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WITHANIA COAGULANS AS A POTENTIAL ANTHELMINTIC IN-VIVO AGAINST NEMATODES IN CHAKUR *(ALECTORIS CHUKAR)* HOSTS

Ajmal Nisar Mengal[1], Muhammad Hassan Saleem[2*], Aneela Zameer Durrani[3], Aftab Ahmad Anjum[4], Muhammad Oneeb[5]

[1,2*,3]Department of Veterinary Medicine, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore - Pakistan
[4]Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore - Pakistan
[5]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore - Pakistan

***Corresponding author:** Muhammad Hassan Saleem,
*Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore – Pakistan. Email: dr_mhs@uvas.edu.pk

Abstract

The Chukar partridge (Alectoris chukar), a prominent species in the Phasianidae family within the Galliformes order, stands as the national bird of Pakistan and Iraq. These birds, operating within a semi-scavenging system, contend with a diverse array of helminth parasites. To treat helminthiasis, the repeated utilization of anthelmintic has inadvertently nurtured a global prevalence of anthelmintic resistance. In response to this challenge, the trend of employing botanical anthelmintics has gained momentum as a viable strategy to counter anthelmintic resistance. A notable contender in this realm is *Withania coagulans*, a plant indigenous to Balochistan and other regions, member of the *Solanaceae* family. Following the procurement of fresh leaves and fruits, the subsequent drying process culminated in the transformation of the dried plants into a finely ground powder. A total of 50 birds were evaluated, out of which 15 Chakur birds with elevated egg per gram (EPG) counts exceeding 150 were selected. The unveiled compelling results, with mean efficacy rates of 87.43% for Fenbendazole, 98.2% for Piperazine, and 96.6% for Withania coagulans. Notably, nematodes such as Ascarids and Heterakis gallinarum exhibited susceptibility to Piperazine and Withania coagulans, while concurrently demonstrating resistance to Fenbendazole, indicative of the evolution of drug resistance over time. The core objective of the study centered on evaluating the effectiveness of *Withania coagulans* in combating nematodes within the native Chakur bird population of Pakistan. The research findings were fortified by the presence of withanolides in the methanolic extract of *Withania coagulans*, which demonstrated anti-nematodal activity. The observed decline in EPG counts following treatment with *Withania coagulans* hints at the plausible role of bioactive compounds such as withanolides and alkaloids. In light of these findings, the study calls for a more extensive exploration of the potential of *Withania coagulans* and analogous herbal products as a means of tackling helminth infestations. The insights gleaned from these herbal interventions could catalyze the development of robust, sustainable strategies to confront and overcome anthelmintic resistance among avian populations.

Keywords: Chukar partridge, Anthelmintic resistance, *Withania coagulans*, nematodes.

Introduction

The Chukar partridge (*Alectoris chukar*), belonging to the Phasianidae family and Galliformes order, holds the distinction of being the national bird of Pakistan and Iraq (Shivambu *et al.* 2020). Spanning from Pakistan to Afghanistan in Asia, the Chukar partridge (*Alectoris chukar*) thrives within its natural habitat. Notably resembling the red-legged partridge (A. rufa), which inhabits western regions; the Chukar partridge was initially introduced as a game bird to North America in 1893. Its distribution spans across Pakistan, encompassing regions such as Punjab, Sindh, Balochistan, Khyber Pakhtunkhwa, FATA, as well as areas including Lower and Upper Dir, Malakand Agency, Swat, Chitral, Margalla hills, Kurram mountain, Kohistan, and Gilgit Baltistan (Khan *et al*. 2021). Its presence is observed across diverse regions including Palestine, Turkey, Iraq, Iran, Lebanon, India, Central Nepal, the Middle East, the Western Himalayas, as well as Afghanistan and Pakistan (Farooq *et al.* 2019). Spanning from Pakistan to Afghanistan in Asia, the Chukar partridge (*Alectoris chukar*) thrives within its natural habitat (Afrasiab *et al.* 2013). Breeding Chukar partridges in captivity is a straightforward process, and they are regarded as more manageable to rear in captivity compared to other species of game birds (Hawramany *et al*. 2007).

Birds, like other animals, suffer from various diseases. Birds, in the semi-scavenging system are subjected to various forms of parasites, which include various protozoan, helminths, lice, termites, ticks, and fleas (Rahman *et al*. 1989) Avian species inhabiting open environments exhibit a wider range of parasites, primarily consisting of gastrointestinal protozoans and helminths. This category of parasites, characterized by average infection rates approximately centered on 50%, is predominantly composed of gastro-intestinal protozoans and helminths (Pandey VS and Demey F. 1993). Gastrointestinal nematodes (GINs) infection is one of the main components to face against well-organized feed conversion. These infections not only cause production losses as well as increase the management and treatment cost of birds in Pakistan. They are extensive in prevalence and are found in tropical and subtropical areas of the world and have anxious the production probable by causing numberless deaths and indirect reasonable losses (Terefe *et al*. 2012).

Helminthiasis are commonly treated through commercially available anthelmintic. Firstly, these drugs are either expensive or inaccessible to the poor rural population of the developing countries (Hammond *et al*. 1997). Secondly, repeatedly usage of commercial available anthelmintic paved a path for anthelmintic resistance worldwide (Waller *et al*. 2003).The potential cause of developing resistance is the number of organisms which survives the drug action and contribute to next generations. Although all helminths don't multiply in their mammalian hosts. But resistance becomes an issue until we have a mass systemic therapy (Köhler *et al*. 2001). Resistance of greater concern which was first reported in Australia in 1995. It was the increase in resistance to triclabendazole. It's been the best drug to treat fluke infections because of its high activity against the migrating immature stages (Overend *et al*. 1995). In the same period resistance also noticed in The Netherlands, UK and Ireland, respectively. An epidemic surge of flukes erupt as a result of climate change and due to mild wetter weather (Mitchell *et al*. 2002). In all species of helminths anthelmintic resistance is developed to all available drugs to some extent and almost reported from all over the world. This situation poses a great danger to livestock industry (Lalchhandama *et al*. 2010). Previously, stated factors are responsible to discover the anthelmintic potential of herbal products (Fajimi *et al*. 2005). Evaluation of botanical anthelmintic are in trend now a days to combat these problems (Alawa *et al*. 2003). Plants always played an important role in discovering of new therapeutics. They were always been a good source of medicinal compounds like morphine, digitalis and quinine.

Withania coagulans dunal, commonly known as English rennet (English), Paneer dodha (Punjabi) Piyon pulli (Balochistan), and Khamazor (pashto). This shrub is Member of Solanaceae family commonly found in Afghanistan, East India and Pakistan. This is an erect grey 60-110 cm high; leaves are lanceo-late with greyish tomentum on both sides (Maurya *et al*. 2010). Withanolides from

Withania coagulans have anthelmentic activity (Kuroyanagi *et al*. 2012). Ether extract and essential oils from fruit showed antibacterial activity against Micrococcus pyogens var. aureus, Vibrio cholera and staphylococcus aureus (Khan *et al*. 1993). 17β-hydroxy withanoloied k and withanolide F have anti-fungal activity against highly pathogenic fungi (Choudhary *et al*. 1995). Extracts of fruits of *Withania coagulans* and aerial parts of *Withania coagulans* is been reported to have anthelmintic activity (Jabbar *et al*. 2006; Khare. 2008; Gaind *et al*. 1967).

The treatment of nematodes is huge constraint; many attempts have been made to find new effective compounds derived from herbal extracts. So the present study is designed to evaluate the anthelmintic efficacy of *Withania coagulans* against nematodes.

Material and Methods

Study Area

The current study was designed to evaluate the effect of anthelmintics against nematodes in bird from district Nushki, Balochistan. Collected samples were analyzed at Disease Investigation Laboratory, Nushki, Balochistan.

Geographical map of study area

Collection and preparation of *Withania coagulans* plants

Whole Plants were collected from Nushki district Balochistan. Fresh plant leaves and fruits were dried in shade to prevent it from sun light, rain and other environmental effects. The process of drying took almost 10 days, drying was conformed through breaking of leaves. Whole Dried plants were grounded into powder by using a clean grinder and further stored in polythene zip bags to avoid the effect of moisture.

In-vivo anthelmintic activity of *Withania coagulans*

Chukar partridge birds were selected for screening of nematodes. The fecal samples were collected through cloacal swabs, packed and transported in plastic bags. The samples were taken to the Disease Investigation Laboratory for Coproscopic examination using direct smear method. Samples were then processed with flotation technique for the nematodes, each sample was triturated in

saturated salt solution (specific gravity 1.18 - 1.2) in a pestle and mortar then the suspension was sieved and examined for nematodes eggs by using different techniques (Soulsby 1982; Tsotetsi *et al*. 2013).

The positive samples were projected to McMasters slide for quantitative analysis, Egg per Gram (EPG) was calculated with the formula and multiplication factor as given below (Egg count in chamber 1 + egg count in chamber 2) * 50 = Eggs per Gram (EPG) (Coles. 1992; Zajac. 2006; Mooney *et al*. 2009; Cringoli *et al.* 2010). Sodium Chloride concentrated solution was used as floatation solution. About 330gms of Sodium Chloride powder (United Chemicals, Quetta) was added to 670ml of distilled water. Floatation solution of 28ml was measured in measuring cylinder and poured in a beaker. Fecal sample of 2gm was weighted with digital balance and added to the beaker containing floatation solution. The mixture was sieved via a sieving metal net into another beaker. Each chamber of McMaster slide was filled with the sieved solution (filtrate of faecal sample+ floatation solution) and examined under microscope at 10x. EPG was counted by method from top right to bottom left of the McMaster slide in each chamber and noted (Foreyt *et al*. 2013).

Experimental Trial

After initial screening of the birds, 15 Chukar partridge birds with EPG >150 were included in study trials. The birds were divided into three groups (05 bird / group). Group A was treated with Fenbendazole (FBZ), Group B was treated with Piperazine (PIP), and Group C was treated with a crude powder of *Withania coagulans* (Khan *et al*. 2017).

Statistical design

Microsoft excel used for descriptive statistics, graphs and charts. Data were statistically analyzed through One-way Analysis Of Variance (ANOVA) keeping significance level of 5% ($p<0.05$) and the results were mention in term of means and standard error (SE) by using Statistical Package for the Social Science (SPSS) version 20.0. Variables having $P<0.05$ was considered significant.

Results

This study was conducted in district Nushki on Chukar partridge (*Alectoris chukar*). A total of 15 positive birds were included in the study. The faecal samples collected in sterile containers were brought to the Disease Investigation Laboratory Nushki. The first sampling was conducted in September 2021 to screen the birds for the presence of Ascarids. A total of 50 birds were screened. Out of 50, 15 birds were selected randomly for the trial and divided into three groups each comprising 05 birds in a group. Anthelmintic used in this trial were Fenbendazole (FBZ), Piperazine (PIP), and *Withania coagulans* (Herbal product).

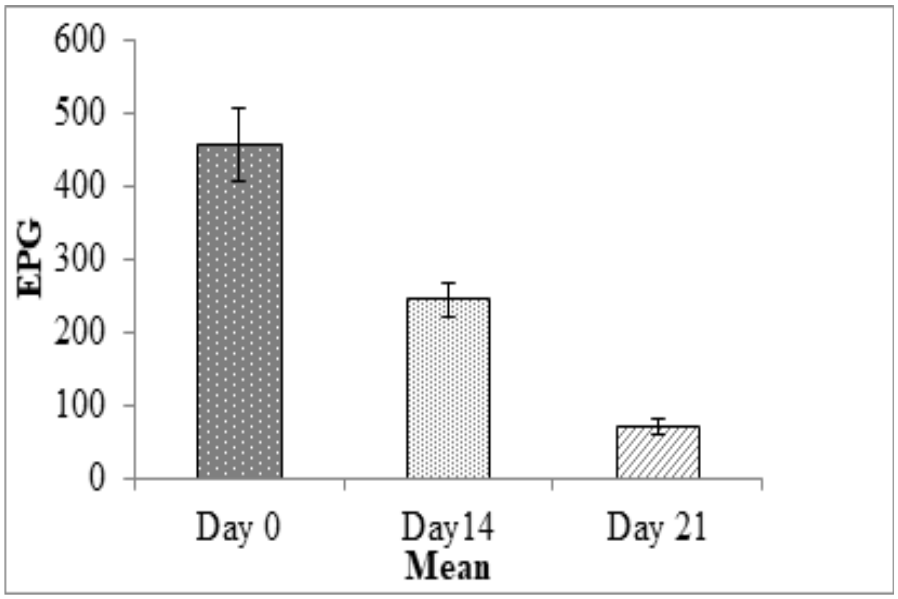
Faecal egg count reduction test

Mean efficacy of Fenbendazole (FBZ), Piperazine (PIP), and *Withania coagulans* was found to be 87.43%, 98.2%, *96*.6% respectively (Table 4.1). According to WAAVP guidelines, these results revealed that Ascarids in Chukar partridge population under study were resistant to Fenbendazole (FBZ) as the mean efficacy of Fenbendazole (FBZ) at day 14 and 21 is less than 95% while nematodes were still susceptible to Piperazine (PIP) and *Withania coagulans*. Details are described in table 4.1.

Table: 1 Mean fecal egg counts at day 0, 14 and 21 days after treatment

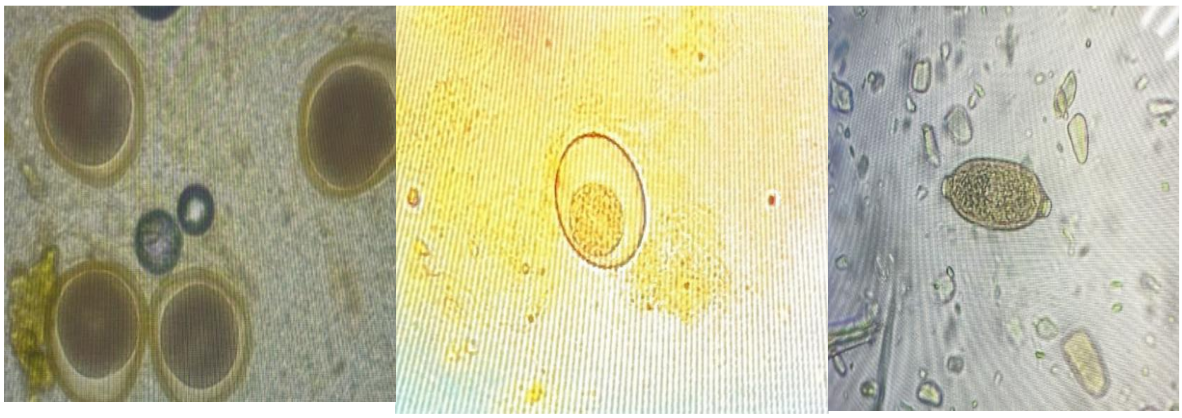
Anthelmintic	**Pre-treatment EPG mean**	**Post-treatment EPG mean N=15**		**% Efficacy**		**Remarks**
	Day 0	Day 14	Day 21	Day 14	Day 21	
Fenbendazole (FBZ)	915±101	95±24.09	105±28.33	89.62%	88.52%	Resistance
Piperazine (PIP)	820±70	5±5	15±7	*98*.00%	98.00%	Susceptible
Withania coagulans	950±144	5±5	0±0	96.00%	96%	Susceptible

Parasitic burden



Microscopy images of various species of gastrointestinal parasites

Microscopically, various species of gastrointestinal nematodes were identified. The *Ascridia galli* was among the highest followed by *Heterakis gallinarum*.



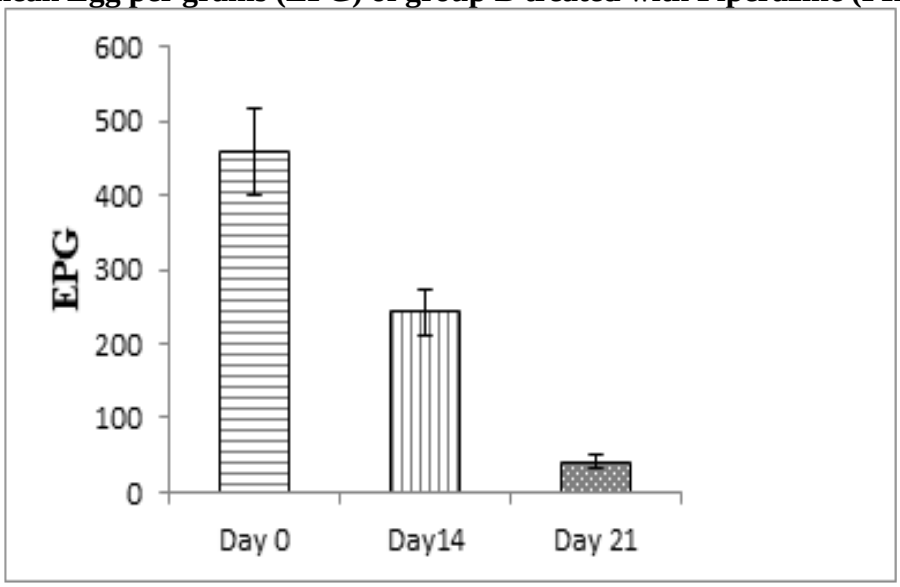
Sampling wise results of birds treated with Fenbendazole (FBZ) at the dose rate of 10mg-50/kg body weight (B.W.)

Birds in group A were treated with Fenbendazole (FBZ) at the dose rate of 10mg-50mg/kg b.w. faecal sample of all birds were collected at day 0, 14, and 21 post treatment. The samples were processed through McMaster technique for the counting of EGG PER GRAM. The data was subjected to SPSS and one way ANALYSIS OF VARIANCE was applied as shown in figure 4.1. The p value was significant ($p<0.05$).

Bar showing mean Egg per grams (EPG) of group A treated with Fenbendazole Sampling wise results of birds treated with Piperazine (PIP) at the dose rate of 10-20mg/kg body weight (B.W.)

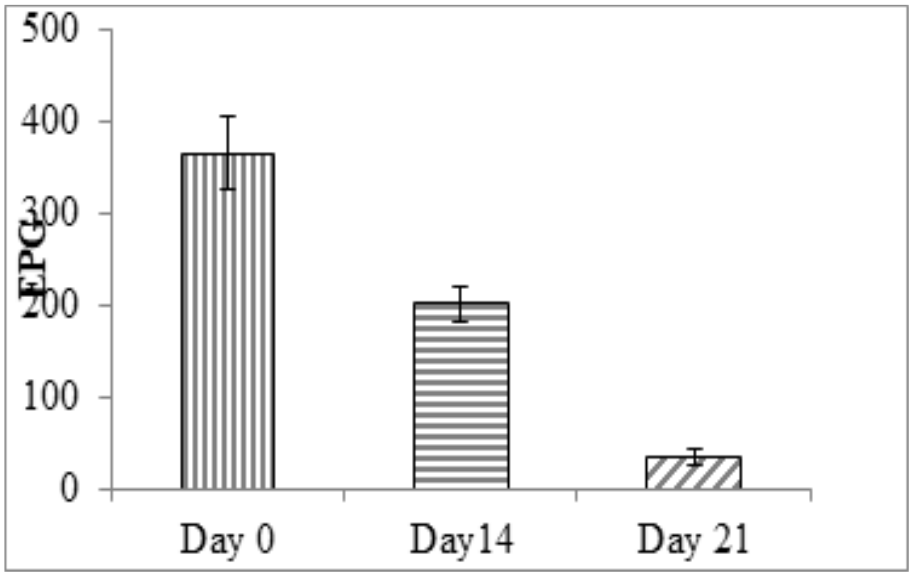
Birds in group B were treated with Piperazine (PIP) at the dose rate of 10-20mg mg/kg b.w. Faecal sample of all birds were collected at day 0, 14, and 21 post treatments. The samples were processed through McMaster technique for the counting of EGG PER GRAM. The data was subjected to SPSS and one way ANALYSIS OF VARIANCE was applied as shown in figure 4.3. The p value was significant ($p<0.000$).

Bar showing mean Egg per grams (EPG) of group B treated with Piperazine (PIP).



Sampling wise results of birds treated with *Withania coagulans* at the dose rate of 5mg/10kg body weight (B.W.)

Birds in group C were treated with *Withania coagulans* at the dose rate of 1gm/10kg b.w. faecal sample of all birds were collected at day 0, 14, and 21 post treatments. The samples were processed through McMaster technique for the counting of EGG PER GRAM. The data was subjected to SPSS and one way ANALYSIS OF VARIANCE was applied as shown in figure 4.4. The p value was significant ($p<0.000$).



Bar showing mean Egg per Grams (EPG) of group C treated with *Withania Coagulans*

Discussion

The Chukar partridge (*Alectoris chukar*) holds the distinction of being the national bird of Pakistan and Iraq (Shivambu *et al*. 2020). Gastrointestinal nematodes (GINs) infestation is one of the key factors which not only cause the death of birds and animals but also causes production losses (Terefe *et al*. 2012). There are different kinds of control strategies to overcome nematodal infestations. In chemical strategy different anthelemtics drugs i.e, Albendazole, Mebendazole .Oxfendazole Fenbendazole (FBZ) and Ivermectic are currently used. Due to the resistance of these anthelmintics drugs alternative strategies are used to cope with this helminthiasis. In which different plant extracts and powders are used for prevention of drug resistance (Jaeger *et al*. 2017).

Many plants in the world exhibit various characteristics specially the natural plants available in sub-continent. Some the plants possess anthelmintic activities while others have antibiotic activity. Many researchers around the globe have scientifically proven various plants with anthelmintic activity (Akhtar *et al*. 2000). *Withania coagulans* plant has anthelmintic activity (Jabbar *et al*. 2006). Also an Indian researcher studied its anthelmintic effects (Khare. 2008). In the result of that study *Withania coagulans* extracts possess a good anthelmintic activity against GIT Nematodes.

In the current study plant powder of *Withania coagulans* was tested against Chukar partridge nematodes. Up-to 21 days post treatment the efficacy was about 96% observed in the EPG of nematodes compared with the EPG at first day of treatment. The withanolides in the methanolic extract were proved with anti-nematodal activity (Khodaei *et al*. 2012). Though extend of the EPG decline was considerable compared to the commercial anthelmintic Piperazine (PIP). One potential reason for the observed decline in EPG with *Withania coagulans* treatment could be the presence of bioactive compounds (withanolides and alkaloids) within the plant that possess anthelmintic properties. Other authors also reported anthelmintic activity of *Withania coagulans* (Jabbar *et al*. 2006; Khare *et al*. 2007). The activity of the *Withania coagulans* of the plant in decreasing the EPG was significantly better than that of treated group with Fenbendazole (FBZ). In contrast, Fenbendazole (FBZ), as a commercial anthelmintic drug, may have a narrower spectrum of action or could face resistance issues among the targeted nematode population may be due to the irrational use of the drugs (APVMA. 2020).

Among chickens, the intestinal nematodes *Ascaridia galli, Heterakis gallinarum, and Capillaria spp.* stand out as the most prevalent and pathogenic species. (Kaufmann *et al*. 2011; Shifaw *et al*. 2021; Thapa *et al*. 2015). A research conducted in Iraq revealed the existence of two nematode species, namely *Ascaridia numidae* and *Heterakis gallinarum* (Khoshnaw *et al*. 2013). Same species of nematodes including (*H. gallinarum* and *A. galli*) with prevalence of 54.3 % reported in Chukar partridge Malakand divison Pakistan by (Ullah *et al*. 2022). In our ongoing study conducted in Nushki, Balochistan, Pakistan, two species of nematodes were identified, specifically *Ascaridia galli*, that was common in both areas of Pakistan while *Heterakis gallinarum* remains consistent in both regions of Pakistan as well as Iraq. The underlying cause for this consistent prevalence of nematodes is worth considering. In Iraq and Pakistan could be attributed to similar ecological conditions that favor the survival and propagation of this nematode species. Environmental factors, such as temperature, humidity, and the presence of suitable intermediate hosts, play a significant role in the distribution and prevalence of parasites. If both Iraq and Pakistan share comparable ecological conditions that are conducive to the life cycle of *Heterakis gallinarum,* it's likely that the nematode has successfully established itself in both regions.

In vivo experiments have also been undertaken to assess the anthelmintic efficacy of different plant-derived substances, including *Withania coagulans*. These trials encompass the examination of their potential to expel nematode worms from their host organisms. (Asuzu *et al*. 1994 And Desta. 1995). Alternatively, these trials involve evaluating the capacity of plant-derived substances, such as *Withania coagulans*, to decrease the count of eggs per gram of feces (EPG) expelled by infected hosts when compared to animals treated with commercial anthelmintics (Akhtar *et al*. 2000). In a separate investigation, the author documented the effectiveness of *Withania coagulans* against earthworms. The steam volatile oil derived from the petroleum ether of *Withania coagulans* was identified to exhibit a lethal impact on earthworms (Budhiraja and Sudhir. 1987).

In a separate investigation, the application of M. azedarach fruit powder at a dosage of 20mg/kg, along with its corresponding water extract, methanol extract, ethanol extract, and Piperazine (PIP) at 200mg/kg, demonstrated varying degrees of efficacy in reducing Egg Per Gram (EPG) counts in chickens infected with *Ascaridia galli*. Specifically, the reductions observed were as follows: M.

azedarach fruit powder at 57.8±2.4%, its water extract at 15.7±4.3%, its methanol extract at 18.5±1.8%, its ethanol extract at 67.8±4.6%, and Piperazine (PIP) at 75±2% (Akhtar and Riffat, 1985).

While in our present study the efficacy of *Withania coagulans* was 96% and Piperazine (PIP) efficacy was 98% recorded against nematodes infestation in Chukar partridge birds. But the plant extract were different from out plant while Piperazine (PIP) result little bit rise from (Akhtar *et al*. 2000)

Still Piperazine (PIP) and Fenbendazole (FBZ) showed better activity mainly Piperazine (PIP) against nematodes infestation in birds. But the worms have developed resistance against the drug through times. Anthelmintic resistance is the emerging issue of the veterinary world. Conclusion of the present study was that the traditional remedy used for the cure of helminthes *Withania coagulans* an herbal medicinal plant exhibit the anthelmintic activity against birds nematodes. In conclusion, the present study has established that the traditional remedy utilizing *Withania coagulans*, an herbal medicinal plant, possesses anthelmintic activity against nematodes in birds. Considering these findings, it is recommended that further research be conducted on the potential of *Withania coagulans* and other herbal products as remedies against helminth infections. Expanding our understanding of these herbal interventions could offer valuable insights for the development of effective and sustainable strategies to combat anthelmintic resistance in avian populations.

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