

## ***In vitro* evaluation of essential oils and their binary mixtures against the blacklegged ticks *Ixodes scapularis* (Acari: Ixodidae)**

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**Thematic section:** Biological activities of natural products: *in vitro* and *in vivo*.

**INTRODUCTION:** *Ixodes scapularis* ticks are known to transmit several infectious agents that cause illness in humans. The most important agent is the bacterium *Borrelia burgdorferi* responsible of causing Lyme disease. In the absence of effective vaccines against major tick-borne diseases, controlling tick population using acaricides and repellent products remain the main method to protect animals and humans. Synthetic acaricides are commonly used for tick control. However, new active ingredients for tick management have been explored, such as essential oils (EOs). Previous studies have demonstrated that mixtures of different plant secondary metabolites have synergistic effect against target pest. Lemongrass (*Cymbopogon flexuosus*) and geranium (*Pelargonium x asperum*) EOs exert significant repellent activity against ticks, while thyme (*Thymus saturejoides*) and white thyme (*Thymus zygis*) EOs are effective acaricides. **OBJECTIVE:** In this study we explored the acaricidal effect of selected EOs (lemongrass, geranium, thyme, and white thyme) alone and in binary mixture against *I. scapularis* adult ticks. In addition, we studied the effects of interactions (synergistic, additive, or antagonistic) of mixtures against ticks, when equal concentrations of two EOs were combined. **METHODOLOGY:** EO samples were analyzed via gas chromatography-mass spectrometry (GC-MS) to determine chemical composition. An *in vitro* bioassay (Adult Immersion Test) using *I. scapularis* unfed female ticks was carried out to determine the lethal concentration 50 (LC<sub>50</sub>) of each EO alone. Binary combinations of EOs were prepared by mixing equal concentrations, ranging from 0.25-, 0.5-, 1-, 2-, and 4-fold of their respective LC<sub>50</sub> value. The CompuSyn software was used to make qualitative assessments of the effects (synergistic, additive, and antagonistic) by calculating the combination index value (CI). **RESULTS AND DISCUSSION:** Significant acaricidal activity was reported against *I. scapularis* female adult ticks, particularly for thyme and white thyme EOs with an LC<sub>50</sub> of 28.0 and 11.0 µg/µL, respectively. Lemongrass and geranium EOs reported a LC<sub>50</sub> of 49.0 and 39.7 µg/µL, respectively. The significant acaricidal activity observed might be related to the presence of carvacrol (26.05%) and thymol (53.6%) in the thyme and white thyme EOs composition, respectively. The acaricidal activities of carvacrol and thymol have been reported to be effective against *Amblyomma cajennense*, *Dermacentor nitens*, *Rhipicephalus microplus* and *R. sanguineus*. The chemical analysis of lemongrass and geranium indicated geranial (48.4%) and citronellol (54.4%) as major components, respectively. These active ingredients are known to be effective repellents against arthropods. The interaction of EO compounds can lead to different outcomes, such as additive, antagonistic, or synergistic effects. An additive effect occurs when the combined effect is equal to the sum of the individual effects. Antagonism is observed when the effect of one or both compounds is less when they are applied together than when individually applied. A synergistic effect is observed when the combined effect is greater than the sum of the individual effects. Among the combination of two EOs in the dose-response study, lemongrass + geranium (LC<sub>50</sub>: 61.3 µg/µL) and geranium + thyme (LC<sub>50</sub>: 89.5 µg/µL) resulted to have an antagonist interaction. Interestingly, the combinations of thyme + white thyme (LC<sub>50</sub>: 40.2 µg/µL), lemongrass + thyme (LC<sub>50</sub>: 59.7 µg/µL), and geranium + white thyme (LC<sub>50</sub>: 33.1 µg/µL) showed synergism only in the 0.5xLC<sub>50</sub> concentration. The combination of lemongrass + white thyme (LC<sub>50</sub>: 14.1 µg/µL) indicated synergistic interaction in the 0.25-, 0.5-, 1-, and 2-LC<sub>50</sub> concentrations. When a synergistic combination is observed, it may offer a valid alternative to reduce the concentration of the individual substances and yet maintaining the same level of pest control. Presented results particularly for the combination of lemongrass and white thyme may be used as a reference for the development of future acaricide formulations. **CONCLUSION:** Among the tested EOs, thyme and white thyme had a significant acaricide effect on *I. scapularis* unfed female adult ticks. These compounds could be employed for the development of formulation with acaricidal and repellent activities that may contribute to control the spread of tick-borne diseases.

Keywords: *terpenoids, essential oils, synergistic, antagonistic, LC<sub>50</sub>*.

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