Research Article

In vitro anthelmintic activity of Leuceana leucocephala (Lam.) De Wit. (Mimosaceae) and Gliricidia sepium (Jacq.) Kunth ex Steud (Fabaceae) leave extracts on Haemonchus contortus ova and larvae

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ABSTRACT

High cost of conventional anthelmintic products associated sometimes with their unavailability on all part of Burkina Faso orders that alternative solutions based on the use of medicinal plants are investigated to help poor rural smallholder farmers. For that purpose, the aqueous extracts of two forage plants (Leuceana leucocephala and Gliricidia sepium) were tested on Hæmonchus contortus parasite of goats to estimate their potential anthelminthic properties. In this study, six concentrations of the aqueous extracts of plants (1,562-50 mg / ml) were used in comparison with a control (distilled water) to lead the tests of egg hatching and larval development. The percentage inhibition of two extract plants showed a dose-dependent anthelminthic effect ($P<0.05$) on an inhibition of egg hatching and larval development. In egg hatching test, only the concentration of 50 mg/ml of G. sepium extract of presented a significant inhibition effect ($P < 0.05$) compared to the control. The obtained effective dose 50 (ED_{50}) were 18.6 mg/ml for G. sepium extract against 44.9 mg/ml for leucocephala L. extract. In larval development test, all concentrations extract of G. sepium and presented a significant inhibition effect ($P < 0.05$) compared to the control, contrary to those of leucocephala L. extract (6,25 - 50 mg / ml). The found ED_{50} were 25 mg/ml and 91.5 mg/ml for extracts of G. sepium and L. leucocephala, respectively. These results denote the existence of natural compounds in the two plant extracts and whose anthelmintic properties could justify their use in the control of gastrointestinal nematode in small ruminant by smallholder farmers. However, these properties may be verified by in vivo procedure among small ruminants.

Keywords: Gliricidia sepium; Leuceana leucocephala; Hæmonchus contortus; anthelmintic activity; eggs; larvae.
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INTRODUCTION

In Burkina Faso, livestock sector holds an important place in the state economy. It occupies about 80 % of the active population [1]. Unfortunately, majority of smallholder farmers practise the traditional system, exploiting local natural grazing that favours pathologies as gastrointestinal nematode parasitism. This pathology is very important in Burkina Faso because this disease is present everywhere in the country [2-3] and its impact on the family economy of smallholder farmers [4]. In rural area, it reduces small ruminant productivity, causes animal mortalities, loss of income and costs of sick animal treatment. In the country, gastrointestinal nematode parasitism constitutes a constant preoccupation for smallholder farmers, for whom mall ruminant raising is the best source of food safety and cash income [5-6]. However, conventional anthelmintic products and sometimes the costs of therapeutic acts recommended to control gastrointestinal nematode parasitism are two expensive for smallholder farmers. This

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situation drove them to explore local alternative solutions thought the use of medicinal plants to fight against gastrointestinal nematode parasites of small ruminants. Thus, the veterinary traditional medicine is liveliness to develop itself progressively in the rural area among smallholder farmers [7-8]. Currently, many smallholders (50%) use the traditional medicine to control gastrointestinal parasitism in small ruminant [8]. In this setting, the interest of this work was to study fodder medicinal plants presented anthelmintic effects. Current study was carried out to explore the potential anthelmintic activity of *Leuceana leucocephala* (Lam.) De Wit. (Mimosaceae) and *Gliricidia sepium* (Jacq.) Kunth ex Steud (Fabaceae) by *in vitro* tests on *Haemonchus contortus* parasite. The two plants are native of Central America. In traditional medicine, *L. leucocephala* is used in the treatment of eye disorders, gonorrhea and as vermifuge [9]. For G. sepium, previous works revealed that it possesses antiparasitic effects [10] (Peraza-Sanchez *et al.*, 2005). In addition, it is used as an antihistaminic, antipyretic, expectorant and diuretic in Mexico [11]. in the treatment of the mange, the wounds, the rheumatisms and the eczema [11].

EXPERIMENTAL SECTION

1- Plant materials
Leaves of *L. leucocephala* and *G. sepium* were collected from experimental station of Kamboinsé of "Institut de l'Environnement et de Recherches Agricoles (INERA)" in Burkina Faso. They were identified in the botanic herbarium of "Centre National de la Recherche Scientifique". The leaves of both plants were dried in shade at ambient temperature, ground and milled to powder by electrical blender (THERMOSI SR 3000) and kept in colored bottles until used.

2- Parasites
Mature worms of *H. contortus* were collected freshly from the abomasums of slaughtered goats in the abattoir of Ouagadougou, Burkina Faso. The female parasites were selected and crushed gently to liberate eggs. These eggs were filtered through different sieves (20 mm, 1 mm and 38 µm) for collection before washed and stored in distiller water for biological tests in the laboratory.

3- *Methodology of study*
3.1- Preparation of extracts
Aqueous extraction was performed by soaking 25 g of each dry powder of two plants in 500 ml of distilled water and shaking for 3 h using electric shaker. The suspensions were filtered through muslin gauze and the filtrates were macerate in deep freezer for 24 h and then evaporated to dry by freeze dryer at 50°C for four days and stored at 4°C until used.

One quarter of hour before the tests, the extracts were dissolving in distilled water to obtain six increasing concentrations (1.562 - 50 mg/ml).

3.2- Egg recovery
The eggs recovery method as described previously by Jabbar et *al.* [12] was used in this study.

3.3- *In vitro* test procedures
The achieved procedures are the adaptation of the techniques described by Jackson and Hoste [13] for egg hatch and larval development tests.

3.3.1- Egg hatch test (EHT)
For EHT, collected eggs were adjusted to approximately 250 eggs per 1 ml in Eppendorf tube (5 ml). Then, the tubes were submitted to different treatments composed of six extract concentrations of each extract plant and negative control using distilled water. Four replicates for each concentration of extract and control were performed. All tubes were incubated under humidified condition at ambient temperature (27°C) for 48 h. After, three drops of Lugol`s iodine solution was added to each well to stop further hatching and all the un-hatched eggs (percentage of inhibition) and larvae L_1 in each well were counted under an inverted microscope.

3.3.2- Larval development test (LDT)
Collected eggs were adjusted as described in egg hatch test in Eppendorf tube (5 ml) and incubated under humidified condition at ambient temperature for 48 h in the obscurity to obtain egg development in larvae of first stage (L_1). Then, 200 µL of nutritive media (nutrient Agar, DIFCO: 2%) was added into each tube before submitted at the same treatments in the previous test. There were three replicates for each concentration of both extracts and control. All tubes were further incubated for 7 days. Further development was stopped by addition of three drops of Lugol`s iodine solution. All larvae's L_1 and L_3 in each tube were counted under an inverted microscope.

3.3- Statistical analysis

Data's from egg hatch (EH) and larval development (LD) tests were transformed to log (x +1) before submitted to one-way analysis of variance and the means values obtained were compared by Student Neuwmann-Keuls test. The means of extract concentrations were also submitted to the non parametric-test of Kruskall-Wallis. All analysis was made with CoStat (Version 6.204) at significance level of 5%.

For EHT and LDT, effective dose (ED_{50}) was calculated as the concentration of extracts producing 50% inhibition of eggs hatching and of larvae development by probit-analysis using SPSS (Version 10.0.5) program for Windows.

RESULTS

1- Egg hatching

The results of *H. contortus* egg inhibition submitted to aqueous extracts of both plants are presented in Figure 1 and Table 1. Percentage Inhibition of egg hatching increased significantly ($P<0.05$) with the increase of the concentrations of both plant extracts (fig. 1). However, all concentrations of two extracts did not inhibit significantly ($P<0.05$) the egg hatching of *H. contortus* in the same manner compared with the control (distilled water) which the mean percentage of egg hatching was 71.9 %.

Only the maximal concentration of L. *leucocephala* extract (50 mg/ml) was presented significant level ($P<0.05$) compared to the control. For *G. sepium* extract, the concentration of 12.5 to 50 mg/ml showed statistically a high inhibition ($P<0.05$) more that the control.

The table 2 presents the effective doses (ED_{50}) of extracts tested on egg hatching of *H. contortus*. Comparing the ED_{50} obtained by probit system, *G. sepium* extract (18.6 mg / ml) was more ovicidal than *L. leucocephala* extract (44.9 mg/ml).

Table 1: **Inhibition mean percentages on *H. contortus* egg hatching at different concentrations of two aqueous extracts plants**

Concentrations (mg /ml)	Extracts	
	L. leucocephala	*G. sepium*
50	56,2 ± 4,4 a A	66,2 ± 6 a B
25	38,6 ± 2,1 b A	54,2 ± 6,1 a B
12,5	15,3 ± 10,0 c A	53,7 ± 5,4 a B
6,25	7,5 ± 4,5 c A	26,6 ± 7,6 b B
3,125	8,4 ± 5,4 c A	15,1 ± 3,8 c A
1,562	3,5 ± 4,1 c A	1,3 ± 1,8 d A
Control	28,0 ± 10,8 b	28,0 ± 10,8 b

(abcd) Small letters compare means in the columns and capital letters in the lines. Different letters indicate significantly different values (P<0.05).

Table 2: Effective dose 50 (ED $_{50}$) values (mg/ml)) on egg hatching and larva development of *H. contortus* of two aqueous extracts plants

Tests	ED $_{50}$ (LCL – UCLS) [a]	
	L. leucocephala	*G. sepium*
EHT	44,9 (33,3 - 68,3)	18,6 (15,2 - 23,4)
LDT	91,5 (54,5 - 206,3)	25,0 (19,3 - 34,9)

([a]): Values at 95% confidence intervals, LCL: lower confidence limit, UCL: upper confidence limit

2- Larva development

Table 3 presents the results of the mean percentages of larvae development inhibition of *H. contortus* submitted to both aqueous extracts. The percentages of larvae development inhibition of *H. contortus* L1 and L2 increased statistically ($P<0.05$) with the increase of the concentrations of two extracts plants (Figure 2). The mean percentage of larvae development inhibition of *H. contortus* first stage was 17.7 % for the control (distilled water). This percentage of inhibition was significantly low ($P<0.05$) compared with those of all concentrations of *G. sepium* extract and only with doses from 6.25 to 50 mg/ml for *L. leucocephala* extract.

Effective doses (ED_{50}) of two extracts plants showed that *G. sepium* extract (25 mg/ml) was more larvicidal than *L. leucocephala* extract (91.5 mg/ml) (table 2).
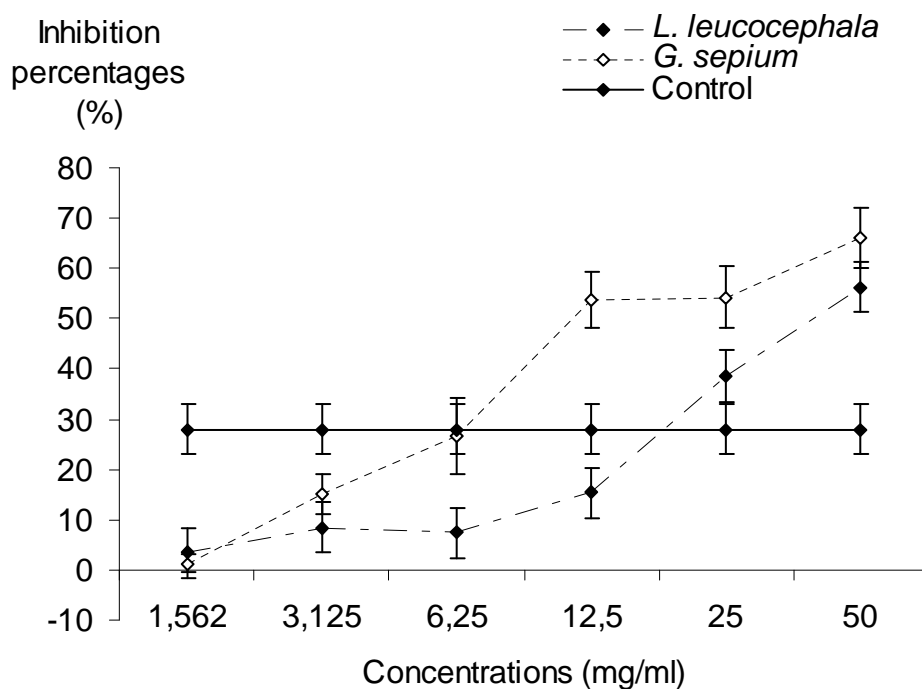

Figure 1: Dose-response profile of egg hatching of *H. contortus* submitted at six concentrations (1.562, 3.125, 6.25, 12.5, 25, and 50 mg/ml) of *L. leucocephala* and *G. sepium* extracts.
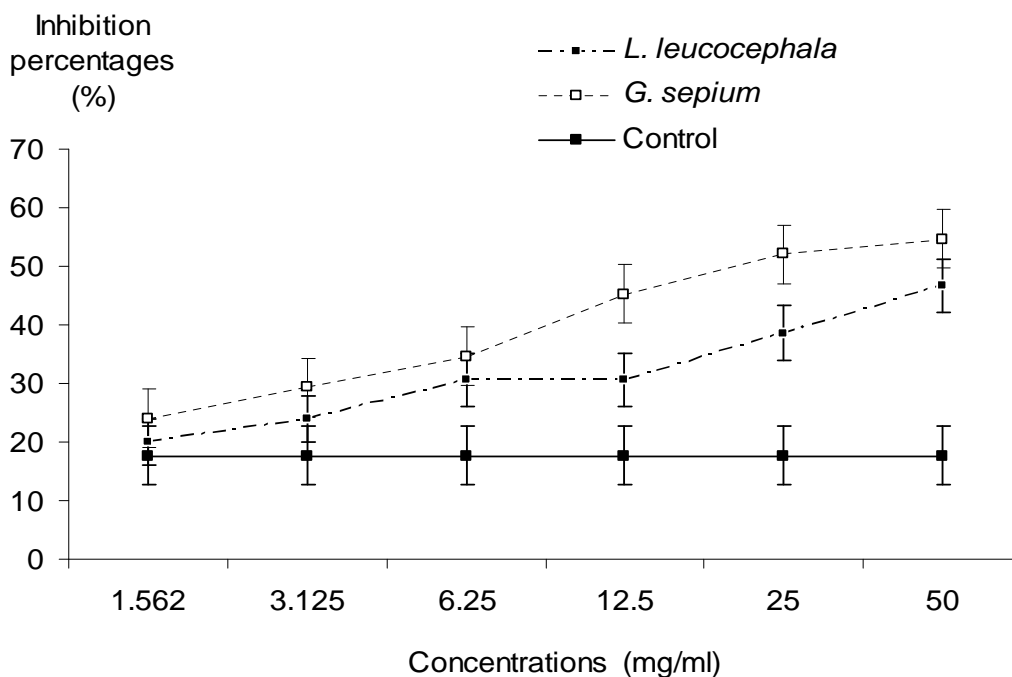

Figure 2: **Dose-response profile of larvae development of *H. contortus* submitted at six concentrations (1.562, 3.125, 6.25, 12.5, 25, and 50 mg/ml) of *L. leucocephala* and *G. sepium* extracts.**

Table 3: Inhibition mean percentages on larvae development of *H. contortus* at different concentrations of two aqueous extract plants

Concentrations	(mg /ml)	Extraits	
		L. leucocephala	*G. sepium*
50		46,6 ± 4.6 a B	54,6 ± 2,3 a A
25		38,6 ± 4.6 ab B	52,0 ± 0 a A
12,5		30,6 ± 4.6 bc B	45,3 ± 2,3 a A
6,25		30,6 ± 4.6 bc A	34,6 ± 4,6 b A
3,125		24.0 ± 4.0 cd A	29,3 ± 4,6 bc A
1,562		20.0 ± 4.0 d A	24,3 ± 6,9 c A
Control		17,7 ± 0,9 d	17,7 ± 0,9 d

(abcd) Small letters compare means in the columns and capital letters in the lines. Different letters indicate significantly different values (P<0.05).

DISCUSSION

The objectives pursued through this study were to establish the ovicidal and larvicidal effects of *L. leucocephala* and *G. sepium* on *H. contortus* parasite. The methods of assessment used are adapted to those usually adopted to value the resistance of gastrointestinal nematodes parasites to conventional anthelmintic products [14-15]. In the two tests, *H. contortus* is chosen because it is the most dominant nematode parasite in all areas of the country of Burkina Faso [16] and the animal model commonly used to verify anthelmintic activity of medicinal plants [12; 17].

The two plants were chosen in this study because their anthelmintic activity in ruminants was not study in Burkina Faso. In addition, these are natural fodder plants that exist in all agro-ecological areas of country. Presently, the problem of animal food arises with acuteness in dry season (8-9 months). They could be exploited in the fields of cultures to realize harvests of feeds for animals and to maintain the soils fertility.

In the study, the results obtained show that both extracts inhibit egg hatching and larvae development of *H. contortus*. However, the inhibitory effects of two plants vary with the extract concentrations and the *H. contortus* life stage compared with to the control (distiller water). Indeed, the significant ovicidal effect was observed at the concentrations of 12.5-50 mg/ml for *G. sepium* and only at 50 mg/ml for *L. leucocephala* extract. The significant larvicidal effect was observed with all concentration of *G. sepium* and at 6.25-50 mg/ml for *L. leucocephala* extract. Towards the obtained results in the two tests demonstrate that *G. sepium* extract is more ovicidal and larvicidal on *H. contortus* parasite than *L. leucocephala* extract. This comparison is attested by the results of effective doses and denotes that the leaves of both plants would contain active substances with anthelmintic effect. Indeed, the leave extracts of both forage plants contain tannins, particularly condensed tannins (1.80 %MS for *L. leucocephala* and 0.30 %MS for *G. sepium*), triterpens and flavonoids [18]. The anthelmintic effects of those actives substances were reported by several authors [19-20]. Some studies related similar results of extracts efficiency of *Spigelia anthlemia* on eggs and larvas of *H. contortus* by Assis et *al.* [21]. Besides, the comparison of effective doses in the two tests suggests that the two extracts are more active on eggs than on larvas. Consequently, we can suppose that active substances contained in our two extracts would cross more easily the shell of eggs than cuticles of larva's to pull the death by food deprivation [22] or by paralysis [23]. Kaboré et *al.* [24] ended the same conclusion further to their works on eggs and larvas of *H. contortus* with aqueous extracts of *Anogeissus leiocarpus* and *Daniellia oliveri*. Wabo et *al.* [25] made also the same observations on *Ancylostoma caninum* parasite with the extract of stem barks of *Canthium mannii*.

For *L. leucocephala* plant extract, our results confirm those of Adémola et *al.* [26] who observed an anthelmintic activity of polar fraction of the plant on *H. contortus* L3. According to these authors, the anthelmintic activity of polar fraction of the plant is bound to the presence of flavonoids and tannins. However, this forage plant contains mimosine, a toxin of the group of nonprotein amino-acids which the products of degradation are harmful and causes alopecia and fiber shedding in sheep [27]. According to the variety of *L. leucocephala* plant and the part used, Smith [28] reported 0.3 to 7.1 % of mimosine concentrations. Contrary to *L. leucocephala*, tannins are only harmful substances observed in *G. sepium* whose tannins contents vary between 2 – 2.3 % in the leaves [28]. According to Woodward and Reed [29], these contents are lower to the admitted average (4% of dry matter) that improves the consumption of fodders.

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CONCLUSION

According to the obtained results, two extract plants inhibit the egg hatching and larvae development of *H. contortus*. However, this inhibition efficiency is significantly more evident with *G. sepium* extract than *L. leucocephala* extract. Analysis of effective doses values (DE_{50}) shows that both extracts act more on eggs than on larvaes. In all case, this behaviour of two extracts on *H. contortus* justifies their uses by smallholder farmers to control gastrointestinal nematode parasite in small ruminant and to limit the infestation of natural pastures. However, it is necessary to evaluate the level of toxicity of two extract plants and to achieve studies in infestation condition in small ruminants of country in order to secure the smallholder farmers in their use.

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