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Laboratory assessment of the potential acaricidal properties of the herb *Artemisia alfa* (Greenginger or wormwood) against the larvae of three tick species

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ABSTRACT

Ticks constitute major pests of domestic animals through sucking of blood and transmission of many parasitic diseases. The search for alternative acaricides as ticks develop resistance to the ones in use is an ongoing process. This study was carried out to test potential acaricidal properties of the wormwood (*Artemisia alfa*) in Olive oil/Trilene formulation against larvae three tick species *Boophilus decoralatus, Ripicephalus appendiculatus and Rhipicephalus evertsi*. The study was carried at the Central Veterinary laboratory, Harare, Zimbabwe, from November to December 2011. The tick larvae were exposed to four concentrations (25, 50, 75, and 100%) of the wormwood leaf extract using the Larval Packet Test (LPT). All leaf extract concentrations induced mortality and mortality increased with concentration. Larvae of *Boophilus decolaratus* were the most sensitive to the leaf extract with mortalities of 51.7% in 100% leaf extract and larvae of *Rhipicephalus evertsi* were the least sensitive (40% mortality) at the same concentration. Tick species and leaf extract concentration had significant effect on tick mortality (P<0.05). The study opens a potential weapon to deal with ticks especially for the poor upcoming farmer with limited resources

Introduction

There are at least 840 tick species in two major families namely the Ixodidae (hard ticks) comprising approximately 80% and Argasidae (soft ticks) approximately 20%.Ticks are important obligate haematophagous ectoparasites of domestic and wild animals, and tick infestations economically impact cattle production worldwide. Ticks transmit viral, rickettesial, bacterial and protozoal diseases affecting domestic animals and wild animals and humans.

Control of cattle tick infestations has been primarily by application of acaricides. Towards the end of the 19th century a complex of problems related to ticks and tick-borne diseases of cattle created a demand for methods to control ticks and reduce losses of cattle. The discovery of arsenical solutions in dipping vats for treating cattle to protect them against ticks revolutionized tick and tick-borne disease control programmes. Arsenic dips were used about forty years before the evolution of resistance of ticks to the chemical and the development and marketing of synthetic organic acaricides after World War II provided alternative products (George et al, 2004).

An acaricide is a pesticide designed to control harmful species of Acari (ticks and mites). The use of acaricides has increased substantially over the past half of the 20th century. Since the first serious and widespread outbreaks of spider mites populations, during the 1950s, organophosphorous and other neuroactive insecticides were replaced by specific acaricides that is compounds exclusively or primarily effective against mites. Several generations of structurally diverse synthetic acaricides, directed against various biochemical and physiological targets, have been commercialized until now. Besides specific acaricides, a number of insecticides with considerable acaricidal activity (pyrethroids, avermectins, and benzoylureas) have also been used, while some older neuroactive compounds are still available for the control of phytophagous mites (Jeppson *et al.*, 1975; Knowles, 1997; Dekeyser, 2005; van Leeuwen *et al.*, Most of the modern acaricides exert their effects through disruption of respiratory processes. Another approach in the development of synthetic acaricides launched compounds that act on growth and development (Dekeyser, 2005; Krämer & Schirmer, 2007). On the other hand, various natural bioactive products with acaricidal activity (botanical and microbial pesticides, essential oils, horticultural spray oils, and mycopesticides) have become important alternatives to synthetic acaricides (Copping & Duke, 2007; Faria & Wraight, 2007). The use of medicinal plants or natural bioactive products has brought the development of effective alternatives to acaricides and antibiotics. However the development of resistance to acaricides remains a challenge and search for alternatives has made tick-control a quite expensive task for both peasant and commercial farmers.

The resistance of ticks to acaricides is an inherited phenomenon. It results from exposure of populations of ticks to chemical parasiticides (acaricides) and survival and reproduction of ticks that are less affected by the acaricide. The higher reproductive rate of ticks that have heritable resistance factors and the resulting increase in the proportion of the population of ticks that carry genes for these factors is known as selection. Resistance to a given acaricide or insecticide can be described as a reduction in susceptibility of a parasite to the acaricide or insecticide when it is used at the recommended concentration and according to all of the recommendations for its use. In most cases, it is likely that genes that confer resistance are already

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present at very low levels in the tick population before the introduction of a new acaricide. The rate at which a resistant allele becomes established in the population and the time it takes for the control of ticks to break down is dependent upon many factors. These include the frequency of the original mutation in the population before treatment, the mode of inheritance of the resistant allele (dominant, co dominant or recessive), the frequency of acaricide treatment, the concentration gradient of the acaricide and the proportion of the total tick population that is not exposed to the acaricide (refugia) (Allen and Uilenberg, 1994).Although the frequency of resistant genes initially only increases slowly, by the time declining efficiency of dipping or treatment is noticed, the rate of increasing frequency of resistance genes is usually high.

In the initial phase, the frequency of heterozygous resistant individuals (single allele mutation) within the population is low and the rate of increase in the frequency of the resistant allele is low. In the next, emerging phase, given continued exposure to a drug, the frequency of heterozygous resistant individuals within the population increases. Finally, the sustained selection pressure results in increasing numbers of homozygous resistant individuals, which ultimately predominate in the population. Usually, lower concentrations are used than are necessary to kill any ticks present on the host. It is likely that this also contributes to an increase in the rate of the evolution of resistance to acaricides in the ticks. It is increasingly common for livestock producers to experience multispecies resistance in parasite populations exposed to acaricides or insecticides (Morales *et al*., 1999). This sometimes involves multiple tick species or multiple taxa.

A lack of standardized techniques for diagnosing acaricide resistance appears to be the main difficulty in creating and maintaining a tick resistance monitoring system. A search for alternatives to the quickly expiring acaricides because of development of resistance is the only hope for farmers. Botanical acaricides are a possible avenue to alternative acaricides. The herb *Artemisia alfa* (wormwood) is one potential acaricidal herb that can be assessed for acaricidal activity.

The word wormwood has been used more frequently than green ginger. Wormwoods are members of the great family of compositae and belong to the genus Artemisia, a group consisting of 180 species. *Artemisia alfa* is a bitter herb and has been used more often than the other members of the genus. With the exception of Rue, Wormwood is thought to be the bitterest herb known. Wormwood grows naturally in the wild. It likes a shady situation and is easily propagated by division of roots in autumn, by cuttings or by seeds sown in the autumn after they are ripe (Leung and Foster, 1996). The history of wormwood dates back to biblical times .The herb has been known in the past to treat stomachic problems, nervous disfunctions and it acts as a direct stimulant of cortex cerebri. These effects show that Artemisia is a potential antimicrobial and as such it is worthwhile an effort to explore the potency of this herb as an alternative acaricide.

In developing countries like Zimbabwe many commercial and peasant farmers find it costly to purchase all the required and proper medicine to treat their livestock and this has resulted in poor beef production and low grade livestock. This has had a huge impact on our Agro-based economy. It is therefore in the researcher's interest to explore and find possible alternatives to the expensive livestock medicine (acaricides) by considering wormwood. Some peasant farmers with small quantities of livestock have relied on ethno veterinary science to treat their cattle and other livestock but this knowledge is partial and only applied by those who have received it orally. Some ethno veterinary tools have however not been scientifically proven and the wormwood herb is one such tool. Since there is no written data on the use and application of the wormwood herb in treatment of livestock, it is in the researcher's interest to make this project a first step towards that goal.

Ticks transmit viral, rickettsial, bacterial and protozoal diseases affecting wild, domestic animals and humans. The genera *Hyaloma*, *Boophilus*, *Rhipicephalus*, *Argas* and *Ornothodoros* are commonly infesting farm animals. They are known to transmit infectious agents to humans and domestic animals such as Dohori, Thogoto and Wanowrit viruses, the rikettesia such as *R. conori* and several protozoa.

More and more chemical acaricides have been synthesized and tick resistance to those chemical pesticides poses a serious threat to most farmers worldwide. This has caused farmers to spend a large percentage of their time and money on the management of ticks and tick-borne diseases. Because of the above mentioned reasons it is necessary for research to look for botanical pesticides as an alternative new safe method for tick control. Botanical pesticides have different properties from plant to plant. They are processed in various ways which include preparation of the crude plant materials, plant extraction by use of different solvents or resins, purifying the chemicals isolated from plants and identifying their structure. Botanical pesticides have various effects against ticks such as reducing tick feeding, molting, fecundity and viability of eggs. Thus botanical pesticides have many advantages over synthetic chemicals. Advantages include minimal mammalian toxicity, minimal impact on pollinators and natural enemies, minimal environmental pollution, less expensive and easily available. The major objective of this study was thus to investigate the potential acaricidal activity of *Artemisia alfa* (Wormwood) against the larvae of three tick species *Boophilus decoralatus, Ripicephalus appendiculatus and Rhipicephalus evertsi*. The study also aimed investigating the feasibility of the use of the extract in Olive Oil and trilene formulation as well as determining the most effective concentration using the Larval Packet Test.

Materials and Methods

The study was carried out at the Central Veterinary Laboratories, Harare, from November to December 2011.

Tick collection and rearing

Engorged female ticks of the three species *Boophilus decoralatus, Ripicephalus appendiculatus and Rhipicephalus evertsi* were collected from farms around Harare and Chitungwiza Towns, Zimbabwe. The ticks were held in small transport vials supported with moist Whatman filter paper. These were then washed in distilled water to remove any eggs laid during transport. All the engorged female ticks were permitted to attach to rabbit host's ear pinnae for feeding until fully engorged. When fully engorged the ticks were transferred to rearing tubes with saline and kept at 27 ^{0}C and 80-90 % Relative Humidity (R.H) in a glass jar incubators until egg laying and larval hatching were completed. Hatched larvae were maintained under the same conditions.

Preparation of the leaf extracts (potential herbal acaricide)

Freshly picked leaves of the herb wormwood were washed under running water and weighed, giving a leaf mass of 50 grams. The leaves were then crushed using a pestle and mortar to give a colloid which was further blended using an electronic blender. The liquid extract obtained was then used to prepare herbal extract solution by mixing with a mixture of olive oil and trilene mixed in the ratio 1:2 by volume to make a volume of 16ml. This solution was serially diluted in 4ml volumes as represented by Table 1 below to concentrations of 25, 50, 75 and 100 % respectively. Three Whatmann 541 filter papers per concentration per tick species were impregnated with each one of the prepared extract concentrations and allowed to dry in a film hood for three hours.

Table 1. Formulation of chemicals to different concentrations for acaricidal test.

Tube number	1	2	3	4	5
Stock (ml)	0	1	2	3	4
Extract O/T	4	3	2	1	0
Concentration (%)	0	25	50	75	100

Bioassay

The three tick species were exposed to four extract concentration as treatments. For each treatment 100 larval ticks were wrapped in larval packets impregnated with leaf extract. The four treatments were 0, 25, 50, 75 and 100% leaf extract. The Filter paper packets were then sealed with bulldog clips so as to securely wrap the larvae. The closed packets were placed in a tray and kept in an incubator overnight at 27 ± 1 °C and a relative humidity of 80-90 %. This procedure was done for each of the three tick species, *Rhipicephalus evertsi, Boophilus decoralatus and Rhipicephalus appendiculatus* and each treatment was replicated three times.

After 24 hours, observations for mortality were made by literally counting the dead and live larvae. All non-motile tick larvae were considered dead. The data obtained were expressed as percentage mortality at each concentration of acaricide.

Data Analysis

A two way ANOVA (Analysis of variance) was conducted that examined the effect of concentration and species on tick mortality. The dependant variable mortality was normally distributed for the groups formed by the combination levels of concentration and species as assessed by the Shapiro-Wilk test. There was homogeneity of variance as assessed by Levine's test for equality of error variances. Data was organized and represented in the form of descriptive tables, histogram and line graphs to assist in the analysis of data.

Results

All experimental treatments induced mortality. Dead ticks were distinguished from live ones by their inability to move. Mortality was thus evidenced by inability to move. For all tick species every extract concentration level and replicate induced mortality (Table 2). No mortality was recorded for all species in the control treatments.

Table 2. Mortalities induced by different concentrations of *A. alfa* extract on tick larvae in the three replicates used during the experiment at respective extract concentration.

Tick species	Concentration (%)				
	0	25	50	75	100
B .decoralatus	0,0,0	4,4,5	25,28,23	31,34,28	50,53,52
R.appendiculatus	0,0,0	7,4,5	16,21,19	27,23,25	36,43,41
R. evertsi	0,0,0	4,5,5	15,21,18	28,24,23	42,41,45

For all tick species, an increase in leaf extract concentration resulted in a general increase in mortality (Figure 1). *B decoralatus* showed the highest susceptibility followed by *R. evertsi* and *R. appendiculatus* was the most resistant of the three. There was a consistent zero percent mortality observed in the control experiments for all the three species.

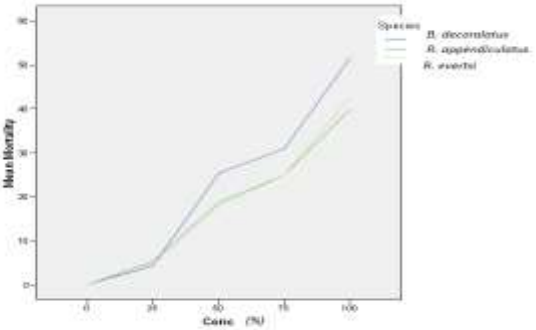

Figure 1. A comparative line plots for the three ticks' mortality figures

There was a general trend for the three mortality plots which reflects an increase in mortality with increasing extract concentration. However the mortality plot for tick species one, *Boophilus decoralatus* is fairly higher than that for the other two tick species, *R. appendiculatus* and *R. evertsi*. The susceptibility level of *B. decoralatus* is thus higher than the other two tick species.

From the zero concentration to the 25% mark, a small count of mortality was observed for all the three species but there was a marked difference between the 25% and 50% concentrations. A slight increase in mortality was observed after increasing the extract concentration from 50 to 75%.

Table 3. Mean mortality figures for tick larvae.

Tick species	Concentration (%)				
	0	25	50	75	100
B. decoralatus	0	4,3	25,3	31.0	51,7
R.appendiculatus	0	5,3	18,7	25.0	40.0
R .evertsi	0	4,7	18.0	25.0	42,2

The highest mortality of 51, 7 was achieved for *B. decoralatus* larvae and the least mortality of 40% was recorded for *R. appendiculatus* (Table 3). The susceptibility trend for each of the species shows a rise in mortality with increase in concentration. There were a small differences recorded between the 75% and 50 % concentration. The herbal leaf extract was more effective against *B. decoralatus* species while slightly less potent against *R. evertsi* and *R. appendiculatus.*

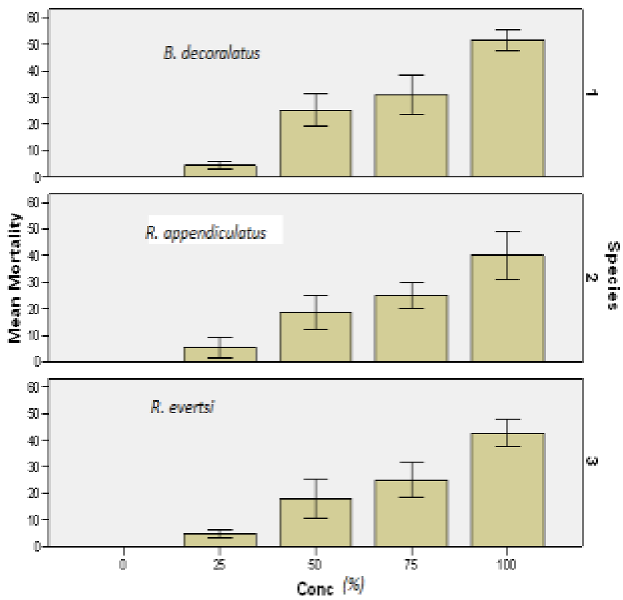

Figure 2. Mean mortalities (with error bars: 95% CL) for the three species of tick larvae at various extract concentrations.

Anova results showed that mortality was highly dependent on concentration ($P<0.05$). The data summerised in Figure 2 above shows that an increase in concentration from 25 to 50% resulted in a significant rise in mortality in all tick species. There were no significant difference between the mean mortality for the 50 and 75% concentration. There were however significant differences between the mean mortalities observed at 75% and that of 100% for all three species.

Analysis of data also showed that mortality was significantly affected by species. There were significant differences between the mean mortalities observed for *B decoralatus* and *R. appendiculatus* ($p=0.012$) and that between *B. decoralatus* and *R. evertsi* ($p=0.018$). There was however no significant differences in mean mortalities observed for *R. appendiculatus* and those for *R. evertsi* ($p=0.984$).

Discussion

Roughly four thousand five hundred (4500) tick larvae were collected and examined for their susceptibility to wormwood (*A. alfa*) herb leaf extracts with the larval packet test (L.P.T). Leaf extracts induced mortality in all experimental treatments. There is enough evidence at the experimental level to suggest that *A. alfa* is a potential herbal acaricide against *B. decoralatus, R. appendiculatus* and *R. evertsi*. The L.P.T is a contact method of pesticide control and thus the mode of action of herbal leaf extracts was through contact. Active ingredients on the larval packets had contact with the larval ticks during the 24 hours of incubation.

Toxicological studies on wormwood by other scholars show that the herb contains extraordinarily bitter compounds called absinthins. Alpha-thujone is said to be the principal active ingredient of wormwood oil and toxic principle in absinth (Arnold 1988). It is also the active ingredient of wormwood oil and some other herbal medicines and is reported to have antinociceptive, insecticidal, and anthelmintic activity (Arnold 1988). The content of betathujone often exceeds that of alpha-thujone depending on the plant source, but the betadiastereomer is generally of lower toxicity. The plant *Artemesia absinthium* and wormwood oil have insecticidal properties (Grainge and Ahmed, 1988), and alpha-thujone was one of the two most toxic monoterpenoids tested against western corn rootworm larvae (Lee *et al*. 1997)

It is mostly likely that one of these active compounds had a toxic effect on the tick larvae on contact with the treated larval packets. There was an increase in mortality with increase in extract concentration (Table 1) in all the experimental treatments. This could have been attributed to the increasing concentration of active ingredient and thus indicating that toxicity is dosage dependant. There was a similar noted trend with *B. decoralatus* larvae but with a significantly higher susceptibility level, mortalities of above 50% percent being recorded in all three replicates. For *R. appendiculatus* and *R. evertsi* the general trend of increase in mortality with increasing extract concentration was evident but at a lesser level of susceptibility. The highest mortalities recorded for *R. evertsi* and *R. appendiculatus* showed little difference, 45 and 43% respectively. It was apparent that mortality was species dependant and differences in mortality arose as a result.Differential susceptibility showed differential resistances among the different species and within different species of the same genera. The resistance of *B. decoralatus* was lower than that of *R. appendiculatus* which in turn was less than that of *R. evertsi*. Slight differences were seen in the highest mortalities recorded for *Rhipicephalus* species. This might have been due to closely related mechanisms of resistance. Differences in mortality might also have been due to differences in mode of nutrition and tolerance levels. Some larval ticks might not have been able to tolerate adverse conditions in the larval packets during the incubation period.

Resistance to acaricides in ticks is a result of multiple factors. The strength of acaricide used as well as the frequency of application (Southerst and Comins, 1979). A very effective acaricide applied frequently will result in rapid elimination of susceptible ticks. This will result in higher selection rates and as a result higher incidence of interbreeding among resistant members giving rise to genetically resistant strains (Southerst and Comins, 1979). Ticks with a shorter life cycle will also have a higher resistance (Spickett, 1998). This is because the selective elimination of a large number of the susceptible individuals in that population.

In conclusion it can be said there is enough evidence at the 5% significance level to support the hypothesis that the herb *Artemisia alfa* is a potential herbal acaricide, confining the validity of this statement to the tick larvae of *Boophilus decoralatus, Rhipicephalus appendiculatus* and *Rhipicephalus evertsi.* This notion is supported by the mortality data obtained when tests were run exposing ticks to the leaf extract of *A. alfa.*

The use of natural products for plant and crop protection dates back to times long before the introduction of synthetic pesticides which imposed themselves as the main means for suppression of harmful organisms. In recent times, the significance of natural pesticides is constantly growing, primarily in organic agriculture, but also in the framework of Bio-rational pest control programs which insist on use of environmentally-friendly pesticides and exploitation of novel biochemical modes of action (Isman, 2006; Isman & Akhtar, 2007). Some of the natural products are substances that have significant acaricidal effect.

Conventional acaricides have been commonly used in the control of ticks for a long time; however, apart from being expensive, they have detrimental effects of creating residues in animal products and its destructive effects to the environment (Laffont *et al*., 2001). Also, ticks tend to develop resistance against them (Latif and Jongejan, 2002). In addition, the indiscriminate use of conventional acaricides in cattle tick control has caused the loss of enzootic stability to tick-borne diseases. Ethno-veterinary remedies tend to reduce the density of tick population to an equilibrium level below that which results in appreciable economic or clinical effects. They promote enzootic stability to ticks and tick-borne diseases (Laffont *et al.*, 2001). The use A alfa extract will present an easily available, cheap and hence sustainable and environmentally alternative to conventional acaricides. The use of whole extract is highly recommended as more research is undertaken to evaluate the use of alternative diluents and formulations.

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