

TITLE: INTERFERENCE OF BLACK PEPPER ESSENTIAL OIL ON MIXED BIOFILM FORMATION OF *Salmonella* Typhimurium, *Listeria monocytogenes* AND *Pseudomonas aeruginosa*

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ABSTRACT:

Essential oils (EO) are aromatic substances that have antimicrobial action and safe application in food preservation. Thus, the study aimed to evaluate the interference of the EO of Black Pepper (*Piper nigrum*) - (OEP) on the formation of mixed biofilms of *Salmonella* Typhimurium, *Listeria monocytogenes* and *Pseudomonas aeruginosa* on polypropylene surface. The minimum inhibitory concentration of EO (MIC) against the combination of the three microorganisms was determined by broth microdilution. Biofilm formation was performed in triplicates with two repetitions, under the interference of the OEP at a concentration of 100mg/ml, corresponding to the MIC value. The isolates were adjusted to 0.5 on the Mc Farland scale in TSB-YE broth supplemented with 1% TWEEN 80 in flasks containing polypropylene coupons (1x1cm), incubated at 10°C for up to 72h under agitation. After 24 and 72h incubation, the coupons were washed with 10 ml of phosphate buffered saline (PBS) to remove planktonic cells and submitted to ultrasonic bath (40Hz, 90s) followed by vortex homogenization for 90s to remove sessile cells. Dilutions were then made and aliquots were inoculated in specific and nonspecific agar to evaluate the individual populations of each microorganism and total population count, respectively. The results were expressed as Log CFU/cm². The interaction of the three microorganisms was able to form biofilm on the polypropylene surface with counts of 6.51 and 6.70 Log of CFU/cm² in 24 and 72h, respectively. When evaluating the action of 100mg of OEP after 24 and 72h of incubation, the total population showed 90.66% (4.44 log CFU/cm²) and 80% (5.92 log CFU/cm²) reduction. In 72 h, the OEP showed better action on *L. monocytogenes* (3.17 log CFU/cm²) followed by *Salmonella* Typhimurium (4.17 log CFU/cm²). The counts of *P. aeruginosa* presented the lowest values of growth in the control biofilm 4.28 and 4.51 CFU/cm² in 24 and 72h, respectively, evidencing a microbial competition. These results demonstrate that the OEP was able to reduce the counts of sessile cells during the formation of mixed biofilms, having its best action in the long term, thus it is possible to evaluate the application of the OEP as a possible adjunct to be used in the food industry in the control of bacterial biofilms.

Keywords: antibiofilm, bioactive compounds, contamination control

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