



## **EFFECT OF CARVACROL AND THYMOL ON A *Rhipicephalus sanguineus* sensu lato TICK CELL LINE**

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Control of ticks on livestock and companion animals in Brazil currently relies on application to the host of toxic chemicals to kill the ticks - acaricides - which are expensive and environmentally damaging. Moreover, ticks can rapidly develop resistance to acaricides, necessitating a continual ongoing search for novel compounds to replace those that are no longer effective. It would be very cost-effective if such compounds were initially screened for activity against tick cell lines, before proceeding to tests of promising candidates on whole ticks, thereby reducing costs and use of laboratory animals for tick feeding. As a first step in developing an *in vitro* screen, we tested two compounds known to have acaricidal effect on ticks *in vivo*, carvacrol and thymol, for their effect on the viability and morphology of a cell line derived from embryos of the tick *Rhipicephalus sanguineus* sensu lato, an important ectoparasite of dogs in Brazil. Replicate cultures of the cell line RML-15 were exposed to different concentrations of carvacrol (0.3, 0.06, 0.012 and 0.0025  $\mu\text{L}/\text{mL}$ ) or thymol (70, 14, 2 and 0.5  $\mu\text{L}/\text{mL}$ ) for 24, 48 and 72 hours. At each time-point, total and viable cell numbers were determined by trypan blue staining and cell morphology was analysed in Giemsa-stained cytocentrifuge smears. Both compounds caused a dose-dependent *in vitro* killing effect on the *R. sanguineus* s.l. cells. All cells exposed to the highest concentration of carvacrol died within 72 hours, while minimal effect on cell viability and morphology was seen at the lowest concentration. Higher concentrations of thymol caused up to 20% cell mortality by 72 hours; cells exposed to all concentrations exhibited increased cytoplasmic vacuolation and nuclear distortion. Further studies are needed to determine the mode of action of these acaricidal compounds at the cellular and molecular level. Our results demonstrate the potential application of tick cell lines for initial screening of putative acaricidal compounds. New protocols are required to fully utilise *in vitro* cell systems to study acaricidal effects, which will in turn support broadening of the search for novel products and refining their killing activity.

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