1982).

2.2.2 Assam

Devi et al. (2012) while studying the prevalence of *Fasciola* infection in fecal sample of cattle of killing village of Assam reported 3–34% infection of *F. gigantica*. Prevalence of *Fasciola* infection in wild elephant in Assam was reported by Islam (1997). He reported that in wild elephant an overall prevalence rate was 33.7% while in captive elephants it was in between 18.18 and 62.28%.

2.2.3 Andaman and Nicobar Islands

The climatic condition of Andaman and Nicobar Islands is ideally suited for the multiplication of parasite *F. gigantica* as well as their host snails. Rai et al. (1996) in an epidemiological study on bovine fasciolosis reported that in Andaman and Nicobar incidence of *F. gigantica* was in between 12.1% and 70%. The incidence of fasciolosis was higher in September to April. Seroepidemiology of the cattle indicated the prevalence of *F. gigantica* infection of about 5.50–11.50% in Andaman and 10–10.42% in Nicobar region (Kundu et al. 2010).

2.2.4 Arunachal Pradesh

Bos frontalis is known as Mithun, cattle of hilly region (Shisode et al. 2009; Bam et al. 2012). In India, semi-domesticated *B. frontalis* are kept by several ethnic groups living in Arunachal Pradesh. Chamuah et al. (2014), while studying the prevalence of fasciolosis in Mithun at different geographical locations of Nagaland and Mizoram, reported *F. gigantica* infection. Biswas et al. (2014) noted 19.23% infection of *F. gigantica* in Mithuns of Arunachal Pradesh.

Joken et al. (2012) observed a seasonal variation of *F. gigantica* infection in Mithuns of Kanieng and Tawang districts of Arunachal Pradesh. Overall 4.08% of *Fasciola* infection was observed in Mithuns of these districts. Human fasciolosis has

been reported by Ramachandran et al. (2012) in a 55-year-old lady from Arunachal Pradesh. The infestation was with *F. gigantica* or a *F. gigantica* like hybrid. Hemadri and Hiremath (2011) in publication Vision 2030 of “Project directorate of animal disease monitoring and surveillance,” reported that prevalence of *F. gigantica* infection in hills, tarai, and plains of North India was 10.79, 13.90, 2.78, and 2.35% in cattle, buffaloes, sheep, and goats, respectively.

2.2.5 Sikkim/Manipur/Nagaland/Mizoram

Fecal analysis of cattle and goats in Sikkim indicates that *F. gigantica* caused 83% and 81% fasciolosis infection (Katiyar et al. 1983). Later Ansari et al. (1989), Katiyar et al. (1991), Rahman et al. (2010), and Goswami et al. (2013) also reported *F. gigantica* infection in Mithun and buffaloes in Sikkim and adjoining areas. Seroprevalence of fasciolosis was found in Mithuns of different geographical location of Nagaland and Mizoram. About 19.23% fasciolosis tests in bloods were positive. Out of this 25.84% were from free range conditions and 10.44% were recorded from semi-intensive conditions. Laha et al. (2016) noted 10.16% *Fasciola* infection in cattle population of Sikkim.

F. gigantica were detected in Imphal, Kohima, and Gangtok districts of this region. *F. gigantica* was predominant and well diversified and the species was thought to be distributed in Eastern India. *F. gigantica* population in this area were categorized into two haplogroups A and B. Population belonging to haplogroups A have dispersed from the west side of the Indian subcontinent to Eastern India, whereas haplogroup B has spread from Myanmar to Eastern India with domestic buffaloes, *Babalus bubalis* (Hayashi et al. 2015).

2.3 Tropical Steppe

2.3.1 Gujarat

Pethkar and Hiregaudar (1972) reported 12–18% infection of *F. gigantica* in cattle and buffaloes of Gujarat. Pandya et al. (2015a) identified the presence of *F. gigantica* in the liver of Anand and Ahmedabad district. In another study, Pandya et al. (2015b) found the prevalence of *F. gigantica* in fecal and liver sample of buffaloes of both the districts. They noted highest prevalence rate in the month of December (25.9% fecal and 33.3% liver samples) in Anand district, whereas in Ahmedabad the highest prevalence rate was recorded in the month of February (26.98%). Prevalence of *F. gigantica* infection was 42% in fecal examination of cattle and 36% in buffaloes in and around Junagadh, Gujarat was noted by Maharana et al. (2016).

2.3.2 Rajasthan

In the livestock rich state of Rajasthan, a large number of animal fairs are held every year at different places. The animal fairs held at Gogameri, Nagaur, Bharatpur, Tilwana, and Jhalrapatan are the important fairs of Rajasthan. Godara et al. (2003) in 2000–2001 studied the helminthes infection in cattle brought at the animal fairs. They noted that 2.4% of cattle were infected with *F. gigantica*.

2.4 Tropical Semi-Eric Steppe

2.4.1 Maharashtra

Bedarkar et al. (2000a) conducted study on fecal samples of cattle, buffaloes, goats, and sheep, and found the prevalence of *F. gigantica* infection round the year in Marathwada region. They found highest (31.87%) infection in sheep followed by buffaloes (23.87%), goats (19.41%) and lowest in cattle (17.51%). Sheep are more prone to *F. gigantica* infection. Females of all ruminant species had higher incidence than males. Age-wise analysis indicated peak infections during maturity age. Breed-wise analysis proved the existing fact that local breeds get less affected than crossbreeds. Bedarkar et al. (2000b) further noted the *F. gigantica* infection in different region of Marathwada districts on the basis of presence of river/free flowing water as source of drinking water (biotype I) and lake/pond or stagnant water as a source of drinking water (biotype II). Biotype II, i.e., stagnant water drinking cattle (19.35%), buffaloes (24.02%), sheep (33.91%), and goats (20%) were more infected than biotype I, i.e., flowing water drinking cattle (16.54%), buffaloes (23.79%), sheep (30.70%), and goats (19.10%). Seasonal prevalence of *F. gigantica* infection was higher in rainy season. Infection of *F. gigantica* in rainy season was 44.5% in buffaloes and 40–90% in sheep. Bedarkar et al. (2000b) also found that maximum-minimum temperature, bright sunshine, and wind velocity deterred the prevalence while rainfall, humidity, and vapor pressure favored the prevalence. In rainy season, *F. gigantica* infection was highest in buffaloes (44.52%) followed by sheep (40.90%), goats (28.74%), and cattle (28.28%). In winter season *Fasciola* prevalence was less (buffaloes—21.3%, sheep—40.2%, goats—21.17%, and cattle—22.44%) than rainy season. Ratnaparkhi (1991) in his M.V.Sc. work studied the incidence of fasciolosis in domestic animals in Parbhani district of Maharashtra. According to them, highest incidence of *F. gigantica* infection 29.2, 20.0, 14.0, 44.3, and 31.42% was noted in buffaloes, cattle, crossbreed cattle, sheep, and goats, respectively. Whereas, lowest incidence was recorded in the month of May (12.0%) in buffaloes, January (5.0%) in indigenous cattle, March (8.0%) in crossbreed cattle, October (16.0%) in sheep, and April (9.41%) in goats.

2.4.2 Tamil Nadu

An outbreak of mixed infection of *F. gigantica* and *Dictyocaulus filaria* in lungs of Nilgiri sheep in Tamil Nadu was reported by Saundararajan and Iyne (2005). They observed that sudden death of Nilgiri sheep in between December 1998 and May 1999 was due to immature *F. gigantica* in liver parenchyma. Fifty percent of the sheep were died due to immature *F. gigantica* in lungs. *F. gigantica* infection was also reported in animals of Perambur in Chennai (Jeyathilakan et al. 2010).

2.4.3 Karnataka

Muraleedharan (2005), while studying the prevalence of gastrointestinal parasites of livestock in central dry zone of Karnataka reported very low infection of *F. gigantica* (0.14%) in cattle. There was no *Fasciola* infection in sheep. Further, Mamatha and D'souza (2006) noted that in and around Bangalore *F. gigantica* infection was 2.4% and 1.5% in cattle and buffaloes, respectively. In 2015, Krishnamurthy and D'souza evaluated the prevalence of fasciolosis in bovines based on carpological examination and postmortem liver examination of slaughtered cattle and buffaloes. They noted 9.9% and 13.3% prevalence of fasciolosis in cattle and buffaloes, respectively.

2.4.4 Andhra Pradesh

F. gigantica infection in ruminants of Andhra Pradesh is endemic causing heavy economic loss (Mahajan 1942). Rao and Madhubala (1998) made a study in outbreaks of hepatopulmonary fasciolosis in sheep of Andhra Pradesh in March and April 1992. Prevalence of *F. gigantica* in fecal examination of cattle and buffaloes of Anantapur district was 3.8%. The incidence was higher in monsoon season (Sreedhar et al. 2009).

2.4.5 Kerala

Outbreaks of subacute Bubaline fasciolosis have been reported in Wayanad, Kerala. In 2012, 6 buffalo calves of 6–9 months died due to Bubaline fasciolosis. Snails collected from nearby water bodies released cercaria, indicating the source of infection (Jyothimol et al. 2013).

2.5 Tropical Savanna Region

2.5.1 Madhya Pradesh

Bannerjee and Agrawal (1992) studied the prevalence of *F. gigantica* infection in between November 1987 to June 1988 at Jabalpur district of Madhya Pradesh. In Jabalpur, annual rainfall were 140–160 cm and a minimum temperature of 7.5 °C and maximum temperature of 42.5 °C. Prevalence of *F. gigantica* infection was 21% and 18% in buffaloes and cattle, respectively Bannerjee and Agrawal 1992. Agarwal et al. (2004) studied the *F. gigantica* infection in eight districts of Madhya Pradesh and reported that the *Fasciola* infection was in between 1.8 to 3.24 to 6.02% out of which highest (6.02%) infection of *Fasciola* was noted in cattle followed by buffalo (3.51%) and goat (3.24%). Recently, Solanki et al. (2016) have reported 22.66% of fasciolosis. In young goat, prevalence was 13.86% while in female prevalence was 25.33%. The prevalence of complete 1 year in relation of season were observed in rainy season (58.6%) followed by winter (19.48%) and in summer (10%).

2.5.2 Chhattisgarh

Pal et al. (2001) noted that parasitic infection particularly on liver fluke in Chhattisgarh is less reported. According to them, *F. gigantica* prevalence in cattle and buffalo was 0.3% and 0.6%, respectively. However, intensity of amphistomes infection was higher in fecal samples of buffaloes (16.1%) followed by cattle (8.0%).

2.6 Mountain Climate

2.6.1 Himachal Pradesh

Systematic survey on the fluke infection in dairy animals of 12 villages in the Kangra valley of Himachal Pradesh was noted in the year 1986–1990 and 1993–1997. Endemic fasciolosis in buffaloes was observed throughout the year, but high peak was noted between July and September (Jithendran and Bhat 1999). *Fasciola gigantica* infection was 36% and 48.7% in cattle and buffaloes during 1986–1999 observation. Between 1993 and 1997, the infection percentage was lower, i.e., 6.3% and 20.6% in cattle and buffaloes. Prevalence of *Fasciola* infection in sheep and goat was 9.6% and 8.8%, respectively (Jithendran 2000).

Krishna et al. (1989), Jithendran and Krishna (1990), HPKV annual report (1989–1998), and Agnihotri et al. (1992) reported severe infection of *Fasciola gigantica* in Kangra, Kullu, Mandi, and Chamba district's cattle and buffaloes. Jithendran (1998) reported severe infection of *Fasciola hepatica* in Kangra district's cattle liver. The intermediate snail is *L. auricularia*. Severe *F. gigantica* was also noted in goat and sheep of Kangra, Kullu, Shimla, Mandi, and Chamba districts (Mathur 1986; Jithendran 1994, 1998).

2.7 Temperate/Subtemperate Region

2.7.1 Jammu and Kashmir

Dhar et al. (1988) reported higher prevalence (18.7–55.6%) of fasciolosis in animal fecal examination. Cattle infection of *Fasciola* was highest in different areas of Kashmir valley. In necropsy examination, they found 100% prevalence in cattle. Later, Sharma et al. (1989) reported necropsy examination of cattle and indicate higher prevalence (85.1%) of fasciolosis in the same area of Kashmir valley.

The temperate climate of Kashmir is very congenial for the propagation of helminth parasites. *F. gigantica* infections in cattle of Kashmir valley in farm and field management were 4.46% and 19.41%, respectively (Pandit et al. 2004). Sharma et al. (2007) while studying the gastrointestinal helminthes in sheep noted that in winter season *F. gigantica* infection was 5% (minimum temperature was 13.35 °C and relative humidity was 58.25%), while in summer season it was 15% in 2003 (minimum temperature was 21.5 °C and relative humidity was 46.25%), respectively. Yadav et al. (2006) noted the prevalence of *F. gigantica* infection (2002–2004) in rainy season was highest (4.16%) in comparison to winter (2.0%) and summer (2.8%). Temperature prevalent in Jammu was >18.3 °C throughout the year, which is optimal for development of *Fasciola*. However, rainfall (>50 mm/month) during rainy season also favors higher prevalence. Yadav et al. (2004) noted the *F. gigantica* infection distributed in 26 villages of R.S. Pura tehsil of Jammu. According to them, *F. gigantica* infection in cows/buffaloes was highest (6.92%) in rainy season, while in winter and summer seasons it significantly reduced to 5.54% and 2.65%.

Khajuria and Kapoor (2003) studied the prevalence of parasites in sheep and goats at Kathua in Jammu. They noted that *Fasciola* species infection in sheep and goats of Kathua was 26.1–73.8% and 38.09–61.9%, respectively. Sheikh et al. (2006) in his study reported the presence of *F. gigantica* and *F. hepatica* in crossbreed Jersey cattle. The reason for overall higher prevalence of *Fasciola* in animals in Kashmir region was apparently due to the fact that agroclimatic conditions prevailing in the valley for most of the year are of a temperate nature.

The mithun (*Bos frontalis*), a rare bovine species is part of a rich biodiversity in the forests of north-east India (inhabiting high altitudes of 1000–3000 m above mean sea level) and in parts of Bhutan, Myanmar, China and Bangladesh, plays an important role in the socioeconomic and cultural life of the tribal population of this region, being primarily used as a beef animal. Fasciolosis caused by *F. gigantica* was reported previously in the mithun in India (Chamuah 2005; Rajkhowa et al. 2005). Molecular characterization of the flukes using ITS-2, 28S rDNA, and nad1 sequences with partial characterization of the parasite haplotypes has shown that the parasite species infecting the mithun in the north-east India was *F. gigantica* (Chamuah et al. 2015). Reports on the molecular characterization of the *Fasciola* species prevalent in domestic ruminants in the different parts of India are limited (Prasad et al. 2009, 2011; Raina et al. 2015; Hayashi et al. 2015). Identification of the *Fasciola* species prevalent in different domestic animals in this country using

molecular tools has gained relevance because of the prevalence of *F. hepatica* reported in sheep in the north-west Himalayan region (Sharma et al. 1989) and *F. gigantica* in cattle and buffaloes in the north-east Himalaya and the plains of India (Gupta and Singh 2002; Prasad et al. 2009, 2011; Hayashi et al. 2015). The geographical co-existence and even the presence of hybrid forms of these two species have been described in several countries (Huang et al. 2004; Itagaki et al. 2005; Peng et al. 2009) but not in India. Therefore, molecular characterization of the *Fasciola* species prevalent in the different hosts in this country is required for proper description of the species, hybrid and parthenogenetic forms of the parasite.

Hemadri and Hiremath (2011) in their writing “Vision 2030” of project directorate on animal disease monitoring and surveillance reported the prevalence rate of *F. gigantica* in hills, tarai, and plains region of North India. It was 10.7, 13.9, 2.7, and 2.3% in cattle, buffaloes, sheep, and goats, respectively. Animals in tarai had the highest prevalence of fasciolosis followed by those in the hills and plains, respectively. *F. gigantica* infection in cattle and buffaloes was highest during the winter (11.84% cattle, 15.5% buffaloes) followed by summers and rains. The seasonal infection trends in sheep and goats were reverse, with the peak prevalence during rains (4.6% sheep, 2.7% goats). Abattoir studies reveal a higher prevalence in buffaloes (31.1%) than in sheep and goats. Screening of *Lymnaea auricularia* snails reveal that 5.4% of the snails harbored larval stages of *F. gigantica*. Snails in the tarai had higher infection rate (7.2%) compared to those in the plains (1.5%).

Variation in prevalence of fasciolosis among different domestic animals and incrimination of different snail species in different geographical area suggest that genetic factors play role in such variation (Chowdhary 1994; Agarwal and Banerjee 2007). India is well known for its rich biodiversity, where animals are resistant to many pathogens (including *Fasciola*), and hence able to survive under adverse condition. No serious attempt has been made to identify resistant breeds of animals either for one or more helminthic infection. There is circumstantial evidence that Garole sheep of sundarban area in West Bengal is resistant to fasciolosis despite grazing in marshy land. A more study is required for confirming the phenomenon with work for isolating resistant gene. A study on host snail is required involving parasitological and biochemical studies to understand habitat, biological variations, and susceptibility status, which affect the epidemiology of *Fasciola* infection in the country.

Several cases of human fasciolosis from different states in India have been reported so far based on the incidental findings on imaging or endoscopy. Most of these reports have documented the presence of flukes in the bile duct and liver and the presence of ova in stools. No epidemiological data on the prevalence or endemicity of this zoonotic parasite in humans in India is available. Most of the human cases of *Fasciola* infection reported the species as *F. hepatica* (Kumar et al. 1995; Narain et al. 1997; Kumari et al. 2013; Ashdhir et al. 2014), with only a few reports incriminating the species as *F. gigantica* (Gandhi et al. 2010; Ramachandran et al. 2012). No molecular markers were used in these studies to determine the species of the parasite; the species being identified on the morphometric analysis of the ova/adult flukes. Ribosomal and mitochondrial markers are well established for

differentiating the *Fasciola* species and identification of the hybrid or parthenogenetic forms of the flukes. *F. hepatica* has not been reported till date either in the animals or in snail intermediate hosts in the plains of India but for a single report of *F. hepatica* from the high altitudes of Kashmir valley (Sharma et al. 1989). Recently, Ghildiyal et al. (2014) reported the presence of *F. hepatica* infection in the skin of 27-year-old women living in Lucknow, Uttar Pradesh. Therefore, the reports of human *F. hepatica* infections in India lack scientific confirmation. Also, human fasciolosis cases in India could be higher than reported in the medical journals, as a higher rural population size is at the risk of infection due to the close contact of human with the animals and snail infested water bodies, lack of healthcare workers familiarity with the diagnosis, and public unawareness of the risks of waterborne trematode diseases in the rural India.

With the increase in urbanization and industrialization, agricultural land is shrinking and availability of the community ponds, lakes, and other water bodies to the grazing animals is also reducing. These factors along with stall feeding practices adopted by dairy farmers have resulted in an overall decline in the prevalence of *Fasciola* infection along with other snail-borne trematode infections in cattle, buffaloes, sheep, and goats during the past few decades. Also, several dams, canals, and improvised irrigation facilities for intensive crop cultivation have come up within the country in the last few decades. This has changed the epidemiology of fasciolosis in different parts of the country with the addition of new pockets of infection in these regions. The importance of livestock in this country makes it essential to prioritize adequate control measures against fasciolosis. A judicious anthelmintic treatment scheme to control the disease is recommended to be applied in the endemic regions along with awareness of the framers for avoiding grazing of animals along the snail infested water bodies.

Kumar et al. (2006) while studying the economic impact of helminthes infestation on milk production of cattle noted that posttreatment of *Fasciola* infected cattle caused increase of 18.18% milk production in 4 weeks. No exact economic loss to milk/meat production as well as other aspects of livestock in India is estimated so far. However, Australian center for international agriculture research (Copeman and Copland 2008) estimated the economic loss on the basis of number of infected cattle and buffaloes with *Fasciola*. According to them, ranges of total losses are in between 8128–9713 millions of Australian dollars in India.

A concentrated effort is needed to generate data with respect to geographical, climatological, and environment/biological factors controlling the fasciolosis. Mapping of fasciolosis prevalence throughout the country is required. So that, forecasting system should be developed for different regions of the country for control of fasciolosis.

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Distribution and Ecology of Lymnaeidae/Planorbidae Snails in India

3

Abstract

Lymnaeidae/planorbidae snails are the intermediate hosts of *Fasciola hepatica* and *Fasciola gigantica*. Distribution of these vector snails explains the infection of fasciolosis as per geography and climatic condition of place. It also plays a vital role in the pattern of fasciolosis transmission. India is a large country, having varied climatic conditions in different states, so that in the present chapter distribution and habitat of snails in different states of India is given, which represents the statewise consolidated information to explore the vector snail presence.

Freshwater gastropods of family Lymnaeidae are vectors of *Fasciola* (Mas-Coma et al. 2019). Bargues et al. (2001) suggested that molecular studies on lymnaeids and hybridization phenomena between the *F. hepatica* and *F. gigantica* have raised the fasciolid – lymnaeid specificity in different parts of the world (Mas-Coma et al. 2009a, b). Lymnaeid vectors explain the distribution of fascioliasis according to geography and climate/environmental condition of place in the same way as orthopods play in transmission of many infectious diseases (Mas-Coma et al. 2019). The lymnaeid vector species represents the vital role in transmission pattern of fasciolosis. Various species of lymnaeids inhabit different ecological, ethological conditions. Presence of different type of water bodies, population dynamics of snail in that water body, variation of seasonal condition as well as susceptibility of lymnaeid snails to infective larval stage of *Fasciola* are the major key points, which affect the distribution of fasciolosis (Mas-Coma et al. 2019).

Gastropods are important intermediate host of various trematode parasites. More than hundred species of freshwater gastropods are reported to serve as intermediate hosts for schistosomes, liverflukes, intestinal and lung flukes (Morphy et al. 1969; Bali et al. 1986; Rao Subba and Mitra 1991). Freshwater gastropods can be grouped

in two subclasses, Prosobranchata and Pulmonata. Out of 100 species noted as intermediate hosts, only 18 are prosobranchs and rest belongs to pulmonates. Among pulmonates, family *Lymnaeidae* and *Planorbidae* are most important as they are the host of maximum helminth parasites in India.

3.1 Family: *Lymnaeidae*

Lymnaeidae is very important family of pulmonate snails. In India, it is represented by genus *Lymnaea* (Lamarck, 1799). Rao Subba (1989) reported 18 species in India. Due to variable shell characters, a large number of species and subspecies forms were noted by various workers (Annandale and Rao 1925).

Burch and Lindsay (1973) explained that classical taxonomic revisions within the family based on the shell characters are more authentic.

3.1.1 Subgenus *Pseudosuccinea*

Lymnaea acuminata (Lamarck, 1822) occurs in permanent water bodies with full of vegetation. In this species, nine forms were noted: form *typica*, *patula*, *chlamys*, *rufescens*, *gracilior*, in which shell is thin, ovate, or elongate; spire exerted; body whorl large; aperture oval, columella spirally twisted; mantle not extended over the shell. These forms were distributed all over the India, whereas form *hians* and form *malleata* were reported in south and north-west India and Punjab/Manipur, respectively. Form *brevissima* and *pseudohorae* were reported in Nagpur and Burma, respectively (Rao Subba and Mitra 1991; Mitra et al. 2005).

Form *typica* Lamark, 1822

Shell thin, ovate, spire short, acuminate, inflated body whorl, a little angular below having a large aperture (Choubisa and Zulfiya 2013) (Fig. 3.1).

Form *brevissima*, Annandale and Rao 1925

This species is found in vegetation with permanent water bodies. *Lymnaea* (*Pseudosuccinea*) *acuminata* form *brevissima* (Rao Subba 1989). Shells are regularly ovate; spire having three whorls and broad; sculpture with fine curved vertical lines.

Fig. 3.1 *L. acuminata*
f. *typica* dorsal and ventral,
size: 20.10 × 11.20 mm

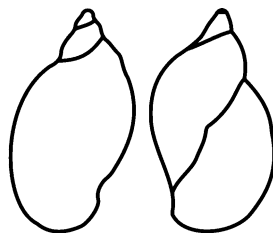


Fig. 3.2 *L. acuminata*
f. *brevissima* dorsal and
ventral, size: 13.4 × 11.2 mm

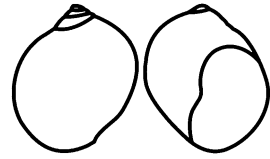


Fig. 3.3 *L. acuminata*
f. *chlamys* dorsal and ventral,
size: 16.7 × 11.9 mm

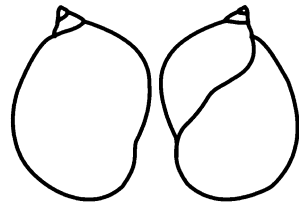
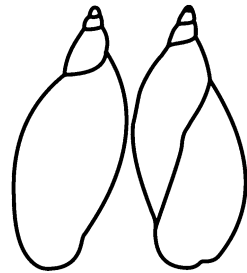


Fig. 3.4 *L. acuminata*
f. *gracilior* dorsal and ventral,
size: 22.95 × 9.90 mm



This form is very closely related to *L. acuminata typica* Lamarck except the depressed spire. It is found in Maharashtra, State of India (Fig. 3.2).

Form *chlamys* Benson, 1836

Lymnaea (Pseudosuccinea) acuminata form *chlamys* (Rao et al. 2004). Shell spire a little large but narrower in comparison to *typica*, columella more twisted, golden yellow in color.

Distribution: Widely occurs throughout India (Fig. 3.3).

Form *gracilior* Martens, 1881

Lymnaea (Pseudosuccinea) acuminata form *gracilior*: (Mitra et al. 2005). Shell is linear with a long narrow spire; shell color varies between greyish to light pink.

It is found in West Bengal: Bardhaman, Kolkata, Hugli, North 24 Parganas, Purulia; Assam, Jharkhand, Kerala, Maharashtra, Orissa, Uttar Pradesh (Fig. 3.4).

Form *hians* Sowerby, 1873

Lymnaea acuminata form *hians*: (Rao Subba 1989). Shell is ovate dwarf, spire high and narrow; body whorl inflated and little angular; aperture large.

It is found in Kerala; Malabar; Mizoram states of India (Fig. 3.5).

Fig. 3.5 *L. acuminata*
f. *hians* dorsal and ventral,
size: 10.80 × 5.40 mm

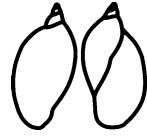


Fig. 3.6 *L. acuminata*
f. *malleata* dorsal and ventral,
size: 20.80 × 13.45 mm

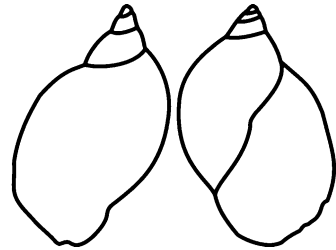
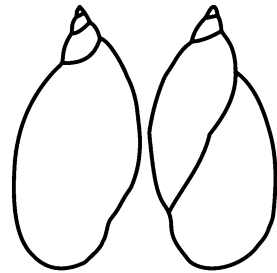


Fig. 3.7 *L. acuminata*
f. *patula* dorsal and ventral,
size: 22.90 × 11.0 mm



Form *malleata* Annandale and Rao 1925

Lymnaea (*Pseudosuccinea*) *acuminata* form *malleata* (Rao Subba 1989). Shells are very similar to the *hians* but can be readily distinguished by its typical texture and well-defined malleated sculpture of the body whorl. It is found in Punjab; Manipur; Mizoram states of India (Fig. 3.6).

Form *patula* Troschel, 1837

Lymnaea (*Pseudosuccinea*) *acuminata* form *patula*: (Rao Subba 1989). Shell is narrower than in *typica*, spire relatively large, tapering anterior extremity of aperture. Found throughout India (Fig. 3.7).

Form *rufescens* Gray, 1822

Lymnaea (*Pseudosuccinea*) *acuminata* form *rufescens* (Mitra et al. 2005). Shell is narrower than in typical form, spire longer, aperture uniformly less expanded; columellar fold is feebly developed; reddish in color. Found throughout India (Fig. 3.8).

All the above species are intermediate host of *Fasciola gigantica*, *F. hepatica*, *Schistosoma indicum*, *S. nasalis*, *S. spindalis* and *Clinostomum*, *giganticum*, *Echinostoma revolutum*, *F. malayanum*, and *Orientobilharzia turkestanicum*.

Fig. 3.8 *L. acuminata*
f. *rufescens* dorsal and ventral,
size: 22.65 × 13.45 mm

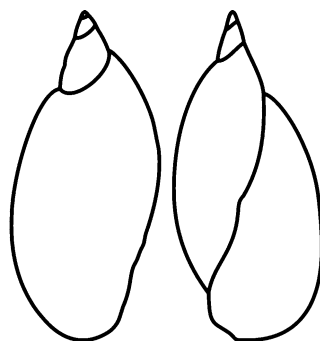
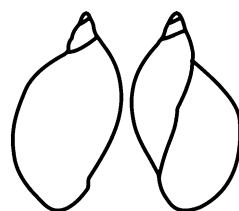


Fig. 3.9 *L. biacuminata*
dorsal and ventral, size:
17.85 × 9.9 mm



3.1.2 *Lymnaea (Pseudosuccinea) biacuminata*: (Mitra et al. 2005)

Shell is thin, fragile, narrow, and elongate spindle shaped; aperture large ovoid; columella slightly twisted and has a broad fold; umbilicus completely occluded; sculptured with curved longitudinal striae; pale luteous in color. This species differs from *L. acuminata* in having the outer lip pointed below resulting in the shell being spindle shaped or acuminate at both ends.

L. biacuminata (Annandale and Rao 1925) is found in Andhra Pradesh: Secunderabad, Hyderabad; Uttaranchal: Kumaon, Nainital (Fig. 3.9).

3.1.3 *Lymnaea luteola* Lamark, 1822

Lymnaea luteola Lamark, 1822 is not very particular about its habitat. Sometime this species is noted in temporary water bodies. It is less variable than *L. acuminata* and considered as pest of paddy in West Bengal. It has six forms, namely *typica*, *Ovalis australis*, *impura* found throughout India, *Succinea* in South and North India and Kashmir, *Siamensis* at Burma border.

Form *typica* *Lymnaea (Pseudosuccinea) luteola*: (Mitra et al. 2005)

Shell is less inflated, thin and glossy, relatively smaller and laterally compressed, spire gradually tapering and more produced, aperture narrow. It is found throughout India (Fig. 3.10).

Fig. 3.10 *L. luteola* f. *typica*
dorsal and ventral, size:
24.65 × 14.10 mm

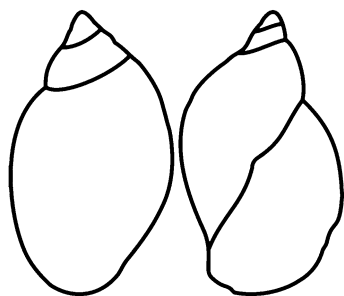


Fig. 3.11 *L. luteola*
f. *australis* dorsal and ventral,
size: 15.80 × 9.25 mm

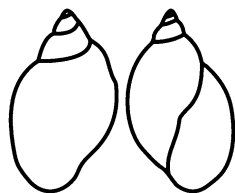


Fig. 3.12 *L. luteola*
f. *impura* dorsal and ventral,
size: 16.20 × 9.40 mm

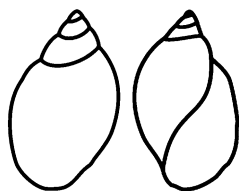
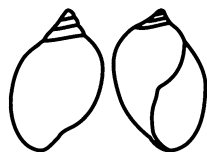


Fig. 3.13 *L. luteola* f. *ovalis*
dorsal and ventral, size:
12.6 × 8.35 mm



Form *australis* *Lymnaea* (*Pseudosuccinea*) *luteola*: (Mitra et al. 2005)

Shell is smaller than *typica*, spire comparatively longer, last whorl well rounded, suture shallow. Reported commonly throughout India (Fig. 3.11).

Form *impura* *Lymnaea* (*Pseudosuccinea*) *luteola*: (Mitra et al. 2005)

Shell is smaller than *typica*, spire comparatively longer, last whorl well rounded, suture shallow. Found in Andhra Pradesh, Assam, Himachal Pradesh, Kerala, Maharashtra, Nagaland and Orissa, India (Fig. 3.12).

Form *ovalis* *Lymnaea* (*Pseudosuccinea*) *luteola*: (Mitra et al. 2005)

Shell is larger than in preceding species, last whorl much inflated without any compression, spire short and abruptly acuminate. Commonly found throughout India (Fig. 3.13).

Fig. 3.14 *L. luteola*
f. *succinea* dorsal and ventral,
size: 18.42 × 8.45 mm

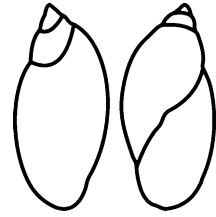
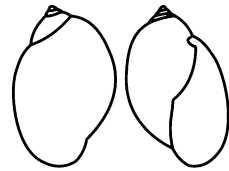


Fig. 3.15 *L. ovalior* dorsal
and ventral, size:
14.45 × 9.25 mm



Form *succinea* *Lymnaea* (*Pseudosuccinea*) *luteola* (Rao Subba 1989)

Shell is larger than in preceding species, last whorl much inflated without any compression, spire short and abruptly acuminate; columellar fold ordinarily pronounced; inner lip of different width. Found in Andhra Pradesh: Godavari Town, Hussain sagar, Kurnool, Secunderabad; Jharkhand: Ranchi; Hazaribagh; Kashmir: Harawan, Kokarnag, Srinagar; Kerala: Malabar Coast; Maharashtra: Andheri (Mumbai); Punjab, Rajasthan: Udaipur; Tamil Nadu: Chennai (Coonoor, Palani hills); Uttar Pradesh: Baijnath, Sarnath (Fig. 3.14).

Lymnaea luteola is the intermediate host of *Schistosoma indica*, *S. nasalis*, *S. spindalis*, *S. suis*, *S. incognitum*, *Fasciola gigantica*, *F. hepatica*, *Clinostomum giganticum*, *F. revolutum*, *Orientobilharzia datta*, *Echinoparyphium bugulai*.

3.1.4 *Lymnaea ovalior*, Annandale and Prashad 1921

Lymnaea ovalior (Rao Subba 1989). Shell is globose, Oviform with a short spire. The base of the spire as broad as upper portion of the body wall. Found in Manipur (Bishenpur) (Fig. 3.15).

3.1.5 *Lymnaea horae* Annandale and Rao 1925

Lymnaea (*Pseudosuccinea*) *horae*: (Rao Subba 1989). Shell is with well-developed and broader spire, with an extra half whorl of the apex; sculptured minutely decussated with numerous, close set of longitudinal striae; radula well developed; teeth shorter and broader. Found in clear water with muddy substratum, on rotten twigs of trees which were floating in water or partly embedded in mud at the bottom.

Distribution: Assam (Fig. 3.16).



Fig. 3.16 *L. horae* dorsal and ventral, size: 6.0×2.7 mm

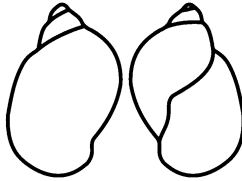


Fig. 3.17 *L. gedrosiana* dorsal and ventral, size: 15.4×10.2 mm



Fig. 3.18 *L. mimetica* dorsal and ventral, size: 6.20×2.40 mm



Fig. 3.19 *L. shanensis* dorsal and ventral, size: 10.3×4.85 mm

3.1.6 *Lymnaea gedrosiana* (Annandale and Prashad 1921)

Lymnaea gedrosiana (Annandale and Prashad 1921) is found in salt range, Punjab and Baluchistan (Fig. 3.17).

3.1.7 *Lymnaea mimetica* Annandale, 1918

Lymnaea mimetica Annandale, 1918 is found in Burma, known as local variant of *Lymnaea acuminata* (Fig. 3.18).

3.1.8 *Lymnaea shanensis* Annandale, 1918

Lymnaea shanensis Annandale, 1918 is found in Shan states, Burma (Fig. 3.19).

Fig. 3.20 *L. bowelli* dorsal and ventral, size: 8.75 × 5.40 mm



Fig. 3.21 *L. laticallosa* dorsal and ventral

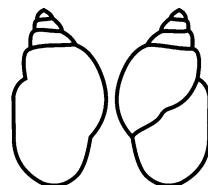


Fig. 3.22 *L. hookeri*, dorsal and ventral, size: 38.5 × 20.0 mm

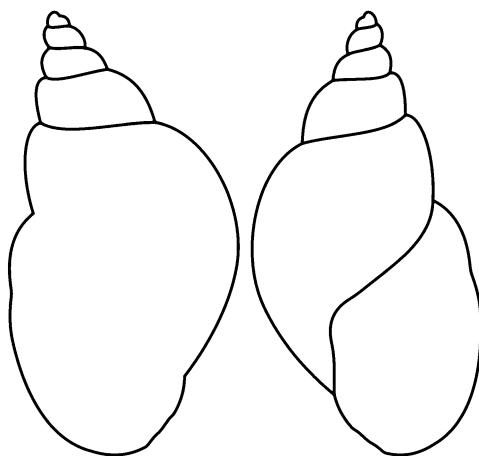


Fig. 3.23 *L. andersoniana*, dorsal and ventral, size: 9.65 × 6.35 mm



3.2 Subgenus Galba

Shell medium narrow with long spire; body whorl not expanded, out lip not inflated; columellar fold with broad and coarse.

Lymnaea bowelli Preston, 1909 is found in Tibet. It is reported at altitudes ranging from 13,120 ft. to 16,000 ft. (Annandale and Rao 1925) (Fig. 3.20).

Lymnaea laticallosa Annandale and Rao 1925 has reported in Burma (Fig. 3.21).

Lymnaea hookeri Reeve, 1850 is found in North side of Sikkim, Himalaya, Tibet (Annandale and Rao 1925) at height of 13,000 to 18,000 ft. (Fig. 3.22).

Lymnaea andersoniana Nevill, 1881 is distributed in Shimla hills, Kashgar, Kangra Valley, Ladakh, Manipur (Fig. 3.23).

Fig. 3.24 *L. truncatula*,
dorsal and ventral, size:
8.30 × 3.60 mm



Fig. 3.25 *L. persica*, dorsal
and ventral, size:
25.70 × 15.10 mm

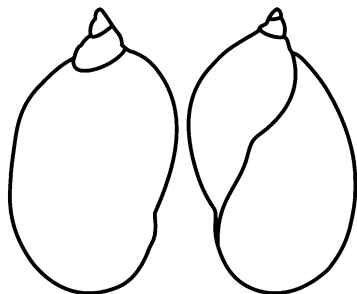
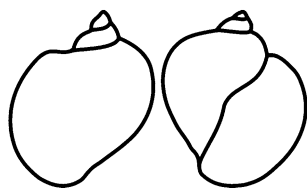


Fig. 3.26 *L. auricularia*,
dorsal and ventral, size:
15.65 × 13.0 mm



Four forms of *L. andersoniana* viz. *typica*, *simulans*, *intermedia*, and *turbinicola*. *Typica* is found in stagnant waters in the valleys of Himalayas, whereas *simulans* is noted in streams of Yunnan. *Intermedia* and *turbinicola* are found in western Himalayas/Naga hills of Assam and hills of Manipur valley.

***Lymnaea truncatula* Mueller, 1774**

Annandale and Rao (1925) and Rajagopal and Rao (1968) reported its distribution at altitude of 2730 M to 3365 M in Kashmir, Leh and Chitral. This snail is mud-loving and amphibious. It serves as an intermediate host of *Fasciola hepatica* (Fig. 3.24).

3.3 Subgenus Radix Montfort

Lymnaea persica Issel, 1865 is noted in Kashmir, Kumaon lakes, Kangra valley in India (Annandale and Rao 1925) although Rajagopal and Rao (1968) did not record this species from Kashmir (Fig. 3.25).

Lymnaea auricularia Linnaeus, 1750 is reported in Kashmir valley in India (Annandale and Rao 1925; Rajagopal and Rao 1968). Only *Orientobilharzia turkestanicum* and *Fasciola gigantica* are reported parasites in this snail (Fig. 3.26).

Lymnaea brevicauda Sowerby, 1873 is noted in Kashmir (Annandale and Rao 1925; Rajagopal and Rao 1968).

Lymnaea peregra Mueller, 1774 was reported in Kashmir and Tibet in India. According to Annandale and Rao (1925), there are eight forms in this species. They are *F. striata*, *F. costulata*, *F. solidissima*, *F. subdisjuncta*, *F. solidissima*, *F. subdisjuncta*, *F. bactriana*, and *F. defilippi*. All the forms are restricted to lakes or ponds in the Himalayas.

Lymnaea lagotis (Schrank) Annandale and Rao (1925) reported six forms of *L. lagotis*, which occur in Kashmir and Tibet. Except form *bactriana* all forms are found in Himalayas.

3.3.1 Subgenus *Lymnaea*

Lymnaea stagnalis Linnaeus, 1758 in India is confined to Kashmir (Annandale and Rao 1925; Rajagopal and Rao 1968). Two forms were reported in Kashmir, i.e., *F. minor* and *F. kashmirensis*. Shell ovately turreted, compressed, umbilicated, thin, yellowish horny, spire produced and sharply acuminate. Five to six whorls convex at upper part and ventricose, striated in direction of line of growth. Lip broadly dilated over the umbilicus (Ramakrishna and Day 2007) (Fig. 3.27).

3.3.2 Subgenus *Stagnicola*

Lymnaea tungabhadraensis Ray 1967. Although it is a cold water subgenus yet it is noted in Andhra Pradesh and South India (Ray 1967). Its presence in such a warm place in Kurnool is puzzling (Ray 1967) (Fig. 3.28).

Fig. 3.27 *L. stagnalis* (Linnaeus) dorsal and ventral, size: 42.60 × 20.80 mm

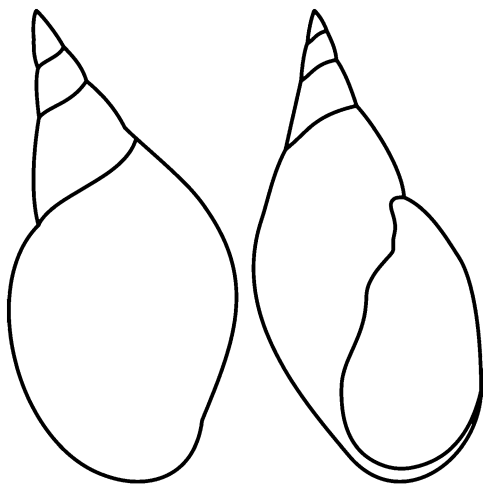


Fig. 3.28 *Lymnaea tungabhadraensis* dorsal and ventral, size: 18.85 × 8.25 mm

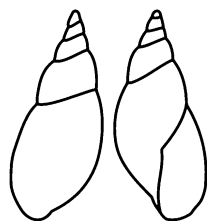


Fig. 3.29 *Indoplanorbis exustus*, size: 15.70 × 5.95 mm



3.3.3 Family Planorbidae

Snails of family planorbidae are widely distributed, found in shallow water and littoral regions. Majority of snails are confined to small ponds or ditches that dried up in summer. Indian planorbids have not received much attention (Annandale and Prashad 1921). This family is divided into five subfamilies but the Indian species are found in three subfamilies, i.e., Bulininae, Planorbinae, and Segmentininae.

Subfamily Bulininae includes ovoid or elongate and acuminate as in *comptoceras* or disc shaped as in *Indoplanorbis*, sinistral, when disk-shaped distinguished from cells of other subfamily by the shape of the last large whorl dilated forward to the aperture.

3.3.4 Genus *Indoplanorbis* Annandale and Prashad 1921

Indoplanorbis exustus is found throughout the plains of India and Jammu and Kashmir. Annandale and Prashad (1921) discussed the impact of ecological changes on this species. There are several studies on its life cycle, ecology and its host parasite relationship (Matta and Raj 1971; Dutta and Bali 1977; Biswas and Subramanian 1978a, 1978b; Islam 1997) (Fig. 3.29).

Trematodes of horse, goat, sheep, camel, dog, buffalo, and other cattle develop to cercarial stage in this snail. The large number (more than 57) of cercariae are recorded from this snail. The important parasite use *I. exustus* as intermediate host are *Schistosoma indicum*, *Fasciola gigantica*, *Paramphistomum* species, and *Echinostoma* species (Fig. 3.30; Table 3.1).



Fig. 3.30 Distribution of Lymnaeidae/Planorbidae snails in different States of India

Table 3.1 Distribution of different species of Lymnaeidae/Planorbidae snails in India

S. no.	Name of the Lymnaeidae snails	Distribution	Habitat occurrence
1.	<i>Lymnaea acuminata</i> Lamarck Nine forms were recognized under these species	Throughout India	Lentic habitat-permanent water bodies with abundant vegetation
	form <i>typical</i>	Throughout India, River Ganges	Lentic habitat
	form <i>patula</i>	Throughout India, River Ganges	Lentic habitat
	form <i>chlamys</i>	Throughout India, River Ganges	Lentic habitat
	form <i>rufescens</i>	Throughout India, River Ganges	Lentic habitat
	form <i>gracilior</i>	Throughout India and Burma	Lentic habitat
	form <i>hians</i>	South and North-West India	Lentic habitat
	form <i>malleata</i>	Punjab and Manipur	Lentic habitat
	form <i>bravissima</i>	Nagpur, Madhya Pradesh	Lentic habitat
	form <i>pseudohorae</i>	Burma	Lentic habitat
The above snails are intermediate host of <i>Fasciola gigantica</i> , <i>F. hepatica</i> , <i>Schistosoma indicum</i> , <i>S. nasalis</i> , <i>S. spindalis</i> , and <i>Clinostomum giganticum</i> and <i>Echinostoma</i> species.			
2.	<i>Lymnaea biacuminata</i>	Hyderabad, Andhra Pradesh, Kumaon, Nainital	Stream, canals
3.	<i>Lymnaea luteola</i> Lamarck Species has six forms	West Bengal	Stream, canals
	form <i>typica</i>	Throughout India	River, Stream, canals
	form <i>ovalis</i>	Throughout India specially in Andaman	River, Stream, canals
	form <i>australis</i>	Throughout South India	Stream, canals
	form <i>impure</i>	Throughout South India, River Ganges in North India	Stream, canals
	form <i>succinea</i>	Malabar Coast, South and North India	Stream, canals
	form <i>sinensis</i>	Burma Border of India	Stream, canals
The above snails are intermediate host of <i>Schistosoma indica</i> , <i>S. nasalis</i> , <i>S. spindalis</i> , <i>S. suis</i> , <i>S. incognitum</i> , <i>Fasciola gigantica</i> , <i>Clinostomum giganticum</i> , <i>Echinostoma revolutum</i> , <i>Orientobilharzia datta</i> , <i>Echinoparyphium bugulai</i>			
4.	<i>Lymnaea ovalior</i>	Manipur	Freshwater lake
5.	<i>Lymnaea horae</i>	Assam	River stream, creeks
6.	<i>Lymnaea gedrosiana</i>	Punjab	River stream
7.	<i>Lymnaea mimetica</i>	Indo-Burma Border	Permanent water bodies

(continued)

Table 3.1 (continued)

S. no.	Name of the Lymnaeidae snails	Distribution	Habitat occurrence
8.	<i>Lymnaea shanensis</i>	Indo-Burma Border	Permanent water bodies
9.	<i>Lymnaea bowelli</i>	Tibet (13,120 ft. to 16,000 ft.)	Warm spring
10.	<i>Lymnaea laticallosa</i>	Burma	Freshwater lake
11.	<i>Lymnaea hookeri</i>	Sikkim, Tibet (13,000 ft. to 18,000 ft.)	Streams, rivers, and lakes
12.	<i>Lymnaea andersoniana</i>	Shimla hills, Kangra Valley, Ladakh, Manipur	Stagnant freshwater
13.	<i>Lymnaea truncatula</i>	India-Kashmir, Leh, Chitrae (9000 ft.)	Freshwater rivers and lakes
Intermediate host of <i>Fasciola hepatica</i>			
14.	<i>Lymnaea persica</i>	Kashmir, Kumaon lakes, Kangra valley	Freshwater lake
15.	<i>Lymnaea auricularia</i>	Kashmir	Freshwater lake
16.	<i>Lymnaea brevicauda</i>	Kashmir	Freshwater Lakes Slow running streams.
17.	<i>Lymnaea lagotis</i> Six forms in this species form <i>striata</i> form <i>andreae</i> form <i>costulata</i> form <i>solidissima</i> form <i>subdisjuncta</i> form <i>bactriana</i> form <i>defilippi</i>	Kashmir, Tibet Except form <i>bactriana</i> all forms are found in Himalayas	Small streams
18.	<i>Lymnaea stagnalis</i>	Kashmir	Ponds, slow moving streams having abundant vegetation
19.	<i>Lymnaea tungabhadraensis</i>	Andhra Pradesh	Ponds and lakes
Planorbidae			
1.	<i>Planorbis exustus</i> <i>Indoplanorbis exustus</i>	Malabar Coast Throughout the plains of India, Jammu and Kashmir	Permanent rivers, ponds and lakes Permanent rivers, ponds and lakes

These snails are parasites of *Fasciola*, *Paramphistomum*, *Schistosoma*, *Gastrodiscus*, *Plasmiorchis*, etc.

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Abstract

Fasciolosis control requires proper diagnosis of the disease, elimination of flukes from infected animals, and vaccination. Different diagnostic procedures such as clinical, blood parameters, enzyme activity, parasitological and immune diagnosis are discussed. Vaccination is one of the efficient methods to control fasciolosis. Development of vaccine against *Fasciola hepatica* and *Fasciola gigantica* by fatty acid-binding proteins, cysteine peptidase, leucine aminopeptidase, and glutathione S-transferase is given in the present chapter. Use of various drugs as flukeicide is also reviewed.

The efficient control of fasciolosis can be done by the appropriate integrated approaches of several measures. Prevention and control of fasciolosis is associated with health plan of livestock as well as human beings. Primarily, it requires the proper diagnosis of disease, elimination of flukes from infected animals, and vaccination. Control of intermediate host snail population is one of the most effective methods to control fasciolosis. Snail control method is discussed separately in Chap. 5. Here, we are discussing the other aspects of fasciolosis control.

4.1 Diagnosis

Diagnosis of fasciolosis is a difficult task. Primarily diagnosis is performed by studying different signs, symptoms, test results and thereafter, analyzing the observation. Fasciolosis is primarily detected by fluke eggs in fecal samples within 10 weeks of infection in cattle and 9 weeks in buffalos (Sanyal 1998) for *F. gigantica*. Coprological confirmation of the disease prior to application of strategic antifleuke medication seems to be a little significance to avoid heavy economic

losses. Immunological techniques provide the advantages of being applicable during all stages of the disease (Hansen et al. 1999). Various serological tests have been used for human diagnosis. Non-invasive diagnostic techniques such as radiology, radio-isotope scanning, ultrasound, computer tomography, and magnetic resonance can be used for diagnosis of fasciolosis in human. We can divide the procedure of diagnosis in the following steps:

1. Clinical diagnosis.
2. Blood parameters and enzyme diagnosis.
3. Parasitological diagnosis.
4. Immunodiagnosis.

4.2 Clinical Diagnosis

Clinical diagnosis of fasciolosis is not a very effective tool. It depends to some extent on the experience of clinician, until some death occurs in livestock. Poor growth and reduced grazing is a potential problem concerned to fasciolosis. In acute form of fasciolosis, previous history of animal grazing in endemic area and their sudden death may be the sign of fasciolosis. Animal's lethargyness, reduced grazing, and reluctant to run may be the primary sign of fasciolosis. In subacute fasciolosis, same symptoms may occur. Infected animal body condition is poor and becomes anemic. Chronic fasciolosis can occur in any time in year, but very common in winter and spring seasons. There is progressive loss of condition, weak and edemas in some part of the body, particularly in abdomen and submandibular region. This clinical diagnosis is supported by fecal examination of animal for the presence of fluke eggs.

4.3 Blood Parameters and Enzyme Diagnosis

Blood parameters and levels of hepatic enzymes are useful tool in diagnosis of fasciolosis. These parameters measurement depends on the sensitivity, specificity, and stability in the plasma of blood (Rowlands and Clampitt 1979). Higher level of aspartate aminotransferase, glutamate dehydrogenase, and γ -glutamyl transferase in the blood sample is the index of damaged liver condition of the fluke infected animal. Dargie et al. (1979) and Sykes et al. (1980) found that there is a significant reduction in intake of animal with the infection of liver fluke, it may be in between 15% and 50% or more depending upon the severity of infection. Hypoalbuminemia and hyperglobulinemia are also significant parameters in fluke infected animals.

4.4 Parasitological Diagnosis

Detection of fluke eggs in feces of affected animals is one of the important tools in measurement of chronic fasciolosis. Direct fecal analysis or sedimentation/floating techniques are used in egg's identification of infected animals. Among all, sedimentation technique is mostly used to observe eggs of fluke with the help of methylene blue stain.

4.5 Immunodiagnosis

Enzyme linked immunosorbent assay (ELISA) and western blots are the two main techniques extensively used in serological diagnosis of fasciolosis in animals and can detect serum antibodies to *Fasciola* antigens from adult fluke extract or excretory secretory products (ES products) (Adedokun et al. 2008). Use of molecular biology tools in detection of fluke infection is one of the efficient methods to check the fluke infections in farm animals (Khan et al. 2017). Immunodiagnosis method can detect the fluke infection as early as 2 weeks post infection but unable to give specific result, when the animals are infected with multihelminth parasite species.

In India, intradermal test with the use of adult fluke antigen against all infected cattle was used in diagnosis of *Fasciola* infection in buffaloes (Swarup and Pachauri 1987). Gupta and Yadav (1993, 1996), Yadav and Gupta (1995) develop immunogenic and diagnostic fractions of antigen of *F. gigantica* and explored the antibody level in infected animal. In extension of this study, they have isolated the antigen by affinity chromatography (Gupta and Yadav 1996). Rao et al. (1996) reported that *F. gigantica* contains an immunodominant epitope PC (Phosphocholine), which is present on various antigens with molecular weight of 200–300 kD. This PC containing antigen can detect circulating *Fasciola* antigen in both naturally infected cattle and goat. In Gupta 2001, Gupta by using antigen consisting of two polypeptides in molecular range of 26–30 kD against sera by ELISA, developed an early detection of experimental fasciolosis in infected cattle. Ghosh et al. (2005) isolated a glycoprotein (27 kD) from crude somatic antigen of *F. gigantica* with the help of two-step affinity chromatography and used in detection of experimental fasciolosis in cattle by indirect ELISA and in dot-ELISA formats. According to them, dot-ELISA was more convenient in field application.

Jayraw (2005) used PCR as an epidemiological tool for screening of intermediate host for the parasite infection and sensitive immunological detection of *F. gigantica* infection in the bovine host. Detection of antigen targeting 52 kDa antigens by sandwich ELISA was carried out to found that antigen could be detected in circulation as early as 10 dpi. *Fasciola* specific primers amplified a 124 bp fragment in PCR, when the genomic DNA preparation from *F. gigantica* and the snail intermediate host *Lymnaea auricularia* infected with the parasite were used in the assay.

Velusamy et al. (2006) evaluated metacercarial antigen for early immunodiagnosis of bovine fasciolosis with the help of ELISA and western blot. In ELISA, *F. gigantica* infection was detected within 2 weeks after infection. In western blot,

sera from infected calves recognized one distinct polypeptide of 21 kD in fractionated metacercariae antigen after the tenth day of infection.

Saxena et al. (2006) have performed immune diagnosis of fasciolosis by DOT-ELISA. DOT-ELISA was found to be 100% sensitive and 52.5% specific, whereas plate ELISA was 90.74% sensitive and 42.5% specific, when compared to bile egg sedimentation technique. They suggested that DOT-ELISA using low molecular weight protein fraction of *F. gigantica* can detect the disease and can be used under field conditions.

Pal and Dasgupta (2007) studied the antigens of *F. gigantica* of buffalo origin by double immuno-diffusion (DID), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotting. DID showed two precipitation lines in *F. gigantica* against anti *F. gigantica* rabbit hyperimmune sera. SDS-PAGE revealed nine polypeptides viz. 97, 75, 69, 59, 46.5, 41, 33.5, 29, and 12 kDa molecular weight of *F. gigantica*.

Khan et al. (2017) identified some of immunodominant proteins from the excretory, secretory (ES) product of *F. gigantica*. They used these proteins for further characterization and early detection of infection as well as vaccine candidates. The polypeptide profile of ES products identified as 24 polypeptides out of which 12 immunogenic polypeptides were identified by western blotting. The dot blot SA proves the utility of ES products for detection of *F. gigantica* in field. Aghamolaei et al. (2020) used the antigenic recombinant multi-epitope (rMEP) construct of these protein epitopes (linear/conformational B-cell epitopes) of the *Fasciola*. They have used *F. hepatica* cathepsin-L1, saposin-like protein 2, and 16.5-kDa tegument-associated protein. This antigenic multi-epitope is helpful in serodiagnostic kit in diagnosis of human/ruminants fasciolosis.

4.6 Vaccination

Fasciola species are cosmopolitan distribution (Mas-Coma et al. 2005). With the continuous use of antiflukeicides such as triclabendazole, oxcylozanide; rafoxanide and nitroxinil *Fasciola* develop resistance, which ultimately affect the control of fasciolosis.

Vaccination is another method to control the fasciolosis. Haroun and Hillyer (1986) suggested the approach of vaccine development with help of radiation—attenuated metacercaria, crude somatic parasite extracts, and mixture of secreted parasite proteins. Further in development of vaccine against both species of *Fasciola*, immunological mechanism was used to neutralize the parasite. Spithill et al. (1997), McMenus and Dalton (2006) and Kumar et al. (2011) identified three prototype antigens candidate molecules of *F. hepatica* and *F. gigantica*. There are fatty acid-binding proteins (FABP), across protective antigen of *F. hepatica*; the glutathione S-transferase (GST), a *fasciola* molecule; cathepsin L, the *fasciola* molecules constituting cysteine proteases. It has been noted that among this *F. hepatica* cysteine protease induces high level protection up to 70% in sheep and 50% in vaccinated cattle. Simultaneously, it has been also noted that cathepsin L

from *F. gigantica* is not able to protect the same homologous infection in cattle (Kumar et al. 2011).

4.7 Fatty Acid-Binding Proteins

Fatty acid-binding proteins (FABPs) are major components of whole *Fasciola* body extract. One of the FABP 12 kDa (nFh12) is obtained from gel permeation separation of adult liverflukes for immune-prophylactic potential against liver flukes. It was used as vaccine in mice (Hillyer 2005). However, its recombinant part rFh15 molecule is very effective vaccine in rabbits against *F. hepatica* exposure. In different studies, 40–70% protection was noted in rabbit. Vaccination also caused reductive effect on fluke size, egg productions, and pathology (Muro et al. 1997; Casanueva et al. 2001). Martinez-Fernandez et al. (2004) later observed that nFh12 and rFh15 vaccination has same protection level against *F. hepatica* exposure. Further, they found that adjuvant is important in which vaccine is prepared (Ramajo et al. 2001; Martinez-Fernandez et al. 2004). When vaccine was prepared in Freund's adjuvant, there was no reduction in size and development of *Fasciola*, but significant reduction in number of eggs was found in fecal matter of sheep. Formulation with saponin and emulsifier (Montanide) vaccine caused significant lower development and egg counts in feces.

4.8 Cysteine Peptidase

Adult liver fluke secretes huge amount of 24.5 kDa protein cathepsin L cysteine peptidases from gut epithelial cell lining. This protein is used in the degradation of the blood meal in the gut of liver fluke (Collins et al. 2004). Digested contents of gut come outside the body of the fluke in the host body. In host intestine, these proteins help in penetration of new parasites in intestinal wall as well as protect them from host immune system (Dalton and Mulcahy 2001; Dalton et al. 2003a, b). Cathepsin L1 and Cathepsin L2 are identified as most effective vaccine against liver flukes in sheep and cattle. Both vaccines provide 38–68% protection against liver fluke infection. Combination of both the vaccines is more effective than single use. These combinations caused protection of liver fluke in range of 51–72% (Dalton et al. 1996, 2003b). These vaccines also affect the liver fluke egg production as well as their viability.

4.9 Leucine Aminopeptidase

Another protein leucine aminopeptidase is also secreted in gut of liver fluke, which helps in food digestion (Piacenza et al. 1999). Its use as vaccine caused higher protection against liver fluke (89.6%). Its combination with other vaccine such as cathepsin L1 and cathepsin 2 is not as effective as its single treatment.

4.10 Glutathione S-Transferase

Glutathione S-transferase (GST) vaccine is tested with different adjuvants and caused 29% and 45% protection in sheep and cattle, respectively (Spithill et al. 1999a, b). Sixty five percent reductions in egg numbers were noted after vaccination. Adjuvant squalene montanide/QuilA used with glutathione S-transferase is very effective in cattle, but not in sheep. It indicates that combination of adjuvant is species specific. DeBont et al. (2003) observed that *S. bovis*-derived GST used with adjuvants QuilA, Alum, and Freund's is not effective in cattle challenged in liver fluke *F. hepatica* metacercariae.

Thioredoxin peroxidase (TPx), saposin like or NK-lysin-like in primary study is effective as vaccine against liver fluke infection (McGonigle et al. 1997; Reed et al. 2000; Espino and Hillyer 2003; Donnelly et al. 2005). Instead of it recombinant protein FhSAP-2 (Espino et al. 2005), cathepsin L (Harmsen et al. 2004), and cathepsin L DNA (Kofta et al. 2000; Wedrychowicz et al. 2003) were also very effective in controlling fasciolosis.

4.11 Treatment

In the beginning of twentieth century, carbon tetrachloride and hexachloroethane were used in treatment of the fluke infection. These drugs were highly toxic to the animals and have low efficacy. Later in second half of the twentieth century, halogenated phenols such as hexachlorophene and bithionol were used as flukeicide. In 1960s, oxyclozanide, brotianide, closantel, and clioxanide were developed as flukeicide.

In the present era, albendazole was recommended for control of fasciolosis (McKellar and Scott 1990). Flubendazole, fenbendazole, oxfendazole, and mebendazole were used as common flukeicide. Triclabendazole (Fasinex) is considered as the most common drug due to its high efficacy against adult as well as juvenile flukes. Triclabendazole is used in control of fascioliasis of livestock in many countries. Nevertheless, long-term veterinary use of triclabendazole has caused appearance of resistance in *F. hepatica*. Considering this fact, scientists have started to work on the development of new drug. Nitazoxanide is considered to be a good alternative to triclabendazole. It has showed its efficacy against human fasciolosis in Egypt (Kabil et al. 2000). A new fasciolicide was successfully tested in naturally and experimentally infected cattle in Mexico. This new drug is called "Compound Alpha" and is chemically very similar to triclabendazole (Ibarra et al. 2004).

Artemether and artesunate have a broad spectral activity against *Fasciola* (Hien et al. 2008; Duthaler et al. 2011). Another drug such as the ozonides (Wang et al. 2011) has significant trematocidal activity. Ozonide OZ78 is potential flukeicide against fasciola species (Keiser et al. 2010).

Usually it has been noted that vaccines reported in fasciolosis protection also reduce the number of fecal egg counts of liver fluke. Most studies reveal that vaccination effect on egg production of liver fluke is more pronounced than their

effects on worm burden in host body. Since an adult liver fluke produces about 20–50 thousand eggs per hour, a single miracidium infection in intermediate host snail gives rise to approximately 600 infective metacercariae (Whitfield 1979). It is believed that a vaccine reducing fecundity of liver fluke more than 70% in host body ensure reduction of parasite transmission. Majority of eggs produced after vaccinations are not viable in natural environment (Dalton et al. 1996). In this way vaccination is effective in controlling the fasciolosis transmission by disrupting the liver fluke life cycle. Liver fluke vaccination against the animals will be helpful in suppressing the burden of their infection both in primary and secondary intermediate host in endemic regions. Human fasciolosis prevention in endemic area should be followed by chemotherapy, so that it can be effectively controlled.

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Abstract

Snail control is one of the most efficient methods to reduce fasciolosis. Snails are the intermediate host of fluke *Fasciola hepatica* and *Fasciola gigantica*. Control of host snail population is best method to delink the life cycle of fluke. Various snail control methods such as biological, mechanical, chemical control and use of plant-derived molluscicides are discussed. Possibility of using bait formulations with various attractants against host snails is also given in the present chapter. Various recent methods of snail control, such as joint action of molluscicides and synergists, cow urine and phytotherapy of infected host snails by various plant products to kill the fluke larvae inside the snail body, without killing the snail are also extensively discussed. Use of different spectral color of visible sunlight as attractant and chlorophyllin, a product of chlorophyll, as molluscicide against host snails or larvicide against different larvae of *Fasciola* are also described. Control of snail population by reducing their reproductive capacity is also given in the present chapter.

Snail control by using molluscicide is an important preventive strategy against schistosomiasis and fasciolosis, associated with chemotherapy, ecological and biological control methods. Certain aspects of the ecology of the snails are also important to the dynamics of the parasites transmission. The whole ecology of the involved snail species must be thoroughly understood in the planning of any control methods (Slootweg et al. 1994). Procedures of snail control must depend on knowledge of the sites of transmission and the seasonal patterns (McCullough 1986). The control of snails by chemical, environmental, and/or biological means can still play a significant supporting role in many endemic situations. An effective disease and snail vectors control strategy has to integrate four basic elements: chemical control, biological control, environmental management, and medical treatment (WHO 1988;

Hespanhol 1996). The molluscan pests may be destroyed by means of synthetic molluscicides or alternatively with the molluscicides from plant sources (WHO 1965, 1983, 1992; Amin et al. 1976; Thomas 1973; Marston and Hostettmann 1985; Sahay et al. 1991; Singh and Singh 1995a, 2008a, b; Singh et al. 1996a, 1997, 2010b, c, 2012a, b, c, d, 2017a, b, 2018, 2018a, b; Rao and Singh 2000, 2001, 2002; Rao et al. 2003; Srivastav and Singh 2005; Kumar and Singh 2006; Tiwari and Singh 2007; Jaiswal and Singh 2008; Jaiswal et al. 2009; Srivastava et al. 2010; Agrahari and Singh 2010; Kumar and Singh 2010a, b; Tripathi et al. 2010; Hanif and Singh 2012, 2013a, b; Soni and Singh 2015, 2017, 2019).

Many experts advocated that snail control is the best way of trematode control (Godan 1983; Marston and Hostettmann 1985, 1987; Singh et al. 1996a, 2005; Singh and Singh 1996b, 2000a, b, c, 2003a, b, c, 2018a, b; Allam 2000; Tripathi and Singh 2001; Rao and Singh 2002; Tiwari and Singh 2004a, b; Tripathi et al. 2004; Jaiswal et al. 2008; Kumar et al. 2009; Agrahari et al. 2012). One of the sure ways to tackle the problem of schistosomiasis and fasciolosis is to destroy the carrier snails and remove an essential link in the life cycle of the flukes. The molluscan pests can be destroyed by the use of molluscicide (WHO 1965, 1970, 1983, 1984, 1992, 1993, 2007; Thomas 1973; McCullough 1986; Singh and Singh 2001a, b, 2003a, b, 2009a, b; Rao and Singh 2001, 2002; Sukumaran et al. 2002, 2004; Rao et al. 2003; Singh et al. 2008). This may be achieved with the aid of synthetic products or alternatively with the molluscicides from plant sources (Marston and Hostettmann 1985; Hostettmann and Lea 1987; Singh et al. 1996a).

Agarwal and Singh (1988), Singh et al. (1996a), Jagtap (2000), El-Deeb and Ismai (2007) have extensively reviewed the status of snail control. Harmful gastropods control can be categorized in mechanical control, biological control, chemical control, control by plant-derived molluscicide, control by joint action of molluscicide, control by cow urine, and control by phytotherapy of snails.

5.1 Biological Control

Biological control is an appealing alternative snail management strategy because of its low cost, technological simplicity, and potential for self-renewal (Ferguson 1977; Jobin et al. 1984; Combes and Cheng 1986). This control method has provided only limited success (Madsen 1990). Predator and competitor snails have thus shown most promise in this regard (Ferguson 1977; Jobin et al. 1977; Pointier and McCullough 1989; Pointier et al. 1989). *Euglandina rosea* is a predatory snail feed on *Achatina fulica* (Cowie 2000; Barker and Efford 2002). A predatory millipede orthomorpha species is found to predating on the giant African snail *Achatina fulica*. The millipede secretes hydro-cynic acid from its sink glands, which helps in paralyzing the prey prior to feeding (Srivastava and Srivastava 1967). *Procambarus clarkii* is omnivorous and is known to consume large number of freshwater snails (Huner and Barr 1983). The biological control of the snail *Biomphalaria glabrata* by using *Pila ovata* and *Marisa cornuarietis* is reported by Hofkin et al. (1991). Pulmonates snails can be eaten away by some carnivorous

snails. *Achatina fulica* was controlled in the USA by introducing carnivorous snail *Gonaxis* in the environment (Peterson 1957). Another carnivorous snail *Marisa cornuarietis* actively preys upon *Bulinus truncatus*, *Biomphalaria alexandrina*, and *Lymnaea cailliaudi* the intermediate host of *Fasciola* and *Schistosoma* (Chernin et al. 1956; Godan 1983). The production of cercariae of *Schistosoma mansoni* in *Biomphalaria glabrata* is affected by *Helisoma duryi*, which is suitable for biological control (Frandsen and Christensen 1977). Pulmonates are also controlled by many groups of vertebrates, such as fishes, snakes, birds, and small mammals (Pelseneer 1935). Two species of birds *Dendrocitta vagabunda* and *Centropus sinensis* are predator on land snail *Achatina fulica* (Raut and Ghose 1984). Snails *Zonitoides* species and *Murex* species have been controlled by using viruses (Ryder and Bowen 1977). The predaceous decollate snail (*Rumina decollata*) has been released in southern *California citrus* for control of the brown garden snail (*Helix aspersa*) and is providing very effective biological control (Flint 2003).

5.2 Mechanical Control

A number of mechanical methods to prevent gastropod damage have been used with more or less positive results in different parts of the world. Handpicking of snail and slugs and squashing was the only control measure in practice up to the middle age. Common salt was then employed for overproduction of mucus by gastropods. Collection of snails and slugs is done before dawn or after dusk. Collected gastropods are killed in strong solution of common salt or in boiling water and then buried at a depth of 70–80 cm in the field away from populated area. The eggs are collected and destroyed by crushing as well. Burning over is a quick method of cleaning land before planting, which is often carried out in tropical countries. Burning over field heavily infested with *Theba pisana* Mull is effective in South Africa (Joubert and Walters 1951) and *Achatina fulica* in Africa and in Guam (Mead 1961). Today, burning is discouraged because of the damage that it causes to the environment. It has been suggested to locate and collect aestivating snails from endemic pockets in the management of snail control program (Raut 1978). Protective barriers of dehydrating chemicals such as salt, caustic soda, quick lime, and kainite are used around seedbeds. Studies on the use of dehydrating chemical barriers for the control of snails *Cryptozonia belangiri* on bean crop and slug, *Laevicaulis alte* on cabbage crop indicate that the barrier strip of 5 cm around the crop with copper sulfate powder is most effective amongst 8 dehydrating substances tested (Jagtap et al. 1990). Creating conditions unfavorable for snails and slugs brings down gastropod population. Keeping area under crops free from weeds, creating a belt of cleaned land around garden or farm about 6 m wide make it difficult for the snails and slugs to crawl up to the cropped field. Clearing of the edges of fields and irrigation ditches after the harvest reduces the level of infestation (Jagtap 2000).

5.3 Chemical Control

Greater attention has been given to chemical control of snails. The important molluscicides are pentachlorophenol (McMullen et al. 1948; Nolan et al. 1953; Meyling et al. 1962; Meyling and Pithford 1966), niclosamide (Gonort and Schraufstatter 1959; Singh and Agarwal 1984a, 1986c; El-Deeb and Ismail 2007), metaldehyde (Lange and MacLeod 1941; Stringer 1946; Coloso et al. 1998; Rao and Singh 2002), and gramoxone (Ebenso et al. 2005). Molluscicidal activity of carbamates, organophosphate, and pyrethroid pesticides and their synergists (Singh and Agarwal 1981, 1983a, b; Singh et al. 1993a; Ebenso et al. 2005) have also been well documented. Among the inorganic chemicals, copper sulfate and zinc sulfate (El-Gindy et al. 1991; Rawi et al. 1994, 1995) have been used widely. Niclosamide is recommended by WHO in snail control programs (WHO 1993; Sturrock 2001).

5.3.1 Metaldehyde

A solid polymer of acetaldehyde is the most popular and generally recommended bait poison for use against terrestrial gastropod pests (Gimingham 1940). The molluscicide metaldehyde primarily acts on the mucous cells; it causes major disruption of the water balance physiology of the molluscs, resulting in their desiccation (Triebskorn and Ebert 1989). Metaldehyde has a secondary neurotoxic effect, contributing to loss of motor activity (Coloso et al. 1998). Three percent and five percent pellets of metaldehyde were used as bait against *Achatina fulica* (Srivastava et al. 1968). Metaldehyde products come in various forms including liquid, granular, and bait formulations (Robert 2007).

5.3.2 Niclosamide

(2,5-dichloro-4-nitrosalicylanilide) is effective against all developmental stages of snails and schistosomes (Takougang et al. 2006). Niclosamide serves as a potent molluscicide against various snails (Azare et al. 2007). Niclosamide is recommended by WHO in snail control programs (WHO 1993). To ensure its practical application, 50% niclosamide ethanolamine salt wettable powder, which has higher dissolution, has been applied widely in China (Yang et al. 2010). The molluscicidal potency of the synthesized derivatives of niclosamide against *Biomphalaria alexandrina* was studied by Soliman et al. (2005).

5.3.3 Carbamate

Compounds are esters of carbonic acid and are proved to be most potent class of molluscicide (Singh and Agarwal 1989; Radwan et al. 1992). The molluscicidal

activity of carbamates relates to their disruption of the neurotransmitter cholinesterase (Frain 1982). In molluscs, the toxicant causes paralysis and loss of muscle tone (Godan 1983). The principal carbamates used to control terrestrial molluscs pests are carbaryl, isolan, mexacarbate, cloethocarb, methiocarb, and thiodicarb. The molluscicidal activity of this class of compounds against terrestrial molluscs has been evaluated from aromatic N-methyl carbamates, i.e., carbaryl, mexacarbate, and methiocarb (El-zemity 2006). Singh and Agarwal (1983a, b, 1989), Singh et al. (1982), Tripathi and Agarwal (1997, 1998) used aldicarb, carbaryl and mexacarbate against *L. acuminata* and reported that these carbamates were potent molluscicides. The breakdown of this substance is strongly dependent on acidity and temperature. The toxicity of carbamate pesticide furadan was effective against the African giant snail *Limicolaria aurora* (Ebenso et al. 2005). Some monoterpenoids and their corresponding new N-methyl carbonate derivatives were potent molluscicides against *Biomphalaria alexandrina*, the snail vector of *Schistosoma mansoni* in Egypt (Radwan et al. 2007).

5.3.4 Organophosphorous

Compounds are powerful inhibitor of acetylcholinesterase (Singh and Agarwal 1983a, b) allowing acetylcholine to transfer nerve impulses indefinitely and causing a variety of symptoms such as weakness or paralysis. It inhibits the nervous enzyme acetylcholinesterase at the synapses (Taylor 1980). Organophosphorous pesticides are species specific. Singh and Agarwal (1982a, b, 1983a, b) reported that under laboratory conditions phorate, formothion, and trichlorofon were very effective against *Pila globosa* and *L. acuminata*. Amongst the three, trichlorofon was found to be the most toxic while phorate was least toxic to both the snails.

5.3.5 Synthetic Pyrethroids

Synthetic pyrethroids are modified version of natural chemical, pyrethrin isolated from chrysanthemums (Go et al. 1999). Several workers have reported it as potent molluscicides. Primarily it acts on the nervous system. On the nerve membrane it changes the permeability of sodium and potassium ions, which causes repetitive discharge of nerve at the synapse and neuromuscular junctions (Narahashi 1983). Pyrethroids are generally solids, having water solubility and they act as neurotoxin in very similar way to DDT. They are readily biodegradable but can bind to particles in soil and sediments, which can be persistent in these locations. Singh and Agarwal (1986a, b, c, 1987a, 1991), Sahay et al. (1991, 1995), Sahay and Agrawal (1997), Singh et al. (2008); and Singh and Singh (2009a, b) studied the toxicity of cypermethrin, permethrin, fenvalerate, and deltamethrin singly and with the synergist piperonyl butoxide against *L. acuminata* and *Indoplanorbis exustus*. The major target site of cypermethrin is the sodium channel of the nerve membrane. A sodium channel exposed to cypermethrin can remain open much longer even up to several seconds (Velisek et al. 2006).

5.4 Plant-Derived Molluscicides

Greater attention has been given to chemical control of snails. Several limitations are experienced in mixing the synthetic molluscicide with water in which the snails live. They are contact poison and therefore are toxic also to non-target animals and may cause long-term detrimental effect on environment. An alternative approach is the evaluation of molluscicidal properties of plant extracts, which should be easier to handle, comparatively safer for environment and non-target organisms. Singh et al. (1996a) have reported that there are several plants, which contain active compounds that are toxic to gastropods at doses much lower than synthetic molluscicides. Plant products have the additional advantage that they are biodegradable and cause lesser contamination to aquatic environment.

The WHO has tested several thousands of synthetic compounds for the control of the snail host. Although effective, these molluscicides have so far not proved themselves to be entirely satisfactory. With a growing awareness of environmental pollution, efforts are being made to discover molluscicidal products of plant origin. Being products of biosynthesis, these are potentially biodegradable in nature. Snail control with plant molluscicides has been one of the effective methods used for rapid and effective control of fasciolosis. Plant molluscicides are gaining wide attention because they are effective, cheaper, safer to non-target organism, easier to handle, and environmentally acceptable (Marston and Hostettmann 1985; Rao and Singh 2001; Singh et al. 2010a, b; Singh and Singh 2016a; Soni et al. 2020; Soni and Singh 2019). The active components present in plants such as saponins, flavonoids, alkaloids, sesquiterpene lactones, glycosides, steroids, phenol, lectins, and triterpenoids (WHO 1983; Marston and Hostettmann 1987; Marston et al. 1996; Singh et al. 1996a) are responsible for their toxic effects. These active compounds present in various plants have been found to be toxic to target organisms at acceptable doses ranging from <1 to 100 ppm (Adewunmi and Sofowora 1980; Marston and Hostettmann 1985, 1987; Okunji and Iwu 1988; Okunji et al. 1991; Jaiswal and Singh 2008; Jaiswal et al. 2008, 2009, 2010).

It has been well acknowledged that certain plant-derived extracts and phytochemicals such as alkaloids, saponins, tannins, phenols, terpenoids, flavonoids, steroids, lactones and glycosides, etc. are lethal to snails (Adewunmi and Sofowora 1980; Marston and Hostettmann 1985, 1987; Okunji and Iwu 1988). Singh et al. (1996a) have extensively reviewed the molluscicidal activity of several groups of compounds present in plants of 56 families of angiosperm, which might possibly be used for snail control. With growing awareness of environmental contamination and ecological disturbances caused by synthetic pesticides, efforts are being focused on to the search of molluscicides of plant origin. In past few years, large numbers of plants have been screened out for their molluscicidal activity. The investigations of molluscicidal properties of plants have been greatly expanded, with more than 1400 species studied so far (Marston and Hostettmann 1985; Kloos and McCullough 1982, 1987; Kuo 1987; Jurberg et al. 1989; Singh and Agarwal 1988, 1992; Shueb et al. 1993, 1996; Abdel-Hamid and Mantawy 1997; Schall et al., 1998; Singh and Singh 1994, 2000a, b, c, 2003a, b, 2004; Rao and Singh 2001, 2002;

Tripathi and Singh 2000, 2001; Tripathi et al. 2004; Singh et al. 2003, 2017b; Tiwari and Singh 2004a, b).

The potential use of plant molluscicides against schistosomiasis was reviewed in 1983 meeting of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease Scientific Working Group on Plant Molluscicides (Mott 1987). Freddy and Ritchie (1980) reported that a Puerto Rican Plant *Solanum nodiflorum* has been used as a source of natural molluscicide. “Endod” is Ethiopian name for soapberry plant *Phytolacca dodecandra* (Phytolaccaceae). Its unripe berries have been used as a botanical molluscicide (Lemma 1965; Kloos and McCullough 1982; Lambert et al. 1991). Okunji and Iwu (1988) have demonstrated the molluscicidal activity of total 112 extracts from 53 plant species used in Nigerian traditional medicine against *Bulinus globosus*, *Biomphalaria pfeifferi*, and *Lymnaea natalensis*. The methanol extracts of the leaves of *Polyscias dolichostachya* plant have strong molluscicidal activity against *Biomphalaria glabrata*, the intermediate host of *Schistosoma mansoni* (Hostettmann et al. 1982). Viera and Kubo (1990) observed that dichloromethane extract of the stem of *Galipea bracteata* have sufficient molluscicidal activity against *Biomphalaria glabrata*. They believed that the use of *Dracaena* berries for the control of schistosomiasis might prove to be a low cost alternative to the synthetic molluscicides.

Singh and Agarwal (1984a, b, 1987b) reported that plants belonging to the family Euphorbiaceae contain potent molluscicidal components in their latex. The plants of Euphorbiaceae family are reported as potent molluscicides against harmful snails (Singh et al. 1996a; Oliveira-Filho et al. 1999). The lattices of certain plants belonging to this family are hundred times more toxic than organic pesticides (Singh and Agarwal 1988). The latex of Euphorbiales has a quick knockdown molluscicidal activity that is even stronger than the pesticides malathion and mexacarbate (Singh and Agarwal 1984b, 1987a, 1988). The latex of *Euphorbia royleana* and *Euphorbia antisyphilitica* was shown to possess anticholinesterase activity (Singh and Agarwal 1983b, 1984b, 1987b). Studies on the kinetics of inhibition of AChE by the latex of euphorbiales demonstrated that these are non-competitive inhibitor of AChE in *Lymnaea acuminata*. Besides this, it profoundly affects the metabolism of biogenic amines in the snail nervous system (Singh and Agarwal 1984b). Jurberg et al. (1985) observed that the aqueous solution of the latex of *Euphorbia tirucalli* (Alveolus plant) has molluscicidal activity against *Biomphalaria glabrata* with LC₅₀-850 ppm, whereas Mendes et al. (1992) performed the similar experiment in a lentic habitat and found that there is 100% mortality of *Biomphalaria glabrata* at a concentration between 5 and 12 ppm. Baptista et al. (1994) reported that the latex of *E. splendens* in 12 ppm concentration cause 100% mortality of *Biomphalaria tenagophila* population in a lotic habitat. Singh et al. (2005) have reported toxic effect of stem bark and leaf of *Euphorbia hirta* plant against freshwater vector snail *Lymnaea acuminata*.

Different plant extracts of *E. cotinifolia* tested against *Biomphalaria glabrata* (Pereira et al. 1978), alcoholic extract of *Bridelia atroviridis*, *B. ferruginea* (Ene-Obong et al. 1991), acetone extract of dried latex of *Synadenium grantil* tested against *B. alexandrina* and *Bulinus truncatus* (El-Sayed 1993), aqueous and ethanol

extracts of *Phyllanthus nummularifolius* tested against *Biomphalaria pfeifferi* and *Lymnaea natalensis* (Chifundera et al. 1993), methanol and n-butanol extract of *Jatropha gossypifolia* unripe seeds tested against *L. luteola* and *I. exustus* (Sukumaran et al. 1995), and aqueous extract of *Euphorbia splendens* against *Biomphalaria glabrata*, *Biomphalaria pfeifferi* (Schall et al. 1998) are found to exhibit molluscicidal properties.

Asteraceae plant families have been observed to have sufficient molluscicidal activity. A herbaceous plant *Ambrosia maritima* was detected in Egypt, where it is known as a very popular drug “Damsissa” (Sidhom and Geerts 1984; Geerts et al. 1991, 1992; Alard et al. 1991; Belot et al. 1993). The first study of its effect on snail was reported by Sherif and El-Sawy (1962). Molluscicidal activity of plant has been tested against *Biomphalaria alexandrina* (El-Sawy et al. 1981, 1984, 1987), *B. glabrata* (Sidhom and Geerts 1984; Belot et al. 1986, 1988), *B. pfeifferi* and *B. globosus* (Belot et al. 1991). *Ambrosia maritima* displays a significant molluscicidal activity against the snail species *Biomphalaria*, *Bulinus*, and *Lymnaea*, examined both in lab (Geerts et al. 1992) and in field (El-Sawy et al. 1984; Belot et al. 1993). The plant also shows ovicidal activity, which is due to sesquiterpene lactones, mostly the ambrosin and damsine found in the plant extract (Fahmy and Darwish 1949; Jakupovic et al. 1987; Alard et al. 1991; Geerts et al. 1991; Triest et al. 1992). The LC₅₀ for *Biomphalaria alexandrina* and *B. truncatus* varied between 8.5 and 13.5 mg/l (24 h exposure) (Marchant et al. 1984; Geerts et al. 1991).

Leguminosae family plants are potential source of molluscicides. The chloroform extract of *Milletia thonningii* seeds is more effective in snail control. Molluscicidal activity of this plant is due to the presence of isoflavonoid alpinumisoflavone (Perrett et al. 1995). Of the 153 crude extracts of Panamanian plants of different families, *Hymenaea courbaril* (Leguminosae) is more effective against *Biomphalaria glabrata* (Marston et al. 1996). The extract of *Tamarindus indica* fruit pulp is effective against *Bulinus truncatus*. Saponins are the active moiety responsible for the molluscicidal activity (Imbabi and Abu-Al-Futuh 1992).

Agavaceae family has been demonstrated out for its molluscicidal value. *Agave attenuata* shows effective molluscicidal activity against *Biomphalaria alexandrina*, *Bulinus truncatus*, and *Lymnaea cailliaudi* snails (Shoeb et al. 1993). Sukumaran et al. (1994) have evaluated the molluscicidal activity of *Agave americana* and found that it is more potent against all developmental stages of snail. Crude saponins isolated from *Yucca aloifolia*, provide good control to snails, *Bulinus truncatus* and *Biomphalaria alexandrina* with LC₉₀ 30 and 35 ppm, respectively (El-Sayed 1994). Steroidal glycosides isolated from the methanolic extracts of *Dracaena mannii* fruit pulp (Okunji et al. 1991) have also shown promise for the control of vector snails.

Phenolic and anacardic acid isolated from *Anacardium occidentale* (Laurens et al. 1997) and *Spondias mombin* (Carthout et al. 1994), respectively, of the family Anacardiaceae, show pronounced molluscicidal activity against the snails *Biomphalaria glabrata*. Members of the family Cruciferae are shown to contain compounds that are highly toxic against *Biomphalaria glabrata* (Cepleanu et al. 1994). *Garcinia kola* has been observed to possess molluscicidal activity against

Bulinus globosus snail (LC₅₀—8 ppm) (Okunji et al. 1991). Kubo et al. (1992) reported that sesquiterpene lactone isolated from the leaves of *Podocarpus andina* (Podocarpaceae) is effective against *Biomphalaria glabrata*.

Aqueous and alcoholic extracts of *Asparagus racemosus* (Chifundera et al. 1993) and *Urginia apigea* leaves (Amusan et al. 1997) of the Liliaceae family exhibit high mortality rate (100%) against *Lymnaea natalensis* (LC₅₀, 1.0 mg/l) and *Bulinus africanus* (LC₅₀—100 ppm), respectively. Tripathi et al. (2004) reported that sublethal in vivo 24 h exposure to active fragment of *Punica granatum* bark and *Canna indica* root caused competitive inhibition of acetyl cholinesterase, acid/alkaline phosphatase, and Na⁺, K⁺ ATPase activity in the nervous tissue of *L. acuminata*. Singh and Singh (2001a) reported that the seed and leaf of the *Annona squamosa* (Annonaceae) has also molluscicidal activity against *Lymnaea acuminata*. Crude water leaves extract of *Alternanthera sessilis* (Amaranthaceae) is very effective in killing the snail *Bulinus globosus* (Azare et al. 2002, 2007).

Singh et al. (1996b) have reported that neem (*Azadirachta indica*) oil, bark, leaf, cake, and neem-based pesticide Achook and Nimbecidine are potent molluscicides against *Lymnaea acuminata* and *Indoplanorbis exustus*. Neem-based pesticide, viz. Rakshak, Neem gold, Multineem, and Neem Azal, caused nearly same mortality in the snail population like the synthetic molluscicides (Singh and Singh 1997a, b). An herbal product “Pestoban” (mixture of three plant extracts, viz. *Cedrus deodara* oil, *Azadirachta indica* oil, and *Embelia ribes* powder) has high molluscicidal activities against *Lymnaea acuminata* (LC₅₀— 6.5×10^{-3} mg/l) and *Indoplanorbis exustus* (LC₅₀— 5.9×10^{-3} mg/l), (Singh et al. 1995). Bark of *Nerium indicum* is an important source of botanical molluscicides (Singh et al. 1993c; Singh and Singh 1997c, 1998a, 1999).

Singh et al. (2003) and Singh and Singh (2008a) in his review on pharmacological effects of spices extensively discussed the molluscicidal activity against *L. acuminata* and *I. exustus*. The water extract of garlic bulb has high molluscicidal activity against *L. acuminata* and *I. exustus* (Singh and Singh 1993a, b). Singh and Singh (1995a) reported that allicin is the active molluscicidal component in garlic bulb. Stored garlic bulb (11 month old) is more toxic to *L. acuminata* and *I. exustus* than fresh garlic bulb (Singh and Singh 1996b). Further, Singh and Singh (1996a) have demonstrated that toxic effect of allicin is due to the inhibition of enzymes, viz. lactic dehydrogenase, acetylcholinesterase, and alkaline phosphatase in the nervous tissue of the snail *L. acuminata* (Singh et al. 1999b).

Molluscicidal activity of seed and root of *Abrus precatorius* and seed of *Argemone mexicana* was reported by Singh and Singh (1999). The active molluscicidal components were protopine and sanguinarine in the seed of *Argemone mexicana* and abrin and glycyrrhizic acid in *Abrus precatorius* seed and root (Singh and Singh 1998b, 1999).

The plant *Ferula asafoetida* (Umbelliferae), *Syzygium aromaticum* (Myrtaceae), and *Carum carvi* (Umbelliferae) are potent molluscicides (Kumar and Singh 2006). Crude extract of *Ferula asafoetida* dried root latex powder, *Syzygium aromaticum* flower bud powder, and *Carum carvi* seed powder are potent molluscicide against *L. acuminata*. Toxicity of *Syzygium aromaticum* flower bud powder (96 h LC₅₀—

51.98 mg/l) was more pronounced than that of root latex powder of *Ferula asafoetida* (96 h LC₅₀—82.71 mg/l) and seed powder of *Carum carvi* (96 h LC₅₀—140.58 mg/l). Active molluscicidal component of these spices, i.e., ferulic acid, umbelliferone; eugenol and limonene significantly reduced the reproductive capacity of the snail *L. acuminata* (Kumar et al. 2010b). They reported that treatment of these active components increases the length of hatching period of snails as well as a significant reduction in fecundity. Withdrawal experiment of 96 h indicates a significant reproductive recovery. According to Kumar et al. (2009), ferulic acid, umbelliferone, and eugenol are competitive and limonene is a competitive-non-competitive inhibitor of alkaline phosphatase. Ferulic acid and umbelliferone are competitive, whereas eugenol and limonene are competitive-non-competitive and uncompetitive inhibitors of acetylcholinesterase, respectively.

Srivastav and Singh (2005) demonstrated that the plant *Cinnamomum tamala* “tejpat” (family—*Lauraceae*) is potent molluscicide. The toxicity of leaf powder of *Cinnamomum tamala* against *L. acuminata* and *I. exustus* (24 h LC₅₀—1273.83 mg/l and 24 h LC₅₀—1371.53 mg/l) was time and dose dependent. The ethanol extract of leaf powder was more toxic against *L. acuminata* and *I. exustus* (24.44 mg/l and 34.02 mg/l) than other solvent extracts. They identify that eugenol and terpenoids were the active molluscicidal component found in leaf of *Cinnamomum tamala* (Srivastav and Singh 2005).

It has been reported that *Azadirachta indica* oil, oleoresin from *Zingiber officinale*, bulb powder of *Allium sativum* and *Polianthes tuberosa* are potent molluscicide (Singh et al. 1997, 1996b, 1998a, b, c, 1999a, 2004; Singh and Singh 1995a, b, c). Singh et al. (2010a) observed that variant abiotic factors can significantly alter the toxicity of oleoresin of *Z. officinale* against *L. acuminata*. The most suitable period to control *L. acuminata* is June and July. The addition of the synergist piperonyl butoxide or MGK-264 enhances the efficacy of these plant-derived molluscicides against the aquatic snail *L. acuminata* (Singh et al. 1998a, c). Binary combination of these plants-derived molluscicides and their tertiary combination with synergist piperonyl butoxide or MGK-264 are very effective (Shukla et al. 2005, 2006). These molluscicides reduce the reproductive capacity of snail *L. acuminata* (Singh et al. 2005). The molluscicidal activity of *Annona squamosa* seed and *Lawsonia inermis*, leaf, bark, and seed alone and their combination with other plant-derived molluscicides have been reported to be more effective than synthetic pesticides (Singh and Singh 2001a, b).

Rao and Singh (2002) studied the molluscicidal effect of *Azadirachta indica*, *Cedrus deodara* oil, *Allium sativum* bulb powder, and *Nerium indicum* bark powder alone and in binary combination against the giant African snail, *Achatina fulica*. They have also studied their effect on the reproduction and survival of young snails of *Achatina fulica* and related these to biochemical changes in ovotestis of this species. They observed that these molluscicides significantly reduced the fecundity of *Achatina fulica*. In further study, they observed that single and binary combination of plant-derived molluscicides caused a significant alternation in phospholipid level and rate of lipid peroxidation in the ovotestis of *Achatina fulica* and enzyme

activity in the nervous tissue of *L. acuminata* (Rao and Singh 2000; Rao et al. 2003; Srivastava et al. 2012).

Rao and Singh (2001) have reported the toxicity of binary combination of *Azadirachta indica* and *Cedrus deodara* oil with piperonyl butoxide, MGK-264, and *Embelia ribes* against *L. acuminata*. Singh and Singh (2003c) have reported the toxicity of binary combination of plant-derived molluscicides with piperonyl butoxide and MGK-264 against *L. acuminata*. Singh and Singh (2003a, 2004) reported the effect of herbal molluscicides and their combinations on the reproduction of the snail *L. acuminata*. Molluscicidal activity of different combination (single, binary, and tertiary) of plant product against harmful snail *L. acuminata* and *I. exustus* is reported by Shukla (2004). Effect of binary combination of plant-derived molluscicides with MGK-264 or piperonyl butoxide on the reproduction of the snail *L. acuminata* is extensively discussed by Singh et al. (2005).

Jaiswal and Singh (2008) have reported the molluscicidal activity of seed and lyophilized latex powder of *Carica papaya* and seed powder of *Areca catechu* against the vector snail *L. acuminata*. The toxicity of these plant products was time and dose dependent. The toxicity of *C. papaya* lyophilized latex powder (LC₅₀ at 96 h: 8.38 mg/l) was more pronounced than that of *A. catechu* seed powder (LC₅₀ at 96 h: 12.32 mg/l) and *C. papaya* seed powder (LC₅₀ at 96 h: 61.56 mg/l). Ethanolic extracts of *C. papaya* seed and *A. catechu* seed were more toxic than their other extracts. The ethanolic extract of *A. catechu* seed (LC₅₀ at 24 h: 17.21 mg/l) was more effective than the ethanolic extract of *C. papaya* seed (LC₅₀ at 24 h: 53.38 mg/l). The LC₅₀ of column-purified fraction of *A. catechu* seed at 96 h was 3.99 mg/l, whereas that of *C. papaya* seed was 7.06 mg/l. *C. papaya* and *A. catechu* may be used as potent molluscicides since the concentrations used to kill the snails were not toxic for the fish *Colisa fasciatus*, which shares the same habitat with the snail *L. acuminata*. Jaiswal et al. (2008) reported the molluscicidal activity of *Myristica fragrans* against the vector snail *L. acuminata*.

Pandey and Singh (2008) have noted the toxic effect of dried berries powder of *Piper cubeba*, dried fruit powder of *Piper longum* and *Tribulus terrestris* against snail *L. acuminata*. Toxicity of these plant products were time and concentration dependent. The toxicity of *Piper longum* fruit powder (96 h LC₅₀—48.99 mg/l) was more effective than fruit powder of *Piper cubeba* (96 h LC₅₀—54.01 mg/l) and fruit powder of *Tribulus terrestris* (96 h LC₅₀—83.49 mg/l). Ethanol extracts of these plants were more effective than other organic extracts. 96 h LC₅₀ of column-purified fraction of *Piper cubeba* against *L. acuminata* was 3.57 mg/l, where 96 h LC₅₀ of the column-purified fraction of *Piper longum* and *Tribulus terrestris* was 5.03 mg/l and 13.53 mg/l, respectively. These plants may be used as potent source of molluscicide against the snail *L. acuminata* (Pandey and Singh 2009).

Singh et al. (2010b) have noted that oleoresin of *Zingiber officinale* is a potent molluscicide against *L. acuminata*. This snail is the vector of *Fasciola* species, which cause endemic fascioliasis in eastern Uttar Pradesh. As this snail breeds and maintains their population constant throughout the year, so that in this study temperature, pH, dissolved oxygen, free carbon dioxide, conductivity of the water in control, as well as molluscicide-treated water were measured simultaneously.

LC₅₀ value of oleoresin was determined in each month of the year. Toxicity of oleoresin in June–July (24 h LC₅₀ 16.54–14.28 mg/l) is highest. Acetylcholinesterase, acid and alkaline phosphatases activity in the nervous tissue of the snails treated with sublethal concentration of oleoresin was simultaneously measured. Significant positive rank correlation, in between the acetylcholinesterase or acid phosphatase activity and LC₅₀ of oleoresin was observed. The present study conclusively shows that variant abiotic factors can significantly alter the toxicity of oleoresin of *Zingiber officinale* in *L. acuminata*. The most suitable period for control of *L. acuminata* is June–July.

Singh and Singh (2009a, b) have reported the molluscicidal activity of bark powder of *Saraca asoca* and leaf powder of *Thuja orientalis* against the snail *L. acuminata*. The 96 h LC₅₀ of *T. orientalis* leaf powder against *L. acuminata* was 250.5 mg/l. Ethanol extracts were more toxic than other organic extracts. The ethanol extract of *T. orientalis* leaf (24 h LC₅₀: 32.74 mg/l) was more effective than that of *S. asoca* bark (24 h LC₅₀: 82.38 mg/l). The 24 h LC₅₀ of column-purified fraction of *T. orientalis* leaf and *S. asoca* bark powder was 29.25 and 64.89 mg/l, respectively. Saponin and thujone were identified as active molluscicide components in the bark of *S. asoca* and leaf of *T. orientalis*, respectively. The product of *S. asoca* and *T. orientalis* may be used as potent molluscicides.

Jigyasu and Singh (2010) and Jigyasu et al. (2010a, b) noted that abiotic factors significantly alter the reproductive behavior of *L. acuminata*. The maximum reproductive capacity and developmental process of snail was observed in between the month of March and May. They have also reported that water quality has also profound effect on the reproduction of snail and suggested that the control of fasciolosis in India is more suitable between months of March and May. In further study they had reported seasonal variation in abiotic environmental factors altered the phospholipid level and rate of lipid peroxidation in ovotestis of *L. acuminata* (Jigyasu et al. 2010a).

Upadhyay and Singh (2011a) have reported time- and concentration-dependent molluscicidal activity of *Sapindus mukorossi* and *Terminalia chebula* fruit powder against the vector snail *L. acuminata*. The molluscicidal activity of *T. chebula* fruit powder (96 h LC₅₀: 93.59 mg/l) was more pronounced than that of *S. mukorossi* fruit powder (96 h LC₅₀: 119.57 mg/l). Ethanolic extract of *S. mukorossi* and *T. chebula* fruit powder were more toxic than their other organic solvent extracts. The molluscicidal activity of ethanolic extract of *S. mukorossi* fruit powder (24 h LC₅₀: 2.75 mg/l) was more effective than the ethanolic extract of *T. chebula* fruit powder (24 h LC₅₀: 124.06 mg/l). The 96 h LC₅₀ of column-purified fraction of *S. mukorossi* fruit powder was 5.43 mg/l, whereas those of *T. chebula* fruit powder was 7.49 mg/l. Column, thin layer, and high performance liquid chromatography analysis demonstrate that the active molluscicidal component in *S. mukorossi* and *T. chebula* is saponin (96 h LC₅₀: 1.31 mg/l) and tannic acid (96 h LC₅₀: 1.64 mg/l), respectively. These plants may be used as potent source of molluscicides against the snail *L. acuminata*. Upadhyay and Singh (2011b) studied the mode of action of the active components and found that they inhibit acetylcholinesterase, acid and alkaline phosphatase in the nervous tissue of treated snails. Further, Upadhyay et al. (2013)

studied the molluscicidal activity of *Moringa oleifera* leaf and *Momordica charantia* fruits. They reported that the molluscicidal activity of *M. oleifera* leaf and fruit powder of *M. charantia* is due to benzyle amine and momordicine, respectively. They inhibit acetylcholinesterase, acid/alkaline phosphatase in the nervous tissue of *L. acuminata*.

Soni and Singh (2015, 2016, 2017, 2019) have reported the molluscicidal activity of *Terminalia arjuna* bark and its different organic extract against *Lymnaea acuminata* and *Indoplanorbis exustus*. Results of their findings demonstrate that toxicity of column-purified fraction was higher among all the treatments of *T. arjuna* bark against *L. acuminata* (96 h LC₅₀—3.12 mg/l) and *I. exustus* (96 h LC₅₀—22.49 mg/l). The TLC analysis demonstrates that the column-purified fraction (0.80) and active component arjunolic acid (0.80) have the same R_f values. It indicates that active molluscicide in *T. arjuna* is arjunolic acid. Toxicity of arjunolic acid against *L. acuminata* at 24 h and 96 h LC₅₀ were 8.00 mg/l and 1.30 mg/l, respectively. 24 h and 96 h LC₅₀ of *T. arjuna* against *I. exustus* were 30.80 mg/l and 14.53 mg/l, respectively.

The toxicity of 96 h column-purified fractions of *Tamarindus indica* bark against *L. acuminata* was 96 h LC₅₀—11.50 mg/l and against *I. exustus* was 96 h LC₅₀—18.07 mg/l, respectively. TLC analysis and HPLC fingerprint profile of the column-purified fraction of *T. indica* bark showed R_t value 2.84 min, whereas pure saponin R_t value was 2.58 min. It indicates the presence of saponin in column-purified fraction of *T. indica*. The seed of *T. indica* was more toxic than its bark against both the snails. The 24 h and 96 h LC₅₀ of ethanolic extract of *T. indica* seed were 5.22 mg/l and 1.18 mg/l against *L. acuminata* and 24 h and 96 h LC₅₀ of ethanolic extract of *T. indica* seed against *I. exustus* were 65.95 mg/l and 33.57 mg/l, respectively. The toxicity of column-purified fraction against *L. acuminata* was (24 h LC₅₀—1.74 mg/l and 96 h LC₅₀—0.71 mg/l) and against *I. exustus* was (24 h LC₅₀—14.08 mg/l and 96 h LC₅₀—21.37 mg/l), respectively (Soni et al. 2020). The identification of active molluscicidal component in column-purified fraction of *T. indica* seed was performed by thin layer chromatography analysis. The R_f value of spot of column-purified fraction of *T. indica* seed (0.77) showing same R_f value corresponding to procynadine (0.77) indicates the presence of procynadine in column-purified fraction of *T. indica* seed. The 96 h LC₅₀ of procynadine against *L. acuminata* was (0.31 mg/l) and against *I. exustus* was (9.37 mg/l) (Soni 2016).

Soni et al. (2017) reported that maximum inhibition in AChE (60.17% of control), ACP (34.66% of control), and ALP (24.01% of control) activity was observed in snail nervous tissue exposed to 80% of 96 h LC₅₀ of arjunolic acid (Soni 2016). They reported that inhibition of AChE was non-competitive, ACP uncompetitive, and ALP competitive-non-competitive by arjunolic acid of *T. arjuna* bark (Soni et al. 2017, 2020). They reported that inhibition of AChE by column-purified fraction and saponin is competitive, while inhibition of AChE by column-purified fraction and procynadine of *T. indica* seed is uncompetitive. Inhibition of ACP by column-purified fraction and saponin were uncompetitive, whereas

ACP Inhibition was competitive. Inhibition of ALP by both of treatments was competitive (Soni and Singh 2019).

Nerium indicum (Kaner) and other members of family Apocynaceae are potent molluscicides. Molluscicidal activity of the crude latex, bark, and leaf of *Nerium indicum* is higher than synthetic molluscicides like phorate, fenvalerate, formothion, and mexacarbate (Singh et al. 1993c; Singh and Singh 1997c, 1998a). Singh and Singh (1998a) have reported that bark of *Nerium indicum* is an important source of botanical molluscicides. Molluscicidal activity of seed and root of *Abrus precatorius* and seed of *Argemone mexicana* was reported by Singh and Singh (1998b). The active molluscicidal components were protopine and sanguinarine in the seed of *Argemone mexicana* and abrin and glycyrrhizic acid in *Abrus precatorius* seed and root, respectively (Singh and Singh 1998b). These active molluscicidal components caused a significant decrease in the levels of protein, free amino acid, DNA and RNA in the nervous tissue of *L. acuminata*. A significant reduction was also found in the phospholipid levels and a simultaneous increase in the rate of lipid peroxidation in the nervous tissue of the treated snails (Singh and Singh 1999, 2000c). Singh et al. (2003) in his review article pharmacological effects of spices has extensively discussed the molluscicidal activity of certain spices. Srivastava (2004) has reported that the two spices *Piper nigrum* (Black and white) and *Cinnamomum tamala* are potent molluscicides. They suggested that piperine interacts with the lipid environment inside the snail body, which leads to increase in permeability of intestinal cells, which is one of the causes for mortality of treated snails (Srivastava et al. 2009, 2010).

Singh et al. (2012c) characterize the molluscicidal activity of *Bauhinia variegata* and *Mimusops elengi* against *L. acuminata*. Saponin and quercetin were identified as active molluscicidal component in bark of *M. elengi* and leaf of *B. variegata*. Further, Singh et al. (2012d) used the binary combination of *B. variegata* and *M. elengi* with piperonyl butoxide/MGK-264 against *L. acuminata* and noted that these combinations were 360–720 times more effective than their individual components. They found that these combinations inhibit acetylcholinesterase, acid/alkaline phosphatase in the nervous tissue of snail *Indoplanorbis* (Singh et al. 2015, 2016). Srivastava and Singh (2013), Srivastava et al. (2013) and Srivastava and Singh (2015) reported that variant abiotic factors in the aquatic habitat can significantly alter the toxicity of thymol, eugenol, and quercetin against *L. acuminata*.

Kumar et al. (2018a) reported that dried root powder of *Potentilla fulgens* acts as active molluscicide against *L. acuminata*. Ninety six hour LC₅₀ of dried root powder against *L. acuminata* was 133.62 mg/l, whereas column-purified fraction of dried root powder was 28.96 mg/l, Further, Kumar et al. (2018b) noted that 96 h toxicity of dried powder of *Potentilla fulgens* against another vector snail *Indoplanorbis exustus* was 104.29 mg/l and column-purified fraction 96 LC₅₀ was 33.75 mg/l.

5.5 Cow Urine as Molluscicides

Although there are a large number of plants, which are rich source of bioactive molluscicidal components, yet several more might still be lying unexplored. The concerted efforts are needed to make the available compounds more potent against harmful snails as pesticide and safe to the environment by way of improved formulations. Successful strategies for identification and investigation of molluscicidal agents from combination of different product used in ethnotherapy and folklore are required. In this connection cow urine, dung, and its binary and tertiary combination with other plant-derived molluscicides are very effective in snail control.

5.5.1 Cow Urine

The Animal Welfare Board of India emphasizes cow protection and goshala movement in the country and the Board has been succeeded and set up many milestone for protection and conservation of cows and its progenies because the cows are treated as “maata” and protector of Indian Ecology, Economics, and Environment. They are advocating the cow revolution, viz. the large-scale use of biofertilizers, biopesticide, and biogas (Lodha et al. 2004). Urine of cow has pesticidal property. It has got the effectiveness against certain harmful pests (Lodha et al. 2004). Use of cow urine as pesticide is advantageous as it does not have the poisonous hazards of chemical pesticides. It will produce uncontaminated and poison-free food grains, which will automatically take care of the health of the human race, thereby saving billions of rupees, which are spent on health programs and also saving the misery and pain which the sick people undergo as a result of consuming poisonous and contaminated food grains (Lodha et al. 2004). The United States of America patent (No. 6410059) and trade office granted a patent for an Indian innovation. It has been proved that cow urine can make antibiotics, antifungal agents, and also anticancer drugs, which are more effective.

5.5.2 Pesticidal Use of Cow Dung/Urine

Three liters to five liters of cow urine is mixed with equal amount of cow dung and the mixture allowed fermenting for 4 days in closed tank. The slurry is filtered through a diaphanous cloth and 200–250 g of quicklime is added to solution in order to neutralize the acidity. The stock solution thus obtained is diluted with 50–80 l of water and is sprayed on one acre of land. The mixture helps in reducing the pest load, since the moth shows no preference to lay the eggs. The spray apart from discouraging the infestation also contributes to the crop health and flower retention of the crop stand by means of trace elements present in it. It is always advisable to spray after 3.30 p.m. Spraying two to three times produces better results (Kashyapa 2003). Two kilograms of neem seeds are ground well and diluted with water and

then filtered. To this filtrate, 10 kg of fresh cow dung are added. This will ensure protection of banana plants from the damage by nematodes (Spirit of Anonymous Creativity; Getting Nematodes out of the way 2003). Aavootam is a pesticide, mix cow dung, urine, milk, ghee, and yoghurt together and pour onto soil and plant. This concoction helps build resistance in plants and keeps disease away. Natural pesticide changes the smell of green to make them desirable to insects and thus keep them away used as natural pesticides (Sujata 2003).

The world's first molluscicidal activity from cow urine is an outcome of diligent effort of Tripathi et al. (2006). The toxicity of cow urine kept for 15 days in sunlight (8 h/day) or ambient laboratory conditions against the snail was time and concentration dependent. The pH of fresh cow urine is 7.4 which changes to 10.9 when kept for 15 days in laboratory; exposure to sunlight for the same period alters the pH to 10.85. Binary combinations (1:1) of freeze-dried cow urine kept for 15 days separately with each of *Allium sativum* (Liliaceae) bulb powder, *Azadirachta indica* (Meliaceae) oil, *Annona squamosa* (Annonaceae) seed powder, *Ferula asafoetida* (Apiaceae) root latex and tea leaves, and *Camellia sinensis* (Theaceae) were more toxic to the snail than treatment with urine alone. Additives to cow urine in sunlight were more effective than those under laboratory conditions (Tripathi et al. 2006).

Sublethal treatment (20% and 60% of 24 h LC₅₀) with freeze-dried cow urine powder and their combination with plant-derived molluscicides, viz. *Allium sativum* bulb powder, *Annona squamosa* seed powder, *Azadirachta indica* oil, *Camellia sinensis* leaves, *Ferula asafoetida* root latex powder and dung + CaCl₂ on the reproduction of snail *L. acuminata* have been studied. It was observed that these combinations caused a significant reduction in fecundity, hatchability, and survival of snails. Withdrawal of snails to freshwater after the above treatments caused a significant recovery in fecundity of *L. acuminata*. Sublethal treatment with cow urine formulations significantly altered level of different biochemical parameters in ovotestis of *L. acuminata*. Maximum reduction in protein (48.29% of control), total free amino acid (9.15% of control), and phospholipid (13.23% of control) levels in ovotestis were observed in the snails exposed to 60% of 96 h LC₅₀ of cow urine + *C. sinensis*, cow urine + *A. sativum* bulb powder, and cow urine + *A. squamosa* seed powder, respectively. Treatment with 60% of 96 h LC₅₀ of cow urine + *A. sativum* bulb powder caused a highest increase in the rate of lipid peroxidation (323.66% of control). Maximum inhibition in acid and alkaline phosphatase activity in ovotestis was observed in snails exposed to 60% of 96 h LC₅₀ of cow urine + *A. squamosa* seed powder (14.81% of control) and cow urine + *F. asafoetida* (12.04% of control), respectively. Withdrawal experiments showed that these changes were reversible (Tripathi et al. 2010).

Molluscicidal activity of different formulations of freeze-dried cow urine (LCU) of different Indian breeds such as *Shahiwal*, *Geer*, and *Tharparkar* were studied against the snail *Lymnaea acuminata* (Kumar et al. 2011a, b, d). The toxicity of different breeds of cow urine kept for 15 days in sunlight (8 h/day)/ambient laboratory condition, as well as boiled urine samples were tested against the *L. acuminata*. Toxicity of all the samples was time and concentration dependent. Toxicity of *Tharparkar* breed urine kept for 15 days in sunlight (24 h LC₅₀—604.85 mg/l)

and laboratory condition (24 h LC_{50} —519.71 mg/l) was higher than *Sahiwal* (24 h LC_{50} —sunlight, 650.70 mg/l; laboratory, 666.98 mg/l) and *Geer* (24 h LC_{50} —sunlight, 619.01 mg/l; laboratory, 674.33 mg/l). Molluscicidal activity was also observed in boiled cow urine. Boiled urine of *Geer* (24 h LC_{50} —519.75 mg/l) and *Sahiwal* (24 h LC_{50} —519.88 mg/l) was more toxic than *Tharparkar* (24 h LC_{50} —664.17 mg/l). Further in his extensive study, Kumar et al. (2013a, b, c, d, f) noted that different combinations of freeze-dried cow urine of Indian breeds are also effective in controlling the reproduction of snail *L. acuminata*.

5.6 Combined Action of Molluscicides

The degree and duration of action of many biological active compounds are closely associated with their rate of metabolism. The metabolism of typical drug occurs in two major steps, which is referred as primary and secondary detoxification. Primary detoxification constitutes the initial metabolic attack at the molecular level, and usually involves a biotransformation reaction in which a polar, reactive group is either added to or unconverted in the chemical compound. The products of primary metabolism are sometimes directly excreted, but more often they undergo one of several secondary reactions that result in their conjugation with a variety of endogenous materials. The major types of reactions by which primary detoxification is affected are oxidation, reduction, hydrolysis, dehydrochlorination, and group transfer. Oxidation which is the most important reaction is affected by mixed-function-oxidase (MFO) system, which appears to play a ubiquitous role in detoxification throughout the plant and animal kingdom (Parke 1968). The MFO system is located mainly (though not exclusively) in the liver of mammals, fish, and birds and in gut, fat body, and malpighian tubules in the insects (depending on the species concerned) (Wilkinson and Brattsten 1972). The rate of detoxification of a drug can be altered by giving them in combination with other chemicals which may have promoting or inhibiting action on the detoxification process of the principal drug.

The biological activity of a chemical can be modified by prior or simultaneous exposure of the organism to another chemical agent, which may act at the same site, two different sites, or at sites which are present in tandem to each other, this is known as interaction. This interaction has been studied in a number of ways and procedures to analyze toxicity data, which merge when two chemicals are given in combination. This interaction may result from events taking place at possible loci, including absorption, distribution, and metabolism site of toxic action, excretion and repair. Interaction can vary with route of administration, age, gender, health, nutritional status, etc.

Compounds that enhance the toxicity of drug with which they are combined, are known as synergists. Metcalf (1967), Casida (1970), Wilkinson (1976), Duke and Moore (1976) reported that synergism can theoretically occur through interaction of synergist with any of several processes that determined that ability of drug to penetrate the organism and to be subsequently transported to the target site at a concentration, sufficient to cause a toxic effect.

The study of toxicity mechanism of individual toxicant yields invaluable insights into events at the molecular level and may provide indications of possible mechanism for interaction of toxicant.

Additive interaction requires independent mechanism on that the rate or effect limiting step in process not to be saturated by any of the toxicant acting alone. Antagonism activity is less than that of expected from the toxicity of the separate mixture components. Synergistic action is that, having the toxicity effect more than that of additive. Finney (1971) defined the combined action of different drugs into four types.

Simple similar action can be defined as “The action of two closely related chemicals whose mode of action on test animals are nearly and which consequently have parallel regression lines of probits on log doses.”

Simple independent action can be defined as “the two components are different in their chemical nature so that they affect the organism in entirely distinct ways. In such case death, may be the same for both, yet the two drugs may affect different systems of the body of the animal.”

Correlated independent action can be defined that “When an animal receives a dose of a mixture, it will respond when one of the constituents is present in excess of the animal tolerance for that chemical”. Thus, while the two chemicals may not be acting in conjugation yet they influence the activity of each other (Plackett and Hewlett 1948).

The three types of joint actions discussed above are called “non-interactive” (Plackett and Hewlett 1952) as they do not affect the transport and final concentration of each other at the site of action (Finney 1971).

Interactive joint action is “The action of a mixture in which one component of the mixture affects, either the amount or the effect of the other, at the site of action” (Hewlett and Plackett 1964; Plackett and Hewlett 1967). This includes two classes of joint action, i.e., synergism and antagonism. In case of interactive joint action, if the action of the mixture is greater than when the first is absent, the first is said to synergize the second, the first is said to antagonize the second.

A theoretically attractive way to utilize the synergistic activity of certain groups (synergophores) is to directly incorporate them into the drug structure or, addition to such chemicals which act as synergists (Bliss 1939; Wilkinson 1976; Hodgson et al. 1995).

Compounds that enhance the toxicity of drug with which they are combined, are known as synergists. Metcalf (1967), Casida (1970), and Wilkinson (1976) reported that synergism can theoretically occur through interaction of synergist with any of several processes that determined the ability of drug to penetrate the organism and to be subsequently transported to the target site on the concentration, sufficient to cause a toxic effect. There are two types of synergists, one which are not commercially

produced, but whenever combined with other toxicants, produced synergistic effects.

The most important oxidase inhibitors which are registered as synergists are methylenedioxyphenyl compounds (MDP) (Esacc and Casida 1969; Casida 1970; Wilkinson 1976), piperonyl butoxide, sulfoxide, safrole, isosafrole, propyl isomer, tropitol, and MGK-264 are known MDP synergists (Casida 1970). During past few years, piperonyl butoxide is widely used as synergist (Lange and Feuerhake 1985; Hinks and Spur 1991; Sahay et al. 1991; Bagwell and Plapp 1992; Levot 1992; Saito et al. 1992; Varsano et al. 1992; Kennaugh et al. 1993; Kotze and Sales 1994; Tripathi and Agarwal 1997; Singh et al. 1998a, b). The next group of synergistic compounds is those, which have not any toxic effect and are commonly prepared for synergistic activity. The chemical nature of a synergist for any given insecticide depends on the nature of the enzyme, which is responsible for its detoxification. Among many hundreds of compounds, which were tested for synergistic activity, only five of them (Piperonyl butoxide, Sulfoxide, Propyl isomer, Tropitol, and MGK-264) are registered for commercial use in the United States (Wilkinson 1976). These compounds result in inhibition of microsomal oxidation and are capable of synergizing to some extent the action of all insecticides metabolized by this system. Synergistic action varies with both the synergists and the insecticides. Casida (1970) and Wilkinson (1976) reported that the degree of synergism with most insecticides observed is directly related to the rate of metabolism of insecticides, and this depends on the structure of insecticide and metabolic capacity of organism.

Singh and Agarwal (1983a) studied the *in vivo* and *in vitro* synergism with anticholinesterase pesticides carbamate and organophosphorus against the snail *Lymnaea acuminata* and reported that three synergist viz. piperonyl butoxide, sulfoxide, and dimethyl amino aniline synergises the toxicity of these pesticides many folds (70 times) (Singh and Agarwal 1983b, 1986b). Further, in 1989, Singh and Agarwal extensively studied the effect of PB + carbaryl synergism on the metabolism of snail *L. acuminata*. They observed that there was a significant difference in between the effect of equivalent concentration of carbaryl and carbaryl + PB in AChE, phosphatase activity, glycogen, protein, lactic acid, reducing sugar and amino acid level.

The pesticidal activity of pyrethroids is limited by detoxifying oxidases and esterases (Casida and Ruzo 1980). Inhibitors of these enzymes may prolong the stability and enhance the activity of pyrethroids in insects (Ishaaya and Casida 1980; Gaughan et al. 1980). Brown and Bryson (1996) reported that the monooxygenase inhibitor, propyl aryl ether is a potent synergist of pyrethroid, permethrin, which then become toxic to resistance strain. It has been demonstrated that PB increases the toxicity of pyrethroids, permethrin, and cypermethrin, against snail, *L. acuminata* when given in a ratio of 1:5. The synergistic ratio of cypermethrin was 37–60 times while that of permethrin was 34–38 times (Singh and Agarwal 1986b, c). Sahay et al. (1991) reported that the toxicity of pyrethroid deltamethrin increases four- to sixfold after addition of PB in a 1:5 ratio. In case of another pyrethroid fenvalerate, PB increases the toxicity eight- to tenfolds during an exposure period ranging from 24 to 96 h. In another study, Sahay and Agrawal (1997) reported that MGK-264 was

more effective in case of deltamethrin as compared to fenvalerate against the snail *L. acuminata* when given together in a ratio of 1:5. The synergistic ratio of deltamethrin + MGK-264 ranged between 19 and 44 as compared to 4–6 of deltamethrin + PB. The synergistic ratio of fenvalerate and MGK mixture ranged between 7 and 4 after an exposure period of 24–96 h. Synergism in binary combination of pesticides was studied by Tripathi and Agarwal (1997) against *L. acuminata*. According to them, 24 h toxicity of a binary mixture of deltamethrin+sevin was 1.39 mg/l). MGK-264, the esterase inhibitor is whenever mixed with this binary mixture in 1:46:5 ratio (decis, sevin, MGK), lethality of this tertiary mixture (24 h LC₅₀—0.073 mg/l) was found to be much higher than the binary mixture of decis and carbaryl. Tripathi and Agrawal (1998) studied the toxicity and in vivo inhibition of acetylcholinesterase (AChE) by organophosphate nuvan (Dichlorvos) and nuvan with a pyrethroid deltamethrin in a snail *L. acuminata*. It was found that this nuvan act synergistically with deltamethrin when mixed in 1:46 ratio. Tertiary mixture of nuvan, deltamethrin, and PB in 1:46:5 ratios was also found to have higher toxicity in comparison to the binary combination of Nuvan + PB or Nuvan + decis. Tripathi and Agrawal (1998) have performed a new experiment on tertiary mixture of synergistic action, by mixing the known synergists PB and MGK in the binary mixture of pesticides of also known synergistic action and they found out that deltamethrin + carbaryl + MGK-264 (1:46:5) and Nuvan + Deltamethrin + PB (1:46:5) are more effective than their corresponding mixture (Tripathi and Agarwal 1997, 1998).

Tripathi and Singh (2001) studied the molluscicidal activity of *Punica granatum*, *Canna indica* and their binary combination with other plant-derived molluscicides against *L. acuminata* and *I. exustus*. They reported that the maximum synergism of 879.55 times was observed in binary combination (1:1) of *A. indica* + *C. deodara*. Singh and Singh (2001a, b) studied the molluscicidal activity of binary combination of *Lawsonia inermis* with other plant-derived molluscicides against *L. acuminata* and *I. exustus*. Highest increase (48.28 to 43.32 times) in toxicity was observed when *L. inermis* seed powder + *C. deodara* oil (1:1) were tested against both the snails.

Synergism in combination of different plant molluscicides were reported by Singh et al. (1995). According to them, the fruit powder of *Embelia ribes* is non-toxic against snail *L. acuminata* and *I. exustus*, whereas oil of *Cedrus deodara* (24 h LC₅₀ against *L. acuminata* and *I. exustus*—5.92 and 9.86 mg/l) and *Azadirachta indica* oil (24 h LC₅₀ against *L. acuminata*—17.35 and 8.80 mg/l) were toxic against both the snails. It was observed that when all the three, i.e., *E. ribes* powder, *C. deodara* oil, and *A. indica* oil were mixed, the toxicity against both the snails was 10³ times more than either of the two toxic oils.

Rao and Singh (2002) studied the molluscicidal effects of *Azadirachta indica*, *Cedrus deodara* oil, *Allium sativum* bulb powder, and *Nerium indicum* bark powder alone and in binary combination against the giant African snail, *Achatina fulica*. They have also studied their effect on the reproduction and survival of young snails of *Achatina fulica* and related these to biochemical changes in ovotestis of this species. They observed that these molluscicides significantly reduced the fecundity of *A. fulica*. These single and binary combinations of plant-derived molluscicides

resulted in a significant ($p < 0.05$) decrease in phospholipid level and a significant elevation in the rate of lipid peroxidation in the ovotestis of *A. fulica* (Rao and Singh 2000). Rao et al. (2003) reported that single and binary treatments of plant-derived molluscicides, viz. *A. indica*, *C. deodara*, *N. indicum*, and *A. sativum* caused a significant inhibition of acetylcholinesterase, lactic dehydrogenase and phosphatase activity in the nervous tissue of *A. fulica*.

Combinations (1:1) of *Annona squamosa* seed powder with oils of *Cedrus deodara* or *Azadirachta indica*, powder of *Allium sativum* and oleoresin of *Z. officinale* were found to be more toxic against the snail *L. acuminata* (Singh and Singh 2001a). Maximum synergism about 225 folds was observed in (1:1) combination of *Annona squamosa* seed powder + *Cedrus deodara* oil (Singh and Singh 2001a). In continuation to this study, Singh and Singh (2003a) have observed that sublethal treatment of these combinations effectively reduced the reproductive capacity of the snail *L. acuminata*.

Parmar and Dutta (1987) reported that a number of oils, viz. mahua, neem, karanj, undi, kokum, gombage, dhupa fat and rubber oil generally synergized malathion toxicity, when malathion and oils were tested at 1:1 and 1:5 levels. Synergistic action is probably due to disulfuration of Malathion into Malaoxon by the oils, because of their oxidizing nature.

The combination of *Annona squamosa* and *Lawsonia inermis* seed with *Cedrus deodara* and *Azadirachta indica* oil, bulb powder of *Allium sativum* in 1:1 ratio was more toxic against snail *L. acuminata* than the individual components (Singh and Singh 2001b). These combinations of plants-derived molluscicides were very effective in controlling the reproductive capacity of snail *L. acuminata* (Singh and Singh 2004). The molluscicidal activity of binary combination of *Annona squamosa*, *Punica granatum*, and *Argemone mexicana* with synergist piperonyl butoxide and MGK-264 was reported by Singh and Singh (2004). They reported that these combinations were more toxic against *L. acuminata* than their single treatment.

The binary combination of ethanolic extracts of *Polianthes tuberosa* powder, hecogenin, tigogenin, *Trachyspermum ammi* seed powder and thymol with synergist piperonyl butoxide and MGK-264 in 1:5 ratios was more effective than the independent toxicity of these plant molluscicides (Singh et al. 1999a). Singh et al. (1998a, b) prepared a binary combination of oil of *Azadirachta indica*, *Allium sativum* bulb powder, oleoresin from *Zingiber officinale* and their active molluscicidal components, i.e., azadirachtin, allicin, and [6]-gingerol with piperonyl butoxide and MGK-264 enhanced the toxicity of all the test compound. The sublethal concentrations of these combinations were effective in controlling the fecundity, hatchability, and survival of young snails (Singh and Singh 2003a).

Binary combination (1:1) of freeze-dried cow urine kept for 15 days separately with each of *Allium sativum* (*Liliaceae*) bulb powder, *Azadirachta indica* (*Meliaceae*) oil, *Annona squamosa* (*Annonaceae*) seed powder, *Ferula asafoetida* (*Umbelliferae*) root latex, and *Camellia sinensis* (*Theaceae*) were more toxic to the snail than treatment with cow urine alone (Tripathi et al. 2006, 2010). The molluscicidal activity of binary combination root latex of *Ferula asafoetida* (*Umbelliferae*), flower bud of *Syzygium aromaticum* (*Myrtaceae*), and seed powder

of *Carum carvi* (*Umbelliferae*) were found effective in killing the snail *L. acuminata* and *Indoplanorbis exustus* (Kumar and Singh 2007).

Shukla et al. (2006) reported the single, binary, and tertiary combination of few plant-derived molluscicides alone or in combination with synergist inhibit various vital enzymes in the nervous tissue of the freshwater snail *L. acuminata*. Shukla et al. (2006), while studying the effect of single, binary, and tertiary combination of few plant-derived molluscicides alone or in combination with synergist on acetylcholinesterase (AChE), lactic dehydrogenase (LDH), and acid/alkaline phosphatase (ACP/ALP) in the nervous tissue of the freshwater snail *L. acuminata* noted that sublethal in vivo 24 h exposure to 40 and 80% of LC₅₀ of *Azadirachta indica* oil (AI), oleoresin of *Zingiber officinale* (OL), *Cedrus deodara* oil (CD), *Allium sativum* (AS), and *Polianthes tuberosa* (PT) bulb powder singly, their binary combination of AI + OL, AS + CD, AS + PT, CD + OL, CD + PT, OL + PT, and tertiary combination of these binary combinations with the synergist piperonyl butoxide (PB) or MGK-264 significantly altered the activity of these enzymes. Tertiary combination with PB or MGK was very effective. Combination of CD + PT + MGK was more effective against AChE, whereas the combination of CD + OL + PB, CD + AS + PB and CD + PT + PB were more effective against LDH, ACP, and ALP, respectively.

Singh et al. (2010b) studied the molluscicidal activity of leaf/bark of *Saraca asoca*, leaf/fruit of *Thuja orientalis* and their active components/column-purified fraction with synergist Piperonyl butoxide (PB) and MGK-264 (ENT 8184) in binary combination (1:5) against *L. acuminata*. Combination of *S. asoca* leaf/column extract of *S. asoca* leaf or bark with PB or MGK-264 is more toxic than their single treatment. Highest degree of synergism, i.e., 323 times was observed in the combination of *S. asoca* leaf with PB or MGK-264 at 96 h exposure period. Combination of *T. orientalis* leaf/thujone or fruit powder/column extract of *T. orientalis* fruit with PB or MGK-264 indicate that it enhances the toxicity up to 189.02 times. Toxicity of binary combination was increased hundred folds than their individual components indicating synergistic action.

Srivastava et al. (2010) noted the effect of sublethal treatment (40% and 80% of 24 h LC₅₀) of *Piper nigrum* fruit and *Cinnamomum tamala* leaf/bark and their different organic solvent extract, purified fraction singly and binary combination with synergist PB/MGK-264 (1:5) on level of different biochemical parameters, viz. protein, amino acid, nucleic acids and phospholipids and rate of lipid peroxidation in nervous tissue of *L. acuminata*. Treatment of 80% of 24 h LC₅₀ of piperine (active component of *P. nigrum*) caused maximum reduction in protein (12.95% of control), total free amino acid (10.33% of control), DNA (12.70% of control), and RNA (9.17% of control) levels in nervous tissue of *L. acuminata*. Maximum reduction (18.64% of control) in phospholipid levels and elevation of rate of lipid peroxidation (273.17% of control) were observed in the nervous tissue of snails treated with 80% of 24 h LC₅₀ of piperine. Treatment of 80% of 24 h LC₅₀ of purified fraction of *Cinnamomum tamala* leaf/bark caused significant reduction in protein (41.98% of control), total free amino acid (30.06% of control), DNA (43.71% of control) and RNA (16.42% of control), phospholipids (40.86% of control) level and increase in

the rate of lipid peroxidation (272.69% of control) in nervous tissue of *L. acuminata*. Binary combinations (1:5) plant products with PB or MGK-264 caused significant decrease in the different biochemical parameters. Alternations in the levels of protein, amino acid, nucleic acids, phospholipids and rate of lipid peroxidation in nervous tissue were time and concentration dependent.

Hanif and Singh (2012) noted the molluscicidal activity of *Morus nigra*. According to them, molluscicidal activity of *M. nigra* is due to quercetin, apigenin, and morusin. Hanif and Singh (2013a, b) studied the effect of papain and piperonyl butoxide/MGK-264 on the reproduction and development of *L. acuminata* and they have suggested that these combinations were effective in controlling reproduction of host snail *L. acuminata* even at sublethal exposure. These treatments are not only effective in killing the snails but also possess a capability of making them sterile besides this it also kills the eggs, causes death of the embryo during developmental stages thereby inhibiting its hatching and increases the mortality of the hatched miniature snails. Further, Hanif et al. (2013) noted that bait pellets containing apigenin are strongest attractant (89.1% after 2 h) against *L. acuminata* in comparison to morusin (80.2% after 2 h) and quercetin (72.5% after 2 h) at 5% concentration in bait.

5.7 Bait Formulations in Snail Control

Mostly conventional snail control program is failed because the lack of contact between molluscicides and target snail population due to meshy vegetation, dilution of upwelling sewage water. The evaluation of a molluscicide is based on tests of its toxicity, persistence of effectiveness, attractiveness to gastropods, and also the chances of recovery of affected slugs or snails. Snail control could play an important role in fasciolosis control, if methods of molluscicides delivery could be improved, so as to ensure contact between the molluscicide and the target snail population. One such approach to improve the molluscicide delivery is the development of bait formulation containing both an attractant and a molluscicide, which would be ingested by the snails. It is generally recognized that bait formulation is an important technology relying heavily on plant material, primarily bran as the carrier for the active ingredient and as the principle attractant (Frain 1982). Freshwater snails inhabit an environment containing macrophytes, algae, and bacteria (Thomas et al. 1986). These organisms release copious amount of chemicals, such as carbohydrates and amino acids, into the surrounding water (Kpikpi and Thomas 1992). On the other hand, many investigations proved that snails like other gastropods molluscs use chemical sense as the principal modality for locating food source (Lombardo et al. 1991; Abdel-Hamid and Madsen 1995; Tiwari and Singh 2004a, b). Madsen (1992) showed that freshwater pulmonates are voracious feeder, grazing on epiphytes, algae, decaying macrophytes and fine detritus. Consequently, use of combination of attractants and toxicants has been advocated as an effective tool for pest management. This technique releases less molluscicides in aquatic environment and kills the specific snails. In this way, it is more economical, less hazardous

in the aquatic system. Baits are the best approach for long-term management of slugs and snails (Singh and Singh 2008b).

The bait may either be food, which is then eaten by the target animal (as in vertebrates and molluscs) or an attractant. Baits are highly suitable for controlling terrestrial slugs and snails in agriculture and horticulture (Schnorbach and Wirth 2002; Wirth et al. 2004). The physical and chemical formulation of the pest control composition includes bait with broad spectrum performance against insect pests, many general arthropod pests, and also snails and slugs (Lloyd and Kintz-Early 2006). The use of bait carriers to control fire ants, termites, slugs, snails, mole crickets, household ants and cockroaches is known in the art (David and Hooven 2002).

Bait will generally include an attractant that attracts the target pest and a pesticide (Tiwari and Singh 2007). Attractant comprises of cellulose, a sugar component, a plant starch, instant nonfat dry milk, dried egg yolk, a sterol compound, uric acid, and a plant-based oil (Tiwari and Singh 2004a; Maria et al. 2005). Wirth et al. (2004) reported a new slug and snail baits consisting of at least one magnesium salt, calcium salt and/or iron salt, lignosulfonate, finely divided cereal meal optional binders, and one or more additives. In Malaysia, farmers control the snails simply by using rotten jackfruit as bait (Bidin 2002). Ebenso and Okafor (2002) reported that the chopped green pawpaw (*Carica papaya*) fruits rolled in solution of furadan were used as bait (which was intended to act as both an attractant and feeding stimulant).

The selective molluscicides are also mixed inside the snail attractant pellets, which attract the target snail population. Few works have been done on the bait formulation against harmful snails. It has been reported that snails are attracted towards some chemical compounds such as carbohydrates, amino acids, dead animal matters and decaying plants wastes (Uhazy et al. 1978; Thomas and Assefa 1979; Thomas et al. 1985, 1989; Thomas 1989; Masterson and Fried 1992; Kpikpi and Thomas 1992, 1993; Abdel-Hamid 1996a, b; Tiwari and Singh 2004a, b). Haniotakis et al. (1991) reported the use of a combination of feeding stimulant and toxicant as a good tool for the pest management. It has toxicological and ecological advantage over the use of conventional pesticide program.

Bait formulation is unique in its requirement for approximately balanced concentrations of attractant such as protein, carbohydrate, fat, and toxicant (Maria et al. 2005; Tiwari and Singh 2007; Tiwari et al. 2008). Bait is commonly foodstuff, such as a sugar, protein hydrolyzate, or cereal products, which may be mixed with a toxicant. Bait was usually applied in clumps but not always with success, because insufficient attention was paid to behavior, learning capacity, habituation, and activity rhythm of the slugs and snails. Food preference and their attraction vary from species to species in snails. So, various types of attractant are used inside the snail attractant pellets.

Barnes and Weil (1942) tested carbohydrates such as wheat bran, fruit pulp, boiled potatoes and their attractiveness in mixture with molluscicides. Different types of carbohydrates are used in snail attractant pellets with molluscicides (Abdel-Hamid 1996a, b; Tiwari and Singh 2004b). MacInnis et al. (1974) show that *Biomphalaria glabrata*, the molluscan vector of *S. mansoni*, contributes amino

acids to the aqueous environment, this elicits chemotactic and chemokinetic responses by miracidia of this parasite. For the success of bait formulation it is important to study the feeding behavior of the host snails, to ensure that this strongest attractant should be mixed with particular molluscicides and then bait formulation was applied in the infested area.

Gastropods display a characteristic, coordinated sequence of stereotyped responses to the presence of chemical stimuli from food. Chemoreception is the most important sensory modality informing gastropods to the presence of food in the environment (Kohn 1983). Recent studies have increasingly documented the importance of distance reception of chemical stimuli from food in aquatic and terrestrial, herbivorous, and carnivorous gastropods. Gastropod feeding responses are subjected to modification by experience, including hunger, habituation, sensitization, satiation, quality of food, intensity of the feeding chemostimulus, and associative learning (Kohn 1983). Chemoreception in the gastropods molluscs has been implicated in social, homing, and feeding behaviors (Thomas 1982; Croll 1983). It has been investigated that the snails use chemical senses as the principle modality for locating food sources as like other gastropods molluscs (Kpikpi and Thomas 1993; Abdel-Hamid and Madsen 1995; Tiwari and Singh 2004a, b).

The basic feeding apparatus of a gastropod consists of the terminal mouth, an oral tube, and a buccal cavity containing the projecting buccal mass. The buccal mass and the odontophore to the posterior end of the mouth opened simultaneously. The radula is then stretched tightly over the odontophore and pressed to the substrate through the posterior portion of mouth. Food is scraped into the mouth as the odontophore with the radula is rotated in an anterior direction (retraction) through the open mouth. As the radula reaches the anterior portion of the mouth, the jaw begins to close from the posterior (Kohn 1983; Kpikpi and Thomas 1992). The cerebral and buccal ganglia contain the central pattern generator and modulatory neurons that control the feeding behavior (Benjamin et al. 2000).

Williams et al. (1983) reported that food preferences in certain pulmonates, opisthobranch and prosobranch molluscs significantly altered by experience and memory of their diet in the recent past. The food preference of the giant African snail *Achatina fulica* on leaves of different plants was reported by Rao and Singh (2002). Croll and Chase (1980) fed groups of juvenile *Achatina fulica* exclusively on either carrot or cucumber for varying lengths and then tested their preference in an olfactometer 12–16 h later. The snails are attracted towards the food they had previously eaten, particularly in the case of cucumber (Croll and Chase 1980). *Lymnaea stagnalis* is an excellent model molluscan system to use in elucidating the causal neuronal and molecular mechanisms underlying both the associative acquisition of a new behavior (i.e., learning) and its subsequent consolidation into long-term memory (LTM) (Ito et al. 1999; Benjamin et al. 2000; Lukowiak et al. 2003; Sakakibara 2006).

The freshwater snails generally feed on the aquatic plants and these aquatic plants play an important role in the positive rheotactic behavior of the snails (Pimental and White 1959; Sturrock 1974). Feeding movements in rasper groups which include member of the genera *Helix*, *Helisoma*, *Limax*, and *Lymnaea* tend to be highly

stereotyped, consistent with the relatively homogenous nature of the food they consume. Straub et al. (2004) demonstrate that chemical appetitive conditioning in *Lymnaea* affects the central, but not the peripheral processing of chemosensory information. Ribeiro et al. (2005) show that the mitogen-activated protein kinase (MAPK) can be detected in the *Lymnaea* central nervous system (CNS), and that MAPK activation by phosphorylation is necessary for food-reward classical conditioning. Phosphorylated MAPK was detected in the nuclei of neurons in the feeding circuitry located in the buccal ganglia and in neurons and neuropile located in other parts of the CNS, including the lip nerves. These lip nerves contain the axons of primary chemosensory neurons located in the lips and tentacles (Straub et al. 2004). Conditioned taste aversion (CTA) in the pond snail *Lymnaea stagnalis* has been widely used as a model for gaining an understanding of the molecular and behavioral mechanisms underlying learning and memory (Sugai et al. 2006).

Chemical appetitive conditioning in the pond snail *Lymnaea stagnalis* is an established model system for the study of the cellular mechanisms that underline associative memory formation (Kemenes et al. 2002; Ribeiro et al. 2003; Fulton et al. 2005). Elliott and Susswein (2002) have examined the feeding behavior of gastropod molluscs and the properties of the nervous system. Sugai et al. (2007) have clarified the causal mechanism of long-term memory (LTM) formation. They used a conditioned taste aversion (CTA) procedure on individuals of the pond snail *Lymnaea stagnalis* and analyzed their subsequent behavior. *Lymnaea* exhibit appetitive (Kemenes et al. 2006) or avoidance (Sakakibara et al. 2005) classical conditioning; as well as operant conditioning of either their aerial respiratory behavior (Parvez et al. 2006; Lowe and Spencer 2006).

Cardarelli (1977) reported that the control release technology for dispensing pesticides provided new and exciting possibility for developing baits, attractant, or other chemostimulants, which will enhance the efficacy of these formulations. Aquatic animals utilize waterborne “chemical signals” (chemical stimuli) to identify and orient towards potential food sources, to escape predators and locate mates. These specific chemical signals are recognized in spite of the chemical complexity of aquatic environments. Therefore, the chemical environment of aquatic animals is vitally important, both physiologically and behaviorally, to understand the status and role of animals in the aquatic environment. The function of specific chemical signals becomes even more significant in a managed biological system (i.e., aquaculture ponds or tanks) that is optimized for production of a single aquatic species since these chemical signals regulate feeding behavior and possibly control reproduction. Because feeds are a significant expense in most aquaculture operations, the need to maximize feeding rates and reduce wasted feed, thereby lowering production costs and the possible lowering of bacterial/viral infections is paramount to economic success. The importance of chemoattractants and/or feeding stimulants in improving both initial palatability and overall feeding rates as a means to reduce wasted feed is now fully recognized. The feed quality and environmental conditions (i.e., water quality and current patterns) have direct effects on the effectiveness of feed attractants and feed stimulants. For these reasons, food detection and feeding stimulation ultimately determine the commercial value of an aquatic feed.

Trapping of the snails by specific chemicals released from food sources or the snails themselves has yet not been identified. The utilization of attractants, arrestants, phagostimulants and toxic factors in control release formulations or bait formulation designed to remove trematode host snails from the freshwater environment is cost effective and ecologically acceptable (Thomas et al. 1980). In the case of feeding responses to particular attractants and arrestants may be regarded as essential components of a complex series of events. The bait formulation process was applied for *Biomphalaria glabrata*, the snail host of *Schistosoma mansoni*, by various substances, especially food sources (Cardarelli 1977). The snails crawled in various directions, but never directly to any of the substances. It was only chance that they encountered one of the baits and ate from it. It now appears that so called attractants used in baits do not appeal to all gastropod species, but only to those which feed on starch rich diet such as potatoes, bulbs and tubers, etc. (Godan 1983).

There are so many chemical molluscicides present, which are repellant at high concentration but are attractive at very low concentration; between these two different extremes the responses to them are indifferent (Godan 1958, 1959). It has been observed that molluscicides pellets achieve a satisfactory high mortality rate only during the first three days of application as a result of great attractiveness; thereafter their effectiveness dwindles rapidly established in the case of metaldehyde bran bait (Barnes and Weil 1942). The attractiveness of the bran bait is increased by the addition of protein (Carcass meal) as a carrier base. Casein is also attractive to gastropods, although it is to certain species; according to the Thomas (1948), casein increased its capacity to catch *Milax budapestensis* and *Deroceros reticulatum* but not of *Arion hortensis*. It has been demonstrated that the beer that is used in bait formulation is attractive to *Deroceros reticulatum* and *Vaginulus plebeius* (Selim 1974; Smith and Boswell 1970).

It was believed that the attraction of the snails towards carbohydrates is high because all these sugars are present in the aquatic plants, which the snail feeds. Abdel-Hamid and Madsen (1995) reported that the snail *Biomphalaria alexandrina* and different stage of the snail attracted towards carbohydrates and amino acids. They found that the snails were highly attracted towards starch, maltose, and glycogen. Abdel-Hamid (1996a, b) and Tiwari and Singh (2004a, b) reported the behavioral responses of snail *B. alexandrina* and *L. acuminata* against different amino acids and carbohydrates. The greater attraction of the snail *L. acuminata* to starch and maltose in the bait is possibly due to fact that in natural starch is the major carbohydrate stored in aquatic plants and maltose is released by some epiphytic algae, which form a part of the snail modular system where snails are found (Madsen 1992; Tiwari and Singh 2004a, b). Sugai et al. (2006) first demonstrated that snails have the capacity to recognize sucrose and carrot juice as distinct appetitive stimuli. Sucrose or carrot juice, both of which increase the feeding response, was used as the conditioned stimulus (CS), whereas KCl, which inhibits feeding behavior, was used as the unconditioned stimulus (US) (Sugai et al. 2006).

The snails are able to detect distance of food sources (Lombardo et al. 1991). It is known that the amino acids the building blocks of the protein and essential for existence of life on the earth, like other chemical compounds are released from dead

and living aquatic organism into the modular system of the snails (Thomas 1982; Sterry et al. 1985; Thomas et al. 1989; Kpikpi and Thomas 1992; Abdel-Hamid and Madsen 1995; Tiwari and Singh 2004a, b). Amino acid serine attracts more *L. acuminata* than other amino acid (Tiwari and Singh 2004a). The higher attraction to serine by *L. acuminata* is due to its more rapid diffusion in different zone since its molecular weight is lower than that of other amino acid. However, Abdel-Hamid (1996a) suggests that proline is most preferred amino acid and highly attracts the snails because it is released from the snails into the surrounding water as a signal (MacInnis et al. 1974). It may be possible that differences in behavioral responses between *L. acuminata* and other snails may be due to differences in the feeding behavior and metabolism of other species or it may be due to variation in receptors that detect the attractants. It has been also reported that the snails are highly attracted towards the bait formulations of plant origin and this behavioral response of the snails towards plant product was because of the presence of some compound that may be essential dietary requirements for the snails in their habitat. Snails commonly encounter these compounds when they are released by epiphytic algae that form part of the snail's model habitats (Thomas 1982).

Singh and Singh (2008b) have studied that the snail control is one of the important methods in the campaign to reduce the incidence of fascioliosis and schistosomiasis. In order to achieve this objective, the method of bait formulation containing an attractant and a molluscicide is an appropriate approach to lure the target snail population to the molluscicide. Snail attractant pellets (SAP) were prepared from binary combination of carbohydrates (10 mM) and amino acids (20 mM) in 2% agar solution. These were tested on *L. acuminata*, an intermediate host of the digenean trematodes *Fasciola hepatica* and *F. gigantica*. The behavioral responses of snails to these binary combinations were examined. The fraction of snails that was in contact with the SAP at different times was used as a measure of attraction. Among all the binary combination of carbohydrates; (sucrose + starch)—72.9%, binary combination of amino acids; (proline + serine)—48.0% and binary combination of carbohydrates and amino acids; (sucrose + serine)—69.5%, emerged as the strongest attractant pellets. Toxicity of these SAP containing different concentrations of molluscicides are very effective bait formulation against the snail, *L. acuminata*. Thymol containing SAP emerged as the strongest bait formulation (96 h LC₅₀ 0.540%, 0.318%, and 0.305%) against *L. acuminata*.

Kumar et al. (2010a, b), Kumar and Singh (2009, 2010a, b) have reported that snail control is one of the important methods in the campaign to reduce the incidence of fascioliasis. However, in order to achieve this objective, the method of bait formulation containing an attractant and molluscicide is an appropriate approach to lure the target snail population to the molluscicide. Snail attractant pellets (SAP) were prepared from binary combination (1:1 ratio) of 20 mM amino acids in 2% agar solution. Attraction of snails to different combinations was studied in glass aquaria having diameter of 30 cm. Among all the binary combination of amino acids valine + aspartic acid, with molluscicide ferulic acid, attract 42.36% of snails after 2 h, which was significantly different from their control. SAP containing

umbelliferone emerged as the strongest bait formulation (96 h LC₅₀ 1.09%) against *L. acuminata*.

Kumar et al. (2010a, b, c) have prepared the snail attractant pellets (SAP) from binary combination (1:1) of amino acid (20 mM) in 2% agar solution. Attraction of snails to different binary combinations with molluscicides was studied by using clear glass aquaria. The fraction of snails that were in contact with the SAP at 1 h and 2 h were used as a measure of attraction. The behavioral responses of snails to these binary combinations of amino acid were examined. The fraction of snails that were in contact with the SAP at different times was used as a measure of attraction. Among all the binary combinations of amino acid alanine + valine with molluscicides 0.7% eugenol attract maximum (39.50%) snail after 2 h. Toxicity of these SAP containing different molluscicides was time and concentration dependent. SAP containing alanine+valine and limonene emerged as the strongest bait formulation (96 h LC₅₀ 1.50%) against *L. acuminata*. Kumar et al. (2011a, b) noted that binary combination of starch+histidine with molluscicide limonene caused significant attraction (54.71%) and mortality (96 h LC₅₀, 0.74%) against *L. acuminata*. Further, they observed that molluscicidal components significantly alter different biochemical parameters in the snail body (Kumar et al. 2011a, b, c). Different binary combinations (1:1 ratio) of carbohydrates+amino acids were used as attractant in the bait formulations against the snail *L. acuminata* (Kumar et al. 2011a, b, e). Snail attractant pellets (SAP) containing 10 mM concentration of carbohydrate + amino acids in 2% agar solution were prepared. Attraction of snails to different combinations was studied by using clear glass aquaria having diameter of 30 cm. Each aquarium was divided into four concentric zones; zone-3 (central zone), zone-2 and zone-1 (middle zone) and zone-0 (outer zone) had a diameter of 13, 18, 24, and 30 cm, respectively. The fraction of snails that was in contact with the SAP at different time was used as a measure of attraction. Among all the binary combinations, glucose + histidine (81.33%) and starch + methionine (70.82%) shows highest attraction against snail *L. acuminata* after 2 h.

Kumar et al. (2012a, b, c, 2013a, e) observed that bait containing carbohydrate (10 mM glucose, starch) and amino acid (10 mM methionine, histidine) in 100 ml. of 2% agar solution plus sublethal doses eugenol, ferulic acid, umbelliferon, and limonene cause a significant inhibition in alkaline phosphatase (20% of control) and acetylcholinesterase (49.49% of control) activity in the nervous tissue of *L. acuminata*. Further, they noted that these bait formulations are more effective in controlling the reproduction capability snail *L. acuminata* (Kumar et al. 2014a, b, c).

Agrahari and Singh (2010) have formulated the development of bait formulations containing both an effective attractant and molluscicide, to ensure good levels of contact between the molluscicide and the target snail populations. Attractiveness of *L. acuminata* (an intermediate host of the digenetic trematode *Fasciola gigantica*) of potential components of snail-attractant pellets was investigated. Carbohydrates (glucose, maltose, sucrose, or starch, each at 10 mM) and amino acids (citrulline, tryptophan, proline, or serine, each at 20 mM), were tested in aquaria, with the snails initially placed 22.5, 30, or 45 cm from an agar pellet containing the component

under test. Under these conditions, starch and proline emerged as the strongest attractants for *L. acuminata*, followed by maltose and serine.

Agrahari et al. (2012) demonstrated that variant abiotic factors (temperature, pH, dissolved oxygen, carbon dioxide and electrical conductivity) in surrounding water can significantly alter the attraction and toxicity of eugenol in snail attractant pellets to *L. acuminata*. They suggested that the use of molluscicide eugenol and attractant starch or proline in SAP for the control of *L. acuminata* is more potent and cost effective than releasing eugenol directly into the water. Agrahari and Singh (2013a, b) reported that variable abiotic factors affect the lethality of a bait containing both limonene and attractant starch/proline. Agrahari and Singh (2016) noted that various electrolytes have a significant effect on control release of biomolluscicides loaded in alginate as a cross-linked matrix. The cross-linking technique plays an important role in defining the film surface morphology and consequently its diffusion property (Singh et al. 2007). This cross-linking has pronounced influence on the drug release. Alginate matrix containing ferulic acid with three alginate, CaCl_2 , BaCl_2 , and AlCl_3 shows that CaCl_2 containing formulations exhibit the highest release of drugs. Srivastava et al. (2014) reported that baits formulation of starch or serine in 2% agar-agar solution with papain the active molluscicidal component of *Carica papaya* (40% and 80% of 24 h LC_{50}) caused a significant reduction in fecundity, hatchability, and survival of young snails.

5.8 Phytotherapy of Snails to Control Fasciolosis

Chemical anthelmintics are commonly used to treat and prevent fasciolosis in animal as well as human. Commonly used anthelmintics are halogenated phenols, salicylanilides, benzimidazoles, phenoxyalkanes and praziquantel, nitazoxanide, triclabendazole and compound Alpha (Overend and Bowen 1995; O'Brien 1998; Mitchell et al. 1998; Rossignol et al. 1998; Savioli et al. 1999; Ibarra et al. 2004). Constant use of these drugs may result resistance (Moll et al. 2000).

Plant with anthelmintic activity has been extensively reviewed by Akhtar et al. (2000). Anthelmintic activity of some plants has been reported as *Sorghum* (Iqbal et al. 2001), *Allium sativum*, *Zingiber officinale*, *Cucurbita mexicana* and *Ficus religiosa* (Iqbal et al. 2001), *Artemisia brevifolia* (Iqbal et al. 2004), *Calotropis procera* (Iqbal et al. 2005), *Nicotiana tabacum* (Iqbal et al. 2006a), and *Butea monosperma* (Iqbal et al. 2006b). There is a long list of plants having anthelmintic activity against various helminthic parasites. However, plant-derived products as anthelminthic are less tested specifically against *Fasciola* species.

Kumari and Singh (2011), Kumari et al. (2013a, b, c, d, e, f, 2016, 2017) for the first time developed a safe and effective method to control the population of *Fasciola* by phytotherapy of infected snails (having sporocyst, redia, and cercaria. These snails are one of the important component in the aquatic ecosystem. Release of molluscicide in aquatic system for snail control also affects the other non-target organism. Sublethal treatment of plant-derived molluscicides against infected snails, which contain sporocyst, redia, and cercaria in division phase of *Fasciola*, can kill

the larvae and reduce the rate of infection without killing the snails. In this continuation, Kumari and Singh (2011) have given sublethal treatment of active molluscicidal components citral, ferulic acid, umbelliferone, azadirachtin, and allicin to kill the redia and cercaria larva of *F. gigantica* inside the body of snail *L. acuminata*. They have designated it as new method, i.e., phytotherapy of snails to control fasciolosis without killing the vector snail. The life cycle of parasite can be interrupted by killing the vector snail or *Fasciola* larva (sporocyst, redia, cercaria) inside the body. Kumari et al. (2013c), reported that sublethal treatment of umbelliferone significantly killed the sporocyst, redia, and cercaria larva of *F. gigantica* inside the body of vector snail *L. acuminata*. Kumari et al. (2013a, b) noted that in-vivo killing of *Fasciola* larva is achieved by different binary combination of plant-derived components such as citral, ferulic acid, umbelliferone, azadirachtin, and allicin. Combination of azadirachtin plus allicin was highly toxic against redia and cercaria (8 h LC₅₀, 0.007 and 0.006 mg/l, respectively). Further, Kumari et al. (2013a, b, c), Kumar and Singh (2014) elaborated the *Fasciola* control methods by phytotherapy of host snail *L. acuminata*, by using allicin, ferulic acid and their different binary combinations to kill *Fasciola gigantica* larvae inside the snail body without killing the snail. Kumari et al. (2014) reported that varying abiotic factors can significantly alter the in vivo and in vitro toxicity of citral against sporocyst, redia, and cercaria larva of *F. gigantica*. The lowest toxicity of citral was noted in between November and April. Further, in 2015 Kumari et al. and Kumar et al. (2016b) noted that binary combination of phytocercaricide including citral, ferulic acid, umbelliferone, azadirachtin, and allicin significantly inhibit the acetylcholinesterase (AChE) and cytochrome oxidase activity within 4 h of treatment. They have also observed that there was no adverse effect of these active cercaricides on the vector snails, as AChE activity in nervous tissue of treated snail was not significantly altered. It is suggested that sublethal phytotherapy of snails is an effective tool for the control of larvae of *Fasciola*, and ultimately fasciolosis.

5.9 Photosensitivity of Host/Parasite and Molluscicide in Control of Fasciolosis

The effect of light on animal behavior has attracted the attention of biologists for many years. Gastropods molluscs are model system for the study of the neuronal basis of learning and memory (Sahley and Crow 1998). In basometophorans eyes of gastropods like *L. acuminata*, cornea, lens, microvillar layer, pigmented layer, cell layer and photoreceptors work in well-organized manner and respond to photic stimulation (Crisp 1972; Eakin and Ferlotte 1973; Eakin 1990; Messenger 1991; Meyer-Rochow 2001; Sakakibara et al. 2005). Cercaria larval stage is found inside the infected snail *Lymnaea*. Eye spots are present and well developed in cercaria and consists of one or two cup-shaped pigment cells surrounding the parallel rhabdomeric microvilli of one or more reticular cells. The mitochondria of the reticular cells packed in a mass near the rhabdomere. Rhabdomeres are photoreceptors, the cup shape of pigment cells allows the cercaria to distinguish light direction (Brooker 1972; Schmidt and Roberts 2000). Light could stimulate

cercarial emergence in diverse species. Light is a powerful stimulant, and the exposure of snail to light source is a common method of stimulating cercarial emergence (Asch 1972; Cable 1972).

Tripathi and Singh (2013), Tripathi et al. (2013a, b), Tripathi et al. (2014a, b), Singh and Singh (2015, 2016a, b; Singh et al. 2017a, b; Chaturvedi and Singh 2016; Chaturvedi et al. 2017) develop a method to control *Fasciola* infection. They have involved the different monochromatic light of seven spectral colors along with chemo-attractant and photo-sensitive molluscicide chlorophyllin developed by natural chlorophyll. Involvement of all these factors simultaneously controls the snail population as well as *Fasciola* larva inside the snail body.

Tripathi and Singh (2013) and Tripathi et al. (2014a, b) have used the photosensitivity of snails for trapping them by different photo- and chemostimulants. The photosensitivity of snail *L. acuminata* towards different monochromatic life wavelength is time dependent. Significant variation in attraction of left/right as well as both eye ablated snails towards different monochromatic light clearly indicate that type and number of photoreceptors, i.e., “A” and “T” type receptors are not same in left and right eyes. They established that snails can be killed by any of the established snail control methods by attracting them. Further, Tripathi et al. (2013a) reported that maximum number of snails was attracted (50–60%), when exposed to photo- and chemostimulants simultaneously, rather than when only chemo control (18–20%) or photo control (14–19%) stimulus were given. Maximum change in acetylcholinesterase in nervous tissue was observed when red monochromatic light was used (258.3% of white light control) as opposed to blue (243.44% of white light control) and orange (213.37% of white light control). The exposure of light directly stimulated the photoreceptors light which transmit the signals through nerves to the brain and snails respond accordingly. In this signal transmission AChE is one of the important enzymes involved.

Singh and Singh (2015) developed a new method for the control of fasciolosis by killing the larva with the help of chlorophyllin formulation under irradiation of visible light. Chlorophyllin is prepared by the chlorophyll found in leaves of spinach. They reported that in vivo phytotherapy of snails by chlorophyllin formulations in red light caused highest toxicity against redia (8 h LC₅₀—7.88 mg/10 ml) and cercaria (8 h LC₅₀—11.99 mg/10 ml), whereas lowest toxicity against redia and cercaria was noted in green light (redia 8 h LC₅₀—32.12 mg/10 ml and cercaria 8 h LC₅₀—43.80 mg/10 ml). Phytotherapy of snails by chlorophyllin in darkness and without treatment of chlorophyllin caused no mortality of redia and cercaria larva. Binary combination (1:1) of chlorophyllin and freeze-dried cow urine were more toxic against cercaria (8 h LC₅₀, 9.6 mg/l) than single treatment with chlorophyllin (8 h LC₅₀, 12.6 mg/l) in sunlight (Singh and Singh 2016a). Singh and Singh (2016b) noted that in in vitro condition binary combination of (1:1) of cow urine+chlorophyllin in sunlight is more toxic against redia larva (8 h LC₅₀—0.03 mg/ml) than cercaria (8 h LC₅₀—0.06 mg/ml). Further, Singh and Singh (2016c) and Singh et al. (2017a) also noted that pheophorbide a potent photobiologically active agent can kill the larval stages of *Fasciola* efficiently at 650 nm irradiation of visible light. Kumar et al. (2016a, 2017) used chlorophyllin

bait formulations against infected and uninfected snails at a different spectrum of visible light and they reported that snails have capacity to monitor photo- and chemostimulus. At higher intensity snails attracted by red light caused the alteration of reproductive capacity with bait containing chlorophyllin. Singh and Singh (2016a, b, 2018a, b) and Singh et al. (2016, 2017b) noted that chlorophyllin and pheophorbide are toxic against *L. acuminata* both in sunlight and laboratory conditions. They inhibit acetylcholinesterase activity in the nervous tissue of *L. acuminata*. Chaturvedi et al. (2017) and Chaturvedi and Singh (2017) demonstrated that phytodynamic chlorophyllin is a powerful molluscicide and it is due to the presence of two types of chlorophyllin, i.e., “a” and “b,” which show great potential of photosanitization prospective for the control of endemic fasciolosis. Ninety six hour LC₅₀ of pure and extracted chlorophyllin was 6.54 mg/l and 939.65 mg/l, respectively in visible spectral band of light at fixed intensity (500 w/m²). In 2018, Singh and Singh noted that treatment of chlorophyllin, chlorophyllin + freeze-dried cow urine significantly inhibited the key enzymes acetylcholinesterase and cytochrome oxidase in the cercaria larvae of *F. gigantica*, thus causing the larval death inside the snail body without affecting the snail itself. This new tool, i.e., phytotherapy of snail by chlorophyllin can efficiently manage the fasciolosis without killing the vector snail.

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