In vitro effects of maize silage extracts on *Haemonchus contortus,* gastrointestinal nematode parasite in Red Maradi Goats

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1 ABSTRACT

In tropical environments, goat rearing faces numerous challenges especially those related to poor feeding and gastrointestinal nematodes infections. This present study aimed at assessing the *in-vitro* effects of maize silage on *Haemonchus contortus*, a gastrointestinal nematode in Red Maradi goats in Benin. After planting and harvesting the maize at 6 and 8 weeks old, the Maize stalks were used to produce silage. The silage was then reduced into powder and extracted using distilled water, hydroethanol and chloroform. Extracts solutions were then prepared at different concentrations and put in contact with *H. contortus* infesting larva and adult worms. Both 6- and 8-week-old maize silages significantly inhibited *H. contortus* larva migration (P<0.05) and adult worms' motility (P<0.05). The effect on the larval migration depended on the solvents (P<0.05) and the concentrations (P<0.05) but was only concentration-dependent (P<0.05) on the adult worm motility. The highest larval migration inhibition rate (52.25%) was recorded with the 8-week-old maize silage. Among 6-week-old maize silage extracts, the best larval migration inhibition (LMI) rates were recorded at 1200 µg/mL of the hydroethanolic and chloroformic extracts (72.46% and 67.00% respectively). As regard the 8-week-old maize silage, the best LMI rates (72.49% and 71.07%) were respectively observed in the aqueous extracts (600 µg/mL) and the hydroethanolic extracts (150 µg/mL). The inhibition rate of adult worms' motility, 6 hours after incubation, ranged from 33.33% to 66.67% and was of 100% after 12 hours of incubation. These inhibitory effects are attributed to chemical compounds contained in the extracts such as alkaloids, anthocyanins, reducing compounds, mucilages and triterpenoids. This potentiality of the maize silage could be further considered while designing a sustainable programme to control *H. contortus* in goats, particularly in dairy goats as Red Maradi goats.

2 INTRODUCTION

Small ruminants farming is a traditional activity practiced by 60 to 87% of local populations, either as a main activity or as a secondary activity. Goats are important in subsistence farming because of their unique ability to adapt and maintain themselves under difficult environmental conditions. As sources of raw materials for the agricultural industries, their manure is also used in biogas production (Adua and Hassan, 2016) and fertilizers for plant production. Beside their use in teaching and research, small ruminant also play important sociocultural roles that are difficult to quantify in monetary terms (ceremonies and festivals, sacrifices and rituals), serve as insurance against poor crop harvest (Hassan *et al.*, 2013). Due to their short reproductive internal compared to cattle, goats are recognized for their meat production capabilities for human consumption which is not subject to any religious taboos. Despite all these benefits, low performance (meat and milk production) and high rate of mortality are the most important difficulties that face West Africa farmers nowadays. In rural areas, health problems are the more evident and prevalent in goat production, particularly those related to gastrointestinal nematodes (GINs) (Hounzangbe-Adote *et al.*, 2005) whose infections impair animal health, welfare and productivity for their presence results in increased mortality rate, poor growth and reproduction (Andrea *et al.*, 2011; Mirhadi *et al.*, 2011). Among those gastrointestinal nematodes, *Haemonchus contortus* is the most important (Jiménez *et al.*, 2010; Khan *et al.*, 2010) causing significant growth reduction rates in goats in tropical environments (Bizimenyera *et al.*, 2008; Mirhadi *et al.*, 2011). Red Maradi goats, a foreign breed of goats newly introduced in Benin Republic, are more affected by GINs infections due to their low adaptation to the local environmental conditions. In northern Benin Republic, as in many parts of the world, the repeated use of chemical drugs is the usual and main mean by which GINs infections are controlled in goats (Knox *et al.*, 2006; Torres-Acosta and Hoste, 2008) as well as in Red Maradi goats. However, the inaccessibility to those chemical drugs for subsistence and small-scale livestock farmers in developing countries and the resistance development of the parasites against chemical drugs are the major factors that limit the use of chemical drugs. For many decades, alternatives methods of control have been identified and are still under investigations. Biological control, vaccine, improvement of food quality of host and the use of traditional medicinal plants are approaches that have been investigated by researchers from different parts of the world (Knox *et al.*, 2006; Wabo *et al.*, 2012). The effect of energy source supplements such as maize grains and forage maize on the development of resistance and resilience in goats against GINs has already been assessed in several studies; but very few was conducted on the potential anthelmintic properties of maize silage. Mainly used as feed resource for dairy ruminants such as Red Maradi goats, maize silage is well recognized for its nutritional values that enhance dairy productivity in ruminants. Its use in controlling GINs might present a double benefit (feeding and deworming) for subsistence and small-scale farmers. Therefore, the objective of this current study was to assess the *in-vitro* effects of maize silage on *Haemonchus contortus* collected from artificially infested Red Maradi Goats.

3 MATERIALS AND METHODS

3.1 Framework of study: The culture of larvae of *H. contortus* and the *in-vitro* biological tests took place at the Laboratory of Ethnopharmacology and Animal Health (LESA) of the Faculty of Agronomic Sciences (FSA), University of Abomey-Calavi (UAC) that is located in Abomey-Calavi (Altitude 17.4 m, 06°24'N, 02°20'E) in southern Benin Republic. The town is characterized by a Guinean type climate with two dry seasons (mid-November to mid-March and mid-July to mid-October) and two rainy seasons (mid-March to mid-July and mid-October to mid-November).

3.2 Maize silage production: For this study, maize seeds (variety EVTD) were purchased at the International Institute of Tropical Agriculture (IITA) at Abomey-Calavi. EVDT is an improved variety of maize developed by the West African Agricultural Productivity Project (WAAPP-Benin) through its Regional Centre of Specialization in Maize. Today, this variety is one of the most used by smallholding farmers in Benin Republic. On the same site, at the Production and Research Farm of the Faculty of Agricultural Sciences located at Sékou (Southern Benin Republic), two maize farms were established according to the harvesting stage considered: 6- and 8-week-old maize farms (plate 1). At these stages, the maize stems and leaves were harvested, chopped and cut into small pieces, then dried for 48 hours under shade in the laboratory for reducing the moisture content. Then, they were separately put in containers for the silage production phase, which lasted three weeks. The maize silages obtained were dried using an oven and ground to get the powders (plates 2 and 3).

Plate 1: Six week-old maize farm (left) and eight week-old maize farm (right)

Plate 2: Harvested maize leaves and stems (left) and leaves and stems cut and shopped into small pieces (right)

Plate 3: Chopped leaves and stems in the hole (left) and maize silage powder (right)

3.3 Animal management and prophylaxis: Ten young Red Maradi goats (average weight 12 kg) were acquired and used as donors both for larva culture and the adult worm collection. They were properly housed in a sheepfold and dewormed with albendazole (7.5 mg/kg body weight). Throughout the experiment, the goats were fed hay of *Panicum maximum* var. C_1 in the morning, concentrates (wheat brans, cotton seeds and palm kernel cake) with cassava peels in the afternoon, and were all provided lick stone and ad-libitum water. Faeces were collected every three days and analysed to establish the parasitic burden of the goats using the Mini-Flotac technique. Deworming was ended when the number of eggs per gram of faeces was null (equal to zero). The animals were then artificially infested with 2000 pure-source larva of *Haemonchus contortus*. Twenty-one (21) days after artificial infestation, faeces were collected daily using a specific clothe for larva culture in the laboratory using the Baermann device.

3.4 Phytochemical analysis of maize silages: Samples of maize silage powders and extracts were subjected to phytochemical analysis at the Laboratory of Pharmacognosy and Essential Oils of Porto-Novo, southern Benin Republic.

3.5 Biological tests techniques

3.5.1 Extraction technique: From each lot of maize silage powder, fifty grams (50g) was collected and mixed with 500 mL of the solvent. The volumes and solvents used were 500 mL of distilled water, 500 mL of chloroform and a 70:30 mixture of ethanol and distilled water (350 mL and 150 mL respectively). The extractions were carried out by maceration. The silage powders and solvents were mixed and macerated using a magnetic stirrer for two hours. Then, the mixtures (powder and solvents) were filtered using absorbent cotton and the filtrates were collected and evaporated under vacuum using an evaporator. The aqueous phases obtained were put in an oven at 40°C for 2 days for drying by evaporation. The extracts obtained were put in a box and kept in the laboratory at room temperature.

Plate 4: Maize silage extracts (obtained from the oven)

3.5.2 Larval Migration Inhibition Test (LMI)

3.5.2.1 Preparation of the larvae solutions (L_3): The L_3 larvae were obtained by culture from faeces of the donor goats previously infested artificially by pure strains of *H. contortus*, kept in culture in the laboratory for 10 days. They were then collected from the device, counted and stored in small boxes in fridge.

3.5.2.2 Test of Larval Migration Inhibition (LMI): 2000/mL of L_3 larvae solution was in contact, for 3 hours at 20°C, with the extracts at four different concentrations (150, 300, 600 and 1200 µg/mL) with 6 replicates per concentration. Negative (PBS buffer, pH=7 and 0.15M) and positive controls (Levamisole at 250µg/mL) were used to evaluate larval migration without the extracts. 200 µL of the mixture (larvae and extract solution) were collected from each replicate for determining the number of larvae. Then, the following formula was used to calculate the percentage of *larval migration inhibition (LMI in %)* according to Alowanou (2016):

$$LMI = \frac{T - M}{T} x100$$

Where **T** is the total number of L_3 larvae that have been in contact with PBS and **M** the number of L_3 larvae in contact with silage extracts or Levamisole.

3.5.2.3 Adult worm motility test: The solutions of the extracts were prepared using Phosphate Buffered Saline (PBS) in six (06) different concentrations (75; 150; 300; 600; 1200 and 2400 µg/mL) with 3 replicates per concentration. Two (02) Red Maradi goats were selected among those artificially infested after faeces analysis, then slaughtered. The adult worms were directly collected from their abomasum and poured into a physiological liquid. The worms having good motility were each placed in 1 mL of physiological fluid in wells of NUNC plates and put in oven at 37°C. After one hour, a volume of 800 µL of physiological fluid was removed and replaced by the extracts. Negative (PBS solution) and positive controls (Levamisole at 500; 250 and 125 µg/mL) were constituted. After introducing the worms into the solutions, the motility was observed with a magnifying glass every 6 hours. The observation stopped when the immobility of all the worms contained in the PBS was observed.

3.6 Statistical analyses: The different values collected (data on the rates of larval migration and adult worms' motility inhibition) were analysed using a two-step custom-made analysis of variance model (concentrations/doses and extracts) following the general linear model (GLM) in Minitab version 18. The average inhibition rates per concentration and extract were separated using LSD test. The level of significance of the differences was considered at 5%.

4 RESULTS

4.1 Phytochemical analysis of maize silages: Table 1 below shows the results of the phytochemical analysis of both the maize silages powders and extracts. Several chemical compounds were found in the maize silage powders: alkaloids, mucilages, triterpenoids, reducing compounds, cyanogenic derivatives and anthocyanin. However, the alkaloids were more abundant in the 6-week-old maize silage than that of 8 weeks. Mucilages and triterpenoids were abundant in both silages while the anthocyanins were only observed in the 6-week-old maize silage. The reducing compounds were only present in the 8-week-old maize silage while the cyanogenic derivatives were observed in abundance only in the 6-week-old maize silage. However, tannins, flavonoids, steroids and saponosides were totally absent in both maize silages powders. On the other hand, the phytochemical analysis of the extracts revealed the presence of alkaloids, reducing compounds, mucilages and triterpenoids in all the extracts even though the alkaloids were more abundant in the 6-week-old maize silage extracts and the reducing compounds more abundant in the 8-week-old maize silage extracts. Mucilages and triterpenoids were abundant in all the extracts. However, tannins were only present in the 6-week-old maize silage extracts with higher concentration in the hydroethanolic extract. Furthermore, the steroids were present in all the extracts except in the chloroformic extract of the 6-week-old maize silage. Finally, the anthocyanins were abundant in all the extracts except in the aqueous extracts of both maize silages.

Table 1 Phytochemical composition of maize silages powders and extracts

Chemical compounds searched	Maize silage powders		Maize silage extracts					
	6-week-old	8-week-old	6CHL	6DW	6DWE	8CHL	8DW	8DWE
Galician tannin	-	-	-	+	++	-	-	-
Catechin tannin	-	-	-	-	-	-	-	-
Anthocyanins	+	-	++	-	++	++	-	++
Leuco-anthocyanins	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-
Alkaloids	++	+	++	++	++	+	+	+
Reducing compounds	-	+	+	+	+	++	++	++
Mucilages	++	++	++	++	++	+	++	++
Saponosides	-	-	-	-	-	-	-	-
Cyanogenic derivatives	++	-	-	-	-	-	-	-
Triterpenoids	++	++	++	++	++	++	++	++
Steroids	-	-	-	+	+	+	+	+
Cardenolides	-	-	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-	-	-
Quinone derivatives	-	-	-	-	-	-	-	-
Athracenics free	-	-	-	-	-	-	-	-
O-Glycosides	-	-	-	-	-	-	-	-
C-Glycosides	-	-	-	-	-	-	-	-

Legends: -: Absent; +: Present; ++: Abundant.
6DW: Aqueous extract of 6-week-old maize silage; 6DWE: Hydroethanolic extract of 6-week-old maize silage; 6CHL: Chloroformic extract of 6-week-old maize silage;
8DW: Aqueous extract of 8-week-old maize silage; 8DWE: Hydroethanolic extract of 8-week-old maize silage; 8CHL: Chloroformic extract of 8-week-old maize silage.

4.2 Larval migration inhibition Test (LMI): The maize silage extracts as well as the positive control (Levamisole) exhibited a significant *in-vitro* inhibitory effect on the migration of *H. contortus* larva (P <0.05). Extracts and tested concentrations (150, 300, 600 and 1200 µg/mL) had all significant effects on larva migration (P<0.05). The inhibition rate regardless of the extract ranged from 17.80% to 72.46% with the 6-week-old maize silage and from 22.69% to 72.49% with the 8-week-old maize silage. The inhibition rate was 100% in the positive control (Levamisole). The average inhibition rates of *H. contortus* larva migration were 52.25% and 47.51% respectively for the 8- and 6-week-old maize silages. A significant effect of the interaction between extracts and concentrations tested was also recorded (P<0.05) (figure 2). At 1200 µg/mL, the hydroethanolic and chloroformic extracts of the 6-week-old maize silage had given the best inhibition rate (72.46% and 67.00% respectively) followed by aqueous extracts of 8- and 6-week-old maize silages powders (respectively 51.61% and 36.53%). At D600, the highest inhibition rate (72.49%) was recorded with the aqueous extract of 8-week-old maize silage. While at 300 µg/mL, the inhibitory effect remained the same with the highest levels recorded in hydroethanolic extracts of the 8-week-old maize silage (66.89%) and the chloroformic extracts of 8- and 6-week-old maize silages (63.43% and 62.36% respectively). Also, at 150 µg/mL, the inhibitory effect remained almost the same as the previous concentration (D300) except the aqueous extract of 6-week-old maize silage which showed a significant increase in the inhibition rate compared to 300 µg/mL (56.23% against 27.06%). Besides, inhibition of larval migration was solvent-dependent (P<0.05). Regardless of the concentrations, the chloroformic extract of 6-week-old maize silage and the hydroethanolic extract of 8-week-old maize silage showed the best inhibition rate (56.30% and 55.44% respectively).

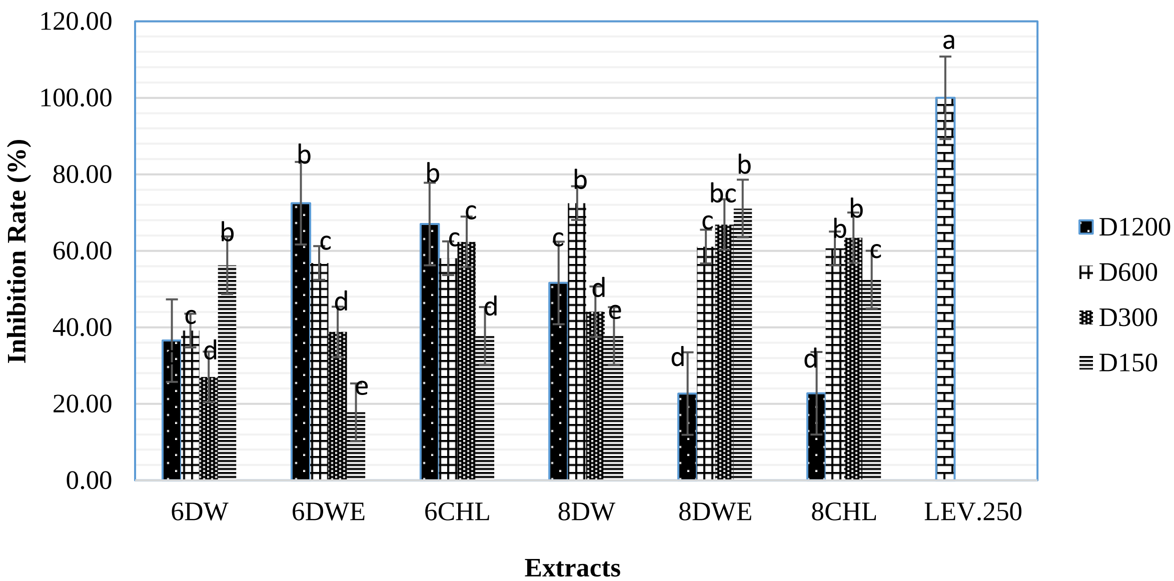

Figure 2: Variation of *Haemonchus contortus* larvae migration inhibition according to the extracts concentrations

a, b, c, d and e: means bars with different superscripts (letters) are significantly different à 5%.

6DW: Aqueous extract of 6-week-old maize silage; 6DWE: Hydroethanolic extract of 6-week-old maize silage; 6CHL: Chloroformic extract of 6-week-old maize silage; 8DW: Aqueous extract of 8-week-old maize silage; 8DWE: Hydroethanolic extract of 8-week-old maize silage; 8CHL: Chloroformic extract of 8-week-old maize silage and LEV.250: Levamisole at the concentration of 250 µg/mL.

4.3 Test of inhibition of adult worms' motility: Adult worms of *H. contortus* in contact with maize silage extracts at different concentrations (2400; 1200; 600; 600; 300; 150 and 75 µg/mL) were all immobilized after 6 hours of incubation, except the chloroformic extract of 6-week-old maize silage (Table 2). In contrast, during the same time, complete inhibition of worms' motility was observed in the levamisole at all tested concentrations (500, 250 and 150 µg/mL). Inhibition of adult worms' motility was highly influenced by the concentration ($P<0.05$) while the extracts as well as their interaction had no significant effects ($P>0.05$). However, both maize silages significantly inhibited the motility of adult worms of the parasite compared to the negative control (PBS). In the PBS solution, 66.67% inhibition of the adult worms' motility was recorded after 24 hours of incubation and all the worms were immobilized 36 hours after incubation. At 6 hours of incubation, a reduction in motility of adult worms was observed only in the hydroethanolic extract of 6-week-old maize silage while all the extracts of 8-week-old maize silage showed an inhibitory effect on motility of adult worms during the same time period (Table 2). This effect was concentration-dependent ($P<0.05$). Regardless of the type of silage and extract (solvent), only the high concentrations (2400, 1200 and 600 µg/mL) had significant inhibitory effects on the motility of adult *H. contortus* worms. The rate of inhibition of motility of adult worms ranged from 33.33% to 66.67% 6 hours after incubation. However, at the level of the 8-week-old maize silage, only 2400 and 600 µg/mL had significant inhibitory effects on the motility of adult worms of the parasite. The chloroformic extract of the 8-week-old maize silage at 600 µg/mL reduced the motility of adult worms to 33.33%. This same extract, but at 2400 µg/mL had a similar inhibition rate (33.33%). It can therefore be concluded that the chloroformic extract of the 8-week-old maize silage at the 600 µg/mL and the ethanol extract of the 6-week-old maize silage at the same concentration (600 µg/mL) showed the best result in terms of *in-vitro* effects on the motility of adult worms of *H. contortus*.

Table 2 Effects of extracts of maize silages on H. contortus adult worms' motility during 36 hours of incubation

Treatments	Solvents	Con. (µg/mL)	Times (Hours)					
			6	12	18	24	30	36
PBS	-		100	100	100	33.33	33.33	0
LEV.	-	500	0	0	0	0	0	0
		250	0	0	0	0	0	0
		125	0	0	0	0	0	0
6MS	Aqueous extracts	2400	33.33	0	0	0	0	0
		1200	66.67	0	0	0	0	0
		600	100	0	0	0	0	0
		300	100	0	0	0	0	0
		150	100	0	0	0	0	0
		75	100	0	0	0	0	0
	Hydroethanolic extracts	2400	33.33	0	0	0	0	0
		1200	33.33	0	0	0	0	0
		600	66.67	0	0	0	0	0
		300	100	0	0	0	0	0
		150	100	0	0	0	0	0
		75	100	0	0	0	0	0
	Chloroformic extracts	2400	100	33.33	0	0	0	0
		1200	100	0	0	0	0	0
		600	100	0	0	0	0	0
		300	100	0	0	0	0	0
		150	100	0	0	0	0	0
		75	100	0	0	0	0	0
8MS	Aqueous extracts	2400	66.67	0	0	0	0	0
		1200	100	0	0	0	0	0
		600	100	0	0	0	0	0
		300	100	0	0	0	0	0
		150	100	0	0	0	0	0
		75	100	0	0	0	0	0
	Hydroethanolic extracts	2400	33.33	0	0	0	0	0
		1200	100	0	0	0	0	0
		600	100	0	0	0	0	0
		300	100	0	0	0	0	0

		150	100	0	0	0	0	0
		75	100	0	0	0	0	0
	Chloroformic extracts	2400	66.67	0	0	0	0	0
		1200	100	0	0	0	0	0
		600	66.67	0	0	0	0	0
		300	100	0	0	0	0	0
		150	100	0	0	0	0	0
		75	100	0	0	0	0	0

Legends: PBS =Phosphate Buffered Saline; LEV. =Levamisole; 6MS =Silage of 6-week-old maize; 8MS =Silage of 8-week-old maize; Con. = Concentrations.

5 DISCUSSION

5.1 Phytochemical composition of maize silage: The phytochemical analysis of the maize silage powders and extracts revealed different results. Indeed, the phytochemical of the extracts showed more chemical compounds than the powders. This might be due to the presence of plant debris in the powders since the extraction process allows to purify the powder before evaporating the solvent. Since both the maize plants were produced on the same soil (types and fertility) following the same production techniques and their silages made using the same materials and following the same process, differences between extracts could due to the age of the plants. The 6-week-old and 8-week-old belong respectively to the vegetative stage and the pre-flowering stage of the maize variety "EVTD". This may affect their contents in secondary metabolites. The 8-week-old maize silage extracts contained more reducing compounds than those of 6-week-old maize silage while the alkaloids were more abundant in the 6-week-old maize silage extracts. In addition, tannins were only found in the 6-week-old maize silage extracts. Based on this result, it can be concluded that in maize, tannins are only present in young plants and its concentration in the extract is solvent-dependant.

5.2 Larval migration and adult worms' motility inhibition tests: The principles of larval migration and adult worms' motility inhibition tests have been used in this study for determining the effects of maize silage on *H. contortus* although data on the anthelmintic properties of maize silage are scarce in the literature. The tests conducted revealed that both 6- and 8-weeks-old maize silages reduced significantly *H. contortus* larvae migration and adult worms' motility. On the larvae migration, this effect was solvent and concentration-dependent whereas the effect on the adult worms' motility were only concentration-dependent. The overall result shows that 8-week-old maize silage extracts gave the highest inhibition rate (52.25%) despite the fact that its extracts contained no tannins and less alkaloids. This could be explained by the abundance of the reducing compounds in 8-week-old maize silage extracts; which are also known for their pharmacological activities (Nene Bi *et al.*, 2009). As regard to the adult worms' motility inhibition, the 8-week-old maize silage chloroformic extract and the hydroethanolic extract of 6-week-old maize silage at 600 µg/mL showed the best results. These effects on the adult worms' motility could be attributed to the presence of families of chemical compounds identified in the extracts such as tannins, alkaloids, mucilages, triterpenoids, reducing compounds, cyanogenic derivatives and anthocyanin. Indeed, antiparasitic potentials of the alkaloids and terpenoids family compounds have been reported by several studies (Kayser *et al.*, 2002; Fernandez *et al.*, 2010; Sylvester and Ignatius, 2017; Widiarso *et al.*, 2018). Widiarso *et al.* (2018) reported significant anthelminthic properties of condensed tannins and alkaloids extracts isolated from seven different plant species on goats' parasites while Olounladé *et al.* (2017) reported the role of tannins from *Newbouldia laevis* and *Zanthoxylum zanthoxyloides* on GINs in sheep. Dedehou *et al.* (2014), Olounladé *et al.* (2017), Alowanou (2016) and Bogning *et al.* (2016) also reported the effects of tannins and alkaloids extracted from several medicinal plants on *H. contortus* larvae migration and adult worms' motility. This study also revealed a significant effect of the extracts and the concentrations on larval migration. The 8-week-old maize silage extracts showed the highest rate of inhibition of larvae migration and this result might be due the abundance of reducing compounds in the 8-week-old maize silage. The lowest rate of inhibition has been observed with the 6-week-old maize silage despite its high content of alkaloids compared to 8-week-old maize silage extracts. This could be due the presence of undesirable particles in the 8-week-old maize silage powder that might hide the identification of alkaloids during the phytochemical analysis and the type of solvent used (Dedehou *et al.*, 2014; Olounladé *et al.*, 2017; Alowanou, 2016). Several researches conducted on plant materials extracts in controlling gastrointestinal nematodes parasites in small ruminants revealed almost similar results. Hounzangbe-Adote *et al.* (2005) reported a reduction of eggs excretion and the fertility of *H. contortus* adult female worms with daily consumption of Fagara leaves in sheep while Olounladé *et al.* (2011) reported an *in-vitro* inhibitory effect of extracts of *Newbouldia laevis* and *Zanthoxylum zanthoxyloïdes* on *H. contortus* larvae migration. Minaflinou *et al.* (2016) observed a significant inhibition effect of the same plant on *H. contortus* larvae migration but a non-significant inhibition effect on the adult worms' motility with the highest rate recorded at the highest concentration (2400 µg/mL).. However, of this study revealed contrary results with the best *H. contortus* adult worms' motility reduction rates observed with the highest concentrations. According to the same author (Minaflinou *et al.*, 2016), that inhibitory effect recorded is associated to the high presence of tannins in the plants. As regard to concentration-dependent effect of the extracts on *H. contortus* larval migration inhibition recorded in this study with the best results showed by the hydroethanolic and chloroformic extracts, similar results were found by Olounladé *et al.* (2011) when they reported the dose-dependent effects of extracts of *Newbouldia laevis* and *Zanthoxylum zanthoxyloïdes* on *H. contortus* larvae migration with more effect recorded with the hydroethanolic extracts especially at high doses. In addition, Dedehou *et al.* (2014) also reported an effect of acetonic and hydroethanolic extracts of *Pterocarpus erinaceus and Parkia biglobosa* on *H. contortus* larvae migration with high efficiency variations depending on the dose used. Administrating leaves powders of *Zanthoxylum zanthoxyloides* and *Newbouldia laevis* to sheep in station Minaflinou *et al.* (2015) revealed the similar results with this study.. These two plants were therefore found to disturb the prolificity of *H. contortus* while the application of their methanolic extracts alone or in combination, reduce their viability. In that study, the tannins, the flavonoids, the alkaloids and the phenolic compounds were the most suspected. The rates of inhibition of *H. contortus* adult worms' motility recorded in this study with both maize silages were higher than those obtained by Minaflinou *et al.* (2016). This might be due to the difference in the type of test (direct administration of the extracts to the animals for three consecutive days) and extracts used. Regardless the extracts, the average inhibition rates of *H. contortus* larval migration recorded in this study (52.25% for the 8-week-old maize silage and 47.51% while 6-week-old maize silage) were higher than those reported by many other studies. In an *in-vitro* research, Zangueu *et al.* (2016) revealed an inhibitory effect of aqueous extract of *Crassocephalum crepidioides* on *Haemonchus contortus* larvae migration up to 35.30% at 1200 µg/mL. The same way, Zangueu *et al.* (2018) concluded an *in-vitro* inhibition rate of 37.77% of *H. contortus* larvae migration using the aqueous solution of the extract of *Maytenus senegalensis* stem bark at 2400 µg/mL. This might be explained by the differences in the doses used which is the double of the highest dose used in this study (1200 µg/mL). However, at that same dose (2400 µg/mL), Zangueu *et al.* (2018) recorded almost the same the inhibition rates with that of the aqueous extract of 6-week-old maize silage but a higher rate compared to those observed with the hydroethanolic and chloroformic extracts of the 8-week-old maize silage. In contrary, Botura *et al.* (2011) had reported no significant effect of *Agave sisalana* on *H. contortus* worm burden in goats. On the other hand, another set of studies have revealed higher inhibitory effects on both gastrointestinal parasites larvae migration and their adult worms' motility compared to the extracts of both maize silages. Indeed, Mbogning *et al.* (2014) also recorded hatching inhibition rates ranging from 60.3 to 92.8% on *H. contortus* eggs with aqueous and hydroethanolic extracts of *Moringa oleifera*. In an *in-vitro* study, Zinsou (2015) showed a higher rate of inhibition of *H. contortus* larvae migration using the aqueous extract of *Pleiocarpa pycnantha* compared to the chloroformic, aqueous and hydroethanolic extracts of both maize silages tested in this study. Similar way, the i*n-vitro* assessment of the effects of different types of extracts of *Bridelia ferruginea*, *Combretum glutinosum* and *Mitragyna inermis* carried out by Alowanou

(2016) revealed various inhibition rates ranged from 17.36% à 67.52% with *B. ferruginea*, 14.79% to 68.17% with *C. glutinosum* and 21.22% to 61.74% with *M. inermis* on *H. contortus* larvae migration at the highest tested doses (300; 600 and 1200 µg/mL). Even these minimum inhibition rates are higher than those recorded with maize silage extracts while the maximum inhibition rates are lower than those recorded with the extracts of the maize silages at the same concentrations. These plants were also found to have a significant reduction effect of the motility of *H. contortus* adult worms (Alowanou, 2016). A higher inhibition rate of the motility of *H. contortus* (74%) has been reported by Zubair *et al.* (2014) at the same concentration (0.47 mg/mL) using aqueous extracts of *Azadirachta indica, Dalbergia sisso* and *Morus alba* while higher inhibition rate of *H. contortus* larvae migration (90%) has been observed by Widiarso *et al.* (2018) using extracts of *Apus bamboo* leaves at the concentration of 0.47 mg/mL. Recently, Zaheer *et al.* (2019) using the hydroethanolic extract of *Camellia sinensis* and *Albizia lebbeck* found a higher in-vitro rate of 88% and 95% mortality of *H. contortus* adult worms respectively at 6 and 8 mg/ml after 8 hours of treatment. All these studies showed and confirmed the anthelmintic potentialities of vegetable plants which could be valued in the control of the GINs in small ruminants in general and goats in particular. As a vegetable plant, maize plant also has the properties to reduce worms burden in goats. However, all the noticed differences in the results compared to the previous studies might be due to several factors but mainly the plant species and parts used (phytochemical composition), environmental location, the solvents used and the tested concentrations.

6 CONCLUSION

The objective of this study was to assess the anthelminthic effects of maize silage on *Haemonchus contortus,* the most prevalent and important gastrointestinal nematode in goats in West Africa regions. The results revealed that maize silage has anthelmintic effects on both the larval migration and the motility of adult worms of *H. contortus*. These effects were associated with the presence of some chemical compounds well recognized for their anthelmintic activities such as alkaloids, mucilages, triterpenoids, reducing compounds, cyanogenic derivatives, anthocyanin and tannins even though they were just present in trace in the maize silages extracts. Therefore, in addition to its nutritional value in small ruminant production, maize silage could also be considered while designing programme of controlling *H. contortus* infections among goats, especially Red Maradi goats, imported breed that are more sensitive to local strains of GINs.

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