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Comparative study of the effect of *Tephrosiavogelii*'s leaves ethanolic extract and Alfapor® (Alpha-cypermethrin) on *Amblyommavariegatum* Borgoucattle

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Abstract

Ticks are responsible for significant economic losses in Africa and the world. This study compares the effect of ethanolic extract of *Tephrosiavogelii* (tv) and Alfapor® on *Amblyommavariegatum*. For this purpose, fifty (50) Borgou bulls of 1-2 years were divided into 10 lots of five. Lot 0 (control) was not treated. The other treatments have been from one treatment to two treatments (seven days after the first one) with the extract of tv in direct application. The spraying form of has been used with one treatment to two treatments (seven days after the first one). The mortality rate of ticks in the Lot 1 between days 2 and 7 ranged from 59.49% to 87.76% and 84, 89% to 100% in lot 9 ($P < 0.05$). The perineum has the highest infection rate (35.31%) with a significant difference compared to the scrotum (26.92%) and dewlap (24.89%) ($P < 0.05$). At day 7 which is the last day count before the second treatment, Lot 5 who received a single treatment of the extract of *Tephrosiavogelii* at 4 ml / L water spray has the highest rate mortality ticks (88.97%) with a significant difference compared to the other groups ($P < 0.05$). It appears that the ano-genital area was the most infested by *Amblyommavariegatum*; the ethanolic extract of tv used in direct application of 8 ml on *Amblyommavariegatum* is effective at 98.51%; Alfapor® used 1ml / L of water spray is as effective as ethanolic extract of tv direct implementation in 8 ml of *Amblyommavariegatum*.

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Introduction

In most African occidental countries such as Benin, ruminant livestock is one of the main activities in animal production. Since 2004, Benin has estimated cattle at 1,717,900 heads. This herd is experiencing an annual growth rate of about 3.6% and is composed of the bullfighting races lagoon, Borgou and Somba (31%) of M'bororo zebu, Goudali and White Fulani (7.7%), as well as subjects and their crossbreeds (61.3%) (MAEP, 2004). These animals offer tremendous benefits to producers on livestock, economic and socio-cultural (Pamoet *al.*, 2002). Unfortunately, their productivity is still low, many diseases that affect their growth and reproduction constituting one of the main causes.

Indeed, like cattle in other countries of West Africa, those of Benin undergo various parasitic attacks including, ticks occupy a prominent place. Diseases transmitted by these ticks are numerous. Some of these diseases include heartwater, anaplasmosis, theileriosis, babesiosis. Infestation by ticks also causes the appearance of dermatophilosis a more or less serious bacterial skin condition (Frebling, 2005). In addition, each of these diseases has risks of immunosuppression and negative impacts on the biochemical parameters of the body static.

To overcome these difficulties, synthetic acaricides are used in intensive production systems to combat and control these ectoparasites (Pamoet *al.*, 2002). However, the impact on man and his environment, the presence of resistant strains of mites to acaricides and the high cost of good quality products at local markets require a search for alternatives because ticks can cause a variety of health conditions ranging from harmless to serious (de Castro *et al.* 1997).. so the aim of this study is to compare the effect of Alfapor® and the ethanolic extract of leaves of *Tephrosiavogelii* on *Amblyommavariegatum* tick in bovine *BosTaurus* Borgou

Materials and methods

Study Area

The work was carried out from May to August 2012

on the farm of breeding Bétécoucou (FEB) in the Collines department, Commune of Dassa-Zoumé in the District of Akoffodjoulé. This farm covers an area of 11,127 ha. According to the 1982 vertical aerial photography and satellite image Spot No. 64335 of April 12, 1986, FEB is between the two meridians 20 ° and 2 ° 28 east longitude and parallels 45 ° and 7 ° 7 52 north latitude. The climate is intermediate type between maritime subequatorial with two rainy seasons and two dry seasons and Sudano-Guinean climate with a dry season and rainy. The average annual temperature is 27.4 ° C. Lower temperatures range from 20 ° to 21 ° C in December and January and the highest range from 35 ° C to 37 ° C between February and March. The average annual rainfall calculated over a period of 10 years has an average of 1072.95 mm.

Animal and plant material

The work took place on over 50 bull Borgou aged of 1 to 2 years, all naturally infested by *Amblyommavariegatum*. Plant material is *Tephrosiavogelii* (Figure 1).

Fetching ethanolic extract of *Tephrosiavogelii*

Young plants of *Tephrosiavogelii* were harvested in areas Bohicon and Ketou then dried at ambient laboratory temperature for 10 days. The dried leaves were ground into powder and mixed with ethanol to 50 g powder per 500 ml of ethanol.

Groups of experimental animals and treatment

The treatment took place for two weeks. The animals were regrouped into 10 groups of 5 cattle with a control group that received no treatment. A comparison lot was treated with Alfapor® and the others were with the ethanol extract of *Tephrosiavogelii*. The lot 0 (control) is infected by ticks without treatment with the extract of *Tephrosiavogelii* or Alfapor®; lot 1, infected cattle treated with the extract of *Tephrosiavogelii* direct application at 4ml / animal (one treatment: Do); lot 2, infected cattle treated with the extract of *Tephrosiavogelii* direct application at 4ml/animal (two treatments a week apart: Do and D7); lot 3,

infected cattle treated with the extract of *Tephrosiavogelii* direct application at 8 ml / animal (one treatment: Do); lot 4, infected cattle treated with the extract of *Tephrosiavogelii* direct application at 8 ml / animal (two treatments a week apart: Do and D7); lot 5, infected cattle treated with the extract of *Tephrosiavogelii* in spraying because of 4ml / L of water (one treatment: Do); lot 6, infected cattle treated with the extract of *Tephrosiavogelii* in spraying because of 4ml / L of water (two treatments a week apart: Do and D7); lot 7, infected cattle treated with the extract of *Tephrosiavogelii* in spraying because of 8ml / L of water (one treatment: Do); lot 8, infected cattle treated with the extract of *Tephrosiavogelii* in spraying because of 8ml / L of water (two treatments a week apart: Do and D7); lot 9, infected cattle treated with a spray Alfapor® at 1 ml / L of water (two treatments a week apart: Do and D7)

In total, two treatments were carried out at 7 days intervals in lots 2, 4, 6, 8, 9 and one treatment for lots 1, 3, 5, 7. Counting ticks took into account five regions namely: dewlap, perineum, scrotum, ears and

other parts (bun members, ear conch, neck, spine, tail toupillon, flank and abdomen).

Methods of Statistics Analysis

The number of ticks was recorded by body regions per animal and lots. Proc Freq of SAS (Statistical Analysis System, 1996) was used to calculate the frequency of ticks by dead body regions and lots. The Z test was used to compare different frequency pairs. The confidence interval for each frequency was calculated using the formula :

$$IC = P \pm 1,9 \sqrt{(pq)/n}$$

With P = the frequency of ticks, p = the probability of a tick Q = the probability of the event or otherwise p (1-p) N = sample size.

Results

Rate of infestation of cattle by *Amblyommavariegatum* according to body regions

The perineum has the highest infection rate (35.31%) with a significative difference compared to the scrotum (26.92%) and dewlap (24.89%) (P < 0.05). As for other regions, they are slightly infested *Amblyommavariegatum* (Table 1).

Table 1. Rates of infection in experimental animals by regions.

Regions	Number of ticks	Infestation rate (%)
Fanon	712.00	24.89 ^b
Ears	123.00	4.30 ^c
Perineum	1010.00	35.31 ^{to}
Scrotum	770.00	26.92 ^b
Other	245	8.56 ^c
Total	2860	100

*The figures are wearing the same letters are not significantly different between regions (P < 0.05).

Evolution of mortality ticks

At the lot 0 which was the control and received no treatment, the mortality rate is zero ticks and goes down over days. The number of ticks gradually increases to inverse lots treated with Alfapor® or ethanol extract of *Tephrosiavogelii*. The mortality rate of ticks at Lot 1 between days 2 and 7 ranged from 59.49% to 87.76% and 84, 89% to 100% at Lot 9 (P < 0.05). The highest mortality rate of ticks was recorded on day 14 (table 2).

Comparison of the effects of the ethanol extract of *Tephrosiavogelii* and Alfapor spray & Dip® on *Amblyommavariegatum*

At day 7 which is the last day count before the second treatment, lot 5 who received a single treatment of the extract of *Tephrosiavogelii* at 4 ml / L water spray has the highest rate mortality ticks (88.97%) with a significant difference compared to the other groups (P < 0.05). Then follows lots: 1 (4 ml of extract *Tephrosiavogelii* direct application), 9 (1 ml / L

of Alfapor water spray) and 8 (8 ml / L of water extract of *Tephrosiavogelii* spray) with respective mortalities of 87.76%, 84.89% and 84.21%. They show no significant difference between them ($P > 0.05$). At Day 14, Lot 9 (1 ml / L of water Alfapor spray) leads with a 100% mortality of ticks and has a difference significant at $P < 0.05$ compared to Lot 4 (8ml extract *Tephrosiavogelii* direct application)

which follows with a mortality rate of 98.51%. This lot has in turn a significant difference compared to lots 6 and 8 (respectively 4 ml / L and 8 ml / L water spray extract *Tephrosiavogelii*) ($P < 0.05$). Alfapor® gave the best mortality rate is 100%, followed by the extract of *Tephrosiavogelii* at 8 ml direct application (98.51%) on *Amblyomma variegatum* the 14th day, so the last day of the experiment (Table 3).

Table 2. Mortality of ticks.

Lots	Do	J2		J7		J9		J14	
		%	CI	%	CI	%	CI	%	CI
0	208	-8,65 ^a	3,82	-10,58 ^a	4,18	-12,98 ^a	4,57	-21,63 ^b	5,60
1	237	59,49 ^a	6,25	87,76 ^b	4,17				
2	357	49,58 ^a	5,19	68,91 ^b	4,80	88,52 ^c	3,31	96,08 ^d	2,01
3	259	49,42 ^a	6,09	79,15 ^b	4,95				
4	336	61,31 ^a	5,21	82,14 ^b	4,10	91,37 ^c	3,00	98,51 ^d	1,29
5	390	64,62 ^a	4,75	88,97 ^b	3,11				
6	372	45,70 ^a	5,06	75,27 ^b	4,38	89,78 ^c	3,08	97,85 ^d	1,47
7	233	38,20 ^a	6,24	78,54 ^b	5,27				
8	190	51,05 ^a	7,11	84,21 ^b	5,18	95,79 ^c	2,86	98,42 ^c	1,77
9	278	66,55 ^a	5,55	84,89 ^b	4,21	98,56 ^c	1,40	100,00 ^c	0,00

* Figures with the same letter are not significantly different between regions ($P < 0.05$)

CI = Confidence Index

Lot 0 (control) : No treatment (or extract *Tephrosiavogelii* or Alfapor®)

Lot 1 : 5 infected cattle treated with the extract of *Tephrosiavogelii* direct application due to 4ml/animal (one treatment : Do)

Lot 2 : 5 infected cattle treated with the extract of *Tephrosiavogelii* direct application due to 4ml/animal (two treatments a week apart: Do and D7)

Lot 3: 5 infected cattle treated with the extract of *Tephrosiavogelii* direct application at a rate of 8 ml / animal (one treatment : Do)

Lot 4 : 5 infected cattle treated with the extract of *Tephrosiavogelii* direct application at a rate of 8 ml / animal (two treatments a week apart : Do and D7)

Lot 5 : 5 infected cattle treated with the extract of *Tephrosiavogelii* in spraying because of 4ml / L of water (one treatment : Do)

Lot 6 : 5 infected cattle treated with the extract of *Tephrosiavogelii* in spraying because of 4ml / L of water (two treatments a week apart : Do and D7)

Lot 7 : 5 infected cattle treated with the extract of *Tephrosiavogelii* in spraying because of 8ml / L of water (one treatment : Do)

Lot 8 : 5 infected cattle treated with the extract of *Tephrosiavogelii* in spraying because of 8ml / L of water (two treatments a week apart : Do and D7)

Lot 9 : 5 infected cattle treated with a spray Alfapor® at 1 ml / L of water (two treatments a week apart : Do and D7).

Discussion

Distribution of Amblyomma variegatum in function of the body regions of cattle

The amount of ticks counted varied significantly

between body regions of cattle with the anogenital region with the highest parasite load. Similar results were reported by Farougouet *al.* (2006) who explained that the preferential sites fixation of ticks

are regions ano-genital and abdominal. Same observations were made by Achukwi *et al.*(1997) who reported that the ventral genital area was the most infested in Namchi and Goudali cattle of Ngaoundere in Cameroun and by *Amblyommavariegatum*. These

results are also consistent with those of Gueye *et al.*(1993) on ticks and hemoparasites of cattle in Senegal. Ticks therefore identify preferred areas for attachment and this could be explained by the degree of blood supply to the body part in question.

Table 3.Comparative effects of ethanol extract of *Tephrosiavogelii* and Alfapor Spray & Dip ® on *Amblyommavariegatum*.

Lots	Do	J2		J7		J9		J14	
		%	IC	%	IC	%	IC	%	IC
0	208	-8,65 ^d	3,82	-10,58 ^d	4,18	-12,98 ^c	4,57	-21,63 ^d	5,60
1	237	59,49 ^{ab}	6,25	87,76 ^{ab}	4,17				
2	357	49,58 ^b	5,19	68,91 ^c	4,80	88,52 ^b	3,31	96,08 ^c	2,01
3	259	49,42 ^b	6,09	79,15 ^{bc}	4,95				
4	336	61,31 ^a	5,21	82,14 ^b	4,10	91,3 ^b	3,00	98,51 ^b	1,29
5	390	64,62 ^a	4,75	88,97 ^a	3,11				
6	372	45,70 ^{bc}	5,06	75,27 ^c	4,38	89,78 ^b	3,08	97,85 ^{bc}	1,47
7	233	38,20 ^c	6,24	78,54 ^{bc}	5,27				
8	190	51,05 ^b	7,11	84,21 ^{ab}	5,18	95,79 ^{ab}	2,86	98,42 ^{bc}	1,77
9	278	66,55 ^a	5,55	84,89 ^{ab}	4,21	98,56 ^a	1,40	100,00 ^a	0,00

* Inside the columns%, figures with the same letters are not significantly different between batches. (P < 0.05).

Legend :

CI = Confidence Index

Lot 0 (control) : No treatment (extract *Tephrosiavogelii* or Alfapor ®)

Lot1 : 5 infected cattle treated with the extract of *Tephrosiavogelii* direct application at 4ml /animal (one treatment : Do)

Lot 2 : 5 infected cattle treated with the extract of *Tephrosiavogelii* direct application at 4ml/animal (two treatments a week apart : Do and D7)

Lot 3 : 5 infected cattle treated with the extract of *Tephrosiavogelii* direct application at 8 ml / animal (one treatment : Do)

Lot 4 : 5 infected cattle treated with the extract of *Tephrosiavogelii* direct application at 8 ml / animal (two treatments a week apart : Do and D7)

Lot 5 : 5 infected cattle treated with the extract of *Tephrosiavogelii* in spraying at 4ml / L of water (one treatment : Do)

Lot 6 : 5 infected cattle treated with the extract of *Tephrosiavogelii* in spraying at 4ml / L of water (two treatments a week apart : Do and D7)

Lot 7 : 5 infected cattle treated with the extract of *Tephrosiavogelii* in spraying at 8ml / L of water (one treatment : Do)

Lot 8 : 5 infected cattle treated with the extract of *Tephrosiavogelii* in spraying at 8ml / L of water (two treatments a week apart : Do and D7)

Lot 9 : 5 infected cattle treated with Alfapor ® in spraying at 1 ml / L of water (two treatments a week apart : Do and D7).

Effects of extract of Tephrosiavogelii on Amblyommavariegatum

The parasite load of the animals treated with the extract of *Tephrosiavogelii* experienced a gradual decline during the experiment. This is what explains

the increase in the mortality rate of these ticks in cattle. These results are similar to those obtained by Kalumeet *al.*(2012) who reported that the toxicity of the ethanolic extract of *T.vogelii* on the tick *Rhipicephalus appendiculatus* with a dose of 2.5 - 5

mg / ml in the Democratic Republic of Congo. Christopher *et al.*(2009) in their study on the use of *T.vogelii* in tick control in dairy cows, also stipulated that from 1st to 5th month of experience, there is a decline in the burden of ticks at 50 g of *T.vogelii* by 100-200 ml of water. Matovu and Oliva (2007) meanwhile, demonstrated in their study of the activity acaricide extracts of *Tephrosiavogelii* on nymphs and adult ticks in Uganda, extracts of *Tephrosiavogelii* with chloroform, methanol (alcohol), petroleum ether and water can be effectively used in the field to tick control all genres. Also the tiquicide effect of this plant in aqueous solution was reported in the quarterly newsletter and information CIRAD(2002) and in the book " What to do without veterinary "Published by CIRAD (2002). Similarly, the insecticidal activity of this plant has been implemented by Munyuli and Mushambanyi(2003) in the fight against pests including weevils, beetles, weevils, the Larger Grain grain and lucite in the Democratic Republic of Congo. By referring to the composition of this plant, we can say that the acaricide *Tephrosiavogelii* activity is probably related to the presence of various retinoid compounds (rotenone and degueline) contained in this plant and are known for their acaricidal and insecticidal activity. Thus, according to Kalumeet *al.* (2012). The acaricide activity sheets *T.vogelii* could be due to its high content degueline. But the exact mode of action of degueline on ticks remains poorly understood and should be studied. According to these authors, rotenone, which is also present in the plant, is at very low levels by blocking the mitochondrial respiratory path of devastating target through specific inhibition of the oxidation of NADH to ATP. This leads to a gradual reduction in nerve conduction and death of the parasite.

In addition, the plant extract has also maximized its effect according to the work of Matovu and Olida (2007) who reported that the leaves of *Tephrosiavogelii* collected in Uganda accumulate relatively high active ingredients from roots quantities.

In addition, cattle in the control group who received noacaricide treatment during the experiment have been a gradual increase in the number of ticks. Parasite load was especially pronounced in these animals especially since the study was conducted during the rainy season that is to say during the month of June. These results are consistent with those of Farougouet *al.*(2006) who explained that there is a positive linear correlation between rainfall and the number of ticks collected from cattle in Benin. From such observations were made by Achukwiet *al.*(1997) comparative study of the infestation of Namchi and Goudali cattle in Ngaoundere by adult *Amblyommavariegatum*. According to previous studies on population dynamics of *Amblyommavariegatum* adult Goudali zebu in the Adamawa Plateau in Cameroon by Stachurskiand *et al.*(1993), the number of ticks of animals experiencing an increase in the beginning of the rainy season.



Fig. 1. *Tephrosiavogelii* plant.

Comparative effects of *Tephrosiavogelii* and Alfapor ® on *Amblyommavariegatum*

We found out in this study that the ethanolic extract of *Tephrosiavogelii* actually has an insecticidal effect on *Amblyommavariegatum*. Indeed the acaricide activity of this plant has been compared to that of Alfapor ® (Alpha-cypermethrin) is a synthetic acaricide used by farmers in Benin. The results showed that the effect of *Tephrosiavogelii* to 8 ml of the extract direct application *Amblyommavariegatum* is similar to that of Alfapor® (1 ml / L of water spray). *Tephrosiavogelii* therefore similar to that of other synthetic acaricides as Alfapor ® in this study, Milbitaz ® acaricide effect work Kalumeet *al.*(2012) Triatix D ® and in the work

of Christopher *et al.*(2009). This suggests that *Tephrosiavogelii* could represent an alternative to the uses of which are usually expensive and difficult to obtain in many parts of the world synthetics.

Conclusion

Following this study, it appears that the *Amblyomma variegatum* effectively colonizes multiple body regions of cattle. But the anogenital region compared to other regions is infested by this tick. Furthermore, the acaricide effect of ethanol extract of leaves of *Tephrosiavogelii* has been proven on *Amblyomma variegatum*. Effectiveness of this extract was demonstrated when applied directly on the tick with 8 ml. At this dose, the extract could effectively replace the product standard Alfapor ® commonly used at a dose of 1 ml / L of water spray.

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