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Anthelmintic and Anticoccidial Activities *of Mangifera indica* and *Leucaena leucocephala* on Helminths (*Ascaridia galli and Heterakis gallinarum*) and Coccidia of Local Hens in Cameroon

Fokam Tagne Achille Bernard[1], Marie Claire Komtangi[2], Julius Awah Ndukum[3], Fossi Josue[1], Djamen Tchantchou Chamberlin[1]

[1]Département de production animale, University of Dschang, Dschang, Cameroun
[2]Animal Production Technology, University of Bamenda, Bamenda, Cameroon
[3]Département de biologie animale, University of Dschang, Dschang, Cameroun
Email: achillefokam14@gmail.com

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Abstract

A trial to determine the anthelminthic and anticoccidial activities of *Mangifera indica a*nd *Leucaena leucocephala* on helminths (*Ascaridia galli* and *Heterakis gallinarum*) and coccidian in local hens was conducted on 96 one-day-old Brahma chicks weighing an average of 31.33 g and hatched from 140 fertilised eggs. These chicks were randomly divided into 12 experimental units, comprising 4 replicates of 8 subjects, *i.e.* 4 females and 4 males. In each experimental unit, the chickens were divided into batches with equivalent infestations: a control batch (untreated), a batch treated with aqueous extracts of *Leucaena leucocephala* leaves at a rate of 15 g of extract per litre of water and a batch treated with aqueous extracts of *Mangifera indica* bark at a rate of 15 g/litre. While in general the number of eggs and oocysts per gram of faeces (OPG) post-treatment always increased in the control batch, it decreased in the treated batches infested with *Ascaridia galli* and *Heterakis* and *Eimeria sp* during the two weeks of treatment. Thus, the reduction rates obtained during the first week of treatment with the aqueous extract of *Mangifera indica* were 72.13%, 80.03% and 77.51% respectively for *Ascaridia galli*, *Heterakis gallinarum* and *Emeria sp.* During the same week, OPG reduction rates of 78.04%, 88.28% and 67% were recorded for the respective parasites of *Ascaridia galli, Heterakis gallinarum* and *Emeria sp* treated with the aqueous extract of *Leucaena leucocephala*. In addition, the percentage of appearance of lesion scores

(+0 Absence of lesion) of coccidia was lower in treated batches compared with the control batch, and the presence of severe and extremely severe lesions was only observed in the control batch. These results attest to the therapeutic effects of *Leucaena leucocephala* leaves and *Mangifera indica* bark against gastrointestinal parasitosis in chickens.

Keywords

Aqueous Extract, *Leucaena leucocephala* Leaf, *Mangifera indica* Bark, *Ascaridia galli, Heterakis gallinarum* and *Emeria sp*, Lesion Scores

1. Introduction

The poultry industry plays an important role in the supply of proteins of animal origin (meat and eggs) to humans and also plays an important role in the national economy, as a source of revenue [1]. In most low-income, food-deficit countries, around 20% of the protein consumed comes from traditional poultry farming, which accounts for 70% of the poultry population [2]. However, chicken production is limited by a number of extrinsic factors, including malnutrition, poor management, lack of feed, lack of biosecurity, poor genetics due to lack of selection and predation by other animals, and diseases including gastrointestinal parasites [3] [4], and parasitoses are particularly important because of the losses they cause in animal productivity: reduced growth, lower egg production, anaemia and death of the young [5] [6]. These parasites are usually treated with imported veterinary products, which are generally beyond the reach of small-scale livestock farmers [7]. In addition, the control of parasitic diseases is becoming complex due to the emergence of parasites that are resistant to many conventional anthelmintics [8]. Numerous cases of resistant parasites have been reported around the world, and these have taken on considerable importance in tropical countries [9] hence the importance of veterinary pharmacopoeia in traditional livestock farming in Africa [6]. Faced with these animal health problems, medicinal plants could provide a therapeutic response adapted to the financial resources and socio-cultural environment of the populations. Plant-based remedies are an alternative in primary care systems and therefore a promising avenue for the development of improved traditional medicines.

2. Materials and Methods

2.1. Study Area

The trial was conducted at the University of Dschang Research and Application Farm (FAR) between November 2023 and January 2024. The farm is located at latitude 05˚26'N, longitude 10˚26'E, at an average altitude of 1,420 m in the agro-ecological zone of the highlands of western Cameroon. The prevailing climate is equatorial, of the high-altitude Cameroonian type, with two seasons. A rainy

season from mid-March to mid-November and a dry season from mid-November to mid-March. The average rainfall is 2000 mm per year and the temperature varies between 14˚C (July-August) and 25˚C (February). The average annual sunshine is 1873 hours and the average relative humidity is 76.8%.

2.2. Plant Material

Mango tree (*Mangifera indica*) bark and *Leucaena leucocephala* leaves were harvested in the Ménoua department, at Batsingla for the mango tree bark and at the University of Dschang campus for the *Leucaena leucocephala* leaves. The plants were harvested fresh, washed down with plenty of water, cut up and dried separately in the shade for a fortnight, then ground using a blender and sieved (mesh size: 0.5 mm). The various powders thus obtained were stored in hermetically sealed opaque jars, then labelled.

2.3. Livestock Equipment and Management

96 one-day-old Brahma chicks with an average weight of 31.33 g from the incubation of 140 fertilised eggs. The chicks were hatched at a small hatchery in Obala in the central region of Cameroon. These chicks were randomly divided into 12 experimental units, comprising 4 replicates of 8 subjects, *i.e.* 4 females and 4 males were used.

2.4. Accommodation and Equipment

For our trial, the chicks were reared entirely under litter. From day 1 to day 30 at a density of 25 chicks/m^2 for start-up and 10 chicks/m^2 for finishing. Two weeks before the arrival of the chicks, the rearing building and the various pieces of equipment were cleaned and disinfected using bleach, cresyl and TH4. The dressing rooms were subdivided to a surface area of 1 m^2, each equipped with a 100-watt incandescent bulb used as a brooder, a linear feeder capable of holding 2 kg of feed and a 5-litre first-age drinker.

2.5. Prophylaxis

The birds were vaccinated against infectious bronchitis (H120®) and Newcastle disease (Hitchner B1®) on day 7 with a booster on day 18th using Multivax® and against Gumboro disease (IBA Gumboro®) on day 10. An anti-stress agent was added to the drinking water for the first 3 days, as soon as the chicks entered the brooding house and each time before and after vaccination and weighing of the birds. No anti-coccidial or anthelmintic treatment was administered to the chicks as a preventive or curative measure. A foot bath was placed at the entrance to each rearing building and the disinfectant, consisting of bleach and cresyl, was renewed every three days.

2.6. Food and Experimental Equipment

The animals were given water and feed ad libitum. The starter and finisher feeds

were formulated from several single feeds. The starter feed was fed from day 1 to day 42, and the finisher feed from day 43. These chicks were randomly divided into 12 experimental units, comprising 4 replicates of 8 subjects, *i.e.* 4 females and 4 males.

2.7. Preparing the Inoculum

The inoculum was prepared according to the method of [10] from the faeces of 10 naturally infested local hens, then on the basis of the conformity of the shape and dimensions of the eggs [11] [12].

2.8. Assessment of the Oocyst Load and Cyst Load of the Inoculum

Evaluation of the oocyst load and cyst load of helminths in the inoculum. The oocyst and cyst load was useful in assessing the average stock of sporulated oocysts and embryonated eggs contained in the inoculum. The aim was to determine the average stock of oocysts and cysts contained in 1.8 ml of suspension. The method used was that of Brumpt cited by [13] [14]. This method is used to count eggs and oocysts in stools. It was chosen because of its simplicity. Using a 3 ml graduated pasteur pipette, we took 1.8 m 1 of suspension and then introduced it into the 12 fields on a Mc Master slide. Finally, in order to enumerate all the helminth oocysts and eggs contained in this preparation, the entire slide had to be methodically cycled during observation using a microscope with a 40X objective. The oocyst load and helminth egg load were assessed using the following formula

$$[(n1 + n2)/2] \times 100$$

n1: sum of eggs counted in each column of chamber 1;

n2: sum of eggs counted in each column of chamber 2.

The oocyst and cyst load of the inoculum was evaluated at 1708 oocysts, 192 *Ascaris galli* eggs and 159 *Heterakis gallinarum* eggs per 1m1 of inoculum

2.9. Evaluation (Scoring) of Lesions and Parasite Load

On the 78th day of age (2 weeks after treatment), 8 chickens per treatment (04 males and 04 females) were slaughtered and the intestines were dissected. Samples were taken to assess lesion scores. Scoring was done using the technique of [15] described as follows:

The lesions were scored using the REID and JOHNSON scale, which ranges from 0 to +4. After the lesions have been scored, the sum of the points for each portion is averaged for each sample: this is the "mean lesion index = MLI". It is used to establish the extent of coccidiosis lesions and their type (intestinal or cecal coccidiosis), where 0 = no lesions; +1 = light lesions; +2 = moderate lesions; +3 = severe lesions and +4 = extremely severe and violent lesions.

2.10. The Parasite Load

The parasite load was determined through the intensity of the infestation before

and after treatment. This was carried out as follows: On the eve of each sampling session, the previously washed and dried tarpaulins (bags) were placed in each cubicle to prevent the faeces (samples to be collected) from coming into contact with the soiled bedding. 5 faeces samples were collected individually from each batch using the pooled sample technique, 1 day before the sampling session. Treatment (pre-treatment sampling) and 1 and 2 weeks after treatment (post-treatment sampling). The samples were placed in plastic stool boxes that had been labelled (date, treatment, number). The samples collected were brought back to the laboratory and stored in a refrigerator at +4˚C. These samples were examined within a maximum of seven days. The number of opg was determined in a McMaster cell according to [11] (reading threshold: 50 opg). As follows:

Two (2) grams of faeces were triturated in a beaker with a small amount of saturated sodium chloride (NaCl) solution and made up to 50ml. After sieving out the coarse elements, the two cells of the Mac MASTER slide were filled, avoiding the formation of air bubbles, and left to rest for five (5) minutes before observation under a light microscope and counting the parasite elements. The number of Eggs Per Gram (OPG) was determined according to the following formula:

$$OPG = (n1 + n2) \times 100$$

n1: sum of eggs counted in each column of chamber 1;

n2: sum of eggs counted in each column of chamber 2.

2.11. Preparing the Inoculation

Each of the 96 30-day-old chicks, declared not infested after coproscopic examination and deprived of feed for one night, received 1ml of inoculum introduced into the oesophagus using a pipette. The beak was held closed for a few seconds to prevent rejection of the product. 7 and 30 days after inoculation, faeces samples were taken and examined microscopically using the flottaison enrichment method in saturated NaCl solution [11]; this was to ensure the effectiveness of the infestation for the first collection and to determine the average intensity of infestation using McMaster's method [11].

2.12. Confirmation Parasite Inoculation in Chicks

7 days after inoculation, faeces samples were pooled from each batch and examined microscopically using the NaCl saturated solution flotation enrichment method [11]. To ensure that infestation was effective. A second sample was then taken on day 30ième (pre-infestation sampling). Treatment not only confirmed the infestation, but also determined the average infestation intensity before treatment.

2.13. Preparation of Aqueous Extracts

The young leaves of *Leucaena leucocephala* and the bark of *Mangifera indiça* were harvested fresh, then washed with plenty of water, cut up, dried separately in the shade for two weeks, then ground using a mill and sieved (mesh size: 0.5 mm).

The various powders thus obtained were used to prepare the extracts as follows: using an ABDPRO-TB-600 brand balance, 100g of powder from each plant was weighed, then introduced separately into 2 containers, each with a capacity of 3L, containing 1l of distilled water beforehand. The whole was homogenised and left to stand for 48 hours. The different infusions were filtered using Wattman paper (3 MM). The filtrate obtained was dried in an oven at 45˚C until all the water had evaporated. The various powders obtained were stored in hermetically sealed opaque jars, then labelled and stored at room temperature until use.

2.14. Precautions Taken to Avoid Degradation of Active Ingredients

The various extracts obtained were placed inside sterile, dry plastic bags (because in a damp environment the active principle contained in the plants could be degraded by hydrolysis) and then placed in a separate opaque box that does not let sunlight in (photolysis) because heat denatures the proteins. It is also advisable to store it at room temperature (25˚C) away from the sun. However, it has been stored for less than 1 year.

2.15. Preparation of Different Product Concentrations

The aqueous extracts of *Mangifera indica* and *Leucaena leucocephala* were used at a concentration of 15 g per litre of drinking water administered for 3 consecutive days. This concentration (dose) was prepared with reference to the results obtained by [14]. On the efficacy of aqueous and ethanolic extracts of *Cylicodiscus gabunensis* bark in the treatment of coccidiosis in broiler chickens.

2.16. Parameters Measured and Statistical Analysis

The parameters measured were the intensity of infestation assessed by the number of oocysts and cysts per gram of faeces (OPG) described by [11]. and [16], the parasite load and the rate of reduction of OPG between samples taken before and after treatment, the evaluation (scoring) of lesions as written [11].

2.17. Statistical Analysis of Data

All the parameters studied were subjected to one-way analysis of variance (ANOVA). Where there was a significant difference between treatments, Duncan's test was used to separate the means at the 5% significance level [17]. The Mann-Whitney test and the Kruskal-Wallis test were used to compare infestation intensities between the different characteristics. SPSS 20.0 (Statistical Package for Social Sciences) was used for the analyses.

3. Results and Discussion

Effect of aqueous extracts of *Mangifera indica* and *Leucaena leucocephala* on variation OPG of helminths and coccidia **Table 1** shows the variation in eggs per gram of faeces (OPG) over time and as a function of the aqueous extracts of

Leucaena leucocephala leaves and *Mangifera indica* bark administered to the chickens. This table shows that, after treatment, the OPG of the different parasites decreased from the control batch (R0-) to the treated batches (RM; RL). In fact, for helminths, these values went from 267 ± 43.29 (*Ascaris sp*) to 80.67 ± 15.306; 67.00 ± 11.593 (cyst load of Ascaridia treated respectively with aqueous extracts of *Mangifera indica* and *Leucaena leucocephala*); then from ; 224 ± 37.863 (*Heterakis sp*) to 49.50 ± 11.589; 39.17 ± 6.494 (cyst load of *Heterakis sp* treated with aqueous extracts of *Mangifera indica* and *Leucaena leucocephala* respectively) between day 0 (Jo) and 2 weeks (D14) post-treatment in the treated batches. The same phenomenon was observed with oocyst OPGs. This rate decreased from 2644.83 ± 290.796 to 624.17 ± 61.532 and 960.33 ± 88.344 respectively for batches treated with *Mangifera indica* and *Leucaena leucocephala*. During the same period. On the other hand, it remained more or less constant in the control batch. The differences between the average OPG of the treated batches and those of the control were significant ($P < 0.05$). These results are comparable to the work of [18]. where the use of 120 g/Kg PV and 30 g/kg PV of fresh *Leucaena leucocephala* leaves on *Haemonchus contortus* larvae in small ruminants resulted in a 92% reduction in *Haemonchus contortus* eggs; the work of [19]. where the use of 6 mg/mL mimosine resulted in a 77.52% reduction in the number of eggs laid. These results are also similar to those of [20], where the use of 2.5 mg/ml of *Mangifera indica* condensed tannin (CT) resulted in a 39.2% reduction in the number of *Haemonchus contortus* eggs laid in sheep.

Table 1. Effect of *Mangifera indica* and *Leuceana leucocephala* aqueous extracts on OPG variation.

Collection of faeces	Treatments									p
	R0⁻			RM			RL			
	Ascaris sp	*Heterakis sp*	*Emeria spp*	*Ascaris sp*	*Heterakis sp*	*Emeria spp*	*Ascaris sp*	*Heterakis sp*	*Emeria spp*	
day 0 (before treatment	267 ± 43.29	224 ± 37.863	2644.83 ± 290.796	269.67 ± 50.820	216.17 ± 51.211	2601.33 ± 254.77	268.00 ± 45.795	219.33 ± 37.066	2696.33 ± 309.096	0.995
1 week after the start of treatment	278.5 ± 40.913	225.33 ± 36.631	2669.33 ± 316.866	75.50 ± 14.460	43.17 ± 10.088	585.00 ± 69.296	58.83 ± 10.128	25.67 ± 5.354	862.83 ± 98.959	0.000
2 weeks after the start of treatment	265 ± 41.290	250.00 ± 40.863	2744.83 ± 299.776	80.67 ± 15.306	49.50 ± 11.589	624.17 ± 61.532	67.00 ± 11.593	39.17 ± 6.494	960.33 ± 88.344	0.000

R0-: negative control that received no antiparasitic treatment (anticoccidial and anthelminthic); RM: treatment with aqueous extract of *Mangifera indica*; RL: treatment with *Leucaena leucocephala.*

3.1. Effect of Aqueous Extracts of *Mangifera indica* and *Leuceana leucocephala* on the Parasite Load and Rate of Reduction of *Ascaridia galli* and *Heterakis sp* Worms in Chickens

The average parasite loads and the rates of worm reduction following the various extracts are shown in Table 2 below. This table shows that for both extracts used, a significant reduction in the average parasite load was observed after autopsy compared with the control (R0-) for *Ascaridia galli*. However, the parasite load of

Heterakis gallinarum was statistically comparable between the different treatments (RM and RL). The reduction rates of *Ascaridia galli* worms were 53.33% and 73.33% in the batches treated with the RM and RL aqueous extracts respectively. In addition, the reduction rates for *Heterakis gallinarum* worms were 75% and 100% respectively for batches treated with the aqueous extracts of RM and RL. The reduction in parasite load was thought to be due to the presence of bioactive molecules such as mimosin and Mangiferin contained in Leucaena leaves and *Mangifera indica* bark respectively. These results are similar to those of [19], who obtained an inhibition of development of *Caenorhabditis elegans* larvae by 92.22% and a 77.52% reduction in the number of eggs laid at the respective concentrations of 0.11 mg/mL and 3.6 mg/mL of mimosine. In addition, these results are consistent with the work of [21], whose use of 100 mg/ml of aqueous extract of *Mangifera indica* on *Strongyloides stercoralis* larvae in humans resulted in inhibition of larval development of worms of the order of 100% after 6h of exposure; the work of [22], where the use of 0.125% mangarifine on *Haemonchus contortus*, *Trichostrongylus* and *Chabertia* and *Teladorsagia/Ostertagia* larvae significantly reduced larval development.

Table 2. Effect of aqueous extracts of *Mangifera indica* and *Leucaena leucocephala* on parasite load and reduction rate of Ascaridia galli and Heterakis worms.

parameters	Treatment						P
	R0-		RM		RL		
	Ascaris sp	*Heterakis sp*	*Ascaris sp*	*Heterakis sp*	*Ascaris sp*	*Heterakis sp*	
Parasitic load	15 ± 9.971[a]	4 ± 6.047	7 ± 7.928[ab]	1 ± 2.828	4 ± 6.047[b]	0.0 ± 0.0	0.036
Discount Rate (%)	----------	----------	53.33	75	73.33	100	----------

R0-: Negative control that received no antiparasitic treatment (anticoccidial and anthelminthic) RM: Treatment with aqueous extract of *Mangifera indica*; RL: Treatment of *Leucaena leucocephala*; a, b, on the same line, numbers marked with the same letter do not differ significantly ($p > 0.05$).

3.2. Effect of Aqueous Extracts of *Mangifera indica* and *Leucaena leucocephala* on the Rate of Reduction (%) of Faecal Concentrations of Parasite Eggs and Oocysts

Variations in the rate of OPG reduction as a function of extract and treatment period are illustrated in **Figure 1** below. It can be seen that, irrespective of the extract, the rate of OPG reduction increased significantly ($P = 0.002$) during the first week of treatment. Furthermore, during the second week, the rate of reduction of *Emeria sp* oocysts by the aqueous extract of *Mangifera indica* was greater than that of *Leucaena leucocephala* with the doses tested and over time. The reduction rates obtained during the first week of treatment with the aqueous extract *of Mangifera indica* were 72.13%, 80.03% and 77.51% respectively for *Ascaridia galli, Heterakis gallinarum* and *Emeria sp*. During the same week, Opg reduction rates of 78.04%, 88.28% and 67% were recorded for the respective parasites of

Ascaridia galli Heterakis gallinarum and *Emeria Sp* treated with the aqueous extract of *Leucaena leucocephala*. In addition, during the second week of treatment, regardless of the extract used, a decrease in the OPG reduction rate of the parasites was observed. These results are inferior to those of [18], where the use of 120 g/Kg PV and 30g/kg PV of fresh *Leucaena leucocephala* leaves on *Haemonchus contortus* larvae in small ruminants resulted in a 92% reduction in *Haemonchus contortus* eggs, and superior to those of [19]. where the use of 6 mg/mL mimosine resulted in a 77.52% reduction in the number of eggs laid. And to that of [20]. Where the use of 2.5 mg/ml of *Mangifera indica* Condensed Tannin (CT) induced a 39.2% reduction in the number *of Haemonchus contortus* eggs laid in sheep. And lower than that of [23]. where the ethanolic extract of *Mangifera indica* at a concentration of 10 mg/ml revealed 91% efficacy in inhibiting the hatching of *Haemonchus contortus* eggs.

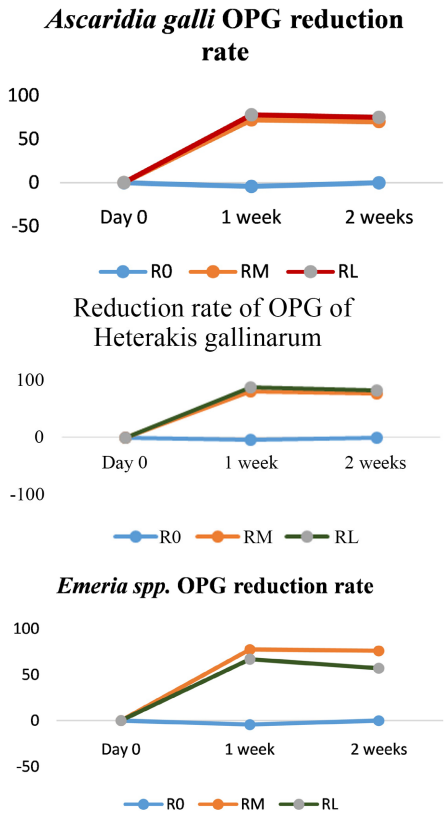

Figure 1. Variation over time in the rate of reduction (%) of faecal concentrations of parasite eggs and oocyts as a function of aqueous extracts of *Mangifera indica* and *Leucaena leucocephala*.

3.3. Effect of Aqueous Extracts of *Mangifera indica* and *Leucaena leucocephala* on Mean Lesion Scores and Percentage Occurrence of Coccidial Lesion Scores

Table 3 shows the mean lesion scores and the percentage of occurrence of lesion scores in each treatment. Severe (+3) and extremely severe (+4) lesions were

observed in subjects receiving no treatment with a respective mean of 0.25 ± 0.463 and 0.13 ± 0.354. On the other hand, severe lesions (+3) were also observed in batches treated with aqueous extract of *Leucaena leucocephala* with a mean of 0.13 ± 0.354. However, no severe or extremely severe lesions were observed in subjects treated aqueous extract of *Mangifera indica*. The percentage of appearance of lesion scores for extremely severe lesions in batches treated with aqueous extracts was lower than in the batch receiving no treatment. However, the aqueous extract of *Mangifera indica* appeared to have a more marked anti-coccidial activity than the extract of *Leucaena leucocephala*. Statistical analysis showed no significant difference between the mean lesion scores of the different treatments ($p > 0.05$). These results are similar to those of [24]. Who demonstrated that wormwood leaf extract used as a feed additive had a protective effect against intestinal lesions produced by the parasite. Recently [25].

showed that wormwood extract significantly reduced oocyst production in hens and restored their weight gain. Furthermore, [26]-[28] reported respectively that oregano essential oil and aqueous extract of *Carica papaya* reduced the expression of coccidiosis in chickens. However, the mechanism responsible for these beneficial effects remains unexplained.

Table 3. Effect of aqueous extracts of *Mangifera indica* and *Leucaena leucocephala* on mean lesion scores and percentage occurrence of coccidial lesion scores.

Lesion scores	Treatment						p
	R0		RM		RL		
	average of lesion score	percentage of appearance of lesion scores	average of lesion score	percentage of appearance of lesion scores	average of lesion score	percentage of appearance of lesion scores	
+0 No lesion	0.00 ± 0.00	25 %	0.13 ± 0.354	54%	0.00 ± 0.00	35%	0.385
+1 few discrete lesions	1.63 ± 0.996	45%	1.25 ± 0.886	41%	1.38 ± 0.996	45%	0.705
+2 moderate lesions	0.63 ± 0.744	16%	0.13 ± 0.354	5%	0.50 ± 1.069	16%	0.425
+3 severe lesion	0.25 ± 0.463	10 %	0.00 ± 0.00	0%	0.13 ± 0.354	4%	0.350
+4 extremely severe lesion	0.13 ± 0.354	4%	0.00 ± 0.00	0%	0.00 ± 0.00	0%	0.385

R0-: negative control that received no antiparasitic treatment (anticoccidial and antihelminthic); RM: treatment with aqueous extract of *Mangifera indica*; RL: treatment with aqueous extract of *Leucaena leucocephala.*

4. Conclusion

At the end of this study on the evaluation of the anthelminthic and anticoccidial activity of *Mangifera indica* and *Leucaena leucocephala* on helminths (*Ascaridia galli* and *Heterakis gallinarum*) and coccidia in local hens, we can conclude that the concentrations of the treating product (15 g/l) significantly reduced the parasite load after inoculation of the germ, as did the percentage of lesion scores. However, the isolation of the active ingredients from the different plants should make

it easier to use the product on the farm. In addition, the study of the mechanisms of action of the active ingredients at larval level in parasite eggs and oocysts should make it possible to better quantify the dose of the products treated.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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