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Anti-coccidian effect of almonds of *Azadirachta indica* (Meliaceae) against *Eimeria* sp. of the dwarf goat

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Abstract

Coccidiosis is a parasitic disease responsible for significant economic losses in farms throughout the world. In order to contribute to the fight against this disease, this study aims to evaluate the effect of extracts of *Azadirachta indica* on coccidia encountered in the dwarf goat in Côte d'Ivoire. Thus, 16 dwarf goats from 4 to 8 months of age, naturally infected with coccidian *Eimeria* sp. were divided into 4 groups of 4 animals. The batches of animals were treated orally during four days. Each animal of the first batch has received, every day, a dose of 300 mg / kg of body weight of Hydro-ethanol extract of *A. indica*. The second batch has received the same dose of Hydro-ethanol extract of leaves of the same plant. The third batch of goat has received a single and daily dose of 150 mg / kg of body weight of a pharmaceutical anti-coccidian. The fourth batch has not received any treatment. The evaluation of the anti-coccidian activity was performed by coprological analysis, determining the percentage of weight gain and the appreciation of the health status of animals. The results showed that the goats treated with *A. indica* almond showed the best performance with an efficiency rate statistically similar to the pharmaceutical anti-coccidian. Also, the percentage of weight gain was positive and much higher than those of other batches. Further studies are needed to evaluate its persistence and toxicity in goats in order for prescription to breeders.

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Introduction

Improving the productivity of dwarf goats is one of the challenges faced by developing countries, particularly Côte d'Ivoire, in achieving self-sufficiency in animal protein. The low productivity of goat meat is mainly due to gastrointestinal parasitosis including coccidiosis (O'callaghan, 1989; Achi *et al.*, 2003; Pilarczyk *et al.*, 2003). This endemic pathology on farms in Côte d'Ivoire affects various animal species including poultry, rabbits, grasses and ruminants (Morel and Inspecteur, 1959 ; Komoin-Oka *et al.*, 1999; Danho *et al.*, 2002 ; Zouh-bi *et al.*, 2013). In small ruminants, the prevalence of coccidiosis is over 64% in the north and 87% in the south of Côte d'Ivoire (Komoin-Oka *et al.*, 1999; Achi *et al.*, 2003). This parasitosis is one of the main causes of mortality in young animals (O'callaghan, 1989; Balicka-Ramisz, 1999; Pilarczyk *et al.*, 2003).

Unlike in Côte d'Ivoire, the poultry producers that have a control program, treatment against coccidiosis is almost nonexistent in livestock farming of small ruminants. This observation is related to the health monitoring failure which subjects these animals, the high cost of anticoccidials and the ignorance of dosages adapted to goats (Mishra *et al.*, 1979 ; Alexandre *et al.*, 2012). In fact, ruminants are not priority recipients of anticoccidials sold in veterinary pharmacies in Côte d'Ivoire.

To solve this problem, one of the exploitable solutions is the use of phytotherapy, especially since medicinal plants offer the advantage of being both less toxic and biodegradable (Koné *et al.*, 2005; Kouakou *et al.*, 2010; Sacramento *et al.*, 2010; Tahiri *et al.*, 2011; Ahmed, 2015). Also, the flora of Côte d'Ivoire having proved very provided in medicinal plants, It seems appropriate to search for new antiparasitic molecules, to increase the chances of controlling animal parasitism.

Among medicinal plants, *Azadirachta indica* has been the subject of numerous studies in agriculture. Leaves extracts of this plant have been effective in controlling arthropod pests (Schmutterer, 1990; Tahiri *et al.*, 2011 ; Raj, 2014).

In the medical field, this plant has been successfully tested against filariasis, (Mukherjee *et al.*, 2014), Malaria (Nagendrappa and Naik, 2013), and gastrointestinal worms (Feroza *et al.*, 2017). In poultry, *A. indica* oil cake reduced oocyst excretion (Dossou *et al.*, 2009). In goat breeding, a mixture of leaves and fresh seeds of this plant reduced oocyst excretion in kids of 2 months of age (Adamou *et al*, 2016). Today, the therapeutic effect of this plant is an obvious. But the problem lies in the nature of the phytochemicals involved in its medicinal properties, the parts of the plant most concentrated in medicinal substances and the search for germs sensitive to this plant.

In this context, this study aims to evaluate the effect of extracts of *Azadirachta indica*. This plant may have an interest in the control of coccidia of the genus *Eimeria* encountered in dwarf goats in Côte d'Ivoire.

Materials and methods

Plant material

Azadirachta indica, commonly known as Neem or Margousier, is a tree with medicinal properties. The choice of this plant is based on its chemical composition already determined by previous studies, toxicity and availability (Gossé *et al.*, 2005; Saxena *et al.*, 2010; Faye, 2010; Tahiri *et al.*, 2011; Djenontin *et al.*, 2012). In order to preserve the plant species, only part of the leaves and fruits were collected.

Animal material

The search for the efficacy of the extracts of *A. indica* on coccidia required the use of 16 dwarf goats from four to eight months of age. Ten of these animals came from the same farm in the town of Bongouanou in east-central Côte d'Ivoire. The remaining six were from Bingerville in the Lagunes region (Southern Côte d'Ivoire).

Harvesting and drying of seeds and leaves of *Azadirachta indica*

To reduce the effects of chemical contaminants leaves and seeds have been collected at a minimum distance of 1 km from communication lines and industrial centers. Organ harvesting of plants took place between 4 pm and 6 pm.

The sampling was carried out so as to avoid contact of the plant organs with the soil. The harvested parts were stored in biodegradable cardboard packages and transported to the laboratory for drying at 18 °C. When the sampling site was very far from the laboratory, drying was achieved in the shade before transport to the laboratory. The harvested leaves and seeds were rinsed and dried until a constant weight of the samples. The leaves were dried for 14 days and the seeds for 43 days. The dried leaves were reduced to powder. The almond seed was separated from the shell prior to being pulverized.

Preparation of leaves and seeds hydro-ethanolic extracts

Hydro-ethanol extraction was carried out by adding 1000 ml of ethanol (90%) to 100 g of each powder obtained. The extraction was carried out under mechanical stirring for duration of 72 hours. After filtration, the ethanol was removed from the extract using a rotary evaporator (Rotavapor). The extract obtained was lyophilized and stored at 18 °C.

Maintenance on animals

For the in vivo tests, 16 goats were randomly distributed into 4 batches of 4 animals. A coproscopy revealed that these animals were naturally infested with various parasites including coccidia of the genus *Eimeria*. Goats have previously been rid of nematodes and cestodes, by an injection of ivermectin (0.2 mg/kg of PV) and oral administration of Oxfendazole combined with Oxyclosamide (20 mg/kg of PV). Then, two spaced 7 days coproscopies were performed to ensure that the goats were completely free from all the digestive parasites except coccidia. In vivo tests began when coccidial loads reached 3000 oocysts per gram (OPG). The four batches of animals differentiated by loops were not separated throughout the experiment. Feeding was similar, consisting of *Panicum maximum* fodder, corn kernels, banana peel, cassava or yam and lickstone.

Treatment of animals

Hydro-ethanolic extracts and anticoccidial were orally administered to animals. Each animal of the first batch received during four days, A total dose of 1200 mg/kg body weight of hydro-ethanol extract of *A. indica* almonds distributed in 300 mg/kg per day. Each animal in the second batch received during four days, the same dose of extract of leaves. Animals of the third batch received individually a total dose of 600 mg/kg body weight of a commercial anti-coccidian named Narcox Plus at 150 mg/kg body weight per day. The fourth batch received no treatment.

Evaluation of anticoccidial activity

Evaluation of anticoccidial activity was carried out two days after treatment and comprised coprological analysis, determination of weight gain and appreciation of the general appearance of the animals.

Coprological analyzes

The faeces of all animals were analyzed first, one day before treatment, then, for six days after treatment. Finally, other analyzes were performed on the 14th and 30th day post-treatment. The Willis flotation method due to its high sensitivity has been used for the detection of oocysts of coccidian (Balicka-Ramisz, 1998; Pilarczyk *et al.*, 2003). The quantification of oocysts was performed using the McMaster method with a saline solution of density 1.3.The genus of coccidian was identified after observation of the sporulated oocysts using a dissection microscope (Gx100). Also, percentages of egg reduction, which also determine product efficacy, were calculated using the following formula.

$$\%E = \left[1 - \left(\frac{T1}{T2} \times \frac{C1}{C2}\right)\right] \times 100$$ (Presidente *et al.*, 1985)

With E% = rate of effectiveness;
T1 = OPG on the nth day after treatment;
T2 = initial OPG of the treated batch;
C1 = OPG on day n after treatment of control batch
C2 = initial OPG of the control batch.

Percentage of weight gain

Weighing animals before treatment and 4 weeks after treatment was used to calculate the percentage of weight gain by the following formula:

$$\%\, Weight\ gain\ = \left(\frac{Pf - Pi}{Pi}\right) X\ 100$$

With Pf = final weight; Pi = initial weight.

Assessment of the health and physical condition of goats

The health status of goats was determined based on observation of the back train of the animals to detect signs of diarrhea. The muscles in the lumbar vertebra, the sternum, the paravertebral muscles, the intervertebral muscles and those around the tail were palpated and analyzed to appreciate the symptoms of slimming (Hervieu *et al*., 1995; Lefrileux *et al*., 1995). Glossary edema, lacrimation, throwing and hairiness were closely monitored for health status.

Statistical Analyses

Statistical analyzes were performed using Microsoft Office Excel and Statistica 7.1 software. OPG values and weight gain percentages of the different groups of animals were compared to each other using the Tukey HSD test at 0.05 probability level.

Results

Health and body condition of goats

The animal health monitoring during the experiment revealed two cases of diarrhea at the 27 and 30 days in the negative control batch of goats. In addition, symptoms of weight loss were observed in the same animals and in those who received the hydro-ethanolic extract of leaves of *A. Indica*. These symptoms were marked by very noticeable joint processes, muscles very imperceptible and a very mobile and flexible skin. For the goats treated with the extract of the almonds of *A. indica* and positive control batch (treated with Narcox Plus), no signs of pathology or weight loss were observed.

Control of oocyst excretion

The coprological analyzes was used to evaluate the efficiency of the two hydro-ethanolic extracts of *A. indica* on reducing the excretion of oocysts of coccidia. These analyze showed a rate of effectiveness of the almonds extract statistically similar to that of the standard anticoccidial until the 30th day after treatment. The mean efficacy rate over the 30 days was 94.8% (min = 76%; max = 98.64%) for almonds and 97.31% (min = 83.83%; max = 100 %), for Narcox Plus.

Moreover, the effectiveness of almonds extract, after four days of treatment persisted until day 30 with a rate of 97.5%. For the leaves, the average effectiveness during the first 30 days was estimated at -21.16% (min = -228.41%, max = 32.28%). The effectiveness of leaves, much less than almonds, increased until the 5th day. After this period, an increase in oocyst excretion resulted in a reduction in the activity of the leaves, which became negative when the parasite load became increasingly important (Fig. 1).

Evolution of body weight

The determination of weight gain percentages highlighted the evolution of the weight of the animals according to treatment received (Table 1). This study revealed that the animals having received the hydro-ethanolic extract of *A. indica* almonds had a positive gain percentage significantly higher than the animals of the other batch, in particular those treated with the Narcox Plus (Fig. 2).

Table 1. Comparison of average of percentage weight gain as a function of the treatments performed on the animals.

Batches of treated animals	*Average percentage of weight gain (%)*
Untreated group	-11,48 ±6,29^{c}
Group treated with Neem seed	8,00±3,13 ^{a}
Group treated with Narcox Plus	0,74 ±0,52 ^{b}
Group treated with Neem leaves	-3,52 ±2,05 ^{b}

Values with the same letter are not significantly different (P<0.05)

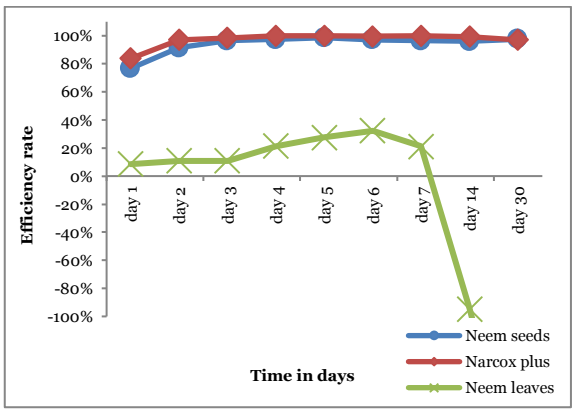

Fig. 1. Comparative efficacy of almonds and leaves of *Azadirachta indica* (Neem) on coccidian of dwarf goats.

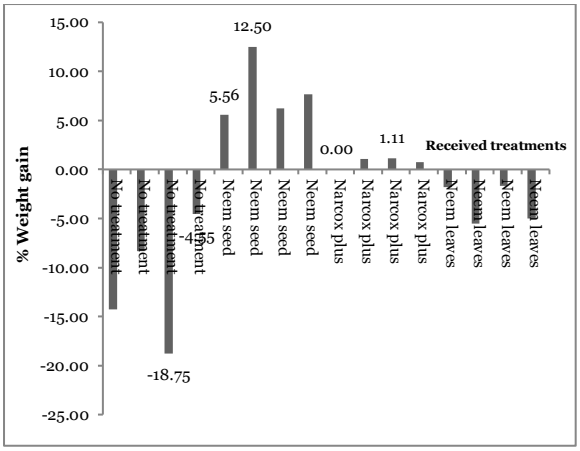

Fig. 2. Evolution of the percentages of weight gain of dwarf goats according to the treatments received.

Discussion

In the study, the hydro-ethanolic extracts of the almonds and leaves of *Azadirachta indica* exhibited in vivo efficacyon the coccidia of the genus *Eimeria*. The rate of effectiveness against coccidia of *A. indica* almonds is greater than 94%. This rate is higher than those obtained by Saratsis *et al.* (2012), on calf coccidia using the methanolic extract of *Onobrychis viciifolia* (Fabaceae). It is also higher than those obtained by Ahmed (2015) with rice water mugwort on coccidia in chickens. The dose of the almonds extract of *A. indica* tested in this study was 1200 mg/kg body weight (300 mg/kg body weight per day). This dose is less than the LD_{50} of the Neem oil between 5000 and 14 000 mg/kg body weight per day in rats and 24000 mg/kg weight in rabbits (Elakovich, 1996; Boeke *et al.*, 2004; Mouffok *et al.*, 2008; Raj, 2014). The observed effectiveness of the almonds may be due to the presence in this extract of a high concentration of bioactive compounds as already reported by several authors (Dossou *et al.*, 2009; Kouakou *et al.*, 2010; Saratsis *et al.*, 2012).

Also the extract of the leaves reduced oocyst excretion, however this activity was low (max = 32.28%). The leaves probably contain low amount of anticoccidial compounds. Adamou *et al.* (2016) have also reached same conclusion while investigating the effect of a mixture of leaves and fresh seeds of *A. Indica*in goatsfrom Niger. The present results are also consistent with those obtained by Dkhil *et al.* (2013) who observed an oocyst reduction of about 58% with the leaves of this same plant.

Indeed, the mixture of leaves, shell and almonds of *A. indica* may have diluted the effect of the almond. Leaves and almonds extracts of this plant species are known to possess insecticidal, bactericidal, fungicidal, antiviral and anti-protozoan properties (Mouffok *et al.*, 2008; Dossou *et al.*, 2009; Saxena *et al.*, 2010; Tahiri *et al.*, 2011; Adamou *et al.*, 2016).

The medicinal properties of this plant are often attributed to the presence in its various parts of several compounds of Terpenoids family including azadirachtin, considered as the main active compound of Neem (Kraus, 1986 ; Elakovich, 1996; Faye, 2010).

The results of monitoring animal health during the experiment corroborate the effectiveness of almonds of *A. indica*. Changes in some body were observed in goats. The animals treated with the hydroethanol extract of the almonds showed no signs of morbidity as those treated with the pharmaceutical anticoccidial. This may be due to the ability of this plant organ to strengthen the immune system of animals.

Also this result demonstrated that *A. indica* not only targets *Eimeria* parasites in hosts, but also exhibited anti-inflammatory activity, thus protecting the host tissues (Dossou *et al.,* 2009 ; Dkhil *et al.*, 2013). Moreover, the product was, able to increase the number of goblet cells of the jejunum of mouse after an experimental infection by *E. papillata* (Dkhil *et al.*, 2013). Morbidity and weight loss symptoms observed in untreated goats confirmed the pathogenicity of the species of coccidia involved.

In addition, the best percentages of weight gain were obtained in the animals treated with the extract of the almonds. This may indicate that, unlike Narcox Plus, Neem almonds contain a growth factor favorable to goat development as observed in chickens (Dossou *et al.*, 2009; Koné, 2010; Sagna ; Dkhil *et al.*, 2013). The decline in performance of the untreated animals may be due to the presence of coccidia. Most coccidiosis are responsible for damage to the intestinal mucosa causing poor absorption of nutrients which reduces the zootechnical performance of animals by decreasing the rate of growth (Lafont *et al.*, 1975).

Conclusion

The hydro-ethanolic extract of the almonds of *A. indica*, seems promising in the fight against coccidiosis in dwarf goats. This plant, known worldwide for its medicinal properties, has significantly reduced the excretion of oocysts from coccidia in naturally infested animals. It has also promoted the growth of treated goats and reduced their morbidity. Further studies would be necessary to assess its persistence and toxicity in goats with a view to prescribing it to breeders.

Conflict of interest

The authors declare that they have no conflict of interest.

The choice of animals and the treatment during the experiment were performed according to the recommendations of the World Association for the Advancement of Veterinary Parasitology and the Veterinary International Cooperation on Harmonization.

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