1. INTRODUCTION

India possesses 135.17 million goats (Livestock census, 2012) which account 26.40% of total livestock population. Small ruminants have enormous potential to boost our economy and play major source of income to marginal farmers. However, various parasitic diseases are responsible for causing harm through production loss, morbidity and mortality.

Gastrointestinal (GI) nematodiosis is considered as a major constraint in livestock productivity round the globe (Githiori et al., 2004). The substantial economic loss due to roundworm infection in small ruminants in India was estimated to be $103.0 million (McLeod, 2004) and in another study Das (2012) estimated economic losses of Rs. 657.51 million in adult goat due to gastrointestinal nematodosis in Madhya Pradesh. The gastrointestinal nematodes of greatest importance in small ruminants are members of the family Trichostrongylidae and Haemonchus contortus is one of the commonest and most pathogenic parasite of small ruminants. One H. contortus female produced up to 10,000 eggs per day. Third stage larvae (L₃) are the longest and resistant stage, grazed by small ruminants in pasture. Both fourth stage larvae (L₄) and adults are voracious blood suckers and suck about 0.05 ml of blood/parasite/day resulting in anemia, hypoproteinaemia, emaciation, oedema, intermittent diarrhoea, weight loss, decreased appetite and occasionally protein loss resulting in fluid accumulation under the jaw known as “bottle jaw”. For control, anthelmintic drugs are the predominant weapon available till date to combat parasite menace in livestock.

The dark side of this story is the emergence of anthelmintic resistance (AR) to three major classes of anthelmintic (Benzimidazole, Imidazothiazoles and Salicylanilides) due to intensive use of drug in livestock industry (Gill, 1993; 1996). The complexity of resistance has been well expressed by Wood (1981). The problem of resistance is multidimensional. It seems clearly to be associated with heavy reliance on chemical control, applied frequently and sometimes haphazardly (Sanyal, 1998). No new anthelmintic with different modes of action are expected on the market in near future (Coles et al., 2006). So the maintenance of efficacy of existing
anthelmintic in combination or development of herbal anthelmintic is therefore essential for continuing animal productivity and welfare. Parasite resistance increases the cost of treatment, decline the efficiency of production and increase the risk of environmental contamination.

There is a need of the hour for judicious use of anthelmintics and also to search for alternative therapy to combat the problem of gastrointestinal nematodosis. Variety of plants has been scientifically validated for their anthelmintic properties in- vitro and in- vivo (Iqbal et al., 2010) and Neem (Azadirachta indica) is one of them. Neem leaves and seeds are utilized to treat different infections and diseases. Active principles found in neem are: Nimbin, Nimbidin, Ninbidol, Gedunin, Sodium-nimbinate, Quercetin, Salannin and Azadirachtin. The use of bioactive forages as a means of nematode control might offer a whole “new” range of anthelmintics in the form of the plant secondary metabolites that provide anthelmintic activity (Hoste et al., 2008).

Therefore, the present work was designed to generate epidemiological information on Benzimidazole (BZ) resistance in gastrointestinal nematodes of goat and their management by using anthelmintic combination strategies and herbal anthelmintic after evaluating their efficacy.

OBJECTIVES:

The present study was designed with the following objectives:

1) To study the prevalence of gastrointestinal parasites in goats of Jabalpur

2) To study the prevalence of Benzimidazole resistance in gastrointestinal nematodes of goat in and around Jabalpur

3) To study the efficacy of Azadirachta indica (Neem) seed powder, Fenbendazole with Piperonyl Butoxide and Methimazole
2. REVIEW OF LITERATURE

2.1 Work done in Madhya Pradesh

2.1.1 Prevalence of gastrointestinal parasites

Gupta et al. (2013) reported 97% prevalence of gastrointestinal parasites in small ruminants in and around Jabalpur. Maximum infection recorded was of strongyles (79.08%) followed by coccidia (45.38%), *Trichuris* (18.19%), amphistome (17.53%), *Strongyloides* (3.91%), *Fasciola* (3.57%), *Moniezia* (2.63%).

Bansal et al. (2015) reported 90.05% prevalence of gastrointestinal (GI) helminths in small ruminants in and around Mhow, Indore. Among various helminths, the highest prevalence was of strongyles (85.40%) followed by amphistomes (21.78%), *Trichuris* spp. (21.70%), *Strongyloides* spp. (12.24%), *Moniezia* spp. (5.77%) and *Fasciola* spp. (4.56%).

Singh et al. (2015) reported 94.48% prevalence of gastrointestinal parasites in goats of Madhya Pradesh, where in coccidia was predominant (82.4%) followed by strongyles (69.27%), amphistomes (22.71%), *Strongyloides* spp. (9.17%), *Trichuris* spp. (3.85%), *Moniezia* spp. (3.02%), *Schistosomes* spp. (2.29%) and *Fasciola* spp. (1.77%). The seasonal incidence was found highest in monsoon (98.06%) and lowest in winter (91.67%). The incidence of gastrointestinal parasitism was found higher in kids (96.25%) in comparison to adult goats (93.89%).

Dixit (2016) examined 2441 goats, 2189 (89.68%) were positive for GI parasites. The maximum prevalence was of coccidia (77.67%) followed by strongyles (41.21%), *Moniezia* spp. (14.58%), amphistomes (10.57%), *Trichuris* spp. (4.75%), *Strongyloides* spp. (1.15%) and *Fasciola* (0.57%).

Gaherwal et al. (2016) studied the incidence of nematodes in goats at five different villages of Barwani district (M.P.). The overall infection on the basis of egg collection was 76.8% during rainy season, 72% during winter season and 66% during summer season. The overall infection on the basis of worm collection was 78% during rainy season, 72.4% during winter season and 66% during summer season.
Singh et al. (2016) studied the prevalence of strongyle infections in goats of Mahakoushal region in Balaghat, Narsinghpur and Chhindwara district of M.P., during the period from July 2011 to February 2012. The larvae of *Haemonchus* spp. (61.63%) and *Strongyloides* spp. (7.5%) were highest in Balaghat, *Trichostongylus* (18.13%) in Narsinghpur, while *Oesophagostomum* spp. (10.50%) and *Bunostomum* spp. (5.75%) were in Chhindwara district.

### 2.1.2 Anthelmintic resistance

Das et al. (2015) reported multiple anthelmintic resistances against Fenbendazole and Levamisole in GI helminthes of goats in the Central India as both the drugs was 87% and 91%, respectively effective with the lower confidence limit was 82% and 87%. Whereas Ivermectin and Closantel were 99% and 100% effective, respectively.

Dixit et al. (2016) studied the status of BZ resistance in GI nematodes of goats using faecal egg count reduction test (FECRT) and egg hatch assay (EHA). Finding of FECRT revealed, fenbendazole reduced the fecal egg count by 23% with a lower confidence interval of -32%. ED$_{50}$ value of 0.335 µg/ml of Thiabendazole confirmed the existence of BZ resistant nematodes. *H. contortus*, *Trichostongylus* spp., *Oesophagostomum* spp., *Strongyloides* spp. and *Bunostomum* spp. larvae were identified in the pre-treatment faecal cultures but post-treatment copro-culture revealed *H. contortus* (92%) as the major nematode exhibiting resistance followed by *Oesophagostomum* (8%).

### 2.1.3 Efficacy of Neem seed powder, Fenbendazole with Methimazole and Piperonyl Butoxide

Dongre et al. (2015) evaluated the efficacy of crude neem (*Azadirachta indica*) leaf powder in goats naturally infected with strongyle infections. The result showed significant reduction (p<0.05) in EPG in goats treated with crude neem leaves powder @ 1g/kg b.wt. on day 15 post-treatment but there was no significant variation in term of EPG in control group. Thus, it can be concluded that crude neem leaf powder has anthelmintic property.
Dixit (2016) studied the efficacy of aqueous leaf extract from Azadirachta indica, Annona squamosa and Nicotiana tabacum against GI nematodes in goats and reported no significant reductions in FEC for any of the groups treated with herbal extracts 10th day post-treatment.

No literature related to combination therapy of Fenbendazole with methimazole and piperonyl butoxide has been found.

2.2 Work done in India

2.2.1 Prevalence of gastrointestinal parasites

Tariq et al. (2010) reported 54.3% prevalence of gastro-intestinal nematodes of goats in the Kashmir valley. The different parasites reported with their respective prevalences (%) were: 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). The mean maximum prevalence of GIN infection, faecal egg counts and average worm burden were found in the summer and lowest in winter.

Khajuria et al. (2013) examined a total of 1920 faecal samples of sheep (960) and goats (960) of stationary flocks of the middle agro-climatic zone of Jammu province, out of which 67.24% animals were positive for helminthic infections. The different nematodes observed were Strongyles (50.1%), Trichuris (12.1%) and Strongyloides spp. (4.2%). Trematode ova recorded were of Amphistomes (8.3%), Fasciola spp. (8.2%) and Dicrocoelium spp. (5.4%). Coproculture studies revealed that Haemonchus contortus (61.18%) predominated during all the seasons, followed by Trichostrongylus spp. (13.67%), Ostertagia spp. (12.17%), Strongyloides spp. (4.14%), Oesophagostomum spp. (3.84%) and Bunostomum spp. (3.83%). Eggs per gram of faeces (EPG) were the highest during monsoon and the lowest during winter. Infection was significantly (P<0.05) higher in young (73.22%) as compared to adults (61.25%).

Sharma et al. (2013) recorded 98.33% prevalence of gastrointestinal helminths of Gaddi sheep (n=240) by faecal examination. The studies revealed that Strongyles (98.33%) were the predominant parasite
followed by *Trichuris* spp. (25.83%), *Anoplocephala* spp. (20.41%), *Strongyloides* spp. (16.66%), *Fasciola* spp. (14.16%) and amphistomes (12.08%). The monthly coproculture studies of pooled samples revealed *H. contortus* to be the dominant species in all the seasons of the year.

Rawte *et al.* (2015) studied epidemiology of GI nematodes in goats maintained under field and farm conditions at Durg situated in the agroclimatic zone of Chhattisgarh Plain by monitoring monthly faecal egg counts and genera of parasites present for a period of one year. While increased egg counts in goats were observed during July to September in goats of both, field and farm conditions. The egg counts were significantly (P<0.05) higher in field goats. Coproculture study revealed that *Haemonchus* spp. was predominant species throughout observation period followed by *Oesophagostomum, Trichostrongylus* and *Bunostomum* spp. in field goats. In farm, apart from *Haemonchus* as a dominant species were also seen during rainy season while *Bunostomum* spp. accompanied *Haemonchus* during summer months.

Das *et al.* (2017) studied the prevalence of GI parasitic infections in goats in hilly region of Meghalaya and recorded the overall prevalence as 28.65%. Season-wise highest infections were recorded during rainy season (34.92%) followed by cool (26.87%), hot (26.62%) and cold (20.39%) seasons. Helminths and protozoa infections were recorded in 63.60% and 23.02% animals, respectively. Among the helminths, strongyles (32.63%) was recorded highest followed by *Strongyloides* spp. (12.55%), *Moniezia* spp. (10.04%) and *Trichuris* spp. (8.36%). Maximum egg per gram and oocyst per gram of faeces were recorded in the month of August (932.4) and September (674.05), respectively. Coproculture of goat fecal samples revealed the presence of *Haemonchus contortus* (72.16%), *Oesophagostomum* spp. (14.41%), *Strongyloides* spp. (8.91%) and *Trichostrongylus* spp. (4.50%) larvae.

Singh *et al.* (2017) studied the prevalence of GI parasitism in small ruminants in western zone of Punjab and out of 603 faecal (391 sheep and 212 goats) samples examined, 501 were found positive for endoparasitic infection with an overall prevalence of 83.08%, consisting of 85.16% and
79.24% in sheep and goats, respectively. The associated risk factors with the prevalence of GI parasites showed that females (85.97%) were significantly more susceptible than males (69.23%). Age wise, the adults were significantly more prone to parasitic infection as compared to young ones. Seasonal variation was recorded throughout the year and was significantly highest during monsoon (90.10%), followed by winter (83.84%) and summer (78.35%).

2.2.2 Anthelmintic resistance

The anthelmintic resistance in *Haemonchus contortus*, in India was first reported by Varshney and Singh (1976), against Phenothiazene and Thiabendazole at state Sheep and Wool Research Station, Pashulok, Rishikesh, Uttaranchal. Since 1990 onwards, there has been a renewed interest in India on this aspect and a considerable number of reports of anthelmintic resistance observed in G.I. nematodes are pouring-in from various parts of the country (Yadav and Garg, 2005).

Benzimidazole resistance of *H. contortus* in goats was also reported from Haryana (Yadav and Uppal, 1993), U.P/Uttaranchal (Yadav et al., 1996; Singh and Yadav, 1997). Laha et al. (1999) reported multiple anthelmintic resistance, involving FBZ and IVM resistant *H. contortus*, in pashmina producing goats.

Ram et al. (2004) reported albendazole-rafoxanide resistant *H. contortus* in goats from temperate zones of Uttaranchal. Chaudhri et al. (2007) conducted a survey on status of anthelmintic resistance in sheep flocks in two different regions (eastern and western) of Haryana and observed resistance in *H. contortus* to FBZ, TEM and MT in 76.9, 66.7 and 83.3% flocks, respectively. Easwaran et al. (2009) reported Benzimidazole and Levamisole resistance from Tamilnadu by using FECRT and EHA from sheep.

Sharma and Raut (2009) observed multiple anthelmintic resistance in gastrointestinal nematodes infecting goats. The efficacy of anthelmintics like albendazole, fenbendazole, morental through faecal egg count reduction test (FECRT) in natural bursate worm infections predominantly of *H. contortus* at organized goat farms of Barbari and
Jamunapari breeds. The reduction in post treatment (14th day after treatment) FEC with albendazole, fenbendazole and morental were 22-85, 78-82 and 79-85 % respectively at Barbari goat farm. The corresponding FECR at Jamunapari farm were 81-83 and 82-86 % for albendazole and fenbendazole respectively. The results showed moderate to high level resistance in H. contortus against commonly used anthelmintics like albendazole, fenbendazole, morental at goat farms.

Maharshi et al. (2011) assessed the status of anthelmintic resistance in GI nematodes (predominantly H. contortus) of sheep maintained at organized farms and farmer’s field in Rajasthan through in vivo FECRT and in vitro EHA. The magnitude of reduction in the faecal egg counts by fenbendazole (@5.0mg/kg b.wt) revealed emergence of benzimidazole resistance in H. contortus of sheep from all the farm and field flocks except in field flocks from north-eastern Rajasthan where 66.7% flocks possessed benzimidazole resistant H. contortus. On egg hatch assay, strains of worms were found susceptible to benzimidazole in farm flocks of northern region while in field flocks prevalence of benzimidazole resistance strongyle worms was 100% in eastern and northern region and 83.33% in north-eastern region.

Sanyal et al. (2014) studied the emergence of AR in ruminants of Chhattisgarh and ED50 for egg hatch was 0.150 in goats of Chhattisgarh Plain and considered resistant while those of Northern Hills Region and Bastar Plateau were susceptible.

Manikkavasagan et al. (2015) investigated AR to GI nematodes affecting goats in 27 unorganized farms in three different agro-climatic zones (Cauvery delta zone, high altitude zone and high rainfall zone) of Tamil Nadu using the FECRT. Results revealed the presence of high level of resistance to both albendazole and LEV. In the high rainfall and high altitude zones, all the farm flocks were found to be resistant to LEV. In the Cauvery delta zone, 13 farm flocks were resistant and four farm flocks showed suspect resistance to albendazole. 15 farm flocks showed resistance and two showed suspect resistance to LEV. Further, morphological characterization of the infective larvae derived from faecal cultures indicated that by far the most predominant GI nematode species found in goats was H. contortus.
2.2.3 Efficacy of Neem seed powder, Fenbendazole with Methimazole and Piperonyl Butoxide

Costa et al. (2006) studied comparative anthelmintic activity of A. indica and Closantel against GI nematodes in sheep. Group I and II received a treatment of A. indica dry leaves mixed in a concentrate at a dose rate of 0.1g/kg and 0.2g/kg b.wt. for 3 months and Group III was treated with Closantel (diantel) at recommended dose once at the beginning of the study. None of the evaluated parameters, EPG, worm burden, weight gain and haematocrit of the treatment groups were statistically different when compared to the control group, demonstrating that, A. indica has no anthelmintic effect compared to significant effects shown by closantel.

Ramteke et al. (2008) evaluated the anthelmintic efficacy of herbal formulation consisted of powder of A. indica bark, seeds of Butea frondosa and Nigella sativa and fruits of Piper longum in equal proportions (w/w) in goat naturally infected with GI parasites. Animals treated with the formulation @ 5 g/animal/day for ten days showed 77.40 per cent and 86.52 per cent reduction in EPG respectively 18th day post treatment.

Kumbhakar et al. (2015) studied the pharmacokinetics of albendazole in goats at a dose of 7.5 mg/kg b.wt. with and without co-administration with piperonyl butoxide at 63.0 mg/kg b.wt. The faecal egg count reduction and lower 95% confidence limit for the group treated with albendazole alone were 97 and 68%, while for co-administration of albendazole and piperonyl butoxide the values were 99 and 97%, respectively. The ED_{50} for egg hatch was 0.196, indicating suspected resistance to benzimidazole anthelmintics. The drug combination proved efficacious against an albendazole-resistant nematode parasite population in goats.

2.3 Work done Abroad

2.3.1 Prevalence of gastrointestinal parasites

Out of a total of 557 faecal samples of sheep and goat in Thessaly region of Greece were examined, helminth eggs were detected in 44 (7.9%) samples. Strongyle-type eggs were found in 19 (3.4%) samples,
Nematodirus spp. eggs in 6 (1.1%) samples, Trichuris spp. eggs in 16 (2.9%) samples, Fasciola hepatica in 3 (0.5%) samples, and Dicrocoelium dendriticum in 1 (0.2%) sample. Coccidian oocysts were found in 36 (6.5%) samples. (Kantzoura et al., 2012)

Owhoeli et al. (2014) examined 213 faecal samples of goats from four abattoirs and households to determine the prevalence of helminthes infections in exotic and indigenous goats in Port Harcourt, South, Nigeria. The study revealed that an overall prevalence of 75.5 per cent was recorded, out of which 57 (76.0%), 55 (70.5%), and 49 (81.6%) were recorded for exotic goat in the months of May–September, 2010, exotic goat in the months October 2010–February, 2011 and for indigenous goats, respectively.

Owusu et al. (2016) recorded the prevalence of gastrointestinal (GIT) parasites in sheep as 98.2%. The total infection rate of GI nematodes and coccidia oocysts were 94.5% and 51.8%, respectively. Strongyle nematode (94.5%) was the most prevalent GI nematode detected, followed by Strongyloides (27.3%). The average nematodal burden was significantly higher in young rams under 1 year (3482.0) than gimmers (1539.0), lamb (825.0), ewes (420.7), and rams over 1 year (313.3). From the studied animals 40%, 6.36%, 48.18% and 5.45% had heavy, moderate, light and no infestation respectively, with GI nematodes.

Zvinorova et al. (2016) studied the prevalence of gastrointestinal parasitic infections in 580 indigenous goats in five agro-ecological regions of Zimbabwe in the dry and wet seasons. Highest prevalence was determined for Eimeria oocysts (43%), Strongyles (31%) and lower levels in trematodes and cestodes. Parasites identified were Haemonchus, Strongyloides and Oesophagostomum. Area, season, sex and age significantly influenced patterns of gastrointestinal infections.

### 2.3.2 Anthelmintic resistance

Among all anthelmintics, BZ resistance is the major problem and extensively reported from different countries.

Chandrawathani et al. (2013a) tested AR on two goat farms in Malaysia using FECRT four anthelmintic from the group; Benzimidazole
(Albendazole), Imidazothiazoles (Levamisole), Macrocyclic Lactones (Ivermectin) and Salicylanilides (Closantel). Results showed that *H. contortus* and *Trichostongylus colubriformis* in these farms were resistant to all four group tested.

Mahieu *et al.* (2014) performed FECRT on 21 goat farms in Guadeloupe. AR to netobimin (BZ) was found in all 15 herds in which it was tested. AR to ivermectin and LEV were also very largely spread (14 out of 17 farms and 7 out of 9 farms, respectively). AR to the final moxidectin (milbemycin) released was already present in 2 out of 9 farms in which it was tested. *Haemonchus* was the dominant genus of GI nematodes and was more frequently found to be resistant to netobimin, IVM and moxidectin than *Trichostrongylus*, the latter appeared to be more often resistant to LEV.

Pena-Espinoza *et al.* (2014) described AR on one of the largest organic small ruminant farms in Denmark. The presence of AR on the investigated farm was initially declared by FECRT and subsequently confirmed by controlled efficacy test and pyro-sequencing. In the FECRT, FBZ treatments were markedly ineffective in reducing FECs in both lambs and kids. IVM resistance was also declared in both animal species. In contrast, Moxidectin (MOX) and LEV were highly effective in the FECRT. BZ-resistant populations of *H. contortus* and *T. colubriformis* were isolated for the first time in Denmark.

Premaalatha *et al.* (2014) evaluated the status of resistance to nematode populations on four major groups of anthelmintics (Benzimidazole, Imidazothiazoles, Macrocyclic Lactones and Salicylanilides) by FECRT. Results showed resistance to benzimidazoles on all farms and the major worm population were *H. contortus*.

### 2.3.3 Efficacy of Neem seed powder, Fenbendazole with Methimazole and Piperonyl Butoxide

Lanusse *et al.* (1995) studied the influence of methimazole on the plasma disposition kinetics of fenbendazole and its metabolites in adult sheep. The anthelmintic was administered by oral drench at 5 mg/kg either alone (control treatments) or together with methimazole given orally at 3
mg/kg b.wt. A modified pattern of disposition, with significantly higher $C_{\text{max}}$ and AUC values for fenbendazole parent drug, and a delayed appearance in plasma with retarded $T_{\text{max}}$ values for the sulphoxide and sulphone metabolites, were the main pharmacokinetic changes observed when the drugs were administered with methimazole.

Benchaoui and McKellar (1996) studied the effect of the cytochrome P$_{450}$ inhibitor, piperonyl butoxide on the anthelmintic efficacy of the benzimidazole compound fenbendazole in sheep and goats. The efficacy of the combination was assessed in sheep against two species of benzimidazole-resistant abomasal nematodes; *Ostertagia circumcincta* and *Haemonchus contortus*. The percentage reduction in the total number of *O. circumcincta* worms was 7.9% (Fenbendazole) and 97.8% (Fenbendazole-Piperonyl butoxide). For *H. contortus*, the percentage reduction was 84.8% (Fenbendazole) and 99.0% (Fenbendazole-Piperonyl butoxide).

Chandrawathani et al. (2013b) evaluated Neem leaves water extract and decoction of neem leaves (NLD) for control of helminths in goats. The efficacy of neem leaves water extract was functional for only 2 weeks after the given treatment. During the following weeks, there was however an increase in faecal egg counts. While for NLD treatment, there was no significant effect on the parasites between the treated and control groups.

Chandrawathani et al. (2013c) evaluated the effectiveness of four types of neem products namely, neem leaf decoction, neem capsule, neem fresh juice and neem extract in controlling natural gastrointestinal helminth infection in goats. Results of feeding these products were variable when compared to untreated control animals, however, a 40-60% worm control was observed especially for neem juice, neem decoction and neem capsule.

### 3. MATERIAL AND METHODS

#### 3.1 Location of work

Madhya Pradesh called Heart of India and it is situated between $17^\circ$ to $25^\circ$ latitude and $72^\circ$ to $85^\circ$ E longitudes. Jabalpur Tehsil of Madhya Pradesh, where the study was undertaken, is situated at 23.17 latitude and 79.57E longitude at 410.87 MSL (Meters above Sea Level)
The present study was carried out in the Department of Veterinary Parasitology, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur (M.P).

3.2 Place of work

Proposed work was carried out on goats of organized and unorganized farm in and around Jabalpur.

3.3 Prevalence of gastrointestinal parasites

3.3.1 Animals

Apparently healthy goats of either sex or age were selected for the study.

3.3.2 Collection of faecal samples

Total of 1675 freshly laid or per rectally collected faecal samples of goat were collected from July 2016 to March 2017 in an individually labelled polythene bags and was carried to the laboratory for further study and examined by qualitative (Floatation and Sedimentation) and quantitative methods (McMaster technique) to determine the worm load (EPG).

3.3.3 Processing and examination of samples

Faecal samples were examined grossly for presence of gravid segments and immature or mature parasites. Later on they were processed qualitatively as well as quantitatively. Faecal samples were kept cool before transportation to the laboratory where they were immediately examined or stored at refrigerated temperature (4°C) till processing.

3.3.4 Examination of faecal samples

3.3.4.1 Qualitative examination

To detect the presence of parasitic eggs in the samples, sedimentation and modified Sheather’s sugar floatation techniques were used as described by Soulsby (1982). Eggs were identified on the basis of size and morphological characteristics (Sloss et al., 1994).
3.3.4.1.1 Floatation method

Approximately 2-3 grams of faecal samples were crushed using pestle mortar and a pasty mass was prepared by adding water drop-wise. About 15-20 ml water was then gradually added to the faecal paste in mortar and well using pestle to prepare a homogenous suspension. The suspension was then strained through a single mesh ordinary nylon tea strainer to remove the coarser particles. The suspension thus obtained, was transferred to a 15 ml centrifuge tube and allowed to stand for 10 minutes after which the supernatant fluid was carefully discarded without disturbing the sediment. The sediment in the centrifuge tube was mixed properly with approximately 10 ml saturated sugar solution. After mixing the content in the centrifuge tube, more saturated sugar solution was added drop by drop till a convex surface was formed at the brim of the tube. A round coverslip was placed on the tube and it was then centrifuged for 3-5 minutes at 1500-2000 rpm. After centrifugation, the tube was removed and the coverslip was taken-off vertically and placed on a clean microslide. The preparation was examined under dry magnifications (10 X) of a compound microscope.

3.3.4.1.2 Sedimentation method

To examine the sediment for the heavier eggs of flukes, the supernatant in the centrifuge tube was discarded. A 5 ml N/10 NaOH and 2-3 glass beads were added to the tube and the contents of the tube were then mixed thoroughly by vigorously shaking the centrifuge tube 2-3 times at 5 minutes interval. About 0.5 ml of well-mixed sediment was transferred to a microslide and examined under low (10 X) dry magnification of the compound microscope.

3.3.4.2 Quantitative method:

Modified McMaster technique (EPG) Sloss et al. (1994) was used for detection of nematode, cestodes and oocysts per gram (OPG). Faecal egg counts were determined at monthly interval to know the EPG.

3.3.4.2.1 McMaster Technique
A combination of 3g of faecal material and 42 ml of floatation solution was mixed to yield a total volume of 45 ml and strained through an ordinary single mesh nylon strainer to remove the coarser particles. The suspension was thoroughly mixed and chambers of McMaster slide were charged with the suspension using a Pasteur pipette, filling all of its spaces and avoiding air bubbles. Keeping the slides for a few minutes and the charged counting chamber was then placed undisturbed on to the platform of a compound microscope for 10-15 minutes. Number of eggs present in each lane of the charged chambers in 1 sq.cm area were counted.

To determine the number of strongyle eggs per gram of faeces.

\[ \text{EPG} = \frac{\text{Total number of eggs in one chamber} \times 100}{3.3.4.3\text{ Coproculture for recovery of Strongyle larvae}} \]

The fresh faecal samples positive for strongyle infection, were pooled and cultured in laboratory by glass tumbler method as per the procedure given by Roberts and O’Sullivan (1949) as described below, to harvest the infective stage (L₃) larvae.

**Procedure**

About 75-100 grams of pooled strongyle positive faecal samples was thoroughly mixed with activated charcoal in 3:1 ratio and water sufficiently to get a pasty consistency and placed in a glass tumbler of 300 ml capacity. After cleaning the inner margin of the tumbler, its mouth was covered with aluminium foil and then it was incubated at 25-28°C for seven days. The culture tumbler was checked once daily in morning for optimum moisture of the sample. On 8th day, the tumbler was taken out of the incubator, its aluminium was removed and lukewarm water was then filled up till a convex surface was formed at the brim. A glass petridish of 4 inch size was placed on to the mouth of the tumbler with precaution that no air bubbles should be trapped within the whole preparation, was then inverted so that the tumbler stood in petridish upside down. Some water, sufficient to submerge the mouth of the tumbler, was now added to the petridish and kept in little slanting position under artificial light. After 4-6 hours, water in the petridish was withdrawn and centrifuged at 1000 rpm for 2 minutes. After discarding the
supernatant, 10% hot formaldehyde was added to the sediment containing larvae so as to preserve then stretched.

3.3.4.4 Identification of Larvae

A drop of preserved sediment containing larvae was placed on a glass slide, mixed with a drop of lugol’s iodine and then the infective larvae (L₃) were identified up to the generic level as per the key of Van Wyk et al. (2004).

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<td>1</td>
<td>No sheath, oesophagus half the length of the body</td>
<td>Strongyloides spp.</td>
</tr>
<tr>
<td></td>
<td>Sheathed larvae, oesophagus short</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Total length &lt;600µ</td>
<td>Bunostomum spp.</td>
</tr>
<tr>
<td></td>
<td>Total length &gt;600µ</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Length of tail sheath &lt;40µ</td>
<td>Trichostrongylus spp.</td>
</tr>
<tr>
<td></td>
<td>Length of tail sheath &gt;40µ</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Length of tail sheath &gt;140µ</td>
<td>Oesophagostomum spp.</td>
</tr>
<tr>
<td></td>
<td>Length of tail sheath &gt;40µ and &lt;140µ</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Chitinous knobs or spots at the anterior end of oesophagus</td>
<td>Cooperia spp.</td>
</tr>
<tr>
<td>6</td>
<td>No such structures at the anterior end of oesophagus</td>
<td>Haemonchus spp.</td>
</tr>
</tbody>
</table>

3.4 Benzimidazole resistance

3.4.1 Detection of Benzimidazole Resistance

3.4.1.1. By in-vivo Faecal Egg Count Reduction Test (FECRT):

The Faecal Egg Count Reduction Test was conducted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for detection of anthelmintic resistance (Coles et al., 1992). The maximum number of animals having uniformity in the nature and intensity of nematode infection, age and weight from each flock were included in the study. The experimental animals were naturally parasitized with mixed species of gastrointestinal nematodes. It was verified that the selected animals had not been treated in the previous 8-12 weeks.
Goats having EPG ≥ 500 were selected for the study and divided into 2 groups with 10 goats in each group. Group-I was treated with Fenbendazole @ 7.5 mg/kg bwt and Group-II was kept as untreated control.

Faecal samples were collected per rectum from each animal individually on day 0 (pre-treatment) and day 10 (post-treatment). The intensity of infection was determined by modified McMaster technique (Sloss et al., 1994). The left over faecal samples from both groups were copro-cultured and third stage larvae was harvested and identified.

The faecal count data were analyzed for faecal egg count reduction test (FECRT%) using RESO calculator (Martin and Wursthorn, 1991). Resistance to anthelmintic was considered if (a) the FECRT is <95% and (b) the lower 95% confidence limit is <90%. If one of two criteria is met, resistance was suspected. (Coles et al., 2006).

3.4.1.2 By in-vitro Egg Hatch Assay (EHA):

Collection of faecal sample

100-150gm fresh faecal samples were collected per rectum from animals. As laboratory was far away from collection site, faecal samples were maintained in anaerobic condition. For developing anaerobic condition faecal samples were collected in sample container containing distilled water and glass beads (Plate 1A). Sample container was screwed tight and shaked vigorously as described by Le Jambre (1976) and as modification by Taylor et al. (2002).

Preparation of faecal slurry

Around 60-70gm faecal sample was put in a large size beaker and ad lib water added in the beaker. It was kept for 20-30 minutes so that faecal matter gets soaked and loosened. Faecal matter was mixed thoroughly with stirrer. After mixing faecal slurry was strained 2-3 times using tea strainer to remove the faecal debris.

Separation of eggs from faecal slurry

Strained faecal slurry was poured in centrifuge tubes and centrifuged at 2000 rpm for 5 minutes. After centrifugation supernatant was
discarded from all centrifuge tubes. Sediment was well agitated and saturated sodium chloride solution was filled up to top in each centrifuge tube for floatation of parasitic eggs. Microscopic Cover glasses were put on each centrifuge tube for adhesion of eggs. After 10 minutes cover glasses were held with forceps and dipped in a beaker containing distilled water. This way eggs were separated from faecal slurry. Two fold serial dilutions of eggs were then made.

**Preparation of Thiabendazole stock solution**

10 mg of Thiabendazole (TBZ) was weighed using precision monopan balance. Thiabendazole was put in a glass vial. 5ml of di-methyl-sulphoxide (DMSO) was added to dissolve the drug. This solution was used as stock solution. By using stock solution five different doses of Thiabendazole was prepared in a two-fold serial dilution method as depicted in the following table.

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of stock solution (ml)</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volume of solution tube 1 (ml)</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volume of solution tube 2 (ml)</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volume of solution tube 3 (ml)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>Volume of solution tube 4 (ml)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>Volume of D.W. (ml)</td>
<td>4.5</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Total Volume (ml)</td>
<td>5.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Conc.TBZ (µg TBZ/10µl)</td>
<td>2.0</td>
<td>1.0</td>
<td>0.5</td>
<td>0.25</td>
<td>0.125</td>
</tr>
</tbody>
</table>

**Charging and incubation of 24 multiwell plate**

A specially designed 24 multiwell plate was used in this technique. An amount of 2ml of fresh egg solution was added in each well of 24 multiwell plate (Plate 1B). 10µl of Thiabendazole solution of varying doses was added in corresponding well. This plate was then incubated in a BOD incubator at 28°C for 24-48 hours.

**Counting of eggs and larvae**

After 24 hrs of incubation embryonation was checked from control wells using 10x objective lens of compound microscope. When larvae
were seen in control well, then further processing carried out. Solution from different wells was pipetted out and poured in separate centrifuge tubes. A drop of lugol’s iodine was added in each tube. These tubes were centrifuged at 2000 rpm for 5 minutes. Supernatant was discarded very carefully. Aliquot of sediment was observed for counting of eggs (Plate 1C) and larvae (Plate 1D) under 10x objective lens of compound microscope.

**Interpretation of Data**

The data was analysed by Probit Analysis to obtain ED$_{50}$ value for egg hatch. ED$_{50}$ value in excess of 0.1mg TBZ/ml was considered as emergence of TBZ resistance (Coles *et al*., 2006).

### 3.5 Efficacy of *Azadirachta indica* (Neem) seed powder, Fenbendazole with Piperonyl Butoxide and Methimazole

#### 3.5.1 Preparation of neem seed powder

For the proposed work, dried Neem (*Azadirachta indica*) seeds were used and procured from the nearby localities. Seeds were dried at room temperature and grinded to fine powder and stored in airtight containers for further use (Plate 2A). A sample of the seed was preserved for the further reference.

#### 3.5.2 Experimental Design:

Forty two goats of either sex were selected to study the comparative efficacy. The animals were grouped into six groups with seven animals in each group. Group-I and II were treated with aqueous crude powder of Neem seed (*Azadirachta indica*) @ 2 g/kg b. wt. and 4 g/kg b. wt. single dose orally, respectively (Plate 2B). Animals of Group-III and Group-IV were treated with FBZ+PBT @ 7.5mg/kg and 63 mg/kg b. wt. and FBZ+MTH @ 7.5mg/kg and 3 mg/kg b. wt., orally respectively. Group-V was treated with FBZ @ 7.5mg/kg b.wt. orally and Group-VI was kept as untreated healthy control (Table 01).
<table>
<thead>
<tr>
<th>Gr.</th>
<th>No. of Animals</th>
<th>Drugs</th>
<th>EPG</th>
<th>Status of FBZ resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7</td>
<td>Crude powder of Neem seed (<em>Azadirachta indica</em>) @ 2 g/kg b.wt. orally single dose</td>
<td>Day 0 and 10</td>
<td>By FECRT &amp; EHA</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>Crude powder of Neem seed (<em>Azadirachta indica</em>) @ 4 g/kg b.wt. orally single dose</td>
<td>Day 0 and 10</td>
<td>By FECRT &amp; EHA</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>FBZ+PBT @ 7.5mg/kg and 63 mg/kg b.wt. orally</td>
<td>Day 0 and 10</td>
<td>By FECRT &amp; EHA</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>FBZ+MTH @ 7.5mg/kg and 3 mg/kg b.wt. orally</td>
<td>Day 0 and 10</td>
<td>By FECRT &amp; EHA</td>
</tr>
<tr>
<td>V</td>
<td>7</td>
<td>FBZ@ 7.5mg/kg b.wt. orally</td>
<td>Day 0 and 10</td>
<td>By FECRT &amp; EHA</td>
</tr>
<tr>
<td>VI</td>
<td>7</td>
<td>Control</td>
<td>Day 0 and 10</td>
<td>By FECRT &amp; EHA</td>
</tr>
</tbody>
</table>

(FBZ- Fenbendazole, PBT- Piperonyl Butoxide, MTH- Methimazole)

3.6 **Statistical analysis**

Suitable statistical technique was performed on prevalence data by applying $\chi^2$ test as per Snedecor and Cochran (1994).
4. RESULTS

4.1 Prevalence

4.1.1 Overall and species wise prevalence of gastrointestinal parasites

Out of 1675 faecal sample of goats examined over a period of 9 month, 1224 (73.07%) were found positive for gastrointestinal parasites. The maximum prevalence recorded was of Strongyles (61.43%) followed by Coccidia (25.97%), Amphistomes (9.73%), Moniezia expansa (8.66%), Trichuris spp. (2.03%), Strongyloides spp. (1.79%) and Fasciola spp. (0.66%).

Overall and species wise prevalence of gastrointestinal parasites in and around Jabalpur of goats has been shown in Table 02 and depicted in Figure 01.

Table 02: Prevalence of gastrointestinal parasites in goats of Jabalpur

<table>
<thead>
<tr>
<th>Location</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Strongyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistomes (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghana</td>
<td>160</td>
<td>97 (60.63)</td>
<td>78 (48.75)</td>
<td>6 (3.75)</td>
<td>0 (0.00)</td>
<td>0.00</td>
<td>6 (3.75)</td>
<td>2 (1.25)</td>
<td>24 (15)</td>
</tr>
<tr>
<td>Panagar</td>
<td>255</td>
<td>166 (65.10)</td>
<td>141 (55.29)</td>
<td>9 (3.53)</td>
<td>1 (0.39)</td>
<td>0.00</td>
<td>11 (4.31)</td>
<td>14 (5.49)</td>
<td>59 (23.14)</td>
</tr>
<tr>
<td>Ramnagara</td>
<td>255</td>
<td>164 (64.31)</td>
<td>132 (51.76)</td>
<td>9 (3.53)</td>
<td>0.00</td>
<td>0.00</td>
<td>6 (2.35)</td>
<td>21 (8.24)</td>
<td>59 (23.14)</td>
</tr>
<tr>
<td>Temarbita</td>
<td>237</td>
<td>159 (67.09)</td>
<td>119 (50.21)</td>
<td>2 (0.84)</td>
<td>2 (0.84)</td>
<td>0.00</td>
<td>30 (12.66)</td>
<td>25 (10.55)</td>
<td>81 (34.18)</td>
</tr>
<tr>
<td>Deori</td>
<td>127</td>
<td>101 (79.53)</td>
<td>74 (58.27)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>3 (2.36)</td>
<td>26 (20.47)</td>
<td>3 (2.36)</td>
<td>55 (43.31)</td>
</tr>
<tr>
<td>Silua</td>
<td>116</td>
<td>86 (74.14)</td>
<td>81 (69.83)</td>
<td>3 (2.59)</td>
<td>3 (2.59)</td>
<td>1 (0.86)</td>
<td>8 (6.90)</td>
<td>3 (2.59)</td>
<td>20 (17.24)</td>
</tr>
<tr>
<td>Adhratal Farm</td>
<td>173</td>
<td>158 (91.33)</td>
<td>146 (84.39)</td>
<td>0 (0.00)</td>
<td>4 (2.31)</td>
<td>0 (1.73)</td>
<td>16 (9.25)</td>
<td>42 (24.28)</td>
<td></td>
</tr>
<tr>
<td>Amanala Farm</td>
<td>336</td>
<td>293 (87.2)</td>
<td>258 (76.79)</td>
<td>1 (0.3)</td>
<td>24 (7.14)</td>
<td>7 (2.08)</td>
<td>73 (21.73)</td>
<td>61 (18.15)</td>
<td>95 (28.27)</td>
</tr>
</tbody>
</table>

Total 1675 | 1224 (73.07) | 1029 (61.43) | 30 (1.79) | 34 (2.03) | 11 (0.66) | 163 (9.73) | 145 (8.66) | 435 (25.97) |
4.1.2 Prevalence in field and farm condition

Prevalence of gastrointestinal parasites under the farm and field condition was recorded as 85.90 and 67.22 per cent, respectively. In farm condition, the overall prevalence was significantly higher (P<0.01) as compared to corresponding value in field condition.

Species wise prevalence was also observed significantly higher (P<0.01) in farm than in field conditions except *Strongyloides* infection which was 2.52% in field and 0.19% at farm.

Prevalence of strongyles, *Moniezia expansa* and Amphistomes infection was recorded significantly high in farm (76.95%, 14.67% and 14.48%, respectively) than at field (54.35%, 5.91% and 7.57%, respectively). There was no significant variation (P>0.05) observed in coccidia infection when compared between field and farm as represented in Table 03 and depicted in Figure 02.

**Table 03: Prevalence of gastrointestinal parasites in goats under field and farm condition**

<table>
<thead>
<tr>
<th>Area</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Strongyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistomes (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm</td>
<td>525</td>
<td>451 (85.9)</td>
<td>404 (76.95)</td>
<td>1 (0.19)</td>
<td>28 (5.33)</td>
<td>7 (1.33)</td>
<td>76 (14.48)</td>
<td>77 (14.67)</td>
<td>137 (26.1)</td>
</tr>
<tr>
<td>Field</td>
<td>1150</td>
<td>773 (67.22)</td>
<td>625 (54.35)</td>
<td>29 (2.52)</td>
<td>6 (0.52)</td>
<td>4 (0.35)</td>
<td>87 (7.57)</td>
<td>68 (5.91)</td>
<td>298 (25.91)</td>
</tr>
<tr>
<td>$\chi^2$ Value</td>
<td>pf=2</td>
<td>63.98**</td>
<td>77.74**</td>
<td>11.14**</td>
<td>41.96**</td>
<td>5.37**</td>
<td>19.6**</td>
<td>34.93**</td>
<td>NS</td>
</tr>
</tbody>
</table>

Significant **P<0.01, NS- Non Significant

4.1.3 Age wise prevalence

Age wise prevalence at Jabalpur revealed that the gastrointestinal parasitic infection was non significantly higher in adults (73.83%) than in young goats (69.71%). Overall age specific species
prevalence showed strongyle and amphistome infection was significantly higher (P<0.01) in adult (64.25% and 11.33%) as compared to young (48.86% and 2.61%, respectively) whereas coccidia and Moniezia expansa infection was observed significantly higher (P<0.01) in young (34.85% and 13.68%) when compared with adults (23.98% and 7.53%, respectively). There was no significant variation in the prevalence of Fasciola and Strongyloides infection as represented in Table 04 and depicted in Figure 03.

Table 04: Age wise prevalence of gastrointestinal parasites in goats of Jabalpur

<table>
<thead>
<tr>
<th>Age</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Stronglyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistomes (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>1368</td>
<td>1010 (73.83)</td>
<td>879 (64.25)</td>
<td>21 (1.54)</td>
<td>21 (1.54)</td>
<td>10 (0.73)</td>
<td>155 (11.33)</td>
<td>103 (7.53)</td>
<td>328 (23.98)</td>
</tr>
<tr>
<td>Young</td>
<td>307</td>
<td>214 (69.71)</td>
<td>150 (48.86)</td>
<td>9 (2.93)</td>
<td>13 (4.23)</td>
<td>1 (0.33)</td>
<td>8 (2.61)</td>
<td>42 (13.68)</td>
<td>107 (34.85)</td>
</tr>
</tbody>
</table>

$\chi^2$ Value

| df=1 | NS | 25.08** | NS | 9.19** | NS | 21.73** | 11.99** | 15.43** |

Significant **P<0.01, NS- Non Significant

Age wise prevalence under field condition at Jabalpur revealed that gastrointestinal parasitic infection was slightly higher in adults (67.72%) than in young (64.85%). Strongyles shows significantly high (P<0.01) prevalence of 56.86% in adult as compared to young (42.57%), whereas Moniezia and Trichuris infection was recorded higher in young (10.89% and 1.98%) than in adult goats (4.85% and 0.21%, respectively). Amphistomes which revealed significantly higher (P<0.01) prevalence of 8.54 per cent in adult as compared to young goats (2.97%) and there was no significant variation in the prevalence of Fasciola and Coccidia was recorded (Table 05 and Figure 04).
Table 05: Age wise prevalence of gastrointestinal parasites in goats under field condition

<table>
<thead>
<tr>
<th>Age</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Strongyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistomes (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>948</td>
<td>642 (67.72)</td>
<td>539 (56.86)</td>
<td>21 (2.22)</td>
<td>2 (0.21)</td>
<td>4 (0.42)</td>
<td>81 (8.54)</td>
<td>46 (4.85)</td>
<td>218 (23.00)</td>
</tr>
<tr>
<td>Young</td>
<td>202</td>
<td>131 (64.85)</td>
<td>86 (42.57)</td>
<td>8 (3.96)</td>
<td>4 (1.98)</td>
<td>0.00</td>
<td>6 (2.97)</td>
<td>22 (10.89)</td>
<td>80 (39.60)</td>
</tr>
</tbody>
</table>

$\chi^2$ Value df=1 NS 13.69** NS 10.04** NS 7.39** 10.91** NS

Significant **P<0.01, NS- Non Significant

In farm condition, age wise prevalence revealed that gastrointestinal parasitic infection was significantly high (P<0.05) in adults (87.62%) as compared to young (79.05%). Species wise variation shows significantly higher (P<0.01) strongyle and amphistome infection observed in adult (80.95% and 17.62%) than young (60.95% and 1.90%, respectively). *Strongyloides* infection showed significantly high (P<0.05) in young ones (0.95%) whereas *Trichuris, Moniezia* and coccidia infection revealed no significant age wise variation as represented in Table 06 and depicted in Figure 05.

Table 06: Age wise prevalence of gastrointestinal parasites in goats under farm condition

<table>
<thead>
<tr>
<th>Age</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Strongyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistomes (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>420</td>
<td>368 (87.62)</td>
<td>340 (80.95)</td>
<td>0.00</td>
<td>19 (4.52)</td>
<td>6 (1.43)</td>
<td>74 (17.62)</td>
<td>57 (13.57)</td>
<td>110 (26.19)</td>
</tr>
<tr>
<td>Young</td>
<td>105</td>
<td>83 (79.05)</td>
<td>64 (60.95)</td>
<td>1 (0.95)</td>
<td>9 (8.57)</td>
<td>1 (0.95)</td>
<td>2 (1.90)</td>
<td>20 (19.05)</td>
<td>27 (25.71)</td>
</tr>
</tbody>
</table>

$\chi^2$ Value df=1 5.10* 18.94** 4.00* NS NS 16.75** NS NS

Significant *P<0.05, Significant **P<0.01, NS- Non Significant
4.1.4 Season wise prevalence

In present investigation, overall seasonal prevalence of gastrointestinal infection showed that prevalence was significantly higher (P<0.01) in monsoon (81.20%) and in post-monsoon (76.66%) as compared to spring (62.60%) and winter (59.47%) season.

Significantly higher (P<0.01) prevalence of strongyles infection were recorded in post-monsoon (69.69%) and monsoon (68.74%) season as compared to winter (50%) and spring (38.17%) seasons. Significantly high infection (P<0.01) of coccidia was observed in monsoon (31.88%) followed by post-monsoon (27.18%) season. Amphistomes were significantly higher in spring (16.79%) and monsoon (14.57%) season as compared to winter (3.52%) and post-monsoon (2.79%) seasons. Trichuris, Fasciola and Moniezia infection showed no significant seasonal variation as shown in Table 07 and represented in Figure 06.

Table 07: Season wise prevalence of gastrointestinal parasites in goats of Jabalpur

<table>
<thead>
<tr>
<th>Season</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Strongyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistomes (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monsoon</td>
<td>803</td>
<td>652 (81.2)</td>
<td>552 (68.74)</td>
<td>27 (3.36)</td>
<td>20 (2.49)</td>
<td>7 (0.87)</td>
<td>117 (14.57)</td>
<td>75 (9.34)</td>
<td>75 (9.34)</td>
</tr>
<tr>
<td>Post Monsoon</td>
<td>287</td>
<td>220 (76.66)</td>
<td>200 (69.69)</td>
<td>0</td>
<td>2 (0.7)</td>
<td>1 (0.35)</td>
<td>8 (2.79)</td>
<td>27 (9.41)</td>
<td>27 (9.41)</td>
</tr>
<tr>
<td>Winter</td>
<td>454</td>
<td>270 (59.47)</td>
<td>227 (50)</td>
<td>3 (0.66)</td>
<td>10 (2.2)</td>
<td>2 (0.44)</td>
<td>16 (3.52)</td>
<td>37 (8.15)</td>
<td>72 (15.86)</td>
</tr>
<tr>
<td>Spring</td>
<td>131</td>
<td>82 (62.6)</td>
<td>50 (38.17)</td>
<td>2 (1.53)</td>
<td>2 (0.76)</td>
<td>1 (0.76)</td>
<td>22 (16.79)</td>
<td>6 (4.58)</td>
<td>29 (22.14)</td>
</tr>
<tr>
<td>χ² Value</td>
<td>df=3</td>
<td>78.8**</td>
<td>81.33**</td>
<td>22.2**</td>
<td>NS</td>
<td>NS</td>
<td>64.51**</td>
<td>NS</td>
<td>39.95**</td>
</tr>
</tbody>
</table>

Significant *P<0.05, Significant **P<0.01, NS- Non Significant

Significantly highest percentage of positive sample was recorded in monsoon (78.31%) season followed by post-monsoon (68.45%) as compared to spring (50.00%) and winter (49.67%) in field condition. Species wise prevalence showed significantly high strongyles infection in monsoon (64.37%) and post-monsoon (59.36%) seasons. Strongyloides and coccidia were also significantly high (P<0.01) in monsoon 4.48 and 30.12 per cent, respectively. No significant variation in seasonal prevalence of Fasciola, Moniezia and Trichuris were observed (Table 08 and Figure 07).
### Table 08: Season wise prevalence of gastrointestinal parasites in goats under field condition

<table>
<thead>
<tr>
<th>Season</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Strongyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistomes (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monsoon</td>
<td>581</td>
<td>455 (78.31)</td>
<td>374 (64.37)</td>
<td>26 (4.48)</td>
<td>4 (0.69)</td>
<td>4 (0.69)</td>
<td>77 (13.25)</td>
<td>34 (5.85)</td>
<td>175 (30.12)</td>
</tr>
<tr>
<td>Post Monsoon</td>
<td>187</td>
<td>128 (68.45)</td>
<td>111 (59.36)</td>
<td>0.00</td>
<td>2 (1.07)</td>
<td>0.0</td>
<td>2 (1.07)</td>
<td>15 (8.02)</td>
<td>54 (28.88)</td>
</tr>
<tr>
<td>Winter</td>
<td>302</td>
<td>150 (49.67)</td>
<td>125 (41.39)</td>
<td>3 (0.99)</td>
<td>0.0</td>
<td>0.0</td>
<td>4 (1.32)</td>
<td>17 (5.63)</td>
<td>50 (16.56)</td>
</tr>
<tr>
<td>Spring</td>
<td>80</td>
<td>40 (50.00)</td>
<td>15 (18.75)</td>
<td>0.00</td>
<td>0.0</td>
<td>0.0</td>
<td>4 (5.00)</td>
<td>2 (2.50)</td>
<td>19 (23.75)</td>
</tr>
</tbody>
</table>

χ²-Value: df=3 85.56**, 86.72**, 18.79**, NS, 55.73**, NS, 20.18**

Significant **P<0.01, NS- Non Significant

Season wise prevalence in farm condition revealed significant high (P<0.05) positive sample in post-monsoon (92%) followed by monsoon (88.74%) season. Strongyles were significantly higher (P<0.01) in post-monsoon (89.00%) and monsoon (80.18%) as compared to other season as shown in Table 09 and represented in Figure 08.

### Table 09: Season wise prevalence of gastrointestinal parasites in goats under farm condition

<table>
<thead>
<tr>
<th>Season</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Strongyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistomes (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monsoon</td>
<td>222</td>
<td>197 (88.74)</td>
<td>178 (80.18)</td>
<td>1 (0.45)</td>
<td>16 (7.21)</td>
<td>3 (1.35)</td>
<td>40 (18.02)</td>
<td>41 (18.47)</td>
<td>81 (36.49)</td>
</tr>
<tr>
<td>Post Monsoon</td>
<td>100</td>
<td>92 (92.00)</td>
<td>89 (89.00)</td>
<td>0.00</td>
<td>0.00</td>
<td>1 (1.00)</td>
<td>6 (6.00)</td>
<td>12 (12.00)</td>
<td>24 (24.00)</td>
</tr>
<tr>
<td>Winter</td>
<td>152</td>
<td>120 (78.95)</td>
<td>102 (67.11)</td>
<td>0.00</td>
<td>10 (6.58)</td>
<td>2 (1.32)</td>
<td>12 (7.89)</td>
<td>20 (13.16)</td>
<td>22 (14.47)</td>
</tr>
<tr>
<td>Spring</td>
<td>51</td>
<td>42 (82.35)</td>
<td>35 (68.63)</td>
<td>0.00</td>
<td>2 (3.92)</td>
<td>1 (1.96)</td>
<td>18 (35.29)</td>
<td>4 (7.84)</td>
<td>10 (19.61)</td>
</tr>
</tbody>
</table>

χ²-Value: df=3 11.15*, 19.79**, NS, 7.85*, NS, NS, NS, NS

Significant *P<0.05, Significant**P<0.01, NS- Non Significant
4.1.5 Month wise prevalence

Month wise prevalence at Jabalpur revealed that overall high percentage of gastrointestinal parasites were found in October (85.35%) followed by August (84.50%) and September (82.08%). Maximum prevalence of strongyles was recorded in the month of October (77.07%) followed by September (73.33%) whereas highest prevalence of *Strongyloides* (4.86%), amphistomes (19.15%), *Moniezia* (11.85%) and coccidia (42.55%) was recorded in the month of August. In January, significant low percentage of positive sample (50.00%) was observed as represented in Table 10 and depicted in Figure 09.

Table 10: Month wise prevalence of gastrointestinal parasites in goats of Jabalpur

<table>
<thead>
<tr>
<th>Month</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Strongyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistomes (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul-16</td>
<td>234</td>
<td>177 (75.64)</td>
<td>153 (65.38)</td>
<td>4 (1.71)</td>
<td>10 (4.27)</td>
<td>2 (0.85)</td>
<td>34 (14.53)</td>
<td>25 (10.68)</td>
<td>41 (17.52)</td>
</tr>
<tr>
<td>Aug-16</td>
<td>329</td>
<td>278 (84.50)</td>
<td>223 (67.78)</td>
<td>16 (4.86)</td>
<td>7 (2.13)</td>
<td>5 (1.52)</td>
<td>63 (19.15)</td>
<td>39 (11.85)</td>
<td>140 (42.55)</td>
</tr>
<tr>
<td>Sep-16</td>
<td>240</td>
<td>197 (82.08)</td>
<td>176 (73.33)</td>
<td>7 (2.92)</td>
<td>3 (1.25)</td>
<td>0.00</td>
<td>20 (8.33)</td>
<td>11 (4.58)</td>
<td>75 (31.25)</td>
</tr>
<tr>
<td>Oct-16</td>
<td>157</td>
<td>134 (85.35)</td>
<td>121 (77.07)</td>
<td>0.00</td>
<td>2 (1.27)</td>
<td>1 (0.64)</td>
<td>5 (3.18)</td>
<td>18 (11.46)</td>
<td>63 (40.13)</td>
</tr>
<tr>
<td>Nov-16</td>
<td>130</td>
<td>86 (66.15)</td>
<td>79 (60.77)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>3 (2.31)</td>
<td>9 (6.92)</td>
<td>15 (11.54)</td>
</tr>
<tr>
<td>Dec-16</td>
<td>175</td>
<td>110 (62.86)</td>
<td>96 (54.86)</td>
<td>3 (1.71)</td>
<td>1 (0.57)</td>
<td>1 (0.57)</td>
<td>2 (1.14)</td>
<td>18 (10.29)</td>
<td>18 (10.29)</td>
</tr>
<tr>
<td>Jan-17</td>
<td>130</td>
<td>65 (50.00)</td>
<td>49 (37.69)</td>
<td>0.00</td>
<td>3 (2.31)</td>
<td>0.00</td>
<td>3 (2.31)</td>
<td>4 (3.08)</td>
<td>32 (24.62)</td>
</tr>
<tr>
<td>Feb-17</td>
<td>149</td>
<td>95 (63.76)</td>
<td>82 (55.03)</td>
<td>0.00</td>
<td>6 (4.03)</td>
<td>1 (0.67)</td>
<td>11 (7.38)</td>
<td>15 (10.07)</td>
<td>22 (14.77)</td>
</tr>
<tr>
<td>Mar-17</td>
<td>131</td>
<td>82 (62.60)</td>
<td>50 (38.17)</td>
<td>0.00</td>
<td>2 (1.53)</td>
<td>1 (0.76)</td>
<td>22 (16.79)</td>
<td>6 (4.58)</td>
<td>29 (22.14)</td>
</tr>
</tbody>
</table>
In field condition, month wise prevalence in goats revealed highest percentage of positive sample in August (82.59%). Prevalence of *Strongyloides* (5.93%), amphistome (19.26%) and coccidia (40.74%) was also highest in the month of September and October, respectively. Lowest prevalence of GI parasites was recorded in the month of January (35%) as represented in Table 11.

**Table 11: Month wise prevalence of gastrointestinal parasites in goats under field condition**

<table>
<thead>
<tr>
<th>Month</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Strongyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistome (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul-16</td>
<td>126</td>
<td>86 (68.25)</td>
<td>74 (58.73)</td>
<td>3 (2.38)</td>
<td>3 (2.38)</td>
<td>1 (0.79)</td>
<td>21 (16.67)</td>
<td>5 (3.97)</td>
<td>17 (13.49)</td>
</tr>
<tr>
<td>Aug-16</td>
<td>270</td>
<td>223 (82.59)</td>
<td>173 (64.07)</td>
<td>16 (5.93)</td>
<td>1 (0.37)</td>
<td>3 (1.11)</td>
<td>52 (19.26)</td>
<td>24 (8.89)</td>
<td>110 (40.74)</td>
</tr>
<tr>
<td>Sep-16</td>
<td>185</td>
<td>146 (78.92)</td>
<td>127 (68.65)</td>
<td>7 (3.78)</td>
<td>0.00</td>
<td>0.00</td>
<td>4 (2.16)</td>
<td>5 (2.70)</td>
<td>48 (25.95)</td>
</tr>
<tr>
<td>Oct-16</td>
<td>107</td>
<td>85 (79.44)</td>
<td>72 (67.29)</td>
<td>0.00</td>
<td>2 (1.87)</td>
<td>0.00</td>
<td>2 (1.87)</td>
<td>14 (13.08)</td>
<td>41 (38.32)</td>
</tr>
<tr>
<td>Nov-16</td>
<td>80</td>
<td>43 (53.75)</td>
<td>39 (48.75)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1 (1.25)</td>
<td>13 (16.25)</td>
</tr>
<tr>
<td>Dec-16</td>
<td>120</td>
<td>68 (56.67)</td>
<td>61 (50.83)</td>
<td>3 (2.50)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>10 (8.33)</td>
<td>14 (11.67)</td>
</tr>
<tr>
<td>Jan-17</td>
<td>80</td>
<td>28 (35.00)</td>
<td>21 (26.25)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1 (1.25)</td>
<td>0.00</td>
<td>14 (17.50)</td>
</tr>
<tr>
<td>Feb-17</td>
<td>102</td>
<td>54 (52.94)</td>
<td>43 (42.16)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>3 (2.94)</td>
<td>7 (6.66)</td>
<td>22 (21.57)</td>
</tr>
<tr>
<td>Mar-17</td>
<td>80</td>
<td>40 (50.00)</td>
<td>15 (18.75)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>4 (5.00)</td>
<td>2 (2.50)</td>
<td>19 (23.75)</td>
</tr>
</tbody>
</table>

In farm condition, the highest positive samples were recorded in the month of October (98%) followed by August (93.22%) and September (92.73%). Strongyles were also high in October (98.00%) followed by September (89.09%) and August (84.75%), whereas coccidia (50.85%) and *Moniezia* (25.42%) found highest in August followed by September. But amphistomes was highest in the month of September (29.09%) followed by August (18.64%) as represented in Table 12.
**Table 12: Month wise prevalence of gastrointestinal parasites in goats under farm condition**

<table>
<thead>
<tr>
<th>Month</th>
<th>No. Examined</th>
<th>Positive (%)</th>
<th>Strongyle (%)</th>
<th>Strongyloides (%)</th>
<th>Trichuris (%)</th>
<th>Fasciola (%)</th>
<th>Amphistomes (%)</th>
<th>Moniezia (%)</th>
<th>Coccidia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul-16</td>
<td>108</td>
<td>91 (84.26)</td>
<td>79 (73.15)</td>
<td>1 (0.93)</td>
<td>7 (6.48)</td>
<td>1 (0.93)</td>
<td>13 (12.04)</td>
<td>20 (18.52)</td>
<td>24 (22.22)</td>
</tr>
<tr>
<td>Aug-16</td>
<td>59</td>
<td>55 (93.22)</td>
<td>50 (84.75)</td>
<td>0.00</td>
<td>6 (10.17)</td>
<td>2 (3.39)</td>
<td>11 (18.64)</td>
<td>15 (25.42)</td>
<td>30 (50.85)</td>
</tr>
<tr>
<td>Sep-16</td>
<td>55</td>
<td>51 (92.73)</td>
<td>49 (89.09)</td>
<td>0.00</td>
<td>3 (5.45)</td>
<td>0.00</td>
<td>16 (29.09)</td>
<td>6 (10.91)</td>
<td>27 (49.09)</td>
</tr>
<tr>
<td>Oct-16</td>
<td>50</td>
<td>49 (98.00)</td>
<td>49 (98.00)</td>
<td>0.00</td>
<td>0.00</td>
<td>1 (2.00)</td>
<td>3 (6.00)</td>
<td>4 (8.00)</td>
<td>22 (44.00)</td>
</tr>
<tr>
<td>Nov-16</td>
<td>50</td>
<td>43 (86.00)</td>
<td>40 (80.00)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>3 (6.00)</td>
<td>8 (16.00)</td>
<td>2 (4.00)</td>
</tr>
<tr>
<td>Dec-16</td>
<td>55</td>
<td>42 (76.36)</td>
<td>35 (63.64)</td>
<td>0.00</td>
<td>1 (1.82)</td>
<td>1 (1.82)</td>
<td>2 (3.64)</td>
<td>8 (14.55)</td>
<td>4 (7.27)</td>
</tr>
<tr>
<td>Jan-17</td>
<td>50</td>
<td>37 (74.00)</td>
<td>28 (56.00)</td>
<td>0.00</td>
<td>3 (6.00)</td>
<td>0.00</td>
<td>2 (4.00)</td>
<td>8 (16.00)</td>
<td>18 (36.00)</td>
</tr>
<tr>
<td>Feb-17</td>
<td>47</td>
<td>41 (87.23)</td>
<td>39 (82.98)</td>
<td>0.00</td>
<td>6 (12.77)</td>
<td>1 (2.13)</td>
<td>16 (35.29)</td>
<td>8 (17.02)</td>
<td>4 (7.84)</td>
</tr>
<tr>
<td>Mar-17</td>
<td>51</td>
<td>42 (82.35)</td>
<td>35 (68.63)</td>
<td>0.00</td>
<td>2 (3.92)</td>
<td>1 (1.96)</td>
<td>18 (35.29)</td>
<td>4 (7.84)</td>
<td>10 (19.61)</td>
</tr>
</tbody>
</table>

4.1.6 Intensity of Strongyle infection

Overall intensity of strongyle infection was higher from August (1204.6) to October (924.8) and the pattern was same in farm condition (1732.0) and (1647.5) and in field conditions, intensity of infection was recorded high in August (1107.8) whereas low level of infection was recorded in the montn of January (163.1) and the pattern was same both in farm (280.0) and field (90.0) condition as represented in Table 13 and depicted in Figure 10.

**Table 13: Month wise intensity of strongyle infection in goats**

<table>
<thead>
<tr>
<th>Month</th>
<th>Overall</th>
<th>Farm</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul-16</td>
<td>677.5</td>
<td>750.0</td>
<td>615.3</td>
</tr>
<tr>
<td>Aug-16</td>
<td>1204.6</td>
<td>1647.5</td>
<td>1107.7</td>
</tr>
<tr>
<td>Sep-16</td>
<td>990.0</td>
<td>1194.5</td>
<td>929.1</td>
</tr>
<tr>
<td>Oct-16</td>
<td>924.8</td>
<td>1732.0</td>
<td>547.6</td>
</tr>
<tr>
<td>Nov-16</td>
<td>413.8</td>
<td>620.0</td>
<td>285.0</td>
</tr>
<tr>
<td>Dec-16</td>
<td>314.3</td>
<td>578.2</td>
<td>193.3</td>
</tr>
<tr>
<td>Jan-17</td>
<td>163.1</td>
<td>280.0</td>
<td>90.0</td>
</tr>
<tr>
<td>Feb-17</td>
<td>230.2</td>
<td>523.4</td>
<td>95.1</td>
</tr>
<tr>
<td>Mar-17</td>
<td>188.5</td>
<td>396.1</td>
<td>56.2</td>
</tr>
</tbody>
</table>
4.1.7 Generic composition of nematode larvae

The infective larvae (L₃) were identified every month up to the generic level and mean generic composition revealed that *Haemonchus* (50.18%) was the predominant nematode followed by *Trichostrongylus* (29.80%), *Strongyloides* (10.71%), *Oesophagostomum* (7.94%) and *Bunostomum* (0.99%) spp. The mean generic composition of nematode larvae is shown in Table 14 and depicted in Figure 11 and 12.

**Table 14:** Month wise generic composition of nematode larvae in goats of Jabalpur

<table>
<thead>
<tr>
<th>Month</th>
<th><em>Haemonchus</em> (%)</th>
<th><em>Oesophagostomum</em> (%)</th>
<th><em>Trichostrongylus</em> (%)</th>
<th><em>Strongyloides</em> (%)</th>
<th><em>Bunostomum</em> (%)</th>
<th>Others (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul-16</td>
<td>151 (45.90)</td>
<td>43 (13.07)</td>
<td>72 (21.88)</td>
<td>54 (16.41)</td>
<td>9 (2.74)</td>
<td>0.00</td>
</tr>
<tr>
<td>Aug-16</td>
<td>212 (52.87)</td>
<td>41 (10.22)</td>
<td>86 (21.45)</td>
<td>61 (12.72)</td>
<td>11 (2.74)</td>
<td>0.00</td>
</tr>
<tr>
<td>Sep-16</td>
<td>228 (56.58)</td>
<td>27 (6.70)</td>
<td>90 (22.33)</td>
<td>42 (10.42)</td>
<td>3 (0.74)</td>
<td>13 (3.23)</td>
</tr>
<tr>
<td>Oct-16</td>
<td>141 (46.69)</td>
<td>26 (8.61)</td>
<td>126 (41.72)</td>
<td>7 (2.32)</td>
<td>2 (0.66)</td>
<td>0.00</td>
</tr>
<tr>
<td>Nov-16</td>
<td>150 (50)</td>
<td>26 (8.67)</td>
<td>91 (30.33)</td>
<td>33 (11)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Dec-16</td>
<td>172 (57.33)</td>
<td>19 (6.33)</td>
<td>75 (25)</td>
<td>34 (11.33)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Jan-17</td>
<td>143 (47.67)</td>
<td>39 (13)</td>
<td>116 (38.67)</td>
<td>2 (0.67)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Feb-17</td>
<td>131 (43.38)</td>
<td>7 (2.32)</td>
<td>149 (49.34)</td>
<td>15 (4.97)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Mar-17</td>
<td>48 (48)</td>
<td>2 (2)</td>
<td>42 (42)</td>
<td>8 (8)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
<td>1719 (50.18)</td>
<td>272 (7.94)</td>
<td>1021 (29.80)</td>
<td>367 (10.71)</td>
<td>34 (0.99)</td>
<td>13 (0.38)</td>
</tr>
</tbody>
</table>

4.2 Prevalence of Benzimidazole (BZ) resistance in gastrointestinal nematodes

4.2.1 *In vivo* Feacal Egg Count Reduction Test (FECRT)

In the present study, prevalence of BZ resistance was detected in field and farm by using *in-vivo* Feacal Egg Count Reduction Test (FECRT) as summarized in Table 15. The study revealed that goats reared in farm condition were resistance to anthelmintic as compared to field. The percent reduction in FEC of Adhartal and Amanala farm was 67.97 and 76.00 per
cent, respectively which was less than 95% as well as their lower confidence limits were also below 90% which indicated resistance. The study indicated that fenbendazole at the recommended dosage were not effective against GI nematodes in goats of these farm.

Study in field condition of goats in and around Jabalpur, observed high susceptibility with BZ treatment and shows excellent efficacy with fenbendazole. Flocks of Temarbita revealed highest FECR% of 99.40 with mean EPG of 1580±261 control group and 10±10 treatment group followed by Devori with FECR% 98.86 and mean EPG on 10th day 1760±336 control group and 20±13 treatment group having 95.1% of lower confidence limit then followed by Silua, Panagar and Ramnagra with FECR% 98.64, 98.60 and 98.18, respectively. In all the villages the lower confidence limit was above 90%.

In Amanala farm, faecal culture revealed *H. Contortus* (72%) and *Strongyloides* spp. (28%) larvae on pre treatment culture and on post treatment the present composition was 92% and 6%, respectively. Whereas in Adhartal farm pre-treatment faecal culture revealed *H. Contortus* (76%) and *Trichostrongylus* spp. (14%) but on post-treatment *H. contortus and Trichostrongylus* spp. were 68% and 30%, respectively. Therefore, the data indicated that the worms are resistance to fenbendazole. In field conditions, post-treatment faecal culture showed no larvae as fenbendazole was effective for gastrointestinal nematodes.

### Table 15: Prevalence of Benzimidazole resistance in field and farm condition using Faecal Egg Count Reduction Test

<table>
<thead>
<tr>
<th>Area</th>
<th>Flock</th>
<th>FEC on day 10 post treatment</th>
<th>FECR %</th>
<th>Upper CL%</th>
<th>Lower CL%</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control group</td>
<td>Treatment group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field</td>
<td>Temarbita</td>
<td>1580±260</td>
<td>10±10</td>
<td>99.40</td>
<td>99.90</td>
<td>94.68</td>
</tr>
<tr>
<td></td>
<td>Silua</td>
<td>1470±248</td>
<td>20±13</td>
<td>98.64</td>
<td>99.68</td>
<td>94.23</td>
</tr>
<tr>
<td></td>
<td>Devori</td>
<td>1760±336</td>
<td>20±13</td>
<td>98.86</td>
<td>99.74</td>
<td>95.10</td>
</tr>
<tr>
<td></td>
<td>Ramnagra</td>
<td>1650±317</td>
<td>30±21</td>
<td>98.18</td>
<td>99.60</td>
<td>91.50</td>
</tr>
<tr>
<td></td>
<td>Panagar</td>
<td>1450±229</td>
<td>20±13</td>
<td>98.60</td>
<td>99.70</td>
<td>94.19</td>
</tr>
<tr>
<td>Farm</td>
<td>Adhartal farm</td>
<td>1280±223</td>
<td>410±76</td>
<td>67.97</td>
<td>81.00</td>
<td>45.00</td>
</tr>
<tr>
<td></td>
<td>Amanala farm</td>
<td>2770±365</td>
<td>670±235</td>
<td>76.00</td>
<td>89.00</td>
<td>47.00</td>
</tr>
</tbody>
</table>
4.2.2 *In vitro* Egg Hatch Assay (EHA)

Prevalence of BZ resistance in field and farm was detected *in-vitro* by using Egg Hatch Assay. Status of the farm shows resistance with benzimidazole group of drug as compared to field conditions. Log probit analysis revealed $ED_{50}$ value of 0.238 $\mu$g TBZ/ml of Thiabendazole in Amanala farm and 0.149 $\mu$g TBZ/ml in Adhartal farm confirmed the existence of BZ resistant nematodes. EHA in field conditions observed $ED_{50}$ value of less than 0.01$\mu$g TBZ/ml of Thiabendazole which shows benzimidazole drugs were effective against nematodes of goats of field condition. Panagar, Silua, Temurbita and Devori having $ED_{50}$ value of 0.053, 0.049, 0.003 and 0.005, respectively as shown in Table 16.

### Table 16: Prevalence of Benzimidazole resistance in field and farm conditions using Egg Hatch Assay

<table>
<thead>
<tr>
<th>No.</th>
<th>Field/Farm</th>
<th>ED$_{50}$ (µg/ml)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ED$_{50}$ Upper limit</td>
<td>ED$_{50}$ Lower limit</td>
</tr>
<tr>
<td>1</td>
<td>Panagar</td>
<td>0.053 0.005 0.103</td>
<td>Susceptible</td>
</tr>
<tr>
<td>2</td>
<td>Silua</td>
<td>0.049 0.018 0.083</td>
<td>Susceptible</td>
</tr>
<tr>
<td>3</td>
<td>Temarbita</td>
<td>0.003 0.002 0.011</td>
<td>Susceptible</td>
</tr>
<tr>
<td>4</td>
<td>Devori</td>
<td>0.005 0.001 0.012</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Amanala</td>
<td>0.238 0.212 0.267</td>
<td>Resistant</td>
</tr>
<tr>
<td>2</td>
<td>Adhartal</td>
<td>0.149 0.100 0.209</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

---

4.3 Efficacy of *Azadirachta indica* (Neem) seed powder, Fenbendazole alone, Fenbendazole with Piperonyl Butoxide and Methimazole.

4.3.1 Based on Faecal Egg Count Reduction Test (FECRT)

Forty two goats of either sex were selected to study the comparative efficacy of different dose of drugs. The animals were grouped into six groups with 7 goats in each group. Group-I and II were treated with crude neem seed powder @ 2 g/kg bwt and 4 g/kg bwt single dose orally, respectively. Animals of Group-VI were kept as untreated control. The faecal samples of the animals were examined for EPG on day 0 (pre treatment) and day 10 (post treatment). The mean EPG of different groups are presented in Table 17 and efficacy of the drugs was observed on the basis of FECR%.
In Group-I, the mean EPG on day 0 was 1500±323 and it increased on 10\textsuperscript{th} day to 2000±532 whereas in Group-II, the mean EPG on day 0 was 1442.85±374 and it reduced to 1171.4±448. There was no reduction of faecal egg count in Group-I whereas 20.4% reduction was recorded in Group-II.

Table 17: Efficacy of drug combination using Faecal Egg Count Reduction Test

<table>
<thead>
<tr>
<th>Gr.</th>
<th>Drugs</th>
<th>Mean EPG</th>
<th>FECR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 Day</td>
<td>10\textsuperscript{th} Day</td>
</tr>
<tr>
<td>I</td>
<td>Crude powder of Neem seed (\textit{Azadirachta indica}) @ 2 g/kg b.wt. orally single dose</td>
<td>1500±323</td>
<td>2000±532</td>
</tr>
<tr>
<td>II</td>
<td>Crude powder of Neem seed (\textit{Azadirachta indica}) @ 4 g/kg b.wt. orally single dose</td>
<td>1442.85±374</td>
<td>1171.4±448</td>
</tr>
<tr>
<td>III</td>
<td>FBZ+PBT @ 7.5mg/kg and 63 mg/kg b.wt., orally</td>
<td>1671.43±337</td>
<td>28.57±18</td>
</tr>
<tr>
<td>IV</td>
<td>FBZ+MTH @ 7.5mg/kg and 3 mg/kg b.wt., orally</td>
<td>1628.57±297</td>
<td>657.14±266</td>
</tr>
<tr>
<td>V</td>
<td>FBZ @7.5mg/kg b.wt., orally</td>
<td>1742.9±304</td>
<td>371.43±152</td>
</tr>
<tr>
<td>VI</td>
<td>Control</td>
<td>1814.3±419</td>
<td>1914.3±378</td>
</tr>
</tbody>
</table>

Animals of Group-III, Group-IV and Group-V were treated with FBZ+PBT @ 7.5mg/kg and 63 mg/kg b.wt, FBZ+MTH @ 7.5mg/kg and 3 mg/kg b.wt and only FBZ @ 7.5 mg/kg b.wt., respectively. The mean EPG of Group-III and Group-IV on day 0 was 1671.43±337 and 1628.57±297 whereas on day 10 it reduced to 28.57±18 and 657.14±266, respectively. For Group-V, mean EPG on day 0 was 1742.9±304 and it reduced to 371.43±152 on day 10. The rates of reduction was higher for Group-III with 98.5% FECR as compared to Group-V and Group-IV with 80.60% and 65.6%, respectively, indicated high efficacy of FBZ+PBT combination against gastrointestinal nematodes resistance to only FBZ anthelmintic as shown in Table 17.
4.3.2 Status of Benzimidazole resistance by Egg Hatch Assay

Observations on *in vitro* egg hatch assay (EHA) to know the status of BZ resistance in gastrointestinal nematodes in goats receiving treatment of Group-III, Group-IV, Group-V and Group-VI are presented in Table 18. The number of unhatched eggs and larvae (L₁) were counted to determine the proportion of eggs hatched. The data were analysed by probit analysis to obtain ED₅₀ value for egg hatch. ED₅₀ value in excess of 0.1μg TBZ/ml was considered as emergence of BZ resistance. As can be seen from Table 16, ED₅₀ for egg hatch of Group-IV and Group-V was 0.458 and 0.445 μg TBZ/ml, indicating BZ resistance. No strongyle egg was detected in 10th day faeces after treatment in goats of Group-III *i.e.*, FBZ and PBT combination, showed high efficacy.

Table 18: Comparative efficacy of drug combination based on Egg Hatch Assay

<table>
<thead>
<tr>
<th>Group</th>
<th>ED₅₀ (μg/ml)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED₅₀</td>
<td>Upper Limit</td>
</tr>
<tr>
<td>III</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>0.458</td>
<td>3.598</td>
</tr>
<tr>
<td>V</td>
<td>0.445</td>
<td>0.734</td>
</tr>
<tr>
<td>VI</td>
<td>0.217</td>
<td>0.410</td>
</tr>
</tbody>
</table>

Larval compositions of gastrointestinal nematodes in pooled faecal samples of goats receiving treatment of FBZ alone, FBZ with MTH, FBZ with PBT and untreated controls subjected to larval culture are presented in Table 19. *H. contortus* (66%), *Strongyloides* spp. (24%) and *Trichostrongylus* spp. (10%) were identified in pre-treatment faecal cultures but post-treatment faecal culture of Group-V revealed *H. Contortus* (40%), *Trichostrongylus* spp. (35%) and *Strongyloides* spp. (25%) where as *H. Contortus* (46%) followed by *Trichostrongylus* spp. (30%) and *Strongyloides* spp. (20%) (Plate 3A and Plate 3B) and co-administration of fenbendazole (FBZ) with piperonyl butoxide (PBT) revealed no larvae, indicating highly efficacious combination.
Table 19: Larval composition (%) of gastrointestinal nematodes in pre and post treatment faecal culture

<table>
<thead>
<tr>
<th>Species</th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FBZ</td>
<td>FBZ+PBT</td>
</tr>
<tr>
<td><em>Haemonchus</em> spp.</td>
<td>66</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td><em>Oesophagostomum</em> spp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Trichostrongylus</em> spp.</td>
<td>10</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td><em>Strongyloides</em> spp.</td>
<td>24</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>
5. DISCUSSION

Small ruminants hold an important niche for sustainable agriculture in developing countries and support a variety of socioeconomic functions worldwide. India has an estimated goat population of 135.17 million, whereas Madhya Pradesh has 8.01 million goats as per 19th livestock census (Livestock Census, 2012). Among the parasitic diseases, gastrointestinal parasitism in goats is of paramount importance because small ruminants’ rearing has been a major source of income especially to the marginal farmers of the country (Pathak and Pal, 2008). These parasites cause both acute infections with a rapid onset and high mortality levels and chronic infections, which are commonly subclinical and may lead to insidious and important economic losses (Singla, 1995) via reduction of live weight gain, reduced wool and milk production, and poor reproductive performance, reduced feed conversion ratio and by way of costs incurred on treatment and control (Sutherland and Scott, 2010). This problem is severe in tropical countries due to highly favourable environmental conditions for helminth transmission (Singh et al., 2013). In the absence of alternate control strategies, control of parasitic gastroenteritis is primarily attempted by use of anthelmintics. The wide spread use, incorrect dosing and increased frequency of treatment have often lead to the development of resistance.

Extensive development of resistance to current chemical classes of anthelmintics poses an undeniable threat to long term ability to control parasitic diseases. One of the most valuable weapons in the battle to conserve susceptibility in nematode population is the ability to detect resistance, while it is still at low level because, accurate diagnosis and measurement of extent of anthelmintic resistance in parasitic populatios are important components in the development of control measures (Donald, 1983). Currently, goat industry appears to be under great threat with widespread, multiple resistance reported throughout the world (Waller, 1997; Mortensen et al., 2003). Therefore, the present study was undertaken to monitor benzimidazole resistance in gastrointestinal nematodes of goats and their management by using anthelmintic combination strategies and herbal anthelmintic to evaluate their efficacy.
5.1 Prevalence

5.1.1 Overall and species wise prevalence of gastrointestinal parasites

In the present study, overall prevalence of gastrointestinal parasites in goats at Jabalpur was recorded 73.07 per cent. Dixit (2016) studied the prevalence of gastrointestinal parasites for a period of 3 years and reported 89.68 per cent of goats infected which was higher than our findings. Similarly, a higher overall prevalence of 90.05 per cent as compared to the present study was reported by Lalbiaknungi (2002) whereas Singh et al. (2017) recorded overall prevalence of 83.08 per cent in small ruminants of western zone of Punjab which may be due to collection of sample from both sheep and goat. Singh et al. (2015) worked on epidemiological studies of gastrointestinal parasitism in goat in Madhya Pradesh and reported a higher prevalence of 94.48 per cent during 8 month study period from July 2011 to February 2012 in different study area among goats of three district viz., Balaghat, Narsinghpur and Chhindwara in Madhya Pradesh. District wise variation is mainly due to agro-climatic condition of study area.

Olanike et al. (2015) studied the prevalence of gastrointestinal parasites of goats in Ibadan, Southwest, Nigeria and observed 75.75 per cent prevalence which is similar to our findings. Das et al. (2017) recorded the low overall prevalence of GI parasitic infections in goats (28.65%) that may be due to study in hilly region of Meghalaya. Pant et al. (2009) also reported 96% prevalence of mixed parasitic infection in sheep and goat from Tarai region of Uttarakhand. These observation are in good agreement with those reported earlier by Bhat et al. (2007), Sonegaokar et al. (2007), Kaur and Kaur (2008), Pathak and Pal (2008) and Tambe et al. (2011).

Among gastrointestinal parasites maximum prevalence recorded was of strongyles (61.43%) followed by coccidia (25.97%), amphistomes (9.73%) and Moniezia spp. (8.66%) in the present study. According to Singh et al. (2015) coccidia was predominant (82.4 %) followed by strongyles (69.27 %) and amphistomes (22.71 %). Dixit (2016) reported the higher prevalence of coccidia (77.67%) followed by strongyles (41.21%), Moniezia spp. (14.58%) and amphistomes (10.57%). However, Das et al. (2017)
reported helminths and protozoa infections were 63.60% and 23.02%, respectively. Among the helminths, strongyle (32.63%) was recorded highest followed by *Trichuris* spp. (12.55%) and *Moniezia* spp. (10.04%) in hilly regions of Meghalaya. A similar findings by Gupta *et al.* (2013) with maximum infection recorded was of strongyles (79.08%) followed by coccidia (45.38%).

### 5.1.2 Prevalence in field and farm conditions

When the prevalence was compared in goats maintained at field and farm conditions, significantly higher (P<0.01) infection was observed at farm (85.90%) as compared to corresponding value in field conditions (67.22%). Similar pattern had been reported by Dixit (2016), with higher prevalence at farm (92.10%) than field (82.75%). Singh *et al.* (2015) also reported higher prevalence of (84.58%) in farm as compared to field (75.23%). Strongyles, *Moniezia expansa* and amphistomes infection were recorded significantly high (P<0.01) in farm (76.95%, 14.67% and 14.48%, respectively) than at field (54.35%, 5.91% and 7.57% respectively). Higher prevalence at farm may be due to emergence of anthelmintic resistant gastrointestinal nematodes and restricted grazing area which reduces the susceptible population in refugia. On the contrary, Pant *et al.* (2009) observed villages has more prevalence (96.15%) than organized farms (96.00%).

### 5.1.3 Age wise prevalence

Age wise prevalence at Jabalpur revealed that the gastrointestinal parasitic infection was slightly higher in adults (73.83%) than in young goats (69.71%). Singh *et al.* (2017) also observed a non-significant difference between young ones (71.42%) and adults (80.79%) similar to our findings. It could be explained that higher nematode prevalence in adults might be due to grazing on larger area of pastures being contaminated with various flocks and different stress conditions such as climate, long daily travelling, and gestation (Radostits *et al.*, 1994). Shakya *et al.* (2017) also found significantly higher infection rate in >1 year-old-goats (50.43%) than <1 year-old-goats (19.31%). Higher incidence of parasitic infection in adults corroborates to the finding of Anene *et al.* (1994) and Dixit (2016). Contrary to this, Singh *et al.* (2015) found incidence of parasites was higher for kids.
(96.25%) in comparison with adult goats (93.89%) that is mainly due to higher prevalence of coccidia (82.4%). Increase in incidence of infection in kids corroborates to the finding of Talukdar (1996) and Bagde (2010), who stated that gastro-intestinal parasitic infection was more in young than older ones in Assam and Nagpur, respectively. Higher prevalence of infection in kids could be attributed to susceptibility of the infection. Likewise, Shirale et al. (2001) also observed higher incidence in kids as compared to adults in Nagpur (India).

Overall age specific species prevalence showed strongyles infection was significantly higher (P<0.01) in adult (64.25%) as compared to young (48.86%) whereas coccidia and Moniezia expansa infection was observed significantly high (P<0.01) in young (34.85% and 13.68%) when compared with adults (23.98% and 7.53%, respectively). Similarly, Dixit (2016) also observed significantly higher (P<0.01) Moniezia infection in kids (21.55%) as compared to adults (13.22%) whereas strongyles were significantly higher in adults (42.65%) as compared to kids (33.83%).

In the present study, Amphistome was recorded significantly higher in Adult (11.33%) as compared to kids (2.61%). However, contrary findings were indicated by Bedarkar et al. (2000) who recorded higher amphistomes incidence in kids as compared to adult goats in the marshy areas of Marathawada region in Maharastra. It is a general practice in villages that the kids below six months of age are not allowed to go out for grazing near to forest area along with adult animal. Having less opportunity of exposure to infection from the field, especially at younger age, the kids, therefore, showed lower incidence that may account for this discrepancy as reflected by the observations of the present study.

5.1.4 Season wise prevalence

In present investigation, overall seasonal prevalence of gastrointestinal infection in goats of Jabalpur showed that, prevalence was significantly higher (P<0.01) in monsoon (81.20%) and in post-monsoon (76.66%) as compared to spring (62.60%) and winter (59.47%) seasons. Pathak and Pal (2008) reported that seasonal prevalence was highest in
monsoon (94.60%), moderate in summer (87.50%) and lowest in winter (63.15%). Low prevalence in winter season was due to reduced grazing hours of the animals, which helps in reducing the chances of contact between host and parasites (Katoch et al., 2000). Singh et al. (2017) observed season wise copro-prevalence of GIT parasitic infections was significantly (p<0.01) highest in monsoon (90.10%), followed by winter (83.84%) and lowest in summer (78.35%). Maske et al. (1990) reported highest helminth infection in goat during rainy season at Nagpur. In prevalence study monsoon season i.e. July to September appear to be more conducive to prevalence of infection as compared to rest of the seasons of the year. On the contrary, Tramboo et al. (2015) recorded highest prevalence in summer (83.00%) followed by spring (78.67%), winter (76.33%), and autumn (70.00%), the difference being statistically non-significant (p>0.05). Singh et al. (2015) observed the maximum prevalence during the monsoon season (98.06 %) while minimum in winter (91.67 %).

In present study, significantly higher (P<0.01) prevalence of strongyles infection were recorded in post-monsoon (69.69%) and monsoon (68.74%) seasons. These observations are in good agreement with those reported earlier by 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. On the contrary, Talukdar (1996) recorded highest strongyle incidence during summer in Assam. This period indicated with heavy rainfall and high humidity in the eastern states of India. Likewise, highest faecal strongyle egg count was also recorded in Sri Lanka (Faizal et al., 1999). The probable reason for high incidence of parasites in monsoon season might be due to suitability of this season for survival, development and dissemination of nematode larvae in pasture, which leads to higher infection in the pasture grazing animal like goat. This findings are in contrast with those described by Manna et al. (1994) that the helminth infection in goat occurs more during summer season than the rest of season in West Bengal while Sahay et al. (1996) reported highest incidence of parasites in goats during winter in West Bengal, India. The probable cause of variation in prevalence of infection could be attributed to
geographical and environmental variation in eastern region of the country. Similarly, Dixit (2016) also observed significantly higher infection in winter (94.91%) and summer (90.41%) season as compared to monsoon (85.77%), due to high prevalence of coccidians (77.67%).

During the course of study carried out for a period of 9 months, an incidence (9.73%) of amphistomes was recorded. Seasonal incidence was high in spring season followed by monsoon and winter. The breeds of goats that in the present study was non-descript, kept on extensive grazing and hence comparatively resistant than the pure and cross breeds. Fritsche et al. (1993) also recorded similar observations. However, workers from Bareilly (Prasad and Verma, 1999), Pantnagar (Yadav, 2000) and Maharashtra (Tamloorkar et al., 2001) have reported incidence of amphistomes throughout the year with the peak incidence of amphistomes during rainy season.

The result of the present investigation indicated that of all the gastrointestinal parasites recorded in goats, coccidia ranked second exhibiting highest (25.97%) rate of infection. The seasonal incidence was highest in monsoon season. The oocyst load was significantly high during monsoon season followed by post-monsoon season. Lloyd and Soulsby (1978), Penjhorn et al. (1994), Parihar et al. (1996) was also observed high incidence of Coccidia and therefore, are in good agreement with those observed in present study. Earlier findings on highest oocyst count and incidence of coccidia infection during rainy season as reported from Udaipur (Sharma, 1984) and dry zone of Sri Lanka (Faizal and Rajapakse, 2001) are in essential agreement with those recorded in the present study.

5.1.5 Month wise prevalence

October (85.35%) followed by August (84.50%) and September (82.08%) revealed overall high percentage of gastrointestinal parasites whereas January reported lowest (50%) infection, in present investigation. Saravanan et al. (2015) studied prevalence of endo-parasitic infection in the delta region of Orathanadu Thanjavur district of Tamilnadu state recorded higher parasitic infection in the month of March (37.24%) followed by April...
(17.44%) and May (12.79%). The probable cause of variation could be attributed to different agro-climatic condition in that region.

In field conditions, month wise prevalence in goats revealed highest percentage of positive sample in August (82.59%) whereas January recorded the month of least positive sample. In farm conditions, the highest positive sample was recorded in the month of October (98.00%) followed by August (93.22%) and lowest in January (74.00%).

5.1.6 Intensity of Strongyle infection

Overall intensity of strongyles infection (EPG) was higher in the month of August (1204.6), September (990) and October (924.8) and lowest in January (163.1) whereas in farm condition, highest intensity was recorded in the month of October (1732) followed by August (1647.5) and in field conditions, intensity of infection was highest in August (1107.8). Das et al. (2017) reported maximum and minimum EPG in the month of August (932.4) and February (208.25), respectively, while maximum and minimum OPG was recorded in the month of September (674.05) and February (118.36), respectively. Singh et al. (2015) reported district wise intensity of strongyles infection in Balaghat, Narsighpur, and Chhindwara district of Madhya Pradesh and recorded the highest intensity of strongyle infection in the month of August-September and lowest in the month of January (192.86). The higher intensity of strongyle infection during rainy season is mainly due to high level of infection acquired by the animals during grazing in the pasture which remains highly contaminated with the infective third stage larvae as the environment condition remains favourable for the survival and development of the infective stage in rainy season.

5.1.7 Generic composition of nematode larvae

The infective larvae (L₃) were identified up to the generic level and mean generic composition observed highest for *Haemonchus* (50.18%), followed by *Trichostrongylus* (29.80%), *Strongyloides* (10.71%), *Oesophagostomum* (7.94%) and *Bunostomum* (0.99%). Singh et al. (2015) observed the overall composition of the coprocultural larvae revealed *Haemonchus* spp. was the predominant (60.88%) nematode, followed by
Trichostrongylus spp. (17.42%), Oesophagostomum spp. (10.13%), Strongyloides spp. (6.83%) and Bunostomum i.e Hookworm (4.75%). Similar observation were recorded by Yadav (2000) from Pantnagar and Nginyi et al. (2001) from Kenya suggested humid tropical environment to be favourable for the development of various species of Strongyle nematodes viz., Haemonchus contortus, Trichostrongylus, Oesophagostomum and Strongyloides spp.

Similar findings by Dixit (2016) revealed Haemonchus is the predominant nematode larvae in faecal culture. Das et al. (2017) revealed the presence of Haemonchus contortus (72.16%), Oesophagostomum spp. (14.41%), Strongyloides spp. (8.91%) and Trichostrongylus spp. (4.50%) larvae throughout the year in the hilly region of Meghalaya. Similarly Tramboo et al. (2015) observed the most predominant strongyle worm was Haemonchus (55%) followed by Trichostrongylus spp. (17.5%) which is similar to our findings. Climatic parameters proposed to play an important role in the development and survival of pre-parasitic stages on pasture. The collective predominance of Haemonchus on copro-culture in the present study was in agreement with those reported by Anene et al. (1994), Parihar et al. (1996) and Faizal et al. (1999).

5.2 Prevalence of Benzimidazole (BZ) resistance in gastrointestinal parasites

In the present study, prevalence of BZ resistance was detected in field and farm by using in-vivo Feacal Egg Count Reduction Test (FECRT). The percent reduction in FEC in farm was less than 95% as well as their lower confidence limits were also below 90% which indicated resistance. The study indicated that Fenbendazole at the recommended dosage were not effective against GI nematodes in goats farms whereas study in the field condition of goats in and around Jabalpur observed high susceptibility with benzimidazole treatment and showed excellent efficacy with Fenbendazole. Larval composition of resistant population revealed Haemonchus contortus as the predominant worms exhibiting resistance in farm.
Shahardar et al. (2014) studied the anthelmintic resistance of GI nematodes of sheep at farms of Kashmir Valley and observed that FECRT revealed moderate resistant to Fenbendazole and Ivermectin. The study on emergence of anthelmintic resistance in ruminants of Chhattisgarh by Sanyal et al. (2014) found resistance against Albendazole and Levamisole in Northern Hills Region of goats. A survey was conducted by Das et al. (2015) to evaluate the efficacy and to know the status of commonly used anthelmintics in goats of Jabalpur and found resistance against Levamisole and Fenbendazole. Similar findings of resistance to Fenbendazole by GIN of sheep has also been reported by Swarnkar et al. (2001); Yadav and Garg (2004); Das and Singh (2005).

Benzimidazole resistance may be due to the fact that the dose recommended for goat may not be sufficient to treat goats for this parasite species. Rapid clearance of anthelmintic from body results in under dosing in goats especially when drug is administered at recommended dose of sheep (Gillham and Obendorf, 1985; Waller, 1987). Hence, FBZ was administered in goats @7.5 mg per kg body weight. In addition, the overuse or misuse of anthelmintics has led to an increase in the incidence of anthelmintic resistance in gastrointestinal nematodes of small ruminants. Jackson (1993) reported that when the predominant resistance species has a high biotic potential and is also highly pathogenic as in case of *H. contortus*, then the risk associated with reintroduction of the drug is very high. Further, Jackson and Coop (2000) reported that reintroduction of the drug can result in a rapid return to resistance state.

The GIN of all farms which were found FBZ resistant by FECRT were also detected resistant by EHA with ED$_{50}$ value greater than 0.1μg TBZ/ml. Observation on *in vitro* EHA test revealed that the ED$_{50}$ of farm strains ranging from 0.149 to 0.238 μg TBZ/ml confirmed benzimidazole resistance in gastrointestinal nematodes of goats. Whereas the ED$_{50}$ of field strains demonstrated were ranging from 0.003 to 0.053μg TBZ/ml indicated susceptibility to benzimidazoles in gastrointestinal nematodes of goats reared under field conditions. This is in consistent with the findings of other researchers (Maharshi *et al.*, 2011; Kumbhakar *et al.*, 2015; Rawte *et al.*, 2015).
2015). The EHA measures the effect of drug directly on hatching, development and motility of parasites without interfering with internal physiological functions of the host and the pharmacodynamics and pharmacokinetics of the drug (Assis et al., 2003). The in vitro experiments were designed to screen and evaluate efficacy of Benzimidazole on egg hatching of gastrointestinal nematodes of goat.

The comparison of results for BZ resistance in strongyle worms based on both FECRT and EHA revealed high agreement. Similar findings were reported by Chartier et al. (1998). From the observations on variation in resistance factor for BZ against different stages of H. contortus, Hall et al. (1978) postulated that following a single dose of a benzimidazole anthelmintic a variable effect would be seen against the eggs, larvae and adults. While comparing the results from these tests Martin and Wursthorn (1991) demonstrated that both tests had similar ability to detect BZ resistance but egg hatch assay provided a better quantitative estimate of the level of resistance.

5.3 Efficacy of Azadirachta indica (Neem) seed powder, Fenbendazole alone, Fenbendazole with Piperonyl Butoxide and Methimazole

5.3.1 Efficacy of Azadirachta indica (Neem) seed powder

In the present study, no significant reductions in post treatment FEC were observed for both of the groups treated with seed powder of Azadirachta indica. Similar to our findings, Iqbal et al. (2010) observed crude powder and crude methanolic extracts did not show significant activity (P>0.05) at the lower dose used but were found effective at 3g/kg with a maximum reduction of 29.3% in eggs per gram of faeces. Many researchers studied earlier on different parts of neem plants by administering fresh leaves (Githori et al., 2004), crude leaf powder (Dongre et al., 2015), powdered A. indica seeds (Hordegen et al., 2003) and their extracts (Iqbal et al., 2010; Dixit, 2016). Costa et al. (2006) observed anthelmintic activity of A. indica against sheep gastrointestinal nematodes and concluded that it had no anthelmintic activity. Dixit (2016) also reported similar finding of 21.40% reduction in faecal egg count when administered as aqueous leaf extract.
The leaves of *A. indica* have been used in folk veterinary medicine as an anthelmintic for ruminants (Jabbar *et al*., 2006); however, Costa *et al*. (2006), Chagas *et al*. (2008), Worku *et al*. (2009) and Igarashi *et al*. (2013) could not endorse the traditional claims of anthelmintic activity of *A. indica* leaves in sheep and goats. In another study Githiori *et al*. (2004) reported higher FEC in sheep than control group on 15th day post-treatment. Hordegen *et al*. (2003) did not observe any anthelmintic action of neem seed extract at 3 mg/kg live weight in sheep artificially infected with *H. contortus* and *T. colubriformis*. Chagas and Vieira (2007), observed no anthelmintic effect of neem at a dosage of 30 g of dried leaves per goat/day given for 5 days.

On contrary to our findings, Nawaz *et al*. (2014) demonstrated that water extract prepared from leaves of *A. indica* was 89% effective in reducing FEC in sheep. Possible factor for the difference in result may be due to origin of plant material. Croom *et al*. (1983) revealed that chemical constituents can vary between individual plants due to genetic or environmental differences, development stages of the plant at harvesting, drying process and storage technique.

Anthelmintic activity of *A. indica* may be attributed to one of the chemical constituent of the plant, Azadirachtin. At 1000 μg concentration of azadirachtin, there was a reduction in motility of L₃ larvae of *H. contortus* up to 92.5% at 24 hrs suggesting that Azadirachtin was able to reduce motility at increased concentrations (Radhakrishnan *et al*., 2007). According to Iqbal *et al*., (2010), Azadirachtin compound has been reported to inhibit glutathione-S-transferase and reduced glutathione and UDP-glucuronol transferase activity in liver, lung, kidney and brain of rats. Dongre *et al*., (2015) revealed that Azadirachtin interferes with the central nervous system of parasite via inhibition of excitatory cholinergic transmission and partly blocks the calcium channel resulting in expulsion of parasites from host body. Costa *et al*., (2006) suggest two hypothesis justifying the absence of anthelmintic effect due to environmental variation (Martin *et al*., 2001) and to treatment duration have been disproven. Studies performed in difference parts of the world, resulted in negative anthelmintic activity, which demonstrates that there was no influence
of the environment on the results. However, further studies are needed with different extracts of neem seed powder with long duration treatment before discarding for its anthelmintic activity.

5.3.2 Efficacy of drug combination by using Faecal Egg Count Reduction Test (FECRT) and Egg Hatch Assay (EHA)

Observations on in vivo faecal egg count reduction test (FECRT) performed as per the guidelines of World Association for the Advancement of Veterinary Parasitology (Coles et al., 1992) to know the status of Fenbendazole resistance in gastrointestinal nematodes in four groups of goats receiving treatment of FBZ alone, co-administration of FBZ and MTH, FBZ and PBT and untreated controls. Resistance to anthelmintic was considered if percentage reduction in faecal egg count (FECR) was <95% and lower 95% confidence limit was <90% (Coles et al., 1992). FECR and lower 90% confidence limit for Group of FBZ alone was 80.60 while Groups of co-administration of FBZ+ MTH and FBZ+PBT were 65.6 and 98.5, respectively. The nematodes were found resistant against FBZ alone and FBZ+ MTH, while susceptible against FBZ+PBT drug combinations. Our findings are in good agreement with Benchaoui and Mckellar (1996) who also studied the effect of the cytochrome P<sub>450</sub> inhibitor, Piperonyl butoxide on the pharmacokinetics and anthelmintic efficacy of the benzimidazole compound Fenbendazole in sheep and goats and reported 99% efficacy of Fenbendazole-Piperonyl butoxide combination as compare to Fenbendazole alone which was only 84.8% effective and was showing resistance.

Pre-treatment of goats with the cytochrome P<sub>450</sub> inhibitor, Piperonyl butoxide caused a greater than three-fold increase in the relative bioavailability of Fenbendazole and Fenbendazole sulphoxide. The AUC of Fenbendazole and the sulphoxide were significantly increased when Fenbendazole was co-administered with Piperonyl butoxide. Combination revealed reduction in benzimidazole-resistant abomasal nematodes. Piperonyl butoxide inhibited significantly the sulphoxidation and sulphonation of Fenbendazole (Benchaoui and Mckellar, 1996).
The hypothesis that modulation of hepatic microsomal sulfoxidation and sulphonation by the cytochrome P450 inhibitor piperonyl butoxide could increase bioavailability of albendazole, to understand the pharmacokinetics of albendazole in goats was tested by Kumbhakar et al. (2015) and reported the faecal egg count reduction and lower 95% confidence limit for the group treated with albendazole alone were 97 and 68%, while for co-administration of albendazole and piperonyl butoxide the values were 99 and 97%, respectively.

Observation on in vitro egg hatch assay (EHA) revealed ED50 for egg hatch was 0.445 with lower and upper limit of ED50 of 0.309 and 0.734, respectively, indicating resistance to benzimidazole group of drug. Kumbhakar et al. (2015) also revealed ED50 for egg hatch was 0.196 with lower and upper limit of ED50 of 0.051 and 0.329, respectively, indicating resistance to benzimidazole group of drug in EHA. The observation on in vitro egg hatch assay (EHA) corroborated with the results of faecal egg count reduction test (FECRT). ED50 value in excess of 0.1μg TBZ/ml was indicating emergence of BZ resistance (Coles et al., 1992) for FBZ alone, FBZ and MTH whereas FBZ with PBT showed ED50 value below 0.1μg TBZ/ml considered susceptibility of drug combination and are in good agreement with Kumbhakar et al. (2015).

Piperonyl Butoxide acts as an insecticide synergist by inhibiting the natural defense mechanisms of the insect, the most important of which is the mixed-function oxidase system, (MFOs) also known as the cytochrome P450 system. The MFO system is the primary route of detoxification in insects, and causes the oxidative breakdown of insecticides such as pyrethrins and the synthetic pyrethroids (Casida, 1970).

Larval compositions of gastrointestinal nematodes in pooled faecal samples of goats receiving treatment of FBZ alone, co-administration of FBZ and MTH and untreated controls subjected to FECRT. Dominant nematode exhibiting resistance was Haemonchus spp. followed by Trichostrongylus spp. and Strongyloides spp. Our finding corroborated with Dixit (2016) who reported H. contortus and Oesophagostomum larval in the post Fenbendazole treated faecal samples of goat.
6. SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

6.1 Summary

Gastrointestinal nematodosis in small ruminants poses a great threat to profitable livestock production in tropical countries where sub clinical form mostly predominates in adults. Gastrointestinal parasitic infections are common in goats causing considerable economic losses as a consequence of mortality in infected animals and reduced weight gain. A combination of treatment and management is necessary to control gastrointestinal parasites.

Anthelmintic resistance is widespread in several states of India. Being ubiquitous nature of the organisms and omnipresent in ruminants, management / control of worms presents a particularly difficult challenge following wide-spread emergence of anthelmintic resistance. Anthelmintics, though singularly blamed for the failure of parasite control programme the world over, cannot be sidelined altogether. They have a definite role to play in sustainable parasite control programme but their frequency of use should be minimized to the extent possible and thus, the available resources of anthelmintics could be used judiciously. There is a need of the hour for judicious use of anthelmintics and also to search for alternative therapy to combat the problem of gastrointestinal nematodosis. Variety of plants have been scientifically validated for their anthelmintic properties in- vitro and in-vivo and neem (Azadirachta indica) is one of them.

Therefore, the present work has been designed to generate epidemiological information on Benzimidazole (BZ) resistance in gastrointestinal nematodes of goat and their management by using anthelmintic combination strategies and herbal anthelmintic after evaluating their efficacy.

Out of 1675 faecal sample of goats examined, 1224 (73.07%) were found positive for different gastrointestinal parasites. The maximum prevalence recorded was of Strongyles (61.43%) followed by Coccidia (25.97%), Amphistomes (9.73%), Moniezia expansa (8.66%), Trichuris spp. (2.03%), Strongyloides spp. (1.79%) and Fasciola spp. (0.66%). When prevalence was compared in goats maintained at field and farm conditions,
significantly higher infection (P<0.01) was observed at farm (85.90%) as compared to field (67.22%) conditions. Age wise prevalence at Jabalpur revealed that the infection was higher in adults (73.83%) than in young goats (69.71%). Overall age specific species prevalence showed strongyles infection was significantly higher (P<0.01) in adult (64.25%) as compared to young (48.86%) whereas Coccidia and *Moniezia expansa* infection was observed significantly high (P<0.01) in kids than adult goats. In farm conditions, age wise prevalence observed significantly high (P<0.05) in adults (87.62%) as compared to young (79.05%) corroborating with the results of field conditions at Jabalpur. Significantly higher infection (P<0.01) were observed in monsoon (81.20%) and post-monsoon (76.66%) seasons as compared to spring (62.60%) and winter (59.47%) seasons. Strongyle infections were significantly higher (P<0.01) in post-monsoon (69.69%) and monsoon (68.74%) season as compared to winter (50.00%) and significantly high infection of coccidia was observed in monsoon (31.88%) followed by post-monsoon (27.18%) season. In field condition, *Strongyloides* and coccidia were significantly high (P<0.01) in monsoon 4.48 and 30.12 per cent, respectively, whereas non-significant in farm condition.

Intensity of strongyle infection (EPG) was higher from August (1204.6) to October (924.8) and lowest in the month of January (163.1). Coproculture study revealed generic composition of infective nematode larvae in goats of Jabalpur was highest for *Haemonchus* (50.18%), followed by *Trichostrongylus* (29.80%) and *Strongyloides* spp. (10.71%) and *Oesophagostomum* (7.94%).

In the present study, prevalence of BZ resistance was carried out in field and farm by using *in-vivo* Feacal Egg Count Reduction Test (FECRT). The percent reduction in FEC of farm was less than 95% as well as their lower confidence limits were also below 90% which indicated resistance in farm. The study indicated that Fenbendazole at the recommended dosage was not effective against GI nematodes in goat under farms condition whereas study in the field condition of goats in and around Jabalpur observed high susceptibility with benzimidazole treatment and showed excellent efficacy with Fenbendazole. Larval composition of resistant population
revealed *Haemonchus* as the predominant worms exhibiting resistance in farm followed by *Trichostrongylus* and *Strongyloides* spp.

The gastrointestinal nematodes of farms which were found FBZ resistant by FECRT were also detected resistant by EHA with ED$_{50}$ value greater than 0.1μg TBZ/ml. Observation on *in vitro* test EHA revealed that the ED$_{50}$ of farm strains ranging from 0.149 to 0.238 μg TBZ/ml confirmed benzimidazole resistance in gastrointestinal nematodes of goats. Whereas the ED$_{50}$ of field strains demonstrated in EHA, were ranging from 0.003 to 0.053μg TBZ/ml indicated susceptibility to benzimidazoles in gastrointestinal nematodes of goats reared under field condition.

Forty two adults goats of either sex were selected to study the comparative efficacy of different dose of drugs. The animals were grouped into six groups with 7 animal in each group, Group-I and II were treated with crude neem seed powder @ 2 g/kg b.wt. and 4 g/kg b.wt. single dose orally, respectively. Animals of Group-VI were kept as untreated control. No significant reductions in post treatment FEC were observed for both of the groups treated with *Azadirachta Indica* on day of post treatment.

Animals of Group-III, Group-IV and Group-V were treated with FBZ+PBT @ 7.5mg/kg and 63 mg/kg b.wt, FBZ+MTH @ 7.5mg/kg and 3 mg/kg b.wt and FBZ @ 7.5 mg/kg b.wt, respectively. The rates of reduction of faecal egg count was higher for Group-III with 98.5% FECR as compared to Group-V and Group-IV with 80.60% and 65.6%, respectively. Resistance to anthelmintic was considered if percentage reduction in faecal egg count (FECR) was <95% and lower 95% confidence limit was <90%, indicated high efficacy of FBZ+PBT combination against gastrointestinal nematodes resistance to only FBZ. Observation on *in vitro* Egg Hatch Assay (EHA) corroborating with the results of FECRT. ED$_{50}$ for egg hatch of Group-IV and Group-V was 0.458 and 0.445 μg TBZ/ml, indicating BZ resistance. No strongyle eggs were detected in 10$^{th}$ day faeces after treatment in animals of Group-III *i.e.*, FBZ and PBT combination, showed high efficacy.

Larval compositions of gastrointestinal nematodes in pooled faecal samples of goats receiving different treatment subjected to FECRT revealed dominant nematode exhibiting resistance was *Haemonchus contortus* followed by *Trichostrongylus* spp. and *Strongyloides* spp.
6.2 Conclusions

- Prevalence of gastrointestinal parasites was significantly higher under farm (85.90%) than in field (67.22%) conditions.

- Age wise prevalence revealed overall infection of gastrointestinal parasites and strongyles were higher in adults (73.83%) than in young goats (69.71%) but reverse is true for Moniezia and coccidia.

- Seasonal prevalence of gastrointestinal infection was significantly higher in monsoon (81.20%) followed by post monsoon (76.66%) season.

- Intensity of strongyle infection was higher from August (1204.6) to October (924.8) and lowest in January (163.1).

- Prevalence of BZ resistance was detected against gastrointestinal nematodes of goats in farm conditions and Haemonchus, Trichostrongylus and Strongyloides spp. were the nematode exhibiting resistance.

- No significant reductions in post treatment FEC were observed for both of the groups treated with seed powder of Azadirachta Indica.

- Gastrointestinal nematodes, resistance to BZ group of anthelmintic can be successfully treated by combination of Fenbendazole and Piperonyl Butoxide.
6.3 **Suggestions for Further Work**

- Experimental trials should be performed for the standardization of the doses of Piperonyl butoxide with Fenbendazole

- Further studies should be performed with long duration dosing of different parts of neem

- Use of combination of herbal extracts on BZ resistant nematodes can be tried

- Further studies should be performed for standardizing the doses with different preparations of neem plant
7. REFERENCES


