

*Tree leaves of Salix babylonica extract as a natural anthelmintic for small-ruminant farms in a semiarid region in Mexico*

**Abdelfattah Z. M. Salem, Mona M. Y. Elghandour, Ahmed E. Kholif, Secundino López, Alberto B. Pliego, Moisés Cipriano-Salazar, et al.**

**Agroforestry Systems**

An International Journal incorporating  
Agroforestry Forum

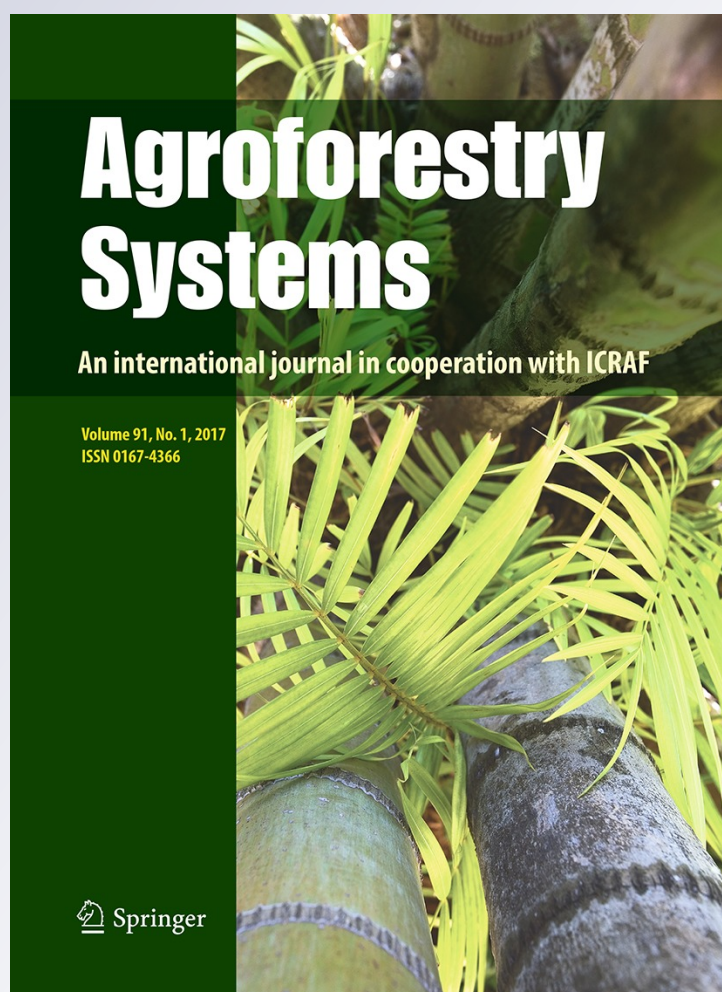
ISSN 0167-4366

Volume 91

Number 1

Agroforest Syst (2017) 91:111-122

DOI 10.1007/s10457-016-9909-z



**Your article is protected by copyright and all rights are held exclusively by Springer Science +Business Media Dordrecht. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at [link.springer.com](http://link.springer.com)".**

# Tree leaves of *Salix babylonica* extract as a natural anthelmintic for small-ruminant farms in a semiarid region in Mexico

Abdelfattah Z. M. Salem · Mona M. Y. Elghandour · Ahmed E. Kholif · Secundino López · Alberto B. Pliego · Moisés Cipriano-Salazar · Juan Carlos V. Chagoyán · Roberto Montes de Oca Jiménez · María U. Alonso

Received: 15 February 2015 / Accepted: 16 February 2016 / Published online: 26 February 2016  
© Springer Science+Business Media Dordrecht 2016

**Abstract** The study aimed to test the potential anthelmintic activity of *Salix babylonica* (SB) extract for the control of gastrointestinal and pulmonary parasites in sheep and goats under field conditions. A representative sample of 20 % of all animals reared in 8 sheep and 7 goat farms was used in the study. Animals from each farm were randomly selected for a total number of 93 sheep and 75 goats. Animals suffered a natural gastrointestinal nematode infection and had never been treated with chemical anthelmintic

drugs. The SB extract (20 mL) was orally administered weekly before the morning feeding to each animal for 60 days. Fecal eggs or oocysts were counted at 0, 1, 20, 40, and 60 days after starting the extract administration. Differences ( $P < 0.01$ ) in the fecal oocyst and egg output of *Eimeria*, *Dictyocaulus*, and *Moniezia* were observed between sheep and goats. In addition, the treatment influenced ( $P < 0.05$ ) egg outputs of *Cooperia*, *Dictyocaulus*, and *Trichuris*. Fecal egg or oocyst counts of *Haemonchus contortus*, *Eimeria*, *Cooperia*, *Chabertia*, *Dictyocaulus*, *Moniezia*, and *Ostertagia* were time-dependent ( $P < 0.05$ ). For sheep, administration of SB decreased ( $P < 0.05$ ) the fecal eggs count of *H. contortus*, *Cooperia*, *Chabertia*, *Dictyocaulus*, *Moniezia*, and *Trichuris*. After 20 days of treatment, *H. contortus*, *Cooperia*, or *Moniezia* were not detected. For goats, SB reduced ( $P < 0.05$ ) the fecal egg counts of *H. contortus*, *Cooperia*, *Chabertia*, and *Moniezia*. Moreover, decreases were observed ( $P < 0.05$ ) for *Chabertia*, *Trichostrongylus*, and *Ostertagia*. Eggs of *H. contortus* and *Moniezia* were not present in the feces after 1 day of administration of the extract. It could be concluded that the weekly administration of SB extract at 20 mL per animal can be used to treat gastrointestinal and lung nematodes of small ruminants in organic and traditional farming systems of tropical regions.

A. Z. M. Salem (✉) · M. M. Y. Elghandour · A. B. Pliego · J. C. V. Chagoyán · R. M. de Oca Jiménez · M. U. Alonso  
Facultad de Medicina Veterinaria y Zootecnia,  
Universidad Autónoma del Estado de México, Tlalpan,  
Mexico  
e-mail: asalem70@yahoo.com

A. E. Kholif  
Dairy Science Department, National Research Centre, 33  
Bohouth St. Dokki, Giza, Egypt

S. López  
Departamento de Producción Animal, Instituto de  
Ganadería de Montaña (IGM) CSIC-Universidad de León,  
Universidad de León, 24071 León, Spain

M. Cipriano-Salazar  
Unidad Académica de Medicina Veterinaria y Zootecnia,  
Universidad Autónoma de Guerrero, Altamirano 40660,  
Mexico

**Keywords** Anthelmintic · Nematodes · Organic farming · *Salix babylonica* extract · Small ruminants

## Introduction

In Mexico, sheep and goats are important resources of cash income, savings, food (meat and milk), wool, fertilizer, and employment of family members, particularly in populations with a low income and where poverty prevails. However, parasitic diseases caused by gastrointestinal nematodes remains as one of the major constraints in sheep and goat production systems in Mexico and in most developing countries (Mejía-Hernández et al. 2014; Cedillo et al. 2015). Nematodes in such humid and sub-humid tropical areas can cause poor growth, production losses, and even mortality in young animals (Ancheta et al. 2004). Globally, small ruminant producers depend on synthetic anthelmintics to achieve control of gastrointestinal nematodes and parasites. However, administration of chemical anthelmintic drugs has been proved to increase the development of parasite resistance towards these treatments (Jabbar et al. 2006; Shaik et al. 2006), and to raise concerns regarding the presence of pharmacological residues in animal products (McKellar 1997). Moreover, chemical treatments are costly when used regularly to prevent helminth parasites in livestock (Jabbar et al. 2006). Consequently, there is an increasing interest in screening phytogetic extracts and medicinal plants as alternatives to the expensive traditional drugs (Olmedo-Juárez et al. 2014; Cedillo et al. 2015; Cervantes-Valencia et al. 2015). It has been reported that medicinal plants not only possess anthelmintic activity, but also have antibacterial and insecticidal properties (Mejía-Hernández et al. 2014). The use of naturally produced plants with anthelmintic properties can also reduce the cost of importing drugs and therefore boost the economic self-reliance in developing countries (Mejía-Hernández et al. 2014; Cedillo et al. 2015; Cervantes-Valencia et al. 2015).

The anthelmintic mode of action of plant metabolites (i.e., tannins, alkaloids, saponins, lactones, lectins) in crude extracts is not completely clear, although it may be a combination of immunomodulatory action and direct antiparasitic effects (Hrckova and Velebny 2013). Some proposed mechanisms include larval motility inhibition, paralysis or death of the worms in the gastrointestinal tract, interference with hatching of eggs, or effects on female worm fecundity (Hrckova and Velebny 2013). Moreover, Wynn and Fougere (2007) stated that the mechanisms

of action of plant metabolites may be additive, synergistic, or antagonistic by acting at single or multiple target sites. Therefore, this work aimed to evaluate the efficacy of a *S. babylonica* (SB) aqueous extract against gastrointestinal nematodes in sheep and goats, and compare the anthelmintic activity of the extract between sheep and goats at different administration times under commercial production conditions in the tropical regions in Mexico.

## Materials and methods

### Animals, treatments, and feeding

The current study was conducted in 8 sheep and 7 goat commercial farms in the State of Mexico, Mexico. The sampling size included 20 % of the animals in each farm. The age of sheep ranged from 10 to 15 months, whereas in goats age ranged from 5 to 7 months. The initial body weight ranged between 25.5–49.6 kg for sheep and 22.2–30.5 kg for goats (Table 1). Animals were randomly selected within each farm. Animals were affected by a natural gastrointestinal nematode infection and had never been treated with chemical anthelmintic drugs. The feeding system consisted of grazing during different hours as well as the supplementation with a concentrate feed in some cases, according to each farm's management system. In all the farms, animals had *ad libitum* access to drinking water. Animals caring and handling were carried out in accordance with the official Mexican standard number NOM-033-ZOO-1995.

A dose of the SB extract (20 mL) was orally administered weekly at 07:00–09:00 h before morning feeding (based on each farm's management system) to each animal during 60 days of the experimental period.

### Parasitology test

The oocyst and egg count technique was performed using the methodology described in Mejía-Hernández et al. (2014). Briefly, fecal samples of each animal were collected individually directly from the rectum before morning feeding on day 0 (pre-extract administration), and thereafter on days 1, 20, 40, and 60 after the first administration of the extract (on day 0). Fecal samples were evaluated for the presence of worm eggs or *Eimeria* oocysts by a salt flotation technique

**Table 1** Animals management systems

Species	Farm number	Sex	Animals (n) <sup>a</sup>	Initial live weight	Age (months)	Feeding strategy
Sheep	1	Females	8	39.9	12	Grazing for 10 h
		Males	2			
	2	Females	14	40.3	12	Grazing for 5 h with corn stalks
		Males	1			
	3	Females	11	41.7	10	Grazing for 5 h
		Males	1			
	4	Females	6	49.6	12	Grazing for 5 h
		Males	5			
	5	Females	9	40.1	12	Grazing for 9 h
		Males	1			
	6	Females	12	25.5	10	Grazing for 3 h with 200 g of ground corn head <sup>-1</sup> d <sup>-1</sup>
		Males	2			
	7	Females	9	40.6	15	Grazing for 10 h
		Males	2			
8	Females	6	40.3	13	Grazing for 10 h	
	Males	4				
Goats	1	Females	9	30.5	7	Grazing for 10 h
		Males	1			
	2	Females	12	22.6	6	Grazing for 10 h
		Males	2			
	3	Females	11	31.0	6	Grazing for 10 h
		Males	1			
	4	Females	7	22.2	5	Grazing for 10 h
		Males	1			
	5	Females	7	25.2	6	Grazing for 10 h
		Males	3			
	6	Females	12	22.1	6	Grazing for 10 h
		Males	1			
	7	Females	6	25.9	7	Grazing for 10 h
		Males	2			

<sup>a</sup> 20 % of the total number of each farm

(MAFF 1979), and afterwards the parasite load was quantified using the McMaster method (Ojeda-Robertos et al. 2008). Fecal pellets were collected, weighed, and 1 g of feces was mixed with 60 mL of a saturated salt (NaCl) solution. The pellets were broken up using a mechanical stirrer, and then strained into a 250- $\mu$ m sieve. The strained solution (10 mL) was used for the determination of fecal egg counts using a 2-McMaster chamber with a limit of detection of 200 eggs g<sup>-1</sup> feces. Fecal cultures were prepared in each sampling time as two replicates of pooled samples from each

animal to allow the development of third-stage larvae from strongylidae eggs in order to identify the genus of the parasite after incubation for 12 days at 27 °C in a chamber with constant humidity and oxygenation. Larvae were then collected from a Baermann equipment, and generic identification of strongylidae nematodes was carried out using identification taxonomic keys (Van Wyk and Mayhew 2013). Mean egg or oocyst counts from each animal within each experimental period were used for statistical comparisons.



### Analysis of secondary metabolites assay

The SB extract was prepared weekly (4 L) as described previously in Salem et al. (2014). Briefly, fresh leaves were collected randomly during summer season from several young and mature trees of *S. babylonica*, chopped into 2–3 cm lengths and immediately extracted soaking leaf material with water (1 g leaf 8 mL<sup>-1</sup> of water). Plant material was soaked and incubated at room temperature of 25–30 °C for 72 h in closed jars of 5 L. After incubation, jars were heated at 39 °C for 1 h, and then immediately filtered. The filtrates collected were stored at 4 °C for further use.

Secondary metabolites in SB extract were determined in triplicate according to the method described in Salem et al. (2014). Briefly, 10 mL of extract was fractionated by funnel separation with a double volume of ethyl acetate to determine total phenolics by drying and quantifying the phenolics layer in the funnel. After phenolics separation, 20 mL of *n*-butanol was added to fractionate the saponins. The remaining solution in the funnel was considered to be the aqueous fraction that has the other secondary compounds such lectins, polypeptides, and starch (Cowan 1999). The SB extract contained (per kg) 12.80 g of total phenolics, 4.80 g of saponins, and 72.53 g of the aqueous fraction.

### Statistical analysis

The experimental design was completely randomized with repeated measures in time, where the animals within each farm were the experimental units. Data of Table 2 were analyzed using the MIXED procedure of Statistical Analysis System (2002) for repeated measures (Littell et al. 1998). The structure of the variance–covariance error matrix employed was unstructured, based on Bayesian criteria observed with several alternative structures. The repeated term was day of sampling. Results reported in tables (Tables 3, 4) and text correspond to the least square means of fixed effects. Tests of simple effects were used to partition (slice) interaction effects by diet in order to test effects of period separately for each diet (Statistical Analysis System 2002).

The statistical model used for the results of Table 2 was

$$y_{ijk} = \mu + S_i + a(S)_{j(i)} + d_k + (Sd)_{ik} + \varepsilon_{ijk},$$

where  $y_{ijk}$  is the value measured at day of sampling  $k$  on the  $j$ th animal of the  $i$ th ruminant species (sheep or goats),  $\mu$  is the overall mean effect,  $S_i$  is the  $i$ th fixed effect of animal species (sheep or goats),  $a(S)_{j(i)}$  is the random effect of the  $j$ th animal within the  $i$ th ruminant species,  $d_k$  is the fixed  $k$ th day of sampling (time) effect when the measurement was taken,  $(Sd)_{ik}$  is the interaction effect between animal species ( $S$ ) and day of sampling ( $d$ ),  $\varepsilon_{ijk}$  is the random error associated with the  $j$ th animal assigned to the  $i$ th farm at sampling time  $k$ . Tukey's test was used for the multiple comparisons among mean values, and linear and quadratic effects of day of sampling were assessed using polynomial contrasts.

Results of proportion of infested animals (eggs detected in feces) were analyzed based on the fecal egg counting. Individuals were classified as negative (no eggs or oocysts detected) or positive (eggs or oocysts found in fecal samples). The proportion of positive animals in the control and treated (receiving the extract) groups were calculated, and the statistical association between treatment with the extract and the presence of eggs of each parasite species in feces was determined by means of Chi square test and contingency tables (using the PROC LOGISTIC of SAS). A statistically significant association was considered with  $P < 0.05$ .

## Results

### Data description

In the current experiment, 75 goats (64 females and 11 males) and 93 sheep (75 females and 18 males) were used. The average initial body weight was 25.6 kg for goats and 39.7 kg for sheep. Goats started the experiment with about 6 months of age versus 12 months for sheep (average ages). All goats and sheep were grazing at different hours according to each individual farm's management system (Table 1).

### Anthelmintic efficacy

Differences ( $P < 0.001$ ) between sheep and goats in the fecal oocysts or eggs counts were identified for

**Table 2** Effect of *Salix babylonica* extract on the oocyst or egg output of protozoan, cestode, or nematode parasites per gram of feces in growing sheep (from 8 farms) and goats (from 7 farms) at 0, 1, 20, 40, and 60 days after the start of the extract administration

Animal species	Day	<i>Haemonchus contortus</i>	<i>Eimeria</i> spp.	<i>Cooperia</i> spp.	<i>Chabertia</i> spp.	<i>Dicyocaulus</i> spp.	<i>Moniezia</i> spp.	<i>Trichostrongylus</i> spp.	<i>Ostertagia</i> spp.	<i>Nematodirus</i> spp.
Sheep	0	64.0	221.0	63.2	218.4	110.5	94.8	78.9	0.0	0.0
	1	20.5	292.3	53.8	182.1	38.5	46.2	23.1	7.7	0.0
	20	0.0	215.4	7.7	115.4	76.9	0.0	38.5	23.1	48.0
	40	0.0	130.8	0.0	46.2	46.2	0.0	23.1	15.4	0.0
	60	7.7	61.5	0.0	38.5	0.0	15.4	0.0	23.1	0.0
<i>P</i> value	Linear	<0.001	0.841	0.002	0.001	0.184	<0.001	0.052	0.111	0.120
	Quadratic	0.418	0.003	0.230	0.567	0.012	0.956	0.047	0.759	0.365
Goats	0	80.0	246.7	60.0	200.0	130.0	10.0	10.0	0.0	0.0
	1	0.0	277.4	9.7	96.8	77.4	0.0	9.7	9.7	89.8
	20	0.0	251.6	19.3	145.2	106.4	0.0	19.3	0.0	0.0
	40	9.7	241.9	0.0	48.4	164.5	0.0	0.0	0.0	0.0
	60	19.3	193.6	38.7	145.2	9.7	0.0	0.0	9.7	74.9
<i>P</i> value	Linear	<0.001	0.884	0.015	0.151	0.490	0.008	0.447	1.000	1.000
	Quadratic	0.054	0.335	0.099	0.022	0.045	0.350	0.637	0.001	0.046
<i>P</i> value										
Species ( <i>S</i> )		0.430	<0.001	0.906	0.461	<0.001	<0.001	0.061	0.079	0.123
Day ( <i>D</i> ):										
Linear		<0.001	0.267	0.004	0.001	0.491	<0.001	0.280	0.340	0.637
Quadratic		0.001	0.011	0.295	0.197	0.003	0.129	0.251	0.065	0.403
<i>S</i> × <i>D</i>		0.880	<0.001	0.015	0.002	0.089	<0.001	0.319	0.910	0.135

**Table 3** Proportion of infested sheep in control and treated (extract-given) groups across 8 farms in Mexico

Sampling day	Treatment	<i>Haemonchus contortus</i>	<i>Eimeria</i> spp.	<i>Cooperia</i> spp.	<i>Chabertia</i> spp.	<i>Dicyocaulus</i> spp.	<i>Moniezia</i> spp.	<i>Trichostrongylus</i> spp.	<i>Ostertagia</i> spp.	<i>Nematodirus</i> spp.
Day 0	Control	0.282	0.641	0.051	0.436	0.077	0.205	0.000	0.000	0.000
	Extract	0.231	0.744	0.231	0.718	0.385	0.205	0.282	0.000	0.000
	P value	0.604	0.328	0.037	0.013	0.003	1.000	0.942	1.000	1.000
Day 1	Control	0.026	0.897	0.051	0.487	0.000	0.026	0.000	0.026	0.026
	Extract	0.051	0.974	0.179	0.590	0.128	0.154	0.077	0.026	0.026
	P value	0.564	0.199	0.095	0.365	0.940	0.081	0.954	0.328	0.959
Day 20	Control	0.000	0.744	0.077	0.359	0.179	0.000	0.051	0.000	0.000
	Extract	0.000	0.718	0.026	0.385	0.256	0.000	0.128	0.026	0.000
	P value	1.000	0.799	0.328	0.815	0.413	1.000	0.250	0.954	1.000
Day 40	Control	0.000	0.359	0.000	0.205	0.179	0.026	0.000	0.000	0.000
	Extract	0.000	0.436	0.000	0.154	0.154	0.000	0.077	0.051	0.026
	P value	1.000	0.488	1.000	0.556	0.762	0.959	0.953	0.943	0.959
Day 60	Control	0.051	0.179	0.000	0.231	0.026	0.026	0.051	0.077	0.026
	Extract	0.026	0.205	0.000	0.128	0.000	0.051	0.000	0.000	0.000
	P-value	0.564	0.774	1.000	0.244	0.959	0.564	0.943	1.000	0.959



**Table 4** Proportion of infested goats in control and treated (extract-given) groups across 7 farms in Mexico

Sampling day	Treatment	<i>Haemonchus contortus</i>	<i>Eimeria</i> spp.	<i>Cooperia</i> spp.	<i>Chabertia</i> spp.	<i>Dichyoctylus</i> spp.	<i>Moniezia</i> spp.	<i>Trichostrongylus</i> spp.	<i>Ostertagia</i> spp.	<i>Nematodirus</i> spp.
Day 0	Control	0.194	0.839	0.065	0.484	0.355	0.097	0.065	0.000	0.000
	Extract	0.258	0.774	0.194	0.645	0.419	0.032	0.032	0.000	0.000
	<i>P</i> value	0.545	0.522	0.148	0.202	0.602	0.324	0.562	1.000	1.000
Day 1	Control	0.097	0.774	0.000	0.323	0.258	0.065	0.097	0.032	0.000
	Extract	0.000	0.839	0.032	0.258	0.258	0.000	0.032	0.032	0.000
	<i>P</i> value	0.954	0.522	0.959	0.576	1.000	0.943	0.324	1.000	1.000
Day 20	Control	0.065	0.903	0.032	0.355	0.290	0.000	0.032	0.000	0.032
	Extract	0.000	0.839	0.065	0.484	0.355	0.000	0.065	0.000	0.000
	<i>P</i> -value	0.943	0.453	0.562	0.305	0.587	1.000	0.562	1.000	0.959
Day 40	Control	0.065	0.742	0.032	0.323	0.355	0.000	0.000	0.000	0.000
	Extract	0.032	0.806	0.000	0.161	0.548	0.000	0.000	0.000	0.000
	<i>P</i> value	0.562	0.545	0.959	0.145	0.128	1.000	1.000	1.000	1.000
Day 60	Control	0.000	0.645	0.065	0.387	0.065	0.000	0.000	0.032	0.032
	Extract	0.065	0.645	0.129	0.484	0.032	0.000	0.000	0.065	0.032
	<i>P</i> value	0.943	1.000	0.399	0.443	0.562	1.000	1.000	0.562	1.000

*Eimeria* spp., *Dictyocaulus* spp., and *Moniezia* spp. The fecal egg or oocyst counts of *H. contortus* (linear effect,  $P < 0.001$ ; quadratic,  $P = 0.001$ ), *Eimeria* spp. (quadratic effect,  $P = 0.011$ ), and *Cooperia* spp. (linear effect,  $P = 0.004$ ) were day-dependent. Furthermore, results showed that the day of administration affected fecal egg counts of *Chabertia* spp. (linear effect,  $P = 0.001$ ), *Dictyocaulus* spp. (quadratic effect,  $P = 0.003$ ), *Moniezia* spp. (linear effect,  $P < 0.01$ ), and *Ostertagia* spp. (quadratic effect,  $P = 0.035$ ).

Interactions occurred ( $P < 0.05$ ) between species and days for the number of fecal oocyst or egg counts of *Eimeria* spp., *Cooperia* spp., *Chabertia* spp., and *Moniezia* spp. For sheep, administration of SB extract decreased the fecal eggs count of *H. contortus* (linear effect,  $P < 0.01$ ), *Cooperia* spp. (linear effect,  $P = 0.001$ ), *Chabertia* spp. (linear effect,  $P = 0.009$ ), *Dictyocaulus* spp. (quadratic effect,  $P = 0.012$ ), *Moniezia* spp. (linear effect,  $P < 0.001$ ), and *Trichostrongylus* spp. (quadratic effect,  $P = 0.047$ ). However, no effects were observed ( $P > 0.05$ ) for *Trichostrongylus* spp., *Ostertagia* spp., and *Nematodirus* spp.; control sheep shed less *Eimeria* spp. fecal oocysts than those animals consumed SB extract. After 20 days of treatment, no *H. contortus*, *Cooperia* spp., or *Moniezia* spp. were detected in sheep feces (Table 2).

For goats, the SB extract administration exerted an effect over only a few species of nematodes ( $P < 0.05$ ). Linearly decreased fecal eggs count of *H. contortus* ( $P < 0.01$ ), *Cooperia* spp. ( $P = 0.015$ ), *Chabertia* spp. ( $P = 0.022$ ), and *Moniezia* spp. ( $P = 0.008$ ) were observed with SB extract administration. In addition, quadratic decreases were observed for *Chabertia* spp. ( $P = 0.022$ ), *Trichostrongylus* spp. ( $P = 0.001$ ), and *Ostertagia* spp. ( $P = 0.047$ ). Eggs of *H. contortus* and *Moniezia* spp. were not present in the feces of goats one day after the administration of the SB extract, in contrast to the shedding of *Trichostrongylus* spp. and *Ostertagia* spp. eggs (Table 2).

The proportions of sheep infected with *Cooperia* spp. ( $P = 0.037$ ), *Chabertia* spp. ( $P = 0.013$ ), and *Dictyocaulus* spp. ( $P = 0.003$ ) were greater in the extract-treated group when compared to the control at the beginning of the experiment (before the administration of the extract). However, the amount of other parasite species did not differ ( $P > 0.05$ ) throughout the distinct sampling times (Table 3).

On the contrary, the proportion of goats infected with different parasites species did not differ ( $P > 0.05$ ) between the treated and control animals throughout all the experiment (Table 4).

## Discussion

### Anthelmintic activity of *S. babylonica*

The main objective of the current study was to validate in vivo, in commercial farms under field conditions, the anthelmintic properties of SB extract that have been observed in other studies (Mejía-Hernández et al. 2014; Cedillo et al. 2015). However, Athanasiadou et al. (2007) stated that both in vitro and in vivo procedures should be carried out in assays that aim to determine the anthelmintic activity of plants. The results of the current study indicated that the weekly administration of the aqueous extract of SB at a dose of 20 mL animal<sup>-1</sup> reduced the fecal egg and oocyst counts of many of the parasitic species in both sheep and goats. It would be reasonable to suggest that the observed anthelmintic activity of the SB extracts against different nematode species could be attributed to the presence of tannins and other active compounds in the plant extract. However, we did not determine tannin content in the extract used in the current study. Valdes et al. (2015) identified 59 chemical constituents in a SB extract which may have anthelmintic effects in ruminants (Mejía-Hernández et al. 2014; Cedillo et al. 2015). It has been reported that SB extract contains saponins, alkaloids, tannins, other polyphenols, non-protein amino acids, lignin, and glycosides; all of which are secondary metabolites with previously demonstrated antiparasitic effects (Guarrera 1999). It is important to point out that tannins may be the major active compounds in the extract. Athanasiadou et al. (2001) demonstrated a direct antiparasitic effect of tannins through different mechanisms. Condensed tannins have the ability to impair vital processes such as parasite feeding and reproduction and can disrupt the integrity of the parasite cuticle (Niezen et al. 1995). This ability is a result of the tannins ability to bind free proteins for larvae nutrition causing larval starvation and death. Moreover, tannins have the ability to affect parasites in the gastrointestinal tract directly through the inhibition of oxidative phosphorylation, causing larval death (Athanasiadou et al.

2001). In addition, tannins can cause autolysis when adult insects or their larvae consume them, because they bind to the intestinal mucosa (Schultz 1989). Condensed tannins may also bind to the cuticle layer of larvae, which is high in glycoprotein (Thompson and Geary 1995) causing their death. Besides, an indirect effect of tannins may be due to the ability of tannins to bind to dietary protein, thus protecting it from rumen degradation and increase protein availability in the small intestine of the ruminant (Mueller-Harvey and McAllan 1992). Increased protein availability has been considered responsible for enhanced immunological responses towards parasites (Coop and Kyriazakis 1999). In addition, condensed tannins can increase digesta flow which may create a hostile gut environment for the intestinal parasites, thus reducing their fecundity and consequently the fecal egg shedding. It has been demonstrated that parasitized sheep grazing herbage high in condensed tannins shed less fecal egg counts and had less worm burdens compared with animals grazing forages with a low condensed tannins concentration (Hoskin et al. 1999). The anthelmintic activity against *Nematodirus spathiger*, *Nematodirus battus*, *Strongyloides* spp., *Strongyloides papillosus*, and *Eimeria* spp. has been reported in vivo, achieving a high efficacy against eggs, oocysts, larvae, and adults of these parasites (Mejía-Hernández et al. 2014).

Secondary metabolites of plants, such as condensed tannins, are polyphenols of high molecular weight and therefore they are not absorbed from the digestive tract. However, some reports have indicated that some types of condensed tannins may depolymerize and can be absorbed (Clausen et al. 1990). Different types of chemical constituents present in plants are responsible for the anthelmintic activity of different plants. Tannins are considered to be the cornerstone of the anthelmintic of such activity. These compounds have a complex nature and different types of tannins (Mueller-Harvey and McAllan 1992); therefore, it is expected that they produce different effects on parasites. Not only tannins are responsible for the anthelmintic activity of SB extract, but it is likely that alkaloids in the plant extract may also contribute to the paralysis and consequent worm death. Alkaloid salts are competitive antagonists at muscarinic acetylcholine receptor sites; hence they prevent binding of acetylcholine. These products have been reported to be physiologically active and possess sedative and

analgesic properties. Furthermore, alkaloids lead to cell excitation and neurological dysfunction (Tarnopolsky and Beal 2001).

Saponins (Francis et al. 2002) and lectins (Ríos-de Álvarez et al. 2012) also have some credit for the anthelmintic activity of the extract. Saponins have been demonstrated to possess anti-protozoan and molluscicidal activity, as well as to probably cause in vivo paralysis that leads to loss of grip of parasites to the gut wall, causing their clearance through feces (Francis et al. 2002).

Taken together, it may be concluded that all plant metabolites possess different degrees of anthelmintic activities; however, they may exert their effect alone or in combination with other metabolites causing additive, synergistic, or antagonistic effects on the gastrointestinal tract parasites.

It is very important to point out that higher concentrations of the active compounds may cause antinutritional effects, such as a reduction in feed intake and performance (Salem et al. 2014). Therefore, it is highly recommended to validate the antiparasitic effects of plant products in relation to their potential antinutritional activity and other possible side effects. It is suggested to carry out further assays and studies to assess the isolation, development, and validation of the effects of these herbal remedies to provide evidence that might support their wider acceptance (Githiori et al. 2006).

#### Effect of *S. babylonica* on sheep versus goats

The anthelmintic activity of the SB extract varied between sheep and goats, as well as throughout time and regarding the parasite species that was affected. In general, goats were more resistant to the parasitic infection; therefore efficiency was higher in goats than in sheep. The reason is not clear; however, this may be related to genetic features of the animal host, as well as to the different characteristics of nematode species between goats and sheep and difference in parasite susceptibility to secondary metabolites of the extract. Perhaps sheep are more immunologically compatible hosts, or it can also be considered that the avoidance behavior of the goat gives it a protective advantage against parasites (Papadopoulos et al. 2006). Another reason for the different response of goats and sheep could be the ability of goats to neutralize tannins and antinutritional factors through specialized saliva and

tannin-resistant ruminal flora (Austin et al. 1989). Moreover, goats are known to have higher metabolic rates (Hennessy 1994) and are more adapted to tannins and other antinutritional factors than sheep (Max 2010). Domke et al. (2013) illustrated that the lower number of parasite egg output in goats as compared to sheep in any region may be related to lower stocking rates particularly during spring grazing and to the practice in many goat flocks to separate adult goats and kids at grazing. Moreover, the age of animals can be a critical factor. Both goats and sheep used in the current study were less than 12 months old; however, some farms had older sheep. It is known that goats and sheep do not develop effective immunity against gastrointestinal nematodes during the first 12 months of age (Peña-Espinoza et al. 2014).

Max (2010) drenched sheep and goats in the tropics with solutions of a commercial tannin preparation, and noted a significant reduction in both fecal egg output and worm burdens in sheep and a slight effect in goats. Moreover, in a survey of Domke et al. (2013), it was shown that there was a higher mean excretion of *Trichostrongylus* fecal egg counts in sheep than in goats at the individual level (392 versus 154 eggs per gram).

#### Effect of the farm (location) within each species

There are many differences between the studied farms regarding their response to the application of SB extract. Many reasons can cause differences. Different animal origin (Ancheta et al. 2004), different farm management practices like breeding (Cabaret et al. 2002), sanitary conditions (Majewskaa et al. 2000), and environmental settings (Coklin et al. 2009) including differences in contamination of the environment with oocysts or eggs of the parasite (Majewskaa et al. 2000). Animal age is another factor (Coklin et al. 2009). Nonetheless, in the current study, information regarding the geographic origin of the animal species included in this study was not available. Furthermore, there was a positive relation between prevalence and the area of pastures available (Cabaret et al. 2002). Ancheta et al. (2004) compared institutional with non-institutional farms at different regions and stated that the proportion of *Trichostrongylus* larvae was higher (47 %) in non-institutional versus institutional (36 %) farms. Moreover, Chandrawathani et al. (1999) noted some differences among different farms in *H. contortus* provenance.

It might be concluded that the weekly administration of an extract of *S. babylonica* at a dose of 20 mL is a promising alternative to conventional anthelmintic therapy in sheep and goats. However, the efficacy in sheep was higher than in goats. The main parasite species detected in sheep were *Eimeria* spp., *Chabertia* spp., and *Dictyocaulus* spp. while *Chabertia* spp. and *Dictyocaulus* spp. were the most common parasites in goats in southern Mexico farms.

Finally, it is suggested to carry out further in vivo experiments that assess the effects of the administration of *S. babylonica*, including purification assays, reproducibility, dosage determination, application regimes, identification of active compounds, and toxicological investigations.

**Acknowledgments** Authors would like to thank the financial support of this research work from GRUPO PRODUCE ESTADO DE MEXICO, A.C. (Projects 3644/2013E and 3645/2013E). Kholif, A.E. thanks the National Council for Science and Technology (CONACyT, Mexico) and The World Academy of Sciences (TWAS, Italy) for his Postdoctoral fellowship at the Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México.

**Conflict of interest** All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

#### References

- Ancheta PB, Dumilon RA, Venturina VM, Cerbito WA, Dobson RJ, LeJambre LF, Villar EC, Gray GD (2004) Efficacy of benzimidazole anthelmintics in goats and sheep in the Philippines using a larval development assay. *Vet Parasitol* 120:107–121
- Athanasiadou S, Kyriazakis I, Jackson F, Coop RL (2001) Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. *Vet Parasitol* 99:205–219
- Athanasiadou S, Githiori J, Kyriazakis I (2007) Medicinal plants for helminth parasite control: facts and fiction. *Animal* 1:1392–1400
- Austin PJ, Suchar LA, Robbins CT, Hagerman AE (1989) Tannin binding proteins in saliva of deer and their absence in saliva of sheep and cattle. *J Chem Ecol* 15:1335–1347
- Cabaret J, Mage C, Bouilhoul M (2002) Helminth intensity and diversity in organic meat sheep farms in centre of France. *Vet Parasitol* 105:33–47
- Cedillo J, Kholif AE, Salem AZM, Elghandour MMY, Vázquez JF, Alonso MU, Barbabosa A, Chagoyán JCV, Reyna AG (2015) Oral administration of Sauce llorón extract to growing lambs to control gastrointestinal nematodes and *Moniezia* spp. *Asian Pac J Trop Med* 8:520–525

- Cervantes-Valencia ME, Alcalá-Canto Y, Salem AZM, Kholif AE, Ducoing-Watty AM, Bernad-Bernad MJ, Gutiérrez-Olvera C (2015) Influence of curcumin (*Curcuma longa*) as a natural anticoccidial alternative in adult rabbits: first results. *Ital J Anim Sci* 14:299–303
- Chandrawathani P, Adnan M, Waller PJ (1999) Anthelmintic resistance in sheep and goat farms on Peninsular Malaysia. *Vet Parasitol* 82:305–310
- Clausen TP, Provenza F, Burritt EA, Reichardt PB, Bryant JP (1990) Ecological implications of condensed tannin structure: a case study. *J Chem Ecol* 16:2381–2392
- Coklin T, Uehlinger FD, Farber JM, Barkema HW, O'Handley RM, Dixon BR (2009) Prevalence and molecular characterization of *Cryptosporidium* spp. in dairy calves from 11 farms in Prince Edward Island, Canada. *Vet Parasitol* 160:323–326
- Coop RL, Kyriazakis I (1999) Nutrition–parasite interaction. *Vet Parasitol* 84:187–204
- Cowan MM (1999) Plant products as antimicrobial agents. *Clin Microbiol Rev* 12:564–582
- Domke AVM, Chartier C, Gjerde B, Leined N, Vatne S, Stuen S (2013) Prevalence of gastrointestinal helminths, lungworms and liver fluke in sheep and goats in Norway. *Vet Parasitol* 194:40–48
- Francis G, Kerem Z, Makkar HP, Becker K (2002) The biological action of saponins in animal systems: a review. *Br J Nutr* 88:587–605
- Githiori JB, Athanasiadou S, Thamsborg SM (2006) Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Vet Parasitol* 139:308–320
- Guarrera PM (1999) Traditional anthelmintic, antiparasitic and repellent uses of plants in central Italy. *J Ethnopharmacol* 68:183–192
- Hennessy DR (1994) The disposition of antiparasitic drugs in relation to the development of resistance by parasites of livestock. *Acta Trop* 56:125–141
- Hoskin SO, Barry TN, Wilson PR, Charleston WAG, Hodgson J (1999) Effects of reducing anthelmintic input upon growth and faecal egg and larval counts in young farmed deer grazing chicory (*Chicorium intybus*) and perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture. *J Agr Sci* 132:335–345
- Hrckova G, Velebny S (2013) Pharmacological potential of selected natural compounds in the control of parasitic diseases. Springer, Wien, pp 29–99
- Jabbar A, Iqbal Z, Kerboeuf D, Muhammad G, Khan MN, Afaq M (2006) Anthelmintic resistance: the state of play revisited. *Life Sci* 79:2413–2431
- Littell RC, Henry PR, Ammerman CB (1998) Statistical analysis of repeated measures data using SAS procedures. *J Anim Sci* 76:1216–1231
- MAFF (1979) Parasitological laboratory techniques, Tech. Bull., No. 18. Ministry of agriculture fisheries and food manual of veterinary. Her Majesty's Stationary Office, London
- Majewskaa AC, Werner A, Sulima P, Luty T (2000) Prevalence of *Cryptosporidium* in sheep and goats bred on five farms in west-central region of Poland. *Vet Parasitol* 89:269–275
- Max RA (2010) Effect of repeated wattle tannin drenches on worm burdens, faecal egg counts and egg hatchability during naturally acquired nematode infections in sheep and goats. *Vet Parasitol* 169:138–143
- McKellar QA (1997) Ecotoxicology and residues of anthelmintic compounds. *Vet Parasitol* 72:413–435
- Mejía-Hernández PM, Salem AZM, Elghandour MMY, Cipriano-Salazara M, Cruz-Lagunas B, Camacho LM (2014) Anthelmintic effects of *Salix babylonica* L. and *Leucaena leucocephala* Lam. extracts in growing lambs. *Trop Anim Health Prod* 46:173–178
- Mueller-Harvey I, McAllan AB (1992) Tannins: their biochemistry and nutritional properties. *Adv J Plant Cell Biochem Biotechnol* 1:151–217
- Niezen JH, Waghorn TS, Charleston WAG, Waghorn GC (1995) Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. *J Agr Sci* 125:281–289
- Ojeda-Robertos NF, Torres-Acosta JF, Aguilar-Caballero AJ, Ayala-Burgos A, Cob-Galera LA, Sandoval-Castro CA, Barrientos-Medina RC, de Gives PM (2008) Assessing the efficacy of *Duddingtonia flagrans* chlamydozoospores per gram of faeces to control *Haemonchus contortus* larvae. *Vet Parasitol* 158:329–335
- Olmedo-Juárez A, Rojo-Rubio R, Arece-García J, Salem AZM, Kholif AE, Morales-Almaraz E (2014) *In vitro* activity of *Pithecellobium dulce* and *Lysiloma acapulcensis* on exogenous development stages of sheep gastrointestinal strongyles. *Ital J Anim Sci* 13:303–307
- Papadopoulos E, Prevot F, Diakou A, Dorchie PH (2006) Comparison of infection rates of *Oestrus ovis* between sheep and goats kept in mixed flocks. *Vet Parasitol* 138:382–385
- Peña-Espinoza M, Thamsborg SM, Demeler J, Enemark HL (2014) Field efficacy of four anthelmintics and confirmation of drug-resistant nematodes by controlled efficacy test and pyrosequencing on a sheep and goat farm in Denmark. *Vet Parasitol* 206:208–215
- Ríos-de Álvarez L, Jackson F, Greer A, Bartley Y, Bartley DJ, Grant G, Huntley JF (2012) *In vitro* screening of plant lectins and tropical plant extracts for anthelmintic properties. *Vet Parasitol* 186:390–398
- Salem AZM, Kholif AE, Olivares M, Elghandour MMY, Melado M, Arece J (2014) Influence of *S. babylonica* extract on feed intake, growth performance and diet in vitro gas production profile in young lambs. *Trop Anim Health Prod* 46:213–219
- Schultz JC (1989) Tannin–insect interactions. In: Hemingway W, Karchesy JJ (eds) Chemistry and significance of condensed tannins. Plenum Press, New York, pp 417–433
- Shaik SA, Terrill TH, Miller JE, Kouakou B, Kannan G, Kaplan RM, Burke JM, Mosjidis JA (2006) *Sericea lespedeza* hay as a natural deworming agent against gastrointestinal nematode infection in goats. *Vet Parasitol* 139:150–157
- Statistical Analysis System (2002) SAS users' guide: statistics. Ver 9.0. SAS Institute, Cary, NC
- Tarnopolsky MA, Beal MF (2001) Potential for creatine and other therapies targeting cellular energy dysfunction in neurological disorders. *Ann Neurol* 49:561–574
- Thompson DP, Geary TG (1995) The structure and function of helminth surfaces. In: Marr JJ, Muller M (eds) Biochemistry and molecular biology of parasites, 1st edn. Academic Press, New York, pp 203–232

- Valdes KI, Salem AZM, Lopez S, Alonso MU, Rivero N, Elghandour MMY, Domínguez IA, Ronquillo MG, Kholif AE (2015) Influence of exogenous enzymes in presence of *Salix babylonica* extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. *J Agr Sci* 153:732–742
- Van Wyk JA, Mayhew E (2013) Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: a practical lab guide. *Onderstepoort J Vet Res* 80:1–14
- Wynn SG, Fougere BJ (2007) Introduction: why use herbal medicine. In: Wynn SG, Fougere B (eds) *Veterinary herbal medicine*. Library of Congress cataloging-in publication data, p 695