DEVELOPMENT OF NANOEMULSION BASED ON *Eugenia sulcata* (SPRING EX MART) ESSENTIAL OIL AGAINST *Aspergillus flavus*

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Introduction

Aspergillus flavus is a species of cosmopolitan fungus that grows naturally on food substrates, such as peanuts, soybeans, corn, and beans. This pathogen can produce aflatoxins, a secondary metabolite with carcinogenic and mutagenic potential, in addition to causing opportunistic infections, such as aspergillosis, resulting from exposure to high levels of the substance through contaminated food [1].

Natural products are commonly used in Asian countries, such as Japan and India, to preserve foods. Furthermore, the effect of essential oils (EOs) from various plants against *A. flavus* has been studied to develop a control of the species that is less toxic to the environment [2]. *Eugenia sulcata* is a biodiversity plant in the Restinga de Jurubatiba National Park, in Carapebus, Rio de Janeiro, popularly known as "murtinha" or "pitangueira silvestre". Its majority component is β-caryophyllene, a sesquiterpene widely distributed in the essential oil of several plants, previously described with antimicrobial potential [3, 4].

However, the incorporation of EOs in aqueous media is difficult, considering that they are made up of mostly lipophilic substances. For that reason, nanoemulsification is considered promising, as it allows the dispersion of nanometric-sized EO droplets, increasing water availability and improving the physicochemical characteristics of the formulation, and can enhance the pharmacological effects of essential oils thus obtaining a better result [5].

Therefore, the objective of this work was to develop nanoemulsions based on *Eugenia sulcata* essential oil or isolated β-caryophyllene and evaluate their inhibition potential against *A. flavus*.

Material and Methods

The essential oil was obtained by hydrodistillation, using a modified Clevenger-type apparatus. Nanoemulsions (NEs) were prepared containing 90% deionized water and 10% oil phase, consisting of *E. sulcata* EO or β-caryophyllene and nonionic surfactant (polysorbate 80). The NEs were prepared using a low-energy input method via homogenization in a vortex shaker, monitored macroscopically in vial-type bottles. The droplet size was determined by dynamic light scattering (DLS). The antifungal activity evaluation was evaluated with the inoculum of *A. flavus* RC 2054 strains, incubated at 27.5ºC for 72h, performing daily measurements of the colony diameter.

Results and Discussion

Nanoemulsions containing both β-caryophyllene (NEBC) and *E. sulcata* EO (NEES) in surfactant/oil ratios (RTO): 5:5, 6:4, 7:3 presented an opaque macroscopic appearance and those in RTO 9:1 presented a transparent appearance. However, there was a difference in the formulations with RTO 8:2, since NEBC had an opaque appearance, while NEES had a translucent appearance, with a slight bluish reflection after preparation. DLS analysis confirms this observation, showing NEBC droplet size with an average diameter equal to 172.6 ± 2.6 nm and 66.8 ± 6.6 nm for NEES.

In *E. sulcata* EO composition has several volatile substances, with different physicochemical behaviors. Thus, one explanation for the greater stability of NEES in the 8:2 ratio, to the detriment of NEBC, is the presence of endogenous components that allow the inhibition of Ostwald Maturation via the Compositional Ripening mechanism, preventing droplets from aggregating and forming larger diameter droplets, thus destabilizing the system. Consequently, NEs in RTO 9:1 and 8:2 were selected to evaluate antifungal activity.

When analyzing the growth inhibition of *A. flavus* colonies, as illustrated in Figure 1, statistical significance was observed ($p<0.05$) concerning the inoculum control after 24h. This result decreases after 48h and remains stable for up to 72h, indicating a fungistatic effect during the incubation period, but not a fungicidal effect. This effect was highlighted in NEES in the 8:2 ratio. Furthermore, there was no significant difference ($p>0.05$) between white NEs (without OE) and the growth control, which indicates that there was no influence of surfactants on colony growth.

This greater inhibition effect in the first hours of nanoemulsions may be due to their greater water bioavailability, causing it to be consumed in the first hours, but maintaining a residual effect in the following hours. As for oils, due to their more lipophilic composition, they may take longer to be consumed, maintaining a longer effect.

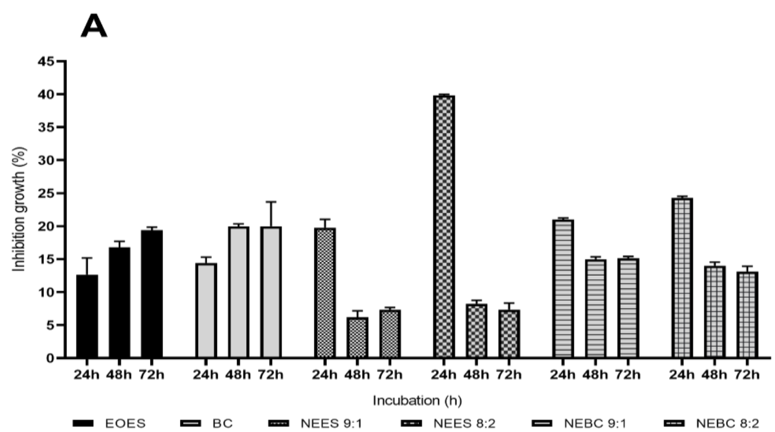

Figure 1. Percentage of growth inhibition against *A. flavus* 24, 48, and 72h of incubation. Treatments were significantly statistically different from the control group (p<0,05)

Conclusion

Overall, a general pattern of inhibition growth was observed as a function of time for NEs, which highlights their importance in increasing the bioactivity of OEES, and improving its penetration and dispersion in the culture medium. Nevertheless, OEES proved to be a potential option for the development of a sustainable product for the antifungal control of this species of *Aspergillus*.

Acknowledgments

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