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Acaricidal Effect of *Commiphora swynnertonii* Stem bark Exudate Against *Rhipicephalus appendiculatus*

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Study Area: Monduli District, Arusha region, Tanzania

Coordinates: 4°24'36"S 37°10'48"E

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Abstract

The objective of this study is to evaluate the acaricidal activity of *Commiphora swynnertonii* (Burseraceae) stem bark exudates against *Rhipicephalus appendiculatus*. *C. swynnertonii* is a small woody plant found in arid and semi-arid environments. Its fruits which are consumed by birds and animals. *R. appendiculatus* is the brown ear tick responsible for the transmission of east coast fever in cattle. This species of tick is commonly found in the savannah and temperate climatic condition. *C. swynnertonii* exudate was prepared using soap solution and evaluated against the *R. appendiculatus* using adult immersion test method (AIT). The percent adult mortality within 15 days and percentage hatching of laid ova were determined at concentrations of 12.5, 25, 50, 60, 70, 80, 90, and 100 mg/ml. The results showed that mortality was significantly high at the concentration greater than 25 mg/ml ($p < 0.05$), while inhibition of laid eggs was found to be significant at 90 and 100 mg/ml ($p < 0.05$). The *C. swynnertonii* exudates found to inhibit completely the hatching of tick's eggs. Conclusively, *C. swynnertonii* exudates have high acaricidal power against *R. appendiculatus* and can be employed as natural acaricides especially by smallholder farmers to control ticks.

Introduction:

In Africa, many societies depend on livestock keeping as a source of food, income and providing power for farming and other domestic benefits (Gonzo *et al.*, 2014). Tick infestation and consequently, tick borne diseases have been a key bottleneck in livestock farming in Africa. Ticks are responsible in the transmission of East Coast Fever (ECF), babesiosis, anaplasmosis, dermatophilosis, and cowdriosis in the Sub Saharan Africa (Kalala *et al.*, 2014; Mkangara *et al.*, 2014). *R. appendiculatus* is hard tick mainly found in the ears of the cattle, buffalo, and antelope. It transmits a number of pathogens including *Theileria parva* (East coast fever), Nairobi sheep diseases virus and brown tick toxicosis (Yessinou, 2016). Tick and Tick borne diseases (TTBD) cause death of livestock, elevate the cost of veterinary services, reduce quality and quantity of the animal product, loss of labor animals and lower reproduction capability (Ilham *et al.*, 2014; Kivaria, 2006; Mkangara *et al.*, 2014). Reducing the incidence of TTBD leads to economic benefits such as increase quality of animal products, fertility, lowers cost of veterinary

services and increase manure production (Kivaria, 2006).

For many years, tick infestations has been controlled through the use of synthetic acaricides (Brito *et al.*, 2011) usually suspended in water. Tick resistance to some common synthetic acaricides, the presence of acaricides residues on animal products and environmental contamination has been reported in the world (Castro-Janer *et al.*, 2010; Krishna *et al.*, 2014; Lovis *et al.*, 2011; Pretty & Waibel, 2005). Resistance to synthetic acaricides has been linked to the multiple use and improper frequency application of synthetic acaricides to livestock (Eshetu *et al.*, 2013). Development of acaricidal agents targeting eggs, larvae, nymph and adults is currently viewed as a sustainable means of controlling ticks (Habeeb, 2010; White *et al.*, 2004). It is in this view that *C. swynnertonii* which is exploited by many African communities for ethnoveterinary use (Miller *et al.*, 2002; Musa, 2008) was evaluated for acaricidal activity. *Commiphora swynnertonii* (family Burseraceae) is a small woody plant which can grow up the height of 2.5 meter (Mukhlesur, *et al.*, 2008; Van Wyk & Wink, 2004). The

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plants is found in the arid and semi-arid environment and grows with tiny leaves and spiny with fruits edible to birds and animals (Paraskeva, *et al.*, 2008). *C. swynnertonii* claimed to be used as veterinary medicine (Deepa, *et al.*, 2015). This paper therefore reports acaricidal activities of *C. swynnertonii* exudates against the *R. appendiculatus*.

Materials and methods:

Plant materials: the plant exudates were collected from the stem bark of *C. swynnertonii* at Mererani in Simanjoro District, Arusha region Tanzania. The *C. swynnertonii* plants were identified by botanist from Tropical Pesticide Research Institute (TPRI) Arusha Tanzania. The voucher specimen CS 001 was deposited in the herbarium at Nelson Mandela African Institution of Science and Technology (NM-AIST) Arusha, Tanzania.

Preparation of acaricides: the extraction was done by using 10 mg/ml of a commercial powder soap OMO (purchased from Uniliver Kenya Ltd) prepared using distilled water. The following dilutions of exudate soap solutions were prepared 100, 90, 80, 70, 60, 50, 25 and 12.5 mg/ml. The dilutions prepared were used to test the ticks' percentage mortality, index of laying eggs, and inhibition of hatching by adult immersion test method (AIT).

Collection of ticks: the ticks were collected from smallholder farmers at Monduli District Arusha region. Ticks collected were stored in a small bottle closed with a muslin cloth to prevent from escape and transported to TPRI for identification. After Identification, the female *R. appendiculatus* were fed on the rabbit to engorge (Drummond *et al.*, 1973; Reghu Ravindran *et al.*, 2011). Ticks were incubated at biological oxygen demand (BOD) incubator at $28 \pm 2^\circ\text{C}$ and relative humidity (RH) 80% to lay eggs. The eggs were incubated and hatch, the larva were fed to nymph and then to adult. The adults were fed on rabbit again to engorge and drop out. The engorged female ticks were transported to Nelson Mandela African Institution of Science and Technology laboratory, washed by distilled water and dried in by absorbent paper ready for the experiment (Abbas *et al.*, 2014). A total of 216 adult engorged female ticks were used for this experiment.

Adult Immersion Test (AIT): the AIT was conducted according to FAO (1984) guidelines with minor modifications (Miller *et al.*, 2002; Ravindran *et al.*, 2014). Four replicates of six ticks were used to test each extract concentration. The ticks were selected randomly and weight of each replicate of the female engorged ticks was recorded. Each replicate were immersed to the respective concentration (10 ml) at room temperature for 10 min in a 50 ml beaker with a gentle agitation according to (Hanus *et al.*, 2005; Ravindran *et al.*, 2014) with minor modifications. Ticks were recovered from the solutions and placed in plastic Petri-dishes. The control group was immersed in soap solution (10 mg/ml). Ticks were placed in a petri dish

over Whatman's filter paper no 1 (purchased from Whatman international Ltd Maidstone England). The treated petri and untreated ticks were kept at room temperature for 24 hours, then the ticks were incubated in biological oxygen demand (BOD) incubators at $28 \pm 2^\circ\text{C}$ and relative humidity $85 \pm 2\%$. The ticks were observed for mortality and oviposition for the period of 15 days. After 15 days the percentage mortality was determined (Hanus *et al.*, 2005). The weights of the eggs laid were recorded and were incubated for additional 30 days to estimate percentage hatching.

The index of laying eggs (IE) = $\frac{\text{Weight of the laid eggs (g)}}{\text{Weight of the females (g)}}$

Percentage inhibition of egg laying = $\frac{(\text{IE control group} - \text{IE treated group})}{\text{IE control group}} \times 100$

After the period of thirty days the number of dead and live larvae and unhatched eggs were determined and percentages hatched eggs were calculated.

Estimated reproductive factor (ERF) and inhibition of reproduction (IR) were calculated using the formula below as described by Drummond *et al.* (1973).

$$\text{ERF} = \frac{20,000 XY}{Z}$$

Where: 20,000 average number of eggs per gram

X = weight (g) of the eggs produced

Y = estimated percentage hatchability of the eggs

Z = weight (g) of the females

Percentage inhibition reproduction (IR) = $\frac{(\text{ERF control group} - \text{ERF treated group})}{\text{ERF control group}} \times 100$

Statistical analysis: Microsoft Excel 2013 computer software was used to obtain regression equation where LC 50 and LC 99 were calculated. One way analysis of variance (ANOVA) was carried out by Genstat software version 10 to determine significant differences among the doses in mortality, index of laying eggs, the percentage inhibition of laid eggs, hatching percentages and inhibition of hatching. The p values less than 0.05 was considered significant.

Results:

Mortality of ticks: *Commiphora swynnertonii* stem bark exudate was evaluated for acaricidal activity against *R. appendiculatus*. The findings emanated from this study are summarized in Table-1. The tested concentrations were prepared by dissolving a known amount of *C. swynnertonii* stem bark exudate in a known volume of diluted soap solution. It was evident that a complete mortality of ticks was observed at 100 mg/ml for the period of fifteen days of the experiment. Mortalities at higher concentrations (60 to 100 mg/ml) were significantly

Table 1. Effect of commiphora Swynnertonii exudates against R. appendiculatus

Dose concentration (mg/ml)	Percentage Mortality	Weight of the laid eggs (g)	Number of eggs incubated	Weights of the females ticks (g)	Index of aid eggs	Percentage inhibition of egg laid
12.5	25cd	0.31b	13592a	1.45a	0.212d	-48a
25	33.3c	0.1a	4413bc	0.67b	0.187cd	-32ab
50	41.7c	0.05cd	2300c	0.56b	0.099abcd	27.2bcde
60	75b	0.16bc	6808b	1.23ab	0.128bcd	15.8abcd
70	87.5ab	0.06cd	2833bc	1.07ab	0.059abc	62.6cde
80	87.5ab	0.08cd	3573bc	1.25ab	0.064abc	48.6cde
90	95.8ab	0.03d	1250c	1.63a	0.025ab	86de
100	100a	0.02d	576c	1.12ab	0.08a	91e
Control	0.0d	0.19b	7128a	1.2ab	0.157cde	0.0abc
Mean	61.11	0.11	4719	1.13	0.104	27.8
CV%	24.40	58.00	58.6	39.3	64.1	159.3
LSD (0.05)	0.001	0.001	0.001	0.05	0.002	0.001
Fpr.	***	***	***	*	**	***

Values are expressed as mean $\pm$ SEM, values with the same superscript within the column means do not show statistically significant differences ($p > 0.05$)

Table 2: Mean estimated reproductive factor and Inhibition of reproduction of R. appendiculatus engorged females subjected to different concentrations of C. swynnertonii stem bark exudates.

Dose mg/ml	0	12.5	25	50	60	70	80	90	100	Mean	Fpr
ERF $\pm$ SEM	314162.6 $\pm$ 51456.6	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	34907 $\pm$ 17427.86	*
IR $\pm$ SEM	100 $\pm$ 0	100 $\pm$ 0	10 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	*	

Values are expressed as mean $\pm$ SEM, * < 0.05 , ERF= Effective reproductive factor, IR= Inhibition of reproduction

higher ($p < 0.001$) than in lower concentrations. The mortality of ticks using synthetic acaricides (Alpha-Cypermethrin) was reported to be 100 percent within two days of exposure (Musa, 2008). The highest tick mortality of the tested *C. swynnertonii* stem bark exudate occurred between day one and day nine post-treatment. The lethal concentrations which can result in mortality for at least 50 and 99 percent (LC₅₀, LC₉₉) of the ticks were established to be 42.9 and 92.8 mg/ml respectively (Fig.-1).

Effects of Commiphora swynnertonii exudates on tick's ability to lay eggs: *C. swynnertonii* exudate was evaluated for its effect on the ability of engorged female ticks to lay eggs. The body weights of engorged female ticks were not significantly different from each other. The engorged female ticks were treated with 25, 50, 60, 70, 80, 90, and 100 mg/ml of *C. swynnertonii*. The weights of the laid eggs were found to decrease as the tested concentration of *C. swynnertonii* stem bark exudates

increased (Table-1). The weight of eggs laid by the treated engorged female ticks was found to be significantly different from those of untreated group ($p < 0.05$).

The index of laid eggs by female ticks was affected by the concentration of *C. swynnertonii* exudates. The female ticks tested with high concentrations found to have lower ability to lay eggs as compared to those tested with lower concentrations of the exudates. The index of laying eggs of treated engorged female ticks with *C. swynnertonii* stem bark exudates at concentration 100 and 90 mg/ml was found to have the significant difference to those untreated group ($p < 0.05$).

Hatching of the ticks eggs: eggs laid by both treated and un-treated engorged female ticks were incubated at 28 ± 2 oC for 30 days. On visual examination of the incubated eggs, it was observed that all eggs laid by ticks treated with *C. swynnertonii* stem bark exudates did not hatch while those eggs laid by untreated ticks were hatched (Table-2). *C. swynnertonii* stem bark exudate was found to interfere the development of eggs. The *C. swynnertonii* stem bark exudate lowers reproduction rates of the tick.

Discussion:

There is an increasing interest on the use of botanicals to control ectoparasite as they are found to be relatively cheaper, eco-friendly, effective, readily available and safe especially for smallholder farmers (Shyma *et al.*, 2014). Plants contain a range of chemically active ingredients

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which can intervene all biological process of the ticks interrupting its life cycle and its dispersal (Habeeb, 2010). In the current study, *C.swynnertonii* exudate was evaluated for acaricidal activity against *R.appendiculatus*. The findings showed that *C.swynnertonii* exudates induces tick mortality, lowers ticks ability to lay eggs and affects the viability of laid eggs from the treated engorged female ticks. Previous phytochemical studies on *Commiphora* spp. reported on the presence of several classes of secondary metabolites namely alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, terpenes, steroids, resin acids and phenolics which found to cause mortality in arthropods (Hanus *et al.*, 2005; Krishna *et al.*, 2014; Musa, 2008; Shyma *et al.*, 2014). Moreover, it was reported that *Commiphora* spp. possessing antibacterial and antifungal activities (Bakari *et al.*, 2012). The acaricidal effect of *C.swynnertonii* exudate was due to the presence of tannins, saponin, terpenes, and flavonoids (Bakari, 2013; Bissinger & Roe, 2010). The observed mortality of ticks may be accounted by several possible mechanisms working separately or working in synergic (Chandler *et al.*, 2011; Kunz & Kemp, 1994). In some cases, insecticides can have both insecticidal and acaricidal properties (George *et al.*, 2014). It is believed that the mechanism of action of terpenoids and flavonoids might be similar to that of the organophosphate compounds. Therefore the acaricidal activities observed in this experiment might be contributed by one or more bioactive compounds present in *C. swynnertonii* exudate.

The mortality of the ticks when immersed in the *C. swynnertonii* stem bark exudates suggests that there is contact effect of the exudates. At higher concentration the effect of the exudates was immediate while at a lower concentration the effect was taking place slowly as the residual on the body stimulate effect. The variation in mortality based on concentration was attributed to the amount of the exudates in contact with the body and penetrates to the inner cells. The amount of bioactive compounds of the exudates penetrates the inner cell is proportional to mortality caused thus exudates with higher concentration results in mortalities earlier than those with lower concentrations. Higher concentration is recommended to be used as lower concentration may result in resistance especially when the activity is not taking place shortly. The mode of action was similar to that of pyrethroids in insect where by it produces hyperexcitation tremors and paralysis followed by mortality (Shyma *et al.*, 2013). The nerve excitations occurs as a result of a change in membrane permeability to sodium and potassium ions (Krishna *et al.*, 2014). The use of OMO (detergent) is recommended as it has lipophilic properties which can remove the outside waxy cuticle layer of the ticks and allow the bioactive compounds to

penetrate to the inner cells. Bioactive compounds revealed to interfere with basic metabolic, biochemical, physiological and behavioral functions of the ticks. The penetration of the bioactive compounds through the outer layer of the ticks causes the mortalities, suppress and reduction in oviposition as well as the development of the eggs hence blocking of the eggs hatching. The bioactive present in the exudates suggests interfering the ovaries as well as the egg formations (Shyma *et al.*, 2014).

This study supports the use *C.swynnertonii* as a potentially acaricidal agent for controlling *R. appendiculatus* and confirm the presence of some factors in the exudates with an acaricidal property. Exudates having acaricidal, anti-oviposition, and inhibition of fecundity properties may make the plant valuable component of developing valuable strategies for integrated tick management.

Conclusion:

The obtained results indicate that *C. swynnertonii* exudates may prove to be effective against control *R. appendiculatus*. Therefore smallholder farmer especially those practice zero grazing, in the remote settings where synthetic acaricides are unaffordable or unavailable, they can use *C. swynnertonii* exudate to control ticks. Further studies should be conducted to determine the mechanism of *C. swynnertonii* exudates mode of actions. However, plants from different parts should be studied to confirm if they have the same activity. Field trial should be conducted to compare the results with laboratory results.

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