Pakistan J. Zool., pp 1-11, 2025. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20240416105757

In Vitro Anthelmintic Efficacy of Two Wild Herbs *Peganum harmala* and *Withania somnifera* Against *Haemonchus contortus*

Muhammad Qarib Hussain[1], Jabar Zaman Khan Khattak[1] and Nousheen Zafeer[2]*

[1]*Department of Biological Sciences, International Islamic University, Islamabad, Pakistan*
[2]*Department of Zoology, University of Gujrat, Gujrat, Pakistan*

ABSTRACT

Peganum harmala and *Withania somnifera* are two medicinally important wild herbs. To explore the use of green harmonious bioproducts, the aqueous and methanolic extracts of these plants were assessed against the eggs, larvae and worms of abomasal blood sucking parasites *Haemonchus contortus* using concentrations of 3.125, 6.25, 12.5, 25 and 50 mg/mL. The tests were performed at a set-temperature (25-30°C) maintained in a newly designed apparatus to mimic abomasal conditions. The investigated concentrations (3.125-50 mg/mL) displayed dose-dependent significant increase ($p < 0.05$) different from both negative (PBS and 3% DMSO) and the positive controls (Albendazole) (12.5 mg/mL). With respect to the highest 50 mg/mL concentration, 100% of the adult worms were found dead in methanolic extracts (MEs) as compared to 80% deaths in aqueous extracts (AEs) of these plants after 8 h of extract exposure. Similarly, higher larval (L1) mortality was observed in MEs of *W. somnifera* (83.7%) and *P. harmala* (82.7%) compared to their AEs (*W. somnifera*=55.7% and *P. harmala*=59%). Interestingly, ME of *W. somnifera* was 90.3% showed higher inhibitory effects on egg hatching as compared to ME of *P. harmala* (79%). Moreover, the AEs of these plants had lower ovicidal activity (*W. somnifera*=72.7% and *P. harmala*=74.3%) than their methanolic counterparts. Considering these results (50 mg/mL) and the lethal concentration (LC_{50}), calculated for adulticidal, larvicidal and ovicidal assays, the AE (31.85 mg/mL, 35.84 mg/mL, 40.34 mg/mL) and ME (24.79 mg/mL, 24.38 mg/mL, 34.67 mg/mL) of *W. somnifera* was found more potent than the AE (39.5 mg/mL, 45.09 mg/mL, 40.74 mg/mL) and ME (26.88 mg/mL, 20.72 mg/mL, 36.04 mg/mL) of *P. harmala*. Taken together, these findings suggest that these herbal extracts are effective and can be used to develop novel, low-cost anthelmintics for *H. contortus* control in goats, sheep and cattle.

Article Information
Received 16 April 2024
Revised 25 June 2024
Accepted 06 July 2024
Available online 15 September 2025 (early access)

Authors' Contribution
QH performed all experimental work and wrote initial manuscript. JZKK supervised all work and analysed data while NZ conceived the idea, designed experimental work and contributed to manuscript writing. All authors have ready and approved the final version of the manuscript.

Key words
Anthelmintic efficacy, *Peganum harmala, Withania somnifera*, Aqueous and methanolic extracts, Ovicidal activity, Larvicidal activity, Adulticidal activity, *Haemonchus contortus*

INTRODUCTION

The genus *Haemonchus* has a dynamic physiological nature. It lives in a faunal host parasitic relationship. The 12 known species of *Haemonchus* infect a wide range of hosts, including goats, sheep, antelopes, giraffes, Cervidae, bovines and wild ruminants (Hoberg *et al*., 2004a; Hoberg and Zarlenga, 2016b). The most vulnerable hosts are domestic ruminants, i.e., goats, sheep and cattle. The infection rate is greater in tropical, subtropical, warm temperate and summer rainfall regions. Hot and humid environmental conditions play a significant role in the spread of infection (Arsenopoulos *et al*., 2021).

Pathologically, *H. contortus* resides in the abomasa of infected host animals. It is actively engaged in sucking blood, which induces anaemia. Sick animal loss their body weight and wool growth. It also effects hormonal levels, the estrous cycle, the absorption of calcium, phosphorus and magnesium, the intake of food, appetite and immunity levels (Besier *et al*., 2016a; Dutta *et al*., 2017; Arsenopoulos *et al*., 2021). Young and adult animals with compromised immune systems are more susceptible to haemonchosis (Zajac, 2006).

According to the 2021-22, economic census, the total populations of sheep, goats and cattle in Pakistan was estimated to be 31.9 million, 82.5 million and 53.4 million, respectively. The overall livestock contribution to the national GDP was 14.04% (FAO, 2022; Pakistan Economic Survey, 2021-22). Progress in the livestock sector can be hampered by infectious diseases. *H. contortus* is the major parasite involved in enormous economic losses worldwide (Charlier *et al*., 2020). Due to

* Corresponding author: nousheenzafeer29@gmail.com
0030-9923/2025/0001-0001 $ 9.00/0

haemonchosis, 29% reduction in milk and 27% in meat production was estimated in Punjab, Pakistan causing a loss of 8800.09 million PkR, annually (Qamar *et al*., 2011). The situation has been worsened by the fact that *H. contortus* has developed resistance against anthelmintic drugs (Bibi *et al*., 2017). This indicates the limited success of both preventive and treatment measures with the help of commercial anthelmintics (Besier *et al*., 2016b).

Medicinal plants have the potential to kill endoparasites and can be used as alternatives to commercial anthelmintics (Lanusse *et al*., 2018; Charlier *et al*., 2022). These therapies are applied in 75-90% of developing countries, while half of the population in the industrialized world uses them (Robinson and Zhang, 2011). In Pakistan, diverse climatic conditions allow rich biodiversity and medicinal wealth. More than 700 species are used as medicines for various purposes, while the remaining plants are being assessed for the efficacy of their active ingredients (Shinwari, 2010; Alamgeer *et al*., 2018). Extracts from different parts of the plant have pharmacological effects on different diseases (Moloudizargari *et al*., 2013).

Peganum harmala, commonly known as the Syrian rue, or harmel is a wild perennial herb. It belongs to the family Nitrariaceae which is distributed in the Middle East and in parts of South Asia, such as India and Pakistan (Miraj, 2016). It is distinguished by its foul smell (Moloudizargari *et al*., 2013) and is traditionally used as a disinfectant and mosquito repellent and for the treatment of many human diseases.

Withania somnifera, also called winter cherry, or Indian ginseng is a wild annual herb. It belongs to the Solanaceae family and abundantly grows in arid areas of the Mediterranean, tropical Africa, South Africa, Canary and Cape Verde Islands, Afghanistan, Pakistan, India, China, Sri Lanka and Nepal. It is also found in Europe, southern Australia and New South Wales (Paul *et al*., 2021). It is considered an important medicinal plant (Meena *et al*., 2020) and is used for treating neurodegenerative diseases (Ale *et al*., 2021).

Helminth parasitism is the major cause of reduced production in livestock worldwide. Wildly grown *P. harmala* and *W. somnifera* are highly esteemed medicinal plants as reported in several studies on various aspects. To exploit their activities as anthelmintics against *H. contortus*, a detailed *in vitro* study was conducted to treat haemonchosis.

MATERIALS AND METHODS

Study area selection for plant collection

P. harmala and *W. somnifera* were collected in May 2022 from the Kala Chitta Range (33°7’34°N and 71°45’73°E), District Attock, Punjab (Pakistan). The study area was selected on the basis of its unique plant biodiversity (Supplementary Fig. 1A, B). The collected plants were washed and taxonomically identified by a plant taxonomist, Dr. Kifayat Ullah, Department of Biological Sciences, International Islamic University, Islamabad (No. FBAS-93) of these plants were preserved at the said department. The washed materials were shade dried for 7 days, the plant materials were subjected to grinding with an electric grinder.

Abomasal working apparatus

The aim of constructing the apparatus was to control the fluctuation in the external laboratory temperature, which possibly affect the accuracy of the adulticidal assay. The apparatus is a specialized chamber designed to generate and maintain a set range of temperatures (25-30°C) and a relative humidity of 70-80%. It works as an artificial abomasal medium, to ensure the viability of temperature-sensitive worms (Supplementary Fig. 2A, B).

In vitro *anthelmintic adulticidal assay*

Abomasa from freshly slaughtered goats were collected from the local slaughterhouse. Highly agile, live and mature *H. contortus* were isolated (Supplementary Fig. 3A, B). The parasites were suspended and washed 2-3 times in PBS. A total of 105 worms were distributed into 7 groups, each in triplicate. Five worms were transferred to each Petri dish. All twenty-one Petri dishes were arranged in an adulticidal working apparatus at 25-30°C. The adjusted range was kept constant for 12 h. Under these controlled conditions, the dead worms were easily detected and separated from the viable worms.

The crude aqueous extract (CAE) and crude methanolic extracts (CME) were prepared by standard methods with partial modifications (Gilani *et al*., 2004). The percentage stock solution was prepared by dissolving 0.5 g or 500 mg of CAE or CME in 100 mL of PBS. The serial dilutions for the 5 tested groups had extract concentrations of 3.125, 6.25, 12.5, 25 and 50 mg/mL. Albendazole was used as a positive control at a concentration of 12.5 mg/mL. For preparing methanolic extract dilutions, 3% DMSO was used. The negative control group did not receive any treatment. The five concentrations of the targeted extracts were applied from groups I to V, whereas the negative and positive control groups were represented by groups VI and VII, respectively.

Worm mortality was observed from 0 to 12 h (Iqbal *et al*., 2006). The number of dead worms was calculated after interval of 2 h. The criterion for declaring mortality during the experiment was the worm’s static appearance. The final confirmation was made by placing the worms

in fresh PBS. If the worm revived in PBS, it was placed back in its respective treatment. The final declaration of worm's death was made using a microscope. The percentage of adult mortality was calculated using the following formula:

$$\text{Adulticidal effects (\%)}: \frac{\text{No. of dead worms after treatment} \times 100}{\text{Total no. of worms before treatment}} \quad \ldots(1)$$

Ovicidal assay

The protocol of Coles (1984) was modified to isolate eggs. Adult female worms were triturated through a mortar and pestle to collect eggs. Eggs were isolated from the debris after filtering through a 100 µm mesh sieve. The egg solution was further diluted by adding some PBS. McMaster slides were then used to count the total number of eggs in the solution (Supplementary Fig. 4). The percentage efficiency of the extract was calculated by using the following formula:

$$\text{Ovicidal activity (\%)}: \frac{\text{No. of unhatched eggs after treatment} \times 100}{\text{Total number of eggs before treatment}} \quad \ldots(2)$$

The 48-wellplates were divided into seven groups of three wells each. Approximately 100 eggs were placed in each well of a microwell plate and PBS was added to create a solution of 130 µL with the help of a micropipette. Egg hatching media was prepared by dissolving 1 g of yeast in a mixture of 90 mL of normal saline and 10 mL of Earle's balanced salt. Then, 20 µL of nutritive media was added to each well. Afterwards, 150 µL of either aqueous or methanolic extracts of both plants were added at the concentrations of 3.125, 6.25, 12.5, 25 and 50 mg/mL in triplicate. Albendazole at a concentration of 12.5µL was used as a positive control, while PBS or 3% DMSO was added to one group as a negative control. All the groups were allowed to incubate for 48 h at 25-30°C. At the end of the incubation period, 1 drop of lugol's iodine solution was added in each group to stop eggs from further hatching. Under a microscope, all dead or unhatched eggs were counted with the help of McMaster slide. The egg hatching method described by Coles *et al*. (1992) was used to evaluate the anthelmintic efficacy of the extracts on the viability of the eggs.

Larvicidal assay

An estimated 100 eggs were suspended in 21 wells of a 48-well plate. Twenty microliters of egg hatching media was added to each well. PBS was further added to make a total volume of 200 µL. This mixture was incubated at 25-30°C for 48 h (Soulsby, 1968; Tariq *et al*., 2008). After completing the incubation period, 200 µL of CAE or CME at concentrations of 3.125, 6.25, 12.5, 25 and 50 mg/mL were added in each well of groups V to I. Albendazole (12.5µL) was added as a positive control in group VII. The negative control group VI was filled with PBS to a total volume of 400 µL.

Larvicidal activity was assessed by counting the number of dead larvae under microscope after 48 h of treatment using McMaster slide (Supplementary Fig. 5). The percentage of extract efficacy was calculated using the following formula:

$$\text{Larvicidal efficacy (\%)}: \frac{\text{Number of larvae killed after treatment} \times 100}{\text{Total no. of larvae before treatment}} \quad \ldots(3)$$

Statistical analysis

The statistical significance of differences between means was determined by using two-way analysis of variance (ANOVA). Furthermore, Tukey's test was used to determine differences between concentrations and solvents. LC_{50} values for each extract and assay were calculated by using probit analysis.

RESULTS

Ovicidal activity

Aqueous and methanolic extracts of *P. harmala* and *W. somnifera* at the concentrations of 3.125, 6.25, 12.5, 25 and 50 mg/mL inhibited the egg hatching of *H. contortus*. The extract potencies of *P. harmala* (AE=74.3%, ME=79%) and *W. somnifera* (AE= 72.7%, ME= 90.3%) at 50 mg/mL significantly increased ($P < 0.05$) in a dose-dependent manner (Table I). AEs of *P. harmala* and *W. somnifera* had similar effects i.e., 74.3% and 72.7% in ovicidal assay. However, significant differences were detected in the activity of the methanolic extracts of *P. harmala* (79%) and *W. somnifera* (90.3%) (Table I). The negative control group (PBS and 3% DMSO) in the AE and ME tests had very little effect on egg hatching, but albendazole (12.5 mg/mL) effectively inhibited (100%) egg hatching (Table I). The estimated LC_{50} values for the aqueous and methanolic extracts of *P. harmala* were 39.50 mg/mL and 26.88 mg/mL, respectively (Table I, Fig. 1). The LC_{50} values of the aqueous and methanolic extracts of *W. somnifera* were 31.85 and 24.79 mg/mL, respectively (Table I, Fig. 1). An evaluation of the ovicidal activity clearly demonstrated that the methanolic extracts of both plant species were better ($P < 0.05$) than the aqueous extracts.

Larvicidal efficacy

Aqueous and methanolic extracts of *P. harmala* and *W. somnifera* at different concentrations (3.125-50 mg/mL) inhibited larval development in *H. contortus* larvae. At 50 mg/mL concentration, the highest activities were observed for the *P. harmala* extracts (aqueous = 59%, methanolic = 82.7%) and *W. somnifera* extracts

Table I. Ovicidal and larvicidal activity (Mean efficacy ± SE) of aqueous extract (AE) and methanolic extract (ME) of *P. harmala* and *W. somnifera* on *H. contortus* eggs.

Groups	Concentrations (mg/mL)	*P. harmala*		*W. somnifera*	
		AE	ME	AE	ME
Ovicidal activity (%)					
Extracts treatments	50	74.33±4.98^{b}	79.00±6.56^{b}	72.67±7.54^{b}	90.33±1.20^{a}
	25	16.00±1.00^{fgh}	74.33±1.67^{b}	46.67±4.18^{d}	61.00±5.69^{c}
	12.5	8.00±1.53^{hi}	24.67±3.84^{fg}	33.00±2.08^{e}	21.67±2.96^{fg}
	6.25	2.67±1.33^{i}	8.33±0.67^{hi}	9.00±2.65^{hi}	11.33±2.73^{ghi}
	3.125	1.33±0.33^{i}	2.33±0.33^{i}	5.00±2.89^{i}	10.00±1.00^{i}
Negative	PBS	1.33±0.33^{i}		1.00±0.58^{i}	
	DMSO3%		1.33±0.67^{i}		2.67±0.33^{hi}
Positive	12.5 ABZ	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
LC_{50}		39.50	26.88	31.25	24.79
Larvicidal activity (%)					
Extract treatment	50	59.00±7.21^{cd}	82.67±0.33^{b}	55.67±5.78^{d}	83.67±1.20^{b}
	25	18.00±3.46^{ghi}	70.33±2.96^{c}	50.33±3.18^{d}	58.67±3.53^{d}
	12.5	6.33±1.76^{jkl}	61.33±2.60^{cd}	37.00±3.79^{e}	30.33±5.84^{ef}
	6.25	7.33±2.03^{ijkl}	13.00±3.21^{hijkl}	27.00±1.73^{efg}	14.00±8.54^{hijk}
	3.125	2.33±0.33^{l}	17.67±2.60^{ghij}	19.67±0.88^{fgh}	26.33±11.05^{efg}
	PBS	2.00±1.00^{l}		9.33±2.85^{hijkl}	
Negative	DMSO3%		5.33±1.86^{kl}		10.33±5.17^{hijkl}
Positive	12.5 ABZ	100.000±0.00^{a}	100.000±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
LC_{50}		45.60	20.72	35.84	24.38

All the values are expressed as Mean ± SE. The difference in superscripts indicate significant differences ($p < 0.05$).

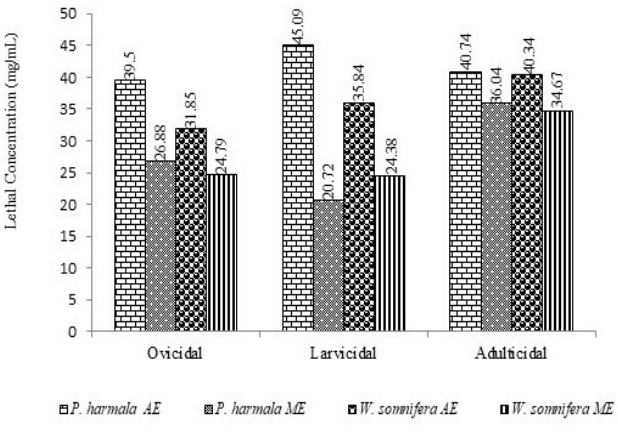


Fig. 1. LC_{50} of aqueous (AE) and methanolic extracts (ME) of *P. harmala* and *W. somnifera*. The bars clearly demonstrate that the methanolic extract of both plants are better than the aqueous extracts in ovicidal, larvicidal and adulticidal treatments.

(aqueous = 55.7%, methanolic = 83.7%) (Table II). There was a dose-dependent increase in the activity of the extracts. Similarly, the AEs of *P. harmala* and *W. somnifera* showed significantly lower activity than the MEs of these plants. PBS and DMSO (3%) were used as negative controls. They have very minor effects on the inhibition of larval development. Albendazole (12.5 mg/mL), the positive control, displayed 100% inhibition of larval development (Table II). The calculated LC_{50} values for the aqueous and methanolic extracts of *P. harmala* were 45.09 mg/mL, and 20.72 mg/mL, respectively (Table II, Fig. 1). However, 35.84 mg/mL and 24.38 mg/mL were the LC_{50} values calculated for the aqueous and methanolic extracts of *W. somnifera*, respectively (Table II, Fig. 1). According to the results of the larvicidal assay, the ME of both plants had greater ($P < 0.05$) efficacy than their aqueous counterparts.

Table II. Adulticidal activity (Mean mortality ±SE) of aqueous extract (AE) and methanolic extract (ME) of *P. harmala* and *W. somnifera* on adult worms of *H. contortus*.

Extracts	Concentrations (mg/mL)	Exposure time						LC 50
		2 h	4 h	6 h	8 h	10 h	12 h	
P. harmala								
AE	50	0.00±0.00^{m}	0.00±0.00^{m}	2.00±0.58^{ghij}	4.00±0.58^{abcd}	4.67±0.33^{ab}	5.00±0.00^{a}	40.74
	25	0.00±0.00^{m}	0.00±0.00^{m}	2.00±0.58^{ghij}	3.00±1.15^{defg}	3.33±0.88^{cdef}	3.67±0.88^{bcde}	
	12.5	0.00±0.00^{m}	0.00±0.00^{m}	1.33±0.33^{ijkl}	1.33±0.33^{ijkl}	2.00±0.58^{ghij}	2.67±0.33^{efgh}	
	6.25	0.00±0.00^{m}	0.00±0.00^{m}	1.00±0.58^{jklm}	1.33±0.33^{ijkl}	1.67±0.33^{hijk}	2.33±0.33^{fghi}	
	3.125	0.00±0.00^{m}	0.00±0.00^{m}	0.67±0.33^{klm}	1.00±0.58^{jklm}	1.67±0.33^{hijk}	1.67±0.33^{hijk}	
Control	Negative PBS	0.00±0.00^{m}	0.00±0.00^{m}	0.00±0.00^{m}	0.00±0.00^{m}	0.33±0.33^{lm}	0.67±0.33^{klm}	
	Positive ABZ	0.00±0.00^{m}	0.00±0.00^{m}	2.00±0.58^{ghij}	2.67±0.33^{efgh}	4.67±0.33^{ab}	5.00±0.00^{a}	
ME	50	0.00±0.00^{m}	1.00±0.58^{jklm}	3.33±0.33^{cdef}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}	36.04
	25	0.00±0.00^{m}	0.00±0.00^{m}	1.67±0.33^{hijk}	3.00±0.58^{defg}	4.00±0.58^{abcd}	4.33±0.33^{abc}	
	12.5	0.00±0.00^{m}	0.00±0.00^{m}	1.00±0.00^{jklm}	2.00±0.58^{ghij}	2.33±0.33^{fghi}	2.67±0.33^{efgh}	
	6.25	0.00±0.00^{m}	0.00±0.00^{m}	1.00±1.00^{jklm}	1.67±0.67^{hijk}	2.33±0.33^{fghi}	2.33±0.33^{fghi}	
	3.125	0.00±0.00^{m}	0.00±0.00^{m}	1.33±0.67^{ijkl}	1.33±0.67^{ijkl}	2.00±0.58^{ghij}	2.33±0.67^{fghi}	
Control	Negative DMSO3%	0.00±0.00^{m}	0.00±0.00^{m}	0.00±0.00^{m}	0.67±0.33^{klm}	0.67±0.33^{klm}	0.67±0.33^{klm}	
	Positive ABZ	0.00±0.00^{m}	0.00±0.00^{m}	2.00±0.58^{ghij}	3.33±0.33^{cdef}	5.00±0.00^{a}	5.00±0.00^{a}	
W. somnifera								
AE	50	0.33±0.33^{lm}	0.33±0.33^{lm}	1.67±0.33^{hijk}	4.00±0.58^{abcd}	4.33±0.67^{abc}	4.33±0.67^{abc}	40.34
	25	0.33±0.33^{lm}	0.33±0.33^{lm}	1.33±0.33^{ijkl}	2.33±0.33^{fghi}	3.00±0.00^{defg}	3.00±0.00^{defg}	
	12.5	0.33±0.33^{lm}	0.33±0.33^{lm}	1.33±0.33^{ijkl}	2.00±0.58^{ghij}	2.00±0.58^{ghij}	2.67±0.67^{efgh}	
	6.25	0.00±0.00^{m}	0.00±0.00^{m}	0.00±0.00^{m}	0.33±0.33^{lm}	0.67±0.33^{klm}	1.00±0.58^{jklm}	
	3.125	0.00±0.00^{m}	0.00±0.00^{m}	0.67±0.33^{klm}	0.33±0.33^{lm}	0.67±0.67^{klm}	1.00±0.58^{jklm}	
Control	Negative PBS	0.00±0.00^{m}	0.00±0.00^{m}	0.67±0.33^{klm}	0.67±0.33^{klm}	0.67±0.33^{klm}	0.67±0.33^{klm}	
	Positive ABZ	0.33±0.33^{lm}	0.67±0.67^{klm}	2.00±0.58^{ghij}	4.33±0.67^{abc}	5.00±0.00^{a}	5.00±0.00^{a}	
ME	50	0.00±0.00^{m}	1.33±0.33^{ijkl}	3.00±0.58^{defg}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}	34.67
	25	0.00±0.00^{m}	0.00±0.00^{m}	1.33±0.33^{ijkl}	3.33±0.33^{cdef}	3.67±0.33^{bcde}	4.00±0.00^{abcd}	
	12.5	0.00±0.00^{m}	0.00±0.00^{m}	1.33±0.33^{ijkl}	2.33±0.67^{fghi}	2.67±0.33^{efgh}	2.67±0.33^{efgh}	
	6.25	0.00±0.00^{m}	0.00±0.00^{m}	0.67±0.33^{klm}	2.00±0.58^{ghij}	2.33±0.33^{fghi}	2.67±0.33^{efgh}	
	3.125	0.00±0.00^{m}	0.00±0.00^{m}	0.00±0.00^{m}	0.00±0.00^{m}	0.33±0.33^{lm}	1.00±0.58^{jklm}	
Control	Negative DMSO3%	0.00±0.00^{m}	0.00±0.00^{m}	0.00±0.00^{m}	0.33±0.33^{lm}	0.33±0.33^{lm}	0.67±0.33^{klm}	
	Positive ABZ	0.00±0.00^{m}	0.67±0.33^{klm}	3.00±0.58^{defg}	4.33±0.33^{abc}	5.00±0.00^{a}	5.00±0.00^{a}	

All the values are expressed as Mean ± SE. The difference in superscripts indicate significant differences ($p < 0.05$)

Adulticidal effects

The anthelmintic activity of the crude methanolic extracts of both plants against adult *H. contortus* had significantly higher ($P < 0.05$) activity at all concentrations from 3.125 to 50 mg/mL as compared to AEs (Table II). ME of *P. harmala* and *W. somnifera* at 50 mg/mL concentration resulted in 100% worm mortality after 8 h of exposure. However, after 8 h of exposure, 80% of the worms were killed by AE at the same concentration (50 mg/mL). The lower concentrations (25-3.125 mg/mL) did not show 100% effects even at 12 hrs of extract exposure (Table II). All the extracts had significant ($P < 0.05$) dose- and time-dependent effects on worms compared to the positive control (albendazole) (12.5 mg/mL). No considerable deaths were observed in the negative controls in the aqueous (PBS) and methanolic (3% DMSO) assays. In adulticidal assay, the estimated LC_{50} values for the aqueous and methanolic extracts of *P. harmala* were 40.74

mg/mL and 36.04 mg/mL, respectively Table II, Fig. 1). The LC_{50} values of the aqueous and methanolic extracts of *W. somnifera* were 40.34 and 34.67 mg/mL, respectively (Table II, Fig. 1). The assessment revealed that the methanolic extracts of both plant species were better ($P < 0.05$) than the aqueous extracts.

DISCUSSION

The results of the present study demonstrated that both *P. harmala* and *W. somnifera* had significant ($P < 0.05$) nematocidal activity compared to the negative controls (PBS and 3% DMSO) and the positive controls i.e., albendazole. In this detailed *in vitro* study, the worms, larvae and eggs were exposed to a set range of temperatures (25-30°C) in a self-structured foster abomasal apparatus. The obtained results were more reliable and easier to record than those obtained under open laboratory conditions. In our analysis, *W. somnifera* was found to be more effective than *P. harmala* at all the tested concentrations. The variations in the results were related to the type of plant extract. The anti-haemonchus effects of these extracts are likely to be associated with the presence of secondary metabolites. Presently, the most tested biologically active compounds include terpenes, glycosides, saponins, flavonoids, tannins, and β-carboline alkaloids such as harmine, harmaline, harmalol and harmol (Deore and Khadabadi, 2010; Moloudizargari *et al*., 2013; Qamar *et al*., 2017; Spiegler *et al*., 2017; Danial *et al*., 2018; Manjusa and Pradeep, 2022). Saddiqe *et al*. (2019) revealed that flavonoids, tannins, steroids and saponins in *W. somnifera* extracts are the key secondary metabolites involved in anthelmintic activity. However, harmine, a primary alkaloid in *P. harmala*, plays a significant role in this regard. The potency of *P. harmala* is attributed to the presence of abundant β-carboline alkaloids (Mayad *et al*., 2019). In the present study, ME had better effects than AE. The difference in mortality might be due to differences in polarity and the total phenol and flavonoid contents in the extracts (Saddiqe *et al*., 2019). The combination of alkaloids from *P. harmala* and tannins and saponins from *W. somnifera* extracts could be more effective than either individual effect alone. The present study lacks this aspect but aimed for promising synergistic results in the future.

Ovicidal results revealed that the tested concentrations (50-3.125mg/mL) of both plant extracts induced egg hatch inhibition, statistically ($P < 0.05$) comparable to that of negative controls and the positive controls of albendazole groups. At 50 mg/mL concentration, ME of *W. somnifera* showed highest ovicidal efficacy as compared to its AE and ME and AE of *P. harmala*. Moreover, the highest activity of ME of *W. somnifera* expressed similar potential owned by the reference drug albendazole. Egg hatch inhibition increases with increased plant concentration. These results can be attributed to the presence of active phytochemicals that act as inhibitors on egg hatching. The variations in their inhibitory effects in the tested groups were due to the nature of the extraction. The extract concentration and type of solvent may determine the level of solubility (Saidulu *et al*., 2014). The active compounds potentially involved in egg hatch inhibition were suggested to be saponins and tannins (Marie-Magdeleine *et al*., 2009; Bekiri *et al*., 2023). The presence of these compounds was detected in maximal concentration in methanol and water during phytochemical screening (Saidulu *et al*., 2014). A lesser amount of these compounds might also explain the lower inhibitory effects of the *P. harmala* extract. Saponins and tannins were absent, but alkaloids and flavonoids were abundantly present in its aqueous extracts (Bekiri *et al*., 2023). Three alkaloids (berberine, harmaline and piperine) in *P. harmala* are known to cause inhibitory effects on egg hatching (da Silva *et al*., 2021). We report that *P. harmala* and *W. somnifera* extracts exhibit anthelmintic properties, which contradict the findings of Niaz *et al*. (2015). These authors suggested that the variation in plant effectiveness could be attributed to the differences in the species of host and the parasite.

Another anthelmintic study of three alkaloids (berberine, harmaline and piperine) against gastrointestinal nematodes in goats showed that harmaline had similar effects (80.30%) on egg hatching (da Silva *et al*., 2021). *P. harmala* was also found to be very effective against *Schistosoma bovis* infection. The orally administered capsule of its powder was used to treat infected bovines. The reductions in parasite eggs were similar to those of praziquantal (PZQ) (Niaz *et al*., 2015). In another study, aqueous extracts of six plants (*Peganum harmala*, *Artemisea herba-alba*, *Ricinus communis*, *Olea europea*, *Allium sativum* and *Thymus vulgar*) were tested for their ability to treat Cryptosporidium parasites. The extract of *P. harmala* induced maximum oocysts shedding from the intestine of infected mice (Al-Hamairy *et al*., 2012). Another confirmation of the anthelmintic efficacy of *W. somnifera* was reported by Miaron et al. (2005). The extract was applied to dorper sheep to control tapeworms and effectively reduced the egg count (88%) in the faecal material.

In case of larvicidal results, the effects of MEs of *W. somnifera* and *P. harmala* showed significantly ($P < 0.05$) greater larvicidal potential than their AEs and the negative controls. However, the activity of MEs was significantly ($P < 0.05$) lower than albendazole (positive control). The possible difference between the efficiencies of methanolic and aqueous extracts was due to the solubility of various

phytochemicals in the solvents. The possible cause of this low activity of aqueous extracts was the poor solubility of various phytochemicals in water (Dhawan and Gupta, 2016). Most of the metabolites are aromatic and fat soluble and dissolve more readily in methanol than in water (Forssten *et al*., 2010). Extracts containing a high number of these secondary metabolites had a collective effect on the integrity of the tested larvae. The dead, deformed larvae lose their morphological shape by separating their cuticle from the pharynx, bulb and intestinal cells (Chan-Pérez *et al*., 2016). Tannins are the most studied active compounds investigated for their effects on egg hatch inhibition and larval development in different nematodes and cestodes (Molan and Faraj, 2010).

In the present study, the methanolic extract performed better than the aqueous extracts, which was very close to the findings of Marie-Magdeleine *et al*. (2010), who revealed that the methanolic extract had the highest efficacy on the larval motility of *H. contortus*. The results of the present study agree with the findings of Osman *et al*. (2012), who investigated the anti-cercaria effects of *P. harmala* extracts (aqueous and methanol) against *Schistosoma mansoni*.

In adulticidal study, MEs from *W. somnifera* and *P. harmala* were significantly ($P < 0.05$) more effective than their aqueous counterpart after 8 h of extract exposure. However, at first 2 and 4 h of extract exposure, the difference in adulticidal activities of aqueous and methanolic extracts at all tested concentrations (3.125-50 mg/mL) were negligible similar to that of positive (albendazole) and negative controls groups. The deaths of 100% worms in albendazole and AEs (50 mg/mL) were noticed after 10 and 12 h of extract exposure. The extent of lethality in worms depends on the concentration, duration of the extract exposure at any specific concentration and the ambient temperature of the worms. A dose-dependent increase in concentration creates necrotic spots on worm cuticles and this haemorrhage eventually leads to worm paralysis and death (Kirtiman, 2012). The scarred worms that were lighter in weight and color were the extract-affected worms floating on the surface of the extract. The rest of the worms were separated as live worms retained their motility. Rasool *et al*. (2019) suggested that tannins might be the cause of anthelmintic activity against adult *H. contortus*. The active phytochemical can directly affect the worm size and fecundity of females (Martínez-Ortíz-de-Montellano *et al*., 2010). Several reports have suggested that these biologically active compounds interact with proline-rich surface proteins or internal tissues of helminths. However, due to the unequal distribution of proteins, these effects may vary among different species and developmental stages of nematode species (Spiegler *et al*., 2017).

However, in another study by Osman (2010), the mortality of worms was greater in the presence of aqueous extracts than in the presence of methanolic extracts. Furthermore, the average duration and mortality of the above-mentioned studies involving the extract type and concentration showed similar effects as those described in our study. In comparison with the findings of other nematocidal reports, the plants in the present study also exhibited significant effects on *H. contortus*. In *P. harmala*, different forms of harmine have been shown to have significant antileishmanial effects (Lala *et al*., 2004). Extracts of the aerial parts of *P. harmala* were found to remove *Theileria hirci* parasites from infected lambs (Derakhshanfar and Mirzaei, 2008). Several studies contradicting our findings, such as Astulla *et al*. (2008), examined four alkaloids (harmine, harmaline, vasicinone, and deoxyvasicinone) in the seed extract of *P. harmala* and found that harmine and harmaline had moderate inhibitory effects on *Plasmodium falciparum*. Sun *et al*. (2021) assessed the dose-dependent toxic effects of harmine on *Caenorhabditis elegans*. It was found that harmine interferes with acetylcholinesterase activity and induces neurotoxic effects on the worm nervous system. The worms quickly lose their structural growth, egg laying ability and life.

Lipi *et al*., (2010) investigated the effects of aqueous extracts of the leaves and roots of *W. somnifera* on adult earthworms (*Pheritima posthuma*). Leaf extract was more effective at inducing worm paralysis than root extract. Its ethanolic extract also exhibited similar vermicidal activity against *Pheritima postanauma* (Meeran *et al*., 2019). The traditional nervine and antistress medicine *Aśvagandhādyariṣṭa* is a fermented liquid of *W. somnifera.* The formulation also proved to have anthelmintic potential when tested against *Eisenia foetida,* a human resembling roundworm (Singh *et al*., 2014). These significant findings validate its use in treating worm infections.

A comparison of these studies confirmed that both *P. harmala* and *W. somnifera* can reduce the number of eggs, larvae and worms of *H. contortus*. The variation in results of these *in vitro* studies compared with earlier reports is due to the controlled environmental factors, i.e., temperature. Other factors, such as the type of plant secondary metabolites, polarity of the solvent, nature of extraction, extract exposure time and extent of resistance in worms, are also considered important for evaluating the medicinal significance of plant extracts.

CONCLUSION

Aqueous and methanolic extracts of *P. harmala* and *W. somnifera* exhibited significant ($p<0.05$) anthelmintic

activity against three life stages of *H. contortus*. The *W. somnifera* plant methanolic extract had the overall highest anthelmintic efficacy. On the basis of the LC_{50}, the extracts of *W. somnifera* were found to be more potent than the other extracts. Eggs, larvae and adult worms exposed to a set range of temperatures (25-30°C) could constitute a novel approach for safe and reliable dose formulation during *in vivo* trials. The *in vitro* results suggested that *P. harmala* and *W. somnifera* plant extracts could be used as substitutes for the treatment of extremely prevalent *H. contortus* nematodes.

DECLARATIONS

Acknowledgments

The authors are thankful to the local inhabitants of kala chitta range for searching, medicinal plants material. The authors are also appreciating the support of abattoir's workers for providing abomasa of slaughtered animals and the technical assistance of the personals of respective department to carry out this research work.

Funding

The manuscript is prepared with no external support/ funding.

IRB approval

The Institutional Ethical Review Committee of International Islamic University, Islamabad, Pakistan granted approval of the study (No. IIUI/ORIC/ Bioethics/110, October 22, 2024).

Data availability

All the data used in paper is presented in tables and figures and no extra data is available for e-mail request.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20240416105757

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Alamgeer, Sharif, A., Asif, H., Younis, W., Riaz, H., Bukhari, I.A. and Assiri, A.M., 2018. Indigenous medicinal plants of Pakistan used to treat skin diseases: A review. *Chin. Med.*, **13**: 1-26. https://doi.org/10.1186/s13020-018-0210-0

Ale, Y., Sharma, S., Chaudhary, A. and Singh, A., 2021. A review on therapeutic use of *Withania somnifera* (Ashwagandha). *Annls Rom. Soc. Cell. Biol.*, **25**: 577-585.

Al-Hamairy, A.K., Mugheer, A.H. and Al-Dulaimi, F.H., 2012. Prevalence of *Cryptosporidium* sp. and treatment by using some plant extracts in Al-Hilla City, Babylon Province of Iraq. *Egypt J. exp. Biol. (Zoo)*, **8**: 287-293.

Arsenopoulos, K.V., Fthenakis, G.C., Katsarou, E.I. and Papadopoulos, E., 2021. Haemonchosis: A challenging parasitic infection of sheep and goats. *Animals*, **11**: 363. https://doi.org/10.3390/ani11020363

Astulla, A., Zaima, K., Matsuno, Y., Hirasawa, Y., Ekasari, W., Widyawaruyanti, A., Cholies-Zaini, N. and Morita, H., 2008. Alkaloids from the seeds of *Peganum harmala* showing antiplasmodial and vasorelaxant activities. *J. nat. Med.*, **62**: 470-472. https://doi.org/10.1007/s11418-008-0259-7

Bekiri, S., Idder-Ighili, H., Guezoul, O., Idder, M.A. and Azouaou, K., 2023. Ovicidal activity and phytochemical analysis of some spontaneous plants extracts against *Ectomyelois ceratoniae* (Zeller) Lepidoptera: *Pyralidae. Al-Qadisiyah J. agric. Sci.*, **13**: 113-122. https://doi.org/10.33794/qjas.2022.135860.1083

Besier, R.B., Kahn, L.P., Sargison, N.D. and Van Wyk, J.A., 2016a. The pathophysiology, ecology and epidemiology of *Haemonchus contortus* infection in small ruminants. *Adv. Parasitol.*, **93**: 95-143. https://doi.org/10.1016/bs.apar.2016.02.022

Besier, R.B., Kahn, L.P., Sargison, N.D. and Van Wyk, J.A., 2016b. Diagnosis, treatment and management of *Haemonchus contortus* in small ruminants. *Adv. Parasitol.*, **93**: 181-238. https://doi.org/10.1016/bs.apar.2016.02.024

Bibi, R., Afshan, K., Khan, I.A., Iqbal, Z., Kayani, A.R., Mushtaq, M., Irfan, M., Qayyum, M. and Hassan, M.F., 2017. Phenotyping and prevalence of *Haemonchus contortus* (Nematoda: Trichostongylidae) in ruminants from endemic areas of Pakistan: Influence of host species and geographical area on phenotypic traits of worms. *Pak. Vet. J.*, **37**: 170-174.

Chan-Pérez, J.I., Torres-Acosta, J.F.D.J., Sandoval-Castro, C.A., Hoste, H., Castañeda-Ramírez, G.S., Vilarem, G. and Mathieu, C., 2016. *In vitro* susceptibility of ten *Haemonchus contortus*

isolates from different geographical origins towards acetone: Water extracts of two tannin rich plants. *Vet. Parasitol.*, **217**: 53-60. https://doi.org/10.1016/j.vetpar.2015.11.001

Charlier, J., Bartley, D.J., Sotiraki, S., Martinez-Valladares, M., Claerebout, E., von Samson-Himmelstjerna, G., Thamsborg, S.M., Hoste, H., Morgan, E.R. and Rinaldi, L., 2022. Anthelmintic resistance in ruminants: Challenges and solutions. *Adv. Parasitol.*, **115**: 171-227. https://doi.org/10.1016/bs.apar.2021.12.002

Charlier, J., Rinaldi, L., Musella, V., Ploeger, H.W., Chartier, C., Vineer, H.R., Hinneyg, G., von Samson-Himmelstjernah, B., Băcescui, M., Mickiewiczj, T.L., Mateusk, Lm., Martinez-Valladaresm, S., Quealyn, H., Azaizeho, B., Sekovskap, H., Akkariq, S., Petkeviciusr, L., Hektoens, J., Höglundt, E.R., Morganu, D.J., Bartleyv, E. and Claerebout, E., 2020. Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. *Prev. Vet. Med.*, **182**: 105103.

Coles, G.C., 1984. Recent advances in schistosome biochemistry. *Parasitology*, **89**: 603-637. https://doi.org/10.1017/S0031182000056808

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A. and Waller, P.J., 1992. World association for the advancement of veterinary parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.*, **44**: 35-44. https://doi.org/10.1016/0304-4017(92)90141-U

da Silva, G.D., de Lima, H.G., de Sousa, N.B., de Jesus Genipapeiro, I.L., Uzêda, R.S., Branco, A., Costa, S.L., Batatinha, M.J.M. and Botura, M.B., 2021. *In vitro* anthelmintic evaluation of three alkaloids against gastrointestinal nematodes of goats. *Vet. Parasitol.*, **296**: 109505. https://doi.org/10.1016/j.vetpar.2021.109505

Danial, K., Mehdi, R., Josep, C., Carine, E. and Yann, G., 2018. Antibacterial effect of two Persian traditional natural compounds-Anbar Nesara and Esfand-'S Fume on treatment of the bacterial vaginitis. *Am. J. Phytomed. Clin. Ther.*, **6**: 1-5.

Deore, S.L. and Khadabadi, S.S., 2010. *In vitro* anthelmintic studies of *Chlorophytum borivilianum* Sant. and Fernandez tubers. *Ind. J. nat. Prod. Resour.*, **1**: 53-56.

Derakhshanfar, A. and Mirzaei, M., 2008. Effect of *Peganum harmala* (wild rue) extract on experimental ovine malignant theileriosis: Pathological and parasitological findings. *Onderstepoort J. Vet. Res.*, **75**: 67-72. https://doi.org/10.4102/ojvr.v75i1.90

Dhawan, D. and Gupta, J., 2016. Comparison of different solvents for phytochemical extraction potential from *Datura metel* plant leaves. *Int. J. biol. Chem.*, **11**: 17-22. https://doi.org/10.3923/ijbc.2017.17.22

Dutta, B., Konch, P., Rahman, T., Upadhyaya, T.N., Pathak, D.C., Tamuli, S.M., Phangchoo, C.V. and Begum, S.A., 2017. Occurrence and pathology of *Haemonchus contortus* infection in goats. *J. Ent. Zool. Stud.*, 5: 1284-1287.

Food and Agriculture Organization, 2022. *FAOSTAT dataset: Crops and livestock products.*

Forssten, S.D., Björklund, M. and Ouwehand, A.C., 2010. *Streptococcus mutans*, caries and simulation models. *Nutrients*, **2**: 290-298. https://doi.org/10.3390/nu2030290

Gilani, A.H., Ghayur, M.N., Saify, Z.S., Ahmed, S.P., Choudhary, M.I. and Khalid, A., 2004. Presence of cholinomimetic and acetylcholinesterase inhibitory constituents in betel nut. *Life Sci.*, **75**: 2377-2389. https://doi.org/10.1016/j.lfs.2004.03.035

Hoberg, E.P. and Zarlenga, D.S., 2016. Evolution and biogeography of *Haemonchus contortus*: Linking faunal dynamics in space and time. *Adv. Parasitol.*, **93**: 1-30. https://doi.org/10.1016/bs.apar.2016.02.021

Hoberg, E.P., Lichtenfels, J.R. and Gibbons, L., 2004. Phylogeny for species of Haemonchus (Nematoda: Trichostrongyloidea): Considerations of their evolutionary history and global biogeography among Camelidae and Pecora (Artiodactyla). *J. Parasitol.*, **90**: 1085-1102. https://doi.org/10.1645/GE-3309

Iqbal, Z., Lateef, M., Jabbar, A., Ghayur, M.N. and Gilani, A.H., 2006. *In vitro* and *in vivo* anthelmintic activity of *Nicotiana tabacum* L. leaves against gastrointestinal nematodes of sheep. *Phytother. Res. Int. J. Devot. Pharm. Toxicol. Eval. Nat. Prod. Derivat.*, **20**: 46-48. https://doi.org/10.1002/ptr.1800

Kirtiman, S., 2012. Comparative study of *Withania somnifera* and *Ocimum sanctum* for anthelmintic activity. *ISCA J. biol. Sci.*, **1**: 74-76.

Lala, S., Pramanick, S., Mukhopadhyay, S., Bandyopadhyay, S. and Basu, M.K., 2004. Harmine: Evaluation of its antileishmanial properties in various vesicular delivery systems. *J. Drug Target*, **12**: 165-175. https://doi.org/10.1080/10611860410001712696

Lanusse, C., Canton, C., Virkel, G., Alvarez, L., Costa-

Junior, L. and Lifschitz, A., 2018. Strategies to optimize the efficacy of anthelmintic drugs in ruminants. *Trends Parasitol.*, **34**: 664-682. https://doi.org/10.1016/j.pt.2018.05.005

Lipi, P., Vivek, S., Makode, K.K. and Jain, U.K., 2010. Anthelmintic activity of aqueous extracts of some saponin containing medicinal plants. *Der. Pharma. Lett.*, **2**: 476-481.

Manjusa, A. and Pradeep, K., 2022. Herbal anthelmintic agents: A narrative review. *J. Tradit. Chin. Med.*, **42**: 641-651.

Marie-Magdeleine, C., Hoste, H., Mahieu, M., Varo, H. and Archimède, H., 2009. *In vitro* effects of *Cucurbita moschata* seed extracts on *Haemonchus contortus*. *Vet. Parasitol.*, **161**: 99-105. https://doi.org/10.1016/j.vetpar.2008.12.008

Marie-Magdeleine, C., Mahieu, M., D'Alexis, S., Philibert, L. and Archimède, H., 2010. *In vitro* effects of *Tabernaemontana citrifolia* extracts on *Haemonchus contortus*. *Res. Vet. Sci.*, **89**: 88-92. https://doi.org/10.1016/j.rvsc.2010.01.002

Martínez-Ortíz-de-Montellano, C., Vargas-Magaña, J.J., Canul-Ku, H.L., Miranda-Soberanis, R., Capetillo-Leal, C., Sandoval-Castro, C.A., Hoste, H. and Torres-Acosta, J.F.J., 2010. Effect of a tropical tannin-rich plant *Lysiloma latisiliquum* on adult populations of *Haemonchus contortus* in sheep. *Vet. Parasitol.*, **172**: 283-290. https://doi.org/10.1016/j.vetpar.2010.04.040

Mayad, E.H., Basaid, K., Furze, J.N., Heimeur, N., Senhaji, B., Chebli, B., El Hadek, M., Mateille, T., Hassani, Lai. and Ferji, Z., 2019. Reversible nematostatic effect of *Peganum harmala* L. (Nitrariaceae) on *Meloidogyne javanica*. *J. Agric. Search*, **6**: 29-33. https://doi.org/10.21921/jas.v6i1.14917

Meena, L.K., Meena, A.K., Gupta, J., Patel, M., Khan, S.Y., Kumar, S. and Gupta, A.K., 2020. Ashwagandha (*Withania somnifera* L). In: *Medicinal plants in India: Importance and cultivation*. Jaya Publishing House, Delhi, India.

Meeran, N.M., Saratha, R., Shafeeq, M.K.A.S. and Kumara, K.S.S., 2019. Evaluation of anti-helminthic activity of ethanolic extract of *Withania coagulans* dunal (flower buds). *Res. J. Pharma. Sci.,* **8**: 14-17.

Miaron, J.O., Wahome, P.G. and Mapenay, I.M., 2005. Anthelminthic activity of *Withania somnifera* L. Dunal water extract in sheep. *East East. Cent. Afr. J. Pharm. Sci.,* **8**: 6-9.

Miraj, S., 2016. A review study of therapeutic effects of *Peganum harmala. Der Pharm. Lett.*, **8**: 161-166.

Molan, A.L. and Faraj, A.M., 2010. The effects of condensed tannins extracted from different plant species on egg hatching and larval development of *Teladorsagia circumcincta* (Nematoda: Trichostrongylidae). *Folia Parasitol.* (Prague), **57**: 62-68. https://doi.org/10.14411/fp.2010.008

Moloudizargari, M., Mikaili, P., Aghajanshakeri, S., Asghari, M. and Shayegh, J., 2013. Pharmacological and therapeutic effects of *Peganum harmala* and its main alkaloids. *Pharmacogn. Rev.*, **7**: 199-212. https://doi.org/10.4103/0973-7847.120524

Niaz, S., Akhter, T., AbdEl-Salam, N.M., Ayaz, S., Shams, S., Ullah, R., Hussain, H. and Bibi, S., 2015. Therapeutic potential of *Peganum harmala* against *Schistosoma bovis* in buffaloes. *Afr. J. Tradit., Complement. Altern. Med.*, **12**: 84-91. https://doi.org/10.21010/ajtcam.v12i6.7S

Osman, E.A., Koko, W., Mohammed, Z. and Galal, M., 2012. Anticercarial activity of the plant *Peganum harmala* (Harmal) crude extracts. *Univ. Afr. J. Sci.,* **2**: 126-138.

Osman, I.A.M., 2010. *Anthelmintic activity of extracts from three putative medicinal plants against caprine haemonchosis.* Doctoral dissertation, PhD Thesis University of Khartoum, Sudan.

Pakistan Economic Survey, 2021-22. Economic Adviser's Wing Finance Division Government of Pakistan, Islamabad.

Paul, S., Chakraborty, S., Anand, U., Dey, S., Nandy, S., Ghorai, M., Saha, S.C., Patil, M.T., Kandimalla, R., Proćków, J. and Dey, A., 2021. *Withania somnifera* (L) Dunal (Ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedicinal and toxicological aspects. *Biomed. Pharmacother.*, **143**: 112175. https://doi.org/10.1016/j.biopha.2021.112175

Qamar, M.F., Maqbool, A. and Ahmad, N., 2011. Economic losses due to haemonchosis in sheep and goats. *Sci. Int.*, **23**: 321-324.

Qamar, M.T., Ashfaq, U.A., Tusleem, K., Mumtaz, A., Tariq, Q., Goheer, A. and Ahmed, B., 2017. *In-silico* identification and evaluation of plant flavonoids as dengue NS2B/NS3 protease inhibitors using molecular docking and simulation approach. *Pak. J. Pharm. Sci.*, **30**: 2119-2137.

Rasool, M., Malik, A., Saleem, S., Ansari, S.A., Iqbal, J., Asif, M., Kamal, M.A., Al-Qahtani, M.H. and Karim, S., 2019. Assessment of circulating biochemical markers in mice receiving cinnamon and glycyrrhizin under carbon tetrachloride induced hepatic injury. *Proc. natl. Acad. Sci. India Biol. Sci.,* **89**: 105-111. https://doi.org/10.1007/

s40011-017-0920-6

Robinson, M.M. and Zhang, X., 2011. *The world medicines situation*. *Traditional medicines: Global situation, issues and challenges*. WHO Geneva, 3rd edition.

Saddiqe, Z., Khalid, S. and Maimoona, A., 2019. *In vitro* anthelmintic activity of extracts of *Withania somnifera*. *J. Nat. appl. Sci. Pak.*, **1**: 89-97.

Saidulu, C., Venkateshwar, C. and Gangadhar, R.S., 2014. Preliminary phytochemical studies of medicinal plant drug: *Withania somnifera* Linn. *Biolife*, **2**: 306-312.

Shinwari, Z.K., 2010. Medicinal plants research in Pakistan. *J. Med. Pl. Res.*, **4**: 161-76.

Singh, M., Kaur, N. and Paul, A.T., 2014. Physicochemical standardization, HPTLC profiling, and biological evaluation of Aśvagandhādyariṣṭa: A comparative study of three famous commercial brands. *Anc. Sci. Life*, **33**: 165-171. https://doi.org/10.4103/0257-7941.144621

Soulsby, E.J.L., 1968. *Helminths, arthropods and protozoa of domesticated animals*. Helminths, arthropods and protozoa of domesticated animals.

Spiegler, V., Liebau, E. and Hensel, A., 2017. Medicinal plant extracts and plant-derived polyphenols with anthelmintic activity against intestinal nematodes. *Nat. Prod. Rep.*, **34**: 627-643. https://doi.org/10.1039/C6NP00126B

Sun, Q., Liu, C., Jiang, K., Fang, Y., Kong, C., Fu, J. and Liu, Y., 2021. A preliminary study on the neurotoxic mechanism of harmine in *Caenorhabditis elegans*. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.*, **245**: 109038. https://doi.org/10.1016/j.cbpc.2021.109038

Tariq, K.A., Chishti, M.Z., Ahmad, F. and Shawl, A.S., 2008. Epidemiology of gastrointestinal nematodes of sheep managed under traditional husbandry system in Kashmir valley. *Vet. Parasitol.*, **158**: 138-143. https://doi.org/10.1016/j.vetpar.2008.06.013

Zajac, A.M., 2006. Gastrointestinal nematodes of small ruminants: life cycle, anthelmintics, and diagnosis. *Vet. Clin. Fd. Anim. Pract.*, **22**: 529-541. https://doi.org/10.1016/j.cvfa.2006.07.006

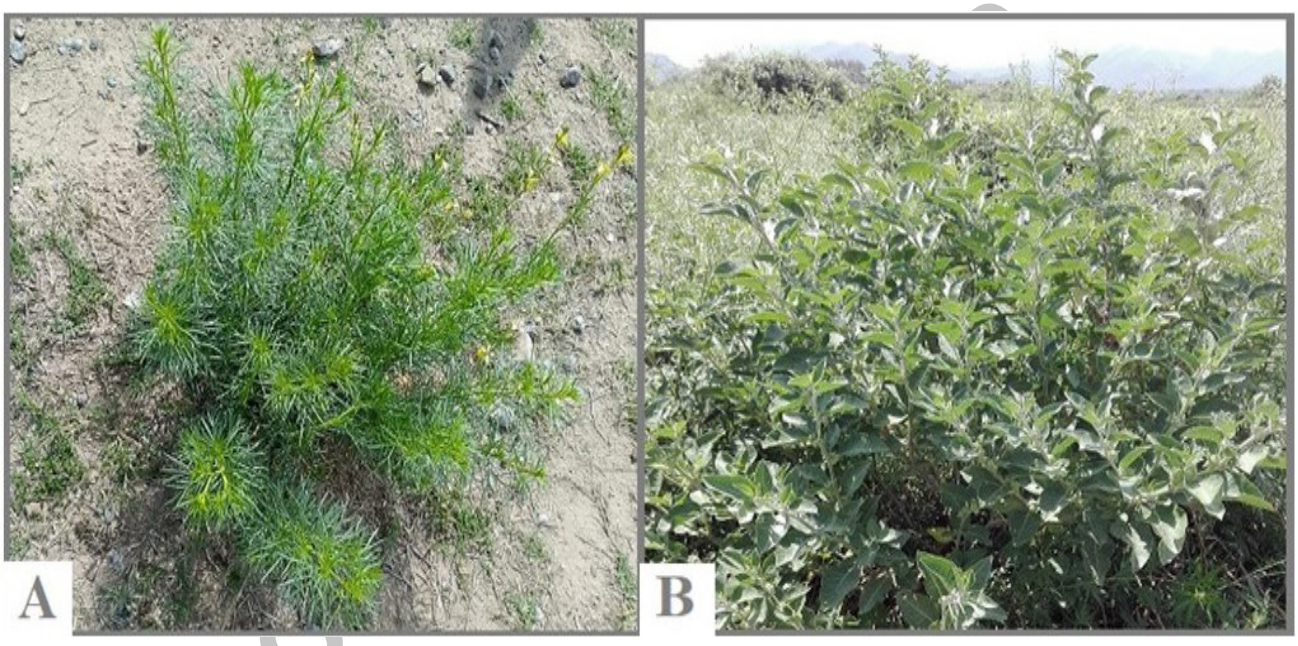
Pakistan J. Zool., pp 1-11, 2025. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20240416105757

Supplementary Material

In Vitro Anthelmintic Efficacy of Two Wild Herbs *Peganum harmala* and *Withania somnifera* Against *Haemonchus contortus*

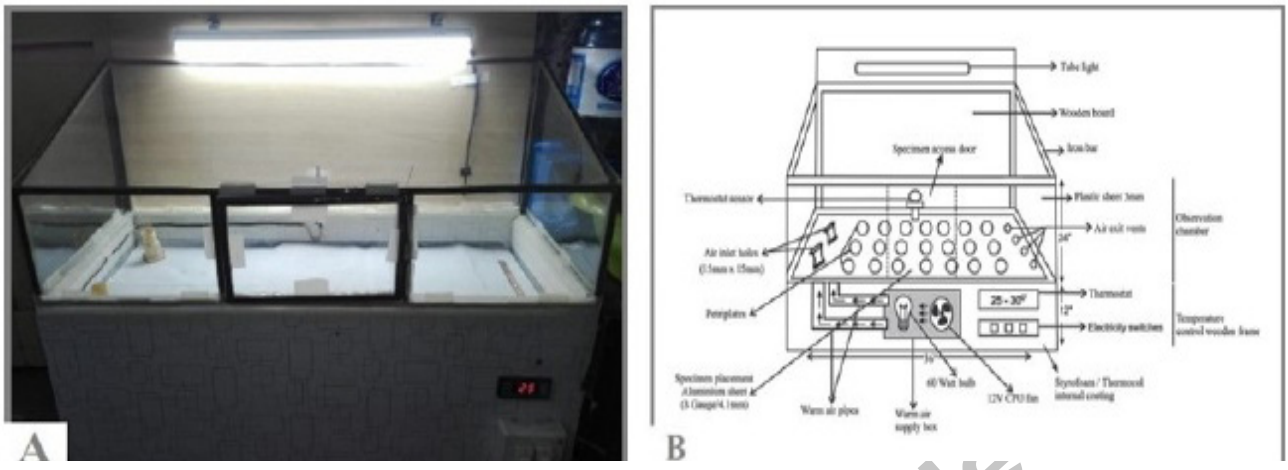
Muhammad Qarib Hussain[1], Jabar Zaman Khan Khattak[1] and Nousheen Zafeer[2]*

[1]*Department of Biological Sciences, International Islamic University, Islamabad, Pakistan*
[2]*Department of Zoology, University of Gujrat, Gujrat, Pakistan*



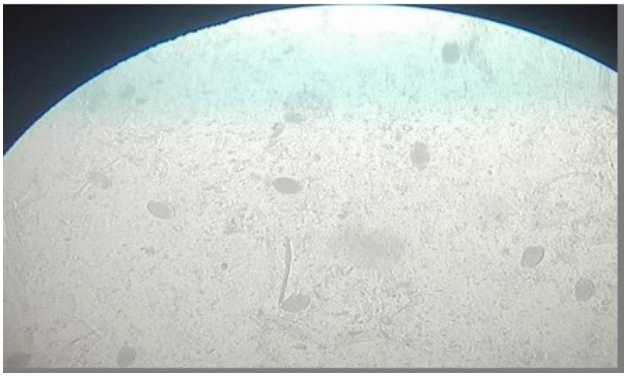
Supplementary Fig. 1. A, *P. harmala*; B, *W. somnifera* in the natural habitat of Kala Chitta Range, Punjab, Pakistan.

* Corresponding author: nousheen.zafeer@uog.edu.pk
0030-9923/2025/0001-0001 $ 9.00/0

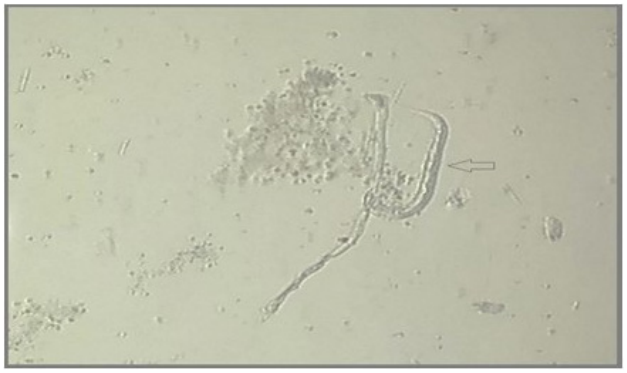


Supplementary Fig. 2. A, the abomasal working apparatus is a self-structured experimental table that was used to maintain a set range of temperature (25-30 ºC) for worms. B, Principle/ layout of abomasal working apparatus showing the observation table used for placing specimen and the electrical circuits used to generate temperature (25-30 ºC) for proper functioning of the apparatus.

Supplementary Fig. 3. Isolation of *Haemonchus contortus*: A, Abomasal incision showing heavy infection of *Haemonchus contortus*; B, highly agile, adult worms of *Haemonchus contortus.*



Supplementary Fig. 4. Microscopic observation of *H. contortus* eggs (10X objective).



Supplementary Fig. 5. Larval (L1) response to plant extract treatment of the extracts (100X magnification).