Full Length Research Paper

Efficacy of chitosan supported organic acaricide extract from *Melia azedarach* leaves on *Rhipicephalus (Boophilus) microplus* ticks

Priscila Fernanda Pereira Barbosa¹*, Pablo Peres de Mendonça¹, Rômulo Davi Albuquerque Andrade², Ana Carolina Ribeiro Aguiar¹, Andréa Rodrigues Chaves³ and Fabiano Guimarães Silva¹

¹Goiano Federal Institute, Rio Verde Goiás, Brazil.  
²Federal Institute of Goias, Luizânia, Goiás, Brazil.  
³Federal University of Goias, Goiânia, Goiás, Brazil.

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The *Rhipicephalus (Boophilus) microplus* tick control is mainly performed by chemicals products, but organic acaricides use has higher advantages compared to conventional products. Brazilian cerrado native plants are known for their bioactive potential. Due to this fact, the objective of this study is to evaluate the acaricide action of native cerrado specie, *Meliaazedarach*, known as Santa Barbara in the tick control. Also, the chitosan nanosphere was evaluated on the extract adsorption and release, as a proposal to raise the acaricide phytotherapic profile. The ethanol extract was obtained by cool extraction from dried leaves of the plant. Chitosan nanospheres were obtained by the phase inversion method. Conductometric titration, ultraviolet-visible (“UV-Vis”) and Fourier transform infrared spectroscopy (FTIR) analysis were conducted with materials to evaluate the chitosan anchoring ability. *In vitro* test was used in engorged females for each treatment, which consisted of control, raw extract and three treatments with increasing concentrations of 0.2; 0.4 and 1%. The organic product effectiveness of 0.2% concentration was found by observing reduction in the eggs mass compared to control group. The nanomaterial proved capable to anchor and release the acaricide gradually in pH between 6 and 7, which makes it feasible for use in cattle, prolonging the exposure time between the tick and acaricide.

**Key words:** Cerrado specie, *Rhipicephalus (Boophilus) microplus* tick, ectoparasites, tick control, acaricide, chitosan nanoparticle.

INTRODUCTION

The tick *Rhipicephalus (Boophilus) microplus* are hematophagous ectoparasites responsible for damage associated with livestock in countries located in tropical and subtropical parts of the world. In Brazil, many cattle...
breeds, especially the Europeans, are susceptible to the
development of tick parasitic stages, and there are
weather changes highly favorable to survival and
development of its non-parasitic stages (Junior and
Oliveira, 2005). The tick control is mainly performed by
chemicals administered by contact or systemically,
aiming to combat its parasitic stages. Improper use of
these acaricides (individual practices of combat, without
applying the recommendations based on appropriate
methods of acaricide management) induces resistance
strains, growth and strengthening the active principles
managed. Thus, perceive the ineffectiveness of some
chemical acaricides, and these leave residues in the
meat and milk hosts (Broglio-Micheletti et al., 2009).

The need for safer and more effective methods to
combat R. (B.) microplus has stimulated the search for
new acaricides from plant extracts. Thus, it is believed
that the plant extracts use isolated or associated to other
application methods can lead to slow development of
resistance, in addition residues reduction in the meat and
milk, because the botanical acaricides are biodegradable
(Iannacone and Sludge, 2002). Santa bárbara (Melia
azedarach) also known as cinamomo is a large tree
belonging to the Meliaceae family, represented in Figure
1. The plant insecticidal activity was valued at over 400
insects species, in which over 100 occur in Brazil. This
plant insecticidal activity is due to the presence of
biologically active compounds, triterpenoids (Brunherotto
et al., 2010).

Engorged females immersion tests and larval package
made with plant extracts from the leaves, green and
mature fruit of this species demonstrated the efficacy in
tick control, because there was larvae oviposition
inhibition and high mortality rates (Sousa et al., 2008).
Thus this plant becomes the subject of many studies
about its bioactive potential, since it presents a variability
of chemical compounds which can cause death or
prevent tick cycle occurrence (Chiffelle et al., 2009).
Besides organic acaricide application, the new acaricide
profile can be improved by using a technology that
promotes the controlled release of the active ingredient
gradually. In this context, the biopolymers use for this
purpose is an attractive proposition, because it is natural
raw materials, degradable and has low cost.

Chitosan is a polymer derived from chitin deacetylation
process by enzyme hydrolysis or alkali treatment. It is
considered a cationic polysaccharide in neutral or basic
pH conditions, contains amino and acetamino groups,
considered a very reactive molecule, which makes it
more susceptible to structural changes. Due to the
chitosan structure, its use becomes very favorable in
compounds binding studies, in addition to controlled
release of these in the desired environment (Liu et al.,
2008). Considering the biotic potential of the specie Melia
azedarach, this study has a goal of verifying the efficacy of ethanol extract from plant leaves in *R. (B.) microplus* control, together with the extract controlled release technology by chitosan nanoparticle surface.

**MATERIALS AND METHODS**

**Nanospheres production**

The chitosan solution was prepared with 5% (v/v) acetic acid; the formed mixture was under stirring until complete homogenisation. NaOH solution was prepared at 10% in 100 ml. The solution was standardized until getting a 0.995 correction factor. Chitosan solution was introduced into a “spray drying” system in which the sample passed through a nebulizer to form an aerosol which was gelled in the chitosan nanospheres form. After obtaining the nanospheres, they were washed until it got to pH 7. Subsequently, they were exposed at room temperature to dry (Dias et al., 2008). This method is known as phase inversion.

**Plant extracts production**

**Plant leaves collection**

The Santa Bárbara leaves were collected from Goiano Federal Institute (Instituto Federal Goiano) at Rio Verde campus, (17° 48’ 16” S, 50° 54’ 19” W, 749 m altitude). After collecting them, they were separated from their stems and weighed. After weighing, they were distributed in paper bags and placed in an oven with forced air circulation at a temperature of up to 40°C to dry the leaves. The paper bags weight were checked daily until the weight remained constant. The obtained dried leaves were ground in four knives mill to facilitate the extraction.

**Extraction**

The extraction process started when the leaves were dried and crushed. They were macerated in 1.5 L of ethanol (Vetec 95%) into an erlenmeyer containing about 300 g of plant mass. The solvent was replaced every 3 days to achieve greater extraction. This process was repeated until the solvent remains translucent. The solvent was volatilized in a rotary evaporator, yielding DCE (dried raw extract). The extract obtained was considered raw extract, but any specific compound was not evaluated by chromatography. It was understood that the *M. azedarach* bioactive potential is due to the presence of triterpenoids.

**Anchoring and conductimetric titration**

For the raw extract anchoring on the chitosan nanosphere surface, 1 g of nanosphere and 0.5 g of raw extract was used. 5% (v/v) hydrochloric acid (Sigma Aldrich) solution in 10 ml was prepared. Besides nanospheres and extract, was added 60 ml of distilled water in the solution. The whole system was stirred for 24 h.A conductivity and pH meter was used to check the system conductivity and pH before and during the titration. The titration started at pH 2, and gradually small amounts (µL) of 10% NaOH was added in the system. As pH and conductivity suffered variations, aliquots were removed for each variation in the conductivity for subsequent analysis in the UV-Vis and FTIR. All procedures were performed in triplicate.

**Fourier transform infrared (FTIR)**

Analyses were performed by FTIR-ATR-NIRA- Frontier PerkinElmer simultaneous with the collection of aliquots during titration. Nothing was added in the samples; the analyses just made from the aliquots collected. The relationship between transmittance and wavelength in the spectra was observed. For comparative purposes, aliquots as extract and chitosan solution at 5% were analyzed and observed, alongside the shift of the energy band.

**Ultraviolet in the visible region (UV-Vis)**

During the conductimetric titrations, the collected aliquots at the points of greatest variation of conductivity were analyzed separately in the UV-Vis Lambda 750 PerkinElmer, which became full scan spectrum (200 - 1000 nm) to obtain the samples gradients absorbance. For this analysis, 200 µL of each sample diluted in 4 ml of ethanol was prepared.

**Scanning electron microscopy (SEM)**

Realized analysis was done by Scanning Electron Microscope (SEM), JEOL JSM - 6610, equipped with EDS, Thermo scientific NSS Spectral Imaging, using metallizer BALTEC SCD 050 for nanoparticles morphological determination.

**In vitro test**

For in vitro experimentation, *R. (B.) microplus* engorged females in naturally infested cattle on a farm near the Rio Verde city, Goiás were collected. They were washed, dried and separated into 5 groups in the laboratory. The study consisted of 5 treatments, the group I was the control group (ethanol), group II corresponded to the raw extract and the groups III until V were the groups in which the raw extract was diluted in ethanol at concentrations of 0, 2; 0.4 and 1%, respectively. For group III to V, 0.5 grams of chitosan nanosphere treatments were added, and the solution was stirred for 24 hours before starting the tests.

The engorged female ticks were immersed in 20 ml of the solutions corresponding to each treatment for 5 minute, this time being established by Leite (1988). After immersion, the engorged females were dried on paper towels and fixed by adhesive on Petri plates, previously identified (Drummond et al., 1973). 6 engorged females for all groups were performed in triplicate. Subsequently, the specimens were dried on tissue paper and placed in identified Petri dishes in the BOD incubator (28 ± 1°C, 80% humidity) for 14 days. After the laying period, the eggs of each tick female for all treatments were transferred to syringes without tips, sealed with cotton, and again sent to the BOD incubator (28 ± 1°C, 80% humidity) where they were kept for 26 days to evaluate the hatching eggs. Treatments were performed in triplicate and the results were obtained from averages. Data were submitted for analysis of variance and means compared using Tukey test at 5% error probability.

**RESULTS AND DISCUSSION**

Figures 2 to 4 correspond with chitosan nanoparticles, the *M. azedarach* extract and the extract anchored in the chitosan nanosphere spectra, respectively. It was observed in Figure 1 bands and stretch peaks for the nanosphere chitosan in the region of 3362 cm\(^{-1}\) and 1024 cm\(^{-1}\), such regions correspond to angular deformation of the N-H, O-H bond and C-O stretching. In Figure 2 the notaries regions are 3377, 2924, 1696 and 1047 cm\(^{-1}\),
Figure 2. Chitosan nanoparticle spectrum in the FTIR.

Figure 3. Santa Bárbara spectrum in the FTIR.
wherein the first region is indicated by the presence of O-H bond in alcohols function, ketones and carboxylic acids, the second region correspond to the C-H bond, the third corresponds to the double stretching of carbon-oxygen and last the stretch C-O of alcohols, esters and others (Aragão and Messaddeq., 2010). All these regions found in M. azedarch extract spectra are related to the major metabolite class found in this specie, the triterpenes. The triterpenes are one of the most structurally diverse groups of terpenes, in this class. There are over 40 thousand different structures, with various compounds which serve as important pharmaceutical agents. The triterpenes structure is considerably large, showing some main organic functions, such as alcohol, ester, ether and carboxylic acid (Silva et al., 2014).

When Meliaazedarch extract is anchored on chitosan nanoparticle, the spectrum takes another form, raises new bands and peaks regarding nanoparticle spectrum without the anchored specie. Figure 4 shows this spectrum, in which the region 3362 cm\(^{-1}\) of Figure 2, undergoes displacement emerging band at 3368 cm\(^{-1}\) region, and the peak 1024 cm\(^{-1}\) also undergoes displacement, showing a peak in the region 1034 cm\(^{-1}\). Furthermore, the other peaks in this figure suffer increase and decrease in the transmittance intensity due to the interactions occurring between the polymer surface and the extract molecules.

During the conductometric titration process aliquots of the reaction medium were collected and analyzed in the FTIR and UV-Vis. It was observed in the FTIR analysis increase and decrease the groups electron density as the pH changed. Figure 5 lists this change in electron density in function of pH change. It was noticed to a greater electron density for spectra were closer to neutral pH, for example at pH 5.95 and 6.43. The peaks near the regions 3500, 1400 and 1000 cm\(^{-1}\) had an increase and decrease in transmittance intensity in function of electron density. The denser the peak the greater the amount of adsorbed extract groups in that pH range. According to the results obtained, the pH at which the extract concentration was higher and chitosan molecule was most stable, was close to neutral, between pH 5 and 6.

Baidu and other colleagues (2007) conducted studies on the sorption capacity of metal ions, arsenic III and arsenic V by chitosan surface. One of the analyses carried out by the group involved chitosan FTIR analysis before and after the sorption. The obtained results pointed to differences in the spectra after sorption, in which there was displacement of chitosan characteristic bands and increased in transmittance intensity. The authors have pointed out a possible explanation for this effect, the amount of functional groups present in chitosan after sorption process (Boddu et al., 2007). The extract absorption and release effects from polymer matrix were also evaluated by UV-Vis analysis, by the aliquots collected in the conductometric titration process. The rising of new peaks is shown in Figure 6, beyond the
increase in absorbance bands according to the pH of the aliquot analyzed.

In the 265 cm\(^{-1}\) region, larger absorbance peaks were observed, in which the values found were 0.65 and 0.60 at pH 2.11 and 2.16, respectively. At these points, the chitosan molecules were protonated due to the acid...
medium. The amine group is responsible for molecule protonation and deprotonation. Therefore, the pH mentioned might have a higher concentration of adsorbed extract. The conductivity solution change is caused by the absorption and release effects, in this case $H^+$ ions or extracts molecules. In general, it is noted that the absorbance peaks decrease as the solution becomes more basic, but the last peak is observed at pH 7.40, in the region 265 cm$^{-1}$ with 0.25 absorbance value. In basic medium occurs the amine group deprotonation, releasing if there is adsorbed extract. At this point, the extract concentration in solution is higher because its release for the medium and chitosan nanosphere structure became stable.

Figure 7 corroborates with the results found in UV-Vis analysis. This graph represents the conductivity versus pH from the NaOH volume added in the extract nanosphere chitosan solution. The conductivity decreases and increases with the sodium hydroxide addition, however at a certain point, the pH and the conductivity values cross each other, and from that the conductivity increases. The meeting point is between pH 6 and 7, at this pH can observe greater concentration of extract released by the analysis discussed above. The images taken by SEM show that the methodology used to produce nanoparticles was efficient, displaying uniformity in particle size, about 589 nm, as shown in Figure 8. Mortality was observed in some individuals due to some treatments, but the mortality rate for treatment II (raw extract) is obvious. The bioassay occurred as 100% death in all individuals. But this acaricide use is infeasible due to the amount of plant material used in obtaining them.

In relation to oviposition, the treatments proved to have great potential in reducing the number of egg masses. According to the graph below (Figure 9), the reduction rate ranged between 30 to 100%, and for the treatment II oviposition did not occurred. The effect of increasing concentration was also noted, as the extract concentration increased the eggs mass reduction rate also increased. These results are partially consistent with those obtained by Borges et al. (2003), who used *Melia azedarach* (Meliaceae) at 0.25% observing in vitro complete oviposition inhibition on engorged female immersed in the raw extract of mature fruits extracted with different solvents. Moreover, they observed high larval mortality and high efficacy on engorged females. Although the extract did not kill adult females, it totally or partially inhibited the eggs production. The female mortality in this bioassay was high, especially for the raw extract and this is different from what have been seen.

Statistical test performed with the data obtained can also see from the graph above the similarity between the treatment I and III, corresponding to control and lowest extract concentration groups. This points out that even the lowest concentration used in this study, the *Melia azedarach* extract, is effective for *R. (B.) microplus* control. Each treatment was performed in triplicate and each bar corresponds one of triplicate for the same treatment. Figure 10 represented by 1, 2 and 3 correspond...
Figure 8. Nanoparticles produced from spray-dry system by gelation method NaOH.

Figure 9. Reduced rate representation in percent of the eggs mass after 14 days from the treatment beginning.

to the control, raw extract and 0.2% concentration, respectively. Clearly, the oviposition for 0.2% concentration treatment decreased considerably compared to the control, and the raw extract did not occur during oviposition, but all the ticks in this treatment died after 7 days of immersion.

Conclusion

The process of production nanoparticles by gelling method in NaOH was effective. It produced a uniform material with 589 nm size. This demonstrates that the spray-drying method is efficient for nanoparticles
production. The chitosan nanosphere proved to be an effective biopolymer adsorption and release *Melia azedarach* extract. This is because, through the spectra of each one and the adsorbed extract on the nanoparticle surface spectrum, two effects were observed: bands displacement and increase of electron density compared with the spectrum before the adsorption. Furthermore, the UV-Vis analysis confirmed this observation. The acid pH extract remained adsorbed, the pH increased was measured and the extract was released by the polymer matrix. The controlled release is more evident in the pH ranges between 6 and 7, which show the efficiency of the material used to raise the organic acaricide profile. More so, the extract performs the *R. (B.) microplus* control, slowly releases the active ingredient, ensuring a longer exposure time between the tick and the acaricide, and preventing its reproductive cycle. Bioassays performed with the ticks for five treatments showed different effects on control, raw extract and the other treatments with increasing concentrations. For raw extract, 100% mortality occurred in all individuals and for some treatments death was observed too, but this was not so significant. Regarding to the eggs mass reduction, the percentage diversified between 30 to 100%, and in most treatments, the percentage was between 80 to 100%.

The treatment of 0.2% was significant at 5% Tukey test, which proves the efficiency of the controlled release technique of the *Melia azedarach* extract and its acaricidal activity.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES


Boddu VM, Abburi K, Talbott JL, Smith ED, Haasch R (2007). Removal of arsenic (III) and arsenic (V) from aqueous medium using chitosan-