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Research Article

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Seed germination and anthelmintic activity of *Cajanus cajan* on sheep

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ABSTRACT

Nematodes parasites of gastrointestinal tract are a major constraint of small ruminant raising in rural farm of Burkina Faso. In the search of endogenous alternatives solutions, this study was initiated to evaluate the potential anthelmintic activity of Cajanus cajan fodder plant in sheep. For this purpose, seed germination test was carried out to produce C. cajan fodder which was used to evaluate adult motility test in vitro and faecal egg counts reduction test in vivo in natural infestation condition. For the first test, applied pretreatments (mechanical scarification and immersion in water for 24 hours) significantly increased the speed of seed germination until the second day after seeding compared to the control (untreated). The results of in vitro anthelmintic activity of C. cajan aqueous extract revealed that the tested concentrations (100, 50 and 25 mg / ml) inhibited H. contortus adult worms during the test. In vivo anthelmintic results of C. cajan fodder administered at 3 g / kg body weight to the animals of treated group (n = 6) showed a decrease in egg excretion (P>0,05), a weight increase and a larger hematocrit levels (P> 0.05) compared to control group (n = 6). These results show that the use of C. cajan fodder is beneficial to small ruminants in infested area of gastrointestinal parasites. Therefore, it could be popularized in north Sudanese area of the country in an integrated struggle approach to improve animal and agricultural production.

Keywords: Germination; Gastrointestinal parasites; sheep; *Cajanus cajan*; Burkina Faso, Anthelmintic activity

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INTRODUCTION

In Burkina Faso, national herd of small ruminants is numerically significant and estimated strengths are 8 243 238 sheep and 12 342 454 goats [1]. Unfortunately, their livestock productions are low related to misconduct and poor health situation of animals like in African subtropic areas [2]. Indeed, practiced raising mode focuses mainly on the exploitation of natural pastures and having direct consequence gastrointestinal parasites that are the cause of digestive pathologies, major constraint of small ruminants in the tropics [3]. Various parasites are responsible for these digestive diseases and *Haemonchus contortus* is among the most dominant in Burkina Faso. This parasite induces enormous socio-economic losses to rural low-income family farmers and small ruminants for which prominently at their farms [4]. Facing the high costs of conventional products and the lack of veterinary officials in some parts of the country, alternative solutions based on the use of medicinal plants have been explored by rural

farmers to control gastrointestinal parasites [5]. In the current context of the country, the legume shrub *Cajanus cajan* is greatly exploited by small rural producers for multiple therapeutic activities [6]. Also, this study was initiated to evaluate the potential anthelmintic activities of the plant fodder to improve the health and nutrition of small ruminants. Pursued objectives through the study were (i) to determine the best pretreatment for good seed germination to produce *C. cajan* fodder and (ii) to verify the anthelmintic activity of *C. cajan* fodder through *in vitro* and *in vivo* tests on gastrointestinal parasites of small ruminants.

MATERIALS AND METHODS

1- Study site

This study was conducted to Saria Experimental Station of the Institute of Environment and Agricultural Research (INERA). This station is located at 12 ° 16' north latitude and 2 ° 09' west longitude and covers an area of 400 hectares. The climate regime is north Sudan type characterized by a short rainy season (June to September) and a long dry season (October to May), which are subject to strong irregularities linked to fluctuations in atmospheric parameters. Temperatures are generally high during the year but with a moderation during rainy season (25-35°C) where the relative humidity are between 60-70%. Soils are tropical ferruginous leached type, very deficient in phosphorus and low in organic matter and shallow [7]. The vegetation is characterized by the presence of a savannah annual grass, trees and shrubs.

2- Materials

2.1- Vegetal material

C. cajan seeds and leaves were used to conduct experimental tests. *C. cajan* seeds were harvested during the dry season of 2010-2011 at the Experimental Station Saria. They were kept in a plastic bag placed in the natural conditions. Meanwhile, a sample of plant forage was collected and washed with water before being dried in dust-free. Then the dried sample was ground to a fine powder to produce a decoction using 100 g powder and diluted in 1 000 ml of distilled water at boiling temperature for 1 hour. Decoction obtained was cooled before being filtered through cotton wool first and then filtered on paper. Resulting aqueous extract was lyophilized after and stored for bioassay *in vitro*. Also, a stock of *C. cajan* fodder was constituted from a parcel sowed of the plant seeds for in vivo *test* during the 2011-2012 winter season.

2.2- Animal material

Haemonchus contortus adult worms have been used for *in vitro* test and Mossi breed sheep for *in vivo* test. *H. contortus* adult worms were obtained from the stomachs of sheep naturally infected. For this, stomachs were purchased for slaughter of Koudougou and transported to Saria Experimental Station to undergo a longitudinal incision. Adult worms were carefully collected manually using forceps and placed in a polypropylene bottle with closure and containing PBS.

Used sheep had a mean weight of 15.8 ± 3.8 kg and came from the fold of the Department of Animal Production Saria Station. In winter, the food needs of animals are covered by exploitation of natural pastures.

3- Applied methods

Three experiments were conducted, namely a germination test and anthelmintic activity of fodder plant through *in vitro* and *in vivo* tests.

3.1- Experiment 1: Germination test

Germination study was performed with intact seeds (free from the pericarp). To this end, three groups were constituted and composed of treated and untreated groups. Treated groups consisted of one group which intact seeds were soaked for 24 h in water and another group whose seeds have been mechanically scarification. Scarification was done by making a small nick in the seed coat on the side opposite pole micropilaire respecting the integrity of the kernel using a razor blade.

Seedlings were carried out in Petri dishes filled with a double layer of filter paper moistened to saturation. Three repetitions of 15 seeds were established for each group. Counting of germinated seeds was performed daily and cumulative germination percentages were determined daily to the in ambient temperature of laboratory. A seed was considered germinated when the radicle becomes visible [8]. The germination percentage was calculated according the following formula:

Germination percentage = (number of sprouts / total number of seeds placed in the Petri dish) x 100

3.2- Experiment 2: *In vitro* Test

Living adult worms of *H. contortus* of small ruminant naturally infected were used to perform *in vitro* test. The technique described by Sharma et *al.* [9] was used with modifications [10]. Briefly, adult worms of *H. contortus* were collected from stomachs of small ruminants freshly slaughtered at the abattoir of Koudougou and cleaned before put in a Petri dish containing PBS. After, three (3) adults worms were exposed to each well of a 24 well plate (Becton Dickinson brand) for the treatments at ambient temperature of laboratory (25-30 ° C). The treatments consisted of three increasing concentrations (25, 50 and 100 mg / ml) of the aqueous extract of *C. cajan* and an untreated group using distiller water. Each treatment was repeated three times. Inhibition of motility adult worms was used as the criterion of anthelmintic activity for each of the above treatments. Motility was observed at intervals of 0, 2, 4 and 6 hours. At the end of the test, the treated worms were put in distiller water for 30 min to observe the recovery of motility worms.

3.3- Experiment 3: *In vivo* test

In vivo study was conducted in natural infestation condition from September to October 2012 on sheep supplemented with *C. cajan* fodder. For this, two groups of six sheep were used. Group 1 was supplemented with *C. cajan* fodder at 3 g / kg body weight of animal and group 2 served as a control (not supplemented).

Following parameters were measured in sheep after treatment application:

- fecal egg count reduction at days 0, 7, 14 and 21. To this end, a quantitative coproscopy was made in two animal groups before treatment (day 0) and after treatment (days 0, 7, 14 and 21). The number of eggs per gram of feces (epg) was determined applying Mac-Master method using saturated NaCl (1 egg = 50).
- weight gain of two groups from day 0 to day 21 using a small scale ;
- packed cells volume was estimated from blood samples taken from the jugular vein of animal in each group using heparinized tubes before (day 0) and after treatment (days 7, 14 and 21). Blood samples were put into capillary hematocrit tubes and centrifuged at 3000 rounds per minute for 15 min and packed cells volume was determinate on the graduated hematocrit reader.
- nutritional values of plant fodder according to AOAC [11].and Van Soest et *al.* [12]. methods

4- Statistical analysis

Collected data were used to calculate the mean (± SD) per group before subjected to analysis of variance (one way) to discriminate the effect of treatment applied. Mean separation was performed by the Tukey-Kramer test at 5%. Previously, data from germination tests and anthelmintic activity were transformed (arc sine and logarithmic) to normalize the distribution. All statistical analyzes were performed with the CoStat software, Version 6.204.

RESULTS

1- Effect of seed treatment

The results of the germination test are shown in figure 1. The behavior germination of untreated seeds shows that the carried seeds in experimentation were good. We notify that one day after sowing, the seeds began to germinate at all groups. However, submerged seeds showed germination rates of 73%, significantly higher ($P < 0.05$) at 2% of the seeds from the control group and 27% of those of the group of seeds having undergone mechanical scarification.

From third day, the germination rate has improved for untreated seeds (82%) and scarified seeds (89%) compared to immersed seeds in water for 24 hours (73%). After 8th day, germination rates were 73%, 89% and 91% respectively for submerged seeds, scarified seeds and control seeds. However, no significant differences ($P > 0.05$) were notified between the three experimental groups from the second day after sowing until the end of the experiment.

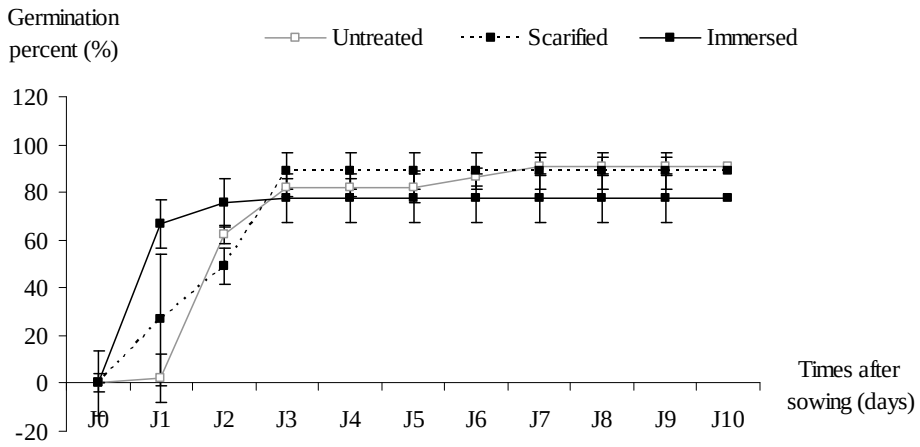

Figure 1: Germination percent evolution of C. Cajan seeds in the three experimental groups

2- Anthelmintic activity of *C. cajan*

2.1- Effect of aqueous extracts on adult worms

The **Figure 2** presents the results of in vitro anthelmintic activity of the plant aqueous extract on the motility or survival of *H. contortus* worms of sheep. Effect of plant extract was dose-dependent. Highest mortality of worms was observed 4 hours post-exposure with 100 and 50 mg/ml. There was 100% mortality of worms in 100 mg/ml concentration after 6 hours post-exposure compared to the extract concentration of 25 mg / ml and control that observed the same mortality percent at this time. There was no mortality of worms kept in distiller water after 6 hours post-exposure.

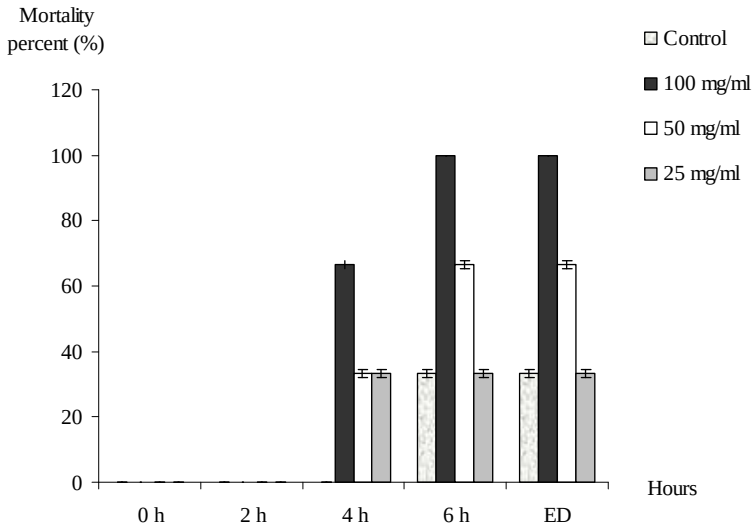

Figure 2:*In vitro* effect of treated (100 mg/ml, 50 mg/ml and 25 mg/ml) and untreated (control) groups during the test

2.2- Effect on excretion of eggs

During the test period, animals have excreted eggs of gastrointestinal parasites in a variable manner after applied treatment (figure 3). Indeed, the percentage of egg excretion in control group has increased (33%) from D0 to D21 unlike the treated group which declined (4.1%).This excretion of eggs ranged from 3316.6 to 3179.1 in treated group and 2662.5 to 3541.6 in untreated group. However, no significant difference ($P>0.05$) of egg excretion percent was notified between the two groups until the end of the test.

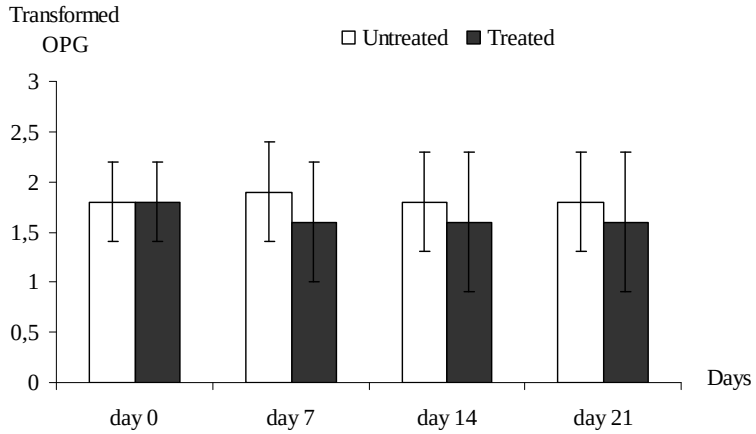

Figure 3: Mean percent of egg excretion in the two groups during ante and post treatments

2.3- Treatment efficacy

Mean percent of egg reduction obtained in the two groups were used to assess the effectiveness of treatment. The results are shown in table 1. The daily distribution of *C. cajan* entailed a decrease of egg excretion at the treated group unlike the untreated group. However, this daily distribution didn't present a statistically different effect (P> 0. 05) between the two groups in these periods.

Table 1: Change in percent (%) of egg reduction in the experimental groups during post treatment period

Groups	Reduction percent (%)		
	day 7	day 14	day 21
Treated	*7.4*	*14.6*	*9.2*
Untreated	*- 1.0*	*- 1.4*	*- 0.6*
Significant	*ns*	*ns*	*ns*

ns: no significant

2.4- Effect on packed cells volume

At the beginning, means of packed cells volume (PCV) were similar in the two groups. After the treatment, PCV increased in the treated (41.1%) and untreated (38%) groups at day 7 before decreasing in the same groups(38.1% and 33.5% respectively) at day 21 (figure 4). However, no significant differences (P> 0.05) were observed between the two groups during post-treatment periods.

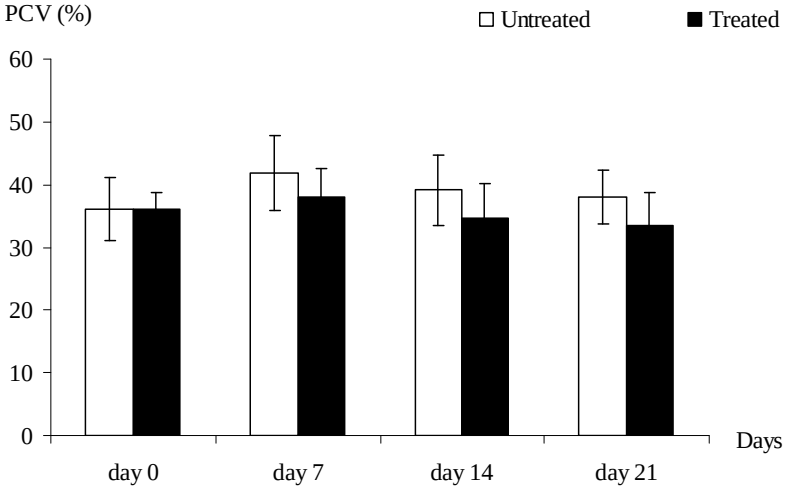

Figure 4: Evolution of PCV in the two groups during the test

2.4- Effect on weight gain

Treated animals consumed all daily quantity of *C. cajan* fodder that were distributed during the test (table 2).

Table 2: Nutritional Values of *C. cajan* distributed to animals in treated group

composition	Content (%)
Dry matter	91,81
Mineral material	8,92
Organic matter	91,08
Total nitrogen	23,36
Crude fiber	25,85

Means of body weight of animals in the two groups knew a variable evolution (figure 5). Animal growth was first decreased from 16 ± 4.1 kg at day 0 to 15.9 ± 3.7 kg at day 7 before increased until day 21 (16.3 ± 4.1 kg) in control group. On the other hand, the treated group increased weight from day 0 (15.7 ± 3.9 kg) to day 14 (16.5 ± 4.0 kg) before declining slightly at day 21 (16.3 ± 4.1 kg). However, statistical analysis didn't reveal a meaningful difference (P>0.05) between the two groups during all post treatment periods. Although the group treated has exhibited high weight gain compared to the control group but there no significant difference (P>0.05) between the two groups at these periods post treatment.

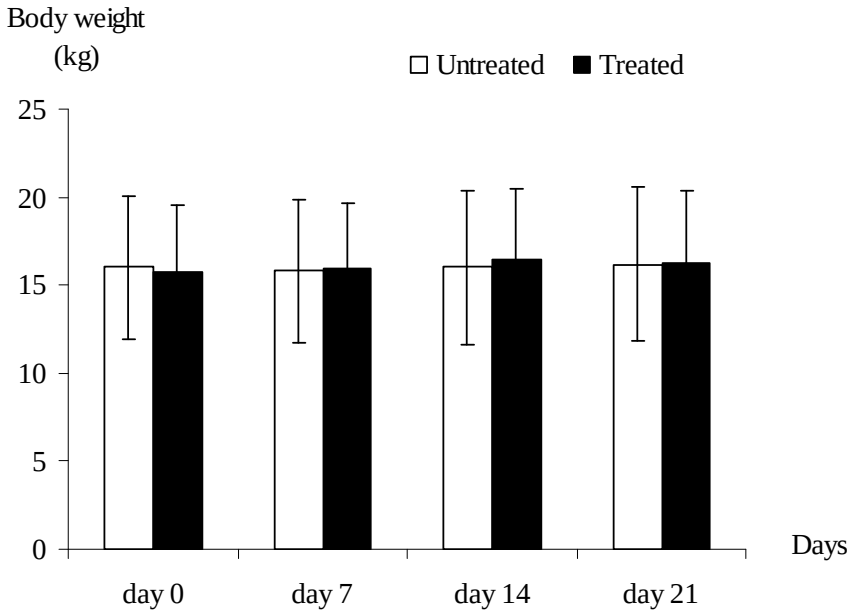

Figure 5: Weight gain evolution in the two groups during the test

DISCUSSION

This study was conducted to find a solution to the problems of management of the gastro-intestinal parasite control in small ruminants that meeting rural famers in Burkina Faso. For these last, animal raising represents a capital solution to fight against poverty and the food insecurity [1]. To help these farmers in their small ruminants activity, the choice of *C. cajan* plant was made because it is drought resistant, an exploitable legume in livestock feed and improving soil fertility under permanent crop conditions [13-14] as rural farming in Burkina Faso. Through this study, we are looking for an integrated solution that takes account of the situation described above. The results of the analysis of the feed of *C. cajan* in our study show that the plant is a legume that can be used as animal additive feed, particularly in ruminants.

Results of the germination test reveal that tested seeds of *C. cajan* have a high germination. This characteristic is similar to that made by Zaman et *al.*[15] with *Tamarisk aucheriana* seeds under desert environment. The fast seed germination (within a day) of scarified and immersed for 24 hours in water lots in our study would be due to the pretreatment applied to the seeds. These treatments weakened the teguments and facilitated water access and oxygen to the embryo and radical protruded. This behavior of *C. cajan* seed treated is specific to several plant species when water is available as strategy to adapt to the stressful conditions. Generally, seeds are dispersed in nature and they germinate immediately if environmental conditions are favorable.

For *in vitro* test, the parasite *Haemonchus contortus* was chosen because it is the animal model commonly used to verify anthelmintic activity of several medicinal plants [16] [17]. Worms' mortality observed during this *in vitro* test shows that aqueous extract of the *C. cajan* leaves would contain chemicals anthelmintic effect. Indeed, the work of Mohanty et *al.* [18] on the phytochemicals characterization of aqueous extract of this plant noted the presence of flavonoids and tannins. However, the anthelmintic activities of these secondary metabolites were demonstrated by Seetharam and Reddy [19] and Hoste et *al.* [20].

Our results obtained *in vivo*, although statistically insignificant corroborate those obtained *in vitro* because leave consumption of *C. cajan* (3 g / kg of body weight) induced a reduction of parasite eggs in the fecal of infested animal. In addition, this consumption leads a weight gain in animal during the period of strong parasitic infestation of the natural pastures compared to the untreated animals. In our context of north Sudanese climate where parasitism leads almost 33% of losses in small ruminants [21], the use of *C. cajan* could be considered in a strategy of gastrointestinal parasitism control among these animals in farming environment to maintain their productivity [22]. Indeed, the feed of the plant is rich in nitrogenous material and therefore it could be indicated to minimize the pathophysiological effects induced by gastrointestinal parasites in animals infected [23]. Furthermore, our belief is reinforced by the fact that *C. cajan* consumption in treated group did not cause a disturbance of PCV during the fodder distribution.

CONCLUSION

In conclusion, the study reveals that the seed has good germination when undergoing a pre-treatment in water for 24 hours or scarification to later produce a good feed for ruminants. Besides, this fodder leads an inhibition of *H. contortus* worm and a reduction of egg parasite excretion in sheep because of secondary metabolites which it contains. Also, the use of forage *C. cajan* could be recommended to fight against gastrointestinal parasites in high parasitic infestation season in farming areas of country. However other studies could be conducted to understand the mechanism of action of the fodder on gastrointestinal parasites to better advise rural herders.

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