Studies on prevalence of gastrointestinal helminths parasites of *Capra hircus* (L) and evaluation of selected ethnoveterinary plants for anthelmintic activity

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Submitted By

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DECLARATION

I hereby declare that the thesis entitled **"Studies on prevalence of gastrointestinal helminths parasites of *Capra hircus* (L) and evaluation of selected ethnoveterinary plants for anthelmintic activity"** submitted to the Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow by me for the award of degree of the Doctor of Philosophy in Applied Animal Sciences is an outcome of my original work and the outcome of my own efforts under the supervision of Prof. Kamal Jaiswal, Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow. It has not been submitted in part or full to this or any other University for the award of any other diploma and degree. This is also declared that the thesis is essentially free from all kinds of plagiarism.

(Jyoti Pandey)

CERTIFICATE

This is to certify that the thesis titled **"Studies on prevalence of gastrointestinal helminths parasites of *Capra hircus* (L) and evaluation of selected ethnoveterinary plants for anthelmintic activity"** submitted by **Jyoti Pandey** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University Lucknow, satisfies all the requirements as stipulated in the Doctor of Philosophy (Ph.D.) regulations -1999 as amended in 2008/2010/2013 and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the university.

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ABBREVIATIONS

S. No	Abbreviations	Full Name
1	NGO's	Non-Government Organization
2	GDP	Gross Domestic Product
3	FAO	Food and Agriculture Organization
4	HCl	Hydro chloric acid
5	DPX	Dextrin Plasticized Xylene
6	SPSS	Statistical Package for the Social Sciences
7	sp.	Species
8	spp.	Multiple species
9	M	Molar
10	Kg	Kilogram
11	g	Gram
11	mg	Milligram
12	µg	Microgram
13	µ	Micron
14	nm	Nano meter
15	cm	Centimeter
16	ml	Millilitre
17	m	Meter
18	mm	Millimetre
19	h	Hour
20	min	Minute
21	PBS	Phosphate Buffer Saline

22	SEM	Scanning Electron Microscopy
23	pH	Potential Hydrogen
24	V/V	Volume by volume
25	OsO_4	Osmium tetra oxide
26	ANOVA	Analysis of Variance
27	LC50	Lethal Concentration resulting in 50% mortality
28	IC50	The half maximal inhibitory concentration
29	SEM	Standard Error of the Mean
30	$FeCl_3$	Ferric Chloride
31	NaOH	Sodium Hydroxide
32	H_2SO_4	Sulphuric acid
33	Na_2CO_3	Sodium carbonate
34	GAE	Gallic acid
35	TAE	Tannic acid
36	$NaNO_2$	Sodium Nitrite
37	$AlCl_3$	Aluminium Chloride
38	BCG	Bromocresol green
39	Fig.	Figure
40	etc.	Et cetra
41	viz.	Namely
42	i.e.	That is
43	e.g.	For example
44	b.wt.	Body weight
45	*et al*.	And others

SYMBOLS

Symbols	Meaning
%	Percentage
°C	Degree Celsius
±	More or Less analogous (Range of Standard error)
χ^2	Chi- square
≤	Less than or equal to
≥	Greater than or equal to
>	Greater than
<	Less than
@	At the rate

Chapter 1

General Introduction and Objectives

1.1. INTRODUCTION

India is predominantly an agricultural-based country major portion of and its economy is based on profitable production of agriculture as well as its allied sectors. Approximately 70 % — 82 % of its rural household population are small and marginal farmers and they still depends on agriculture for their livelihood. In the past few decades, continuous shrinking of agricultural land has been occurred because of the exploitation of land for cultivation, deforestation, lack of water resources or it's purchase by a number of Government and NGOs for the establishment of multiplex complexes, housing societies, and industrial sectors as a part of urbanization and modernization. Hence livestock sector has become prominent in providing livelihood, especially in rural areas (Kumar, 2014).

Livestock is an essential part of the agricultural industry and has played a significant role in the socio-economic sector by providing high-quality foodstuffs such as dairy products, meat, and eggs. India has a rich animal diversity, which is evident of being the World's highest livestock owner with about 512.05 million and also has to its credit of being the sole contributor of the livestock sector with nearly 25.6 % of the total contribution. In India, the overall contribution of the livestock sector in total GDP (Gross Domestic Product) is approximately 4.11 % during 2012-2013 (19^{th} livestock census, 2012). It includes ruminant, poultry, and pig farming system. Among the livestock sector, both large (cattle and Buffalo) and small ruminants play a major role in the Indian economy and it has been approximately, estimated to be 37.28 % are cattle, 21.23 % are Buffalo's population, however, among small ruminants it has been seen that 12.71 % are sheep, 26.40 % are goats and 2.01 % of pigs population (19^{th} livestock census, 2012).

In India, livestock provides a regular additional income and employment not only to the rural people or small-scale farmers but also to the people who are engaged in the livestock business. Livestock also plays a crucial role in the empowerment of the rural poor women by providing occupation and a source of income for their better livelihood. In addition, livestock is also an important source of several value-added by-products such as organic manure, leather, wool, etc. (FAO, 2011; UN Women *et al*., 2012).

Small ruminants are of considerably more important, and they have some characteristic advantages over the large ruminants such as feeding habits, wide range of adaptation to any environmental condition, rearing in small farms or marginal lands, easy management, low investment cost, minimal risk of loss, early sexual maturity, and easy marketing (Devendra, 1979). Sheep and goats are important livestock species and it provides a significant contribution to the sustainable development of the countries, especially for the landless rural people or small scale, marginal farmers. They contribute a large scale of byproducts which ranges from high-quality animal proteins, i.e., milk and meat to fiber and hides, draught power in the highlands, food security, and stable households.

As by the archaeological evidence, among the small ruminants, goats were the first farm animals to be domesticated and have been associated with society in a symbiotic relationship for the very long years (Ensminger and Parker, 1986). Goats distributed all over the World and India has the second position in the population of goats after China (19^{th} livestock census, 2012).

In arid, semi-arid and mountain regions of the World, where the crop farming is not economically valuable, goat livestock rearing can be a fruitful way for the upbringing of the rural livelihood and serves as subsistence for the poor farmers at the time of crisis even during crop failure (Gopalkrishnan and Lal, 1985). Thus, due to their great adaptability to fluctuating environmental circumstances, goats can survive and rear in the most marginal regions of the world and plays an important role in upbringing of the livelihood to millions of resource-poor farmers and landless laborers (Kumar, 2014).

Goats are the cow of poor people producing excellent quality meat for human consumption in very short generation intervals. Moreover, the absence of any religious taboos associated with this animal they can help to fill the gap of protein malnutrition in the consumers (Ozung *et al.*, 2011). Goat livestock is grazed nearby to the soil's surface and to their faeces that mean both the grazing areas as well as living area are joined, which may be an effective source of different types of gastrointestinal helminth parasites including nematodes, cestodes, trematodes, and protozoa. The low prevalence rate of gastrointestinal parasites was observed in that area where the repeated grazing did not occur in the same field.

The stomach of ruminants is divided into four chambers, the rumen, the reticulum, the omasum, and the abomasum. The rumen and reticulum are the abode of varieties of microorganisms which helps in the digestion of cellulose and secrete numerous fatty volatile granules which are the main source of the energy in these animals. The omasum has many folded flaps, which are associated with the absorption of water and nutrients. The abomasum is considered a true stomach and concerned with the digestion of proteins by producing gastric juices and proteolytic enzymes (Mathews, 1998).

The gastrointestinal tract of the animal is one of the most favourable territories for the survival of numerous helminths that cause physical and physiological changes in the digestive tract (Mathews, 1998; Berrilli *et al.,* 2012). During the establishment of the parasite in their microhabitat, a series of vital interactions predominantly physiological and immunological interactions happen in the host-parasite boundary (Simpson, 2000).

Parasites play an important regulating mechanism of population dynamics for the species in their respective ecosystem (Hudson *et al*., 2006; Begon, 2007). Parasites are demarcated as organisms that acquire their nutrients from a living host (Begon, 2007), and they can either be ectoparasite (live on) or endoparasites (within) on the body of the host (Hendrix and Robinson, 2006). Parasitism in goat livestock is an important limiting factor which is responsible for deteriorating the health and productivity of livestock worldwide and has a substantial effect on the profitable production potential (Al-Quaisy *et al.,* 1987; Miller, 2005).

Goats are generally more predisposed to endo-parasites than sheep because goats have a poorer ability to develop an immune response, particularly to helminth parasites as the result of their evolution (McKenna and Watson, 1987; Hoste *et al.,* 2008). Several studies have proved that both the procurement and the manifestation of immune responses against parasitic species are less competent in goats than in sheep (Pomroy *et al.,* 1986; Huntley *et al.*, 1995; and Hoste *et al*., 2008). According to Pomroy *et al.* (1986) when goats are grazing on the same fields as sheep forcefully, the shared helminths may overwhelm the goat livestock even though sheep are less affected.

Parasites are usually host-specific that co-evolved and developed with their hosts (Foreyt, 2001; Begon, 2007). Parasites are classified into two major groups, namely 1) microparasites - parasites of microscopic sizes, such as viruses, bacteria, and protozoa 2) macroparasites - parasites observable to the naked eye, such as trematodes, cestodes, nematodes, and arthropods (Begon, 2007; Sinclair, 2007; Lawrence, 2008).

The intensity of the parasitic load on the host depends on many external and internal factors that can affect the dynamics of the host-parasite relationship (Foreyt, 2001; Hudson *et al.,* 2006). The parasitic infestation is a complex interaction between environmental circumstances and associated determinant factors such as season, age, gender, and internal physiological condition such as metabolic rate, nutrition level, immunity as well as genetic differences of the host (Zuk & Mckean, 1996; Hoberg *et al.,* 2001; Gunn and Irvine, 2003; Body *et al.*, 2011; Milner *et al.,* 2013).

Amongst the parasites, Gastrointestinal (GI) helminth parasites are the serious menace to the profitable goat production in all agro-climatic zones of the World (Bagley, 1997). These gastrointestinal helminth parasites have a significant impact on the goat livestock, causes unalterable damage or even death to the host. Moreover, GI helminths lower the host's immunity and predispose them to other diseases such as a protozoan, bacterial, fungal, viral etc., which lead to great economic losses (Soulsby, 1982; Khan *et al.*, 1989; Garedaghi *et al.*, 2011).

Goat livestock is a reservoir house of different type of helminth parasites for instance *Haemonchus* spp., *Ostertagia* spp., *Nematodirus* spp., *Strongyloides* spp., *Oesophagostomum* spp., *Fasciola* spp., *Trichostrongylus* spp., *Trichuris* spp., *Bunostomum* spp., *Cooperia* spp., *Chabertia* spp., *Moniezia* spp., *Thysaneizia* spp., *Thysanosoma* spp., *Avitellina* spp., *Eimeria* spp., and *Paramphistomum* spp. Helminth parasitic infestation depends on factors such as the infection pressure occur in the environment, i.e., temperature, rainfall, and moisture, which effects on the free-living and intermediate stages of the parasite and also the susceptibility of the host. Additionally, the large number of the susceptible primary and intermediate hosts will increase the parasites ability to reproduce and as a consequence high parasite burden will happen (Torgerson and Claxton, 1999).

The helminths comprise various groups of parasitic worms and are categorized into cestodes, trematodes, and nematodes (Soulsby, 1982).

Cestodes

The main parasite of veterinary importance in this class infecting the ruminants is *Moniezia* spp. Cestodes have an indirect life cycle, i.e., involving one definitive host (human) and one or two intermediate hosts (crustacean and vertebrate) and infections are acquired by feeding on contaminated food or water. Diarrhea is the main clinical manifestation. The gravid proglottids of the worms are passed out through the feces of the definitive host (ruminants) and later on the eggs are eaten by the intermediate host (soil mites). In the alimentary canal of the mites, the eggs hatch out as larvae (oncosphere) and penetrate into the gut wall and enter the haemocoel and develop in to the Cysticercoid stage. Later on, these infected mites are eaten by grazing ruminants and then mature cysticercoid larvae digest out of the mites and finally develop into mature tapeworm (Bashir, 2009). Tapeworms are relatively harmless and asymptomatic, but in heavy infections may cause reduction in weight, diarrhea and intestinal blockages (Elliott, 1986).

Trematodes

Trematodes are commonly known as flukes. They are found in the bile duct, liver or small intestine. The main parasites of veterinary importance include *Fasciola* spp., *Paramphistomum* spp., and *Schistosoma* spp. (Bashir, 2009).

Trematodes also have an indirect life cycle and generally matured eggs of flukes leave the definitive host through the feces. Each egg hatches and produces one motile larva called miracidium. After finding a suitable intermediate host (usually a mollusk, often snails) larvae actively penetrate into it and develops to the next stage, the sporocyst. The sporocyst then produces a redia (larva with a mouth sucker) or more numbers of sporocysts. Finally, rediae produce either more rediae or a motile cercaria larva, which leaves the intermediate host. Still outside a host cercariae can encyst and form metacercariae that are quite resistant to climatic changes. Both cercariae and metacercariae can infest ruminants. The definitive host gets infected by ingesting moist grasses and aquatic plants contaminated with infective metacercariae or cercariae larvae. Further cercariae will migrate to their preferred locations and develops into adult trematode (Junquera, 2017).

Generally, livestock suffers from the mild burden of stomach flukes with no sign of clinical pathogenicity but, at their heavy infections may cause loss of appetite, lethargic, weight loss, diarrhoea, dehydration, and reduced milk production (Lloyd *et al.,* 2007).

Nematodes

The nematodes are the most pathogenic helminths in both tropical and sub-tropical countries. This group contains many parasitic spp. of veterinary importance includes *Haemonchus* spp., *Ostertagia* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Trichuris* spp., *Strongyloides* spp., and *Cooperia* spp. (Agyei, 1997; Zajac, 2012; Degefu *et al.,* 2011; Raza *et al.,* 2014; Molla and Bandyopadhyay, 2016; Rashid *et al.,* 2016).

Gastrointestinal nematodes are major contributors to reduce the productivity in ruminants worldwide. Usually, the host gets infected while grazing and causes a clinical condition known as Parasitic Gastroenteritis (PGE) (Waller and Thamsborg, 2004). Depending on the parasitic burden, common symptoms of PGE include anorexia, diarrhoea, reduced production, anaemia, decreased weight gain and oedema (Kassai, 1999), low milk yield (Diehl *et al.,* 2004) and decreased fertility potential (Ankers *et al.,* 1998; Osaer *et al.,* 1999 and Sultan *et al.,* 2010).

Most gastrointestinal nematodes have a direct life cycle, i.e., no involvement of intermediate hosts. The life cycle has two distinct phases; 1) sexual phase, occur within the definitive host, and 2) asexual phase (free-living stage), where the parasite is developing in the environment.

Adult fertilized female worms lay unembryonated eggs which are passed from the host feaces. Within a few days, the eggs get embryonated and hatch out from the eggs into a first stage free-living larva (L_1). The hatched larva (L_1) undergoes into 2 molts and develops into a second stage larva (L_2), and a third stage larva (L_3). The L_3 larva is an infective stage and needs to survive on the pasture until ingested by the host. Once the host becomes infected while grazing on contaminated pasture the L_3 larval stage migrate through the gastrointestinal tract and penetrate into the intestinal wall and undergo into two more molts L_4 and L_5 and finally, develop into adult worms (Levine, 1968).

Among all the gastrointestinal worms, *Haemonchus* spp. is the most prevalent and pathogenic species in the tropical and subtropical countries (Soetan *et al.,* 2011; Khalafalla *et al.,* 2011). The worm belongs to the family Trichostrongylidae. It is a small, slender and blood-sucking abomasal parasite, and commonly known as 'barber pole worm' (Soulsby, 1982). This parasite causes several diseases in small ruminants like reduction in body growth, loss of appetite, weight loss (Jittapalapong *et al.,* 2011) and anaemia (Qamar *et al.,* 2009), which leads to significant economic loss to farmers (Mwale *et al.,* 2012).

Blood-sucking GI nematodes are also a causative factor to alter the haematological parameters in small ruminants such as a notable decline in Total ErythrocyteCcount (TEC), Haemoglobin (Hb) level, Packed Cell Volume (PCV), Lymphocyte Count (LC) and Mean Corpuscular Volume (MCV) and a remarkable increase in total neutrophil and eosinophil counts, Erythrocyte Sedimentation Rate (ESR), Mean Corpuscular Haemoglobin (MCH), was observed experimentally infected with *Haemonchous contortus* (Rasool *et al.,* 1995).

Due to GI parasitic infection, severe histopathological changes were observed such as mucosal and submucosal haemorrhages, epithelial cells degeneration, hypertrophy, alteration in gastric glands with an increased level of eosinophils, mucous gland hyperplasia, connective tissue proliferation and necrosis (Borji *et al.*, 2010; Tehrani *et al.*, 2012). Destruction in gastrointestinal mucosa caused by GI parasite also results in leakage of plasma proteins with consequent hypoproteinemia (Yadav *et al.*, 1993).

Undeniably, the arrival of broad-spectrum anthelmintics has played a vital role in reducing the effects of parasitism in livestock and increased their productivity. Helminthiasis is treated with a variety of synthetic anthelmintic drugs (Macrocyclic lactones, Benzimidazoles, Imidazothiazoles, and Praziquantel) which have been considered as an effective way for the treatment of helminth infestation.

The last few years, anthelmintic resistance (AR) has become a serious threat to the health and well-being of livestock (Waller, 1987). The emergence of anthelmintic resistance is due to the recurrent use of the anthelmintic medicines and the susceptible population shows loss of sensitivity towards the drug resulting in non-functioning of the drug, even at the higher dose of the drug with no effect on the worms (Prichard *et al.*, 1980; Coles, 2006).

Drudge *et al.* (1957) published the first report of anthelmintic resistance against *H. contortus* which has become resistant to phenothiazine in sheep. This report provided a new path of study for Veterinary scientists all over the world, and many reports from across the world were presented on resistance to various groups of synthetic anthelmintic drugs against gastrointestinal helminths in small ruminants (Hotson *et al.*, 1970; Pichard, 1980; Pomary *et al.*, 1992; McKenna, 1997). From India, Varshney and Singh (1976) were the first to report anthelmintic resistance against the *H. contortus* to thiabendazole and phenothiazine in sheep from Uttarakhand.

During last two decades, the farmers have paid attention towards the resurgence of alternative health care practices all over the World. These practices comprise pasture management, selective breeding, grazing management, nutritional management as well as biological control and herbal treatment (Thamsborg *et al.*, 2010). Vaccination may be an alternative way to control the parasitic infestations, but the antigenic complexity and antigenic variation at various developmental stages of the parasites have reduced the process of vaccine development (Raza, 2013).

The use of anthelmintic drugs for disease control management is always a big challenge due to the unapproachability of drugs, especially in rural areas, lack of money to purchase, misuse of the drug due to scarcity of knowledge, accumulation of toxic residue in the body of animal, environmental pollution and most importantly in drug resistance (Jabbar *et al.*, 2006; Saeed *et al.,* 2007). Lately, many researchers have reported the synthetic anthelmintic drugs are toxic to human health because the residue of these drugs is present in foodstuffs derived from the livestock (Nunomura *et al.*, 2006 and Dewanjee *et al.*, 2007).

Consequently, the control of helminthiasis in the livestock is an immense challenge for veterinarians/producers because of the huge prevalence of AR (Anthelmintic Resistance) which is now a worldwide phenomenon. Therefore, there is an urgent requirement for use of alternate methods to diminish the worm burden, which should be nontoxic, economic, having no side effects, easily accessible, and biodegradable. Due to this reason, researchers have developed a keen interest towards the herbal medicinal plants as an alternative source of synthetic anthelmintic drugs for the treatment of parasitism.

Traditional medicines have been used to treat the diseases of human and livestock since the ancient time. According to the World Health Organization (WHO), about 75% to 80% of the populations of developing countries depend solely on traditional medicines for their primary health care treatments. Medicinal plants are the spine of traditional medicine, and most of the population of underdeveloped countries uses medicinal plants as a regular prophylactic measure (Dobriyal and Narayana, 1998). The plant kingdom is known as an ample of a reservoir for herbal medicines, and several numbers of medicinal plants have been used to combat parasitic infections all over the World.

Herbal plants are the best option to accomplish these prospects (Raje *et al.,* 2003). These plants contain numerous types of the biologically active compound having an important medicinal and nutritional value (Swargiary *et al.,* 2017). Medicinal plants exhibit antiparasitic properties because of their different phytochemical compounds such as alkaloids, phenolic groups, glycosides, saponins and volatile oils in the roots, leaves, bark and seeds of the plants (Anthnasiadou *et al.,* 2000; Wang *et al.,* 2010).

The use of medicinal plants for the prevention and treatment of gastrointestinal parasitism has its origin in ethnoveterinary medicine (Anthnasidou *et al.*, 2007). There are many plants which have been reported in the literature for their anthelmintic property such as *Nerium olender* (Khan *et al.,* 2011), *Eucalyptus staigeriana* (Macedo *et al.,* 2010), *Punica granatum* (Prakash *et al.,* 1980; Pradhan *et al.,* 1992), *Allium sativum* (Ayaz *et al.*, 2008; Orengo *et al.,* 2016), *Acacia catechu* (Patil *et al.,* 2013), *Calotropis procera* (Al-Qarawi *et al.,* 2001;

Iqbal *et al.,* 2005), antifungal property such as *Salvia sclarea*, *Salvia officinalis* and *Rosmarinus officinalis* (Dellavalle *et al.*, 2011), *Calendula officinalis* (Gazim *et al.,* 2008), antibacterial property in plants like *Calendula officinalis* (Iauk *et al.,* 2003), *Bergenia ciliata*, *Jasminum officinale*, and *Santalum album* (Khan *et al.*, 2013) and antimicrobial properties in plants such as *Calendula officinalis* (Kubas, 1972; Tarle and Dvorzak, 1989), *Punica granatum*, *Syzygium* aromaticum, *Zingiber officinale*s and *Thymus vulgaris* (Mostafa *et al.,* 2018), *Aloe vera* (Selvamohan *et al.,* 2012).

Hence, a successful control of parasitic diseases is dependent on the proper use and monitoring of anthelmintic drugs, regular extension program on the epidemiology of helminth parasites, and transforming the proper knowledge about variables such as host resistance, climate, and management data, exploitation and scientific validation of traditional plants having anthelmintic potential are the basic need for effective control strategy against helminthiasis.

In view of the above discussed considerations, this study was designed to achieve the following objectives:

1.2 OBJECTIVES

1. To investigate the prevalence of gastrointestinal helminths parasite in relation to age, sex, month and season.
2. To identify the different types of gastrointestinal helminths parasite.
3. To evaluate the anthelmintic activity of selected ethnoveterinary plants against gastrointestinal helminths parasites *in vitro*.
4. To study the changes in the parasite before and after treatment with the most effective plant(s) extract using light microscopy.
5. To screen the selected (showing high anthelmintic activity) ethnoveterinary plants for phytochemical analysis.

Chapter 2

Review of Literature

Helminths are invertebrate and are a group of parasitic worm. Term helminth is derived from the Greek words ‘‘helmins” meaning a worm. They usually belong to the phylum Platyhelminths (such as flukes and tapeworms) and Nemathelminths (roundworms). Further division is named in reference to the host organ host organ (e.g., Lung flukes, liver flukes, blood flukes, and intestinal helminths).

Cestodes (tapeworms) are ribbon like flatworms. They are hermaphroditic in nature and lives inside the human gastrointestinal tract. Body is divided into 3 parts: 1. Scolex or head, 2. Neck, 3. Strobila (segmented body). Some cestodes are mainly human parasite and others infected animals as well as humans. Trematodes (flukes) are flattened, oval, leaf-shaped or worm-like with length varying from a few millimeters to several centimeters. Trematodes except the blood flukes are hermaphroditic that means having both male and female reproductive organs. They are the endoparasite of phylum Mollusca (intermediate host) and subphylum Vertebrata (definitive host). Nematodes (roundworms) are bilaterally symmetrical and elongated in structure. Usually sexes are separate in most species (bisexual). Their size range from microscopic to 7 meters long and occur as parasites in both animals and plants. Nematode occurs in almost all organs of the body, but the most common sites are alimentary canal, blood vascular system, and respiratory systems. They have successfully adapted to survive nearly in all ecosystems. Most nematodes that are parasitic in humans have two different modes for infection: Direct and skin penetration. Direct infection occurs when the eggs are transmitted directly from anus to mouth (e.g. *Enterobius vermicularis* (pinworm/threadworm) and *Trichuris trichuria* (whipworm) etc. Skin penetration generally is the characteristic of hookworms (*Necator americanus* and *Ancylostoma duodenale*) (Chatterjee, 1969).

A complex condition refers as ‘Helminthiasis’caused by these parasites is a very chronic problem of small ruminants in India and overseas. The prevalence of gastrointestinal helminths in the livestock greatly differs from zone to zone due to fluctuating environmental conditions. Thus, so many works on the incidence of gastrointestinal helminths in sheep and goats have been conducted in different agro-climatic zones of the world (Fakae, 1990; Cheah and Rajamanickam, 1997; Lateef *et al*., 2005; Haile *et al*., 2010) and in India (Katoch *et al.*, 2000; Deshpande *et al.*, 2001; Garg *et al.*, 2003; Khajuria and Kapoor, 2003). Some prevalence and identification studies are being reviewed as under:

2.1 Prevalence and identification of gastrointestinal helminth parasite with associated risk factors (age, sex, month, and season) in Goat.

Manuel and Madriaga (1967) recorded the most common gastrointestinal nematodes in goats in Philippines were *Haemonchus contortus, Oesophagostomum columbianum, Trichostrongylus* spp. and *Trichuris* spp.

Fabiyi (1970) inspected the frequency of nematodes in goats in Nigeria and detected mixed infection of *Haemonchus* spp., *Oesophagostomum columbianum*, *T. colubriformis,* and *Gaigeria pachyscelis*.

Le Reiche *et al.* (1973) accompanied a survey on gastrointestinal helminths of sheep and goats in Cyprus and noticed ninteen species of helminths. They also reported the most recurrent species were *Ostertagia circumcincta* (77 %), *O. trifurcata* (77 %) followed by *Trichostrongulus* spp. (62 %), *T*. axei (53 %), *T. ovis* (55 %), *Chabertia ovina* (38 %), *H. contortus* (23 %), *Bunostomum trigonocephalum* (6 %), *Parabronema skrjabini* (1.6 %) and *Skrajabinema ovis* (9 %).

Sinha and Sahai (1973) conducted a study on incidence gastrointestinal nematode in goats in Bihar and found the highest prevalence for *H. contortus* (74.2%) followed by *H. bispinosus* (67.2 %), *O. columbianum* (50%), *T. ovis* (48 %), and *T. globulosa* (27%).

Cotteleer and Fameree (1978) surveyed 116 goats in Belgium and found that 99 (85.34 %) of 116 goats were infected for *Trichostrongylus* sp. (54 %), *Trichuris* sp. (18 %), and *Nematodirus* sp. (4.3 %).

Assoku (1981) conducted a study on helminths of sheep and goats in Ghana, and reported seven species of nematodes, among which *O. circumcincta*, *T. axei*, and *H. contortus* were the most common species whereas *Cooperia curticei*, *G. pachycelis*, *Marshallagia mashalli*, and *N. filicollis* were the less common nematodes.

Specht (1982) conducted a prevalence study of gastrointestinal helminths with respect to the season in 20 sheep and goats in Southern Mozambique and reported that *H. contortus*, *O. columbianum*, *T. colubriformis*, *Cooperia* sp., *S. papillosus*, and *Trichuris* spp. were the most prevalent nematodes. The mean total egg counts were increasing in October due to the commencement of heavy rains with a higher number of *H. contortus* egg, which reached the peak in January followed by may in both animals.

Islam (1984) conducted a prevalence study on helminth parasites of goats in Zambia and noted highest prevalence of *O. columbianum* (24.73 %) followed by *H. contortus* (23.46 %), *T. ovis* (12.13 %), *T. axei* (7.6 %), *G. pachyscelis* (6.53 %), *C. ovina* (5.75 %), *S. papillosus* (4.26 %), *N. spathiger* (2.53 %), and *C. punctata* (1.06 %).

Jurasek (1986) noted down highest prevalence rate for *Haemonchus* sp. (42.8 %) followed by *Oesophagostomum* sp. (32.6 %) and *Trichostrongylus* sp. (16.3 %), and *Cooperia* sp. (16.3 %) in goats in Mozambique.

Asanji and Williams (1987) carried out a study on the prevalence of gastrointestinal helminth parasites of sheep and goats in Sierra Leone and reported that *H. contortus* was the most frequent nematode species followed by *Oesophagostomum columbianum* and *Oesophagostomum venulosum*. They noticed higher relative worm density in October and lower July. They also found that female and young animals were more prone to worm load than old and male animals.

Okafor (1987) conducted a prevalence study on gastrointestinal nematode in goats in Imo state of Nigeria and reported overall infection rates were 78 %. He also recognized 7 types of nematode species viz., *O. columbianum* (37.61 %), *T. globulosa* (30.74 %), *H. contortus* (35.5 %), *T. ovis* (8.95 %), *G. pachyscelis* (8.66 %), *S. papillosus* (6.56 %), and *T. colubriformis* (6.26 %).

Yadav and Tandon (1989) carried out a prevalence study on gastrointestinal nematodes in goats in India. They found that 86.8 % of goats infected with one or more species of nematodes with the highest prevalence of infection *H. contortus* (52.7 %).

Saha *et al.* (1990) noted down 75.83 % infection of gastrointestinal nematode parasite in goats of West Bengal by showing the highest incidence rate in winter (79.41 %) followed by monsoon (76.40 %) and summer (72.28 %).

Joshi (1994) carried out a prevalence study on gastrointestinal nematodes of goats in Nepal and recorded *H. contortus* (49.4 %) was most predominating species followed by *Trichostrongylus* spp. (43.5 %), *O. venulosum* (4.2 %), *O. circumcincta* (2.5 %), and *B. trigonocephalum* (0.4 %).

Pandey *et al.* (1994) investigated a season-wise prevalence study of gastrointestinal nematodes in goats in Zimbabwe. They reported that *T. axei*, *T. colubriformis*, *H. contortus*, and *O. columbianum* were the most dominating species in the studied area in 88.97 % of the animals followed by *Trichuris* spp. (21 %), *S. papillosus* (9 %), and *Bunostomum* spp. (3 %).

They also noticed the minimum prevalence occurred in the dry season, which, steadily approached highest in the rainy season. They also saw a direct association between the intensity of infection of gastrointestinal nematodes with rainfall.

Beriajaya and Copeman (1997) done an epidemiological investigation on gastrointestinal nematodes of small ruminants (sheep and goats) in West Java. They found that *Haemonchus* and *Trichostrongylus* species were most predominant. The overall infection was found 94% infected with *T. colubriformis*, *T. axei*, *H. contortus*, *O. columbianum*, and *S. papillosus*. Egg counts were significantly decreased during the late dry-early wet season. They also observed that sheep and goats were infected with *H. contortus*, *T. axei*, and *C. curticei* throughout the year, but the occurrence of infective larvae of *T. colubriformis* was maximum throughout the dry season than the wet season and conversely for *O. columbianum* in sheep than goats.

Katoch *et al.* (2000) carried out a study on seasonal dynamics of gastrointestinal nematode infection in Barbari goats in the Mathura region and recorded overall incidence was 21.66 %. They found the highest incidence of *Trichuris* in winter (40 %) followed by the rainy season (17.5 %). Abomasal worm counts disclosed the maximum incidence of *Haemonchus* in the rainy season (80 %) and the minimum in the summer (26.9 %).

Abebe and Esayas (2001) investigated caprine gastrointestinal helminthiasis in the eastern part of Ethiopia throughout the dry season of the year. They found the overall prevalence was 100 %. They also reported the prevalence of different nematode's species viz, *Haemonchus* spp. (96.5 %), *Trichostrongylus axei* (64.3 %), *T.columbiformis* (90.0 %), *Bunostomum* spp. (35.2 %), *Strongyloides* spp. (43.6 %), *Oesophagostomum* spp. (70.8 %), *Trichuris* spp. (48.2 %) and *Skriabinema* spp. (33.4 %) in goats. The cestodes found were *Cysticercus tenuicolis*, *Avitellina* spp., *Moniezia* spp., and *Stilesia* spp.

Tamaloorkar *et al.* (2002) conducted a prevalence on flukes in ruminants namely *Fasciola* spp., *Amphistomes* spp., and *Schistosoma* spp. in the Marathwada region in India. They reported the highest infection in the rainy season, followed by the winter and summer season. The also recorded higher infections in males than females in all host species.

Shahadat *et al.* (2003) reported that the prevalence of *Haemonchus contortus* in goats in Mymensingh was 63.7 %. They also revealed the prevalence was found highest in the rainy season (75.5 %) followed by winter (58.7 %) and summer (57.7 %) season. The prevalence was highest in young animals (68.6 %) than the younger (61 %) and older, (67.6 %) animal.

Pal and Bandyopadhyay (2004) surveyed government goat farms of Sikkim and noted the overall infection rate of gastrointestinal nematodes as 77.13 %. They also observed that the highest (92.01 %) prevalence occurred in November and lowest (53.24 %) in March. The maximum infection rate of *Strongyle* sp., *Strongyloides* sp., and *Trichuris* sp. (65.14 %, 38.46 %, and 14.16 %) is observed in November, May, and July, respectively, however, it was minimum in January (29.16 %), October (0), and December (0). Seasonal incidence of these parasites was found to be highest (89.01 %) in autumn and lowest in winter (59.11 %). The species-wise prevalence of gastrointestinal nematodes recorded highest for *H. contortus* (70 %) followed by *B. trigonocephalum* (51.66 %), *O. columbianum* (43.01%), *T. colubriformis* (28.33 %), *Nematodirus* spp. (21.66 %), *T. globulosa* (10 %), and *T. ovis* (6.66 %).

Dhand *et al.* (2004) reported that 70 goats and 50 sheep of different age groups were suffering from fascioliopsis due to the high prevalence rate of *F. gigantica* in Punjab. They stated that these ruminants were suffering from high fever with diarrhoea and among these 40 sheep and 5 goats died before the investigation.

Chaudhri (2004) reported that the most dominated species of gastrointestinal (GI) helminth parasites infecting small ruminants in India include the GI nematodes (*H. contortus*, *Trichostrongylus* spp., *Oesophagostomum* spp., *Strongyloides* spp. and *Trichuris* spp.), tapeworm (*Moniezia* spp., *Avitellina* spp. and *Thysaniezia* spp.), blood flukes (*Schistosoma spindale* and *S. indicum*), liver fluke (*F. gigantica* and *F. hepatica*), *Paramphistomes* spp. (rumen fluke),

Muraleedharan (2005) conducted a study on the prevalence of gastrointestinal parasites of small ruminants in the central dry zone of Karnataka and reported a higher incidence rate in goats (46.12 %) than sheep (39.34 %). Seasonal observation disclosed that highest (55.13 %) incidence rate in June-September followed by November-December (43.01 %), March-May (41.31 %) and lowest in January-February (36.36 %). He also observed that *H. contortus* was the most prevalent species in small ruminants.

Nasreen *et al*. (2005) reported overall prevalence of gastrointestinal nematode in sheep in Kashmir valley. They noted the highest prevalence in the summer season (67.14 %) and lowest in the winter season (44.31 %). They also revealed species-wise prevalence rate of nematodes such as *Strongyle* (60.78%), *Trichostrongylus* sp. (35.56 %), *Haemonchus* sp. (20.73 %), *Nematodirus* sp. (3.66 %) and *Marshallagia* sp. (1.37 %).

Umur *et al.* (2005) investigated the 100 % overall prevalence of gastrointestinal nematodes in goats in Burdur region, Turkey. They observed that the most dominated nematode species in the goats were *Ostertagia circumcincta* (78 %), *Marshallagia marshalli* (72 %), *Nematodirus abnormalis* (66 %), *Trichuris ovis* (60 %), *N. spathiger* (52 %), *T. skrjabini* (50 %), and *Trichostrongylus vitrinus* (40 %). Seasonal findings revealed that the parasitic burden in the goats was highest in autumn followed by spring, summer and least in the winter. The prevalence of *Trichostrongylus* spp. was highest in spring and autumn while month-wise percentage prevalence of *Ostertagia* spp. was highest in September and for *M. marshalli* in October. The prevalence of *Nematodirus* spp. was found to be increased in January and April while highest in September. They also stated that the occurrence of nematodes such as *Ostertagia* sp., *Trichostrongylus* sp., *Nematodirus* sp., and *M. marshalli* found in all seasons of the year.

Mungube *et al.* (2006) estimated the prevalence caused by *Faciola hepatica* and *Faciola gigantica* in the ruminants of Taveta division of Kenya. They also reported that liver infestation rates differed significantly between ovines, caprines, and bovines ($p \leq 0.05$) for *F. hepatica* (28 %, 22 %, and 0.4 %, respectively) and for *F. gigantica* (5.2 %, 6.6 %, and 26 %, respectively).

Uddin *et al.* (2006) investigated the prevalence of amphistome parasites in Black Bengal goats of Mymensingh district, Bangladesh. They noted the overall prevalence rate was 72.92 %. They have also identified the three species of amphistomes namely *Paramphistomum cervi*, *Cotylophoron cotylophorum*, and *Gastrothylax crumenifer*. The high infection rate was observed for *Paramphistomum cervi* (65.28 %), and low for *Cotylophoron cotylophorum* (36.11 %). Prevalence of mixed infections was observed at 60.42 %. They observed that age has a significant ($p<0.01$) effect on the prevalence of amphistomes and reported a high prevalence rate (89.58 %) in adult animals followed by young animals (78.57 %), and growing animals (45.0 %). They also found a high infection rate in females (75.0 %) than in the males (67.5 %). Season-wise prevalence was found to be reported highest in monsoon (83.64%), then in summer (69.23 %) and lowest in winter (64.0 %).

Mohanta *et al.* (2007) conducted a study to investigate the prevalence and population dynamics of intestinal helminths in Black Bengal goats in Mymensingh district, Bangladesh and reported infection with different species of helminths in 94.67 % of goats. They also identified a total of 5 species of helminth parasites namely *Oesophagostomum columbianum*

(92 %), *Trichuris ovis* (56.66 %), *Schistosoma indicum* (38 %), *Moniezia expansa* (10.66 %), and *Moniezia benedeni* (2.66%). The overall mean burden of the parasite were found to be 34.02±2.20. *Oesophagostomum columbianum* showed the highest (29.91±2.00) mean parasitic burden followed by *Trichuris ovis* (5.70±0.47), *Schistosoma indicum* (4.66±0.42), *Moniezia expansa* (2.59±0.54), and *Moniezia benedeni* (1.00±00). Prevalence of gastrointestinal helminths is found to be significantly ($P<0.05$) higher in winter (100 %) than in summer (89.33 %). The mean parasitic burden was observed to be higher in winter (41.53±3.15) than in summer (25.52±2.57).

Nwosu *et al.* (2007) surveyed the livestock of sheep and goats in the semi-arid zone of North-Eastern Nigeria to determine the prevalence and seasonal abundance of the egg and adult stages of nematode parasites. They revealed that 43.1 % of sheep fecal samples and 55.8 % of goats fecal samples were infected with at least one nematode egg type. They have found *strongyle* egg type (35.4 % in goats and 22.5 % in sheep) being the most prevalent egg type followed by *Trichuris* (4.1 % in goats and 5.9 % in sheep) and *Strongyloides* (4.1 % in goats and 4.9% in sheep) egg types. The overall prevalence of adult nematode parasites was found highest in sheep (60 %) followed by goats (52 %). They identified six species of nematode parasites in both sheep and goat namely *Trichostrongylus*, *Strongyloides*, *Haemonchus*, *Oesophagostomum*, *Trichuris, Cooperia* except *Bunostomum* species which only was noticed in sheep. The percentage prevalence of *Haemonchus* spp. and *Trichostrongylus* spp. was found at the peak during August (sheep), and June (goats) whereas *Strongyloides* species was observed during the whole year in both animals regardless of the season.

Pathak and Pal (2008) conducted a systemic study on the prevalence of gastrointestinal helminths parasite in goats and revealed that the overall prevalence of infection was 85.22 %. They recorded the prevalence of most dominating helminths parasite was *Paramphistomum* spp. (80.68 %) followed by *Cotylophoron* spp. (45.45 %), *Oesophagostomum* sp. (30.68 %), *Trichuris* sp. (27.27 %), *Haemonchus* sp. (26.13 %), *Moniezia* spp. (17.04 %), *Trichostrongylus* spp. (5.68 %), *Bunostomum* sp. (5.68 %), *Avitellina* spp. (3.40 %), and *Cooperia* spp. (3.40 %). Seasonal prevalence was found highest in monsoon (94.60 %) followed by summer (87.50 %) and winter (63.15 %).

Asif *et al.* (2008) investigated the prevalence study on the endoparasite of goat and sheep in and around Rawalpindi and Islamabad and reported the overall prevalence of 65.7 %. They revealed that sheep were more susceptible for the infection and showed higher (72 %) prevalence rate than in goats (63.7 %). They also encountered different species of endoparasites in sheep encompassed *Haemonchus* (80.64 %), *Coccidia* (51.61 %), *Trichuris* (32.25%), *Nematodirus* (29.03 %) and *Fasciola* (4.38 %) while only *Haemonchus* (75 %), *Trichuris* (62.5 %), and *Coccidia* (57.5 %) were found from the fecal samples of goats.

Gadahi *et al.* (2009) conducted a survey on the prevalence of gastrointestinal helminths parasite of goat and sheep in Rawalpindi and Islamabad, Pakistan and recorded the overall prevalence of 63.50%. Among both animals, 53.33 % of sheep and 66.45 % of goats detected positive for gastrointestinal parasites. They identified *Trichuris* sp. (40.00 %), *Haemonchus* sp. (28.88 %), *Coccidia* sp. (27.77 %), *Nematodirus* sp. (11.11 %) and *Fasciola* sp. (4.44 %) found in sheep. In case of goats, the prevalence of *Haemonchus* sp., *Coccidia* sp., *Trichuris* sp., *Nematodirus* sp., *Trichostrongylus* sp., *Strongyloides* sp., and *Faciola* sp. was found 64.19 %, 43.87 %, 35.48 %, 13.00 %, 4.51 %, 3.22%, and 0.66 %, respectively.

Gwaze *et al.* (2009) carried out a survey to determine the parasitic burden of gastrointestinal parasites in goats of Qaukeni (QA) and Nkosana (NK) communities of Eastern Cape Province of South Africa. *Strongyle* egg type was the most frequently detected gastrointestinal eggs in both QA (68.4±8.49) and NK (96.1±12.01), followed by *Coccidial* oocysts (53.3±8.76, 68.8±8.00 in QA and NK respectively). The other identified nematodes were *Trichostrongylus* and *Strongyloides* egg types. Season-wise prevalence of strongyle eggs recorded significantly ($P < 0.05$) higher in the hot-wet and post-rainy seasons, although the other egg types showed highest in the hot-wet season. They also reported the burden of Strongyle egg counts significantly ($P < 0.05$) higher in NK compared to QA.

Bano and Sultana (2009) recorded the incidence of helminth parasites in goats and sheep in Bilhaur area of Kanpur, U.P. and the overall prevalence reported was 33.21% in goats and 34.285% in sheep. *Moniezia* sp. was found to be the most frequently occurring endoparasite in both goats (9.28 %) and sheep (8.57 %). The other major type of parasitic infections occurred in goats were found to be by *Avitellina* sp., *Fasciola* sp., *Trichuris* sp., *Haemonchus* sp., *Dicrocoelium* sp., *Strongyloides* sp., *Stilesia* sp., and *Oesophagostomum* sp. with prevalence rate 5.37 %, 4.28 %, 3.57 %, 3.21 %, 2.14 %, 2.85 %, 1.42 %, and 1.07 %, respectively. In case of sheep, the percentage prevalence of helminth parasites was recorded as 5.35 % (*Fasciola* sp.), 2.50 % (*Dicrocoelium* sp.), 5.35 % (*Avitellina* sp.), 1.78

% (*Stilesia* sp.), 2.85 % (*Strongyloides* sp.), 3.21 % (*Trichuris* sp.), 1.78 % (*Oesophagostomum* sp.), and 2.85 % (*Haemonchus* sp.). The rate of infection was slightly higher in sheep (34.28 %) than in goats (33.21 %). The parasitic burden reported high in the rainy season, moderate in spring and summer seasons while low in the winter season in both animals.

Bagde *et al.* (2010) carried out prevalence studies on the helminth parasites in goats of Nagpur region, Maharashtra. A total of 1500 goats were examined in 10 villages from different localities viz., Ramtek and Katol Tahsils of Nagpur district of Maharashtra and their faecal samples were collected. 60.26 % of faecal samples were found positive for different helminths infection whereas 22.56 % of cases were found positive for mixed helminths (*Strongyloid* sp., *Trichuris* sp., and *Moniezia* sp.) infection. Nematode infection was commonly encountered throughout and after the rainy season.

Tariq *et al.* (2010) investigated the seasonal epidemiological prevalence of gastrointestinal nematodes (GINs) of goats with associated factors (gender and age) of the host in the Kashmir valley. A total of 1267 goats examined, in which 938 goats went for faecal examination (1year: 470; 2year: 468), and 329 went for gastrointestinal (GIT) examination (1year: 175; 2year: 154). The overall prevalence of gastrointestinal nematodes infection in these animals was found to be 54.3% (1year: 54.8%; 2year: 53.8%). Different parasites were identified with their respective nematode prevalence: *Haemonchus contortus* (48.3 %); *Bunostomum trigonocephalum* (30.1 %); *Chabertia. ovina* (29.8 %); *Ostertagia circumcincta* (29.8 %); *Nematodirus spathiger* (25.2 %); *Trichostrongylus spp.* (25.1 %); *Oesophagostomum columbianum* (23.5 %); *Trichuris ovis* (19.0 %); and *Marshallagia marshalli* (16.6 %). Season-wise study is reported a significant ($P < 0.05$) difference between summer and winter and it was also observed that in summer, the average worm burden (333.25 ± 2.25), mean worm burden (GIT examination: 85.3 ± 0.95; faecal examination: 75.6 ± 0.20), and faecal egg counts (2552 ± 85.7) found to be highest and lowest in winter i.e., mean worm burden (faecal examination: 23.2 ± 0.95, GIT: examination 12.7 ± 0.20), faecal egg counts (134.15 ± 9.15), and average worm burden (79.8 ± 52.2).

Sutar *et al.* (2010) examined the prevalence of helminth parasites in goats in Ahmednagar District, Maharashtra. A toatal of 400 faecal samples of goats from different villages were collected and recorded the overall prevalence rate of 62.75 %. The rainy season has the highest infestation rate (77.33 %) followed by winter (60.83 %) and then in summer (51.53 %).

Bandyopadhyay *et al.* (2010) carried out a study regarding the parasitic burden of gastrointestinal parasites of goat in Shillong, Meghalaya. They observed 250 gastrointestinal tracts for adult parasites and recorded the maximum intensity of parasitic infection in the rainy season and minimum during winter. The different helminths parasite noted were *Oesophagostomum venulosum*, *Oesophagostomum columbianum*, *Haemonchus contortus*, *Bunostomum trigonocephalu*, *Trichuris* sp., *Trichostrongylus colubriformis*, *Moniezia expansa*, *Moniezia benedeni*, *Gaigeria pachysalis*, and *Amphistom* sp. They reported an interesting finding i.e., the maximum prevalence of *Haemonchus contortus* mostly observed from reticulum (in 76.8 % of cases).

Mishra *et al.* (2010) examined an overall prevalence rate (57.69 %) of strongyle in goats in Pantnagar with the highest prevalence during monsoon season (46.35 %) followed by summer (33.04 %) and lowest in winter (24.8 %).

Arunachalam *et al* (2011a) scanned 59 gastrointestinal tracts of goats in Tamilnadu and reported that *H. contortus* showed highest incidence rate (62.71 %) followed by *O. columbianum* (28.81 %), *M. expansa* (22.03%), *T. ovis* (15.25%), *O. asperum* (11.86 %), *B. trigonocephalum* (6.77 %), and *S. globipunctata* (1.69 %).

Hassan *et al.* (2011) conducted a research to measure the prevalence of ectoparasite and endopatasites in black Bengal goats (*Capra hircus*) at Pahartali Thana under Chittagong district, Bangladesh. The overall prevalence of gastrointestinal helminths in the goats observed was 63.41 %. The most prevalent species identified was *Strongyloides* spp. (51.74 %) and *Moniezia* sp. whereas *Capillaria* sp. identified to be the least prevalent. Age was manifested to be the risk factor and showed older goats (> 24 months) significantly ($p < 0.05$) are more infested by endoparasites than younger ones (< 24 months).

Rashmi *et al.* (2011) reported the prevalence of *strongyle* sp., *strongyloides* sp., and *coccidial* sp. infection in the goats of Etawah district of Uttar Pradesh. They also found that *H. contortus* (40-60 %) was the most prevalent over the strongyle worm followed by *Trichostrongylus* sp. (15 %) and *Oesophagostomum* sp. (10%) along with the larvae of *Strongyloides* sp.

Nath *et al.* (2011) investigated the prevalence of intestinal helminths in 150 (viscera) black Bengal goats of Chittagong district, Bangladesh and reported 94.67 % of goats infected with intestinal helminths. They identified three species of helminth parasites with their prevalence rate namely *Oesophagostomum columbianum* (92 %), *Trichuris ovis* (56.66 %), *Moniezia* spp. (11.33 %). Season-wise prevalence of intestinal helminth noted significantly higher in winter (100 %) than in rainy season (89.33%).

Tambe *et al.* (2011) conducted an epidemiological study which dealt with the prevalence of helminthic infection in *Capra hircus* in Ahmednager district (M.S.). Out of 300 samples examined 255 were found infected with the helminth parasites and the overall percentage of infection was to be noted 85 %. The seasonal variation of gastrointestinal helminthic infection reported higher in monsoon (95 %) followed by winter (87 %) and summer (73 %). They have also revealed that among the helminth parasites, maximum incidence is observed for cestode parasites in all seasons (48.33 %) followed by nematodes (26.66 %) and trematodes (10 %).

Tavassoli *et al.* (2011) conducted a study to determine the prevalence and intensity of gastrointestinal infections in goats of North-West of Iran. For this they surveyed 403 goats and identified seven different species of gastrointestinal parasites, including *Strongylida* (51.11 %), *Marshallagia* (9.18 %), *Nematodirus* (2.97 %), *Trichuris* (14.39 %), *Cystocaulus* (0.7 %), *Moniezia* (5.7 %) and *Fasciola* (1 %), from their fecal samples.

Lone *et al.* (2012) conducted a comparison study on the prevalence of flukes, tapeworms and nematodes parasitizing the gastrointestinal tract of small ruminants from various regions of District Ganderbal, Kashmir. They examined 284 and 318 gastrointestinal tracts of sheep and goats respectively and found the overall prevalence of gastrointestinal helminths higher in goats than in sheep. The most prevalent nematode species reported were *Haemonchus* (82 %), *Trichuris* (74 %), *Nematodirus* (60 %), *Trichostrongylus* (58 %), *Chabertia* (52 %), *Oesophagostomum* (46 %), and *Strongyloides* (42 %). Among cestode species, *Moneizia* (48 %), *Avitellina* (42 %), and *Thysenezia* (28 %), and among trematodes, *Fasciola* sp. (60 %), *Dicrocoelium* sp. (52 %), and *Paramphistomum* sp. (46 %) reported most prevalent helminths.

Zeryehun (2012) carried out a cross-sectional study on the prevalence of gastrointestinal (GI) helminths in sheep and goats in and around Haramaya, Southeastern Ethiopia. For this purpose, 768 fecal samples (384 sheep and 384 goats) were collected and observed the overall prevalence (61.4 % in the small ruminants, 67.75 % in sheep and 55.47 % in goats). Nematodes were (59.89 %) found to be as the most prevalent helminths in both sheep and goat. The study revealed significantly higher ($p<0.05$) prevalence of helminths in sheep than goats. The season-wise study revealed the infestation rate of helminths significantly higher ($p<0.05$) in the wet season than drier ones. Gender-wise study depicted that young animals significantly are more susceptible than adults. Coproculture method revealed the presence of different types of helminths viz., *Haemonchus* sp., *Trichostrongylus* sp., *Oesophagostomum* sp., *Strongyloides* sp., and *Bunostomum* sp. in both sheep and goats.

Chedge *et al.* (2013) conducted a study on the prevalence of gastrointestinal parasites of two breeds of the goats namely Barberi (24) and Sirohi (22) of the instructional farm, Adhartal. For this research faecal samples of 46 goats (10 males and 36 females) were examined and observed the overall prevalence of parasites and was recorded to be 78 %. Among the helminths parasite encountered in the study, *strongyles* sp. showed the highest (70%) followed by *coccidian* sp. (57 %), *Trichuris* spp. (37 %), *Strongyloides* spp. (4 %), *amphistome* sp. (2 %), *Fasciola gigantica* (2 %), and *Moniezia expansa* (2 %). Age-wise prevalence noticed in young animals was 22 % while 57 % in adult animals. Sirohi breed showed 100% of infection rate while 58 % of prevalence was observed in the Barberi breed. Sex-wise prevalence was observed lowest in males (13 %) than in females (65 %).

Gebeyehu *et al.* (2013) conducted this study to investigate the prevalence and intensity of gastrointestinal (GI) parasites in Korean native goats (*Capra hircus aegagrus*). Faecal samples from 241 goats were collected and examined for parasite eggs/oocysts and reported that 69.3% of goats were infected with one or more GI parasites. The percentage prevalence of gastrointestinal parasite was reported to be 67.6 %, 22.4 %, and 2.1 % for coccidian, nematodes, and cestodes respectively. *Eimeria* spp. was the only coccidian parasite recognized, however, *Strongyle* group (20.7 %) was the most prevalent nematode identified. Additionally, they also found 31.7 % of mixed parasitic infections.

Elshahawy *et al.* (2014) described the occurrence of various gastrointestinal helminths and liver flukes in goats in Egypt. A total of 45 adult goats were selected for necropsy examination and among those 11 animals (24.44 %) were found infected with one or more species of gastrointestinal helminth. *Haemonchus contortus* arose as the most ubiquitous species (15.5 %), with *Moniezia expansa* and *Fasciola gigantica* also being common (11.11

% and 4.4 %, respectively). Additionally, *Paramphistomum microbothrium*, *Cysticercus tenuicollis*, and *Haemonchus longistipes* were also present with low infection rates (2.2 %).

Olanike *et al.* (2015) conducted a prevalence study on the gastrointestinal parasites of goats in Ibadan, South Western Nigeria. A total of 400 faecal samples (103 West African dwarf, and 297 Red sokoto breeds) of goats were examined and out of this 75.75 % were found positive for gastrointestinal parasites. The Red sokoto breed showed a higher prevalence rate of 54.25 % whereas the West African dwarf breed showed a lower prevalence of 21.5%. They also revealed that the female goats observed a prevalence of 35% while male goats have a prevalence of 40.85%. They also identified four gastrointestinal parasites namely *Monieza* spp., *Strongyloides papiillosus*, *Strongyle* spp., and *Coccidia* spp., and observed that *Strongyle* spp. showed a higher prevalence while *Monieza* spp. showed a lower prevalence rate.

Singh *et al.* (2015) carried out a study on epidemiological data of gastrointestinal parasitism of goats in Madhya Pradesh, India. For this a total of 960 samples were collected and observed an overall prevalence of 907 (94.48 %), wherein *coccidia* was predominant (82.4 %) followed by *strongyles* (69.27 %), *amphistomes* (22.71 %), *Strongyloides* sp. (9.17 %), *Trichuris* sp. (3.85 %), *Moniezia* sp. (3.02 %), *Schistosomes* sp. (2.29 %) and *Fasciola* sp. (1.77 %). The seasonal incidence detected highest in monsoon (98.06 %) and lowest in winter (91.67 %). The prevalence of gastrointestinal parasitism reported higher in kids (96.25 %) in comparison to adult goats (93.89 %).

Zvinorova *et al.* (2016) conducted a study to determine the prevalence of gastrointestinal helminths in different age groups and between the gender of goats. A total of 580 native goats randomly selected from the five agro-ecological regions of Zimbabwe. The high prevalence rate noticed for Eimeria oocysts (43 %), and *strongyles* sp. (31%) whereas the lower for trematodes and cestodes. They identified three nematode species namely: *Haemonchus* sp., *Strongyloides* sp., and *Oesophagostomum* sp. They observed that area, season, sex and age have significant ($P < 0.05$) influence on gastrointestinal parasitism. Significantly ($P < 0.05$) high level of helminths and *Eimeria* infections were noticed in the wet season than dry season. Young animals were significantly ($P < 0.05$) more prone to parasitic infections than adult animals. In Gender-wise prevalence, males showed a higher prevalence rate than females ($P < 0.0001$).

Odogu and Okaka (2016) reported the prevalence of gastrointestinal helminths in goats slaughtered in Aduwawa abattoir, Benin City, Edo state. A total of 492 goats examined for the presence of parasites and they identified two different species of gastrointestinal helminths with prevalence namely *Monieza benedeni* (34.76 %), and *Paramphistomum cervi* (21.54 %). The overall prevalence of gastrointestinal parasites observed was 61.59 %. Female goats showed a higher prevalence (63.64 %) than the male goats (61.07 %). The rainy season showed higher prevalence (73.36 %) than the dry season (50 %).

Dixit *et al.* (2017) done a survey on the prevalence of helminths parasite in goats of Jabalpur district, Madhya Pradesh. A total of 632 faecal samples were examined, out of which 82.75 % samples were found positive. Among several helminths, maximum prevalence was noted for *Strongyles* sp. (32.59 %) followed by *Amphistomes* sp. (14.40 %), *Moniezia* spp. (12.50 %), *Strongyloides* spp. (0.79 %), *Trichuris* spp. (0.47 %), and *Fasciola gigantica* (0.32 %). Monsoon season showed significantly ($p < 0.05$) high infections rate (87.97%) as compared to the winter (81.48 %) and summer (79.03 %) season. The season-wise study revealed a significant difference on the infection rate of *Strongyloides* spp., strongyles, *Moniezia* spp., and amphistomes while no significant disparity was noted in the infections of *Trichuris* spp., and Fasciola gigantica. The age-wise prevalence showed non-significant variation and reported high prevalence in kids (83.13 %) than in adults (82.62 %).

Bihaqi *et al.* (2017) carried out a prevalence study on gastrointestinal (GI) helminths of goats at Government Farms of twin districts of Bandipora and Ganderbal and locally reared goats of district Baramulla of the Kashmir Valley. A total of 1016 faecal samples were observed and revealed that the overall prevalence of GI helminths was 74.70 %, with the prevalence of platyhelminths (14.76 %) and nemathelminths (70.07 %). The helminths identified in the study were strongyle worms including *Nematodirus* spp. (68.30 %), *Strongyloides* spp. (5.90 %), *Trichuris* spp. (4.29 %), *Fasciola* spp. (2.75 %), *Dicrocoelium* spp. (3.44%), *Paramphistomes* (3.05 %), and *Moniezia* spp. (9.44 %). Coprocultural examination indicated the the most predominant parasites is *Haemonchus* spp. (71.05%) followed by *Trichostrongylus* spp. (12.86 %), *Chabertia* spp. (8.47 %), and *Ostertagia* spp. (7.62 %). Non- significant ($P > 0.05$) difference was seen in the seasonal prevalence and reported highest in summer (78.03 %) followed by spring (75.39 %), winter (74.90 %), and autumn (70.47 %). The incidence rate of platyhelminths was found non-significantly higher in winter (20.00 %) followed by spring (15.07 %), summer (12.54 %), and autumn (11.41

%). Nematodes prevalence recorded highest in summer (75.68%) followed by spring (71.82 %), autumn (68.50 %) and winter (64.31 %). The overall prevalence of GI helminths was found significantly ($P \leq 0.05$) high in adult goats (77.85 %) compared to young ones (67.42 %), whereas it was found to be higher in females (75.89 %) as compared to males (66.12 %).

Bedada *et al.* (2018) conducted a cross-sectional study to determine the prevalence and identification of gastrointestinal parasites (GI) of sheep and goats in two selected districts of Afar. For this research, 590 (332 goats and 258 sheep) faecal samples were collected from small ruminants managed in pastoral and agro-pastoral production. Out of the total examined feacal samples, 87.8 % (92.2 % of goats and 82.2 % of sheep) were found to be positive for one or more genera of helminth parasites. Helminths parasites identified in both goat and sheep where *Strongyles* sp., *Fasciola* sp., *Strongyloides* sp., *Paramphistomum* sp., *Trichuris* sp., *Ascaris* sp., and *Monezia* sp. The risk of infection with GI parasites was higher in goats than in sheep.

Jena *et al.* (2018) conducted a study and examined 930 goats for the presence of gastrointestinal helminths and of which 801 (86.13 %) were found to be positive. The different gastrointestinal helminths observed were *Fasciola* spp. and *Paramphistomum* spp. in trematodes; *Moniezia expansa* and *Moniezia benedeni* in cestodes; *Strongyloides* spp., *Trichostrongylus* spp., *Haemonchus* spp., *Trichuris* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Ostertagia* spp., *Cooperia* spp., and *Marshallegia* sp. in the nematode. They noticed significant ($P<0.01$) relationship between season, age and sex wise variations with helminth prevalence.

Saiyam *et al.* (2018) examined the 1478 faecal samples of goats to determine the presence of gastrointestinal helminth parasite and 1194 (80.78 %) of samples were found positive for GI helminths. They also demonstrated the percentage prevalence of nematodes, trematodes, and cestode which were 77.47 %, 7.37 % and, 14.75 % respectively. They identified various helminths species namely *strongyles* (75.17 %), *Moniezia* (14.75 %), *Strongyloides* (7.92 %), *Trichuris* (6.16 %), *Amphistomes* (4.59 %), and *Fasciola* (1.76%). A non-significant variation was observed in both season-wise and age-wise prevalence and demonstrated that post monsoon showed higher infection rate (83.16 %) than the monsoon (81.08 %) season whereas higher infection was showed in adults (81.04 %) than in kids (77.68 %).

AL- Amran *et al.* (2018) conducted a one-year long epizootiological survey to investigate the prevalence of gastrointestinal (GI) helminthiasis in goats (*Capra hircus*) of three geographical areas of Bangladesh including S.A. Quaderi Teaching Veterinary Hospital (SAQTVH) of Chittagong, Veterinary Hospital of Bangladesh Agricultural University (VHBAU), Mymensingh, and Central Veterinary hospital (CVH) of Dhaka by means of coproscopical study of feacal samples. They found almost similar overall prevalence rate in all the study areas was 63.88 % (CVH), 62.13 % (VHBAU), and 59.43 % (SAQTVH). The highest prevalence of trematodes infection was found to be 40 %, 6 %, and 2 % in the areas of VHBAU, CVH, and SAQTVH, respectively. They also recorded a higher prevalence rate (66%) for nematode in goats of CVH, and most prevalent nematode observed was *Haemonchus* sp. (39.81 %) in CVH. The Cestode incidence rate was observed remarkably low in all the three study areas.

b 2.2 Status of anthelmintic resistance in small ruminants

Synthetic anthelmintics drugs are used to treat diseases caused by the parasitic worms. Parasitic worms include both flat worms (trematodes and cestodes) and roundworms (nematodes). There are many anthelmintic drugs present in the market and highly effective against helminths species and can be categorized into 4 groups on the basis of their mode of action: 1. Benzimidazoles (BZ): e.g. albendazole, mebendazole, fenbendazole, and oxfendazole 2. Levamisole (LM) 3. Macrolcyclic lactones (ML): such as doramectin, abamectin, moxidectin, and ivermectin, 4. Amino acetonitril derivatives (AD) (Drudge *et al.*, 1964; Shalaby, 2013). Synthetic anthelmintics drugs have great importance for human medicine and for veterinary medicine. The advent of these anthelmintic drugs has played a key role in dropping off the effects of parasitic infestation in human beings and livestock (Shalaby, 2013). However, since few decades parasites have been developing resistance to these anthelmintics that means allowing worms to survive even on the exposure of standard therapeutic dose of the anthelmintic.

Drudge *et al.* from the USA in 1957 gave the first report about anthelmintic resistance in gastrointestinal nematodes of sheep against phenothiazine. From then on, the parasitic worms have developed resistance against all classes of broad-spectrum synthetic anthelmintic medicines. Anthelmintic resistance has become a global problem and was reported in India for the first time by Varshney and Singh (1976) and all over the world (Hotson *et al.*, 1970; Hall *et al.*, 1979; Pichard, 1980; Van Wyke *et al.*, 1980; Middelburg and McKenna, 1983; Giordano *et al.*, 1988; Taylor and Hunt, 1989; Beveridge *et al.*, 1990; Pomray *et al.*, 1992; Nari *et al.*, 1996; Leathwick *et al.*, 2001) etc.

Borgsteede *et al.* (1986) reported that nematode parasite namely *H. contortus*, O. *circumcincta*, *C. curticei* and *T. colubriformis* of farm Angora and Anglo Nubian goats in Netherland noticed resistant against benzimidazole. They found that the efficacy of oxfendazole was 68%, 31%, 21% and 48% against the *H. contortus*, *O. circumcincta*, *C. curticei*, and *T. colubriformis* respectively.

Van *et al.* (1989) reported that *H. contortus* and *O. columbianum* of farm goats in the northwestern part of Sri Lanka showed resistance to febantel, mebendazole, and thiabendazole. They confirmed the anthelmintic resistance of *H. contortus* against benzimidazoles by *in vitro* egg hatch assay.

Craig and Miller (1990) reported resistance to ivermectin and oxfendazole against *H. contortus* in Angora goat in East Texas, USA, @ 0.2 mg/kg b. Wt. orally and @ 5 mg/kg b. wt. orally respectively.

Yadav and Uppal (1992) revealed levamisole resistant *H. contortus* strain in goats in Hissar. They found 64 % faecal egg count reduction and 55 % worm count reduction @ 7 mg/kg b. wt. orally.

Varady *et al.* (1993) recorded multiple anthelmintic resistance in nematodes of goats in Czechoslovakia. They confirmed benzimidazole resistant nematodes by performing egg hatch assay and also found 74 % egg hatch reduction with albendazole, 83 % with ivermectin and 86% with Levamisole. They recognized resistant nematode genera comprised of 54 % *Ostertagia* sp. and 46 % *Trichostrongylus* sp.

Coles *et al.* (1996) reported resistance towards fenbendazole, ivermectin, morantel, and levamisole against *Ostertagia* sp. from angora goats in two farms of U.K. by using Faecal egg count reduction. They were found 63 % and 64 % reduction after fenbendazole (@ 5 mg/kg. b. wt. orally) treatment, 59 % and 65 % after ivermectin treatment (@ 0.2 mg/kg b. wt. S/C), 80 % after levamisole (@7.5 mg/kg. b. wt. s/c) and 54% and 0% after morantel (@ 23.2 mg/kg b. wt., 17.4 mg/kg b. wt. orally, respectively) on one farm.

Barre *et al.* (1997) carried out faecal egg count reduction test after anthelmintic treatment with fenbendazole (@ 22.5 mg/kg b. wt. orally) and ivermectin (@ 0.32 mg/kg b. wt. s/c) on 1057 goats in 19 farms of Gaudeloupe. They reported that *H. contortus*, *Oesophagostomum* spp., and *Trichostrongylus sp*. were resistant to benzimidazole derivative.

Waruiru *et al.* (1998a) reported the anthelmintic resistance towards the benzimidazole, rafoxanide, and levamisole against *H. contortus* and *T. colubriformis* while *Oesophagostomum* sp. showed resistant to levamisole on a goat farm in Kenya using Faecal egg count reduction test.

Laha *et al.* (1999) reported fenbendazole (@ 5 mg /kg b. wt. orally) resistance and susceptible to ivermectin (@ 0.2 mg/kg b. wt. s/c) against *H. contortus* with 25 and 100 percent reduction in faecal egg counts, respectively at organized Pashmina goat farm in Mukteshwar, Uttarakhand.

Hertzberg *et al.* (2000) reported the incidence of benzimidazole resistant gastrointestinal nematodes in 20 sheep and goat farms in Switzerland using faecal egg count reduction test and larval hatch assay. Benzimidazole resistant showed by *Trichostrongyles* sp. in 15 farms and *H. contortus* was detected the predominant species on most of the farms.

Bakunzi *et al.* (2003) conducted a survey on the occurrence of anthelmintic resistance of nematodes in jointly grazed goats in a semi-arid area in South Africa. They evaluated the efficacies of fenbendazole, levamisole, and rafoxanide by faecal egg count reduction (FECR) tests. Efficacy of 80 % is considered a threshold for anthelmintic resistance. The FECR tests showed that rafoxanide was only 31 % effective and in another case fenbendazole was found to be bonly 47 % effective.

Ancheta *et al*. (2004) evaluated the efficacy of (BZ) anthelmintics in the Philippines by *in vitro* larval development assay (LDA) using worm eggs recovered from faeces collected from goats and sheep. They reported resistance to Benzimidazole on *Haemonchus* sp., *Trichostrongylus* sp., and *Oesophagostomum* sp. with mean efficacy of 82 % and 64 % for goats and sheep, respectively.

Das and Singh (2005) surveyed four systematic and two non-systematic sheep and goat farms in Hisar to evaluate the efficacy of anthelmintic resistance in gastrointestinal nematodes by faecal egg count reduction test (FECRT). They reported moderate to severe anthelmintic resistance to fenbendazole in all the farms of sheep and goats studied whereas anthelmintic resistance to Rafoxanide and Morantel were noticeable in University goat breeding farm and in Government goat breeding farm, respectively.

Eysker *et al.* (2006) designed an experiment to asses the anthelmintic efficacy of ivermectin, levamisole, and oxfendazole against the gastrointestinal nematodes of goats on a farm in the Netherlands, using FECRT (faecal egg count reduction test) combined with faecal larval cultures. They observed that Levamisole was 99.9 % effective against *H. contortus*, 81.9 % effective against *T. circumcincta*, and 99.5 % against *Trichostrongylus* spp. Oxfendazole was 75.5 % effective against *H. contortus*, 33.2 % effective against *T. circumcincta*, and 100 % effective against *Trichostrongylus* spp. Ivermectin was 49.1% effective against *T. circumcincta* but 100 % effective against *H. contortus* and *Trichostrongylus* spp. Accordingly, they reported that *T. circumcinta* appears to be resistant to ivermectin and oxfendazole whereas *H. contortus* showed resistance against oxfendazole.

Artho *et al.* (2007) examined the occurrence of Avermectin (AVM) resistant Gastrointestinal Nematodes (GIN) both in Dorper sheep and Boer goats breeds in Switzerland. For this they have investigated a total of 24 Boer goat farms and 12 Dorper sheep farms and according to the faecal egg count reduction test (FECRT) they have confirmed AVM-resistant GIN populations in 7 of the 24 Boer goat farms and 2 of 12 Dorper sheep farms and suspected in a further 8 and 6 of the 24 Boer goat and 12 Dorper sheep farms, respectively. *Haemonchus contortus* and *Trichostrongylus* spp. were observed as the main resistant species according to larval cultures.

Cringoli *et al.* (2007) reported resistance to benzimidazoles, levamisole, and ivermectin in a multi-breed goat farm of southern Italy. Strongyle nematodes studied for anthelmintic resistance on the basis of faecal egg counts, egg hatch assay, and necropsies. They confirmed resistance to anthelmintics evinced only for benzimidazoles and observed *Trichostrongylus colubriformis* as the only resistant strongyle species.

Howell *et al.* (2008) conducted a cross-sectional study to investigate the prevalence of anthelmintic resistance on 46 farms of small ruminants (sheep and goats) in the southeastern United States. They noticed *Haemonchus contortus* was the most prevalent helminth parasites in 44 farms followed by *Trichostrongylus colubriformis*. They demonstrated that *Haemonchus contortus* from 45 farms (98 %) showed resistance to benzimidazole, 25 farms (54%) resistant to levamisole, 35 farms (76 %) resistant to ivermectin, and 11 farms (24 %) resistant to moxidectin. Multiple resistance was observed in three classes of anthelmintic (benzimidazole, levamisole, ivermectin) in 22 (48 %) farms, whereas 8 farms (17 %) revealed multiple resistance to four classes of anthelmintics (benzimidazole, levamisole, ivermectin, and moxidectin).

Jabbar *et al.* (2008) investigated the first report of Anthelmintic Resistance (AR) against gastrointestinal nematode in dairy goats (Dera Din Panah, Pak Angora, and Beetal) in Pakistan. They evaluated the efficacy of selected anthelmintics by faecal egg counts, faecal egg count reduction test, postmortem worm count and copro-culture technique. They reported prevalence of *Haemonchus controtus* and *Trichostrongylus* species of GINs which exhibited resistance against oxfendazole.

Scheuerle *et al.* (2009) performed a study to analyze the reduction in anthelmintic efficacy of eprinomectin and benzimidazoles (albendazole, fenbendazole, and oxfendazole) in two goats and two sheep flocks with the help of the faecal egg count reduction test. They revealed *Haemonchus contortus* as the predominant resistant species and showed resistance to eprinomectin and benzimidazoles (albendazole, fenbendazole, and oxfendazole) in both goats and sheep flock, respectively.

Paraud *et al.* (2009) conducted an experiment to determine the resistance of goat nematodes to different anthelminthics. They found resistance of *Trichostrongylus colubriformis* showed resistant to both levamisole and fenbendazole.

Arunachalam *et al.* (2011b) conducted studies on anthelmintic resistance in sheep (3 farms) and goats (2 farms) in NorthWestern Tamilnadu and observed resistance of *H. contortus* showed resistance to fenbendazole, levamisole, and ivermectin in both goat farms and in two sheep farms. Third sheep farm showed resistance only to fenbendazole and ivermectin, but no resistance was observed against levamisole.

Godara *et al.* (2011) tested the efficacy of Fenbendazole (FBZ), Levamisole (LEV) and Ivermectin (IVM) on naturally infected gastro-intestinal nematode parasites in goats. The study specified that FBZ and LEV were not effective against *H. contortus* in goats even at the recommended dosage while IVM was sensitive with 98.11 % efficacy.

Domke *et al.* (2012) carried out a research to estimate the efficacies of the Benzimidazole (BZ) drug Albendazole (ABZ) and the Macrocyclic Lactone (ML) drug Ivermectin against Gastrointestinal Nematodes (GIN) of randomly selected sheep and goat flock (Sheep = 28 and goat = 28) and non-randomly selected sheep flocks (n=32) in Norway. The efficacy of anthelmintic drugs evaluated by means of the fecal egg count reduction (FECR) test and by post-treatment coprocultures study. They reported 10.5 % and 31.0 % in the randomly and non-randomly selected flocks, respectively and showed resistant (i.e., FECR <95 % and with a lower 95% confidence interval of <90 %) to BZ (Benzimidazole) drug Albendazole. The Macrocyclic Lactone (ML) drug Ivermectin showed 100 % sensitivity in all surveyed sheep and goat flocks. They also manifested *Teladorsagia/Trichostrongylus* sp. and *Haemonchus* sp. both were showed anthelmintic resistance to BZ drug Albendazole in sheep flock while only *Teladorsagia/Trichostrongylus* sp. showed resistance to BZ (Benzimidazole) drug Albendazole in the goat flocks.

Byaruhanga *et al.* (2013) determined the anthelmintic efficacy of Albendazole (ABZ) and Ivermectin (IVM) against Gastrointestinal Nematodes (GIN) in naturally infected goats in the pastoral region of Karamoja, Uganda. Anthelmintic efficiency was determined by using the Fecal Egg Count Reduction Test (FECRT). They reported ABZ (5 mg/Kg b.wt.) and IVM (0.2 mg/Kg b.wt.) reduced FEC by 81.03 % and 96.55 % respectively. They reported that ABZ group shown resistance against nematode parasite. Further, copro culture results revealed only *Haemonchus* spp. showed resistance against albendazole treatment.

Zanzani *et al.* (2014) determined the potency of anthelmintic resistance (AR) against gastrointestinal nematodes (GNIs) in goat flocks in Northern Italy. For this 15 flocks were selected to assess the incidence of AR in GINs by means of the Fecal Egg Count Reduction Test (FECRT). They noticed anthelmintic resistance and suspected resistance which found in 40% of the flocks treated with benzimidazoles whereas 20 % of the flocks were treated with eprinomectin.

Borges *et al.* (2015) demonstrated the efficacy of anthelmintic drugs (levamisole, benzimidazoles and macrocyclic lactones) against gastrointestinal nematodes (GINs) in goats. For this, 18 herds were selected from the Caatinga (n=12), Mata Atlântica (n=6) biomes, Bahia State, Brazil, which grew in common grazing field in the semiarid region. Eight to ten goats from each group were treated with albendazole (ABZ), ivermectin (IVM), levamisole (LEV), moxidectin (MOX), and closantel (CLOS). The anthelmintic resistance was evaluated with the Fecal Egg Count Reduction Test (FECRT) indicated the resistance of *Haemonchus* sp. and *Trichostrongylus* spp. against albendazole (0 to 92 %,), ivermectin (0 to 75 %), levamisole (0 to 91 %), moxidectin (69 to 97 %), and closantel (0 to 85) in the Caatinga biome, and 0 to 59 % for ABZ and 9 to 59 % for IVM in the Mata Atlântica biome.

Sharma *et al.* (2015) conducted an experiment to evaluate the efficacy of regular dewormer used and its comparison with some uncommon anthelmintic drug for the same purpose. For this study, 27 goats were allocated into three groups: Group I- treated with ivermectin @ 0.2

mg/kg b.wt. orally, Group II- treated with fenbendazole @ 5 mg/kg b. wt. orally, and Group III- kept as an untreated control. FECRT percentage revealed 100 % efficacy for ivermectin whereas for fenbendazole treated group the efficacy was reported to be 57.44 % (on day 7) and 70.87 % (on day 14) post-treatment. They revealed low efficacy of fenbendazole and emergence of anthelmintic resistance in *Haemonchus* sp.

Gelot *et al.* (2016) carried out faecal egg count reduction test to determine the efficacy of albendazole (ALB), levamisole (LEV) and ivermectin (IVM) against gastrointestinal nematodes in naturally infected goats in the semi-arid region of Gujarat, India. They revealed the presence of resistance of worms against ALB, LEV, and IVM on the 14^{th} day of post-treatment with reduced faecal egg counts and efficacy varied from 53 % to 76 %. They reported *Haemonchus* spp showed resistance against ALB and LEV drug while IVM showed low efficacy towards *Trichostrongylus* species.

Furgasa *et al.* (2017) surveyed sheep and goat farms to evaluate the status of anthelmintic resistance against Gastrointestinal (GI) parasites in Haramaya University, Ethiopia. A total of 30 black head Ogaden sheep and 30 Hararghie highland goats (6-18 month) were selected and evaluation was done by means of Feacal Count Reduction Test (FECRT). Both sheep and goats categorized were into two treatment (albendazole, ivermectin) and one control groups. They found resistance against albendazole and ivermectin in sheep with percentage reduction of fecal egg for albendazole was 82 % (95 %, CL 60-92), and for ivermectin it was 68 % (95 %, CL 0-90), whereas in goats the percentage reduction in FECs for albendazole was 63 % (95 %, CL 28-81), and for ivermectin it was 41 % (95 %, CL 0-72).

Mickiewicz *et al.* (2017) reported resistance of *H. contortus* to benzimidazole anthelmintic, which is the first such case reported in Polish goats, in Poland.

Babják *et al.* (2018) conducted a study to determine the occurrence of anthelmintic resistance (AR) on 30 goat farms in Slovakia during the pasturing seasons. For this analysis, they categorized goats into treated and control groups in each farm. The treated groups were medicated with recommended dose (5 mg/kg b.wt.) and double doses (10 mg/kg b.wt.) of Benzimidazole (BZ) drug Albendazole (ALB). The estimation of anthelmintic resistance was done by means of comparisons between percent reduction in the faecal egg count reduction test (FECRT), an egg hatch test (EHT), and larval development test (LDT). The FECRT showed percent reductions of 69.2-86.2 % and of 36.3-45.4 % for the single dose and the double dose respectively. The EHT indicated levels of resistance on the basis of low hatching (<15 %) in 13 farms whereas high hatching (>15 %) in 17 farms and also revealed that all farms had BZ-resistant nematodes. The LDT detected low and high levels of resistance against L3 larval stage on seven and 14 farms, respectively.

2.3 Anthelmintic potential of traditional plants against gastrointestinal helminths: General overview

Control of gastrointestinal helminths in livestock is now becoming a very difficult task due to the occurrence of drug resistance and different types of environmental hazards. Hence, there is an urgent need of the alternate control methods to reduce parasitic burden, which should be economical, less toxic, have no side effects, easily accessible, and environmentally friendly. Assessment of traditional plants and isolation of its active compounds responsible for anthelmintic activity is one of the better options to accomplish these goals (Raje *et al.*, 2003). A brief account of the phytochemical screening and evaluation of anthelmintic activity of medicinal plants against gastrointestinal helminths are presented below:

Kalesaraj and Kurup (1962) reported that alkaloid hydrochlorides extracted from the seed of *Butea fundosa* (0.1-20.0 mg/ml in dose) were shown 100 % anthelmintic activity against earthworms within 24 hours.

Ali and Mehta (1970) reported the anthelmintic potential of essential oil of *Piper betle* against earthworms**.**

Sharma *et al.* (1971) have reported significant in vitro anthelmintic effect of the extract of *Calotropis gigantea*, *Cucurbita pepo*, *Juglans regia*, *Musa paradisaca*, *Scindapsus officinalis* and *Momordica charantia* against *H. contortus* of goats.

Girgune *et al.* (1978) reported essential oils *Boswellia serrata* and *Cinnamomum tamala* to have better anthelmintic property than standard drug (Piperazine citrate) against earthworms.

Prakash *et al.* (1980) reported the anthelmintic activity of alcoholic extract of *Punica granatum* and revealed its dose-dependent inhibition activity on the hatching of eggs of *H. contortus.*

Mehta *et al.* (1981) disclosed the anthelmintic potency of *Zanthoxylum alatum* and reported that it showed better anthelmintic efficacy than the piperazine phosphate against earthworms.

Khobragade *et al.* (1994) evaluated in vitro anthelmintic potential of aqueous extract of *Allium sativum* against *Bunostomum trigonocephalum* and observed 100 % death of worms in 200, 100, 50 and 25 mg/ml concentration during 6, 12, 16 and 20 hours, respectively.

Jangde *et al.* (2001) found that aqueous extract of *Artemesia maritima* (root) and *Butea frondosa* (seed) was able to cause the death of H. contortus at different concentration.

Iqbal *et al.* (2001) carried out in vitro anthelmintic efficacy of methanol extracts of *Zingiber officinale*, *Allium sativum*, *Ficus religiosa*, and *Curcurbita mexicana* against *H. contortus* and reported 100 % mortality with Z. *officinale, F. religiosa*, and *A. sativum* at 2, 4 and 6 hours respectively and 83.4 % mortality with C. mexicana at 6 hours.

Pessoa *et al.* (2002) evaluated the anthelmintic activity of the essential oil of *Ocimum gratissium* and its key component eugenol against eggs hatching of *Haemonchus contortus* of small ruminants. They disclosed that at 0.5% concentration of the essential oil and eugenol showed highest eclodibity inhibition.

Alawa *et al.* (2003) evaluated the anthelmintic property of *Annona senegalensis* against *H. contortus* by egg hatch test. They revealed that hot water extract of the plant showed a significant reduction in egg hatching at 7.1 mg/ml. Ovicidal and larvicidal activity of methanolic extract of *Spigelia anthelmia* showed 97.35 and 84.4% respectively against *H. contortus* whereas ethyl acetate extract at 50 mg/ml concentration inhibited 100% and 81.2% of the egg hatching and larval development, respectively.

Iqbal *et al.* (2004) described the *in vitro* anthelmintic activity of *Artemisia brevifolia* in comparison with levamisole. In vitro studies revealed an increase in EPG (Egg per gram of faeces) reduction after the treatment of crude aqueous and methanol extracts of *Artemisia brevifolia* (whole plant) on *Haemonchus contortus*.

Iqbal *et al.* (2005a) reported the anthelmintic potential of Crude Aqueous Extract (CAE) and Crude Methanolic Extract (CME) of flowers of *Calotropis procera* (*in vitro*) and revealed the ability of plant causing mortality of *H. contortus*.

Peter and Deogracious (2006) surveyed twenty-one indigenous plants, out of which seven plants were selected for *in vitro* anthelminthic activity against *Ascaris suum*. Of the 7 plants, five plants namely *Momordica foetida* (16.75mg/ml), *Carica papaya* (12.50 mg/ml), *Cassia occidentalis* (4.13mg/ml), and *Tetradenia riparia* (1.62mg/ml) showed anthelmintic property.

Nwosu *et al.* (2006) evaluated the anthelmintic potential of the aqueous extracts of leaf, stem, and root barks of *Azadirachta indica* (neem) by means of egg hatch test and larval development test against nematode parasites of small ruminants. *In vitro* results revealed aqueous extracts of both leaf and stem bark showed a significant reduction in nematode egg hatch assay whereas aqueous extracts of the leaf and root bark showed a significant reduction in larval survival within 60 minutes.

Bizimenyera *et al.* (2006) conducted an *in vitro* experiment to analyze anthelmintic efficiency of *Peltophorum africanum* against *Trichostrongylus colubriformis*. They reported that inhibition of egg hatching and larval development significantly ($P<0.05$) increased with increasing concentrations of the extract.

Jabbar *et al.* (2007) carried out an *in vivo* study to determine anthelmintic activity of crude powder, aqueous and methanolic extract of both *Caesalpinia crista* and *C. album*. These plants were tested against mixed infections of nematodes in sheep at Faisalabad, Pakistan, and observed 82.2% to 93.9% reduction in faecal egg counts at 3gm/kg b. wt on 13 and 15 days post-treatment.

Eguale *et al.* (2007a) demonstrated the *in vitro* anthelmintic activity of aqueous and hydro-alcoholic extracts of the ripe fruits of *Hedera helix* against eggs and adult nematode parasites *Haemonchus contortus*. They found hydro-alcoholic extract showed better activity against adult parasites compared to the aqueous extract. They noticed significant ($p< 0.05$) reduction in Faecal Egg Count (FEC) and Total Worm Count (TWC) in both treated groups on day 2 of the post-treatment. They also observed significant ($p< 0.05$) reduction detected only for a higher dose of *H. helix* on day 7 post-treatment.

Eguale *et al.* (2007b) carried out the *in vitro* anthelmintic evaluation of crude aqueous and hydro-alcoholic extract of the seeds of *Coriandrum Staivum* on eggs and adult *H.contortus*. Both extracts of *Coriandum sativum* showed 100 % ovicidal activity at a concentration less than 0.5 mg/ml, and hydro-alcoholic extract showed more potent in vitro anthelmintic activity against adult parasites than the aqueous extract.

Sujon *et al.* (2008) carried out an investigation of ten indigenous medicinal plants to find out their anthelminthic potential. They revealed ethanol extract showed more than 70% of efficacy against Labanga, Neem, Karolla, and Pineapple at a higher dose (100 mg/kg).

Lalchhandama (2008) concluded that at higher concentration (20 mg/ml) of plant extract of *Acacia oxyphylla* it showed significant ($P < 0.05$) anthelmintic efficacy against *Ascaridia galli*. Histological observation revealed that at higher concentrations (20 mg/ml) the plant showed extensive structural alterations involving rupture of the cuticle, disruption of the ovaries, the breakdown of the muscular layers, and deformity on the egg membranes.

Costa *et al.* (2008) carried out an *in vitro* study to assess anthelmintic potency of ethyl acetate extract and ethanolic extract of *Azadirachta indica* against *Haemonchus contortus* at concentrations of 0.19 mg/ml, 0.78 mg/ml, 3.12 mg/ml, 12.5 mg/ml, and 50 mg/ml. This study concentrated on evaluation of ovicidal and larvicidal activity. They found 51.31% ovicidal activity for ethyl acetate extract at concentration 3.12 mg/ml while 99.77% ovicidal activity noted for ethanol extracts at concentration 50 mg/ml. 68.10% and 87.11% larvicidal activity noted for both extract at concentration 50 mg/ml.

Kosalge and Fursule (2009) determined the anthelmintic potential of aqueous extract of three plants namely *Cassia auriculata* leaves (Awali), *Erythrina variegate* leaves (Pangora), and *Dioscorea bulbifera* bulbs (Kand) against tapeworms (*Raillietina spiralis*), roundworms (*Ascardia galli*), and earthworms (*Eicinia faeteda*). Various concentrations (10 mg/ml, 50 mg/ml) of each plant extract tested, and results revealed that all the extracts expressed significant anthelmintic activity at the highest concentration (50 mg/ml) as compared to standard drug Piperazine citrate (10 mg/ml).

Saowakon *et al.* (2009) reported anthelmintic potential of crude extract of *Artocarpus lakooch*a on adult *Fasciola gigantica*. They revealed the crude extract of *A. lakoocha* reduced the parasite's motility and also inhibited the larval migration as compared to triclabendazole (TCZ) at all concentrations. Scanning Electron Microscope (SEM) study revealed TCZ and the crude extract leading to chronological changes in the tegument of the parasite including puffiness, followed by blebbing that ruptured afterward, leading to the destruction and exuviation of the tegument layer. They also stated the severity of the damages dependent on increasing dose concentration of the crude extract.

Nedialkova *et al.* (2009) conducted an experiment to screen the phytochemical constituents of *Chenopodium* and reported the presence of phenolics, flavonoids, saponins, ecdysteroids, and triterpenoids secondary metabolites..

Kumar *et al.* (2010) investigated the anthelmintic activity of methanol extract *Amaranthus caudatus* Linn. They reported the methanolic extract showed the dose-dependent manner of anthelmintic activity at the concentrations 60, 80, 100 mg/ml. They also screened the methanol extract for the presence of various phytoconstituents such as steroids, alkaloids, glycosides, terpenoids, phenolic compounds, flavonoids, and carbohydrates.

Parida *et al.* (2010) designed an experiment to determine the anthelmintic efficacy of crude extracts of *Pterospermum Acerifolium* Linn in different fractions from leaves, barks, and flowers against roundworms (*Ascardia galli*), earthworms (*Pheretima posthuma*), and tapeworms (*Raillietina spiralis*) using Piperazine citrate and Albendazole as reference drugs. They found ethyl acetate fraction of all the parts has shown more potent anthelmintic activity followed by n-butanol fractions of those parts, nevertheless at higher doses.

Kamaraj and Rahuman (2011) evaluated the anthelmintic efficacy of dried leaf and seed extracts of five medicinal plants (*Annona squamosa*, *Eclipta prostrata*, *Solanum torvum*, *Terminalia chebula*, and *Catharanthus roseus*). For this, they used different types of solvent extract such as ethyl acetate, acetone, and methanol. The *in vitro* study was done by means of Egg Hatch Assay (EHA) and Larval Development Assay (LDA) on *Haemonchus contortus* at concentrations 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.13 mg/ml with all plant extracts. They reported all extracts (ethyl acetate, acetone and, methanol) of leaf and seed showed complete inhibition (100 %) at the higher concentration (50 mg/ml).

Khan *et al.* (2011) carried out an experiment to categorize the phytochemical constituents and also to evaluate the *in vitro* anthelmintic potency of the aqueous extract of *Nerium olender* flower. The various concentrations (15 mg/ml, 25 mg/ml, 50 mg/ml, and 100 mg/ml) of the aqueous extract were tested and demonstrated that *Nerium olender* has a potent anthelmintic property. The qualitative phytochemical screening of aqueous extracts *Nerium olender* revealed the presence of active biological compounds like tannins, alkaloids, steroids, flavonoids, glycosides, and carbohydrates.

Maithani *et al.* (2011) conducted a phytochemical analysis of the aqueous leaves extract of *A. indica* and found the presence of glycosides, phenolics, alkaloids, saponins, tannins, and carbohydrates.

Marathe *et al.* (2011) evaluated the anthelmintic activity of *Drynaria quercifolia* (L) against the endoparasite of goats (*Capra hircus* Linn.) in Western Maharashtra, India. They reported that *D. quercifolia* (Linn) plant showed anthelmintic property against the endoparasite of goats.

Molefe *et al.* (2012) performed a study to assess the anthelmintic activity of aqueous and acetone extracts of *Mentha longifolia* and *Artemisia afra* by means of Egg Hatch Assay (EHA), Larval Development Assay (LDA), and Larval Mortality Assay (LMA). They disclosed both EHA and LMA inhibited at certain percentages by both plants, however, LDA was totally inhibited at all concentrations (2.5 mg/mL, 5.0 mg/mL, and 7.5 mg/mL) as compared to the control. They also revealed acetone extracts showed high efficacy against parasitic gastrointestinal than the aqueous extract.

Dorwal (2012) evaluated the methanolic and ethanolic leaves extract of *Calendula officinalis* at three different concentrations viz., 5, 10 and 15 mg/ml. The results revealed that both the extracts showed significant anthelmintic potential as compared to the standard drug, Albendazole (10 mg/ml). They also depicted both extracts in dose-dependent manner of anthelmintic activity.

Bairwa *et al.* (2012) screened the phytoconstituents of *Trachyspermum ammi* and reported the presence of phenolic compound, glycosides, carbohydrates, saponins, volatile oil, protein, fat, fiber, and minerals (calcium, phosphorous, iron, and nicotinic acid).

Muley *et al.* (2012) demonstrated the anthelmintic activity of aqueous and ethanolic extract of fruit peel of *Momordica charantia* and illustrated that ethanolic extract of peels of fruits *Momordica charantia* showed better anthelmintic activity than aqueous extract as compared to the standard drug, piperazine citrate.

Vinoth *et al.* (2012) screened phytochemical components in methanol, ethanol, and acetone extracts of *A. indica* and revealed the presence of tannin, reducing sugar, and glycoside in methnolic extract. Reducing sugar, flavonoids, and saponin in ethanolic extract and terpenoids, reducing sugar, and glycoside in acetone extract.

Jiju *et al.* (2013) reported methanolic extract of *Asystasia gangeticum* leaves screened for anthelmintic activity against roundworms (*Ascaridia galli*) and earthworms (*Pheretima posthuma*) at two different concentrations (10-100 mg/ml). They found the extract has significant anthelmintic activity at the highest concentration of 100 mg/ml as compared with the standard drugs piperazine citrate (10 mg/ml). The preliminary phytochemical analysis disclosed the presence of phenols, tannins, alkaloids, flavonoids, terpenoids, and saponins.

El-Sherbini and Osman (2013) evaluated the anthelmintic potential of *Mangifera indica* L. for inhibition of larval development. The results revealed that aqueous extract showed 100 % inhibition of larval development at concentration 100 mg/ml. The phytochemical analysis revealed the presence of tannins and flavonoids.

Krishna *et al.* (2013) evaluated the anthelmintic activity of corm ethanolic extracts of *M. paradisiaca* cv. at Three different concentrations viz., 25 mg/ml, 50 mg/ml, and 100 mg/ml.The results manifested that the corm ethanol extract showed a significant effect at the concentration of 100 mg/ml and took less time (42.33±1.45 min) to get paralyzed at and die (54.00±0.58 min) than the control group (paralysis: 142.67±1.45 min; death:168.00±1.53 min). They stated the corm ethanol extract showed the antihelmintic activity in a dose-depend manner than the standard drug piperazine citrate (paralysis on 39.67±0.88 min and death at 59.00±0.58 min). The results of the qualitative phytochemical screening depicted the different phytoconstituents present in corm extracts namely flavonoids, glycosides, terpenoids, Sterols, and tannins.

Pekamwar *et al.* (2013) evaluated the anthelmintic potential of *Hibiscus rosa-sinensis* by means of time taken to get paralyzed and die. Two different (aqueous and ethanolic) extracts were tested for anthelmintic activity and revealed that both showed comparable anthelmintic activity with standard drug albendazole. Both extracts (ethanolic and aqueous) underwent for phytochemical screening and showed the presence of flavonoids, and tannins compounds.

Mini *et al.* (2013) evaluated that *in vitro* anthelmintic assay of aqueous and ethanol extracts *Aristolochia indica* against *H. contortus* with Egg Hatch Assay (EHA), and Larval development assay (LDA). For the egg hatch assay (EHA), eggs were treated with the concentration 20, 50, and 100 mg/ml were compared with fenbendazole at 1μg/ml and 25 μg/ml. In the larval development assay (LDA), the larvae were treated with the concentrations 5, 20, 40, 80 and 100mg/ml and fenbendazole as the standard drug. Egg hatch assay revealed, 90 % and 70 % inhibition by aqueous, and ethanol extracts of *A. indica*, respectively at a concentration of 100 mg/ml as compared to standard drug (41.05 to 47.04 % inhibition) at 25 μg/ml. They also reported that in larval development assay 60.20 % and 50.83 % of larval inhibition was found at concentration of 100 mg/ml in both aqueous and ethanolic extracts of *A. indica* respectively. The qualitative analysis of both extracts disclosed the presence of flavonoid, tannin, phenol, terpenoid, saponin, and quinine.

Swarnakar and Kumawat (2014) evaluated *in vitro* antiparasitic activity of the alcoholic extract of *Citrullus colocynthis* against amphistome parasites *Orthocoelium scoliocoelium*. The results revealed that parasites showed complete loss of motility followed by mortality at concentration 40 mg/ml after 5 hours of exposure. Histological observation disclosed wide scale distortion in the tegumental structural design of treated parasites with rupture and impartiality in surface tegument.

Apte *et al.* (2014) investigated the anthelmintic activities of alcoholic and aqueous extract of seeds of *Trachyspermum ammi* L. The results revealed that both the extract showed anthelmintic potency at all concentration (10 mg/ml, 20 mg/ml, 40 mg/ml) as compared with standard drug albendazole.

Kumar *et al.* (2014) evaluated the anthelmintic activity of ethanolic extract of *Leucasindicavar nagalopuramiana* at various concentrations (i.e., 10 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml). The results revealed that this plant had an anthelmintic activity greater than standard Phytochemical study revealed the presence of saponins, alkaloids, and carbohydrates.

Aggarwal and Bagai (2014) evaluated the potential of ethanolic and aqueous flower extract of *Calotropis procera* as an anthelmintic against *Gastrothylax indicus*, a trematode parasite, collected from the rumen of goat/sheep. They found death at 0.5± 0.05 h and 0.75± 0.10 h for ethanolic and aqueous extracts, respectively at 50mg/ml as compared to albendazole (0.08±0.01 h) at concentration 80 µg/ml.

Agrawal *et al.* (2015) compared the anthelmintic efficacy of the ethanolic extract of *Aegle marmelos leaves* and flowers of *Hibiscus rosa-sinensis* with various standard drugs such as piperazine citrate, pyrantel pamoate, and albendazole at the concentration of 20 mg/ml. The results revealed that the ethanolic extract of *Aegle marmelos* and *Hibiscus rosa-sinensis* both showed potent anthelmintic activity as shown by standards.

Singh *et al.* (2015) evaluated the *in vitro* efficacy of the aqueous extract of seeds of *Butea monosperma* (Lam.) Kuntze as an anthelmintic against *H. contortus*. The quantitative phytochemical analysis revealed the concentration of flavonoids (238.17 ± 19.14 mg of quercetin/g extract), phenolic (11.93 ± 0.64 mg of GAE/g of extract), and tannin (10.80 ± 0.70 mg of GAE/g of extract) content. The observations revealed the death of the adult *H. contortus* at the concentrations of 100 mg/ml after 6 hours of exposure, of while death occurred at a concentration of 50 mg/ml after the post-exposure of 8 hours. At 25 mg/ml concentration 50 % death was recorded at 6 hours of post-exposure and completed (100 %) at 8 hours of post-exposure as compared to the reference drug (levamisole) which showed 50 % mortality at concentration of 0.5 mg/ml at 2 hours post-exposure and 100 % mortality at 4 hours post-exposure. They have stated this nematocidal activity may be due to the presence of high amount of phytochemical component.

Meenakshisundaram *et al.* (2016) demonstrated *in vitro* and *in vivo* evaluation of anthelmintic efficacy of the ethanolic extract of *Indigofera tinctoria*. They reported that the ethanolic extract showed significant ($p<0.01$) inhibition of egg hatching at concentrations of 40 mg/ml and 80 mg/ml whereas *in vivo* assay depicted extract of *I. tinctoria* decreased the fecal egg count ranging 30.82 % to 47.78 % at various doses (125, 250 and 500 mg/kg).

Balqis *et al.* (2017) demonstrated *in vitro* evaluation of extract of betel nuts, *Veitchia merrillii* on the tegumental surface damage of *Ascaridia galli*. They revealed the ethanolic extract of betel nuts, *V. merrillii* which induced surface changes causing cuticular destruction of *A. galli*. They also found the ethanolic extract of *V. merrillii* betel nuts contains a different type of phytochemical components such as tannins, alkaloids, flavonoids, triterpenoids, and saponins.

Lone *et al.* (2017) performed an experiment to investigate the efficacy of the aqueous and methanolic extracts of *Prunella vulgaris* as an anthelmintic against gastrointestinal nematodes of sheep under both *in vitro* and in *vivo* conditions with faecal egg count reduction percent (FECR %), worm motility inhibition assay, egg hatch assay and assay. They reported 94.44 % significant ($p<0.01$) mean percentage mortality by both methanol extract and aqueous extract of *P. vulgaris.* They also reported in egg hatching assay, the methanolic extract (LC50=2.48 mg/ml) showed higher inhibitory effects as compared to that of aqueous extract (LC50=3.36 mg/ml). Maximum reduction (92.86 %) in faecal egg counts, was recorded for methanolic extract followed by aqueous extract (80.34 %) in *in vivo* assay with dose 2 g/kg of body weight per day 15 post-treatment.

Ngaradoum *et al.* (2017) demonstrated that both aqueous and methanolic extracts of *Ziziphus mucronata* have anthelmintic activity on eggs hatch assay and larval mortality assay of *H. contortus* parasite. The activity may be related to the presence of biological components such as saponins and tannins.

Koorse *et al.* (2018) conducted a study to assess the *in vitro* ovicidal, larvicidal and adulticidal activity of methanolic extract and its fractions from fruits of *Piper longum* against strongyle ova, larvae, and adult amphistomes respectively. The results revealed the n-hexane fraction was potent anthelmintic (IC50 of 1.383 mg/ml) inducing larval mortality, while chloroform fraction inhibited (IC50 of 1.796 mg/ml larval migration). They also reported methanolic extract of *P. longum* showed potent IC50 of 5.493 mg/mL adulticidal activity on amphistomes. The phytochemical analysis revealed the presence of phenolics groups, tannins, flavonoids, glycosides, diterpenes and triterpenes in extract and fractions of *P. longum.*

Muthee (2018) reported the percentage of faecal egg count reduction against the mixed gastrointestinal nematodes as 59%, -11 %, -31 % and 87 % for *Myrsine africana*, *Albizia anthelmintica*, *Embelia schimperi* and albendazole, respectively.

Chapter 3

Prevalence investigation and identification of the gastrointestinal parasite

3. 1 INTRODUCTION

In the Indian agriculture system, goats are the most widely adopted livestock and progressively used to enhance the cash income and improve food security, consequently help in the household livelihood strategies. Its rearing has significant advantage over increased economic value and management issues (such as a low effort requirement, small initial investment, ease selling, and early sexual maturity) over other livestock.

Apart from their valuable importance, goat livestock had been severely suffering from gastrointestinal parasitism which is responsible for lowering productivity, reduced animal performance, involuntary culling, rough hair coat, decreased food intake, increase in weight loss, fast breathing, submandibular oedema (bottle jaw), reduced milk production (Lebbie *et al.*, 1994; Badran *et al.,* 2012; Risso *et al*., 2015), and mortality and morbidity in heavily parasitized animals (Negasi *et al.,* 2012) resulting in severe economic losses. The intensity of the gastrointestinal parasitic infestation in goats might be due to the susceptibility of goats to internal parasites due to their poor immunity as compared to other livestock species.

Helminthiasis is the predominated parasitic diseases affecting goat livestock yield worldwide, especially in hot and dry areas (Torres-Acosta and Hoste, 2008; Calvete *et al*., 2014). Huge numbers of endoparasites and their prevalence in goats have been investigated in different studies from all over the world such as Namibia (Kumba *et al.,* 2003), Turkey (Umur, 2005) Nigeria (Nwosu *et al.*, 2007), Pakistan (Raza *et al.*, 2007), Kenya (Odoi *et al.*, 2007), Ethiopia (Sissay *et al.*, 2007a), South Africa (Gwaze *et al.*, 2009), India (Sutar *et al.*, 2010), Nepal (Karki *et al.,* 2012); Cameroon (Ntonifor *et al.,* 2013); Tanzania (Sharma and Mandal, 2013), Nigeria (Olanike *et al.,* 2015) etc.

The major risk factors of helminthiasis are categorized as– Host factors, including age, gender, genetic resistance and, physiological condition of the animal; Epidemiology species; Environmental factors such as climatic condition, nutrition status, pastoral management, and stocking density (Odoi *et al.*, 2007) which simultaneously have a great influence on the prevalence of gastrointestinal parasitism.

Globally, various parasitic diseases are highly prevalent in goat livestock. In case of host-parasitic interactions, the onset of diseases represents a decline in the resistance of the host and an increase in the infection rate for the hostility of the parasites. Commercial goat livestock rearing is posed with so many constraints. Parasitic diseases, specially helminthiasis leading to a condition known as Parasitic Gastro-Enteritis (PGE), is the major health problem which is the prime reason for non-profitable production all over the world (Chiejina, 1986). The most common helminth infestation seen in the goats and frequently contributing to PGE disease complex are *Haemonchus* spp., *Cooperia* spp., *Bunostomum* spp, *Trichostrongylus* spp., *Oesophagostomum* spp., *Strongyloides* spp., *Trichuris* spp. (Ross *et al.* 1961; Fabiyi, 1970; Ikeme, 1997), *Fasciola* sp., *Paramphistomum* sp., and *Moniezia* spp. (Ikpeze and Nzemeka, 2009; Gupta *et al*., 2013).

Generally, severe gastrointestinal pathogenesis has been accredited to the movement of the infective larval stage after ingestion instead of the adult worms in the intestine (Dube *et al.*, 2002). The occurrences of frequency of parasitic infestation and clinical pathogenicity depend on the various environmental factors of those areas (Sharma *et al.,* 2009). Thus keeping in view the damage caused by the gastrointestinal helminths, priority should be given in successful formulation and implementation of an efficient and effective strategic control through periodic surveillance of the incidence of gastrointestinal helminths parasite within a given environment. The associated risk factors that influence their transmission also requires an in depth study for its control. The detailed understanding of the parasite epidemiology, knowledge of infective stages, and the factors that affect parasite growth are essential for controlling and management of internal parasites (Tembely *et al.*, 1997; Waller and Thamsborg, 2004; Sissay *et al.*, 2007b).

Very few researches on the prevalence of gastrointestinal helminths infection of goats in Uttar Pradesh were carried out and especially in Lucknow no systematic work on these parasites is conducted. Thus the present study, hence is performed at Lucknow, UP to generate a baseline data which will be very helpful in formulating an appropriate strategic parasite control program and also for effective control of these parasitic infestations and further to minimize the economic losses and domestic goat farming.

3.2 MATERIALS AND METHODS

3.2.1 Study area

The prevalence study was carried out during November, 2014 to October 2016 to investigate the epidemiology of gastrointestinal helminth parasites of goats in Lucknow, Uttar Pradesh. Lucknow is the capital of Uttar Pradesh and situated on the Northern Gangetic plains of India. Geographically, Lucknow is situated between latitudes 26"30' to 27° 10 from the Northern side and longitudes 80°30' to 81° 13' from Eastern side and elevation location at the height of 123 meters above sea level. Lucknow is surrounded by so many villages such as Chinhat, Bakshi ka talab, Malihabad, Rahimabad, kakori, Alamnagar, Jugganr, Bijnaur, Banthia, Utrathia, Mohanlalganj, Nagram, Gosainganj, Nigoha etc. The city is bounded by Barabanki on the east, Unnao on the west, Raebareli on the south whereas Sitapur and Hardoi towards north (UPDG, 1959; UNDP, 2004). Warm and humid subtropical climatic condition prevails in Lucknow during three seasons: 1. Torrid and steamy summers (March to June), 2. Cool, shrivelled winters (November to February), 3.Monsoon (July to October) with an average annual rainfall of 896.2 millimetres. An extremely hot temperature (40 °C to 45 °C) is recorded during the months of May to June and very low temperature (3°C to 7 °C) in the month of January. The average annual percentage of humidity is 59.0 % which is higher in the month of August, September and low in the month of April (Singh *et al*., 2012; IMD, 2014a; IMD, 2014b).

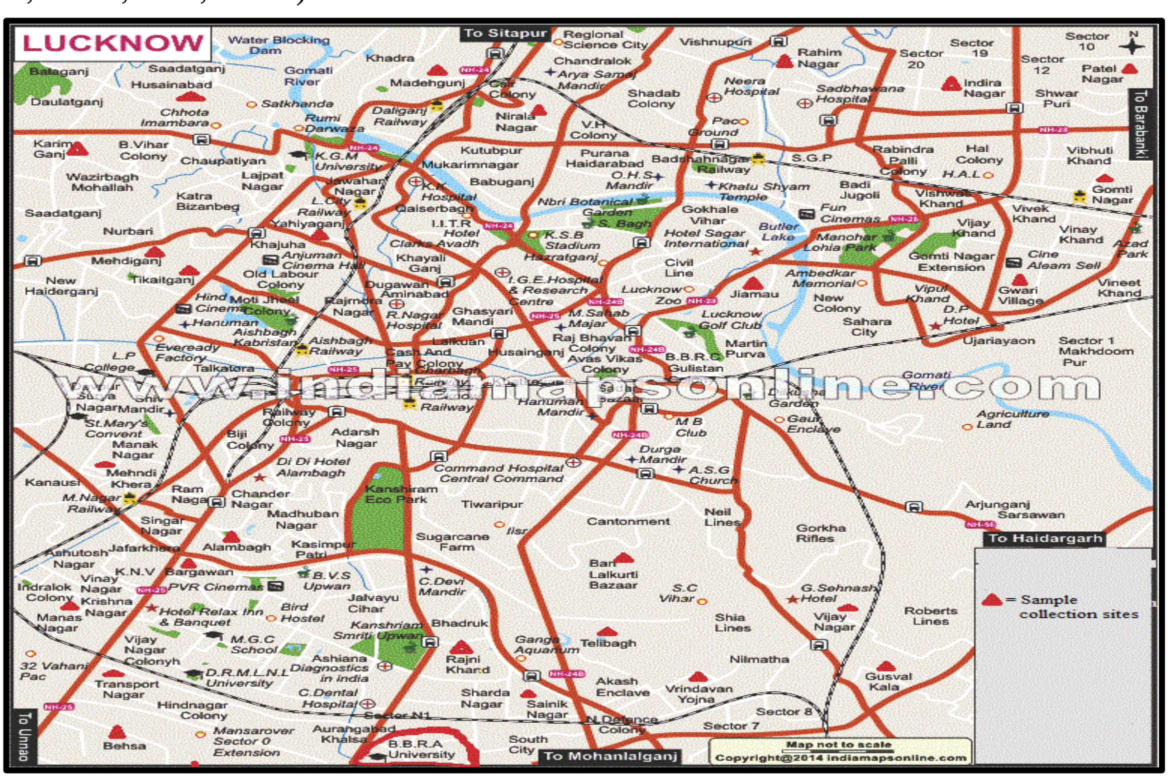


Fig. 3.2.1: Map of study area showing different sample collection sites (source: IND map.com)

3.2.2 Sample collection

A total of 355 gastrointestinal (GI) tracts of goats were collected randomly as per the age and gender of the slaughtered animal from the local slaughterhouses located at of different parts of Lucknow, Uttar Pradesh. The GI tracts were tied at both (to prevent the leakage of internal material) the end and kept in the polythene bags and brought to the Parasitology and Silkworm Pathology Laboratory of Dept. of Zoology (formerly the Department of Applied Animal Sciences) BBAU, Lucknow. Each part of the gastrointestinal tract was examined carefully for the presence of helminth parasites by following the method described by Cable, 1958; Mayer and Olsen, 1975. Collected parasites were counted and transferred to fresh normal saline (0.9 %) (Figure 3.2.2. i, ii, iii, iv, and 3.2.2.v).

Fig 3.2.2.i: Dissection of rumen of gastrointestinal tract of *Capra hircus* (L)

Fig 3.2.2.ii: Dissection of rumen and omasum of gastrointestinal tract of *Capra hircus* (L)

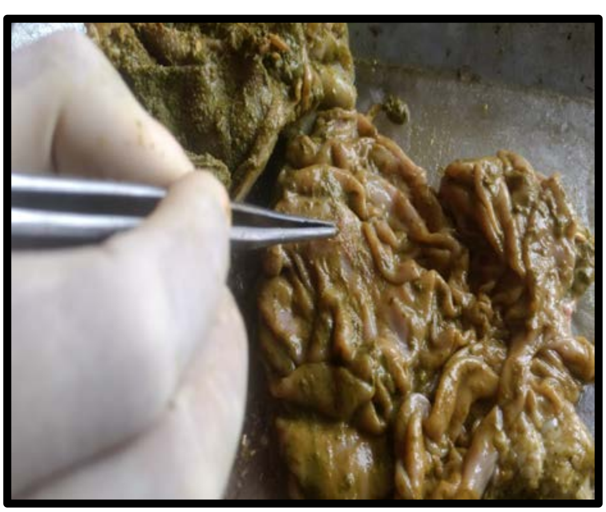


Fig 3.2.2.iii: Dissection of abomasum of gastrointestinal tract of *Capra hircus* (L)

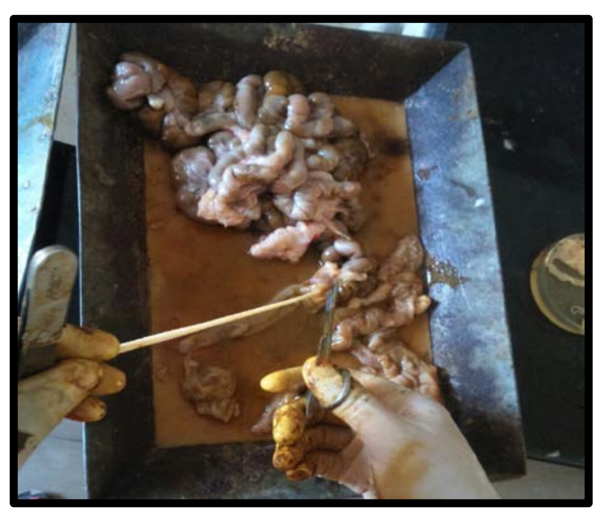


Fig 3.2.2.iv: Dissection of small and large intestine of gastrointestinal tract of *Capra hircus* (L)

Nematodes

Nematodes and Trematodes

Cestode

Fig 3.2.2.v A,B,C: Collection of gastrointestinal helminths parasites of *Capra hircus* (L)

3.2.3 Processing of gastrointestinal helminths parasites

3.2.3.1 Processing of Cestodes and Trematodes

Cestodes and trematodes were processed through the steps as described under-

- **Fixation**

The body parts of the cestode parasite were dissected into smaller region- Neck region (scolex), 2. The immature region, 3. Mature and gravid region, and in case of trematode the whole parasite, were gently pressed between two slides and allowed to fix using the Carnoy's solution as a fixative for 24 hours to one week depending on their size (Mayer and Olsen, 1975 and Cable, 1977).

The Carnoy's fixative is composed of the following reagents given below:

25 ml of glacial acetic acid

75 ml of ethanol (Absolute)

- **Preservation**

After fixation, all parasite specimens were washed in distilled water for 2 to 4 times to remove fixative. The parasites were then processed through 30% → 50% → 70% grades of alcohols and finally preserved in 70% alcohol for further use.

- **Staining**

The preserved parasites (in 70% alcohol) were hydrated through descending grades 70 % →50 % → 30 % of alcohol, washed in distilled water for 2 to 4 times and then placed in aqueous Borax Carmine (a staining reagent) for 10 to 30 minutes depending on the thickness of the parasites and then the parasites were destained using acid water (1-2 ml of concentrated HCl in 100 ml distilled water) to achieve the desired intensity. When the parasites took appropriate stain then they were washed in distilled water and dehydrated with ascending grades 30 % → 50 % → 70 % → 90 % → 100 % of alcohol for 10 to 30 minutes (depending on the size of the parasites) in each grade. After complete dehydration, parasites were transferred into Xylene (clearing agent) for 2 to 5 minutes.

- **Mounting**

The Xylene treated parasites were then mounted with DPX (Dextrin Plasticized Xylene) and a coverslip was carefully lowered over them.

3.1.3.2 Processing of Nematodes

Nematodes were processed through the following steps:

- **Fixation**

Nematodes were thoroughly washed 2 to 3 times with normal saline (0.9 %) and then were fixed by putting them into hot 70 % alcohol for 24 hours.

- **Preservation**

Nematodes were preserved in glycerol-alcohol (70 % alcohol + 5 % glycerine).

- **Clearing**

Parasites were cleared in glycerine.

- **Mounting**

Temporary mounting was done using glycerine. Cleared nematodes were kept on the slide and then glycerine was released in drops on the slide and a coverslip was lowered on them.

3.2.3.3 Microscopy

Microscopic studies were carried out by using Light bright field microscope (Olympus CH20i) under 100X magnification (10Xeye-piece ×10X objective lenses). Photographs were taken using EVOS XL imaging microscope.

3.2.3.4 Identification

All the parasites were identified by studying their morphological characters as described by Soulsby (1982).

3.2.4 Data analysis

The population dynamics of helminthiasis was recorded using the formulae described by Margolis *et al.* (1982) as mentioned under to calculate the Prevalence, Mean Intensity (MI) and Relative Abundance (RA) or Abundance.

$$\text{Prevalence} = \frac{Total\ number\ of\ hosts\ infected}{Total\ number\ of\ host\ examined} \times 100$$

$$\text{Mean intensity}(MI) = \frac{Total\ number\ of\ parasite}{Total\ number\ of\ host\ infected}$$

$$\text{Relative Abundance }(RA) = \frac{Total\ number\ of\ parasites}{Total\ number\ of\ host\ examined}$$

Statistical analysis of data was performed with SPSS (Statistical Package for the Social Sciences) 20.0 software by applying χ^2 test and values of $p \leq 0.05$ was considered as significant.

3.3 RESULT

3.3.1 Identification of different gastrointestinal helminths parasites

On the processing, following gastrointestinal helminth parasites were identified in the samples collected for the research. The identification, systemic position, site of infection and morphology of each parasite is detailed to ensure chalking out prior strategy for their control.

3.3.1.a *Haemonchus* spp.

Of all the helminth parasites identified, *Haemonchus* spp. showed high prevalence rate.

Systematic position
Kingdom: Animalia
Phylum: Nemaatoda
Class: Secernentea
Order: Strongylida
Family: Trichostrongylidae
Genus: *Haemonchus*

Source:wikipedia

Site of infection

Haemonchus spp. is abomasum and very rarely in rumen of the host.

Morphology

The species is easily identified by its 'barber pole' colouration. This characteristic is due to its blood sucking nature of the parasite which filled the intestine with blood, and white ovaries coil around the blood filled intestine. Adult female length ranges from 17 to 29 mm long whereas adult male is 10 to 20 mm long. Prominent Cervical Papillae (CP) is present. Male has a prominent well-developed Copulatory Bursa (CB) having symmetrical Lateral Lobe (LL), asymmetrical Dorsal Lobe (DL) and Y- shaped Dorsal Ray (DR). Female displays a distinct Vulvar Flap (VF) or vulvar knob whereas male is identified Spicule (Sp) having mean length 398 -470µ (Figure 3.3.1.a, a-f).

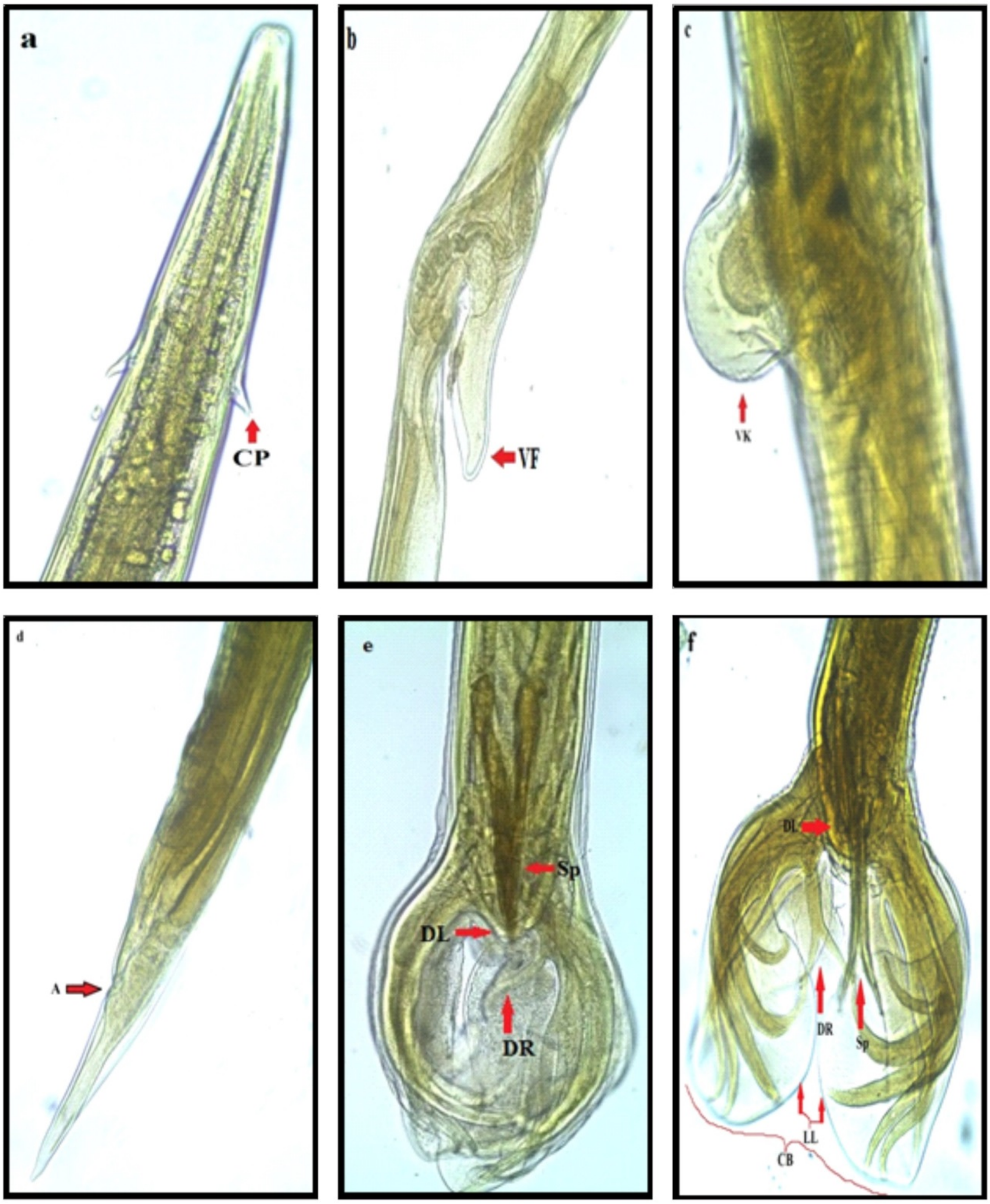


Fig. 3.3.1.a (a-f): Light microscopy of *Haemonchus* spp. a. anterior end of *Haemonchus* spp., ventral view, showing prominent paired lateral cervical papillae (CP). b.and c. Lateral view showing female Vulvar Flap (VF) and Vulvar Knob (VK). d. Female posterior tail region, ventral view showing lateral position of Anus (A). e and f. Ventral view of male posterior region, showing Y-shaped Dorsal Ray (DR), Copulatory Bursa (CB) with very similar lateral lobes (LL), Bursal Ray, Dorsal Lobe (DL) and Spicules (Sp). 100X

3.3.1.b *Bunostomum* sp.

Systematic position
Kingdom : Animalia
Phylum : Nematoda
Class : Secernentea
Order : Strongylida
Family : Ancylostomatidae
Genus : *Bunostomum*

Source:wikipedia

Site of infection

Small intestine

Morphology

Bunostomum sp. is quite thick and white to creamish in colour. Females are larger in size ranging from 18.5 mm to 24.7 mm long whereas size of males range from 11.8 mm to 16.6 mm long. They have a characteristic funnel-shaped Buccal Capsule (BC) opens antero-dorsally bears Chitinous Plate (CP) at its ventral margin and a pair of small Subventral Lancet (SL) at its base. They possess a well-developed Copulatory Bursa (BC) with an asymmetrical dorsal lobe. The Right Dorsal Ray (RDR) commences superior on the dorsal stem and longer than Left Dorsal Ray (LDR). The Vulva (V) opens in the mid of the body of female worm and male has two long Spicules (Sp) (Figure 3.3.1.b 1-3 and figure 3.3.1.b 4-6).

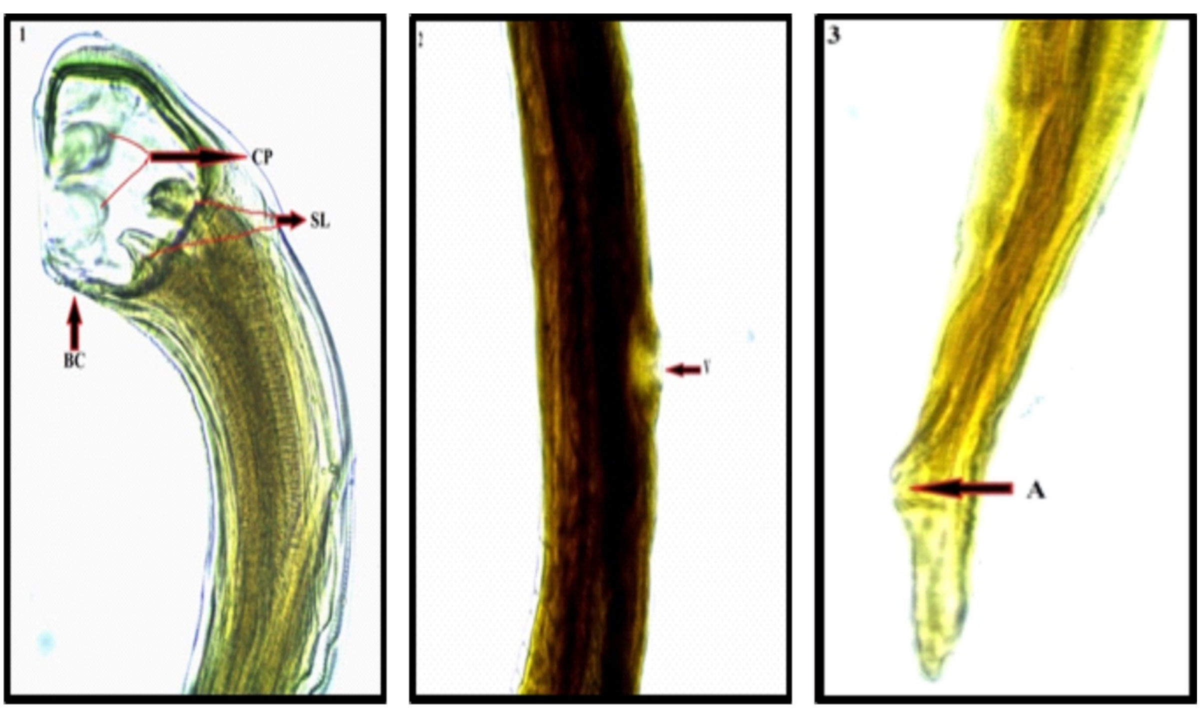


Fig. 3.3.1.b (1-3): Light microscopy of *Bunostomum* sp. 1. Anterior end of *Bunostomum* sp., ventral view, showing large antero-dorsally Buccal Capsule (BC), chitinous plate (CP) at its ventral margin, and a pair of Subventral Lancet (SL). 2 &3. Ventral view, showing lateral opening of Vulva (V) in the middle position and Anus (A) at posterior side in female species. 100X

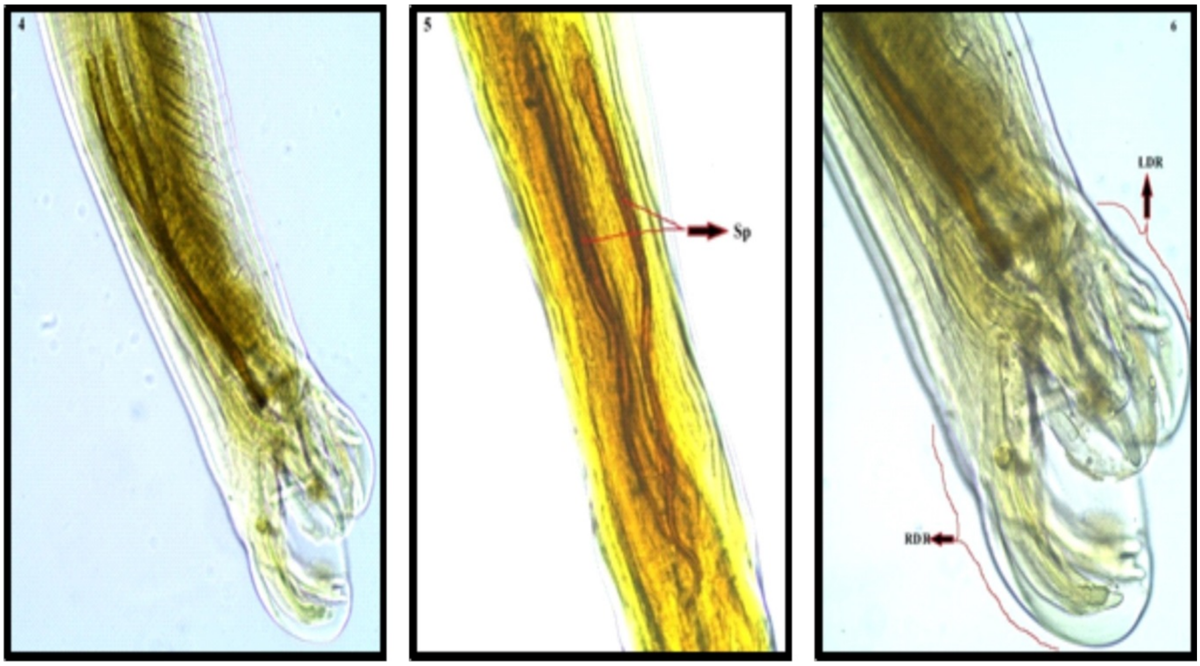


Fig. 3.3.1.b (4-6): Light microscopy of male *Bunostomum* sp. 4. Showing posterior region of male species. 5 & 6. Showing long Spicules (Sp) and well-developed bursa with asymmetrical Right Dorsal Lobe (RDR) and Left Dorsal Lobe (LDR). 100X

3.3.1.c *Oesophagostomum* spp.

Systematic position
Kingdom: Animalia
Phylum: Nematoda
Class: Nemathelminthes
Order: Strongylida
Family: Strongyloidae
Genus: *Oesophagostomum*

Source:wikipedia

Site of infection

Large intestine

Morphology

Oesophagostomum spp. is opaque white in colour and the anterior end is bent dorsally into a hook. Adult female measured around 15 to 21.5 mm long and 0.45 to 0.58 mm in width whereas mature male measured about 12.0 to 15.0 mm long and 0.42 to 0.51 mm in width. Anteriorly, truncated cones like structure known as mouth collar are formed and separated from the rest of the body by constriction. Mouth collar covered by lingering cuticle called as a Cephalic Vesicle (CeV). Prominent structure, i.e.,, Cervical Groove (CG) is encompassing around the ventral surface on to the lateral side of the body. Cuticle just anterior to cervical groove is expanded to form a Cervical Vesicle (CV) while the cuticle below this groove extended along the body is known as lateral alae (LA). The developed Buccal Capsule (BC) is surrounded by External Corona Radiate (ECR) and Internal Corona Radiate (ICR). The mature female worm showed vulva, opening anterior to the Anus (A), covered by Copulatory Cement (CC) and the tail is pointed towards the posterior end. Vagina was very short leading into kidney-shaped vestibule like ovijectors. The mature male worm had well-developed Copulatory Bursa (CB) with large Lateral Lobes (LL) and indistinct dorsal lobe. Bursa constituted with bursal rays viz, bifurcated ventral ray, both Medio-Lateral (ML) and Postero-Lateral (PL) rays that are fused proximally and well separated from the Latero-Ventral (LV) ray. Externo-dorsal rays (EDR) and Dorsal Ray (DR) are found arising together from a common trunk. Dorsal rays are bifurcated into two divergent terminal branches with further split of each branch into short Lateral Twig (LT). A Pair of Pre-Bursal Papillae (PBP) was also present. Spicules were long, equal size, and alate with Blunt Tips (BT) (Figure 3.3.1.c 1-2, Figure 3.3.1.c 3-4, Figure 3.3.1.c 5-6, Figure 3.3.1.c 7-8, and Figure 3.3.1.c 9-10).

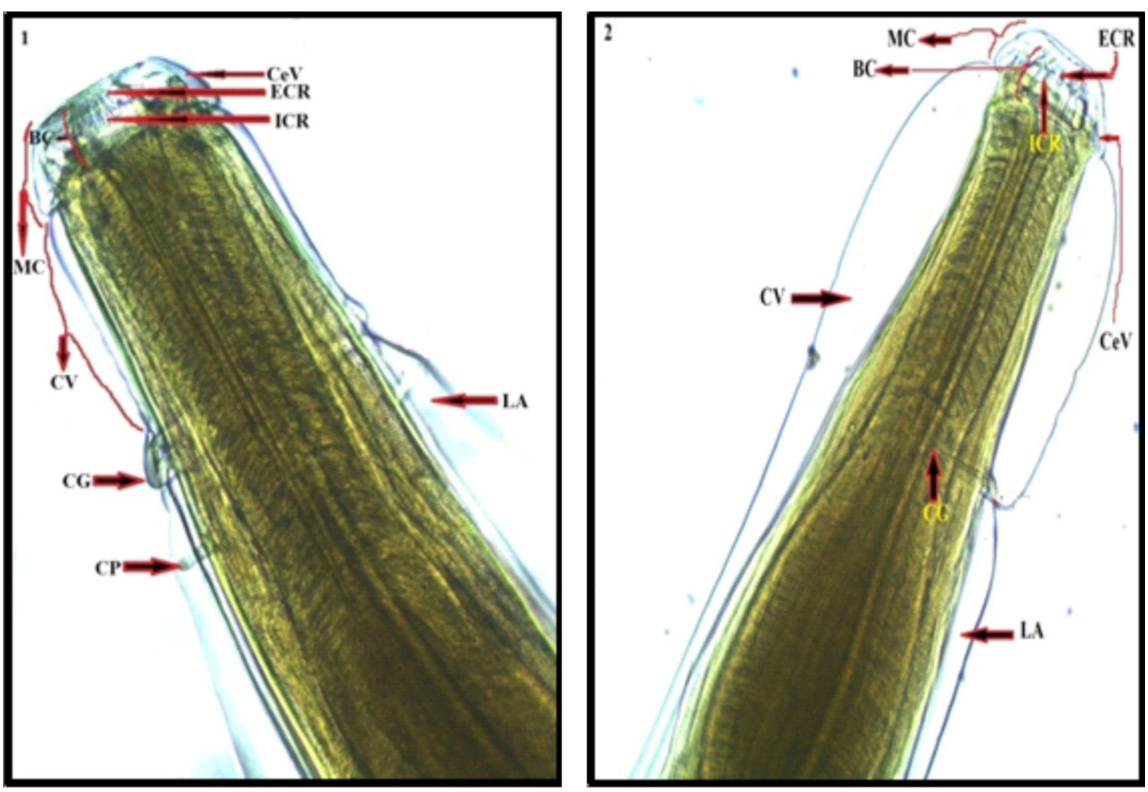


Fig. 3.3.1.c (1-2): Light microscopy of the anterior end of *Oesophagostomum* spp.,ventral view showing CG-Cervical Groove, CV-Cervical Vesicle, CP-Cervical Papillae, Mouth Capsule (MC), Buccal Capsule (BC), ICR-Internal Corona Radiata, ECR- External Corona Radiata, CeV- Cephalic Vesicle, LA-Lateral Alae.100X

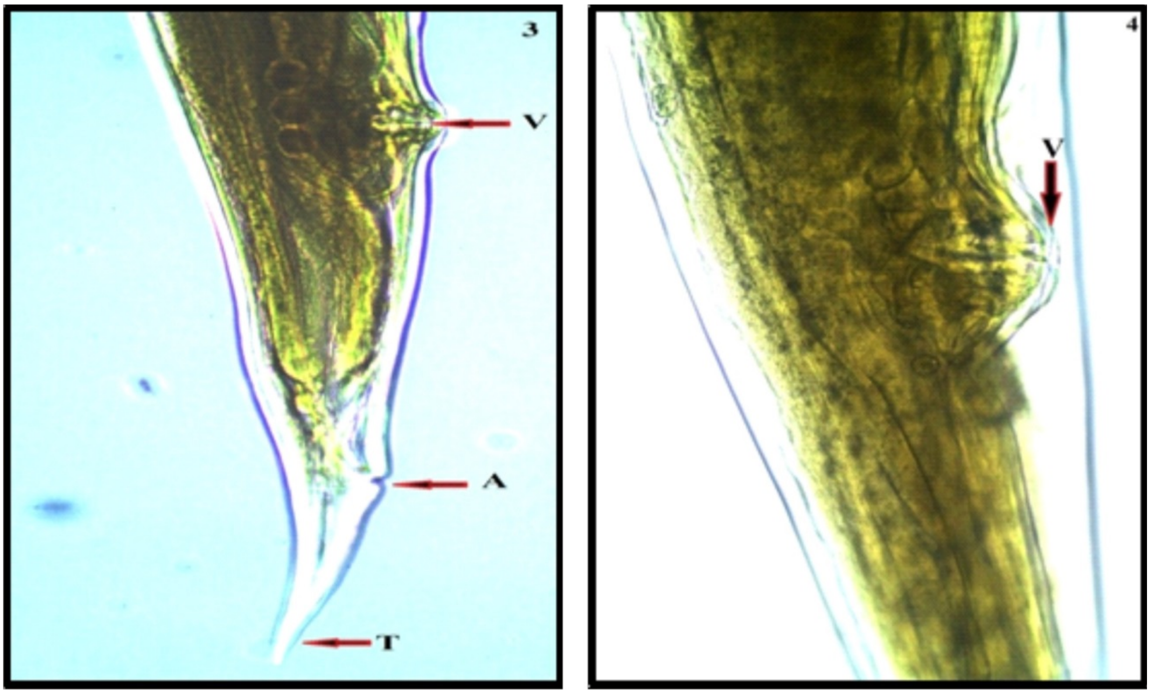


Fig. 3.3.1.c (3-4): Light microscopy of the female *Oesophagostomum* spp., ventral view, showing lateral opening of Vulva (V), a slit like structure i.e.,, Anus (A) and sharp pointed Tail (T).100X

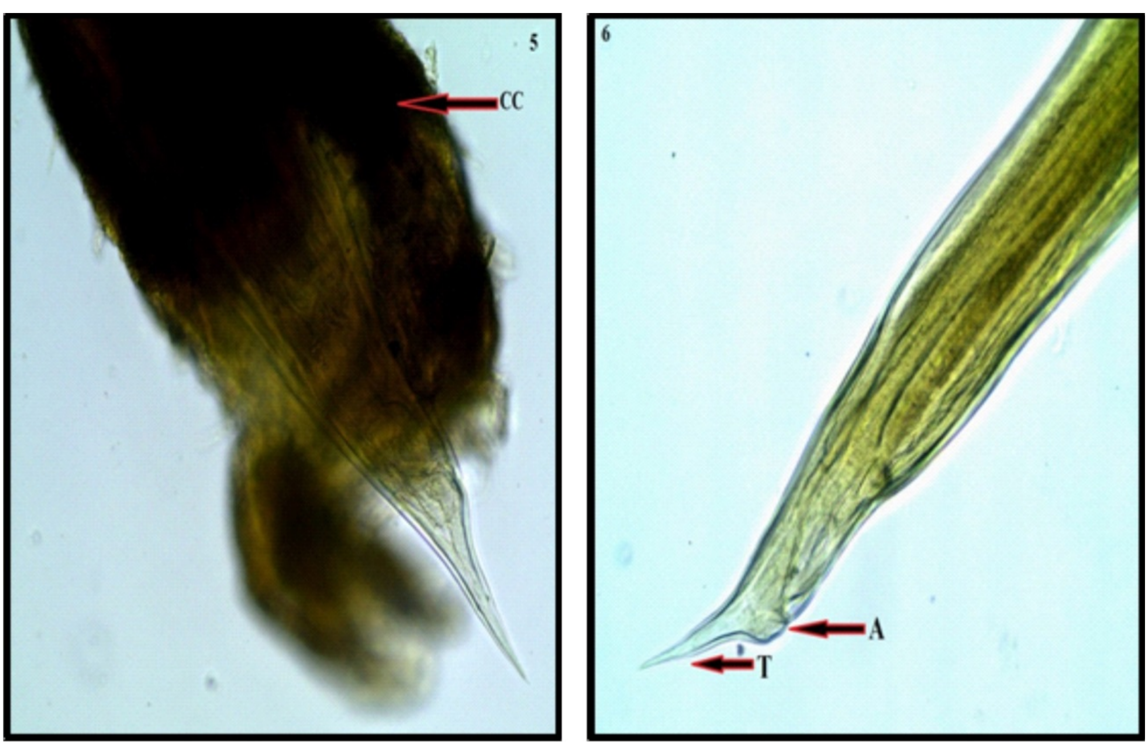


Fig. 3.3.1c (5-6): Light microscopy of the posterior end of female *Oesophagostomum* spp., showing Copulatory Cement (CC) covered the vulva region, the Anus (A) and pointed Tail (T). 100X

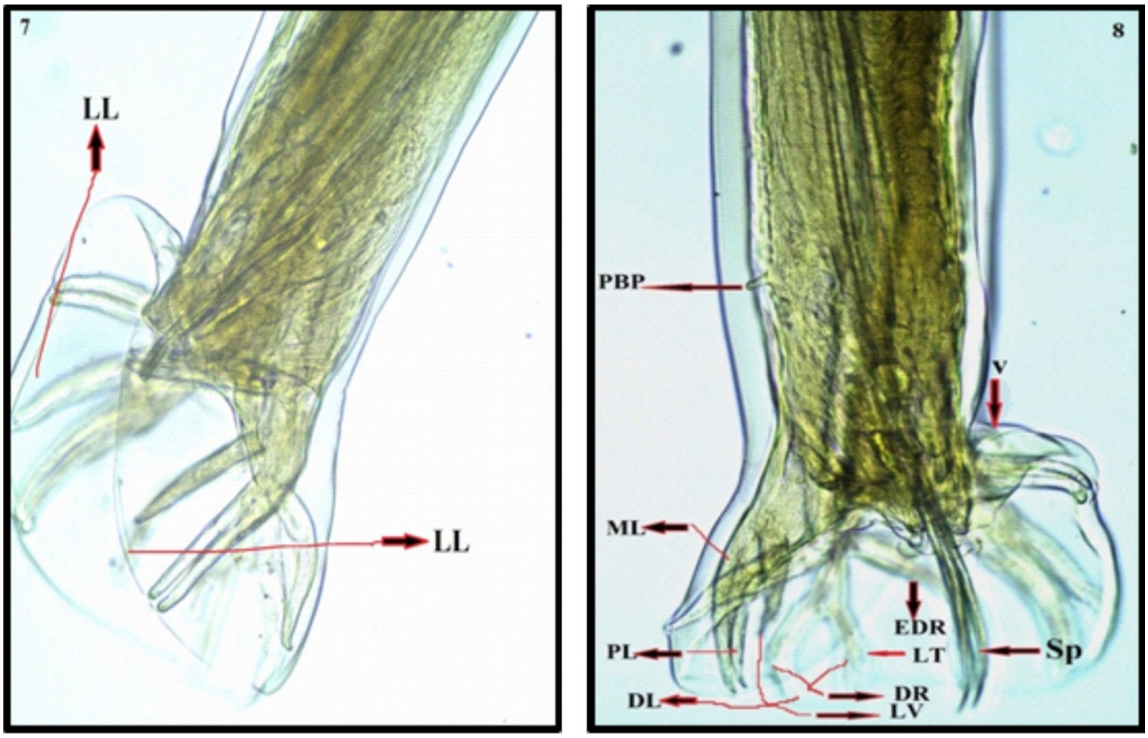


Fig. 3.3.1.c (7-8): Light microscopy of the posterior end of male *Oesophagostomum* spp., ventral view, showing, LL-Lateral Lobe, DL-Dorsal Lobe, DR-Dorsal Rays, Sp-spicules, EDR-Externo-Dorsal Ray, ML-Medio-Lateral, PL- Posteriolateral, LV-Latero-ventral, V-Veneral Ray system, PBP-Pre Bursal Papillae. 100X

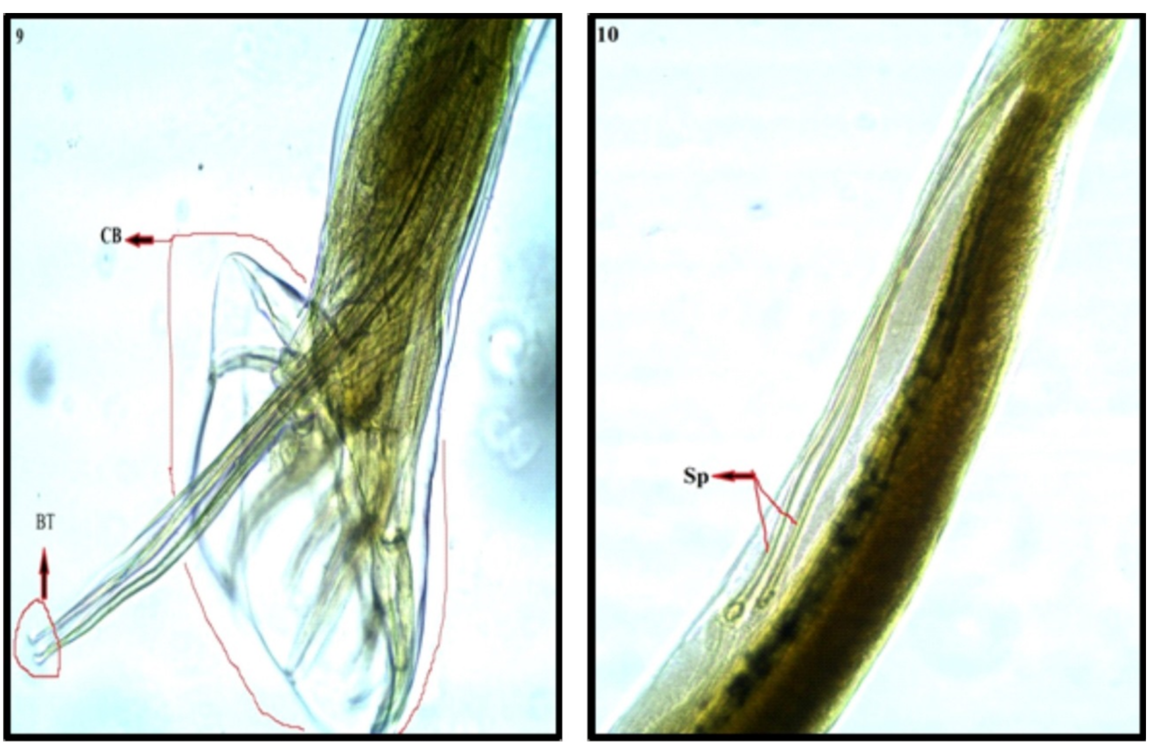


Fig. 3.3.1.c (9-10): Light microscopy of the posterior end of male *Oesophagostomum* spp., showing developed Copulatory Bursa (CB) and long Spicules (Sp) with Blunt Tips (BT). 100X

3.3.1.d *Trichuris* sp.

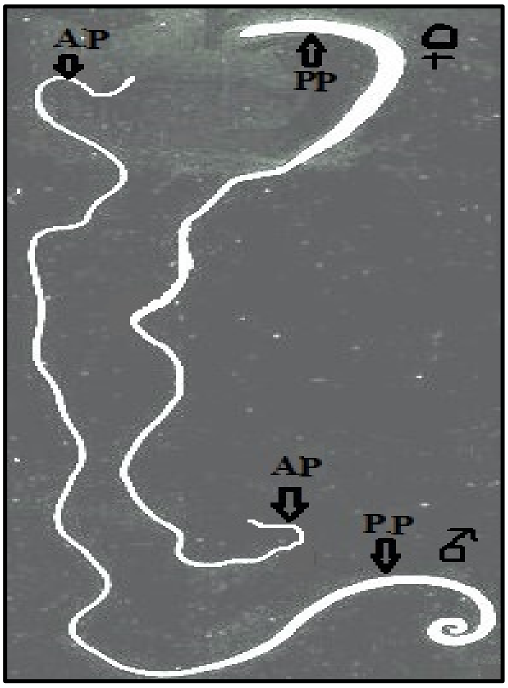


Fig. 3.3.1.d *Trichuris* sp. Male (♂) and Female (♀) Showing Anterior part (AP) and Posterior part (PP) of the body

Systematic position
Kingdom : Animalia
Phylum : Nematoda
Class : Adenophorea
Order : Trichurida
Family : Trichuridae
Genus : *Trichuris*

Source:wikipedia

Site of Infection:

Large intestine

Morphology

Trichuris sp. is off-white to creamish in colour, and commonly known as 'Whipworm'. The Anterior Part (AP) of the body is long and slender, while the Posterior Part (PP) is much thicker. The posterior end of the female was slightly curved whereas male is spirally coiled at the posterior end. The length of adult males varied from i.e.,, 34.5 mm to 69 mm long; with a thin thread like anterior end including about three-fourths of the whole body length. Single spicule was fully evaginated and ranges from 4.4 mm-5.7 mm long along with spicular sheath covered with minute spines (S). Spear shaped buccal capsule (BC) was shown, but without lips. Esophagus (E) was thin-walled. The vulva region of the female was positioned near the esophageal-intestinal junction with the genital tract having completely developed eggs (DE) in a single outline (Figure 3.3.1.d 1-4 and Figure 3.3.1.d 5-6).

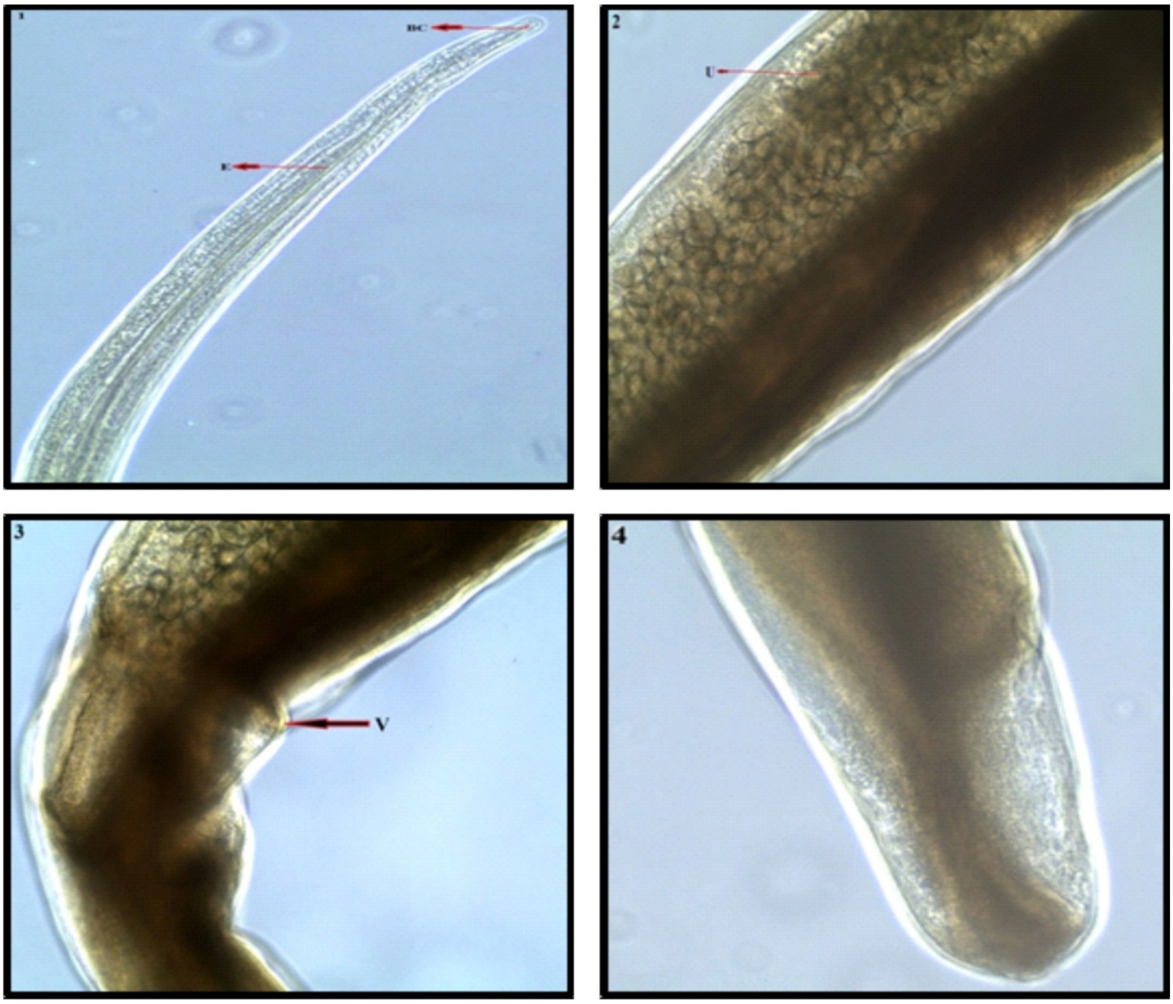


Fig. 3.3.1.d (1-4): Light microscopy of the female *Trichuris* sp., 1. Anterior part showing Buccal Capsule (BC) and Esophagus (E). 2-4. Female worm showing mature Uterus (U) with full of egg, the Vulva (V) and posterior end. 100X

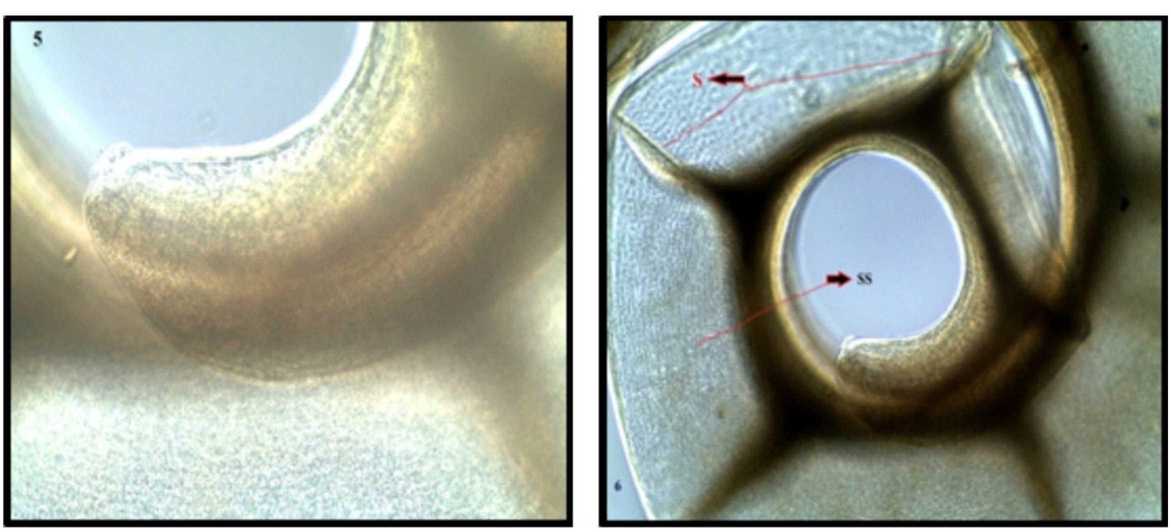


Fig. 3.3.1.d (5-6): Light microscopy of the male *Trichuris* sp., 5-6. Posterior end of showing coiled distal end and Spicular Sheath (SS) with globular expansion which is covered with Spins (S). 100X

3.3.1.e *Paramphistomum* sp.

Systematic position
Kingdom: Animalia
Phylum: Platyhelminthes
Class: Trematoda
Order: Echinostomida
Family: Paramphistomatidae
Genus: *Paramphistomum*

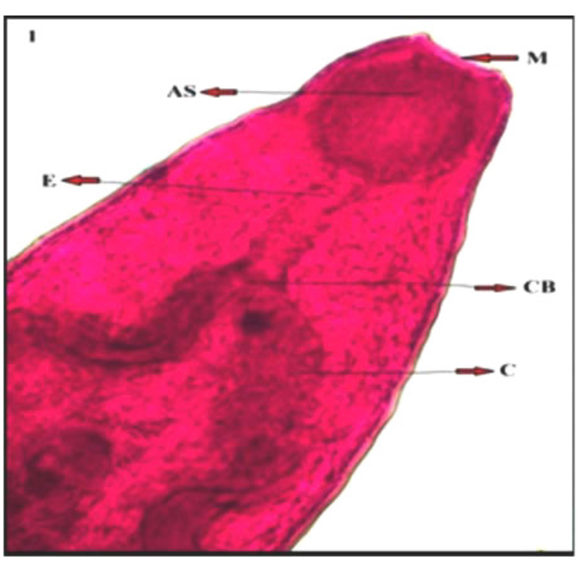
Source:wikipedia

Site of infection

Rumen

Morphology

The adult *Paramphistomum* sp. was light pink in colour and conical in structure i.e.,, the anterior end narrowing and the posterior being broad. They showed variation in size ranging from 3.9 mm to12.1 mm long, and 1.5 mm to 4.4 mm wide. They were concave to a certain extent on their ventral side whereas dorsally convex. Mouth (M) was acquired the terminal position towards the anterior side. The parasite had two prominent suckers, an anterior oral sucker and ventrally large posterior sucker (acetabulum). Esophagus (E) was well-developed and dorsally bent. Genital pore is located at the anterior third part just behind the Caecum Bifurcation (CB). Caeca (C) were wide, going posteriorly through winding passage towards the reached an anterior level of the Posterior Sucker (PS) with blind ends called intestinal caecal end (ICE). Both male and female reproductive systems were found towards the posterior region of the body. Two testes were present, anterior testes and posterior testes and both testes were slightly lobed and situated anterior to the ovary. The ovary (O) was situated just anterior to the posterior sucker and Undulating uterus (U) was found dorsally towards the testes (Figure 3.3.1.e 1-2 and Figure 3.3.1.e 3-5).



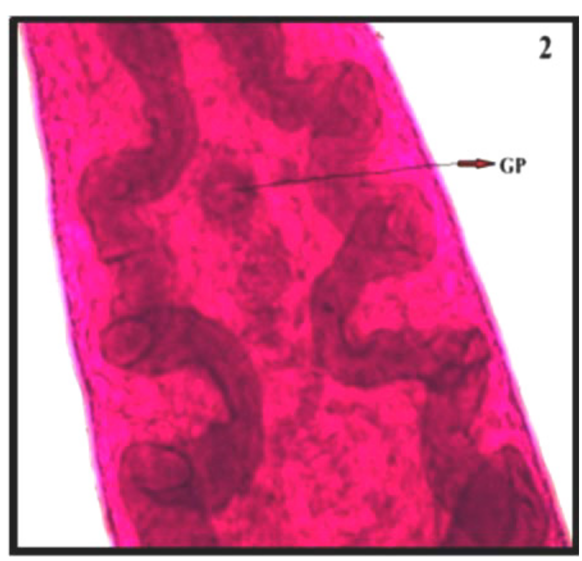


Fig. 3.3.1.e (1-2): Light microscopy of *Paramphistomum* sp., 1. Anterior part of the worm, showing terminal Mouth (M), Anterior Sucker (AS), Esophagus (E), Caecal Bifurcation (CB) and Caeca.2 showing genital pore (GP) and undulating caeca. 100X

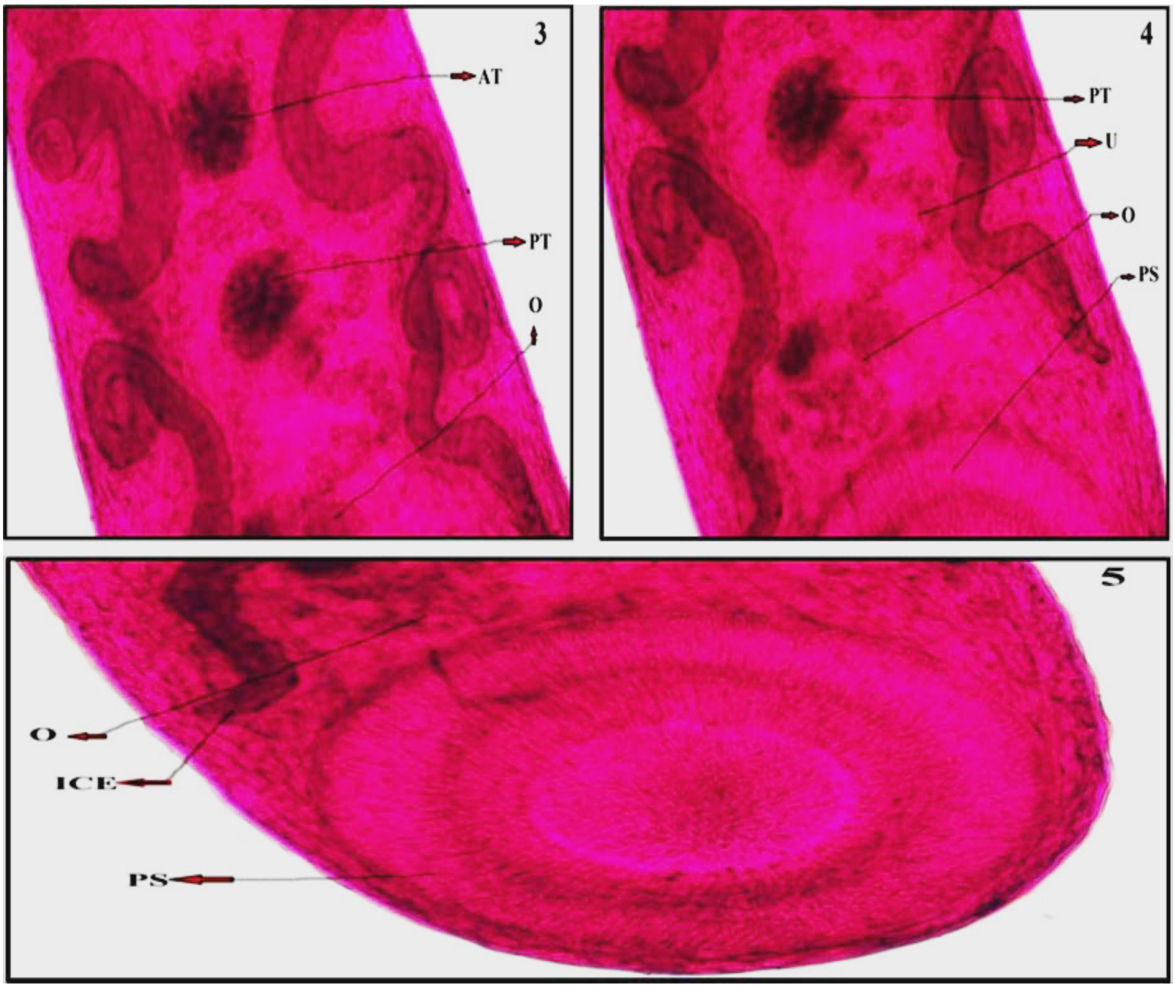


Fig. 3.3.1.e (3-5): Light microscopy of the posterior part of *Paramphistomum* sp., showing pair of testes, anterior testes (AT) and posterior testes (PT) located anterior to the ovary. Ovary (O) is situated between posterior sucker (PS) and posterior testes. Intestinal caecal end (ICE) terminated towards the lateral marginal position and anterio dorsal to posterior sucker. 100X

3.3.1.f *Moniezia* sp

Systematic position

Kingdom:Animalia

Phylum: Platyhelminthes

Class: Cestoda

Order: Cyclophyllidea

Family: Anoplocephalidae

Genus: *Moniezia*

Source:wikipedia

Site of infection

Small intestine

Morphology

Moniezia sp. is a very long tapeworm, and can extend up to 5.8 m to 9.5 m. It has a distinguishing cestode body, consisting of the scolex, followed by the Neck (N) and a highly extended body proper, the strobilus (proglottids). The scolex bears four large Suckers (S) which are oval to round in shape, arrange in two pairs, devoid of rostellar hook and spines. The proglottids have immature, mature, and gravid segments. The inter-segmental region between the proglottids is dotted with a row of Inter-Proglottid Glands (IPG). The mature proglottids are broader than long with concave and convex bilateral margins. One describing prominent characteristic of the genus is that there are two sets of reproductive organs located at the lateral sides with the associated Cirrus Pouches (CP) and the Genital Pores (GP) in each mature proglottid. Ovary (O) is an inverted cup (horse-shoe) shaped and lies on each lateral side of proglottid. The vagina is long tube like, situated posterior to cirrus pouch passing diagonally for a long distance, widens from the receptaculum seminis (RS) and reaches to ootype. Testes (T) are small, oval to round in shape and scattered all over the segments in between the longitudinal Excretory Canal (EC). The Cirrus pouch is small, oval shaped, located in the central margin of the segments. The Genital pores are large, oval in shape, bilateral marginal, and protruded outside proglottids (Figure 3.3.1.f, 1-4 and Figure 3.3.1.f, 5-6).

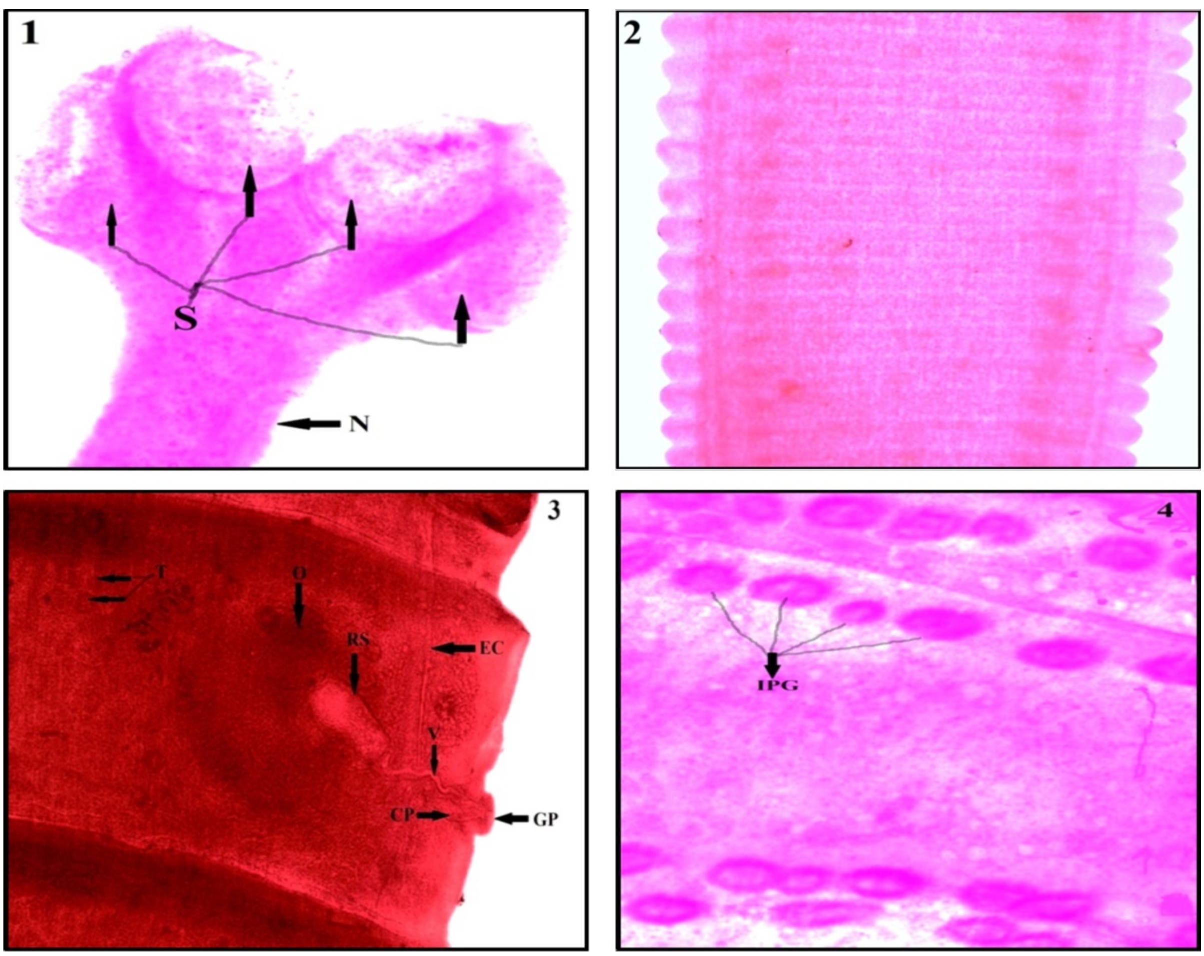


Fig. 3.3.1.f (1-4): Light microscopy of *Moneizia* sp., 1. Anterior part of the parasite showing scolex with four large suckers (S) and neck (N) region.2. Showing immature proglottids. 3. Showing the mature proglottids comprising Testes (T), Cirrus Pouch (CP), Ovary (O), Receptaculum Seminis (RS), Vagina (V), Genital Pore (GP), and logitudinal Excretory Canal (EC). 4. Showing Interproglottid Gland (IPG). 100X

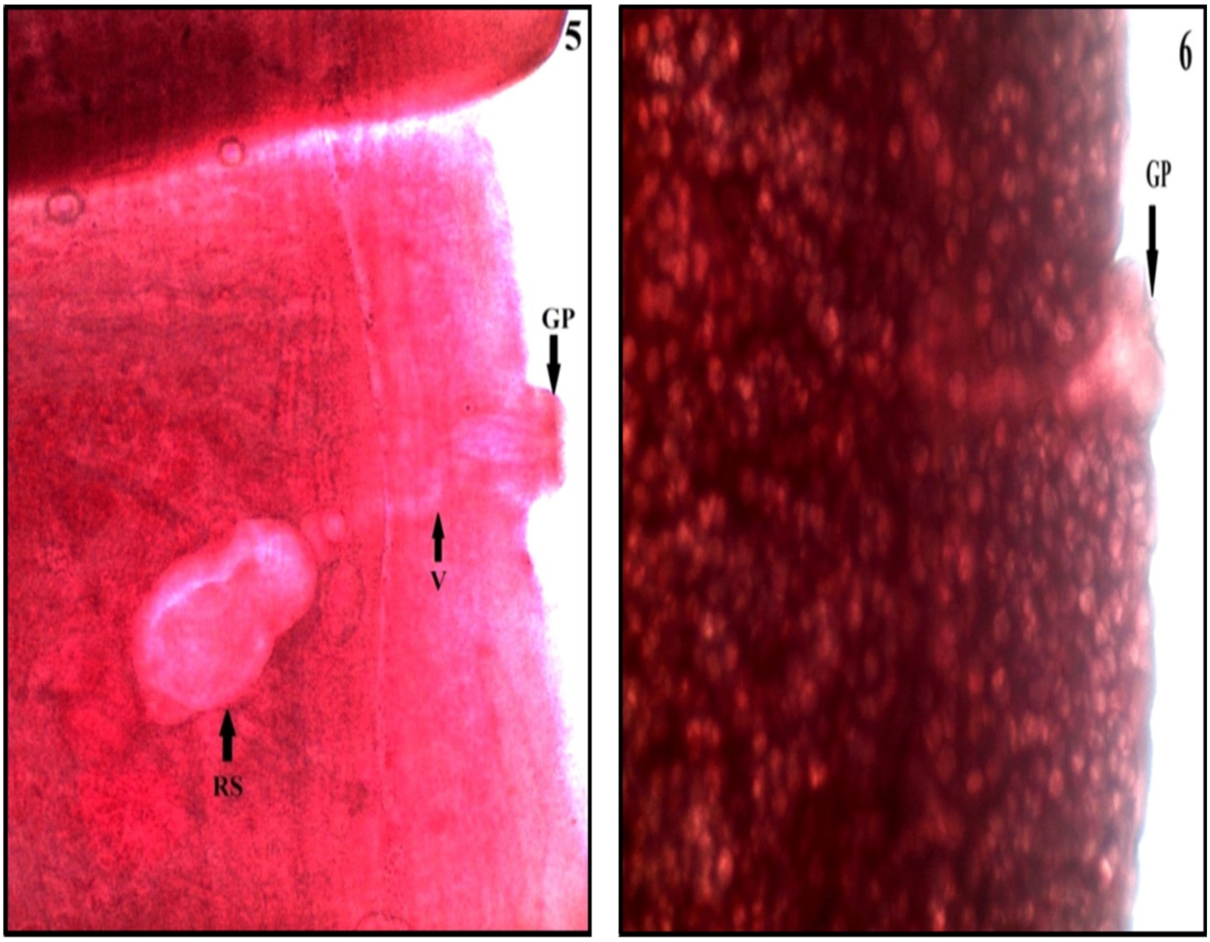


Fig. 3.3.1.f (5-6): Light microscopy of gravid proglottids of the *Moneizia* sp. showing Receptaculum Seminis (RS), Vagina (V), Genital Pore (GP) and mature uterus with full of eggs. 100X

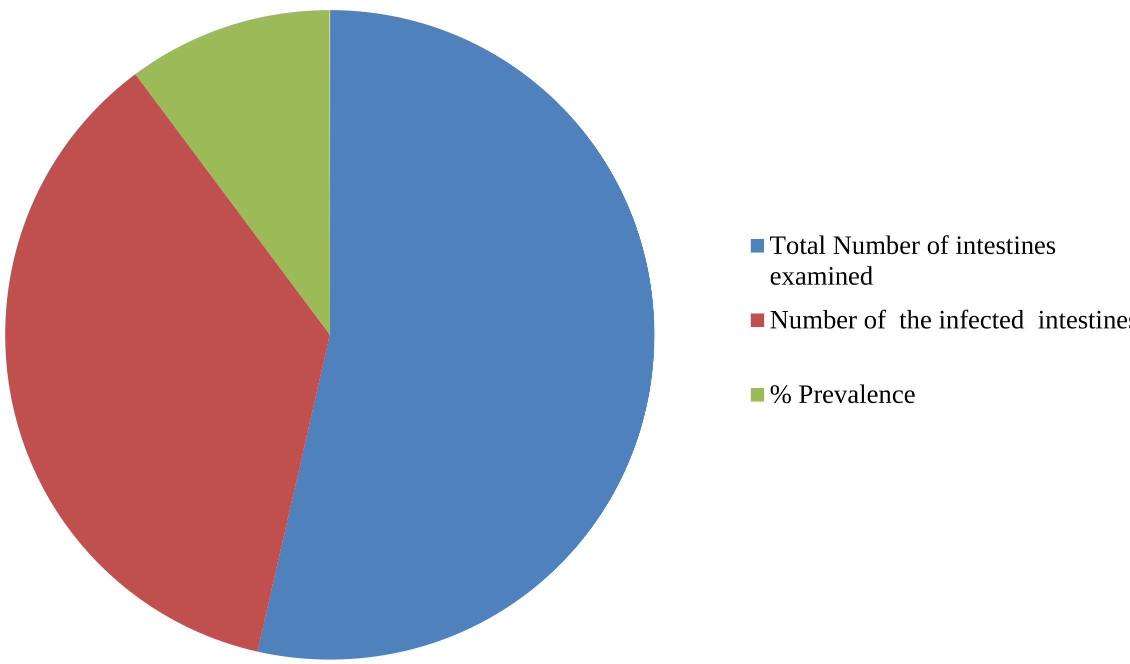
3.3.2 Prevalence of Gastrointestinal Helminths Parasites

3.3.2.a Overall prevalence of gastrointestinal helminths parasites in *Capra hircus*

A total of 355 gastrointestinal tracts were collected and examined during the study period (November 2014 to October 2016) for the infestation and it was found that 240 gastrointestinal tracts were found to be infected with different helminth species indicating 67.6 % prevalence (Table 1, Graph1). Significantly ($P \leq 0.05$) higher prevalence of nematodes (65.1 %) was found in comparison to trematodes (18.6 %) and cestode (15.5 %) (Table 2, Graph 2).

Table 1: Overall prevalence of gastrointestinal helminths parasites in *Capra hircus*

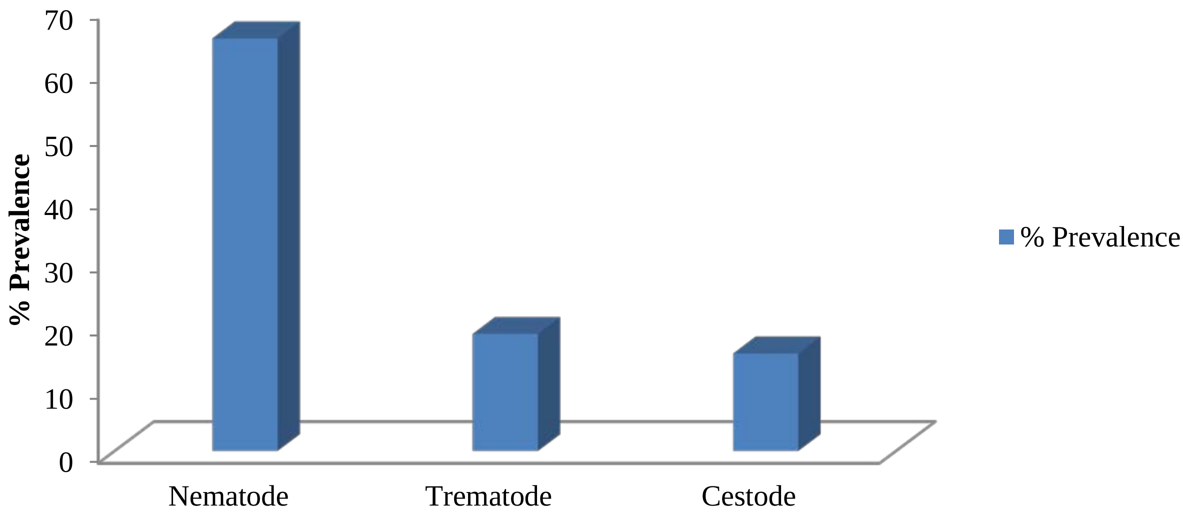
Total Number of intestines examined	Number of the infected intestines	% Prevalence
355	240	67.6



Graph 1: Overall prevalence of gastrointestinal helminths parasites in *Capra hircus*

Table 2: Prevalence of different parasitic helminths genera in *Capra hircus*

Parasitic helminth genera	Total Number of intestines examined	Number of the infected intestines	% Prevalence
Nematode	355	231	65.1
Trematode	355	66	18.6
Cestode	355	55	15.5
X^2	247.486 (P≤0.05)		



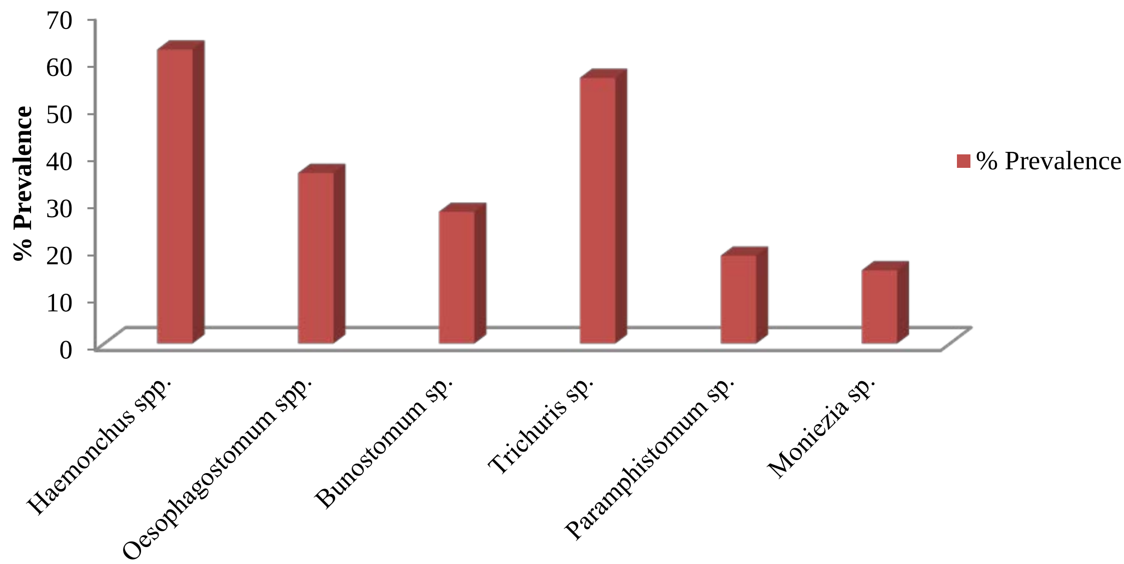
Graph 2: Prevalence of different parasitic helminths in *Capra hircus*

3.3.2.b Prevalence of different genus of gastrointestinal helminths parasites in *Capra hircus*

Six species of helminth parasites were identified and of which four were nematode-(*Haemonchus* spp. 62.3 %, *Trichuris* sp. 56.3 %, *Oesophagostomum* spp. 36.1 %, *Bunostomum* sp. 27.9 %). One species of trematode i.e.,, *Paramphistomum* sp. (18.6 %), and one species of cestode i.e.,, *Moniezia* sp. (15.5 %) were also found to be prevalent (Table 3 and Graph 3). All the gastrointestinal parasites differed significantly (P≤0.05) with their prevalence.

Table 3: Prevalence of different parasitic species in *Capra hircus*

Parasitic species	Total Number of intestines examined	Number of the infected intestines	% Prevalence
***Haemonchus* spp.**	355	221	62.3
***Oesophagostomum* spp.**	355	128	36.1
***Bunostomum* sp.**	355	99	27.9
***Trichuris* sp.**	355	200	56.3
***Paramphistomum* sp.**	355	66	18.6
***Moniezia* sp.**	355	55	15.5
X^2	291.190 (P≤0.05)		



Graph 3: Prevalence of different parasitic species in *Capra hircus*

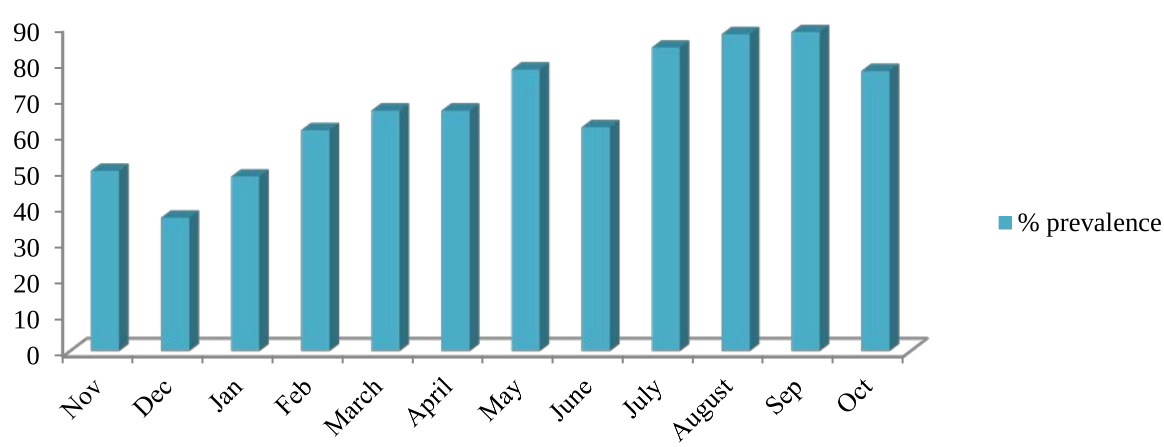
3.3.2.c Month-wise prevalence of the gastrointestinal helminths parasites

Month-wise breakdown of the prevalence of various intestinal parasites is depicted in Table 4 and Graph 4 and the results revealed that the highest incidence rate of these parasites was recorded in the month of September (88.5 %) followed by August (87.9 %) whereas the lowest was reported in the month of December i.e., 37.0 %. In addition, the data depicted in the Table 5 and Graph 5 indicating the month–wise prevalence study of different parasitic species. The data revealed that the nematode parasite namely *Haemonchus* spp., *Trichuris* sp., and *Oesophagostomum* spp. showed a significant ($P \leq 0.05$) increase in the prevalence rate from July (84.4 %, 68.8 % and 43.8 % respectively) to September (88.5 %, 80.8 % and 65.4 %) except *Bunostomum* sp. which showed significantly($P \leq 0.05$) higher prevalence rate in the month of September (61.5 %) followed by July (43.4 %) and August (42.4 %) whereas all these parasite had shown a significant ($P \leq 0.05$) decrease in the prevalence rate in the month of December.

Furthermore, *Paramphistomum* sp. showed a significant ($P \leq 0.05$) discrepancy in the prevalence rate and recorded highest in the month of July (40.6 %) followed by March (33.3 %) and not observed in the month of November and December. Although, in the case of *Moniezia* sp., a significantly ($P \leq 0.05$) higher prevalence rate was noticed only in the month of September (42.3 %) while no prevalence were recorded in the month of November, December and January (Table 5 and Graph 5).

Table 4: Month-wise overall prevalence of gastrointestinal helminths in *Capra hircus*

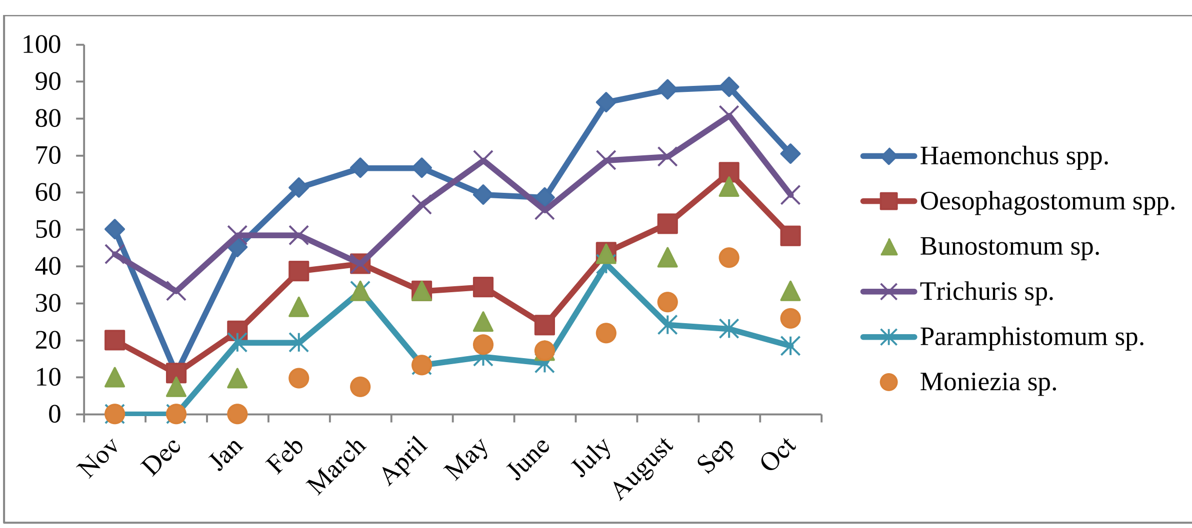
Month	Total number of intestines examined	Number of infected intestines	% Prevalence
Nov	30	15	50.0
Dec	27	10	37.0
Jan	31	15	48.4
Feb	31	19	61.3
March	27	18	66.7
April	30	20	66.7
May	32	25	78.1
June	29	18	62.1
July	32	27	84.2
August	33	29	87.9
Sep	26	23	88.5
Oct	27	21	77.7



Graph 4: Month-wise prevalence of gastrointestinal helminths parasites in *Capra hircus*

Table 5: Month-wise prevalence of helminths species in *Capra hircus*

Month	**Total number of intestine examined**	**% Prevalence of parasitic species (number of infected intestines)**					
		***Haemonc-hus* spp.**	***Oesophagos-tomum* spp.**	***Bunostom-um* sp.**	***Trichuris* sp.**	***Paramphis-tomum* sp.**	***Moniezia* sp.**
Nov	30	50 (15)	20 (6)	10 (3)	43.3 (13)	0 (0)	0 (0)
Dec	27	11.1 (3)	11.1 (3)	7.4 (2)	33.3 (9)	0 (0)	0 (0)
Jan	31	45.2 (14)	22.6 (7)	9.7 (3)	48.4 (15)	19.4 (6)	0 (0)
Feb	31	61.3 (19)	38.7 (12)	29.0 (9)	48.4 (15)	19.4 (6)	9.7 (3)
March	27	66.6 (18)	40.7 (11)	33.3 (9)	40.7 (11)	33.3 (9)	7.4 (2)
April	30	60.0 (18)	33.3 (10)	33.3 (10)	56.7 (17)	13.3 (4)	13.3 (4)
May	32	59.4 (19)	34.4 (11)	25.0 (8)	68.8 (22)	15.6 (5)	18.8 (6)
June	29	58.6 (17)	24.1 (7)	17.2 (5)	55.2 (16)	13.8 (4)	17.2 (5)
July	32	84.4 (27)	43.8 (14)	43.4 (11)	68.8 (22)	40.6 (13)	21.9 (7)
August	33	87.9 (29)	51.5 (17)	42.4 (14)	69.7 (23)	24.2 (8)	30.3 (10)
Sep	26	88.5 (23)	65.4 (17)	61.5 (16)	80.8 (21)	23.1 (6)	42.3 (11)
Oct	27	70.4 (19)	48.1 (13)	33.3 (9)	59.3 (16)	18.5 (5)	25.9 (7)
X^2		60.643 (P≤0.05)	31.012 (P≤0.05)	37.320 (P≤0.05)	24.958 (P≤0.05)	29.400 (P≤0.05)	41.764 (P≤0.05)



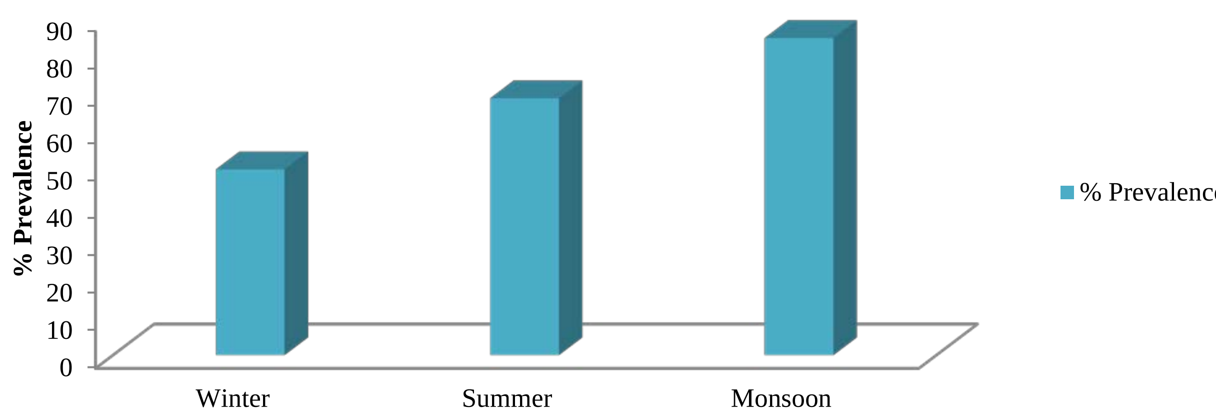
Graph 5: Month-wise prevalence of helminths species in *Capra hircus*

3.3.2.d Season-wise prevalence, mean intensity and relative abundance of the parasite

The prevalence of these parasites across the three seasons (winter, summer, monsoon) were recorded and it was found that the significantly by $P \leq 0.05$ higher incidence rate was seen in the monsoon season (87.4 %), followed by summer (68.6 %) and winter season (49.6 %) (Table 6, Graph 6).

Table 6: Season-wise overall prevalence of helminths parasites in *Capra hircus*

Season	Total Number of intestines examined	Number of the infected intestines	% Prevalence
Winter	119	59	49.6
Summer	118	81	68.6
Monsoon	118	100	84.7
X^2	33.543 ($P \leq 0.05$)		



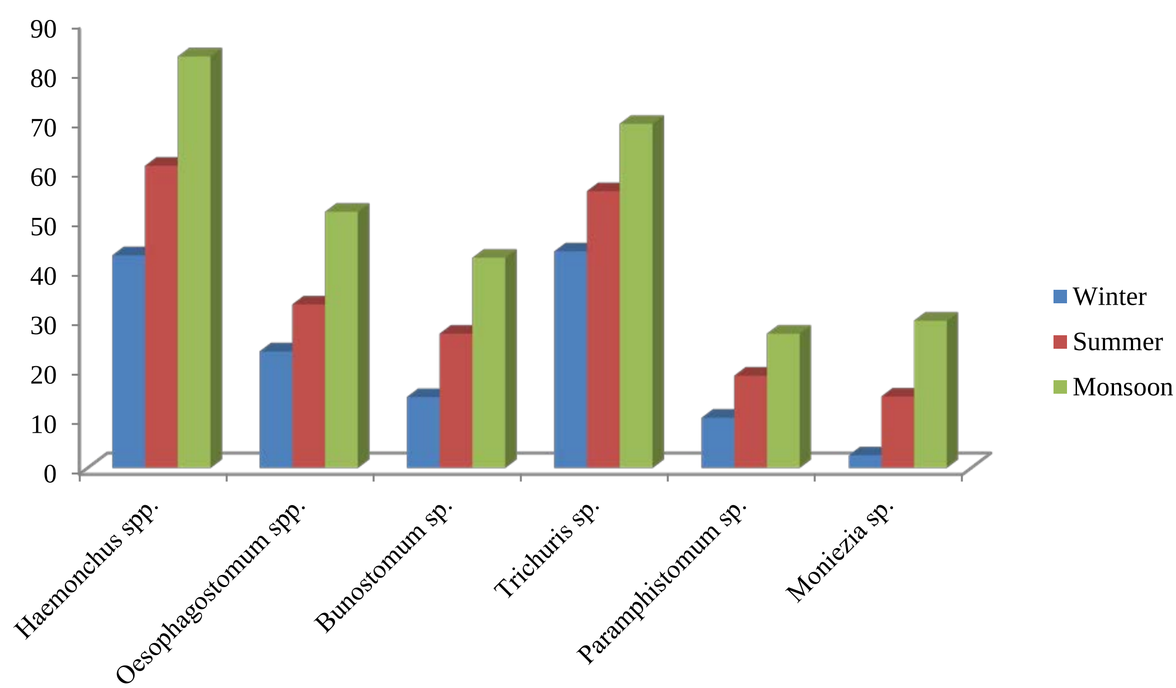
Graph 6: Seasonal prevalence of helminths parasites in *Capra hircus*

Moreover, it was also recorded that all helminth parasites showed a significant ($P \leq 0.05$) disparity throughout all the three seasons and in this respect the highest incidence rate was noted down in the monsoon season (*Haemonchus* spp. 83.1 %, *Trichuris* sp. 69.5 %, *Oesophagostomum* spp. 51.7 %, *Bunostomum* sp. 42.4 %, *Paramphistomum* sp. 27.1 %, *Moniezia* sp. 29.7 %) followed by summer (*Haemonchus* spp. 61.0 %, *Trichuris* sp. 55.9 %, *Oesophagostomum* spp. 33.0 %, *Bunostomum* sp. 27.1 %, *Paramphistomum* sp. 18.6 %, *Moniezia* sp. 14.4 %) and winter (*Haemonchus* spp. 42.9 %, *Trichuris* sp. 43.7 %, *Oesophagostomum* spp. 23.5 %, *Bunostomum* sp. 14.3 %, *Paramphistomum* sp. 10.1 %, *Moniezia* sp. 14.4 %) (Table 7, Graph 7).

Table 7: Season-wise prevalence of different helminths species in *Capra hircus*

Helminth species	% Prevalence (Number of infected intestine)			X^2 (P value)
	Winter N=119	Summer N=118	Monsoon N=118	
***Haemonchus* spp.**	42.9 (51)	61.0 (72)	83.1 (98)	40.849 (P≤0.05)
***Oesophagostomum* spp.**	23.5 (28)	33.0 (39)	51.7 (61)	21.079 (P≤0.05)
***Bunostomum* sp.**	14.3 (17)	27.1 (32)	42.4 (50)	23.294 (P≤0.05)
***Trichuris* sp.**	43.7 (52)	55.9 (66)	69.5 (82)	16.037 (P≤0.05)
***Paramphistomum* sp.**	10.1 (12)	18.6 (22)	27.1 (32)	11.360 (P≤0.05)
***Moniezia* sp.**	2.5 (3)	14.4 (17)	29.7 (35)	33.492 (P≤0.05)

N= Total number of intestines examined

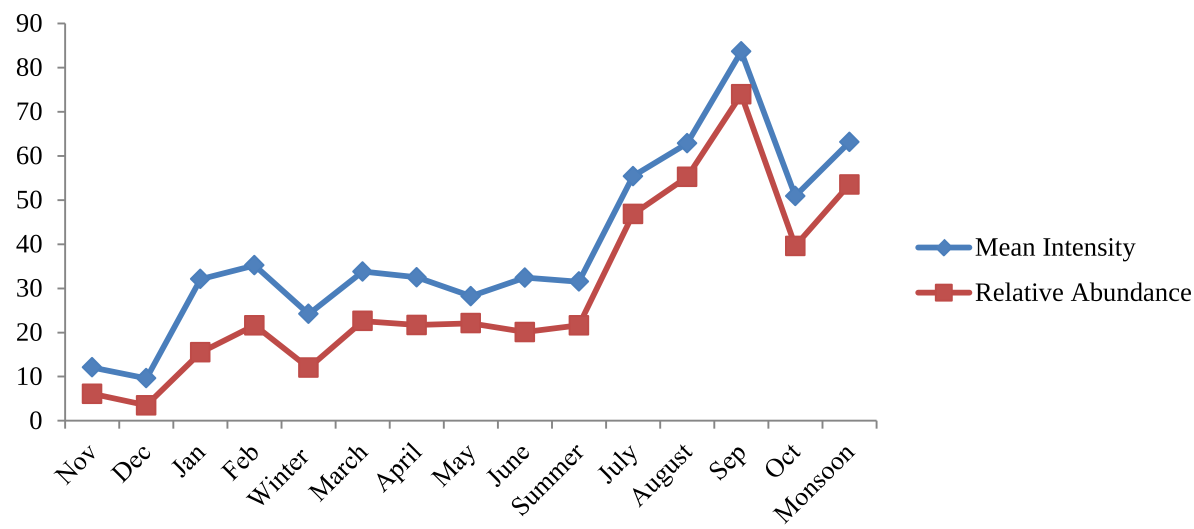


Graph 7: Season-wise prevalence of different helminths species in *Capra hircus*

Furthermore, in the season-wise study, the mean intensity (MI) and relative abundance (RA) were recorded highest during the monsoon (MI= 63.1; RA= 53.5) followed by summer (MI= 31.5; RA= 20.6) and winter (MI=24.2; RA=12.0). Present study also reported higher mean intensity and relative abundance in the month of September (MI= 83.6; RA= 73.9) followed by August (MI= 62.8; RA= 55.5), July (MI= 55.4; RA= 46.8), October (MI= 50.9; RA= 39.6), June (MI= 32.4; RA= 20.1), May (MI= 28.2; RA= 22.1), April (MI= 32.5; RA= 21.7), March (MI= 33.8; RA= 22.6), February (MI= 35.2; RA= 21.6), January (MI=32.1; RA=15.5), November (MI= 12.1; RA= 6.1) and December (MI=9.6 ; RA=3.5) (Table 8, Graph 8).

Table 8: Month-wise and season-wise Mean Intensity and Relative Abundance of gastrointestinal helminths parasites in *Capra hircus*

Month	**Total Number of intestines examined**	**Number of the infected intestines**	**Total number of parasite**	**Mean Intensity (MI)**	**Relative Abundance (RA)**
Nov	30	15	182	12.1	6.1
Dec	27	10	96	9.6	3.5
Jan	31	15	481	32.1	15.5
Feb	31	19	669	35.2	21.6
Winter	**119**	**59**	**1428**	**24.2**	**12.0**
March	27	18	609	33.8	22.6
April	30	20	650	32.5	21.7
May	32	25	706	28.2	22.1
June	29	18	584	32.4	20.1
Summer	**118**	**81**	**2549**	**31.5**	**21.6**
July	32	27	1497	55.4	46.8
August	33	29	1822	62.8	55.2
Sep	26	23	1923	83.6	73.9
Oct	27	21	1070	50.9	39.6
Monsoon	**118**	**100**	**6312**	**63.1**	**53.5**



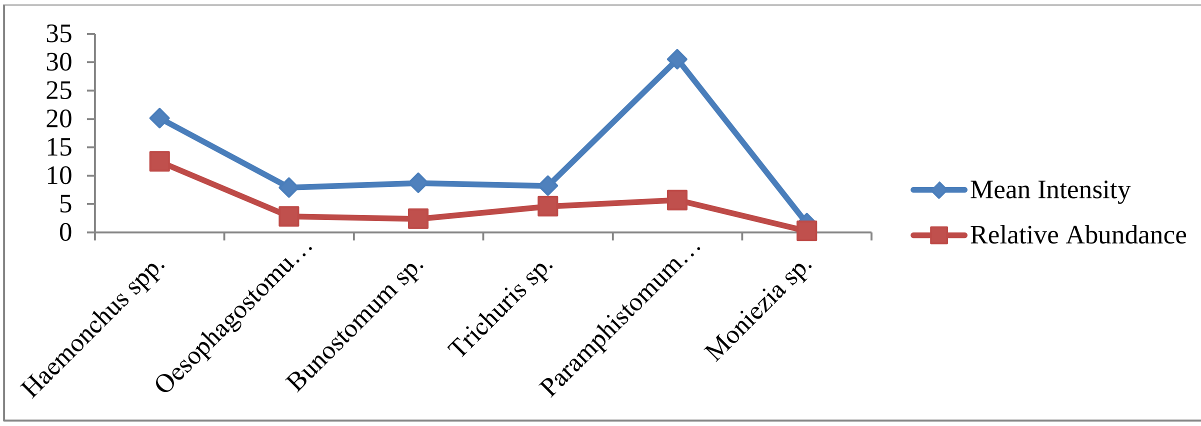
Graph 8: Month-wise and season-wise mean intensity and relative abundance of gastrointestinal helminths parasites in *Capra hircus*

3.3.2.e The overall Mean Intensity and Relative Abundance of different helminths species in *Capra hircus*

The Mean Intensity (MI) of the parasite was recorded maximum for *Paramphistomum* sp. (30.5) followed by *Haemonchus* spp. (20.12), *Trichuris* sp. (8.2), *Oesophagostomum* spp. (7.9), *Bunostomum* sp. (8.7) and *Moniezia* sp. (1.6). At the same time Relative Abundance (RA) was highest for *Haemonchus* spp. (12.5) followed by *Paramphistomum* sp. (5.7), *Trichuris* sp. (4.6), *Oesophagostomum* spp. (2.8), *Bunostomum* sp. (2.4) and *Moniezia* sp. (0.24) (Table 9 and Graph 9).

Table 9: Mean intensity and Relative abundance of different helminths species in *Capra hircus*

Parasitic species	Total Number of intestines examined	Number of the infected intestines	Total number of parasite	Mean Intensity (MI)	Relative Abundance (RA)
***Haemonchus* spp.**	355	221	4448	20.12	12.5
***Oesophagostomum* spp.**	355	128	1005	7.9	2.8
***Bunostomum* sp.**	355	99	863	8.7	2.4
***Trichuris* sp.**	355	200	1643	8.2	4.6
***Paramphistomum* sp.**	355	66	2015	30.5	5.7
***Moniezia* sp.**	355	55	85	1.6	0.24



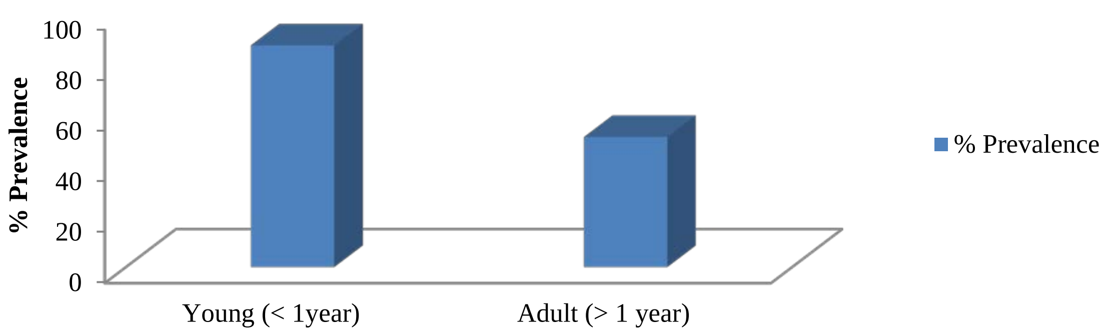
Graph 9: Mean Intensity and Relative Abundance of different helminths species in *Capra hircus*

3.3.2.f Age-wise and gender-wise prevalence of gastrointestinal helminths parasites in *Capra hircus*

It is also evident from Table 10 and Graph 10 that younger goats are significantly (P≤0.05) more susceptible to these parasites (87.5 %) as compared to adults (51.3 %) and the data from the Table 11 and Graph11 also revealed that the prevalence of these parasites is significantly (P≤0.05) higher in females (74.2 %) than males (59.6 %).

Table 10: Age -wise prevalence of helminths parasites in *Capra hircus*

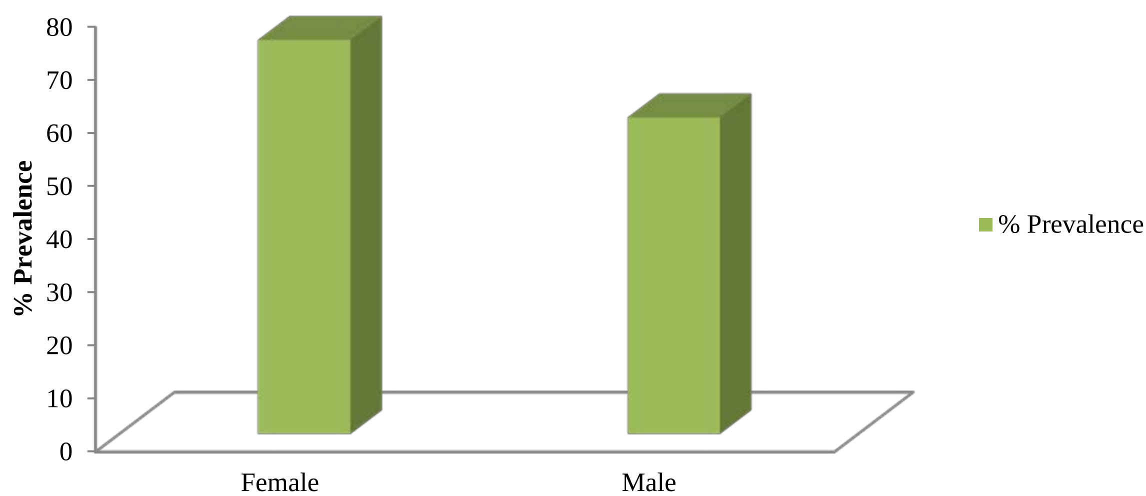
Age	Total Number of intestines examined	Number of the infected intestines	% Prevalence
Young (< 1year)	160	140	87.5
Adult (> 1 year)	195	100	51.3
X^2	52.641 (P≤0.05)		



Graph 10: Age- wise prevalence of helminths parasites in *Capra hircus*

Table 11: Gender -wise prevalence of helminths parasites in *Capra hircus*

Gender	Total Number of intestines examined	Number of the infected intestines	% Prevalence
Female	194	144	74.2
Male	161	96	59.6
X^2	8.563 (P≤0.05)		



Graph 11: Gender -wise prevalence of helminths parasites in *Capra hircus*

3.4 DISCUSSION

Epidemiological pattern of the helminthiasis in the different areas of the world would provide a basis for developing strategic and planned control of these parasites. Nevertheless, the control approaches designed for one geographical region and livestock industries may not necessarily be suitable for all agro-climatic zones and livestock industries due to differences in local environmental factors and management practices. Therefore, it is necessary to investigate the local gastrointestinal helminth parasite fauna and also to demonstrate the associated risk factors that impact the epidemiology of gastrointestinal parasitic infection in goats in different regions of India (Dixit, 2016).

Investigations of causative factors influencing the prevalence rate of helminth infection revealed that the agro-climatic conditions, development of resistance due to frequent use of anthelmintics and unhygienic pasture management were identified to be the vital factors responsible for determining type of parasitic infestation and the levels of infection. The results of the present study revealed 67.6 % of the overall prevalence of gastrointestinal helminths and the findings are at par with the results of the study conducted by Khajuria *et al.,* 2013, who reported that 67.24 % goats were infected with gastrointestinal helminths in Jammu province, Kashmir. The results also were had partial agreement with the findings of Zeryehun (2012), Hassan (2011), Gadahi *et al.* (2009) who noted 61.4 % prevalence in small ruminants in Ethiopia, 63.41% in Black Bengal goat in Chittagong district, Bangladesh and 66.45 % in goats in and around Rawalpindi and Islamabad, Pakistan, respectively. Present findings were also supported by the findings of Gupta *et al.* (2013); Islam *et al*. (2017); Sohail *et al.* (2017) and Jena *et al.* (2018). On the other hand, the findings of this study greatly differed from the observations of Yadav and Tandon (1989); Fritsche *et al.* (1993); Patel *et al.*, (2001), Lima *et al.* (2003), Islam and Taimur (2008), Ntonifor *et al.* (2013) and Yimer *et al*. (2016) who reported 86 %, 96 %, 54.9 %, 82.00 % 74.55 %, 90.4 %, and 26 % goats were infected with GI helminths, respectively. However, the disparity between the present and previous reports might be due to the differences between the geographical conditions of the study area, pasture management practices, grazing habitat, economic condition of the farmer, anthelmintics used, age, sex and strains of the animals examined (Shan and Chaudhry, 1995; Sanyal, 1998; Raza *et al*., 2012; Rashid *et al*., 2016; Ahmed *et al*., 2017).

Infections due to different helminth parasites were also studied in the present research and the highest prevalence rate of nematode (65.1 %) was reported, followed by trematode (18.3%) and cestode (12.7 %). Nearly similar result was also given by Tripathi and Subedi, 2015 where in the prevalence of nematode, trematode, and cestode in goats was found to be 65.3 %, 22.2 % and 11.1 %, respectively while Barua *et al*. (2015) reported 49.1 %, 28.1 % and 20.9 % prevalence rate for nematode, trematode and cestode respectively. Moreover Sukupayo and Rayamajhee (2018) reported higher prevalence rate for nematodes (10.67 %) followed by trematodes (4.67 %) and cestodes (2 %). These findings were also in agreement with the results of Ijaz *et al.* (2008) who divulged the highest infection rate for nematode (42.67 %) followed by trematode (16.67 %) and cestode (4 %) in a study conducted at Pakistan. Likewise, Sheikh *et al*. (2016) also supported the present finding and reported higher prevalence for nematode (26.04 %) followed by trematode (22.91 %) and cestode (15.62 %). The higher prevalence of nematodes could be associated with the fact that this helminth has a relatively short life cycle. Moreover, this parasite is capable of developing resistance against modern synthetic drugs faster than any other helminth species (Radostits *et al.,* 1994; Katoch *et al.,* 1999). Conversely, some of the studies have also reported results in contradiction with the present research like Rashid *et al.* (2016) reported the highest prevalence rate for nematodes (23.0%) followed by cestodes (22.4 %) and trematodes (0.2 %), Opera *et al.* (2005) has reported least prevalence of nematodes (8.7 %) in comparison to trematodes (78.4 %), and cestodes (13 %); (Tambe *et al.,* 2011) reported the highest prevalence of cestodes (48.33%), followed by nematode (26.66 %) and trematode (10 %).

In the present study a total of six species of helminths including four nematodes, i.e., *Haemonchus* spp., *Oesophagostomum* spp., *Bunostomum* sp., and *Trichuris* sp.; one trematode, i.e., *Paramphistomum* sp. and one cestode, i.e., *Moniezia* sp. were identified. These parasites were also reported previously in goat by many researchers (Nwosu *et al.,* 2007; Raza *et al*., 2012; Karki *et al*., 2012; Raza *et al.,* 2014; Barua *et al*., 2015; Nwoke *et al*., 2015; Amran *et al.,* 2018). However, these workers have also reported some other helminths in addition to those recorded in the present study. This study also reported the prevalence rate of these species (*Haemonchus* sp. 62.3 % followed by *Trichuris* sp. 56.3 %) and the results are in agreement with the study carried out by Lone *et al.*, 2012 where in the highest prevalence rate was recorded for *Haemonchus* sp. (82 %) and *Trichuris* sp. (74 %) in Kashmir. Somehow, similar findings were also reported by (Raza *et al.,* 2014) in a study conducted at Pakistan, where. *Haemonchus* sp., showed the highest prevalence (12.8 %) followed by *Trichuris* sp. (7.2 %). In the present study the most dominating and frequently observed species were *Haemonchus* spp. and the same was also reported by other researchers (Bali and Singh, 1977; Grant, 1981; Ahmed and Ansari, 1987; Gupta *et al.,* 1987; Tsotetsi and Mbati, 2003; Bakunzi *et al.,* 2013; Ntonifor *et al.*, 2013). Pathak and Pal (2008) reported the most prevalent parasite as *Paramphistomum* spp. (80.68 %) followed by *Oesophagostomum* spp. (30.68 %), *Trichuris* sp. (27.27 %), *Haemonchus* sp. (26.13 %), *Moniezia* spp. (17.4 %), and *Bunostomum* sp. (5.68 %) contrary to the findings of present study. Similarly, Faran *et al*. (2017) reported the most prevalent (31.08 %) *Strongyles* sp. followed by *Amphistome* sp. (23.31 %), *Trichuris* sp. (15.20 %), *Fasciola* sp. (7.77 %), *Moniezia* sp. (2.70 %) and *Strongyloides* sp. (0.67 %). Contrary to the findings of the present study where it was found that *Haemonchus* spp. showed highest prevalence rate and this could be attributed to that the adult females are capable of producing thousands of eggs per day in their relatively short generation interval, which can initiate immediate larval pasture contamination, and ability to take the advantage of favourable environmental conditions (Grant, 1981; Roeber *et al.*, 2013). In contrary, some researches i.e.,, a study conducted by (Tripathi and Subedi, 2015) recorded *Toxocara* sp. as the most prevalent species (16.66 %) followed by *Fasciola* sp. (15.9 %) in Nepal; Rashid *et al.,* 2016 reported the most prevalent species as *Trichuris* sp. (21.8 %) followed by *Haemonchus* sp. (10.9 %) in Pakistan; Sukupayo and Rayamajhee (2018) reported the highest prevalence rate for *Fasciola* sp. (4.67 %) and least for *Haemonchus* spp. (1.33 %). This difference in the incidence of helminths could be due to the variation in geographical zone, ecological factors, availability of intermediate host and anthelmintic resistance species.

In the present research, monthly fluctuations in the prevalence of gastrointestinal helminths were recorded and higher incidence rate of gastrointestinal helminths was observed in the month of July (84.2 %), August (87.9 %) and September (88.5 %) and lowest in the month of December (37 %) and January (48.4 %). The results are almost at par with the findings of Moiloa, (2017), who observed monthly prevalence of gastrointestinal nematode in Maseru district and Quthing district of Lesotho, and reported lowest prevalence rate in December (54.9 %) and highest in the month of July (98.0 %) in Maseru district where as nematode infections were found lowest during October (64.20 %) and highest in September (79.60 %). Similarly Faran *et al.* (2017) observed higher helminth infection in the month of October (80.76 %), followed by September (80.61 %), August (78.04 %), May (77.27 %), November (75.0 %), June (72.5 %), July (69.44 %), December (66.66 %) and January (63.15 %). These results are also supported by Tripathi (1970) and Maske *et al.* (1990) in goats from Uttar Pradesh and Maharashtra, respectively. Almost similar findings were also observed by Talukdar (1996) from Assam (India), Jitendran (1997) from Himachal Pradesh, Faizal *et al.* (1999) from Sri Lanka, Meshram *et al.* (2007) from Maharashtra, Pathak and Pal (2008) from Chhattisgarh, Singh and Swarnkar (2010) from Rajasthan. Similar study was performed by Yua and Jayaprakash (2013) from Madhya Pradesh, and reported an increased prevalence rate of *Haemonchus* sp. in the month of August-September (54.6 %) as compared to January-February (33.3 %) while there was an increased prevalence rate of *Bunostomum* sp. (38%), *Oesophagostomum* sp. (42.9 %), and *Moniezia* sp. (2.4 %) during January-February as compared to August-September (15 %, 33 %, and 0 % respectively).This finding was in contrary with the result of Minnat, (2014), who recorded highest prevalence rate of gastrointestinal parasite infection in February (93.75 %) and January (93.1 %) and lower infection in June (75 %). Despite of this, species-wise monthly prevalence data revealed that all the identified helminths except *Paramphistomum* sp. showed higher incidence in the months of August and September. The possible reason for the high occurrence of parasites in August to September might be due to the suitable condition of this month i.e., optimal temperature, humidity and rainfall for survival, development and propagation of helminth larvae in pasture, which leads to higher infection on the pasture animals like goat. The finding were supported by the research conducted by Singh *et al.* (2015) who reported that the month wise prevalence of *Haemonchus* sp. was predominant during July to September, *Oesophagostomum* sp. through July to August and in October, while *Bunostomum* sp. was predominant during July to November. The study of Verma *et al.* (2018) also supported the present findings who reported highest prevalence rate of *Moniezia* sp. in the month of September (71.42 %) and lowest in the month of March (6.02 %). In the current study, the highest prevalence of *Trichuris* sp. was observed in the month of September (80.8 %) and lowest in the month of December (33.3 %). Nahar *et al.* (2015) reported highest prevalence of *Haemonchus* sp. in the month of July (82.1 %) followed by August (79.7 %) and lowest in the January (28.3 %) followed by February (38.7 %). The findings are also found to be at par by Faran (2017) who reported maximum prevalence of *Trichuris* sp. occurred in September month (17.72 %) and lowest in the month of January (8.33 %) while Singh *et al.* (2015) reported highest prevalence rate of *Trichuris* sp. was noted in the month of July (9.17 %) whereas lowest in December (0 %).

Month- wise incidence of *Paramphistomum* sp. was also investigated and it was found highest in the month of July (40.6 %) and not observed in the month of November (0 %) and December (0 %). This might be due to the changes in temperature and humidity during monsoon season in India which usually starts in the last week of June. These rapid environmental fluctuations influence the rapid propagation of the parasitic life cycle and produce the paramphistomiasis in the month of July (Pfukenyi *et al.,* 2005; Haridy *et al.*, 2006). Our findings are also corroborated with the reports of Khan (2009) who observed high incidence in the month of July and lowest during the winter. Maitra *et al*. (2014) also supported the result who observed highest infectivity in July (15.20 %) and the lowest in the month of May (1.84 %). The findings of the current study are not supported by the results of Kanwal *et al.* (2014) who reported highest prevalence of *Paramphistomum cervi* in the month of February and lowest in the month of April. These dissimilarities in the month-wise prevalence of gastrointestinal helminths with the earlier reports might be due to the variances in sample size, species variation, temperature and moisture, which in turns favours the hatching of eggs, and viability of encysting larvae and the population of secondary hosts (Rolfe *et al*., 1991; Abrous *et al.* 1999).

The overall seasonal prevalence of the gastrointestinal helminths was also studied in the present research and it was found to be highest in monsoon (84.7 %), moderate in summer (67.7 %) and lowest in winter (50.4 %).The seasonal prevalence of all helminth species were observed higher in the monsoon season (*Haemonchus* spp., 98 %; *Oesophagostomum* spp., 51.7 %; *Bunostomum* sp., 42.4 %; *Trichuris* sp., 69.5 %; *Paramphistomum* sp., 27.1 %; *Moniezia* sp., 29.7 %) followed by summer (*Haemonchus* spp., 61.0 %; *Oesophagostomum* spp., 33.0 %; *Bunostomum* sp., 27.1 %; *Trichuris* sp., 55.9 %; *Paramphistomum* sp., 18.6 %; *Moniezia* sp., 14.4 %) and winter (*Haemonchus* spp., 42.9 %; *Oesophagostomum* spp., 23.5 %; *Bunostomum* sp., 14.3 %; *Trichuris* sp., 43.7 %; *Paramphistomum* sp., 10.1 %; *Moniezia* sp., 2.5 %). These results were supported by the findings of Pathak and Pal (2008), who reported that monsoon season showed the highest prevalence rate (94.6 %) followed, by summer (87.5 %) and winter (63.2 %). Pathak and Pal (2008) also supported the species-wise seasonal prevalence of gastrointestinal helminths and reported high prevalence during monsoon season (*Haemonchus* spp., 29.72 %; *Oesophagostomum* spp., 32.43 %; *Bunostomum* sp., 5.4 %; *Trichuris* sp., 27.07 %; *Paramphistomum* sp., 91.89 %; *Moniezia* sp., 16.21 %) followed by summer (*Haemonchus* spp., 25.0 %; *Oesophagostomum* spp., 34.37 %; *Bunostomum* sp., 5.40 %; *Trichuris* sp., 27.02 %; *Paramphistomum* sp., 34 %; *Moniezia* sp., 16.21 %) and winter (*Haemonchus* spp., 21.05 %; *Oesophagostomum* spp., 21.05 %; *Bunostomum* sp., 5.26 %; *Trichuris* sp., 21.05 %; *Paramphistomum* sp., 63.15 %; *Moniezia* sp., 10.52 %). Similar reports were also observed by Barua *et al.* (2015) for *Haemonchus* sp. (48 %), *Oesophagostomum* sp. (12 %), *Paramphistomum* sp. (26 %) and *Moniezia* sp. (14 %) in monsoon season. The findings of the studies of Khajuria *et al*. (2013); Varadharajan and Vijayalakshmi (2015); Sorathiya *et al*. (2017) findings also in agreement with the present results which revealed that monsoon had higher number of positive cases than during other seasons. Similarly, Yadav *et al*. (2006) described highest prevalence rate during monsoon season (88.5 %) in comparison with summer (83.2 %) and winter (76.0 %) seasons in Jammu Province, Kashmir. Singh *et al.* (2015) in Madhya Pradesh, India also recorded higher prevalence in the monsoon (98.0 %) whereas minimum was recorded during winter season (91.7 %). Likewise, Singh *et al.* (2015) in Punjab, India reported highest incidence rate throughout rainy season (90.10 %), as compared to winter (83.84 %) and summer (78.35 %) season. Additionally, Radostits *et al.,* 1994 from London; Talukdar (1996) from India; Faizal *et al.* (1999) from Sri Lanka; and Gebeyehu *et al.* (2013) from Korea also reported higher prevalence rate during monsoon season. However, the study of Biswas *et al.* (2014) from Bangladesh differed with the result which reported highest prevalence in summer season (84.6 %), followed by rainy (83.6 %) and winter seasons (81.2 %). In contrary, the studies of Saha *et al.* (1996); Gul and Tak (2016) reported *Trichuris* sp. showed higher prevalence in winter season whereas Brahma *et al.* (2015) reported the higher prevalence rate of *Trichuris* sp. in both summer (8.34 %) and winter (8.34 %) but *Moniezia* sp. showed highest prevalence rate in winter (20.0 %). Purja and Maharjan (2017) have given a contrary result and indicating that the higher prevalence rate of *Paramphistomum* sp. (4.54 %), *Moniezia* sp. (19.09 %) in summer while for *Bunostomum* sp. (39.09 %) it was observed in winter.

Present results revealed the overall mean intensity and relative abundance was found highest in monsoon season (maximum in the month of September: 83.6 and 53.5 respectively) i.e., 63.1 and 73.9 respectively whereas lowest in winter (minimum in the month of December: 9.6 and 3.5 respectively) i.e., 12.0 and 24.2 respectively. These findings are supported to a certain extent by Brahma *et al.*, 2015 who reported highest mean intensity during in monsoon season (maximum in the month of September) but lowest in summer season (minimum in May) which is contradictory with present findings. The overall mean intensity was recorded highest for *Paramphistomum* sp. (30.5), followed by *Haemonchus* spp. (20.5) while relative abundance was observed higher for *Haemonchus* sp. (12.5) in comparison to *Paramphistomum* sp. (5.7). The findings were also supported by Abebe and Esyasa (2001); Sheikh (2016) who reported higher relative density and mean intensity for *Haemonchus* sp. The higher prevalence rate, mean intensity as well as relative abundance in monsoon season could be due to favourable climatic conditions, like optimal temperature, relative humidity, and rainfall which collectively support parasitic development and provide a basic favourable environment to increase the availability of infective larval stages. It is well recognized that gastrointestinal parasitism in grazing animals is directly connected to the easy accessibility of larvae on grazing field; weak body condition and the unhygienic condition of pasture (Gaherwal *et al*., 2016). Furthermore, the highest intensity and abundance in monsoon is due to heavy rainfall, which provides appropriate molarity of salts in the soil and may be responsible for moulting (Soulsby, 1966). Besides, high temperature and rainfall lowers the immunity of the host and finally predisposes it to a heavy infection (Hawkins, 1945). Lower the prevalence rate in winter could be related to low temperature which arrested the development of helminth larvae in host (Ogunsuri and Eysker, 1979; Gibbs, 1986) and also it may be due to short duration for grazing of the animals as the days are shorts and which decreases the chances of contact between host and parasites (Katoch *et al.,* 2000).

Further, age-wise prevalence study disclosed that younger goats are more predisposed to higher infection as compared to their adults and this result was supported by Sangvaranond *et al.,* 2010. Similarly, Raza *et al.* (2014) from Pakistan found a higher prevalence in younger goats (80.6 %) than adults (72.8 %). As well, Singh *et al.* (2015) observed high prevalence in younger ones (96.3 %) as compared to adult (93.9 %) in goat, from India. In addition, Zvinorova *et al.* (2016) reported higher prevalence in young animal (76 %) than adults (38 %). On the other hand some researchers observed higher prevalence in adults in comparison with young animal (Pomroy *et al.,* 1986; Jallow *et al.,* 1994; Swarankar *et al.,* 1996; Uddin *et al.,* 2006; Yadav *et al.,* 2006; Hassan *et al.,* 2011; Chedge *et al.,* 2013 Nwoke *et al.,* 2015). Higher prevalence of infection in younger goat could be attributed to underdeveloped immune system and susceptibility to the infection. Lower prevalence of infection in adult animals can obtain immunity against gastrointestinal helminths through frequent encounter and by which they can expel the parasites before their establishment of infection.

The present research also revealed a high prevalence of infection in female goats (74.2 %) compared to their male counterparts (59.6 %). The findings are in agreement with the results of Odogu and Okaka (2016) and Sukupayo and Rayamajhee (2018) who reported females are more prone to helminth infection. This result is also in agreement with Azrul *et al.* (2017) who found higher prevalence rates in female goats (75.42 %) than in the males (56.72 %) in Bangkok, Thailand. Similarly, Lone *et al.* (2012) from India and Singh *et al.*, (2017) from Pakistan recorded a higher prevalence in female sheep and goat. In addition, Asif *et al.* (2007); Raza *et al.* (2012); Raza *et al.* (2014) and Olanike *et al.* (2015) also contradicted with the present findings who reported that male animals are more prone to infection as compared to females. High prevalence in female goats might be due to stress, which is arisen by hormonal disturbance during the estrous cycle, weak immune status during the pregnancy period, and lactation period and further resulting in decreased immunity, which, led to in increase the susceptibility of female to any infection (Lloyd, 1983; Urquhart *et al.,* 1996; Dabasa *et al.,* 2017).

3.5 CONCLUSION

- ✓ The study concluded that nematode has higher prevalence rate in the studied area and among all helminths *Haemonchus* spp. showed highest prevalence rate. Maximum infection occurred in the month of September i.e., during the time of monsoon season. Young animals and female goats were found to be more susceptible than adult animal and male goat.
- ✓ Prevailing agro-climatic conditions, poor grazing management, and unhygienic grazing land provide more favourable conditions for the transmission of the helminth infestation into the host. The results show that nematode was the most prevalent helminths in the studied area and this could be due to the lower immunity of hosts as a result of malnourishment and poor sanitary management. Keeping in view of the results some control measures for preventing gastrointestinal helminth should be adopted to reduce the intensity of the infection in the affected area. In this concern, it is recommended that the practice of well-planned grazing of animals on hygienic land, organizing regular extension programs to educate rural farmers, conducting training programs for proper use of anthelmintics and other management practices should be adopted.

Chapter 4

Study of the in vitro evaluation of the anthelmintic activity of the selected ethno veterinary plants and changes in gastrointestinal parasite before and after treatment with most effective plant extract (s)

4.1 INTRODUCTION

Animal husbandry is the pillar of the rural subdivision of different parts of the world and growth of this sector may boost the livelihood of rural communities. Livestock provides various products comprising animal power, hide, yarn and additional nutrition (Maikhuri, 1992). Despite, environmental pressures and deprivation of secluded areas, the range of livestock diversity in this area is rich and diverse and manifested by the occurrence of various breeds of small ruminants, large ruminants, poultry etc. In these areas modern practices for the therapeutic treatment and early prevention of many diseases are very poor, thus the societies of these areas solely depend on several indigenous veterinary health care management practices maintaining the livestock population (Palni *et al*., 1998; Samal *et al.*, 2004).

The medicinal properties of plants have been first mentioned in “Rig Veda” which was written between 1500 and 1600 B.C. for the treatment of human beings and animals. Thus, alternate control methods have switched over to find out herbal plants effective against parasitic as well as other diseases (Mazid *et al.*, 2012).

Since the ancient time, traditional plants have been used in various parts of the world as a substitute for both precautionary and therapeutic use for both human and livestock. Traditional veterinary medicine was experienced since 1800 B.C., during the regime of King Hammurabi of Babylon, who developed laws for the commercial practice of veterinary indigenous medicine to treat animal livestock (McCorkle *et al.*, 1996).

Traditional veterinary medicine is a specific branch of ethno-botany and basically defined as “The interdisciplinary study of ethnic knowledge, skill, people beliefs about the health care of their animals, medical practices, nutritive food for animals and always being concerned about its practical tactics and applications for the livestock production and livelihood systems with the ultimate goal of raising human well-being via increased profits from livestock development” (McCorkle, 1986; McCrokle, 1995). According to Tabuti *et al.* (2003), ethnoveterinary medicinal plants can generate useful information needed to develop livestock therapeutic practices and methods which are suited to the native environment, add a new idea to develop useful herbal drugs to the pharmaceutical industries and finally contribute to biodiversity conservation.

Ethnoveterinary practices are very common in developing countries due to diverse socioeconomic factors (Rahman *et al.*, 2009). Now it is assessed that more than 80 % of the people living in developing countries depend on indigenous medicine (WHO, 2002; Mbwambo *et al.,* 2007; Sharafzadeh and Alizadeh, 2012; Pfoze *et al.*, 2012) due to its availability and affordability. These medicinal practices are also noticeable in developed countries i.e., Belgium (38 %), China (40%), USA (42 %), Australia (48 %), Canada (70 %), and France (75 %) (Mbwambo *et al.*, 2007).

Traditional knowledge of ethno-veterinary is transmitted vocally from generation to generation (McCorkle, 1986; McCorkle *et al.,* 1995), and vanishing due to rapid environmental, socio-economic, and technological changes (Mathias-Mundy and McCorkle, 1989). The importance of traditional plant-based medicines are due to the strong relation of people with local flora, easy accessibility, eco-friendly, lack of side effects, the simple approach of their use, poor access of rural societies to allopathic medicine and their high costs.

The indigenous plants were used by people without the knowledge of their active constituents and took the crude extracts of these plant materials including seeds, berries, roots, leaves, barks or flowers (Oreagba *et al.,* 2011) which may contain some toxic constituents (Egwaikhinde *et al.,* 2009). They developed knowledge of medicinal plants by means of trial and error (Okigbo *et al.,* 2008; Qureshi *et al.,* 2010).

For the last few decades, control of livestock diseases is a big challenge for veterinary scientists due to the emergence of drug resistance and lack of any other effective control method. The recurrent development of resistance to commonly used allopathic drugs, associated with their high cost, and has developed a new interest in traditional medicinal plants as a substitute of anthelmintic drugs. Use of traditional plants is one such option to medicate livestock diseases (Raje *et al.,* 2003). Medicinal plants exhibit anthelmintic properties through different phytochemical compounds such as alkaloids, tannins, glycosides, flavonoids, phenol, and saponins in the roots, leaves, bark, and seeds (Cruz, 2008, Mute, 2009; Sharma *et al.,* 2010).

Therefore, there is an urgent requirement to validate the indigenous knowledge of the traditional plants and use as the alternative methods of control to treat the diseases of livestock. Thus, the present study focused on the evaluation of anthelmintic activity of selected ethno-veterinary plants.

4.2 MATERIALS AND METHODS

4.2.1 Plants

Plants or plant materials were either collected from field or obtained from local market of Lucknow, India. The plants to be assessed were chosen according to literature and their usage in ethnoveterinary medicine in India. In this study eight plants were selected to evaluate their anthelmintic potential (*in vitro*) against gastrointestinal nematode (*Haemonchus* spp.) of goats, namely, *Trigonella foenum-graecum* (seeds), *Curcuma longa* (rhizomes), *Trachyspermum ammi* (seeds), *Coriandrum sativum* (seeds), *Nigella sativa* (seeds), *Azadiracta indica* (leaves), *Hibiscus rosa- sinensis* (leaves), and *Ficus religiosa* (leaves). All plants were identified and authenticated by the Department of Plants Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh. The collected plant materials were put into large cotton bags and brought into the Parasitology and Silkworm Pathology Laboratory of the Department of Zoology (formerly the Department of Applied Animal Sciences) Babasaheb Bhimrao Ambedkar University (B. B. A. U.), Lucknow, Uttar Pradesh.

4.2.1.a *Trigonella foenum-graecum*

Systematic classification; (source: USDA)

Kingdom	:	Plantae
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Family	:	Fabaceae
Genus	:	*Trigonella*
Species	:	*Trigonella foenum-graecum* (L.)

Fig.4.2.1.a.1: ***Trigonella foenum-graecum*** **seeds**

Fig.4.2.1.a.2: ***Trigonella foenum-graecum*** **powder**

Trigonella foenum-graecum (L.) is an annual herb, usually known as Methi and Fenugreek, and belongs to the family Leguminosae, (Amri *et al.*, 2009; Mehrafarin *et al.*, 2011). It is broadly cultivated in many parts of India and throughout the world. Indian traditional systems of medicine viz., Ayurveda and Siddha have mentioned medicinal properties of Fenugreek seeds and used to cure various types of diseases namely anti-microbial, arthritis, dysentery, bronchitis, fever, loss of appetite, and heart diseases, etc., however, in Unani therapy it is used to treat aphrodisiac, diuretic (Nadkarni, 1982; Bahatti *et al.*, 1996; Sadeghzadeh-Ahari *et al.*, 2009). Several prior research divulged the medicinal efficiency of this plant as an anti-cancerous (Shabbeer *et al.*, 2009; Sadeghzadeh-Ahari *et al.*, 2009), antifungal (Dharajiya *et al.*, 2015), antibacterial (Premanath *et al.*, 2011, Kumari *et al.*, 2016), and antidiabetic (Gupta *et al.*, 2001). But very less work is reported on the anthelmintic potential of this plant viz., Khadse and Kakde, 2010 reported against *Pheritima posthuma*, Ghafghazi *et al.*, 1981–82 against *Hymenolepis nana* (cestode) and *Syphacia obvelata* (nematode) in mice, whereas Swarnakar *et al.*, 2014 reported against *Gastrothylax crumenifer* in cattle.

4.2.1.b *Curcuma longa* (L.)

Systematic classification; (source: USDA)

Kingdom	:	Plantae
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Liliopsida
Subclass	:	Zingiberidae
Family	:	Zingiberaceae
Genus	:	*Curcuma*
Species	:	*Curcuma longa* (L.)

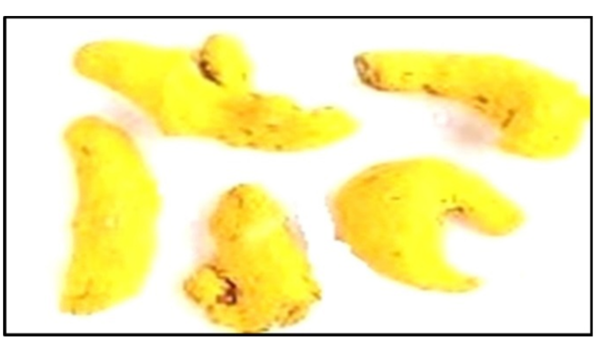


Fig. 4.2.1.b.3: Dry rhizomes of *Curcuma longa*

Fig. 4.2.1.b.4: Powder of *Curcuma longa*

Curcuma longa (L.) (turmeric) is usually known as "Haldi" and belongs to the family Zingiberaceae. It is a perennial herb having tuberous roots, short stem with large leaves. It is widely cultivated in Asian countries like India, Bangladesh, Pakistan, Indonesia, France etc. Turmeric is reported to be an important plant having a wide range of medicinal value due to its phytochemical constituents such as alkaloid, essential oil, curcumin (a polyphenol), and starch grain, (Singh *et al.*, 2011). From the period of times, *C. longa* has been used as herbal medicine for different therapeutic purposes (Maheshwari *et al.*, 2006). Some earlier reports have described the pragmatic effects of *C. longa* in the treatment of soreness, cancers, and heart disease (Akram *et al.*, 2010). Extract of *C. longa* has also been recognized for antiparasitic activities against hydatidosis, leishmaniasis, giardiasis, schistosomiasis, and trypanosomiasis (Morais *et al.*, 2013). Bazh and El-Bahy, 2013 reported the anthelmintic potential of *C. longa* against *Ascaridia galli* both *in vitro* and *in vivo*, whereas Ullah *et al.*, 2013 and Ullah *et al.*, 2017 reported *in vitro* anthelmintic activity of this plant against *Haemonchus* spp., and *Fasciola gigantica*, respectively.

4.2.1.c *Trachyspermum ammi* (L.)

Systematic classification; (courtesy: USDA)

Kingdom	:	Plantae
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Family	:	Apiaceae
Genus	:	*Trachyspermum*
Species	:	*Trachyspermum ammi* (L.)

Fig. 4.2.1.c.5: *Trachyspermum ammi* seeds

Fig. 4.2.1.c.6: *Trachyspermum ammi* powder

Trachyspermum ammi (L.) also known as Ajwain is a small greyish, highly valued medicinally plant, belongs to the family Apiaceae. Ajwain is a native of Egypt, but nowadays primarily grown in South Asian countries' viz., Afghanistan, Bangladesh, Pakistan, India, Iraq etc. In India, it is cultivated in Uttar Pradesh, Maharashtra, Gujarat, Bihar, West Bengal, and Rajasthan (API, 1999–2011). Aqueous extract of ajwain locally pronounced as Ajwan-ka-arak is popular preparation for dysentery (Bentely and Trimen, 2000). *T. ammi* seeds are therapeutically used to treat stomach-ache, carminative, amoebiasis, antimicrobial activity (Sivropoulou *et al.*, 1996), stomach disorder, flatulence, common cold, dyspepsia (Joshi, 2000), and as antifungal (Singh and Singh, 2000) etc. Ajawin oil as was reported for having an active nematicidal component which worked against Pine Wood Nematode: *Bursaphelenchus Xylophilus* (Kwon *et al.*, 2007). Some scientists also evaluated the anthelmintic potency of *T. ammi* against specific helminths, e.g. *Ascaris lumbricoides* in humans and *Haemonchus contortus* in sheep (Jabbar *et al.*, 2006b).

4.2.1.d *Coriandrum sativum* (L.)

Systematic classification; (courtesy: USDA)

Kingdom	:	Plantae
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Family	:	Apiaceae
Genus	:	*Coriandrum*
Species	:	*Coriandrum sativum* (L.)

Fig. 4.2.1.d.7: *Coriandrum sativum* seeds

Fig. 4.2.1.d.8: *Coriandrum sativum* powder

Coriandrum sativum (L.) comes under family Apiaceae, is an annual herbaceous plant and normally known as dhania. *Coriandrum sativum* is originated from Southern Europe and Western Mediterranean area, however currently grown all over the world. In India it is prominently produced in Gujarat, Rajasthan, Madhya Pradesh, and Andhra Pradesh (Shashidhar *et al.,* 2017). The leaves of the plant are irregular in shape, broadly lobed at the base (Mahendra and Bisht, 2011; Zadeh *et al.,* 2016). The plant seeds are spherical, arid schizocarp and light brown in colour (Maroufi *et al.,* 2010). Seeds of *C. sativum* have valued medicinal properties and is best used in treatment of fertility, diabetes etc. It also has best antioxidant properties (Al-Mofleh *et al.,* 2006; Begnami *et al.,* 2010; Sahib *et al.,* 2013). Traditionally *C. sativum* is used to treat ascariasis and hepatitis in humanbeings in many countries (Eguale *et al.,* 2007b). Besides, many scientists from different parts of the world reported anthelmintic potency of this plant against *Pheretima posthuma* (Chandan *et al.,* 2011 from India), *Hymenolepis nana* (Zadeh *et al.,* 2016 from Iran), *Haemonchus contortus* (Macedo *et al.,* 2013 from Brasil).

4.2.1.e *Nigella sativa* (L.)

Systematic classification; (source: USDA)

Kingdom	:	Plantae
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Magnoliidae
Family	:	Ranunculaceae
Genus	:	*Nigella*
Species	:	*Nigella sativa* L.

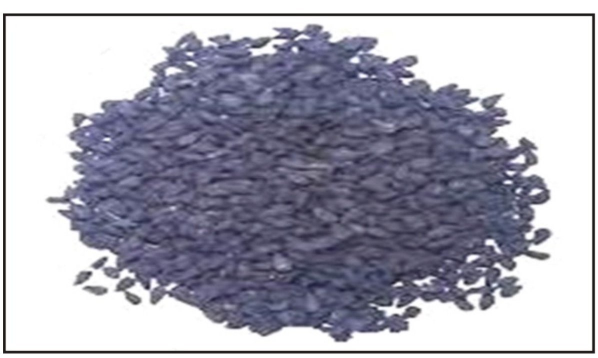


Fig. 4.2.1.e.9: *Nigella sativa* seeds

Fig. 4.2.1.e.10: *Nigella sativa* powder

Nigella sativa (L.) is commonly known as kalonji and comes under family Ranunculaceae. *N. sativa* is native to southern Europe, North Africa, and Southwest Asia, but now cultivated widely in India in different states Bihar, Punjab, Jammu & Kashmir, Himachal Pradesh, and Assam (Ahmad *et al*., 2013; Wani *et. al*., 2013; Yessuf, 2015). The plant height ranges between 20-90 cm, finely alienated leaves, and the leaf section is linear and slender (Sharma *et al.,* 2009; Aftab *et al.*, 2014). The flowers are fragile, usually white, pale blue or light purple in colour, with 5-10 petals. The fruit is a capsule with several united follicles and each follicle contains many seeds. For centuries, the seeds of *N. sativa* are mentioned as an important drug in Ayurveda and Unani (Indian traditional system of medicine) and their seeds have been widely used to treat various diseases viz., skin ailments, bronchitis, dysentery, asthma, and rheumatism all over the world. In addition, *Nigella sativa* has also been shown antimicrobial, diuretic, antihypertensive, antidiabetic, anticancerous, immunomodulatory, antimicrobial, analgesics, anti-inflammatory, antiparasitic, bronchodilator, gastroprotective, hepatoprotective, and antioxidant properties. It is also used as an appetizer, liver stimulant, to boost milk production in lactating mothers, and to assist the immune system (Goreja, 2003; Khaled, 2009; Boskabady *et al.*, 2010; Assayed, 2010; Abel-Salam, 2012; Ishtiaq *et al.*, 2013). Moreover, several researchers have reported its anthelmintic potential against gastrointestinal nematodes (*Trichostrongylus* spp. and *Oesophagostomum columbianum*) in sheep (Al-Shaibani *et al.*, 2008), against trematode (*Fasciola gigantica*) in ruminants (Ullah *et al.*, 2017), against *Bunostomum* spp., *Trichostrongyles* spp., *Oesophagostomum* spp., and *Haemonchus* spp. *in vivo* (Jain and Sahni, 2010).

4.2.1.f *Azadiracta indica* (L.)

Systematic classification; (source: USDA)

Kingdom	:	Plantae
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Family	:	Meliaceae
Genus	:	*Azadirachta*
Species	:	*Azadirachta indica* (L.)

Fig. 4.2.1.f.11: *Azadirachta indica* leaves

Fig. 4.2.1.f.12: *Azadirachta indica* powder

Azadirachta indica (L.) is usually known as Neem, a member of the family Meliaceae. *A. indica* typically cultivated in warm and humid areas and basically native to India subcontinents like Nepal, Pakistan, and Burma (Alzohairy, 2016). Form the ancient time, *A. indica* has been mentioned in Chinese, Ayurvedic, and Unani medicinal system for its therapeutic implications to cure various types of diseases (Kumar and Navaratnam, 2013). Neem is a rapid-growing evergreen plant that can range a height between 15-20 m, but it may shed their leaves under extreme drought condition. The plant has characteristic alternate, pinnate leaves with serrated margins. The branches are extensively outspread. The flowers are white in colour and smooth. It has green colour drupe fruit, differs in shape from elongated oval to closely round in shape (Girish and Neem, 2008). Some previous works have proven the therapeutic potential of *A. indica* such as antioxidant potency (Sithisarn *et al.*, 2005; Ghimeray *et al.*, 2009), antimicrobial property (Hoque *et al.*, 2007; Yerima *et al.*, 2012; Ghonmode *et al.*, 2013), antiviral potency (Badam *et al.*, 1999; Tiwari *et al.*, 2010), antifungal (Mondali *et al.*, 2009; Kumari *et al.*, 2013, Shrivastava and Swarnkar, 2014), anthelmintic activity (Radhakrishnan *et al.*, 2007; Haque and Mondal, 2011; Aggarwal and Bagai, 2014; Jamra *et al.*, 2015).

4.2.1.g *Hibiscus rosa- sinensis* (L.)

Systematic classification; (source: USDA)

Kingdom	:	Plantae
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Dilleniidae
Family	:	Malvaceae
Genus	:	*Hibiscus*
Species	:	*Hibiscus rosa-sinensis* (L.)

Fig. 4.2.1.g.13: ***Hibiscus rosa-sinensis*** **plant**

Fig. 4.2.1.g.14: ***Hibiscus rosa-sinensis*** **powder**

Hibiscus rosa-sinensis (L). is the member of Malvaceae family and commonly known as *gurhal*. It is a native of China but extensively cultivated throughout India. It is a bushy, smooth, evergreen shrub, and ranges height between 5-8 feet. Leaves are simple, alternate, petiolate, ovate, and bright green in colour. Leaves also have unicostate reticulate venation, with the pointed tip and serrated margin (Kindersley, 2008). The plant of *Hibiscus rosa-sinensis* is reported to have antibacterial (Hena, 2010), antioxidant (Bhaskar *et al.,* 2011), aphrodiasic (Alami and Macksad, 1976), emmenagogue (Pardo and Tavera, 1901), anthelmintic (Pekamwar *et al.,* 2013; Agrawal *et al.,* 2015; Nath and Yadav, 2016), analgesic (Sawarkar *et al.,* 2009), antipyretic (Soni and Gupta, 2011), antidiabetic (Venkatesh *et al.,* 2008), Anti-inflammatory (Singh *et al.,* 1978), wound healing (Nayak, *et al.,* 2007), and hair growth (Adhirajan *et al.*, 2003) properties.

4.2.1.h *Ficus religiosa* (L.)

Systematic classification; (source: USDA)

Kingdom	:	Plantae
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Hamamelididae
Family	:	Moraceae
Genus	:	*Ficus*
Species	:	*Ficus religiosa* (L.)

Fig. 4.2.1.h.15: ***Ficus religiosa*** **leaves**

Fig. 4.2.1.h.16: ***Ficus religiosa*** **powder**

Ficus religiosa (L.) or sacred fig is a great perennial tree, and commonly known as peepal. The plant is originated from the Indian-subcontinent and Indo-China and belongs to the family Moraceae. The young plant is glabrous but becomes a robust big tree at maturity. Leaves are bright green in colour while the plant is young and finally changes into dark green at old age and bear 5-7 veins, petiolate, alternate, and heart shaped. Due to the mythological, religious opinion, this plant is popularly known as Bodhi tree and reported its medicinal importance in the traditional Indian medicine system since ancient times (MHFW, 2001; Prasad *et al*., 2006; Chandrasekar *et al*., 2010). Traditionally the plants have been used in various types of ailments i.e. as antibacterial, antiviral, antiprotozoal, antidiarrhoeal, gonorrhea, ulcers, and skin infections (Chopra and Chopra, 1958; Kalpana *et al*., 2009). Uddin *et al*. (2009) from Bangladesh reported its use in the treatment of several diseases such as cancer, inflammation, or infectious diseases. Moreover, some previous reports have described their anthelmintic efficacy for example against *Ascaridia galli* (Kaushik *et al*., 1981) and against *Haemonchus* sp. (Iqbal *et al*., 2001).

4.2.2 Processing of plant material and extracts preparation

The plant materials (leaves, seeds, and roots) were dried in the oven at ambient temperature (45^0C) for 2 to 3 days till the materials are completely dry, and subsequently cleaned of all the scrap (weeds, soil particles). The cleaned material was then ground to a fine powder with the help of electric grinder. 100 g of fine powder of the seeds were subjected to extraction using 500 ml each of water and methanol separately, in a Soxhlet apparatus, for 2 days at room temperature. The extract then using evaporation technique in a water bath is concentrated, and then dried at 20^0C—25 ^{0}C, and the same stored in airtight bottles at 4 ^{0}C until use (Sermakkani *et al.*, 2010; Khyade *et al.*, 2012; Pandey *et al.*, 2018a, 2018b) (Figure 4.2.2.a, Figure 4.2.2.b, and Figure 4.2.2.c).

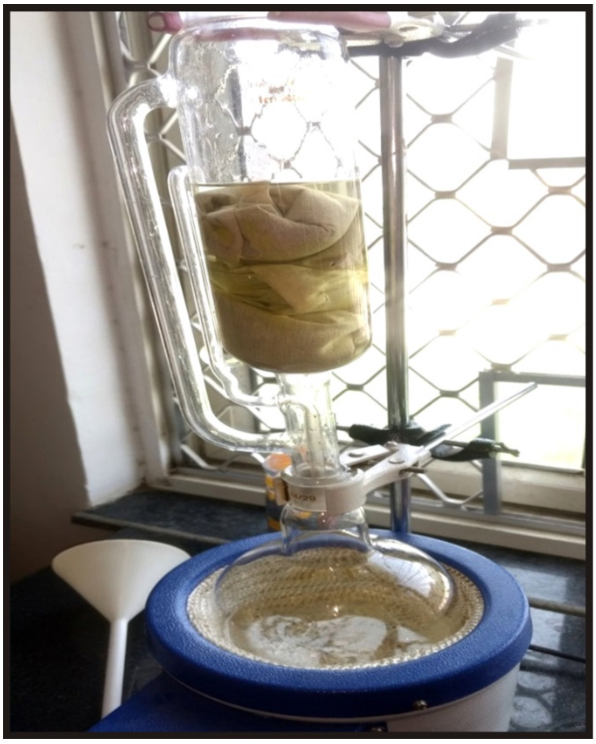


Fig. 4.2.2.a: Soxhlet apparatus unit

Fig. 4.2.2.b: Prepared plant extracts

Fig. 4.2.2.c: Prepared plant extracts

4.2.3 Collection of Test organism

In the present study, the *Haemonchus* spp. was observed more frequently and showed highest prevalence rate. Hence, in the present research anthelmintic assay was carried out by using *Haemonchus* sp. as experimental parasite. For this, adult parasites of *Haemonchus* spp. were collected from abomasum of goats from slaughterhouses of Lucknow, Uttar Pradesh. The abomasum was brought to the laboratory and dissected immediately after arrival. After that motile worms were collected and cleaned with lukewarm water and normal saline (0.9 %) solution. The cleaned worms were transferred in petridishes containing PBS at room temperature (Cable, 1958; Mayer and Olsen, 1975; Pandey *et al.*, 2018a, 2018b).

4.2.4 Preparation of stock solutions and dilution of extracts

One gram of the crude extract of both aqueous and methanolic extract of all selected plants was dissolved in 100 ml of PBS to obtain a 1 % stock solution (10 mg/ml). The stock solutions were prepared in various concentrations, i.e., 1 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml to yield 20 ml volume of each concentration in PBS. Similarly, 1% stock solution of reference drug Albendazole (Ranbaxy- ALBAXY IP-400 mg) was prepared in PBS and from the same 1mg/ml concentration was prepared in a total volume of 20 ml for experimental use.

4.2.5 Observation for *In vitro* anthelmintic activity

During the period of this research the prevalence of *Haemonchus* spp. was found highest as compared to other helminths which were identified in this study. So, the evaluation of ethno-veterinary plants for the anthelmintic efficacy was carried out with this parasite.

The anthelmintic assay with all selected plants was performed by following the standard protocol (Dash *et al.,* 2002; Eguale *et al.,* 2007a; Ullah *et al.,* 2013, Pandey *et al.,* 2018a, 2018b) with lesser modifications. All extract concentrations and drug solution were prepared freshly before starting the assay. Ten actively motile, same sized worms were chosen and placed in petri dishes having 1 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml concentration of both Aqueous Extracts (AE) and Methanolic Extracts (ME) in PBS. PBS was taken as the control (negative control) and standard drug albendazole was used as a reference (positive control). Each concentration was taken on the set of three replicates. The observations were recorded. Time taken to get paralyzed and the death of the individual worms at 1, 2, 3, 4, 5, 6, 7, and 12 hours and readings were noted in minutes. The paralyzed worms were placed in Phosphate Buffer Saline (PBS) for 30 minutes after each interval of time, for observing the possibility of rescue of the parasite motility. After accomplishment of the assay (after 12 hours), alive and dead parasites were counted for each of the experimental groups under a dissecting microscope and recorded. The time taken for paralysis and death was evaluated on the basis of the behaviour of the worm i.e., no recovery in motility even after placing in PBS whereas death was determined on the basis of the complete loss of motility with discoloration in body colour (Iqbal *et al.,* 2001; Dash *et al.,* 2002; Ghosh *et al*., 2005; Pandey *et al.,* 2018a, 2018b). Percentage mortality was calculated as the number of dead worms divided by the total number of worms per petri dish (Figure 4.2.5. A,B,C).

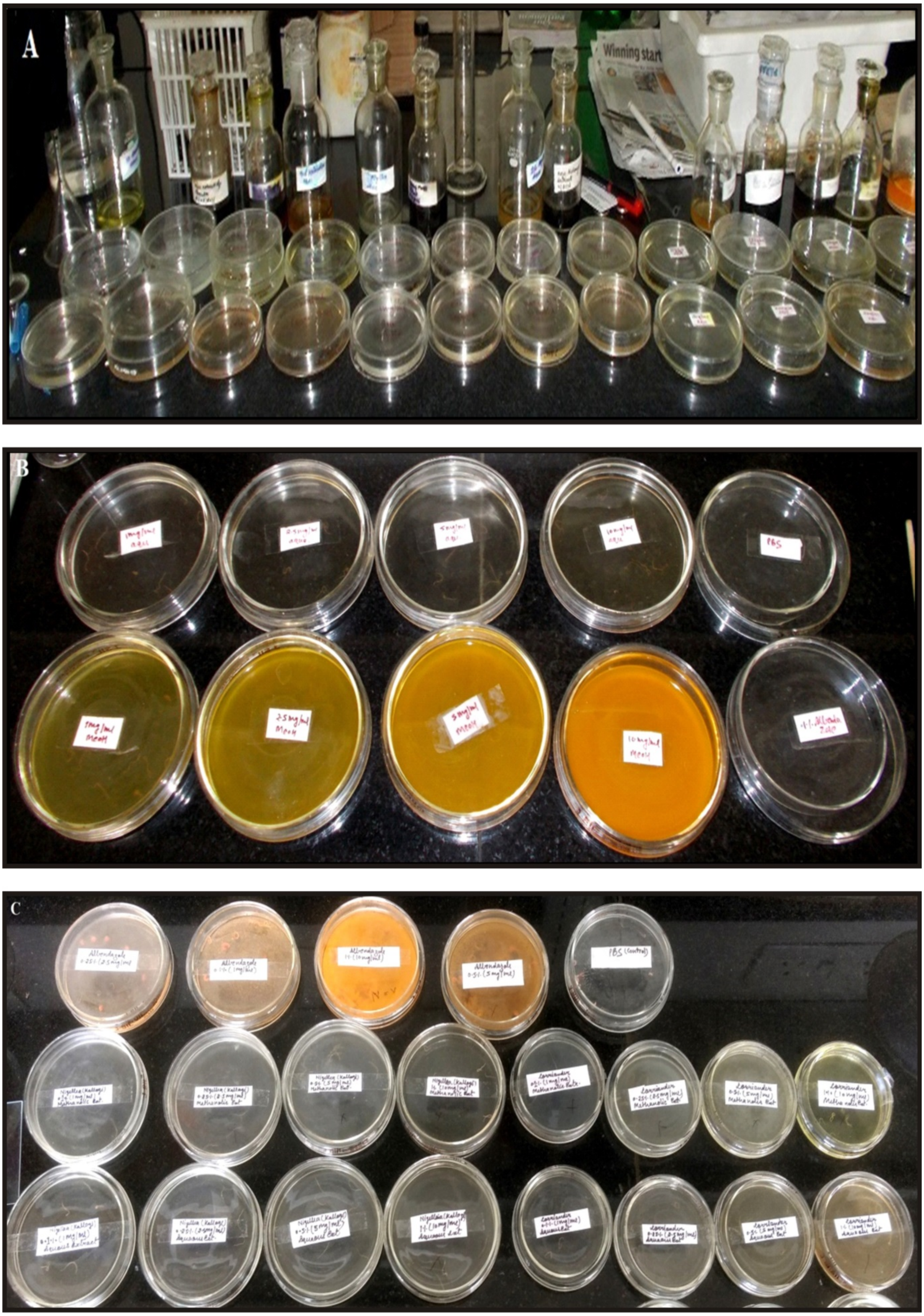


Fig. 4.1.5. A, B, C: photographs showing the *in vitro* anthelmintic assay of the selected ethno-veterinary plants against *Haemonchus* spp.

4.2.6 Histological study

The histological study was performed using the most effective plant(s) to examine the anatomical changes occurred in their tissue morphology and the study was carried out by following the method proposed by Darmawi *et al.* (2013) with slight modifications. Microtomy is the technique of cutting thin sections of desired tissues or organs for microscopic study.

- **Fixation**

For this control and treated parasites were immediately fixed in Bouin's Fixative for 24 hours. The composition of Bouin's Fixative is:

Saturated Picric acid –75 ml

Formaldehyde – 25 ml

Glacial Acetic acid – 5 ml

- **Dehydration**

After fixation, parasites were washed in distilled water till the fixative is removed. They were then dehydrated with ascending grades of alcohol (30 %→ 50 % →70 %→ 90 %→ 100 %) for 10 to 15 minutes in each grade and then cleared in Xylene.

- **Infiltration**

After dehydration, the parasites were subsequently transferred into an equal volume of Xylene + paraffin wax (1:1) for 10 to 20 minutes, followed by a series of molten paraffin wax (wax-1→wax-2) at 50^0C to 55^0C for 20 minutes.

- **Embedding**

Paper boats were used to make a block and hot (55^0C to 58^0C) paraffin was poured to embed the tissues. Further, excess wax was removed by the process of trimming.

- **Section cutting**

After trimming the parasites were taken for transverse section (4µ -5µ) block in the form of ribbon by using a rotatory microtome (WESWOX, DPTIK Model MI-1090A11425). Ribbons were spread on the slide coated with Mayer's albumin (egg white (25 ml) + Glycerine (25 ml) + crumb of thymol crystal) with the help of lukewarm water and incubated at 35^0C to 40^0C.

- **Staining**

Sections were deparaffinised by keeping them in xylene for 3 times, each time ranging from 5-10 minutes, followed by descending grades of alcohols i.e., 100 %→ 90 %→ 70 % →50 % →30 % for 10 to 15 minutes in each grade and then kept in distilled water for 15 minutes. After that, all slides were stained with Haematoxylin (Loba Chemie: Haematoxylin Ehrlich) for 5 to 10 minutes, then washed in running water followed by distilled water for 20 minutes. Over stained slides were destained with acid water (100 ml distilled water + 1ml concentrated HCl). Further, slides were washed in running water for 10 to 15 minutes and kept in distilled water for 10 minutes, followed by 30 %→ 50 % →70 % (10 minutes in each grade) then processed in eosin (Loba Chemie: 1 g eosin powder + 100 ml of 90 % absolute alcohol) for 10 minutes. Over stained slides were destained with acid alcohol (1 ml of concentrated HCl + 100 ml 70 % alcohol) followed by 70 % →90 % →100 % for 10 minutes in each grade. Slides were finally cleared by keeping them in xylene for 4 to 5 minutes.

- **Mounting**

Parasites were finally mounted in DPX (Distyrene Plasticizer Xylene) and observed under the light bright field microscope (100X and 400X), and photographs were taken by Evos XL imaging microscope.

4.2.7 Scanning electron microscope study

Scanning Electron Microscopy (SEM) was performed for the parasite after treating with the most effective plant to study the changes on the surface topography and architecture thoroughly. The Scanning Electron Microscopy (SEM) was done at USIC (University Science Instrument Centre), Babasaheb Bhimrao Ambedkar (A central) University, Lucknow, Uttar Pradesh. The scanning electron microscopy was performed by following method proposed by Undeen, 1997

Steps in SEM:

- **Primary fixation**

Parasites were properly washed in 0.1 M Phosphate Buffer Saline (PBS, pH-7.5) then fixed in 2.5 % (V/V) Glutaraldehyde solution for 4hours.

- **Secondary Fixation**

Prior to the secondary fixation the parasite was thoroughly washed in 0.1 M Phosphate Buffer Saline for 3 to 4 times to remove the primary fixative. After that, the parasite was placed in 1 % Osmium Tetroxide (OsO_4) for post fixation for overnight.

- **Dehydration process**

After post fixation, the parasite was washed properly in 0.1M Phosphate Buffer Saline (PBS) for 3 to 4 times and subsequently, the parasite was dehydrated using ascending grades of Acetone i.e. 30 %→50 %→70 %→90 % →100 % for 15 minutes in each grade and to ensure complete dehydration the parasite was dried in critical point dryer.

- **Mounting**

The critically dried parasite was mounted on specimen stubs and viewed under scanning electron microscope (Jeol, Japan; JSM 6490 LV) and photomicrography was accomplished, at different resolutions.

4.2.8 Statistical Analysis

The data was expressed as Mean ± S.E.M of 10 worms for each concentration. Data analysis was done using one way ANOVA followed by Tukey- post hoc test with the help of SPSS (version 20.00). The difference in the value at $P \leq 0.05$ was set as statistical significance. For the comparison of the efficacy of different plant extracts, lethal concentration (LC50) parasites was calculated by using MS Excel 2010, from the best-fit line obtained from the linear regression (for Y= % mortality and X= concentration) equation for each plant extract.

4.3 RESULT

4.3.1 *In vitro* anthelmintic analysis

The standards for interpretation of results of in vitro anthelmintic assay in the present study were (i) time taken by the worms to paralyse and die (in minutes) in all the tested concentrations of the both aqueous and methanolic extract (ii) % mortality of worms (*Haemonchus* spp.) after 12 hours of exposure to all selected ethno-veterinary plants. All eight selected plants showed the dose-dependent manner of anthelmintic efficacy against the *Haemonchus* sp.; still, a wide dissimilarity was detected in the anthelmintic efficiency among both extracts of all plant species as far as the potency, dose-dependent effects were concerned.

In the entire anthelmintic assay run, albendazole (positive control) showed a significant difference ($P \leq 0.05$) with both the extract of all plants at all tested concentration except the 10 mg/ml concentration of methanolic extract of both *Trachyspermum ammi* (39.6±0.3 minutes) and *Hibiscus rosa-sinesis* (73.6 minutes) and aqueous extract of *Trachyspermum ammi* (44.0±2.0 minutes), where in the time taken to get paralysis was less (32±2.3 minutes). Moreover, use of Albendazole also recorded less time taken by worms to die (57.3±5.5 minutes) and showed a significant disparity ($P \leq 0.05$) at all the concentrations using both the extracts of all plants except 10 mg/ml concentration of methanolic extract of *Trachyspermum ammi* (60.3 minutes). No mortality of the worm was observed in PBS (negative control). However, the highest and lowest anthelmintic efficacy was obtained at a concentration of 10 mg/ml and 1 mg/ml, respectively in all the plant extracts, within 12 hours and within one hour of exposure. It was evident from the Table 4.3.1.a-4.3.1.b, and Graph 4.3.1.a-4.3.1.b that both the extracts showed significant ($P \leq 0.05$) anthelmintic potency as compared to negative control and methanolic extracts of all plants exhibited high anthelmintic efficacy than the aqueous extracts against *Haemonchus* spp., the abomasal nematode parasite of the goats.

Table 4.3.1.a: *In vitro* anthelmintic assay of Aqueous Extract (AE) and Methanolic Extracts (ME) of selected ethnoveterinary plants against *Haemonchus* spp. (Paralysis time)

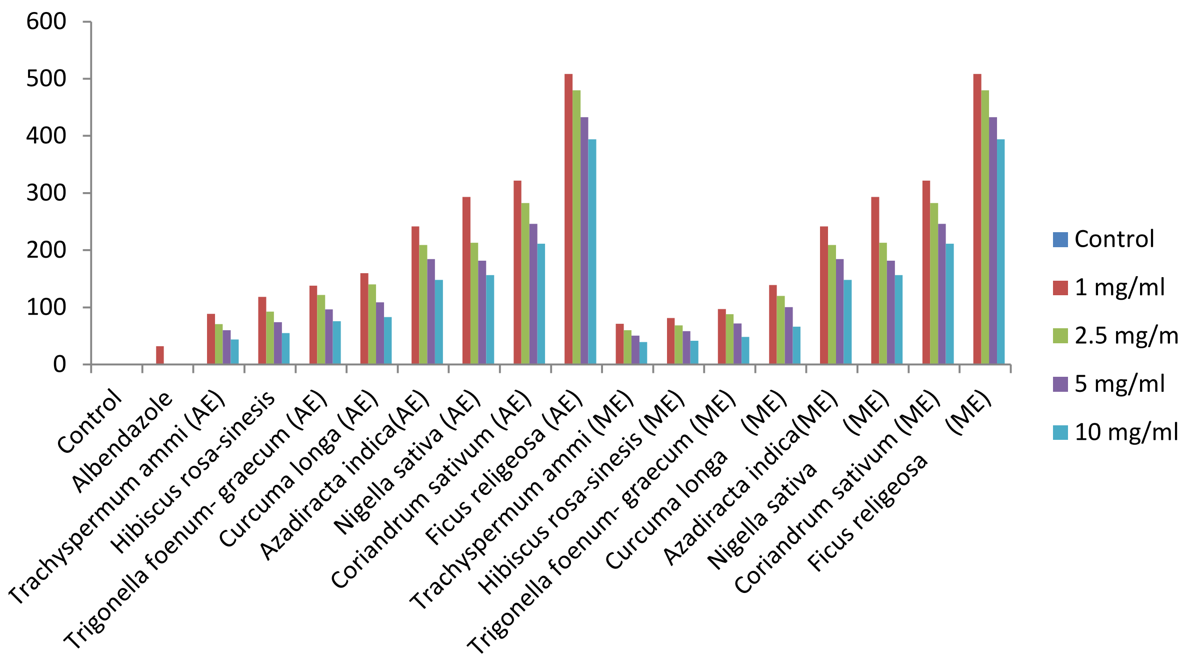
Treatment	Concentration (mg/ml)	Paralysis (min)	
Control	0	0	
Albendazole	1	32±2.3*	
		Aqueous Extracts (AE)	Methanolic Extracts (ME)
Trachyspermum ammi	1	88.6±2.0*^{abcde}	71±1.0*^{abcde}
	2.5	70.6±.08*^{afgij}	60.3±2.7*^{afghi}
	5	60.3±1.4*^{fklmn}	50.3±0.8*^{fjklm}
	10	44.0±2.0*^{Ako}	39.6±0.3*^{Bjno}
Hibiscus rosa-sinesis	1	118.3±1.6*^{pqr}	81.3±1.8*^{bpq}
	2.5	92.6±1.4*^{bstu}	68.6±2.0*^{cgrst}
	5	74.3±1.7*^{hlvw}	58.3±2.0* ^{hkruv}
	10	55.3±1.7*^{ino}	41.3±1.2*^{lnw}
Trigonella foenum-graecum	1	138.0±1.7*^{mpxyz}	97.3±6.3*^{xyz,1,2,3,4}
	2.5	121.6±3.7*^{qx1}	88.0±4.7*^{px1,5}
	5	96.6±2.0*^{cst2,3}	72.0±1.7*^{diqs,6,6}
	10	74.6±1.2*^{dv4}	48.3±2.0*^{Cmouw}
Curcuma longa	1	159.7±2.0*^{5,6}	139.0±0.6*^{7,8,9,10}
	2.5	140.0±1.2*^{y7,8}	120.0±1.1*^{11,12,13}
	5	109.0±1.8*^{r1,2}	100.3±0.9^{*y,2,14,15,16,17}
	10	83.3±0.9*^{ejuw3,4}	66.0±1.5*^{etv}
Azadiracta indica	1	241.6±1.6*^{9,10}	150.6±0.6*^{7,18}
	2.5	209.3±5.8*	133.3±0.8*^{8,19,20}
	5	184.6±2.6*^{11,12}	110.0±1.1*^{11,14,21,22}
	10	148.0±4.3*^{z5,7,}	97.0±1.0*^{z,3,5,15,23}
Nigella sativa	1	293.0±3.5*^{13}	175.0±2.3*^{24,25}
	2.5	213.3±8.8*^{14}	146.0±1.7*^{9,18}
	5	181.6±4.4*^{11}	126.6±1.6*^{12,19,26}
	10	156.3±1.8*^{5,6,8,12}	100.6±1.3*^{4,16,21,23,27}
Coriandrum sativum	1	321.6±6.0*^{9}	192.3±1.8*^{28}
	2.5	282.3±4.3^{*13}	173.6±2.4*^{24,29}
	5	246.3±2.3*	132.0±1.5*^{10,13,20,26}
	10	211.3±2.0*^{10,13}	111.3±1.8*^{17,22,27}
Ficus religeosa	1	508.3±6.0*	291.0±2.0*
	2.5	480.0±2.8*	211.6±2.0*
	5	432.6±2.6*	185.6±1.7*^{25,28}
	10	394.3±1.7*	166.0±1.5*^{29}

Values are Mean±SEM, (n=10), *p≤0.05 as compared to control group, ^{ABC} P≥0.05 as compared to Albendazole and the values with same superscript in a column do not differ significantly at P≥0.05 (one way ANOVA followed by Tukey post hoc test).

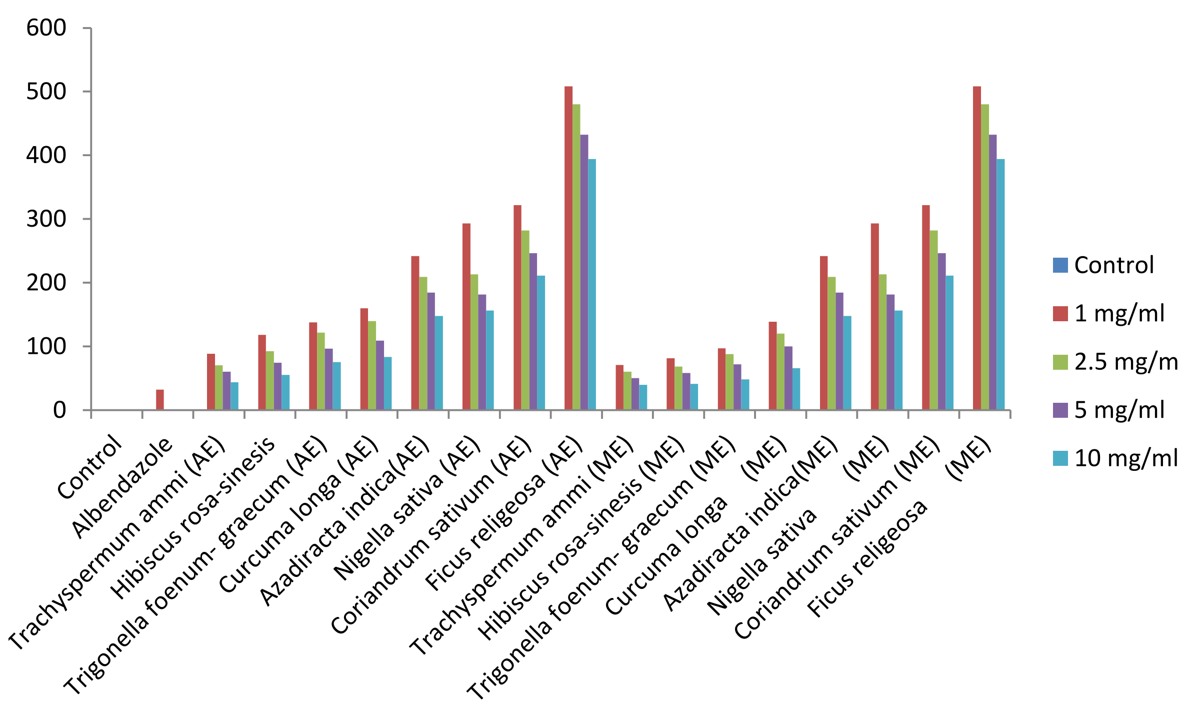
Table 4.3.1.b: *In vitro* anthelmintic assay of Aqueous Extract (AE) and Methanolic Extract (ME) of selected ethnoveterinary plants against *Haemonchus* spp. (Death time)

Treatment	Concentration (mg/ml)	Death (min)	
Control	0	0	
Albendazole	1	57.3±5.5*	
		Aqueous Extracts (AE)	Methanolic Extracts (ME)
Trachyspermum ammi	1	121.6±2.2*^{abc}	91.3±1.3*^{abcd}
	2.5	131.0±0.5*^{ade}	84.3±2.6*^{aefgh}
	5	108.0±1.1*^{bfgh}	76.0±1.0*^{ei}
	10	86.0±3.6*^{hi}	60.3±0.9* ^{B}
Hibiscus rosa-sinesis	1	154.0±2.3*^{jklm}	132.3±1.7*^{j}
	2.5	146.6±0.9*^{jn}	114.3±2.3*^{k}
	5	131.6±2.2*^{copq}	93.6±2.6*^{bfl}
	10	98.6±2.3*^{fr}	73.6±2.4*^{i}
Trigonella foenum-graecum	1	182.0±2.0*^{is}	148±2.0*^{mn}
	2.5	170±1.5*^{kst}	126.7±2.0*^{j}
	5	144.0±2.6*^{dlnopu}	107.7±1.4*^{ko}
	10	106.0±1.6*^{begrv}	90.3±2.6*^{cglp}
Curcuma longa	1	198.0±2.0*	175.0±2.0*^{q}
	2.5	178.7±1.9*^{t}	160.7±1.8*^{rs}
	5	154.3±1.2*^{mu}	147.7±3.5*^{mt}
	10	119.0±2.6*^{ehqv}	96.0±2.1*^{dhlop}
Azadiracta indica	1	378.6±1.8*^{wx}	199.3±2.9*^{uvwx}
	2.5	350.0±3.2*	170.3±1.4*^{y}
	5	334.0±2.6*	158.0±2.0*^{jnrt}
	10	310.3±2.9*	129.3±1.2*
Nigella sativa	1	433.0±2.1*^{y}	226.3±1.3*^{z}
	2.5	408.0±4.4*^{z}	211.0±2.0*^{u1}
	5	393.0±2.6*^{1}	192.0±2.0*^{v2}
	10	370.6±2.2*^{w}	170.3±1.8*^{s}
Coriandrum sativum	1	455.0±1.1*	245.0±2.3*^{qy}
	2.5	433.0±2.0*^{y}	224.3±0.6*^{z}
	5	406.3±4.4*^{z1}	207.3±3.2*^{w1}
	10	388.3±3.8*^{x}	194.6±1.7*^{x2}
Ficus religeosa	1	649.6±2.6*	390.3±3.9*
	2.5	614.6±2.2*	346. ±2.3*
	5	595.0±3.0*	315.7±1.2*
	10	532.6±2.3*	290.3±2.6*

Values are Mean±SEM, (n=10), *p≤0.05 as compared to control group, ^{B}P≥0.05 as compared to Albendazole and the values with same superscript in a column do not differ significantly at P≥0.05 (one way ANOVA followed by Tukey post hoc test).



Graph 4.3.1.a: Anthelmintic assay (worm death) of Aqueous Extract (AE) and Methanolic Extract (ME) of all ethnoveterinary plants as compared to standard drug (Albendazole)



Graph 4.3.1.b: Anthelmintic assay (worm paralysis) of Aqueous Extract (AE) and Methanolic Extract (ME) of all ethnoveterinary plants as compared to standard drug (Albendazole)

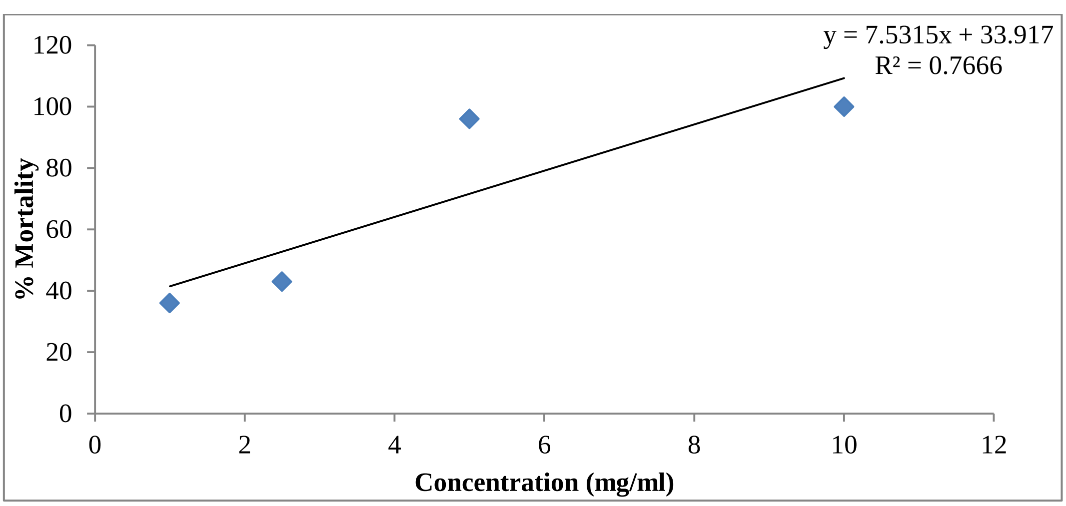
Moreover, on the basis of LC50 values the study revealed that aqueous extracts showed less potency as compared to methanolic extracts (Graph 4.3.1.e). The study ranked the potency of the plant (Table 4.3.1.d) and the ranking was *Trachyspermum ammi* (LC50= 2.76 mg/ml, y = 7.917x + 28.134 and LC50 = 2.13 mg/ml, y = 7.5315x + 33.917 for both aqueous and methanolic extracts respectively), followed in the descending order of their anthelmintic efficacy by *Hibiscus rosa-sinesis* (LC50= 3.81 mg/ml, y = 8.9076x + 16.052 and LC50= 2.62 mg/ml, y = 7.8527x + 29.431both aqueous and methanolic extracts respectively), *Trigonella foenum-graecum* (LC50= 4.31 mg/ml, y = 9.4351x + 9.3628 and LC50= 2.88 mg/ml, y = 8.0535x + 26.752 both aqueous and methanolic extracts respectively), *Curcuma longa* (LC50= 4.90 mg/ml, y = 10.434x — 3.506 and LC50= 3.62 mg/ml, y = 8.6988x + 18.518; R^2 = 0.931 both aqueous and methanolic extracts respectively), *Azadiracta indica* (LC50= 5.43 mg/ml, y = 10.411x — 6.502 and LC50= 4.20 mg/ml, y = 9.4672x + 10.214 both aqueous and methanolic extracts respectively), *Nigella sativa* (LC50= 5.71 mg/ml, y = 11.074x — 13.216 and LC50= 4.65 mg/ml, y = 9.8019x + 4.4163 both aqueous and methanolic extracts respectively), *Coriandrum sativum* (LC50= 5.95 mg/ml, y = 11.299x — 17.256 and LC50= 5.21 mg/ml, y = 10.195x — 3.1539 for both aqueous and methanolic extracts respectively), and *Ficus religeosa* (LC50= 6.10 mg/ml, y = 11.421x — 19.673 and LC50= 5.75 mg/ml, y = 10.513x — 10.473 both aqueous and methanolic extracts, respectively).

On the other hand, in order to rank plants on the basis of dose-dependent efficacy (based on their R^2 value), was found different as compared to anthelmintic potency. The best dose-dependent effects on adult motility were shown by *Azadiracta indica* (R^2 = 0.9914) followed by *Nigella sativa* (R^2 = 0.9912), *Curcuma longa* (R^2 = 0.986), *Ficus religeosa* (R^2 = 0.9718), *Coriandrum sativum* (R^2 = 0.977), *Trigonella foenum-graecum* (R^2 = 0.972), *Hibiscus rosa-sinesis* (R^2 = 0.9463), and *Trachyspermum ammi* (R^2 = 0.8671) in aqueous extract, whereas in methanolic extracts the order was *Coriandrum sativum* (R^2 = 0.9961), *Nigella sativa* (R^2 = 0.987), *Ficus religeosa* (R^2 = 0.9595), *Azadiracta indica* (R^2 = 0.9528), *Curcuma longa* (R^2 = 0.9528), *Trigonella foenum-graecum* (R^2 = 0.8622), *Hibiscus rosa-sinesis* (R^2 = 0.8233) , and *Trachyspermum ammi* (R^2 = 0.7666).

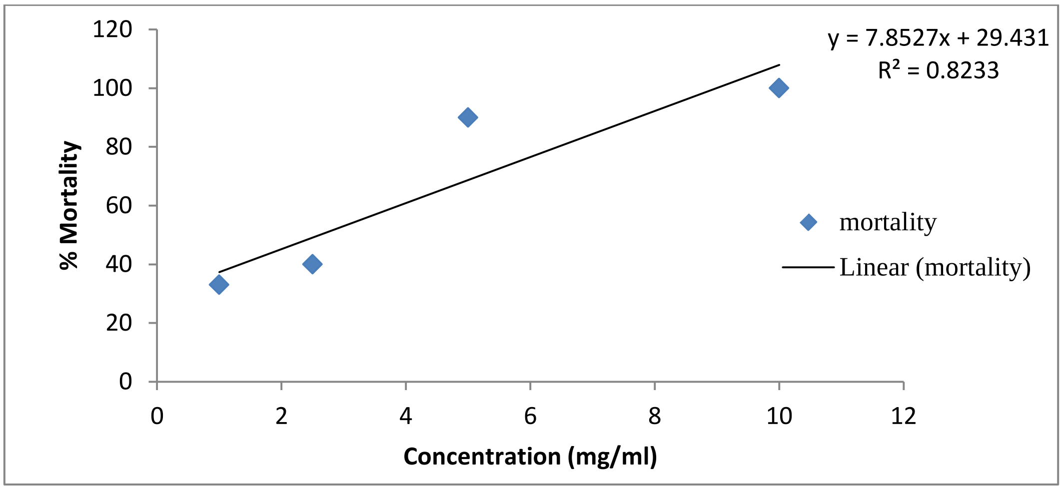
The data of the Table number 4.3.1.c depicted the percentage mortality at different concentration of extracts, LC50, and Graph 4.3.1.c.1 to 4.3.1.c.16 showed the correlation regression values, which indicate the potency and dose dependant effects of both aqueous and methanolic extracts of all plants.

Table 4.3.1.c: % Mortality and LC50 of Aqueous Extract (AE) and Methanolic Extract (ME) of selected ethnoveterinary plants against tested worm

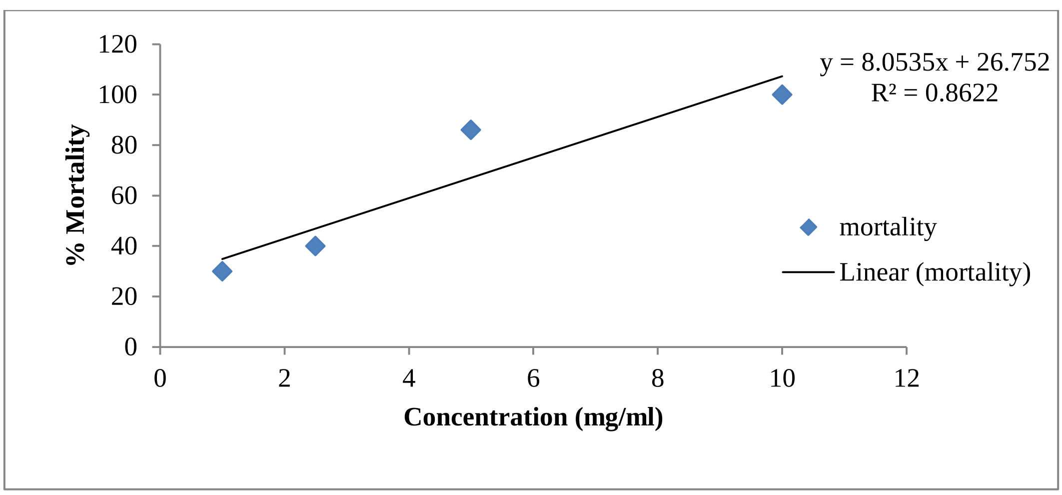
Plants	Aqueous Extract (AE) Mortality (%)				LC50 mg/ml	Methanolic Extract (ME) Mortality (%)				LC50 mg/ml
	1 mg/ml	2.5 mg/ml	5 mg/ml	10 mg/ml		1 mg/ml	2.5 mg/ml	5 mg/ml	10 mg/ml	
Trachyspermum ammi	30	43	86	100	2.76	36	43	96	100	2.13
Hibiscus rosa-sinesis	20	36	73	100	3.81	33	40	90	100	2.62
Trigonella foenum-graecum	16	30	66	100	4.31	30	40	86	100	2.88
Curcuma longa	10	26	53	100	4.90	23	36	76	100	3.62
Azadiracta indica	6.6	20	40	100	5.43	16	30	70	100	4.20
Nigella sativa	0	16	36	100	5.71	13	26	60	100	4.65
Coriandrum sativum	0	10	30	100	5.95	10	20	46	100	5.21
Ficus religeosa	0	6.6	26	100	6.10	6.6	16	30	100	5.75



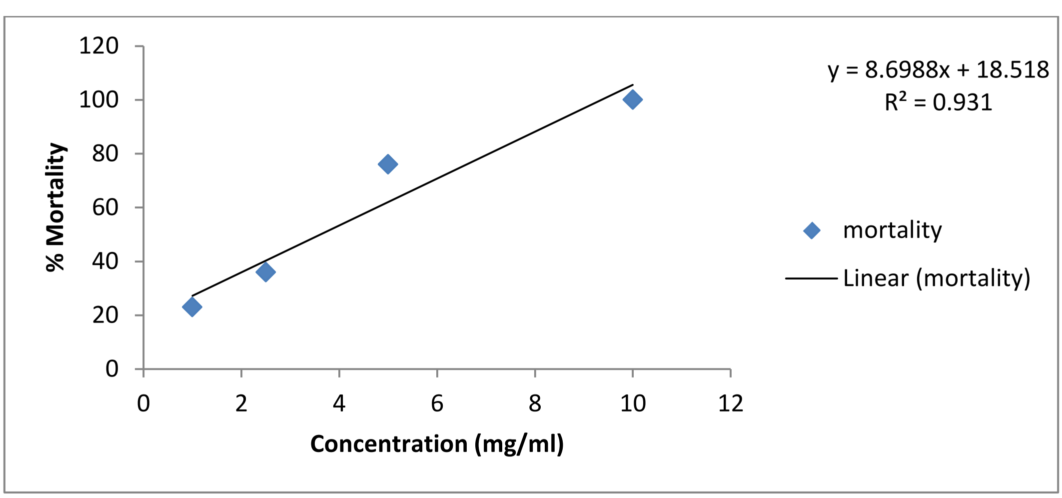
Graph 4.3.1.c.1: Mortality curve of the worm exposed in the ME of *Trachyspermum ammi*



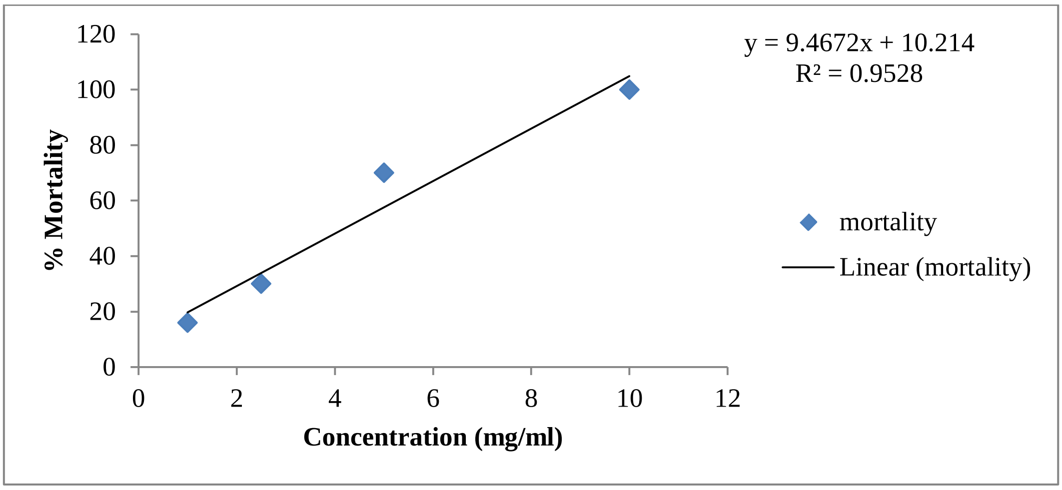
Graph 4.3.1.c.2: Mortality curve of the worm exposed in the ME of *Hibiscus rosa-sinesis*



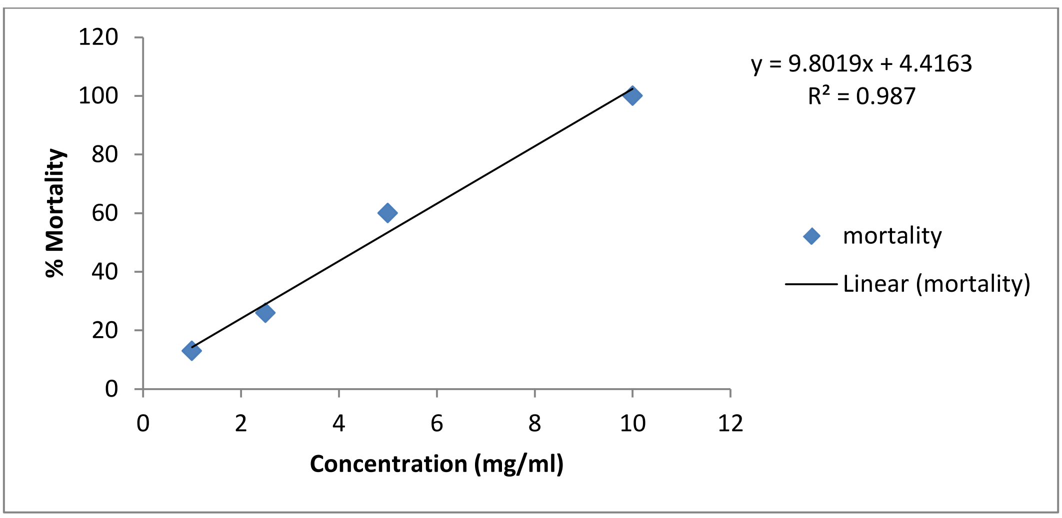
Graph 4.3.1.c.3: Mortality curve of the worm exposed in the ME of *Trigonella foenum-graecum*



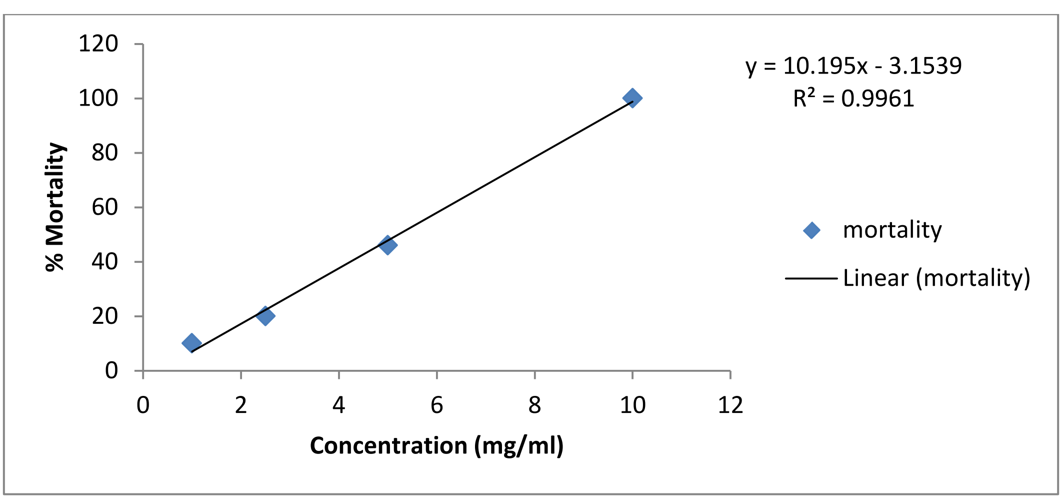
Graph 4.3.1.c.4: Mortality curve of the worm exposed in the ME of *Curcuma longa*



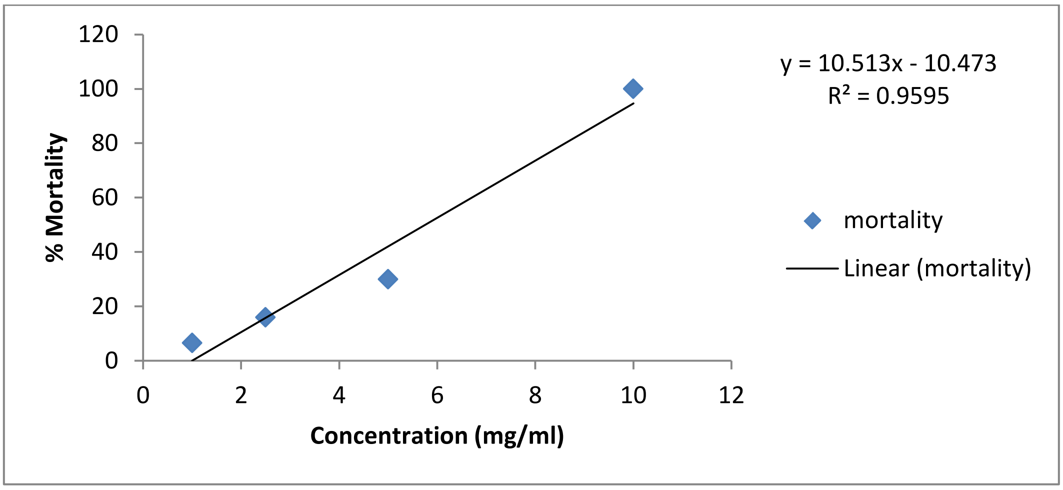
Graph 4.3.1.c.5: Mortality curve of the worm exposed in the ME of *Azadiracta indica*



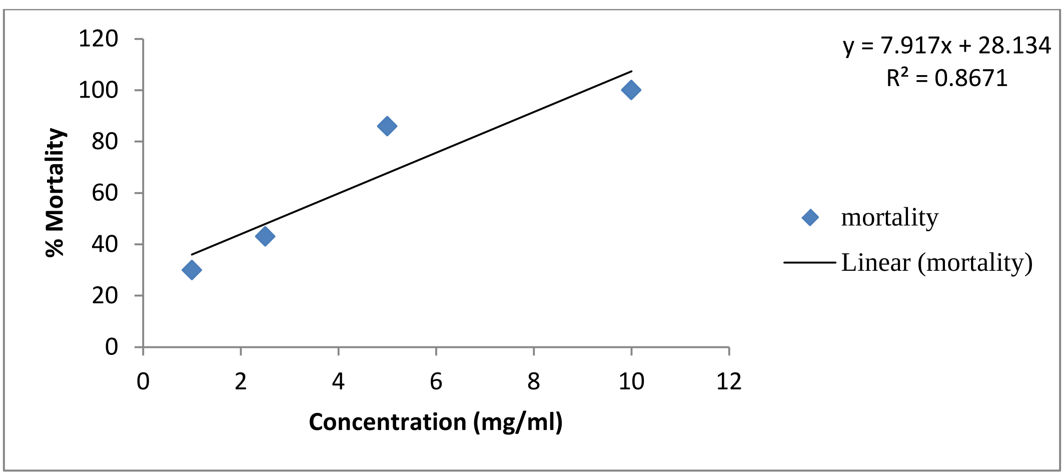
Graph 4.3.1.c.6: Mortality curve of the worm exposed in the ME of *Nigella sativa*



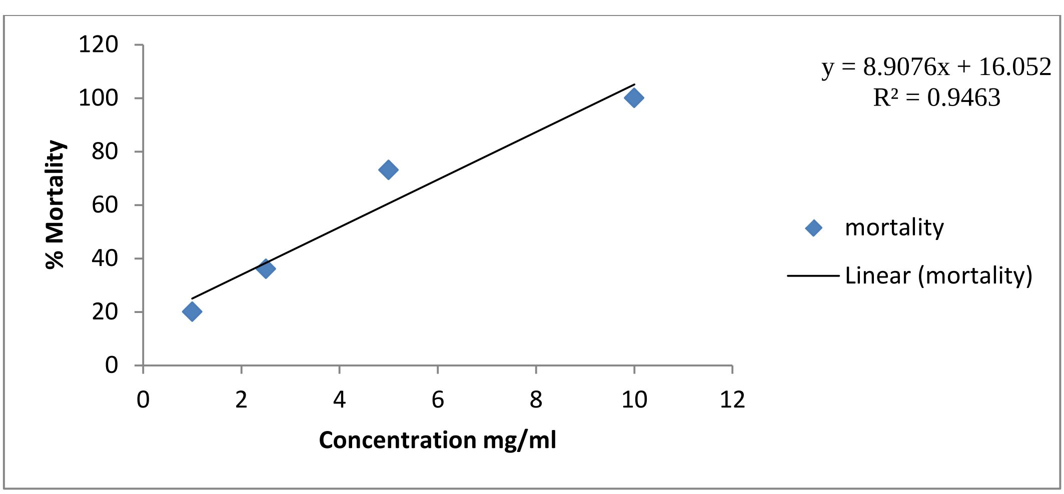
Graph 4.3.1.c.7: Mortality curve of the worm exposed in the ME of *Coriandrum sativum*



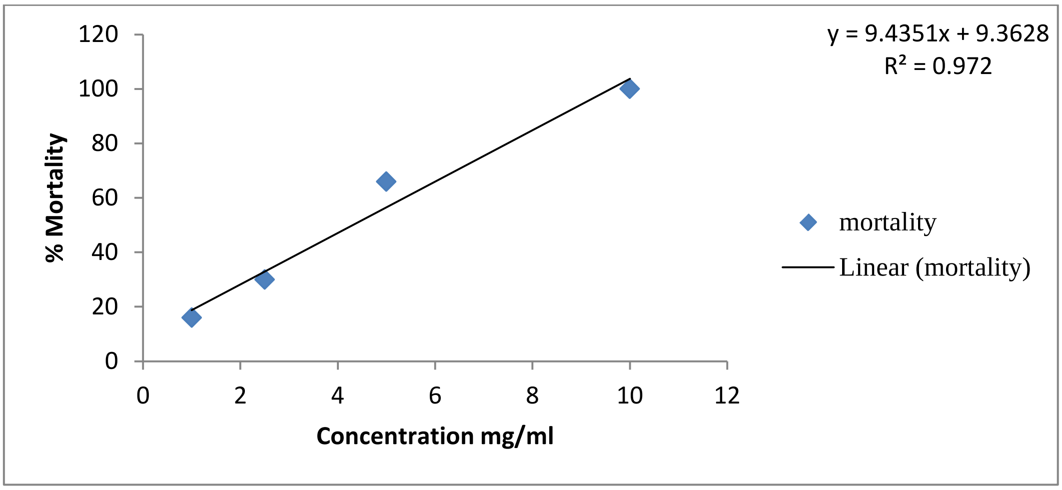
Graph 4.3.1.c.8: Mortality curve of the worm exposed in the ME of *Ficus religeosa*



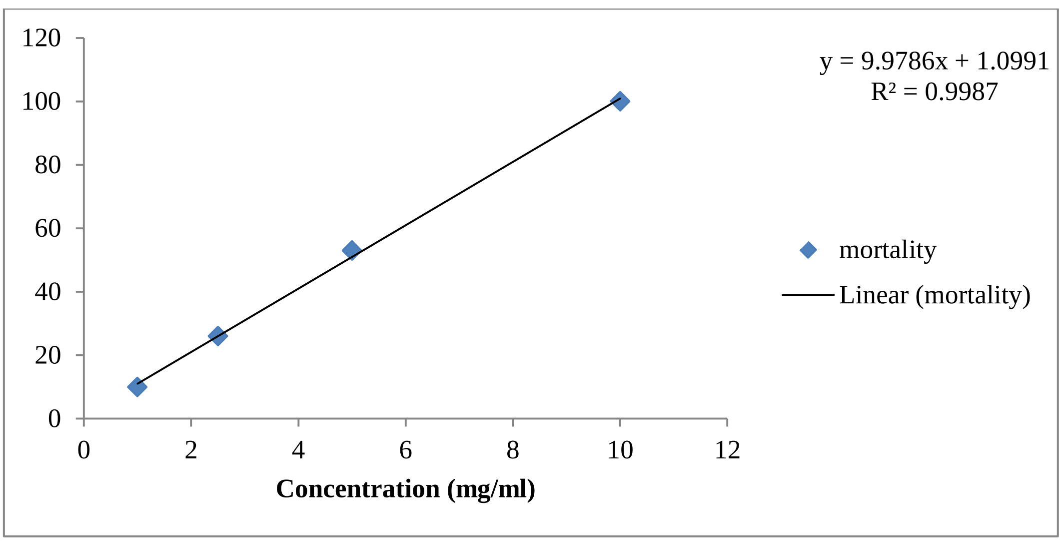
Graph 4.3.1.c.9: Mortality curve of the worm exposed in the AE of *Trachyspermum ammi*



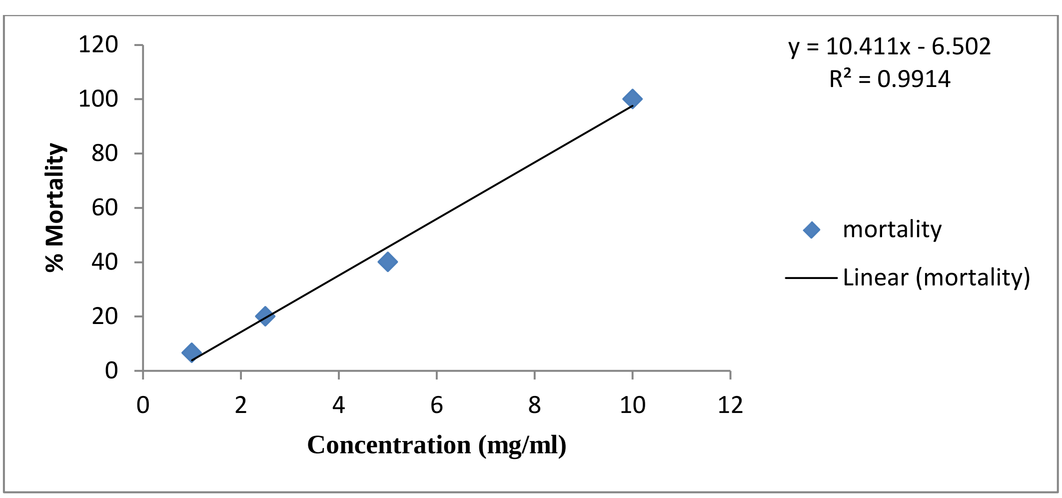
Graph 4.3.1.c.10: Mortality curve of the worm exposed in the AE of *Hibiscus rosa-sinesis*



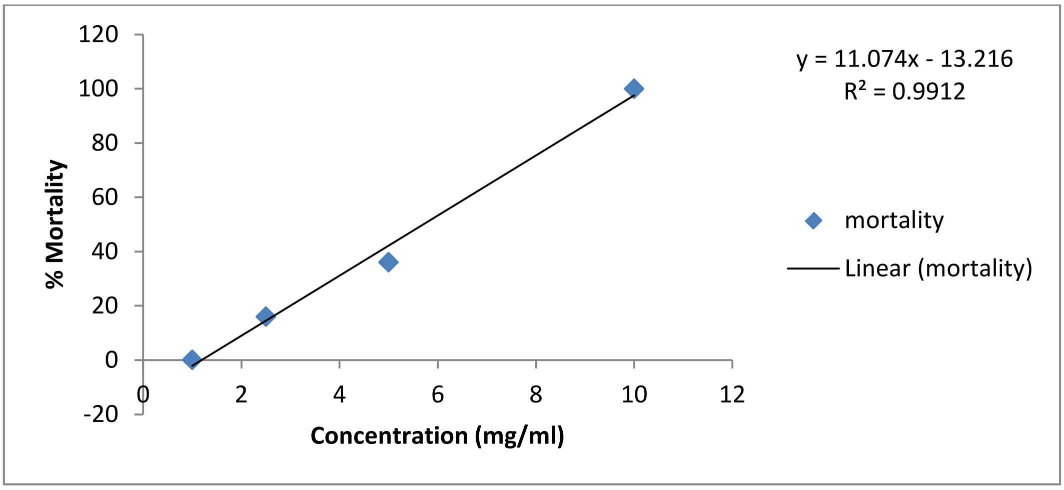
Graph 4.3.1.c.11: Mortality curve of the worm exposed in the AE of *Trigonella foenum-graecum*



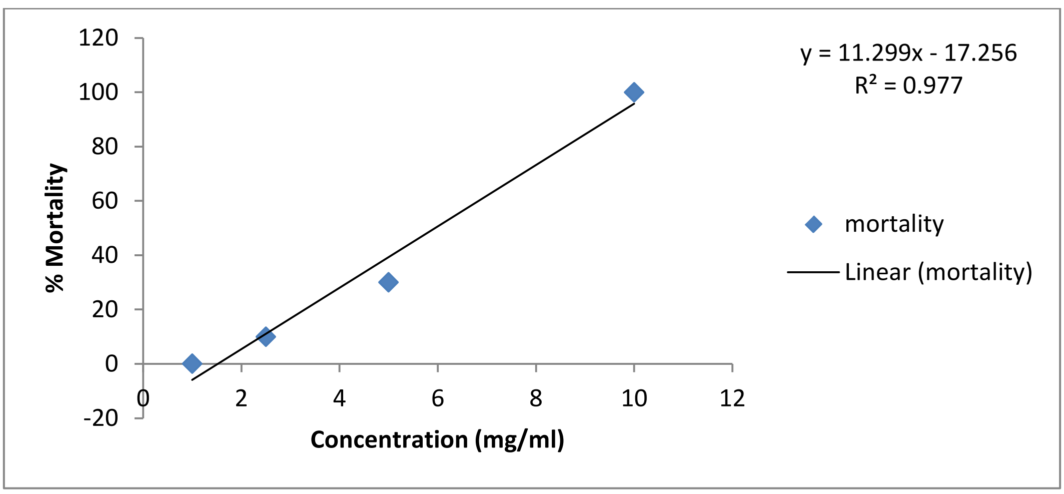
Graph 4.3.1.c.12: Mortality curve of the worm exposed in the AE of *Curcuma longa*



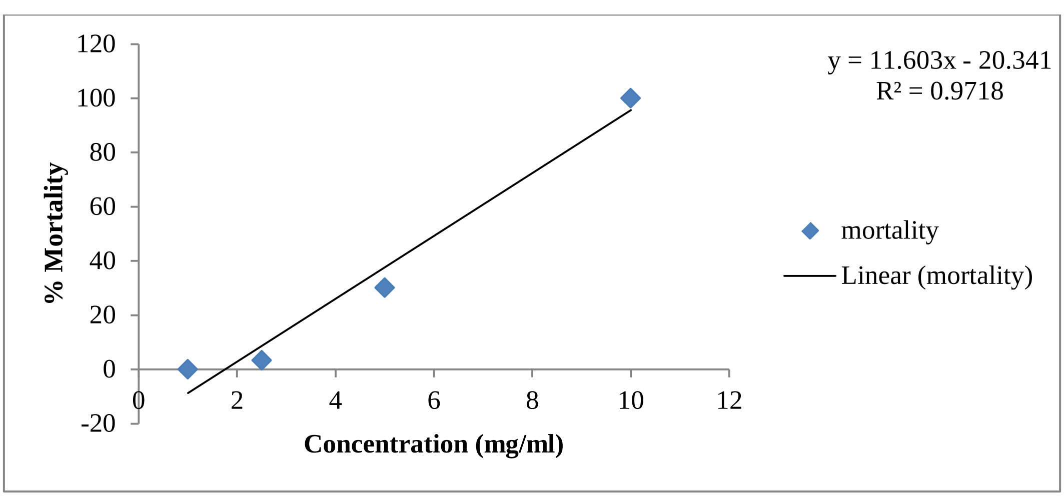
Graph 4.3.1.c.13: Mortality curve of the worm exposed in the AE of *Azadiracta indica*



Graph 4.3.1.c.14: Mortality curve of the worm exposed in the AE of *Nigella sativa*



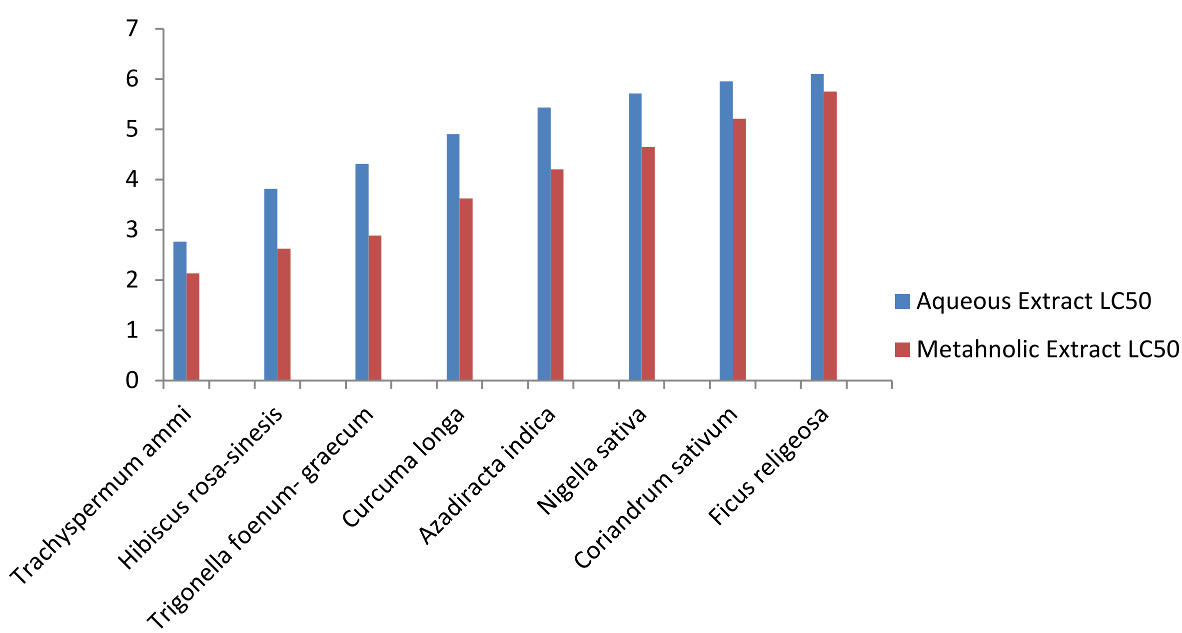
Graph 4.3.1.c.15: Mortality curve of the worm exposed in the AE of *Coriandrum sativum*



Graph 4.3.1.c.16: Mortality curve of the worm exposed in the AE of *Ficus religeosa*

Table 4.3.1.d: Ranking of the selected ethnoveterinary plants on the basis of LC50 value

Plants	Aqueous Extracts (AE)		Methanolic Extracts (ME)	
	LC50 mg/ml	Rank	LC50 mg/ml	Rank
Trachyspermum ammi	2.76	1	2.13	1
Hibiscus rosa-sinesis	3.81	2	2.62	2
Trigonella foenum-graecum	4.31	3	2.88	3
Curcuma longa	4.90	4	3.62	4
Azadiracta indica	5.43	5	4.20	5
Nigella sativa	5.71	6	4.65	6
Coriandrum sativum	5.95	7	5.21	7
Ficus religeosa	6.10	8	5.75	8



Graph 4.3.1.e: Determination of LC50 value of both AE and ME of all selected ethnoveterinary plants

4.3.2 Comparative Light microscopic (histological) observation

On the basis of LC50 value of all selected ethno-veterinary plants, the present study ranked three most effective plants i.e., *Trachyspermum ammi* followed by *Hibiscus rosa-sinesis* and *Trigonella foenum-graecum*. The study also revealed that methanolic extracts exhibited high anthelmintic potential than the aqueous extracts. Based on these observations, the worms kept at higher concentration i.e., 10 mg/ml of methanolic extracts of these plants were taken to observe the comparative histological (anatomical) changes in the worms after *in vitro* treatment.

Histology of normal (control) adult (*Haemonchus* spp.) revealed that the cuticle has a special cuticular modification in the form of longitudinal ridges (synlophe). The ridges are more protuberant in the anterior one-third of the body, gradual reduction in size in the middle region and are nearly absent in the posterior area. In a transverse section, these ridges appear like cuticular elevations (ridges) and covered by a cuticular sheath epicuticle enclosing a spine like core or strurt, which form a sort of exoskeleton to provide a mechanical asset to the worm (Singh, 2012). Below the cuticle, hypodermis is present comprising syncytial epidermis, giving rise to a complex layer of longitudinal muscles towards the outside and connective tissues towards the inner side. The middle portion of the body has a large body cavity called pseudocoel. A large intestine lies centrally in the cavity and runs through the length of the body. Near the intestine, two well-developed ovaries are present in females (Michelle *et al.,* 2010). The comparative histological study of the three plants also revealed that like reference drug Albendazole all these plants also caused the anatomical damage in the worm body but among these plants *Trachyspermum ammi* showed more anthelmintic potency than other two plants. The worm treated with *Trachyspermum ammi* showed extensive damage in the epicuticle along with cuticular wall and cuticular ridges. A severe disintegration was found in ovary wall and oviduct whereas irregular disorganization occurred in the muscle layer and intestinal wall (figure 4.3.2).

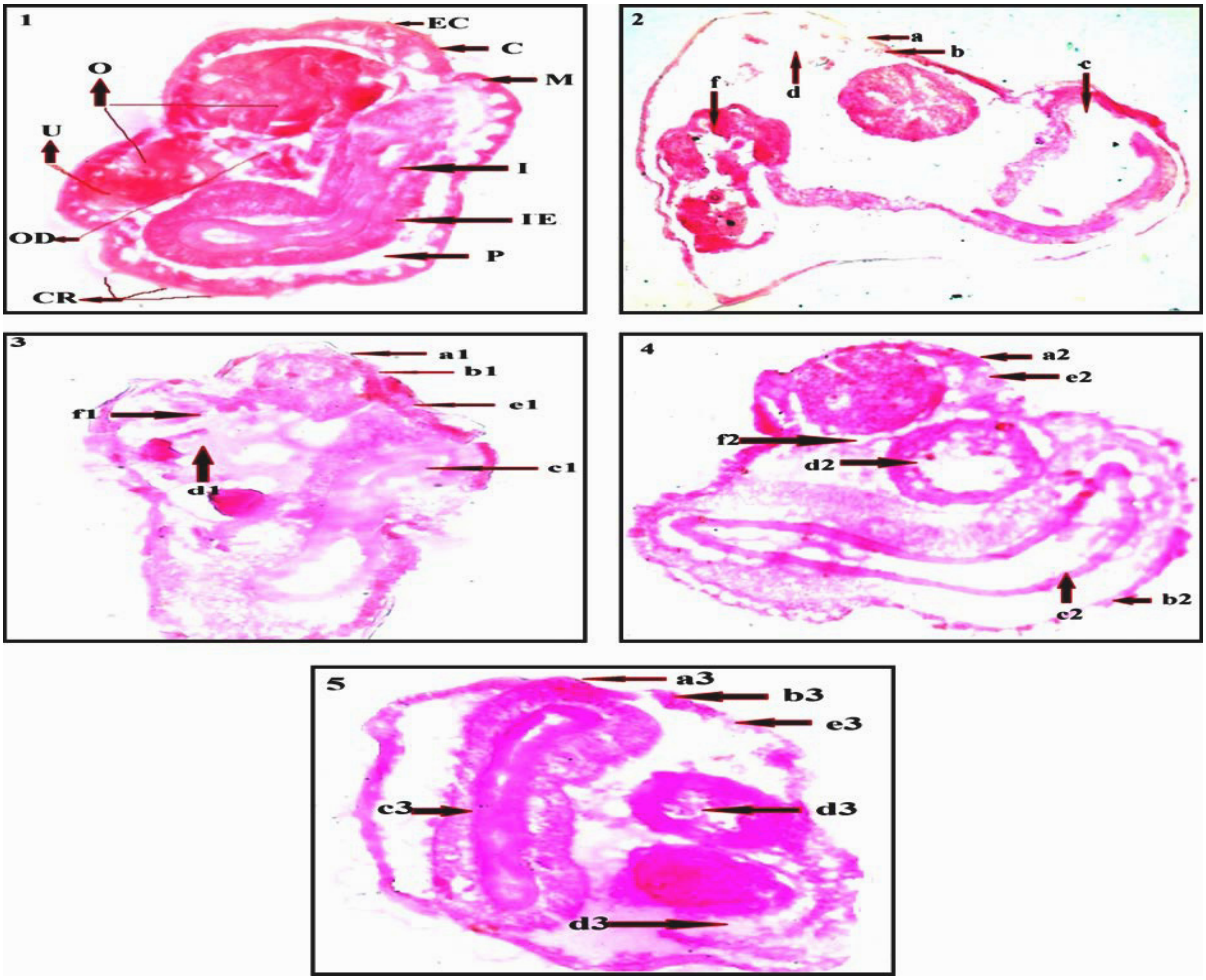


Fig. 4.3.2: Light Photomicrograph (1) shows transverse sections of control female *Heamonchus* spp. having well organized epicuticle (EC), cuticle (C) with prominent ridges and an underlying layer of longitudinal muscle (M), that encompasses pseudocoel (P), where ovary (OV) containing numerous oogonia, oviduct (OD), uterus (U) with maturing eggs (E), and an intestine (I) are situated. Light Photomicrograph (2) shows transverse sections of female *Heamonchu*s spp. treated with Albendazole (1mg/ml) showing depletion of epicuticle (a) along with rupture of ridges and cuticlar wall (b), disintegration of longitudinal muscle layer (M) lies below the cuticle layer, damage of one ovary (d), and uterus (f), extensive damage in intestinal wall (c). Light Photomicrograph (3) shows transverse sections of female *Heamonchus* spp. treated with *Trachyspermum ammi* (10 mg/ml) showing extensive disintegration of epicuticle (a1) along with cuticle (b1) and rupture ridges with evident disruptions and irregular disorganization of the underlying longitudinal muscle layer (e1). The intestinal epithelial cells (c1) appeared diffused and contracted. Intensive damage of one Ovary (d1) and uterus wall (f1). Light Photomicrograph (4) shows transverse sections of female *Heamonchu*s spp. treated with *Hibiscus rosa sinensis* (10 mg/ml) showing damage epicuticle (a3), cuticle wall (b2) along with spine, slight disintegration in longitudinal muscle (e2), intestinal wall (c2). Slight damage occurs in one ovary (d2) and intestinal wall (c2). Light Photomicrograph (5) shows transverse sections of female *Heamonchu*s spp. treated with *Trigonella foenum- graecum* (10 mg/ml) showing damage of epicuticle (a3) cuticle wall (b3) along with ridges and longitudinal muscle (e3), little damage in ovaries (d3), intestinal wall (c3) and uterus wall (f3). 400X

4.3.3 Scanning Electron Microscopic observation

In the present study, the efficacy of most effective plant i.e., *Trachyspermum ammi* (methanolic extracts) was further evaluated through observation of surface morphological changes occurred in treated worm as compared to control. The results through Scanning Electron Microscopy (SEM) of control worms showed semi-circular rudimentary lips and dorsal lancet (DL) worm's blood sucking apparatus. A pair of cervical papillae was protuberant and spine-like. The cuticle was transversally striated and with longitudinal cuticular ridges. Digital end of the body is pointed and straight (4.3.3A)

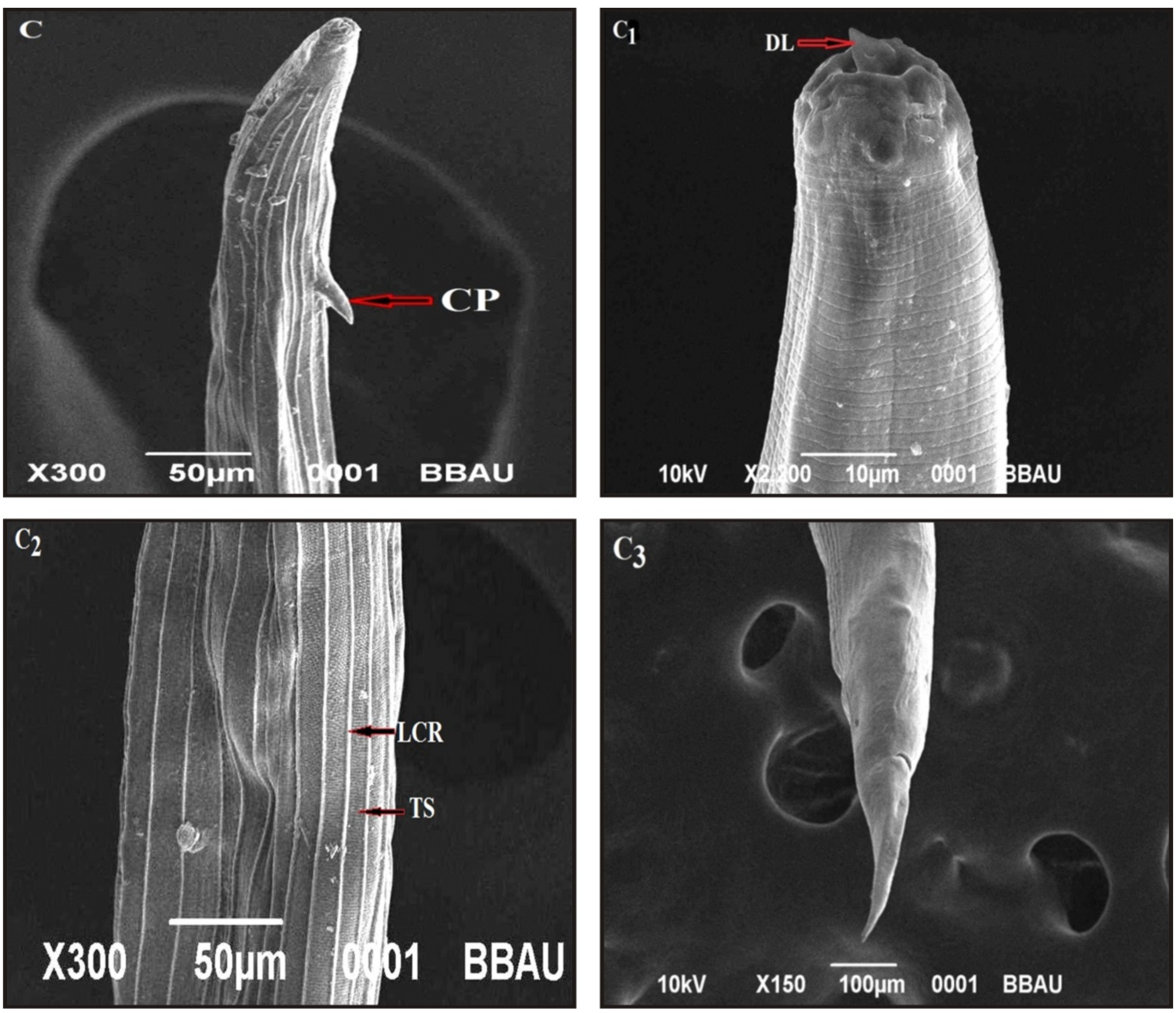


Fig. 4.3.3.A: SEM Photomicrograph (C) showing the of anterior extremity of control female *Haemonchus* spp. displaying the beginning of the longitudinal cuticular ridges short distance behind the anterior extremity and a prominent cervical papillae (CP). SEM Photomicrograph (C1) showing the anterior end of control worm having a clearly visible dorsal lancet (worm's blood sucking feeding apparatus) and well organized semicircular rudimentary lips. SEM Photomicrograph (C2) showing enlarged body surface of control worm with well structured longitudinal cuticular ridges (LCR), and transverse striations (TS). SEM Photomicrograph (C3) showing pointed straight tail of control worm.

The main changes observed in treated worm were, extreme damage to transverse striation and longitudinal cuticular ridges, the cuticle was completely ruptured and buccal capsule showed severe distortion with blebbing of the lips at the anterior end. The posterior end, i.e. the tail was slightly more wrinkled and constricted (4.3.3B).

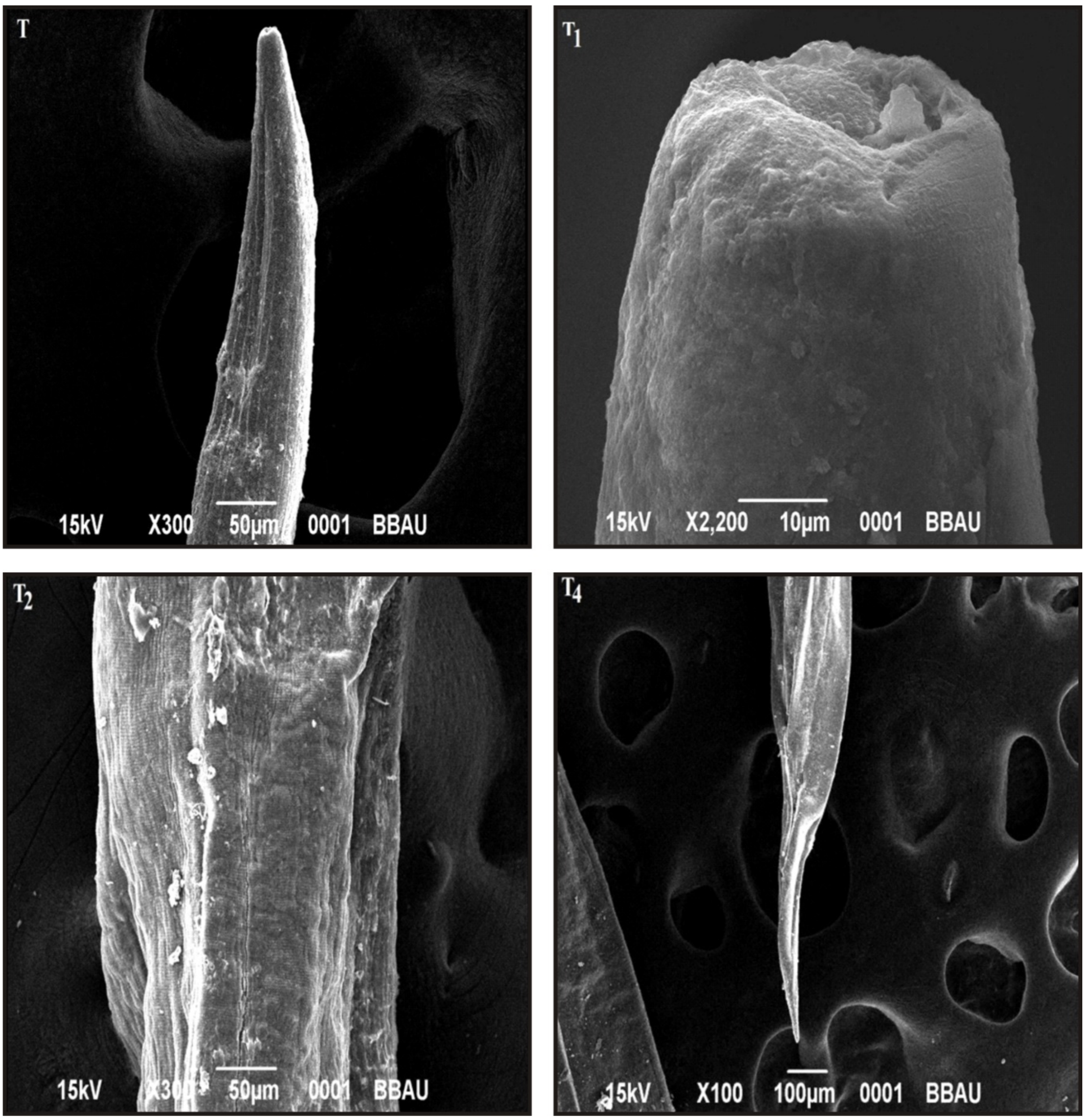


Fig. 4.3.3.B: SEM Photomicrograph (T) showing the of anterior extremity of female *Haemonchus* spp. treated with methanolic extract of *Trachyspermum ammi* (10 mg/ml) diplaying the severe damaged of longitudinal cuticular ridges and cervical papillae (CP). SEM Photomicrograph (T1): the anterior end of worm treated with *Trachyspermum ammi* (10 mg/ml) showing distorted dorsal lancet (worm's blood sucking feeding apparatus) and mouth showed distortion with severe blebbing of the lips. SEM Photomicrograph (T2): enlarged body surface of the worm treated with *Trachyspermum ammi* (10 mg/ml) showing ruptured longitudinal cuticular ridges (LCR), and transverse striations (TS). SEM Photomicrograph (T3) showing distortion and shrinkage at tail region of worm treated with *Trachyspermum ammi* (10 mg/ml).

4.4 DISCUSSION

Parasitic infestations and associated diseases are measured as a serious health nuisance to livestock (Iqbal *et al.*, 2005b). Among different parasitic diseases, helminth infections are not only widespread but recurrently develop resistance against the frequently used synthetic drugs worldwide (Jabbar *et al.*, 2006a; Saeed *et al.*, 2007). In view of the above problems along with the high costs of allopathic anthelmintics and their various side effects, the assessment of alternate treatment based on plants used in traditional healthcare systems is indicated (John *et al.*, 2006).

A primary method frequently applied for evaluating anthelmintic-efficacy is a direct observation of the sustainability of the helminthic worms upon exposure to the anthelmintic agent. Different classes of anthelmintic drugs are recognized to show profound effects on the physical activities, generally ending into the loss of mobility and mortality of helminth parasites (Togo *et al.*, 1992; Xiao *et al.*, 2005). The principal effect of the synthetic anthelmintic drug on the worm, as described by other authors also, was to cause a drooping paralysis that results in the eviction of the worm by peristalsis. The high efficacy of the standard drug Albendazole is attributed to the action that it probably blocks the glucose uptake (by binding itself to free protein in the intestinal tract or on the body surface) which leads to reduced glycogen level in the parasite and finally resulting in the occurrence of the death of the worms (Parvathy, 2012; Pandey *et al*., 2018a).

Results of the present investigation indicate that all eight plants expressed anthelmintic potency against tested nematode species i.e., *Haemonchus* spp. was the most prevalent species in the studied area (Chapter 3). Yet, their efficiency differed with respect to the time, doses, the solvent used for extracts preparation as well as used plant part that means the presence of different kinds of secondary metabolites in that part.

The observed disparity in the anthelminthic efficacy of all the selected plants is affected by the types of solvent used for the preparation of plant extracts (Malu *et al.*, 2009), but in the priority it was determined by the differences in the nature and concentration of their secondary metabolites accountable for killing the parasites (Eguale *et al.*, 2007a). Parasite mortality was directly proportional to the time of exposure and increasing concentration of the plant extracts, demonstrating, time and dose-dependent efficacy. A number of studies evaluating the in vitro anthelmintic activity of different plants were conducted and also reported their concentration and time-dependent effect (Costa *et al.*, 2002; Yadav *et al.*, 2010;

Shekhawat and Vijayvergia, 2011; Ahmed *et al.,* 2012; Moreno *et al.,* 2012; Nalule *et al*., 2013; Singh and Dhuria, 2014).

The present study demonstrates that both aqueous extracts (AE) and methanolic extracts (ME) of all plants showed anthelmintic effectiveness toward the worm's paralysis, which finally progressed to death with increased concentration as compared to control. A similar result was reported by Mali *et al.* (2008) with the alcoholic and aqueous root extracts of the *Baliospermum montamum* against Indian earthworm and *Ascardia galli* as an experimental model. The present study illustrates the higher efficacy of three plants on the basis of their LC50 values i.e., *Trachyspermum ammi* showed higher anthelmintic potential followed by *Hibiscus rosa-sinensis* and *Trigonella foenum-graecum*. The present research also revealed that the methanolic extract of *Trachyspermum ammi* showed high anthelmintic potential i.e. 100% mortality at higher concentration (10 mg/ml) than the aqueous extract in very less time. A similar study was reported by Apte *et al*., 2014 against *Pheritima posthuma*. *Hibiscus rosa-sinensis* also showed high potential at a higher concentration than their aqueous extract and took a little more time for death than *Trachyspermum ammi*. A similar study was carried out by Pekamwar *et al.* (2013) with *Hibiscus rosa-sinensis* against the *Pheritima posthuma* and reported the same result. *Trigonella foenum-graecum* also showed high anthelmintic efficacy in methanolic extract than aqueous and took more time to attain the mortality of worms than the other two effective plants. Khadse and Kakde 2010 also reported a similar result with *Trigonella foenum-graecum* and reported that alcoholic extract has more potent anthelmintic activity than aqueous extract. The higher anthelmintic activity of the methanolic extracts, as compared to the aqueous extract might be due to the presence of high amounts of secondary metabolites as compared to aqueous. Moreover, methanol was found to easily penetrate the cell membrane in extracting the intracellular components from the plant material (Lapornik *et al*., 2002; Wang, 2010).

Other plants in this study i.e., *Curcuma longa*, *Azadiracta indica*, *Nigella sativa, Coriandrum sativum*, *Ficus religeosa* also, have anthelmintic potential were also and evaluated by many researchers from different part of the world such as anthelmintic effect of *Curcuma longa was* evaluated by Morais *et al.* (2013); Bazh and El-Bahy (2013); Ullah *et al.* (2013); Ullah *et al.* (2017), *Azadiracta indica* was assessed by Rahman (2002); Rahman *et al.* (2011); Radhakrishnan *et al.* (2007); Arora *et al.* (2007); *Nigella sativa* was determined by Jain and Sahni (2010); Shah *et al.* (2014), *Coriandrum sativum* was investigated by Chandan *et al.* (2011); Zadeh *et al*. (2016), and *Ficus religeosa* was evaluated by Sawarkar *et al.* (2011)

with different experimental models and all studies supported the present findings in respect to the dose and time-dependent manner of antheminthic efficacy. The motility of the worm inhibited with increased concentration of extracts may be due to saturation of target receptors band that increases with the time exposure (Lullman *et al.*, 1993) and at higher concentration, all binding receptors on the worms were occupied thus leading to hyperpolarization of membranes, limiting excitation and impulse transmission causing flaccid paralysis of worm muscle (Wasswa and Olila, 2006).

The LC50 values of the eight plants were different for both solvents (aqueous and methanol), this might be due to various phytochemical components extracted by the different solvents and their biological effects on the worms (Eloff, 1998). The results of the study reported that methanolic extracts were most effective against *H. contortus* then their aqueous extract and this may be due to lipophilic secondary metabolites which were responsible for the activity against *H. contortus*. Further, detailed profiles of the secondary metabolites of the most effective plants were evaluated in this study and are discussed in detail in Chapter 5. Predominantly, plants may contain alkaloids (lipophilic in nature and poorly soluble in water), tannins (water and fat soluble), phenols (hydrophilic in nature), saponins (hydrophilic in nature), carotenoids (fat soluble), glucosinolates (hydrophilic in nature) and phytosterols (lipophilic in nature) that are responsible for their anthelmintic activity and may have worked independently or synergistic against the helminth parasites (Briskin, 2000).

The results of the current study demonstrate the comparision by using the light microscopy of the most effective plants and revealed the structural alterations in the treated worm, i.e., *Haemonchus* spp. as depicted by photomicrography. This result further illustrated that *Trachyspermum ammi* treated worms showed severe distortion in the internal body structure, disintegrations in the cuticle wall as compared to both plants. Microfilariae worm, i.e., *Onchocerca volvulus* was treated with anthelmintic drugs, namely amocarzine and milbemycin which resulted in breakdowns of the cytoplasm, myofilaments, and mitochondria of the muscle cells, associated with progressive disintegration of the cuticle from the hypodermis (Strote *et al.*, 1992). Tribendimidine drug was also reported by Xiao *et al.* (1989) to cause severe disruption of the cuticle and intestinal epithelium in *Necator americanus*. Disorganization of the cuticle in *Trichinella spiralis* by Cyclosporin A, especially with the hypodermal wall which appeared somewhat thickened and irregular, with destruction in the cuticular ridges (Boulos *et al.*, 1992). A similar result was reported by Lalchhandama (2008) against the *Ascaridia galli* after treating with higher concentration (20 mg/ml) of *Acacia* *oxyphylla* indicating extensive structural alterations involving detachment of the cuticle, the disintegration of the muscular layers, rupture of the ovaries and deformity of the egg membranes. Yadav and Tandon, 1992 also supported these findings by using *Flemingia vestita* against a nematode parasite namely *Ascaris suum* and reported irregular disorganization in the muscle layer. Jeyathilakan *et al.* (2010) also reported the anthelmintic efficacy of citronella oil on the basis of light microscopy against the *Fasciola gigantica* and reported the total disappearance of spines from the cuticle.

Scanning Electron Microscopy (SEM) was performed by using the worms treated with methanolic extract of *Trachyspermum ammi* (most effective plant) and revealed the structural changes occurred on the surface topography due to the interactions between the extract and the cuticle. This has played a very important role in many functions for the survival of the nematode worm in an adverse environment, particularly to maintain structural uniformity thus permitting its proper motility and to absorb nutrients along with osmoregulation in the gastrointestinal system of its host (Page and Winter, 2003; Lee, 1965; Smyth, 1996).The ultrastructural changes found in the adult worm after the *in vitro* exposure are not limited to the cuticle, but also ruptured the buccal capsule. Hoste *et al.* (2006) also described the SEM analysis and reported the cuticular changes along with distortion in longitudinal cuticular ridges and transverse striation after in vitro exposure to walnut extract rich in condensed tannin on *Trichostrongylus colubriformis*. Martínez-Ortíz-De-Montellano *et al*. (2013) also described the occurrence of cuticular wrinkles in adult worm of *H. contortus on* the in vitro exposure of tannin rich plant. SEM of treated worms *H. contortus'* with 50 mg/ml ethanolic extract of *Punica granatum* peel showed the buccal capsule distortion with severe blebbing of the lips. The cuticle was distorted and the transverse striations became less pronounced showing longitudinal wrinkles described by Nabila *et al.* (2017). All these researches supported the findings of the present research with respect to surface topographical alteration on the worm body after exposure of different ethnoveterinary plants. On the basis of the functions of the cuticle, the structural changes in cuticle as described in this study points towards inhibition of the motility of the parasite, as well as cause of osmotic imbalance, thus dealing its interaction with its environment, primarily, as it relates to the disturbance in the nematode's nutrition, which might ultimately lead to worm malnutrition.

4.5 CONCLUSION

- ✓ These types of research provide a baseline data of ethno-anthelmintics which may contribute to further investigations in relation to a professional ethnoveterinary medicinal approach. The plants considered in this study showed anthelmintic efficacy in both aqueous and methanolic extracts and methanolic extracts exhibited high efficacy than aqueous extracts against the tested worm. This study also ranked the selected plant in order of their potency and ranked the *Trachyspermum ammi* as the most effective plant followed by *Hibiscus rosa-sinesis*, *Trigonella foenum-graecum*, *Curcuma longa*, *Azadiracta indica*, *Nigella sativa*, *Coriandrum sativum*, and *Ficus religeosa.*

- ✓ To further support and corroborate the present findings, in vivo studies are required prior to these locally used plant-based therapies can be developed into effective low-cost anthelmintics in the forthcoming future. Besides, these plants need to be broadly studied in depth for isolating the active element responsible for antiparasitic property and estimating its potential for the systematic remedial treatment of the livestock diseases in India and probably even in other parts of the world.

Chapter 5

Phytochemical screening of selected ethnoveterinary plants

5.1 INTRODUCTION

In Indian agriculture system, livestock not only plays a significant role in the rancher's life by providing animal power, rural conveyance, compost, fuel, dairy product, and meat but also plays a major role in the development of rural economy by providing income and employment to the small scale farmers and weaker sections of the rural society. The ethnic knowledge to cure the livestock diseases acquired by traditional plants, used by the older people of the society and orally transformed from one generation to other in non-systematic and less formalized manner (Mathias, 2004).

Various species of helminths, also discussed in the earlier chapters parasitize the livestock and result in considerable pathogenesis and economic losses. For few decades the problem to control the parasitic diseases are further complicated due to the emergence of Anthelmintic Resistance (AR) against the chemical anthelmintics (Tariq, 2018). So researchers paid their interest towards the traditional medicine to cure these veterinary diseases. These traditional medicines often provide an economical option as compared with synthetic drugs and the products are locally accessible and more easily available even to the people of rural society.

Nature provides everything for the well-being of society over the years. In ancient times, people used to depend on plants for the treatment of several diseases. Indian traditional medicine such as Ayurveda, Unani and Siddha used herbal medicines to treat various types of diseases by following traditional approaches (Tambe and Bhambar, 2014). The therapeutic potential of plant origin crude drugs is being used from the time immemorial by simple procedures without isolation of the pure compounds. Even in present day, plant resources remain an important resource for combating ailments, including contagious diseases and many of these plants have been explored for novel drugs or used as prototypes for the development of new remedial agents, food preservatives, agrichemicals, and synthetic chemicals (Borris, 1996).

Ethnoveterinary plants have been attributed to the number of medicinal properties and the medicinal value of these plants are due to different types of phytochemical compounds present in the plant in the form of bioactive substances which not only help in plant defense mechanism but also responsible for the treatment of many pathogenic diseases (Tariq, 2018).

Based on the functions in plant metabolism, these phytochemicals are categorized into two forms viz., primary and secondary constituents. Primary phytochemical compounds include proteins, carbohydrates, and fats that are utilized as food by the animals and humans whereas the huge number of compounds including alkaloids, terpenoids, flavonoids, saponins, phenols, glycosides etc. are considered as secondary constituent which exert definite physiological effects.

Isolation and characterization of several biologically active phytochemical constituents from these medicinally important plants have given the origin of new therapeutic drugs and healthcare products (Mukherjee *et al*., 2002; Ivanova *et al.* 2005; Mandal *et al.* 2007). Additionally, number of previous researches reported that secondary metabolite especially Tannin (Patel *et al.*, 2010), Phenols (John *et al*., 2009), flavonoids (Fitriana, 2008), glycosides (Melzig *et al.*, 2001), and alkaloids (Roy *et al.*, 2010) were responsible for antiparasitic activity. So this study was aimed to carry out the phytochemical screening of the most effective ethnoveterinary plants (evaluated in Chapter 4) for the presence of bioactive compounds responsible for their anthelmintic potency.

5.2 MATERIALS AND METHODS

5.2.1 Plant collection and extraction

Collection and preparation of all plants was done as described in Chapter 4 on page number 83, 91.

5.2.2 Preliminary phytochemical screening (Qualitative analysis)

The preliminary qualitative phytochemical analysis was done for both aqueous extracts and methanolic extracts of all selected plants through different tests to know the presence of active biological active compounds viz., alkaloids, phenolic compounds, flavonoids, saponins, glycosides, tannin, terpenoids, anthraquinone, and carbohydrate by following the standard procedures as described by Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993; Wang *et al*., 2010; Hegde and Joshi, 2010; Sawant and Godghate, 2013; Pandey *et al*., 2018a, 2018b) with slight modification (Figure 5.2.2).

5.2.2.a Detection of carbohydrates

Benedict's test: 5 ml test solutions (extracts) was treated with Benedict's reagent and boiled slightly in the water bath for 2-3 minutes. Formation of red precipitate specifies the presence of reducing sugars.

Fehling's Test: 5 ml of test solution was treated with Fehling's A (7 % Cupric sulphate in distilled water) and B (35 % Potassium Sodium Tartrate and 10 % Sodium Hydroxide in distilled water) solutions and boil mildly in the water bath. Development of red precipitate confirms the presence of reducing sugar.

5.2.2.b Detection of Anthraquinone

5 ml of test solution was added with an equal volume of 10 % ammonia solution and shaken gently. Formation of bright pink colouration in the upper aqueous layer confirms the presence of anthraquinone.

5.2.2.c Detection of Terpenoids

Salkowski's test: 5 ml of test solution was dissolved in 1ml of chloroform and then 1 ml of sulphuric acid was added cautiously. The appearance of reddish brown colour indicates the the presence of terpenoids.

5.2.2.d Detection of Tannins

$FeCl_3$ test: 5 ml of test solution was mixed with an equal volume of $FeCl_3$ solution. A dark green or black colour precipitate indicates the presence of tannins.

5.2.2.e Detection of Alkaloids

Mayer's test: 5 ml of test solution was mixed with a few drops of Mayer's reagent. Formation of cream colour precipitate confirms the presence of alkaloids.

- 5ml of the extract was mixed with an equal volume of distilled water and heated up to $60^{o}C$ and allowed to cool for 10 minutes. Thereafter 5 ml of NaOH (Sodium hydroxide) solution was added and at that time the colour of mixture change to yellow but after addition of 5 ml dilute HCl mixture colour changes from yellow to colourless, indicates the presence of alkaloids.

5.2.2.f Detection of Flavonoids

Alkaline Reagent Test: 5ml of the test solution was taken and treated with 2-3 drops of sodium hydroxide solution (NaOH). Development of intense yellow colour, which becomes disappear on the addition of dilute acid (HCl), confirms the presence of flavonoids.

- 5ml of the test solution was taken and mixed with few drops of 1% ammonia solution; emergence of yellow colour indicates the presence of flavonoids.

5.2.2.g Detection of Saponin

Foam Test: test solution was vigorously shaken with 2 ml of distilled water. If foam produced persists for 10 minutes it confirms the presence of saponins.

5.2.2.h Detection of Phenol

FeCl3 test: 5 ml of plant extract mixed with equal volume 10 % aqueous ferric chloride, Bluish green colour appears, confirms the presence of phenol.

5.2.2.i Detection of glycosides

5ml of the extract was mixed with 2.5 ml of dilute H_2SO_4 and boiled for 10-15 minutes then cooled and counterbalanced with 10 % NaOH. After that Fehling's solution A and B were added. Formation of brick red colour precipitation indicates the presence of glycosides.

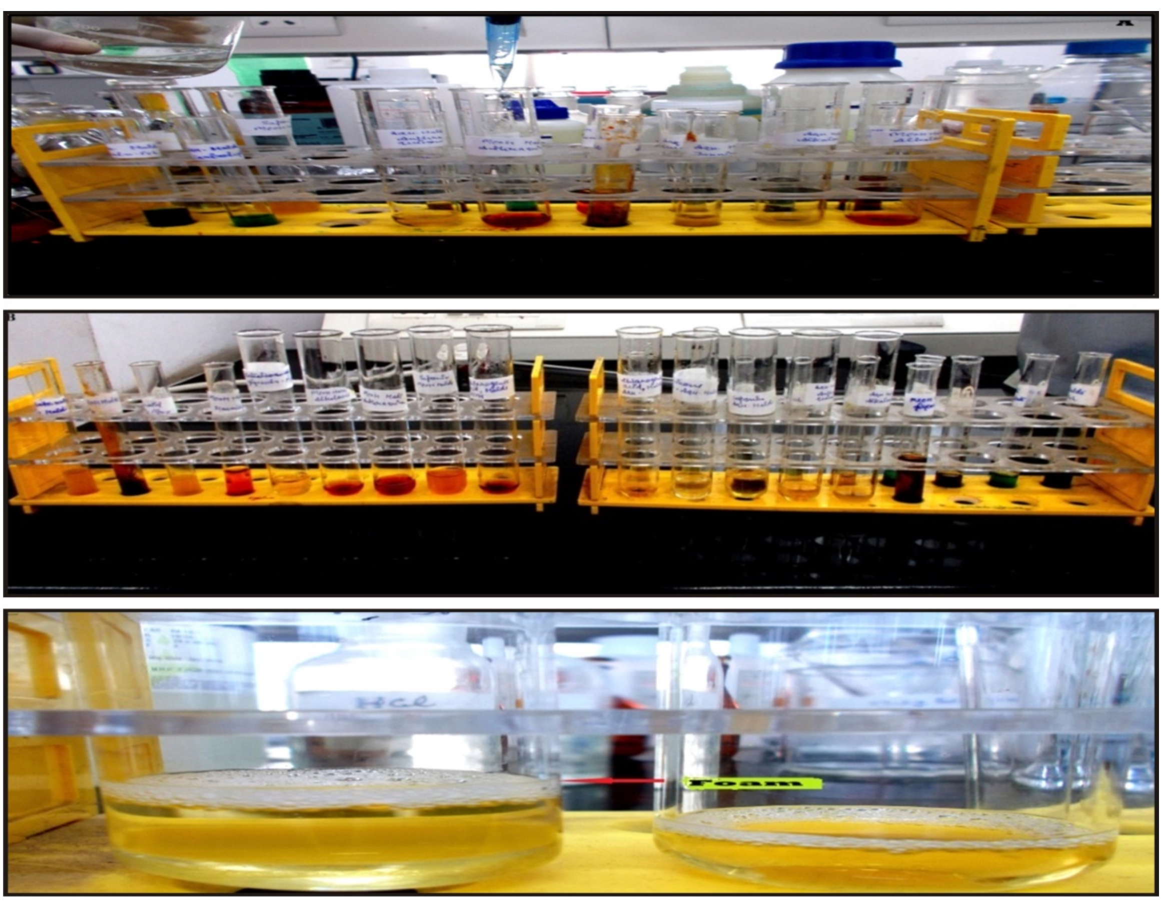


Fig. 5.2.2. A, B, C showing preliminary qualitative phytochemical screening of selected ethnoveterinary plants

5.2.3 Quantitative phytochemical screening

Quantitative phytochemical screening was performed with methanolic extract of the three plants, i.e. *Trachyspermum ammi*, *Hibiscus rosa-sinesis*, and *Trigonella foenum-graecum* which were identified to be more effective. Aqueous Extract (AE) was excluded due to the low anthelmintic potential than Methanolic Extract (ME). This study was done to calculate the total amount of tannins, flavonoids, phenol, and alkaloids because these constituents are previously reported as powerful anthelmintic agents.

5.2.3.a Determination of total Phenol

The quantity of total phenol present in the plant extract was assessed by following the Folin-Ciocalteu colorimetric process (Waterhouse, 2003; Ghasemzadeh *et al*., 2010; Milan, 2011) with slight modification.

Preparation of standard concentration

The stock solution was prepared by dissolving 10 mg Gallic acid powder into a small quantity of methanol and made the volume up to 10 ml by using the same solvent (1 mg/ml stock solution) and used as working standard solution. From this stock solution, 0.01, 0.2, 0.04, 0.06, 0.08 and 0.1 ml volume were taken into separate test tubes.

Sample and blank

0.5 ml of the extract and 0.5 ml of methanol were taken in separate test tubes.

Preparation of test sample

0.5 ml of the Folin-Ciocalteu reagent (1:10) and 4 ml of 7.5 % sodium carbonate (Na_2CO_3) solution was added to each test tube of standard, extract and blank. The volume of each of the test tube was made up-to 5 ml with distilled water. Then the test tubes were covered with silver foil and incubated into a boiling water bath for 30 minutes with intermittent shaking. After that, the test tubes were cooled at room temperature (25^0C- 30^0C) and the absorbance of each test tubes sample was measured at 765 nm into a UV-VIS Spectrophotometer (Perkin Elmer Preasely Lamda 25) against the reagent blank. The calibration curve of the Gallic acid is shown in Figure1 and results were expressed as Gallic acid equivalents (mg GAE/g dried extract).

5.2.3.b Determination of total Tannin

The amount of total tannin present in the plant extract was estimated by following the Folin-Ciocalteu colorimetric method (Singh *et al.,* 2012) with minor modification.

Preparation of standard concentration

The stock solution was prepared by dissolving 10 mg Tannic acid powder into a small amount of methanol and made the volume up to 10 ml by using the same solvent (1 mg/ml stock solution) and used as working standard solution. From this stock solution, 0.01, 0.2, 0.04, 0.06, 0.08 and 0.1 ml volume were taken into separate test tubes.

Sample and blank

0.5 ml of the extract and 0.5 ml of methanol were taken in separate test tubes.

Preparation of test solution

0.5 ml of the Folin-Ciocalteu-phenol reagent and 1 ml of 35 % Na_2CO_3 (Sodium Carbonate) solution were added in each test tube of standard, extract and blank and the samples were diluted up to the volume of 10 ml by using distilled water. The mixtures were shaken well and kept at room temperature for 30 minutes. After that, the absorbance of each test tubes sample was measured at 725 nm into a UV-VIS Spectrophotometer (Perkin Elmer Preasely Lamda 25) against the reagent blank. The calibration curve of the Gallic acid is shown in Figure 2 and the result was expressed as Tannic acid equivalents (mg TAE/g dried extract).

5.2.3.c Determination of total Flavonoid

The amount of total tannin present in the plant extract was estimated by following Aluminium Chloride colorimetric assay (Zhinsen *et al.,* 1999; Hajaji *et al.,* 2010) with the minor modification.

Preparation of standard concentration

The stock solution (standard solution) was prepared by dissolving 10 mg Rutin powder into a small quantity of methanol and made the volume up to 10 ml by using the same solvent (1 mg/ml stock solution). From this stock solution, 0.01, 0.2, 0.04, 0.06, 0.08 and 0.1 ml volume were taken into separate test tubes.

Sample and blank

0.5 ml of the extract and 0.5 ml of methanol were taken in separate test tubes.

Preparation of test solution

2 ml distilled water and 0.15 ml of 5% $NaNO_2$ (Sodium Nitrite) solution were added in each test tube of standard, extract and blank. After 6 min, 0.15 ml 10% $AlCl_3$ solution was added and left for another 6 min. Then, 2 ml 4% NaOH was added solution and the final volume was adjusted up to 10 ml by immediate addition of distilled water and allowed to stand for 15 min. The absorbance was recorded at 510 nm into a UV-VIS Spectrophotometer (Perkin Elmer Preasely Lamda 25) against the reagent blank. The calibration curve of the Rutin is shown in Figure 3 and the result was expressed as Rutin (RE) equivalent (mg RE/g dried extract).

5.2.3.d Determination of total Alkaloid

The total amount of Alkaloid present in the plant extract was determined by following the standard procedure as described by Khandelwal, 2006; Shamsa *et al.*, 2008 with little modification.

Preparation of standard concentration

The stock solution was prepared by dissolving 10 mg Atropine powder into a small amount of methanol and adjusted the volume up to 10 ml by using the same solvent (1 mg/ml stock solution) and used as working solution for the standard. From this stock solution, 0.01, 0.2, 0.04, 0.06, 0.08 and 0.1 ml volume were taken into separate test tubes.

Sample and blank

0.5 ml of the extract and 0.5 ml of methanol were taken in separate test tubes.

Preparation of test solution

2.5 ml pH 4.7 phosphate buffer was added in each test tube of standard, extract and blank. Thereafter, 2.5 ml Bromocresol green (0.04 % BCG in 1:10) solution and 4 ml of Chloroform were added and shaken vigorously. After that, the final volume was adjusted up to 10 ml by adding Chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank. The calibration curve of the Atropine is shown in Figure 4 and the result was expressed as Atropine (AE) equivalent (mg AE/g dried extract).

5.2.3.e Determination of total Saponin

The total quantity of Saponin present in the plant extract was estimated by following the standard procedure as described by Hiai *et al.*, 1976; Baccou *et al.*, 1977; Sim, 2011 with minor modifications.

Preparation of standard concentration

The stock solution was prepared by dissolving 10 mg Diosgenin in a small volume of methanol and final volume was diluted up to 10 ml by using the same solvent (1 mg/ml stock solution) and used as working solution for the standard. From this stock solution, 0.01, 0.2, 0.04, 0.06, 0.08 and 0.1 ml volume were taken into separate test tubes.

Sample and blank

0.5 ml of the extract and 0.5 ml of methanol were taken in separate test tubes.

Preparation of test solution

0.25 ml of 8 % vanillin reagent was added in each test tube containing standard aliquot, extract and blank subsequently 2.5 ml of 72 % H_2SO_4 was mixed gradually from the side of the test tube wall. Thereafter solutions were shaken well and heated in water bath at 60 ^{0}C. After that tubes were kept to incubate for 10 minutes and finally placed at 4^{0}C for cooling for 4 – 5 minutes. The absorbance was observed at 544 nm against blank. The calibration curve of the Diosgenin is shown in Figure 5 and the result was articulated as Diosgenin (DE) equivalent (mg DE/g dried extract).

5.2.4 Statistical analysis

- The spectrophotometric analysis was carried out in triplicates and the results were expressed as Mean±Standard Deviation.
- Calculation of the total amount of the phytochemical compounds (Phenol, Tannin, Alkaloids, Flavonoids, and Saponin) in the plant extracts were done by the formula:

$$TPC = \frac{cV}{M}$$

Where, TPC = Total amount of Phytochemical Compound in mg/ g (in Gallic acid/Tannic acid/ Rutin/ Atropine/ Diosgenin equivalent)
c = Concentration of Gallic acid/Tannic acid/ Rutin/ Atropine/ Diosgenin established from the calibration curve
V= Volume of extract used (ml)
M= Mass of extract used (gram)

5.4 RESULT

5.4.1 Preliminary qualitative analysis

5.4.1.i *Trachyspermum ammi*

The Preliminary qualitative analysis of *Trachyspermum ammi* seeds as shown in Table 5.4.1.a revealed that both aqueous extract and methanolic extract of this plant showed the presence of carbohydrate, alkaloids, flavonoids, terpenoids, tannins, phenols, saponins, glycosides except the anthraquinone. Furthermore, methanolic extract showed the intense colour of precipitate for alkaloids, terpenoids, tannins, and phenols while saponins showed more foam formation than aqueous extract.

5.4.1.ii *Hibiscus rosa-sinesis*

It was evident from the Table 5.4.1.b, the preliminary qualitative analysis of *Hibiscus rosa-sinesis* leaves showed that both aqueous extract and methanolic extract of this plant showed the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, saponins, glycosides except carbohydrate, and anthraquinone. Also, the methanolic extract showed the intense colour of precipitate for alkaloids, tannins, and phenols than aqueous extract.

5.4.1.iii *Trigonella foenum-graecum*

The qualitative analysis of *Trigonella foenum-graecum* seeds showed that the aqueous extract showed the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, saponin, glycosides whereas methanolic extract showed the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, and saponins except glycosides. Moreover the methanolic extract showed the intense colour of precipitate for carbohydrate, alkaloids, tannins, flavonoids and phenols than aqueous extract (Table 5.4.1.c).

5.4.1.iv *Curcuma longa*

The phytochemical analysis of *the Curcuma longa* rhizome extracts as tabulated in Table 5.4.1.d, revealed that the different types of phytochemical constituents were observed in the methanolic and aqueous solvents, i.e., methanolic extract showed the presence of glycosides, alkaloids, flavonoids, tannins, saponins, terpenoids, phenol, anthraquinone, and carbohydrates while the aqueous extract showed the presence of alkaloids, carbohydrate, flavonoids, terpenoids, and saponins. Besides the methanolic extract showed the intense colour of precipitate for carbohydrate, alkaloids, tannins, flavonoids, and phenols while saponin showed more foam formation than aqueous extract.

5.4.1.v *Azadiracta indica*

The result of phytochemical analysis of extract of *Azadiracta indica* leaves was summarized in Table 5.4.1.e. It reveals that the methanolic extract showed the presence of glycosides, alkaloids, flavonoids, tannins, saponins, terpenoids, phenol, and carbohydrates except for anthraquinone. In other hands, aqueous extract showed only the presence of alkaloids, tannins, flavonoids, and phenols.

5.4.1.vi *Nigella sativa*

The preliminary phytochemical analysis of both aqueous extract and methanolic extract of *Nigella sativa* seeds were tabularized in Table 5.4.1.f. This table divulges that the aqueous extract showed only the presence of alkaloids and phenol whereas the methanolic extract showed the presence of carbohydrate, alkaloids, phenol, tannin, flavonoids, and saponins.

5.4.1.vii *Coriandrum sativum*

The preliminary phytochemical analysis of extract of *Coriandrum sativum* seeds was arranged in Table 5.4.1.g. It was manifested from the table that methanolic extract has shown the presence of flavonoids, terpenoids, tannin, phenol, and glycosides while aqueous extract showed the presence of flavonoids, terpenoids and phenol.

5.4.1.viii *Ficus religeosa*

The qualitative phytochemical analysis of leaves extract of *Ficus religeosa* disclosed that the methanolic extract displayed the presence of carbohydrate, alkaloids, tannin, and phenol whereas aqueous extract showed the presence of carbohydrate, terpenoids, and phenols (Table 5.4.1.h).

Table 5.4.1.a: Preliminary phytochemical analysis of both Aqueous Extract (AE) and Methanolic Extract (ME) of *Trachyspermum ammi* seeds

Phytochemical Constituents	Aqueous Extract (AE)	Methanolic Extract (ME)
Carbohydrate	+	+
Anthraquinone	–	–
Alkaloids	+	++
Flavonoids	+	+
Terpenoids	+	++
Tannins	+	++
Phenols	+	++
Saponin	+	++
Glycosides	+	+

Where; +: Positive, ++: Strong positive, -: Negative

Table 5.4.1.b: Preliminary phytochemical analysis of both Aqueous Extract (AE) and Methanolic Extract (ME) of *Hibiscus rosa-sinesis* leaves

Phytochemical Constituents	Aqueous Extract (AE)	Methanolic Extract (ME)
Carbohydrate	-	-
Anthraquinone	–	-
Alkaloids	+	++
Flavonoids	+	+
Terpenoids	+	+
Tannins	+	++
Phenols	+	++
Saponin	+	+
Glycosides	+	+

Where; + : Positive, ++ : Strong positive, - : Negative

Table 5.4.1.c: Preliminary phytochemical analysis of both Aqueous Extract (AE) and Methanolic Extract (ME) of *Trigonella foenum-graecum* seeds

Phytochemical Constituents	Aqueous Extract (AE)	Methanolic Extract (ME)
Carbohydrate	+	++
Anthraquinone	+	+
Alkaloids	+	++
Flavonoids	+	++
Terpenoids	+	+
Tannins	+	++
Phenols	+	++
Saponin	+	+
Glycosides	+	–

Where; + : Positive, ++ : Strong positive, - Negative

Table 5.4.1.d: Preliminary phytochemical analysis of both Aqueous Extract (AE) and Methanolic Extract (ME) of *Curcuma Longa* rhizome

Phytochemical Constituents	Aqueous Extract (AE)	Methanolic Extract (ME)
Carbohydrate	-	++
Anthraquinone	+	+
Alkaloids	+	++
Flavonoids	+	++
Terpenoids	+	+
Tannins	–	+
Phenols	–	++
Saponin	+	++
Glycosides	–	+

Where; +: Positive, ++: Strong positive, - : Negative

Table 5.4.1.e: Preliminary phytochemical analysis of both Aqueous Extract (AE) and Methanolic Extract (ME) of *Azadiracta indica* Leaves

Phytochemical constituents	Aqueous Extract (AE)	Methanolic Extract (ME)
Carbohydrate	-	+
Anthraquinone	-	-
Alkaloids	+	+
Flavonoids	+	+
Terpenoids	+	+
Tannins	–	+
Phenols	+	+
Saponin	-	+
Glycosides	–	+

Where; +: Positive, - : Negative

Table 5.4.1.f: Preliminary phytochemical analysis of both Aqueous Extract (AE) and Methanolic Extract (ME) of *Nigella sativa* seeds

Phytochemical Constituents	Aqueous Extract (AE)	Methanolic Extract (ME)
Carbohydrate	-	+
Anthraquinone	-	-
Alkaloids	+	+
Flavonoids	–	+
Terpenoids	–	–
Tannins	–	+
Phenols	+	+
Saponin	–	+
Glycosides	–	–

Where; +: Positive, - : Negative

Table 5.4.1.g: Preliminary phytochemical analysis of both Aqueous Extract (AE) and Methanolic Extract (ME) of *Coriandrum sativum* seeds

Phytochemical constituents	Aqueous Extract (AE)	Methanolic Extract (ME)
Carbohydrate	+	–
Anthraquinone	–	–
Alkaloids	–	–
Flavonoids	+	+
Terpenoids	+	+
Tannins	–	+
Phenols	+	+
Saponin	–	–
Glycosides	–	+

Where; + :Positive, - : Negative

Table 5.4.1.h: Preliminary phytochemical analysis of both Aqueous Extract (AE) and Methanolic Extract (ME) of *Ficus religeosa* leaves

Phytochemical Constituents	Aqueous Extract (AE)	Methanolic Extract (ME)
Carbohydrate	+	+
Anthraquinone	-	-
Alkaloids	–	+
Flavonoids	–	–
Terpenoids	+	–
Tannins	–	+
Phenols	+	+
Saponin	–	–
Glycosides	–	–

Where; + : Positive, -: Negative

5.4.2 Quantitative analysis

The quantitative analysis was carried out for the methanolic extracts of the plants evaluated to be the most effective for calculating the most important phytochemical compounds viz., tannin, alkaloids, phenol, flavonoids, and saponin which were previously reported as for having anthelmintic potential.

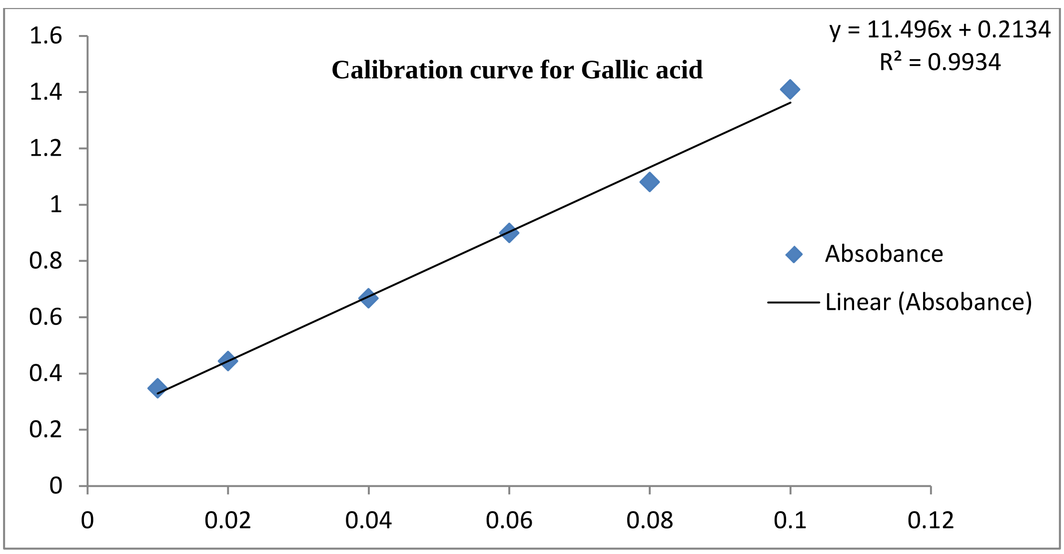
The quantitative analysis data was tabulated in Table 5.4.2 and Graph 5.4.2.f. The amount of total phenol was determined with the Folin-Ciocalteu reagent method using Gallic acid as a standard compound. The total phenols were expressed as mg/g Gallic acid equivalent using the standard curve equation: $y = 11.496x + 0.2134$; Where y is the absorbance at 765 nm and x is total phenolic content in all three plants (Graph 5.4.2.a). It is evident from the Table 5.4.2 that *Trachyspermum ammi* showed the highest amount of total Phenolic compound (52.85±2.9) followed by *Hibiscus rosa-sinensis* (48.53±0.12), and *Trigonella foenum-graecum* (44.65±1.3).

The amount of total tannin was also determined with the Folin-Ciocalteu reagent process using Tannic acid as a standard compound. The total tannin was expressed as mg/g Tannic acid equivalent using the standard curve equation: $y = 7.0764x — 0.0914$; Where y is the absorbance at 725 nm and x is total phenolic content in all three plants (Graph 5.4.2.b). As seen in the Table 5.4.2 and Graph 5.4.2.f, it was found that *Trachyspermum ammi* showed highest amount of total tannin compound (78.63±0.29) followed by *Hibiscus rosa-sinensis* (62.15±1.16), and *Trigonella foenum-graecum* (55.42±0.88).

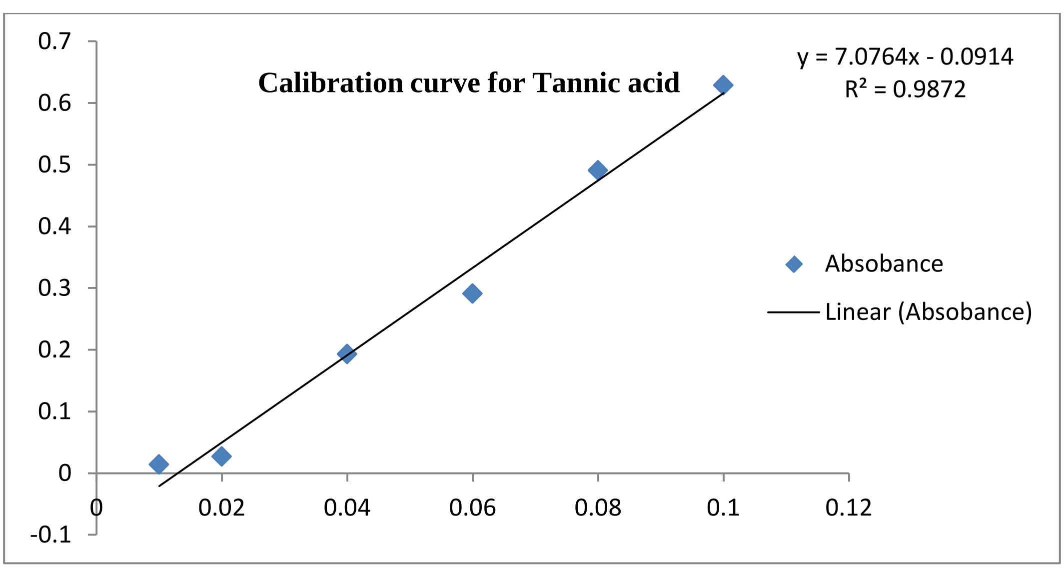
The quantity of total flavonoid content was measured by using $AlCl_3$ (Aluminum Chloride) reagent method and Rutin was used as a standard. The total flavonoid content was manifested as mg/g Rutin equivalent by means of the standard curve equation: $y = 13.129x — 0.1742$; where y is the absorbance at 510 nm and x is total flavonoid content in all three plants (Graph 5.4.2.c). The results revealed that the total flavonoid content was found highest for *Trachyspermum ammi*, i.e. 37.65±0.6 followed by *Hibiscus rosa-sinensis* 18.5±0.69, and lowest in *Trigonella foenum-graecum* 14.4±0.33 (Table 5.4.2 and Graph 5.4.2.f).

The total alkaloids present in plant extract were determined by Bromocresol green-chloroform method. Atropine was used as a standard compound and the total alkaloid content was expressed as mg/g Atropine equivalent using the standard curve equation: $y = 0.4394x - 0.0065$; where y is the absorbance at 470 nm and x is total alkaloid content in the extract (Graph 5.4.2.d). The higher amount of alkaloids contents was observed in *Trachyspermum* *ammi*, i.e. 104.9±2.4 while *Hibiscus rosa-sinensis* showed moderate 73.72±0.54, and low in *Trigonella foenum-graecum* 45.62±1.0 (Table 5.4.2 and Graph 5.4.2.f).

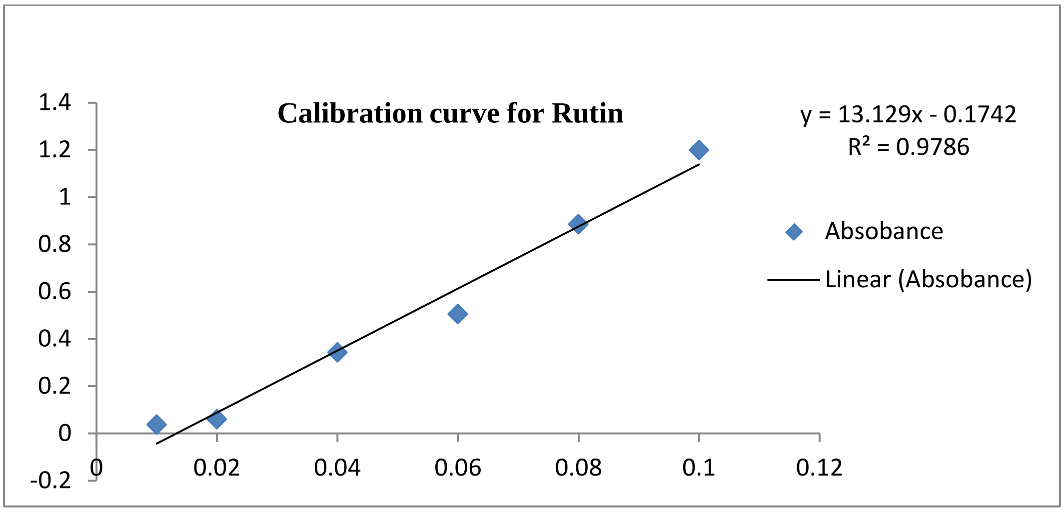
Furthermore, the total Saponin present in plant extract was determined by Vanillin reagent method. Diosgenin was used as a standard compound and the total alkaloid content was expressed as mg/g Diosgenin equivalent using the standard curve equation: y = 0.3726x + 0.0217; where y is the absorbance at 544 nm and x is total Diosgenin content in the extract (Graph 5.4.2.e). Higher amount of saponin was observed in *Trachyspermum ammi*, i.e. (40.66±4.0) followed by *Hibiscus rosa-sinensis* (14.7±2.1), and lowed in *Trigonella foenum-graecum* (8.4±1.3) as depicted from the Table 5.4.2 and Graph 5.4.2.f.



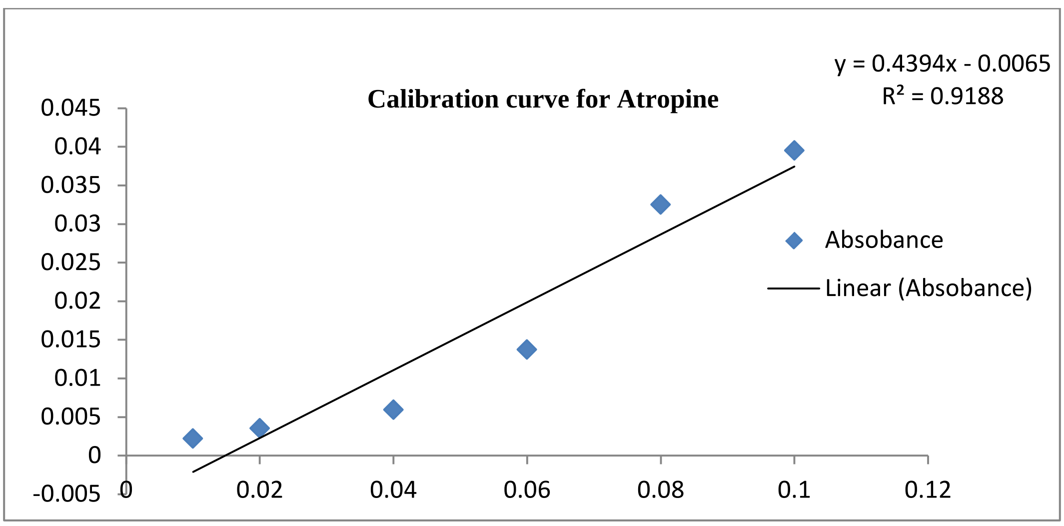
Graph 5.4.2.a: Gallic acid standard curve of total phenolic content.



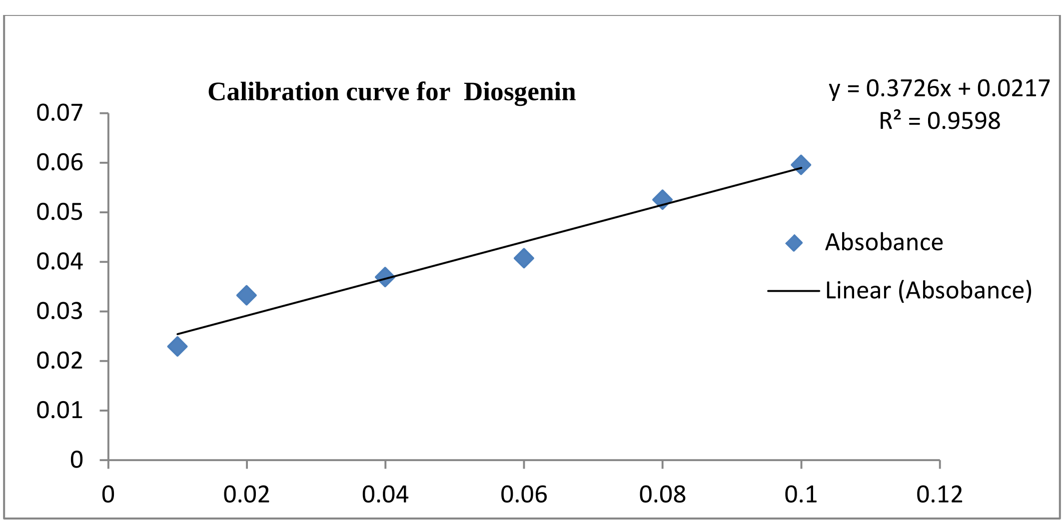
Graph 5.4.2.b: Tannic acid standard curve for total tannin content



Graph 5.4.2.c: Rutin standard curve of total flavonoid content



Graph 5.4.2.d: Atropine standard curve of total alkaloids content

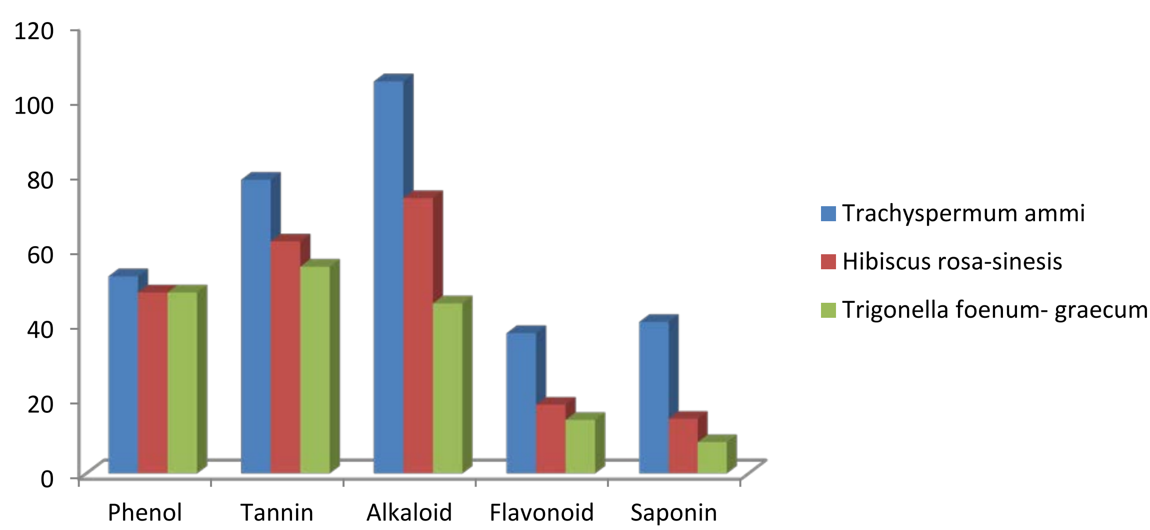


Graph 5.4.2.e: Diosgenin standard curve of total Saponin content

Table 5.4.2: Quantitative estimation of total phenol, total tannin, total alkaloids, total flavonoids and total saponins in Methanolic Extract (ME) of most effective plants

Plant	Phytochemical compound				
	Phenol (mg/g equivalent of Gallic acid)	**Tannin** (mg/g equivalent of Tannic acid)	**Alkaloid** (mg/g equivalent of Atropine)	**Flavonoid** (mg/g equivalent of Rutin)	**Saponin** (mg/g equivalent of Diosgenin)
Trachyspermum ammi	52.85±2.9	78.63±0.29	104.9±2.4	37.65±0.6	40.66±4.0
Hibiscus rosa-sinesis	48.53±0.12	62.15±1.16	73.72±0.54	18.5±0.69	14.7±2.1
Trigonella foenum-graecum	44.65±1.3	55.42±0.88	45.62±1.0	14.4±0.33	8.4±1.3

Values are expressed as Mean±Standard deviation.



Graph 5.4.2.f: Quantitative estimation of total phenol, total tannin, total alkaloids, total flavonoids and total saponins in ME of most effective plants

5.5. DISCUSSION

Herbal plants are the reservoir of natural by-products including various types of the biologically active compounds having important characteristics of medicine or for nourishment (Swargiary *et al.*, 2017). The use of indigenous plants for the impediment and cure of gastrointestinal helminthiasis has its beginning in ethno-veterinary medicine (Athansidou *et al.*, 2007). In ethnoveterinary, medication has played a very important role for a few decades with a range of plants or plant extracts which were suitable for treating almost every parasitic disease of livestock.

The phytochemical analysis of the present study showed a large variation in the phytochemical compounds. The therapeutic value of plants is due to the presence of these biologically active substances. These compounds have a certain physiological action on the living system. Even though, the anthelmintic potency of the plants is primarily due to their phytochemical compounds. These compounds independently or cooperatively may act by creating hindrance in the process of tubulin polymerization and obstructing glucose uptake (Jain and Jain 2011). Any impairment to the cuticular wall (the mucopolysaccharide membrane) of worms will restrict their movement which may finally lead to paralysis and eventually the death of parasite (Chandrashekhar *et al*., 2008). Many previous reports disclosed that many secondary metabolites namely tannin (Bate-Smith, 1962; Kumar and Prabhakar, 1987; Shukla *et al*., 2000; Fransworth, 2011), phenols (Bate-Smith, 1962; Pratap *et al*., 2018), alkaloids (Bate-Smith, 1962; Wang *et al*., 2010; Fransworth, 2011; Pratap *et al*., 2018), flavonoids (Ademola *et al*., 2005; Barrau *et al*., 2005; Fransworth, 2011) and saponin (Melzig *et al.*, 2001; Fransworth, 2011) were responsible for anthelmintic activity.

Qualitative phytochemical screening

The qualitative screening of *Trachyspermum ammi* showed presence of carbohydrate, alkaloids, flavonoids, terpenoids, tannins, phenols, saponin, and glycosides except for the anthraquinone in aqueous and methanolic extract both. Aafreen *et al*. (2017) also performed the qualitative analysis of *Trachyspermum ammi* with both aqueous and methanolic extract and supported this results by reporting the presence of alkaloids, flavonoids, terpenoids, phenols, saponin except tannins in aqueous extract whereas in methanolic extract they reported the presence of alkaloids, tannins, flavonoids, phenols, saponin except for terpenoids. Tacouri *et al.*, (2013) also supported this study by investigating the alkaloids, flavonoids, phenols, saponin, and tannins in the aqueous extract of *Trachyspermum ammi*.

Almost similar results were reported by Mumtaz *et al*., 2014 with the methanolic extract of *Trachyspermum ammi* and reported the presence of alkaloids, flavonoids, phenols, saponin, and tannins except for the glycosides.

The qualitative analysis of *Hibiscus rosa-sinensis* showed the presence of alkaloids, flavonoids, phenols, saponin, tannins, and glycosides except for carbohydrate and anthraquinone in both extracts. Tiwari *et al.* (2015) also reported the similar compounds viz. alkaloids, flavonoids, phenols, saponin, tannins, and glycosides in methanolic extract of *Hibiscus rosa-sinensis*. In a study conducted by Alapati and Shaik (2015), they reported presence of only phenol and resins in the aqueous extract of *Hibiscus rosa-sinensis.* Moreover, findings of the researches of Pekamwar *et al*. (2013) and Prasanna *et al.* (2017) are at par with findings of the present study and reported the presence of alkaloids, flavonoids, phenols, saponin, tannins, glycosides and carbohydrate in aqueous extract.

The preliminary phytochemical screening of *Trigonella foenum-graecum* displayed the presence of alkaloids, flavonoids, phenols, saponin, tannins, glycosides, anthraquinone and carbohydrate in aqueous extract, while methanolic extract indicated the presence of some phytochemical compound same as the aqueous extract except for glycosides which were absent in methanolic extract. This result of the aqueous extract of *Trigonella foenum-raecum* was in agreement with the findings of Kumari *et al.* (2016) except tannins compounds which were absent in the aqueous extract. This result is at par with the findings of Chalghoumi *et al*. (2016) who reported the presence of flavonoids, tannins, saponins, terpenoids and glycosides except for alkaloids, phenolic compounds which were absent in the aqueous extract. Yadav and Chowdhury (2017) also reported similar result to some extent in addition to the presence of alkaloids, flavonoids, phenolic compounds and tannins, carbohydrates with absence of glycosides in the aqueous extract. The methanolic extract of *Trigonella foenum-graecum* also indicated the presence of flavonoids, carbohydrates, terpenoids alkaloids, phenolic compound, tannins, and saponins except for the glycosides. Similar results were reported by Kumari *et al.* (2016) in the methanolic extract and reported same compound as investigated in the present study. Mishra *et al.* (2016) also have reported same findings and reported the presence of alkaloids, flavonoids, phenolic compounds and tannins, carbohydrates and absence of glycosides.

The qualitative phytochemical analysis of *Curcuma longa* also depicted the presence of flavonoids, carbohydrates, terpenoids, alkaloids, phenolic compounds, tannin, and saponins.

The aqueous extract showed the same compound as investigated in the methanolic extract of the present result except for tannin, phenols, glycosides, and carbohydrate. The results are similar to the findings of Gupta *et al*. (2015) who reported the presence of alkaloids and flavonoids in the aqueous extracts of *Curcuma* rhizome but showed the contrast result for the presence of tannins, glycosides, carbohydrates, and phenol. Deb *et al.* (2013) also reported the same result in respect to flavonoids but disagreed with the presence of tannin, alkaloids, terpenoids, and glycosides in aqueous extract of *Curcuma* rhizome. In the other study, Srivastav *et al.* (2018) has screened aqueous extract of *Curcuma longa* rhizome and reported the same result with regard to alkaloids, flavonoids, saponin, and carbohydrate except for tannin. Saxena and Shau (2012) isolated the same compounds, i.e. carbohydrate, glycoside, flavonoid, alkaloid, tannin, and saponin from Methanolic extracts of rhizomes of *Curcuma longa*. Sawant and Godghate, 2013 screened the methanolic extract of *Curcuma* rhizome and reported the similar compound as described in the methanolic extract of present finding.

The phytochemical investigation of aqueous extract of *Azadirachta indica* has shown the presence of alkaloids, terpenoids, flavonoids, and phenol whereas methanolic extract showed the existence of alkaloids, terpenoids, flavonoids, phenol, saponins, glycosides, tannin, and carbohydrate. Same study was performed by Gupta *et al.* (2013) with the aqueous extract and alcoholic extract of *Azadirachta indica* to analyse the phytochemical compounds and reported the presence of same compounds, i.e., glycosides, carbohydrate, flavonoids and terpenoids in aqueous extract except for alkaloids, saponins, and tannin while alcoholic extract showed the presence of glycosides, flavonoids, terpenoids, alkaloids, saponins, and tannin at par with the findings of the present study except for carbohydrate. Raphael (2012) reported the presence of alkaloids, flavonoids, glycosides, terpenoids, and carbohydrates except for tannin in the aqueous extract of the same plant. Dash *et al.* (2017) also carried out the similar study to investigate the phytochemical compounds present in aqueous and methanolic extract and reported the presence of alkaloids, tannins, saponins, glycosides, flavonoids, and reducing sugars.

The preliminary phytochemical analysis of both the aqueous and methanolic extract of *Nigella sativa* showed the variation in the presence of the phytochemical compound. This study revealed that the methanolic extract of this plant showed the presence of carbohydrate, alkaloids, flavonoids, tannins, phenols, and saponins whereas aqueous extract showed the presence of alkaloids and phenol only. Ishtiaq *et al.* (2013) also investigated the phytochemical compound present in the aqueous and methanolic extract of *Nigella sativa* and reported the existence of tannin, flavonoids, glycosides, and saponins in the methanolic extract which is in agreement with the present findings except for terpenoids. They also reported the tannins and flavonoids in the aqueous extract which has not supported the findings of the present result. Aafreen *et al.* (2017) screened the phytochemical compound in both the aqueous and methanolic extract of *Nigella sativa* and supported the findings of the present study by showing the presence of alkaloids, flavonoids, tannins, phenols, and saponins in the methanolic extract. In the aqueous extract, they also reported the presence of alkaloids and phenol at par with the result of the finding of present research but contrary findings were reported indicating the presence of flavonoid, tannin, saponins, and terpenoids in the aqueous extract.

The qualitative phytochemical analysis *Coriandrum sativum* revealed the presence of flavonoids, terpenoids, tannins, phenols and glycosides in methanolic extract whereas aqueous extract showed the presence of flavonoids, terpenoids, phenols, and glycosides. Shrivastava (2017) supported the present findings by reporting the glycosides, terpenoids, saponins, tannins, and alkaloids except for the flavonoids in methanolic extracts whereas in the aqueous extract glycosides, terpenoids, and flavonoid was present and showed the absence of tannins, saponins, and alkaloids. Pathak *et al.* (2011) carried out a similar study and reported the glycosides in the methanolic extract at par with the findings of the present study while aqueous extract showed the absence of flavonoids, terpenoids, phenols, and glycosides.

The qualitative phytochemical analysis of *Ficus religeosa* divulged that the methanolic extract exhibited the presence of carbohydrate, alkaloids, tannins, and phenols whereas aqueous extract showed the presence of carbohydrate, terpenoids, and phenols. Prakash *et al.* (2017) has performed the same experiment and reported that both aqueous extract and methanolic extract showed the presence of carbohydrate, saponins, phenols, flavonoids, tannins, and terpenoids except the presence of alkaloid. Alapati and Shaik (2015) reported that aqueous extract of this plant contains glycosides, saponins, and phenol.

Quantitative phytochemical screening

Quantitative estimation of methanolic extract of most effective plants suggested that the *Trachyspermum ammi* as most potent anthelmintic due to their high amount of biologically active compounds i.e. alkaloids, tannins, flavonoids, saponins, and phenol in comparison to the other two plants. As reported in many previous kinds of research, the main effects of the allopathic anthelmintic drugs on the parasites were to create a relinquishing paralysis that results in the dislodgment of the worm by peristalsis. The high efficiency of standard drug Albendazole is accredited to the action that it possibly blocks the glucose acceptance in parasites (by binding itself to free protein in the intestinal tract or on the body surface) which leads to decrease in the glycogen level and subsequently leading to the death of the worms (Parvathy *et al.*, 2014). Chemically tannins are polyphenolic compounds (Wang *et al*., 2010). Some synthetic phenolic anthelmintics drugs e.g. niclosamide, oxyclozanide, bithionol etc., are reported to impede with energy generation kinetics in helminth parasites by uncoupling oxidative phosphorylation (Hegde and Joshi, 2010). Likewise, the anthelmintic effects of phenolic group and tannins are may be due to its capacity to bind with the free protein accessible for larval nutrition of the parasites and thus dropping the nutrient obtainability and further resulting in larval starvation. It may also through binding to the cuticular or binds to the cuticular glycoprotein and hence consequently creates a hindrance in parasites movement or decline in gastrointestinal metabolism directly through inhibition of oxidative phosphorylation leading to larval death (Scalbert., 1991, Athanasiadou *et al*., 2001; John *et al.*, 2009; Patel *et al*., 2010).

Alkaloids reduce the nitrate generation together with its anti-oxidating properties, hence; obstruct the process of protein synthesis and local homeostasis that is essential for the development of helminths (Borba *et al.*, 2010). Moreover, alkaloids also suppress the allocation of sucrose from the stomach to the small intestine and disturbed the glucose uptake process of the helminths which consequently affect the central nervous system causing paralysis (Roy *et al.*, 2010; Srivastav *et al.*, 2018).

The role of saponin as an anthelmintic compound is revealed in recent researches and reported that the saponin has membrane with permeabilizing and pore formation property similar to two recognizable synthetic anthelmintic drugs such as Praziquantel and Toltrazuril. Due to this property saponin may have an effect on the permeability of the cell membrane of the parasites and causing vacuolization and disintegration of leading to the breakdown of helminths cuticular surface (Roy *et al.*, 2010; Patel *et al.*, 2010). Additionally, saponins chemically are glycosides, which are made by a lipid-soluble aglycon comprising of either a sterol or more commonly a triterpenoid with diverse water-soluble sugar residues. Saponins can also affect eukaryotic organisms that contain steroids in their membrane due to their surfactant properties (Osbourn *et al.* 2011). On the other hand, flavonoids also generate a disruption in the nerve impulse pathway which consequently affects the nervous system, leading to neuron degeneration. Helminth's homeostasis is also disturbed making the worms to get paralyzed and finally leading to death (Fitriana, 2008).

5.6. CONCLUSION

- The results of preliminary phytochemical analysis of the eight selected ethno-veterinary plants showed a huge disparity for the presence of different types of biologically active compounds in both extracts. Methanolic extract showed presence of more phytochemical compounds which may be the main reason for its high potential towards the anthelmintic activity. The major phytochemical constituents responsible for anthelmintic activity i.e. alkaloids, tannins, glycosides, flavonoids, and saponins were present in most of the methanolic extract of the plants.
- Quantitative estimation of the present study concluded that the *Trachyspermum ammi* is most potent anthelmintic plant followed by *Hibiscus rosa-sinesis* and *Trigonella foenum-graecum* due to the variation in the presence of phytochemical compounds. Hence, the plants used in the present study can be comprehended as a potential source of new valuable anthelmintic drugs. Further, the phytochemical characterization, identification, and dose standardization of responsible bioactive compounds are necessary for future study.

Chapter 6

Significance of the Study

SIGNIFICANCE OF THE STUDY

- ✓ The prevalence data obtained from this study concerning with gastrointestinal helminth biology and epidemiological infection patterns in the studied area and other geographical areas with similar ecological conditions could be exploited for developing appropriate control program against gastrointestinal helminthiasis of the small ruminants and this has a prospective to reduce production losses.
- ✓ The *in vitro* anthelmintic assay of this study was aimed togenerate positive, useful data on ethno-veterinary plants that could be certainly used to treat helminth parasitism and its related infestations. The outcome of this study will give some baseline data, which could lead to further research in the forthcoming future, and hopefullythe development of herbal based remedies to control and management of helminthiasis. In the past few years, the importance of plant-based drugs for the treatment of livestock diseases has immensely increased due to their fewer side effects. Therefore, through the frequent use of cheaper and locally accessible medicinal plants could help resource-poor farmers of rural society to improve and maintain the health care of livestock and also increased their production.

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Appendix

PAPERS PUBLISHED IN JOURNALS

S. No.	Title of the paper	Authors	Journal Name/Volume (Issue)/Page Number: ISSN	Year
1	*In Vitro* Anthelmintic Efficacy of *Trigonella foenum-graecum* Seed Extract on *Haemonchus* sp. (An Abomasal Parasite) in Goats	Jyoti Pandey, Suman Mishra, Kamal Jaiswal	International Journal of Advanced Scientific Research and Management/ 3 (11)/188-193: 2455-6378	2018
2	*In Vitro* Evaluation of The Anthelmintic Activity of Rhizome Extracts of *Curcuma Longa* (Linn.)	Jyoti Pandey, Suman Mishra, Kamal Jaiswal	Asian Journal of Pharmaceutical and Clinical Research, 11(12)/ 425-428: 2455-3891	2018