Repellent Activity Of Carvacrol And Timol Encapsulated With Yeast Cell Wall Against *Amblyomma Sculptum* And *Rhipicephalus Sanguineus* (*Sensu Lato*) Nymphs

Jhone Robson Silva Costa
Federal University of Maranhão – UFMA
Tassia Lopes Vale
Federal University of Maranhão – UFMA
Geovane Ferreira Silva
Federal University of Maranhão – UFMA
Naylene Silva Sales Cavalho
Federal University of Maranhão – UFMA
Aldilene Silva Lima
Federal University of Maranhão – UFMA
Lívio Martins Costa-Junior
Federal University of Maranhão – UFMA
Hermes Ribeiro Luz
hermes.luz@ufma.br
Federal University of Maranhão – UFMA

Research Article

Keywords: DEET, Natural repellents, Essential oils, monoterpene, microencapsulation, tick

Posted Date: March 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1436716/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Experimental and Applied Acarology on March 12th, 2024. See the published version at https://doi.org/10.1007/s10493-023-00896-y.

Abstract

The main way to avoid contact with ticks and consequently tick-borne disease is the use of synthetic repellents. The search of new repellent compounds to increase the possibilities of use in strategies controls are necessary. The present study evaluated the repellent activity of two natural terpenes carvacrol and thymol in each one two different formulation (encapsulated and nonencapsulated with yeast cell wall) against the ticks *Amblyomma sculptum* and *Rhipicephalus sanguineus* sensu lato nymphs. Nymphs of *A. sculptum* and *R. sanguineus* s.l. of a single generation were used. The vertical filter paper repellency assay were performed with different concentration of both terpenes encapsulated and nonencapsulated in yeast cell wall. The repellent concentration 50% (CR_{50}) were calculated to each compound formulation. Both carvacrol and thymol (encapsulated and nonencapsulated), had a repellent activity against *A. sculptum* and *R. sanguineus* s.l nymphs. *Amblyomma sculptum* was more sensitive to nonencapsulated carvacrol (RC_{50} values: 0.0032 to 0.0082 mg/cm^2 after 1 and 15 min) ($P < 0.05$), while *R. sanguineus* s.l. was more sensitive to encapsulated carvacrol (RC_{50} values: 0.00008 to 0.0035 mg/cm^2 after 1 and 15 min) ($P < 0.05$). Among tick species, *R. sanguineus* s.l. was more sensitive for most compounds than *A. sculptum* ($P < 0.05$). Although with distinct repellent activities, carvacrol and thymol encapsulated can be a promising alternative to synthetic repellents against *A. sculptum* and *R. sanguineus* s.l.

Introduction

Rhipicephalus sanguineus sensu lato is the vector of *Babesia canis vogeli* and *Ehrlichia canis* for domestic dogs (Dantas-Torres et al. 2010), while *Amblyomma sculptum* (formerly *A. cajennense*) is the vector of *Theileria equi* (Peckle et al. 2013). Furthermore, *A. sculptum* is the main vector of the bacterium *Rickettsia rickettsii*, the causative agent of Brazilian Spotted Fever (BSF) to humans in Brazil, being *R. sanguineus* s.l. a potential vector (Dantas-Torrres et al. 2010; Labruna et al. 2017; Luz et al. 2019). Currently, the use of synthetic repellents is the most used method to avoid ticks infestation and consequently tick-borne disease. The N,N-diethyl-3-methylbenzamide (DEET) is the compound most used (Carroll et al. 2004; Jensenius et al. 2005; Soares et al. 2010; Meng et al. 2016; Ferreira et al. 2017). However, the uncontrolled use of these synthetics can cause numerous adverse effects on animals, humans and the environment, in addition to selecting resistant species (Naqqash et al. 2016).

Studies are focused on finding promising alternatives to synthetic repellents against ticks, particularly those of animal and human importance. In this context, the use of several essential oils (EOs) has low toxicity on animal, human and environmental health. EOs and their constituents are natural products of plant with characteristics of low toxicity, rapid degradation and sustainability to a promising repellent action against ticks (Tabari et al. 2017). The monoterpenes carvacrol and thymol stand out for their repellent effectiveness against a variety of hard tick species (Tabari et al. 2017, Lima et al. 2019; Arafa et al. 2020), except *A. sculptum* and *R. sanguineus* s.l. nymphs.

The monoterpenes are highly volatile reducing their repellent action over time, since they are unstable under high temperature, oxygen, and light. Microencapsulation formulation could preserve the terpenes properties and increase the time of repellent effect. The *Saccharomyces cerevisiae* yeast wall have been used for encapsulating hydrophobic compounds, including EOs and terpenes (Pannell 1990; Paramera et al. 2011; Ciamponi et al. 2012; Lima et al. 2017). Lima et al. (2017) showed encapsulation of carvacrol with *S. cerevisiae* cell walls increased acaricidal activity, up to 2.5 times compared to nonencapsulated carvacrol. In turn, Lima et al. (2019) also demonstrate that the encapsulation process increased the repellent effects of carvacrol by 5.4 times compared to unencapsulated carvacrol at 6 hours post-treatment.

Due to the veterinary and public health importance of *A. sculptum* and *R. sanguineus* s.l., the need for alternative compounds to synthetic ones against these ixodides is notorious. Thus, the present study evaluate *in vitro* repellent activity of carvacrol and thymol (encapsulated and nonencapsulated with yeast cell wall) against *A. sculptum* and *R. sanguineus* s.l., nymphs in order to contribute to the development of repellents with natural compounds.

Materials And Methods

Ticks

The experiments with animals were approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Maranhão, Brazil under protocol number 23115.005443/2017-51. The colony of *A. sculptum* was established of donated specimens by the Laboratory of Parasitic Diseases of the University of São Paulo, São Paulo, Brazil. The colony of *R. sanguineus* s.l. was established from naturally infested dogs in the municipality of São José de Ribamar, Maranhão, Brazil.

For colony maintenance, ticks of *A. sculptum* and *R. sanguineus* s.l. were fed through artificial infestations on rabbits *Oryctolagus cuniculus*, using the technique proposed by Faccini et al. (2021). After natural detachment, engorged larvae, nymphs and adults were collected and kept in a climatized chamber (BOD) (27 ± 1ºC and 80 ± 10% relative humidity. Only nymphs aged between 15 and 25 days of fasting were used in the experiment.

Carvacrol, Thymol and DEET

The Thymol, Carvacrol and DEET were purchased commercially from the Sigma-Aldrich company (St. Louis, MO), with the issuance of certificates of purity ≥ 98%. DEET, carvacrol and, thymol were diluted in a solution containing P.A. ethanol and distilled water, with serial concentrations at 50% starting at 1 mg/cm^2. The tested concentrations of encapsulated and non-encapsulated carvacrol and thymol, as well as DEET, were: 0.5, 0.025; 0.0125; 0.00625; 0.003125; 0.001563; 0.000781; 0.000391; 0.000195; 0.000098 mg/cm^2.All solutions were prepared on the day of the test. As a negative control, distilled water and unencapsulated yeast wall.

Encapsulation of Carvacrol and Thymol

The yeast cell wall were purchased commercially from Biorigin (São Paulo, SP, Brazil). The carvacrol and thymol encapsulation process in *S. cerevisiae* yeast cell wall was performed according to the method of Paramera et al. (2011) and Lima et al. (2017). The yeast cell wall (3g) was washed in 20 mL of distilled water, centrifuged at 3.000 rpm for 10min, and the supernatant discarded. Carvacrol or Thymol (3g) were added to the washed yeast wall and the mixture was stirring at 45°C ± 1 for 4 hours. The material washed by centrifugation at 3.000 rpm for 20 min to remove nonencapsulated terpene. The encapsulated sample were kept at -20°C, and after lyophilized. The yeast cell walls were washed using the same washing procedure and used as negative control. The encapsulation efficiency (%EY) was calculated in mg of terpene encapsulated in yeast cell wall according to Paramera et al. (2011).

Morphological analysis

Carvacrol and thymol encapsulated were placed on carbon coated tape and examined using scanning electron microscopy (Phenom ProX, Phenom-World, Eindhoven, Netherlands) at an acceleration of 15 kV. Two hundred yeasts from each sample were measured using Image J software (National Institute of Mental Health, USA) and were compared through prism 8.0 using the Way-Anova test followed by the Tukey post-test.

In vitro repellence test

Nymphs of *A. sculptum* and *R. sanguineus* s.l. of a single generation (25-day fasting), avoiding the influence of possible genetic variability were used to repellency test. The repellency test on vertical filter paper with the different species of ticks were performed according Carroll et al. (2004). Filter papers (7 x 4 cm), with a central area corresponding to 5 x 4 cm were impregnated with 165µL of different concentrations of encapsulated carvacrol, encapsulated thymol, nonencapsulated carvacrol, nonencapsulated thymol and positive control using DEET. The corresponding 1 x 4 cm upper and lower areas were considered neutral areas (untreated). The papers were dried for 10 minutes at room temperature and suspended vertically by one of the non-impregnated areas in an apparatus. At each observation time 10 specimens of tick were placed in the non-impregnated area at the bottom of the filter paper. The location of specimens in each area of the filter paper was observed at 1, 5, 10 and 15 minutes post initial of the test. As a negative control, unencapsulated yeast wall and water was used. All concentrations tested were carried out with three repetitions.

Statistical analysis

The concentration repellent for 50% of the nymphs (CR_{50}) was calculated using GraphPad Prism Version 8.0 (Graphpad Software, Inc., San Diego, CA, USA). The data were initially transformed to log (X) and normalized; subsequently, nonlinear regression was performed to obtain the CR_{50}. The repellency results for the different treatments and species were compared using the 2way-Anova and Wilcoxon tests, respectively. For all statistical analyses, a significance level of 5% was adopted.

Results

Encapsulation process

Scanning electron microscopy images showed samples containing carvacrol and thymol encapsulated in the yeast wall. Furthermore, the images of encapsulated *S. cerevisiae* show spheroidal or slightly elongated shapes, regular and smooth surfaces with no deformities, suggesting that the encapsulation process did not damage the yeast cell wall. Thus, it is pointed out that the walls remained thick while the encapsulated molecules had contact with encapsulating material (Fig. 1). Morphological analysis by scanning electron microscopy also revealed size differences between control yeast cell walls (mean area 0,138 ± 0.025 μm), encapsulated carvacrol (mean area 0,192 ± 0.031 μm) ($p < 0.05$) and encapsulated thymol (mean area 0,190 ± 0,035 μm) ($p < 0.05$). The encapsulation yield of carvacrol and thymol in yeast cell walls was 74.5% and 85.7%, respectively, showing the encapsulation efficiency of these compounds.

Repellent activity

The RC_{50} of carvacrol and thymol (encapsulated and nonencapsulated), and DEET (positive control) on *A. sculptum* and *R. sanguineus* s.l. are shown in Table 1. All compounds, including the positive control (DEET), had a repellent effect at all concentrations tested against *A. sculptum* and *R. sanguineus* s.l. Negative control showed no repellent activity.

Table 1
Concentration repellent (CR50) of encapsulated and unencapsulated carvacrol, thymol and DEET to 50% of nymphs of *Amblyomma sculptum* and *Rhipicephalus sanguineus* s.l.

Compounds	Tempo	*Amblyomma sculptum*			*R. sanguineus s.l.*		
		RC50	**95% CI**	R2	**RC50**	**95% CI**	R2
Nonencapsulated carvacrol	1	**0.0032bA**	0.0028–0.0037	0.9689	**0.0018cA**	0.0012–0.0028	0.7839
	5	**0.0061bA**	0.0050–0.0075	0.9446	**0.0059cA**	0.0046–0.0076	0.8985
	10	**0.0079bA**	0.0065–0.0096	0.9377	**0.0122dA**	0.0092–0.0160	0.8808
	15	**0.0082bA**	0.0065–0.0103	0.9189	**0.0194dA**	0.0156–0.0242	0.9115
Encapsulated carvacrol	1	**0.0064cB**	0.0061–0.0068	0.9898	**0.00008aA**	0.00005–0.0001	0.8793
	5	**0.0111cB**	0.0102–0.0121	0.9770	**0.0006aA**	0.0005–0.0009	0.9029
	10	**0.0126cB**	0.0118–0.0135	0.9834	**0.0013aA**	0.0009–0.0017	0.9046
	15	**0.0157cA**	0.0148–0.0167	0.9861	**0.0035aA**	0.0026–0.0046	0.9054
Nonencapsulated Timol	1	**0.0026bB**	0.0020–0.0033	0.9167	**0.0006bA**	0.0005–0.0008	0.9195
	5	**0.0105cB**	0.0074–0.0148	0.8081	**0.0037cA**	0.0031–0.0044	0.9501
	10	**0.0156cA**	0.0119–0.0205	0.8622	**0.0041cA**	0.0033–0.0052	0.9212
	15	**0.0237cB**	0.0222–0.0253	0.9602	**0.0041cA**	0.0080–0.0130	0.9100
Encapsulated Timol	1	**0.0062cB**	0.0057–0.0067	0.9825	**0.00033bA**	0.0002–0.0004	0.8989
	5	**0.0088cB**	0.0083–0.0092	0.9919	**0.0014bA**	0.0011–0.0019	0.9095
	10	**0.0108cB**	0.0098–0.0119	0.9765	**0.0029bA**	0.0022–0.0037	0.9156
	15	**0.0195cB**	0.0174–0.0219	0.9498	**0.0058bA**	0.0045–0.0076	0.9097
DEET	1	**0.0017aA**	0.0015–0.0019	0.9769	**0.0004bA**	0.0003–0.0006	0.8929

	5	**0.0019aA**	0.0018–0.0020	0.9938	**0.0012bA**	0.0009–0.0016	0.9057
	10	**0.0028aA**	0.0021–0.0035	0.9184	**0.0032b,cA**	0.0025–0.0042	0.9173
	15	**0.0046aA**	0.0039–0.0054	0.9504	**0.0053bA**	0.0043–0.0066	0.9385

For *A. sculptum* nymphs, the RC_{50} values of carvacrol ranged from 0.0032 to 0.0082 (nonencapsulated) and 0.0064 to 0.0157 (encapsulated) mg/cm^2, thymol from 0.0026 to 0.0237 (nonencapsulated) and 0.0062 to 0.0195 (encapsulated) mg/cm^2, and DEET of 0.0017 to 0.0046 mg/cm^2. The tick *A. sculptum* was more sensitive to DEET than the other compounds, with RC_{50} ranging from 0.0017 to 0.0046 mg/cm^2 ($P < 0.05$) (Table 1). Among the monoterpenes, nonencapsulated carvacrol was the most effective against *A. sculptum* ($P < 0.05$), having a high repellent effect. Carvacrol (encapsulated) and thymol (encapsulated and nonencapsulated) showed low repellent activity, with similar RC_{50} values ($P > 0.05$) (Table 1).

Rhipicephalus sanguineus s.l. nymphs was more susceptible to the repellent effect of encapsulated carvacrol in relation to other compounds, including positive control (DEET) ($P < 0.05$). On the other hand, nonencapsulated carvacrol showed little effectiveness in repelling *R. sanguineus s.l.* nymphs with high RC_{50} values. Encapsulated thymol also showed high repellent activity against *R. sanguineus* s.l. with RC_{50} similar to positive control (DEET) ($P > 0.05$). Nonencapsulated thymol showed also a relevant repellent effect, with RC_{50} close to positive control (DEET), and statistically similar at 1 minute of the test ($P > 0.05$).

Among the species, *R. sanguineus* s.l. exhibited high sensitivity for most compounds compared to *A. sculptum* ($P < 0.05$), except for nonencapsulated carvacrol (5, 10 and 15 minutes) and DEET (10 and 15 minutes) ($P < 0.05$).

Discussion

Amblyomma sculptum and *R. sanguineus* s.l. are among the most important vectors of diseases for animals and humans worldwide (de la Fuente et al. 2008), including the lethal BSF caused by *R. rickettsii*. Although all evolutionary stages of *A. sculptum* and *R. sanguineus* s.l. can parasitize man, the nymph stage is considered the most important due to its greater parasitic success, increasing the chances of BSF transmission (Szabó et al. 2013; Labruna et l. 2017; Luz et al. 2019). One of the main ways of prevention is the use of synthetic repellents, which can cause numerous adverse effects on animals, humans and the environment. The search for alternative compounds (e.g., EOs and its terpenes) against these ixodids is mandatory. Furthermore, EOs and terpenes are highly volatile reducing their repellent effect time, and the microencapsulation should be an alternative to decrease the volatility and increase the effect.

The encapsulation yield of carvacrol and thymol in yeast cell walls in the present study was 74.5% and 85.7%, respectively, showing the encapsulation efficiency of these compound. Furthermore, the encapsulation process did not degrade the yeast cell walls, remaining thick and without deformities after containing the terpenes. Similar efficacy was observed in the encapsulation of extract of curcumin (Paramera et al. 2011) and carvacrol (Lima et al. 2017; 2019).

Overall, carvacrol and thymol exhibited repellent effect against *A. sculptum* and *R. sanguineus* s.l. nymphs. This suggests these monoterpenes as alternative compounds in prevention and control of these ixodids and its tick borne disease. Carvacrol and thymol are known for their acaricidal activity, especially to *Amblyomma*, *Rhipicephalus* e *Ixodes* (Novelino et al. 2007; Daemon et al. 2009; Oliviera Monteiro et al. 2010; Silva Mendes et al. 2011; Carroll et al. 2017; Tabari et al. 2017; Lima et al. 2019; Vale et al. 2021). Furthermore, carvacrol and thymol are considered promising compounds against other arthropods such as mosquitoes, flies and mites (Lindberg et al. 2000; Park et al. 2012; Tababari et al. 2015).

Although both carvacrol and thymol had repellent activity against *A. sculptum* nymphs, nonencapsulated carvacrol was most effective. This result was unexpected, since both compounds are highly volatile and encapsulation with *S. cerevisiae* cell walls tends to increase their repellent effect over time (Bakkali et al. 2008; Shi et al. 2008; Lima et al. 2019). One hypothesis would be methodological, where the time from 1 to 15 minutes was not enough to observe the increase in repellent activity, requiring a longer time for a better release of the compound from the encapsulated and, consequently, having a greater activity. In addition, the species and stage of the tick may have influenced, since studies of the repellent activity of encapsulated monoterpenes (e.g., carvacrol) were carried out on *Rhipicephalus* larvae for up to 6h (Lima et al. 2019). Therefore, larvae would be more sensitive than nymphs for their morphophysiological characteristics (e.g., size, little chitin, and skin respiration). Thus, further studies on the repellent activity of encapsulated and nonencapsuled thymol and carvacrol against *A. sculptum* nymphs are needed (e.g., larvae and adults). Interestingly, carvacrol and thymol have high acaricidal activities against *A. sculputm* immatures, reaching a mortality rate > 90% (Vale et al. 2021), indicating the potential of these compound to control these ixodids.

Rhipicephalus sanguineus s.l. was more sensitive to encapsulated carvacrol when compared to the other compounds ($P < 0.05$), including the positive control (DEET). These results agree with those reported by Lima et al. (2019) for *R.* (*B.*) *microplus* larvae, in which encapsulated carvacrol was also more effective. Nonencapsulated carvacrol also showed high repellent activity against *R. sanguineus* s.l., but with repellent effect 22.5, 9.8, 9.3 and 5.5 less than encapsulated carvacrol. Lima et al. (2019) also reported that the repellent activity of encapsulated carvacrol was 3 to 5 times more potent than nonencapsulated carvacrol against *R.* (*B.*) *microplus*, concluding that the encapsulation technique protected and increased the repellent activity of carvacrol. However, thymol (nonencapsulated and encapsulated) also showed a high repellent activity against *R. sanguineus* s.l., in a greater similarity between DEET and encapsulated thymol. As observed for *A. sculputm*, thymol encapsulated and nonencapsulated had a varied repellent effect against *R. sanguineus* s.l.. Therefore, it is possible that thymol encapsulation with *S. cerevisiae* cell walls does not change its repellent activity over time as seen for encapsulated carvacrol in the present

study and by Lima et al. (2019). Thus, the time from 1 to 15 minutes may have been a limiting factor, requiring further investigation. Importantly, thymol is toxic to a variety of ticks and mites of public and animal health importance, including *Rhipicephalus* spp. (Monteiro et al. 2012; Barimani et al. 2016; Tabari et al. 2017).

The tick *R. sanguineus* s.l. was more sensitive to the repellent effects of most compounds than *A. sculptum* ($P < 0.05$), except for nonencapsulated carvacrol (5, 10 and 15 minutes) and DEET (10 and 15 minutes) ($P < 0.05$) (Table 1). These results confirm the toxic effect of carvacrol and thymol against ticks of the genus *Rhipicephalus* (Araújo et al. 2016; Tabari et al. 2017; Lima et al. 2019), and the need of further investigation of these monoterpenes against ticks of the genus *Amblyomma*. Senra et al. (2013) using a concentration of 2.5 mg/mL of carvacrol and thymol, recorded a lethality of 100% for *R. sanguineus* s.l. nymphs, and of 45.0–62.7% for *A. sculptum* nymphs. It is possible that *Rhipicephalus* spp. tends to suffer more the effects of these monoterpenes than *Ambyomma* spp., indicating a possible natural resistance from this last group to the monterpenes carvacrol and thymol. In addition, *A. sculptum* parasitizes a variety of wild and domestic hosts, and during the non-parasitic phase they are found on the forest vegetation and dirty fields (Barros-Battesti et al. 2010; Guglielmone et al. 2014). Already *R. sanguineus* s.l. parasite the domestic dog, never found in forest areas or fields. Thus, it is possible that the constant contact of *A. sculptum* with botanical compound at the environments has evolved some type of resistance of this species against the toxicity of different plant compounds.

Due to their acaricide and repellent effects against ticks, low toxicity to humans, animals and the environment, EOs and it's terpenes have become promising compounds in the control of these ectoparasites, and in the prevention of transmitted diseases (e.g., Lyme Disease, Tick-Borne Encephalitis and BSF). Although with distinct repellent activities, the results in the present study show a significant repellent efficacy of carvacrol and thymol against nymphs of *R. sanguineus* s.l. and *A. sculptum*. Also, the effectiveness of the yeast wall in encapsulating these monoterpenes confirming that the yeast wall is a promising structure in encapsulating molecules with high volatility (e.g., carvacrol and thymol), acting as a physical barrier against temperature and humidity variations.

Declarations

Conflicts of interest

The authors have no conflict of interest to declare.

Author contributions

All study authors. Material preparation, data collection and analysis were performed by [Jhone Robson da Silva Costa], [Tassia Lopes do Vale], [Geovane Ferreira da Silva], [Naylene Silva Sales] and [Aldilene da Silva Lima]. The review, editing and supervision of this study were performed by [Livio Martins Costa-Junior] and [Hermes Ribeiro Luz]. All authors comment as previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgment

This research was partially supported by IECT Biotechnology through grants from FAPEMA (Maranhão State Research Foundation, Brazil) and FINEP (Funding Authority for Studies and Projects). This study was financed in part by the Coordination for the Improvement of Higher Education Personnel (CAPES/Brazil) – Finance Code 001. CAPES/DPG - “Amazônia-Legal” (Support Program for Postgraduate Studies in the Legal Amazon), number- 088.990.417-09.

References

1. Arafa WM, Aboelhadid SM, Moawad A, Shokeir KM, Ahmed O (2020) Toxicity, repellency and anti-cholinesterase activities of thymol-eucalyptus combinations against phenotypically resistant *Rhipicephalus annulatus* ticks. Exp Appl Acarol 81:265–277. https://doi.org/10.1007/s10493-020-00506-1
2. Araújo LX, Novato TPL, Zeringota V, Maturano R, Melo D, Silva BD et al (2016) Synergism of thymol, carvacrol and eugenol in larvae of the cattle tick, Rhipicephalus microplus, and brown dog tick, *Rhipicephalus sanguineus*. Med Vet Entomol 30:377–382. https://doi.org/10.1007/s00436-016-5113-3
3. Barimani A, Youssefi MR, Tabari MA (2016) Traps containing carvacrol, a biological approach for the control of *Dermanyssus gallinae*. Parasitol Res 115:3493–3498. https://doi.org/10.1007/s00436-016-5113-3
4. Barros-battesti DM, Arzua M, Bechara GH (2010) Carrapatos de importância médico-veterinária da região neotropical: um guia ilustrado para identificação de espécies ICTTD-3 Instituto Butantan. https://repositorio.butantan.gov.br/handle/butantan/3153
5. Ciamponi F, Duckham C, Tirelli N (2012) Yeast cells as microcapsules. Analytical tools and process variables in the encapsulation of hydrophobes in *S. cerevisiae.* Applied microbiology and biotechnology. 95: 1445–1456. https://doi.org/10.1007/s00253-012-4127-8
6. Carroll JF, Solberg VB, Klun JA, Kramer M, Debboun M (2004) Comparative activity of deet and AI3-37220 repellents against the ticks Ixodes scapularis and *Amblyomma americanum* (Acari: Ixodidae) in laboratory bioassays. J Med Entomol 41:249–254. https://doi.org/10.1603/0022-2585-41.2.249
7. Carroll JF, Demirci B, Kramer M, Bernier UR, Agramonte NM, Baser KHC et al (2017) Repellency do Origanum onites L. óleo essencial e constituintes para o carrapato estrela solitária e mosquito febre amarela. Prod Res 31:2192–2197. doi: 10.1080/14786419.2017.1280485
8. Dantas-Torres F (2010) Biology and ecology of the brown dog tick, Rhipicephalus sanguineus. Parasites & vectors 3:1–11. https://doi.org/10.1186/1756-3305-3-26
9. Daemon E, Monteiro CMO, Rosa LR, Clemente MA, Arcoverde A (2009) Evaluation of the acaricide activity of thymol on engorged and unengorged larvae of *Rhipicephalus sanguineus* (Latreille, 1808) (Acari: Ixodidae). Parasitol Res 105:495–497. https://doi.org/10.1007/s00436-009-14269

10. de la Fuente J, Estrada-Pena A, Venzal JM, Kocan KM, Sonenshine DE (2008) Overview: ticks as vectors of pathogens that cause disease in humans and animals. Front Biosci 13(13):6938–6946. DOI: 10.2741/3200
11. Faccini JLH, Almeida TK, Sousa IC, Junior LMC, Luz HR (2021) Temperature effects on the non-parasitic phase of *Amblyomma parvum* (Acari: Ixodidae). Syst Appl Acarology 26:1168–1176. https://doi.org/10.11158/saa.26.6.12
12. Ferreira LL, Filho JG, O, Mascarin GM, León A A P, Borges LMF (2017) In vitro repellency of DEET and β-citronellol against the ticks *Rhipicephalus sanguineus* sensu lato and *Amblyomma sculptum*. Vet Parasitol 239:42–45. https://doi.org/10.1016/j.vetpar.2017.04.021
13. Guglielmone AA, Robbins RG, Apanaskevich DA, Petney TN, Estrada-Peña A, Horak IG (2014) The hard ticks of the world: (Acari: Ixodida: Ixodidae). Springer, London, p 738
14. Jensenius M, Pretorius AM, Clarke F, Myrvang B (2005) Repellent efficacy of four commercial DEET lotions against Amblyomma hebraeum (Acari: Ixodidae), the principal vector of Rickettsia africae in southern Africa. Trans R Soc Trop Med Hyg 99:708–711. https://doi.org/10.1016/j.trstmh.2005.01.006
15. Labruna MB, Krawczak FS, Gerardi M, Binder LC, Barbieri AR, Paz GF et al (2017) Isolation of *Rickettsia rickettsii* from the tick *Amblyomma sculptum* from a Brazilian spotted fever-endemic area in the Pampulha Lake region, southeastern Brazil. Veterinary Parasitology: Regional Studies and Reports 8: 82–85. https://doi.org/10.1016/j.vprsr.2017.02.007
16. Luz HR, Costa FB, Benatti HR, Ramos VN, Serpa MCA, Martins TF et al (2019) Epidemiology of capybara-associated Brazilian spotted fever. PLoS Negl Trop Dis 13:e0007734. https://doi.org/10.1371/journal.pntd.0007734
17. Lima AS, Maciel AP, Mendonça C D J S, Junior LMC (2017) Use of encapsulated carvacrol with yeast cell walls to control resistant strains of Rhipicephalus microplus (Acari: Ixodidae). Ind Crops Prod 108:190–194. https://doi.org/10.1016/j.indcrop.2017.06.037
18. Lima ADS, Landulfo GA, Costa-Junior LM (2019) Repellent effects of encapsulated carvacrol on the Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). J Med Entomol 56:881–885. https://doi.org/10.1093/jme/tjy240
19. Meng H, Li AY, Costa Junior LM, Castro-Arellano I, Liu J (2016) Evaluation of DEET and eight essential oils for repellency against nymphs of the lone star tick, Amblyomma americanum (Acari: Ixodidae). Exp Appl Acarol 68:241–249. https://doi.org/10.1007/s10493-015-9994-0
20. Monteiro CM, O, Daemon E, Silva AMR, Maturano R, Amaral C (2010) Acaricide and ovicide activities of thymol on engorged females and eggs of Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). Parasitol Res 106:615–619. https://doi.org/10.1007/s00436-009-1709-1
21. Monteiro CM, Maturano R, Daemon E, Catunda-Junior FEA, Calmon F, Senra TS et al (2012) Acaricidal activity of eugenol on Rhipicephalus microplus (Acari: Ixodidae) and Dermacentor nitens (Acari: Ixodidae) larvae. Parasitol Res 111:1295–1300. https://doi.org/10.1007/s00436-012-2964-0

22. Naqqash MN, Gökçe A, Bakhsh A, Salim M (2016) Insecticide resistance and its molecular basis in urban insect pests. Parasitol Res 115:1363–1373. https://doi.org/10.1007/s00436-015-4898-9
23. Novelino AMS, Daemon E, Soares GLG (2007) Avaliação da atividade repelente do timol, mentol, salicilato de metila e ácido salicilico sobre larvas de Boophilus microplus (Canestrini, 1887) (Acari: Ixodidae). Arquivo Brasileiro de Medicina Veterinária e Zootecnia 59:700–704. https://doi.org/10.1590/S0102-09352007000300023
24. Pannell NA Microbial Encapsulation.International Patent Application No. EP0242135B1, 12 April 1986.
25. Paramera EI, Konteles SJ, Karathanos VT (2011) Stability and release properties of curcumin encapsulated in Saccharomyces cerevisiae, β-cyclodextrin and modified starch. Food Chem 125:913–922. https://doi.org/10.1016/j.foodchem.2010.09.071
26. Peckle M, Pires MS, Santos TM, Roier EC, Silva CB, Vilela JA et al (2013) Molecular epidemiology of Theileria equi in horses and their association with possible tick vectors in the state of Rio de Janeiro, Brazil. Parasitol Res 112:2017–2025. https://doi.org/10.1007/s00436-013-3360-0
27. Szabó MPJ, Pinter A, Labruna MB (2013) Ecology, biology and distribution of spotted-fever tick vectors in Brazil. Front Cell Infect Microbiol 3:27. https://doi.org/10.3389/fcimb.2013.00027
28. Senra TOS, Calmon F, Zeringóta V, Monteiro CM, O, Maturano R, Matos RS et al (2013) Investigation of activity of monoterpenes and phenylpropanoids against immature stages of Amblyomma cajennense and Rhipicephalus sanguineus (Acari: Ixodidae). Parasitol Res 112:3471–3476. https://doi.org/10.1007/s00436-013-3527-8
29. Shi G, Rao L, Yu H, Xiang H, Yang H, Ji R (2008) Stabilization and encapsulation of photosensitive resveratrol within yeast cell. Int J Pharm 349:83–93. https://doi.org/10.1016/j.ijpharm.2007.07.044
30. Soares SF, Braga RDS, Ferreira LL, Louly CCB, Sousa L, A D D, Silva ACD et al (2010a) Repellent activity of DEET against Amblyomma cajennense (Acari: Ixodidae) nymphs submitted to different laboratory bioassays. Revista Brasileira de Parasitologia Veterinária 19:12–16. https://doi.org/10.4322/rbpv.01901003
31. Tabari MA, Youssefi MR, Maggi F, Benelli G (2017) Toxic and repellent activity of selected monoterpenoids (thymol, carvacrol and linalool) against the castor bean tick, Ixodes ricinus (Acari: Ixodidae). Vet Parasitol 245:86–91. https://doi.org/10.1016/j.vetpar.2017.08.012
32. Vale L, Paula LGF, Vieira MS, Alves S, D G A, Moraes Junior NR, Gomes MDF et al (2021) Binary combinations of thymol, carvacrol and eugenol for Amblyomma sculptum control: Evaluation of in vitro synergism and effectiveness under semi-field conditions. Ticks and Tick-borne Diseases 12(6):101816. https://doi.org/10.1016/j.ttbdis.2021.101816

Figures

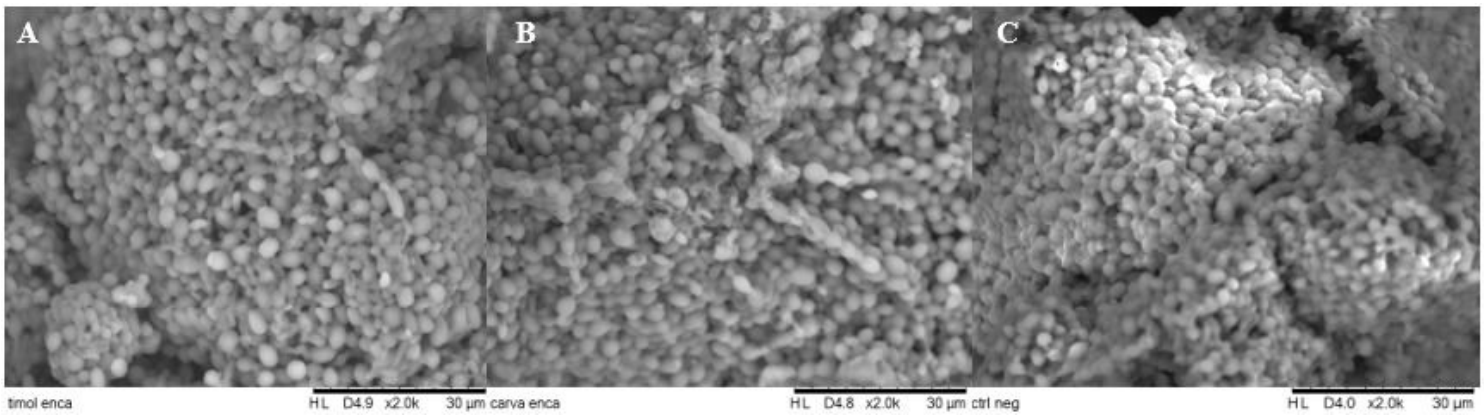

Figure 1

Scanning electron microscopy (SEM) of thymol (A) and carvacrol (B) encapsulated with yeast cell walls and negative control (C).