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Research Article

ANTHELMINTIC EFFICACITY OF BALANITES AEGYPTIACA (L.) DELILE (ZYGOPHYLLACEAE) ALMONDS ON THREE DEVELOPMENTAL STAGES OF HAEMONCHUS CONTORTUS

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ABSTRACT

Gastrointestinal nematodes are a major constraint on small ruminant farming in Burkina Faso. To overcome this, rural farmers use the *Balanites aegyptiaca* plant to combat gastrointestinal parasites on their family farms. The aim of this study was therefore to investigate the *in vitro* anthelmintic efficacy of the aqueous extract of *B. aegyptiaca* almonds powder against the parasitic nematode *Haemonchus contortus*. To this end, *in vitro* tests were carried out to evaluate the biological activity of the aqueous extract using five increasing concentrations over the three life stages of *H. contortus* by performing the tests (i) inhibition of egg hatch, (ii) inhibition of larva development and (iii) mortality of adult female worms. The results showed 21.22-80.4 %, 47.80-88.57 % and 22.20-100 % of ovicide, larvicide and vermicide rates, respectively. The effects were dose and exposition time dependent. The median lethal inhibition concentrations (IC_{50}) were estimated to be 2.20 and 0.84 mg/ mL for the inhibitionof egg and the inhibition of larva, respectively. It can be concluded that *B. aegyptiaca* almonds powder has anthelmintic activity against *H. contortu*s. However, this activity needs to be verified *in vivo* to reassure farmers about its use in small ruminant raising.

Keywords: *Balanites aegyptiaca*, Anthelminthic, *in vitro*, *Haemonchus contortus*, Small ruminant.

INTRODUCTION

The breeding of small ruminants plays a key role in the economy of Sahelian countries. In Burkina Faso, these animals occupy the second position after poultry farming in terms of numbers. With approximately 10,442,000 sheep and 15,635,000goats recorded in 2018, the breeding of small ruminants contributes significantly to meat production (MRA, 2019). It plays an important socio-economic role but also cultural, with the existence of ceremonies and rituals that take place throughout the year (Belem *et al.*, 2000, Zabré *et al.*, 2017). Despite its importance, this sector still faces food and health constraints that limit animal productivity. Gastrointestinal nematodes are a major health constraint in small ruminants, especially in rural areas (Ouattara et Dorchies, 2001; Belem *et al*., 2005a; Belem *et al*., 2005b; Kamaraj *et al*., 2011). The most dominant parasite is *Haemonchus contortus* (*H. contortus*) with a prevalence rate more than 80% (Belem *et*

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al., 2005b). This parasite causes socio-economic losses to low-income rural family farmers for whom small ruminants play an important role on their exploitation (Kaboré *et al.*, 2011). For a long time, the treatment of these parasitoses has been based on the use of synthetic anthelmintics (Hoste *et al.*, 2011). But, faced with the high cost of treatment and the cases of resistance, the use of new substances such as the exploitation of natural plants with anthelmintic properties would be a promising approach in the fight against gastrointestinal strongyles (Kaboré, 2009; Zabré *et al.*, 2017).

This alternative offers an inexpensive means of control that is available and, above all, accessible to livestock farmers in rural areas of Burkina Faso, where the *Balanites aegyptiaca* plant is widely used in traditional veterinary medicine to treat various pathologies. In Burkina Faso's veterinary pharmacopoeia, the plant is cited by livestock farmers for its anti-parasitic properties (Tamboura et *al.*, 1998; Kaboré, 2009; Abdoulaye et *al*., 2017). Therefore, the aim of the present study is to contribute to the development of traditional veterinary medicine in Burkina Faso. Specifically, the aim is to assess the in vitro anthelmintic activity of the aqueous extract of *B. aegyptiaca* almonds on three developmental stages of *H. contortus* (eggs, larvae and adult worms), in order to support livestock farmers in the breeding of small ruminants.

MATERIALS AND METHODS

Sample Collection

Almonds of *Balanites aegyptiaca* were collected from traditional practitioners in Ziniaré (Burkina Faso) on May 19, 2020 and stored in plastic bags. These almonds were then ground using a mini-grinder at the Institute for Research in Health Sciences (IRSS) and 500 g of the powder was packaged in a transparent plastic bag for biologicals test. To obtain the adult worms of the *H. contortus* parasite, small ruminant abomasums were collected in the slaughterhouses of the municipality of Saaba and taken to the laboratory to collect the worms in order to carry out the in vitro tests. As for the *H. contortus* parasite, small ruminant abomasums were purchased from the slaughter area of the municipality of the rural commune of Saaba in Burkina Faso before being transported to the laboratory to collect adult worms in order to carry out the in vitro tests.

Preparation of extract

Plant extract was prepared in accordance with the practice of farmers in rural areas. To this end, an aqueous maceration was prepared with *B. aegyptiaca* almonds powder using 1 g of powder which was then macerated in 20 mL of distilled water. The mixture was homogenised on a hot plate to obtain a stock solution of 50 mg/mL. From this stock solution, cascade dilutions were made to obtain five increasing concentrations (0.625 - 1.25 - 2.5 - 5 and 10 mg/mL) for the in vitro tests.

Collection *H. contortus* eggs

The test was carried out according to Jabbar *et al* (2006b). Abomasums purchased from the slaughter area were and kept in a cooler before placed in Petri dishes in the laboratory and washed with water before being lengthwise. The worms contained in the abomasum were emptied into another Petri dish to identify the *H. contortus* females before sorting and rinsing with PBS. These selected females were then placed in a porcelain mortar and lightly crushed with a pestle to release the eggs. The obtained ground was diluted with PBS and filtered through 1 mm and 100 µm sieves. The released eggs were collected in a 38 µm sieve, rinsed several times with PBS before being collected in a 15 mL tube. 110 µL of the egg solution were placed on a slide and observed under a microscope (x40) in order to count the number of eggs. The egg solution was adjusted to approximately 200 eggs/mL solutions.

Collection of adult worms

Adult worms were collected after making a longitudinal cut in sheep abomasums collected at the slaughter area in the rural commune of Saaba. The abomasums were then rinsed with distilled water to collect the adult worms in a Petri dish where the *H. contortus* females were identified and used for the biological test on the adult worms.

Biological tests applied

The biological tests to inhibit egg hatching and larval development and the adult worm mortality test were carried out as follows:

Egg hatch inhibition test (EHT)

Egg hatch inhibition test were carried out according to Coles et *al.*(2006). For this, 100 µL of egg suspension was put in the wells of a 24-well plate.100 µL of aqueous extract of *B. aegyptiaca* almonds powder of increasing concentrations (1.25 - 2.5 - 5 - 10 and 20 mg/mL) was added to each well. A control was used as negative with PBS (Phosphate Salt Buffer, pH: 7.2). The plates were closed and placed in an incubator at 27°C for 48 h. After 48 h of incubation, three drops of formalin (10%) were placed in each well to stop the eggs hatching. Three replicates were performed per used sample as well as the control. The number of L1 larvae and eggs was counted under the microscope (x 10) and the percentage hatching was calculated using the formula:

$$\text{EHT} = \frac{nomberofL1}{\text{nomber of eggs} + \text{nomber of L1}} * 100$$

Larval Development Inhibition Test(LDT)

The method used is that of Vernerova *et al.* (2009). 100 µL of egg suspension was placed in each well of a 96-well plate and incubated at 27°C for 24 h. After 24 hours of incubation, 50 µL of a nutrient solution and 100 µL of

aqueous extract of *B. aegyptiaca* almonds powder of increasing concentrations (0.625 - 1.25 - 2.5 - 5 and 10 mg/mL) were added to the larval solution. Plates were incubated at 27°C for 6 days. A reference control (distilled water) was constituted. After six days of incubation, the test was stopped by adding two to three drops of formalin (10%) to each well. Each used samplewas repeated 3 times as well as the control. The percentage of larval development was calculated according to the formula:

$$\mathbf{LDA} = \frac{\boldsymbol{nomberofL3}}{\mathbf{nomberofL1 + L3}} * \mathbf{100}$$

Adult Worms Mortality Test (AMT)

Mortality test was performed according to Jackson and Hoste (2010). The test was carried out using Petri dishes containing 3 adult worms per concentration. In each Petri dish, worms were brought into contact with increasing concentrations of *B. aegyptiaca* (0.625 - 1.25 - 2.5 - 5 and 10 mg/mL) plus a reference control of distilled water. Dishes were incubated at room temperature and read using microscope at 0h, 2h, 4h and 6h to enumerate dead and alive worms according to Skantar et *al.* (2005). They were considered dead when they did not show any movement and as alive when there were at least some tails, head or pharyngeal movements (during 10 s of observation).

Statistical Analyses

Data collected was put on Excel version and underwent a logarithmic transformation (log(x+1)) to normalise them before being analysed statistically. Then, the means ofpercentages of inhibition were calculated before being subjected to a variance analysis with SPSS software at the significance level of 5%. The effect of concentrations was determined by the non-parametric Kruskall-Wallis test. The 50% inhibitory concentrations (IC_{50}) were calculated by Probit-analysis with Polo plus Software (Version 1.0) for Windows. The data collected were formatted in Excel and underwent a logarithmic transformation (log(x+1)) to normalise them before being analysed statistically. Then data were used to calculate the mean percentages of inhibition before being subjected to analysis of variance for comparison at the significance level of 5%. The effect of tested concentrations was determined by the non-parametric Kruskall-Wallis test at 5%. The 50% inhibitory concentrations (IC_{50}) were calculated by Probit-analysis with Polo plus Software (Version 1.0) for Windows.

RESULTS AND DISCUSSION

The use of bioactive plants as an alternative treatment to chemical anthelmintics is one approach that could reduce the development of parasite resistance. Thus, the present study was conducted with the aim of finding a palliative solution to the problems of gastrointestinal parasitosis of small ruminants in Burkina Faso. In this study, aqueous extracts of *B. aegyptiaca* almonds powder were used to evaluate their anthelmintic activity on eggs, larvae and adult female worms of *H. contortus* through three *in vitro* tests: egg hatch inhibition test (EHT), larval development inhibition test (LDT) and adult worms mortality test (AMT). Figure 1 shows the percentage of inhibition of egg hatching at different concentrations for aqueous extract of *B. aegyptiaca* almond powder which vary from 21 to 80%. These concentrations of aqueous extract of *B. aegyptiaca* significantly inhibited ($p<0.05$) eggs hatching of *H. contortus* compared to the control (14.61 ± 2.8%).

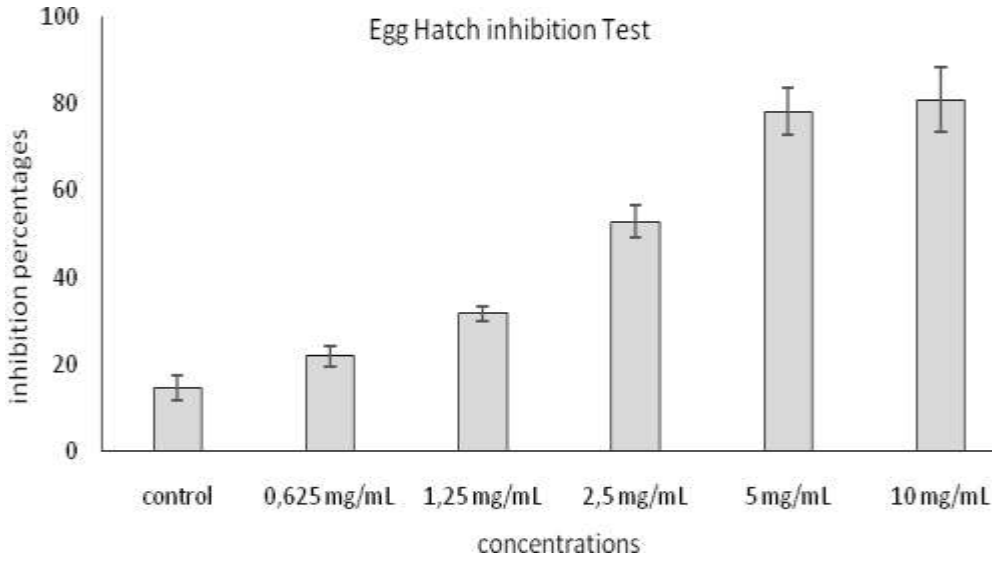

Figure 1 .Effects of five aqueous extract concentrations of *B. aegyptiaca* almond owder and the negative controlon egg hatch inhibition test (EHT).

In addition, they acted in a dose-dependent manner (n = 5; ddl = 4;$p<0.05$) by applying the Kruskall-Wallis test. This dose-dependent behaviour of the aqueous extract of *B. aegyptiaca* almond in the study is similar to the observations of Shaddad *et al.* (2013) with the aqueous extracts of *B. aegyptiaca* almond. Similarly, Assefa et *al.* (2017) recorded a dose-dependent effect with the plant's condensed tannins. Figure 2 shows the percentages of inhibition of larval development (LDT) at different concentrations for aqueous extract of *B. aegyptiaca* almond and the negative control. The aqueous extract of *B. aegyptiaca* significantly inhibited larval development ($p<0.05$) compared to the negative control (15.76 ± 5.84%) with percentages varying from 47 to 88%. Concentrations of 5 and 10 mg/mL showed a percentage of inhibition more than 80%. The percentages of inhibition increased significantly ($p<0.05$) with increasing concentrations (n = 5; df = 4; $p<0.05$).

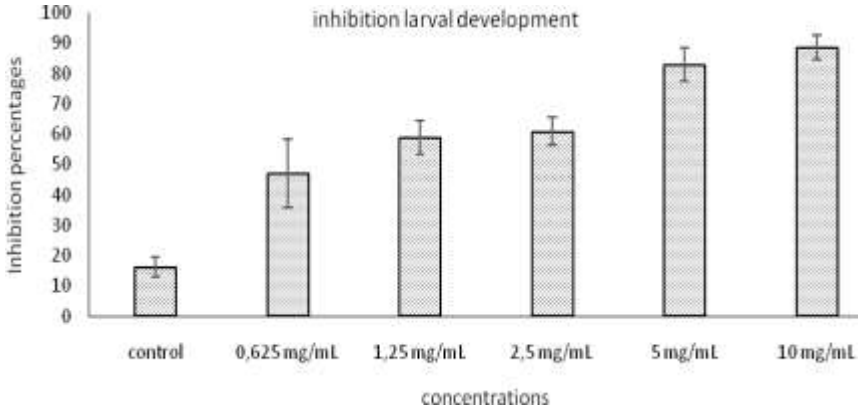

Figure 2. Effects of five aqueous extractconcentrations of *B. aegyptiaca* almond powder and the negative control on inhibition larval development (IDL).

The larval development inhibition test showed that larvae were more sensitive to *B. aegyptiaca* almond powder extract compared to eggs and adult worms. This sensitivity would be linked to their physiology. We remarkable dose dependent larval development inhibition in the study. Also, efficacy of concentrations revealed potential larvicid activity compared to the results of much previous study for other plant. In fact, Maciel *et al.*, 2006 recorded activity on larvae (67.90 ±39.55%) in the strongest concentration (50mg/mL). Marie-Magdeleine *et al.*, 2009 recorded larval development inhibition at all concentrations of *C. moschata* seed extracts compared with negative control. Assis *et al.*, 2003 tested *Spigelia anthelmia* extracts on larval development of *Haemonchus contortus* and the result show that at 50 mg ml−1, the ethyl acetate and methanolic extract inhibited respectively 81.2% and 84.4% of the larval development. Figure 3 shows the mortality rates of adult *H. contortus* worms incubated with the concentrations of aqueous extract of *B. aegyptiaca*. The extract showed a significant increase ($p<0.05$) in the mortality rate of adult worms compared to the control (distilled water). From 0 h and 2 h, no mortality was recorded in the group excepted the concentration of 10 mg/mL with 11% of mortality. The highest mortality rates (100%) were obtained after 6 h of incubation with concentrations of 5 and 10 mg/mL.

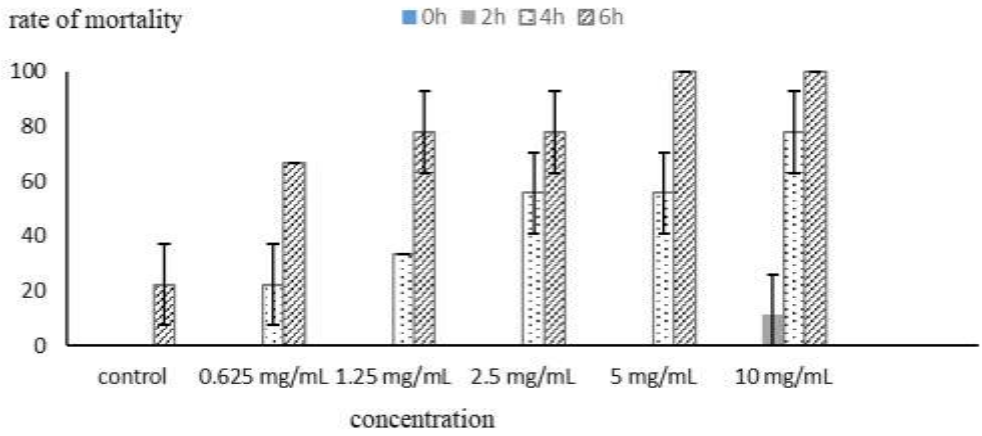

Figure 3. Percentage of adult *H. contortus* mortality at different concentrations.

In our study, no mortality was observed from 0 h to 2 h after incubation of adult worms with *B. aegyptiaca* extract. Our results are different from those of Ibrahim (1992) who obtained at 2.5 mg/mL, 45% of mortality after 2 hours of incubation with aqueous extract of leaves and almonds of *B. aegyptiaca* on *Caenorhabditis elegans*. By incubating the adult worms with the different concentrations, a high mortality parentage was recorded from the 4th hour, especially for the two highest concentrations (5 and 10 mg/mL), thus showing a vermicidal activity of *B. aegyptiaca* almond powder aqueous extract. Previous work has shown the anthelmintic efficacy of *B. aegyptiaca* almond on gastrointestinal strangles (Gnoula, 2007). Shaddad *et al.* (2013) also reported adult worm mortality rates of 20%, 40%, 40% and 90% for exposure times of 1, 3, 6 and 12 hours respectively, to the aqueous and methanolic extract of *B. aegyptiaca* almond. Table 1 presents the inhibition concentrations (IC_{50}) for egg hatch and larval development. These IC_{50} obtained varied according to the tests carried out. For the EHT,IC_{50} was 2.199 mg/mL and for the LDT, IC_{50} was 0.838 mg/mL. In general, IC_{50} were less than 2.5 mg/mL that mean the efficacy of the plant.

Table1. Inhibition concentrations (IC_{50}) for EHT and LDT (mg/mL).

Tests applied	IC_{50} (mg/mL)		
	Lower limit	IC_{50}	Upper limit
EHT	1,804	2,199	2,659
LDT	0,502	0,838	1,168

EHT = Egg hatch inhibition test LDT = Larval development inhibition test

In the three test of the study, the larval development inhibition test showed that larvae were more sensitive to *B. aegyptiaca* almond powder extract compared to eggs and adult worms. This sensitivity would be linked to their physiology. Indeed, worms and eggs have coatings capable of protecting them against external aggressions. Phytochemical studies have shown that *B. aegyptiaca* almond contains secondary metabolites such as tannins, saponosides, flavonoids (Hosny *et al.*, 1992; Gnoula, 2007).Condensed tannins have been identified as the main compounds responsible for the anthelmintic effect observed in many plants (Assefa *et al.,* 2017; Koffi *et al.,* 2018). Several studies have shown the mode of action of condensed tannins on the stages of development of gastrointestinal strongyles. Indeed, Perry (2002) showed that condensed tannins would be able to bind to the lipoproteins of the eggshell membrane to inhibit osmotic pressure in egg, thus compromising hatching. According to Lem *et al.* (2014), the condensed tannins would be able to penetrate the nematode cuticle and prevent the absorption of glucose, or block the post-synaptic receptors, thus paralyzing the larvae. On adult worms, condensed tannins would act on the digestive epithelium of the parasite to inhibit the functions of nutrition and lead to the death of the latter (Min *et al.,* 2003). All these différences observations suggest that the anthelmintic activity of the extract in the study could be attributed to the individual or combined action of the bioactive compounds contained in the almond.

CONCLUSION

The objective of this study was to contribute to improving the health status of small ruminants through the use of a peasant innovation based on *Balanites aegyptiaca* almond in Burkina Faso. The results obtained after the *in vitro* tests showed an ovicidal, larvicidal and vermicide activity of *B. aegyptiaca* almond on *H. contortus*, the larvae having been more sensitive than eggs and adult worms. This effectiveness has been attributed to the presence of secondary metabolites like condensed tannins, flavonoids, saponosidesetc contained in the extract. Thus, we can say that the incorporation of *Balanites aegyptiaca* almond could be recommended as an alternative and sustainable solution in the management of gastrointestinal parasitosis in Burkina Faso.

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