Oregano–Rosemary Essential Oil Combinations as Potential Growth-Promoting Alternatives in Poultry: Evidence from Bacterial Time–Kill Curves

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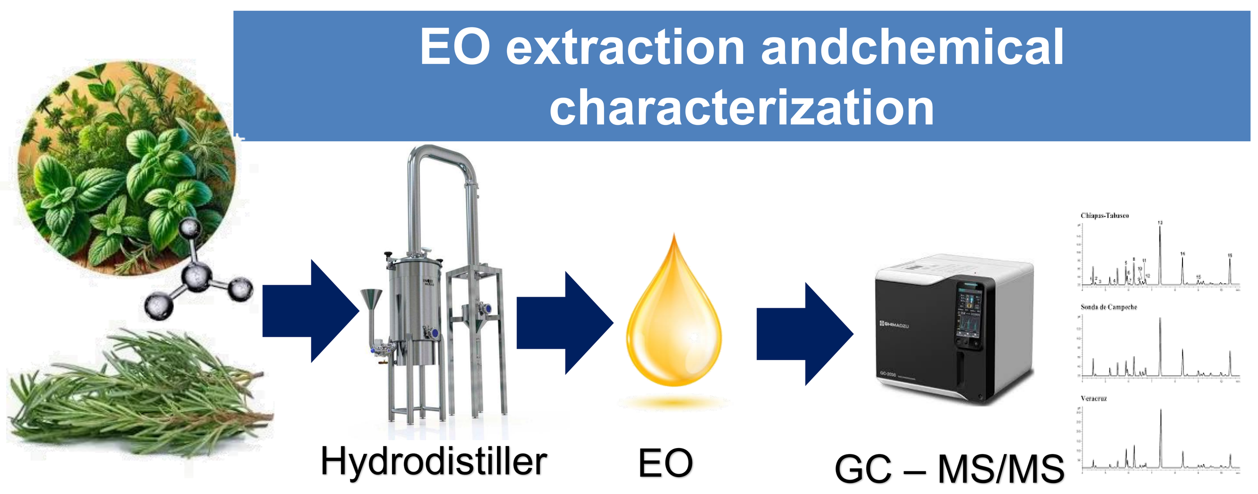
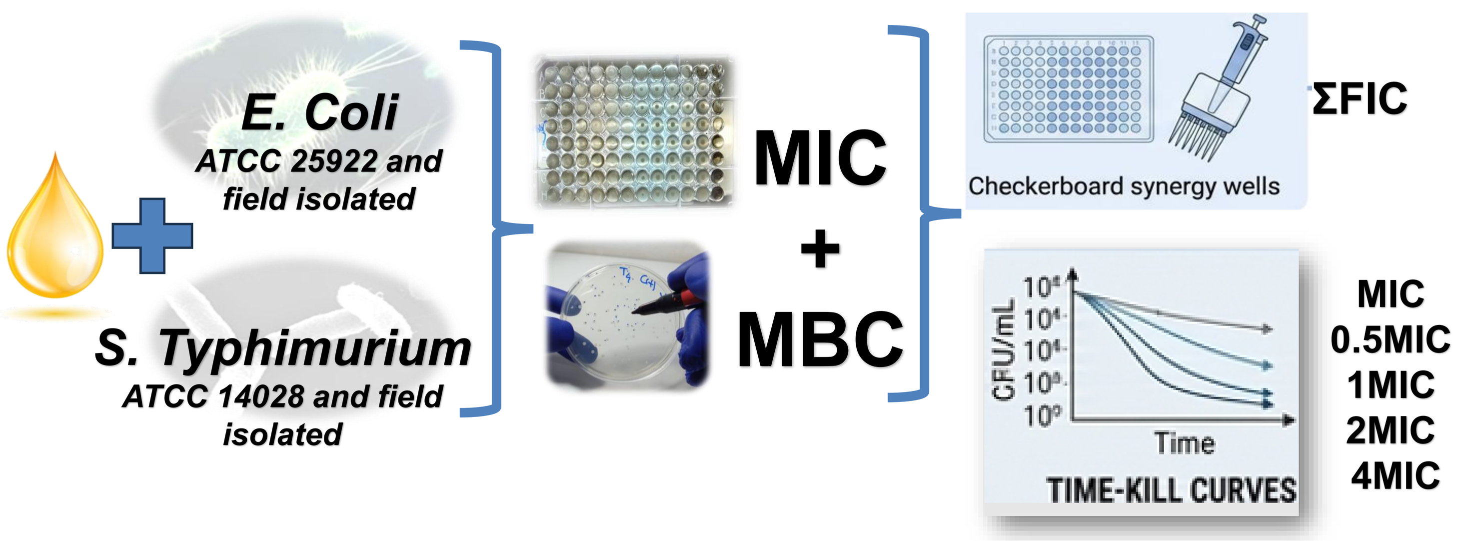
BACKGROUND

- ✓ The use of antibiotics as growth promoters in poultry production has contributed to the emergence and dissemination of antimicrobial resistance, including resistant zoonotic pathogens. Therefore, the development of effective non-antibiotic strategies is a priority within a One Health framework.
- ✓ Essential oils (EOs) obtained from aromatic plants have shown antibacterial activity against foodborne and poultry-associated bacterial pathogens.
- ✓ Among them, oregano essential oil (OEO) and rosemary essential oil (REO) contain bioactive compounds with recognized antimicrobial effects.
- ✓ However, their antimicrobial activity may vary according to bacterial species, strain origin, concentration, exposure time, and gastrointestinal pH conditions.

MATERIALS AND METHODS



- ✓ **OEO** and **REO** were evaluated individually and in combination (**OEO + REO**).
- ✓ Assays were conducted under two pH conditions simulating the avian gastrointestinal tract **(pH 5.0 and 7.4)**.
- ✓ The assays included *E. coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, and poultry field isolates (n = 2 per bacterial species).



- ✓ **The Antibacterial effect** was quantified using the **E index**:

$$E = \log_{10}(n_{t\text{-}final}) - \log_{10}(n_{t\text{-}0})$$

- ✓ The antibacterial effect was interpreted as **Bacteriostatic activity:** E = 0, **Bactericidal activity:** E ≤ −3, **Virtual eradication:** E ≤ −4

OBJECTIVE

To evaluate the ***in vitro* antibacterial activity** of OEO, REO, and their combination against reference strains and poultry field isolates of *Escherichia coli* and *Salmonella Typhimurium*, using MIC/MBC determinations, checkerboard assays, and bacterial time–kill curves under pH conditions representative of the avian gastrointestinal tract.

RESULTS

- ✓ Both EOs exhibited rapid and concentration-dependent antibacterial effects against *E. coli* and *S. Typhimurium*. At higher multiples of the MIC (2x-4xMIC), bacterial counts decreased markedly during the first minutes of exposure (10-20 min) (Fig. 1 and 2).
- ✓ OEO showed strong killing activity, consistent with the known antimicrobial potential of OEO.
- ✓ REO also displayed antibacterial activity, which was enhanced when combined with OEO.
- ✓ The selected OEO + REO combination improved antibacterial activity compared with the individual oils.
- ✓ The combination reached bactericidal or eradication thresholds faster and, in several conditions, at lower concentrations than those required for the individual EOs (Fig. 3 A, B).
- ✓ This effect was observed against both reference strains and poultry field isolates.
- ✓ The antibacterial effect was maintained at both pH 5.0 and pH 7.4.

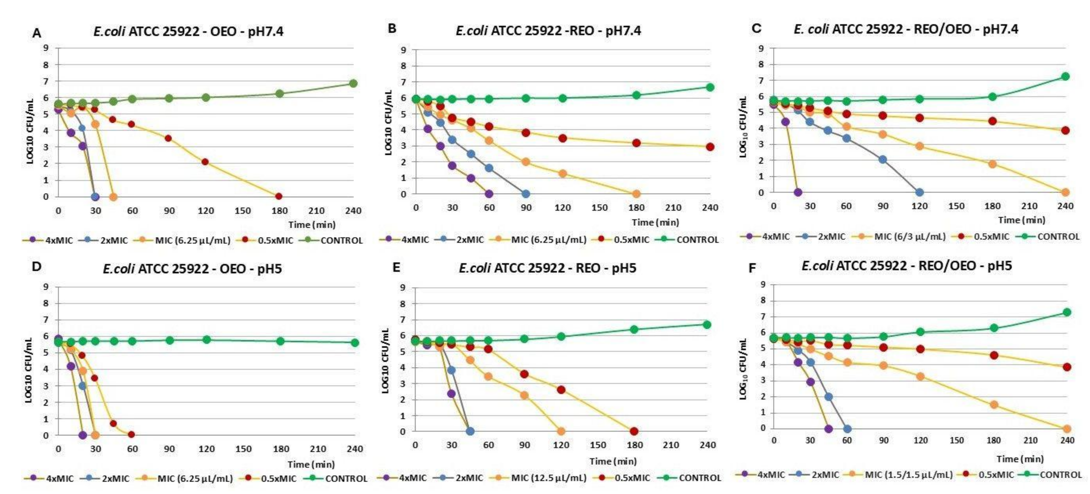


Figure 1. Time–kill curves showing the antibacterial activity of OEO (A,D), REO (B,E), and the OEO + REO combination (C,F) against *E. coli* strains under pH 7.4 (A-C) and pH 5.0 (D-F).

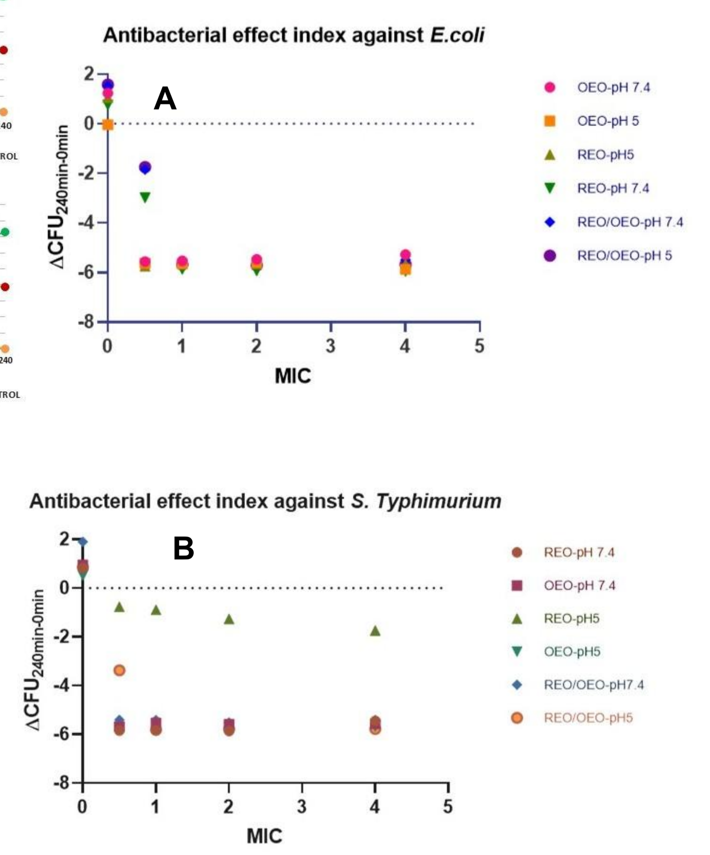


Figure 3. Antibacterial effect index values obtained after exposure to OEO, REO, and the OEO + REO combination under pH 7.4 and 5.0. **A:** *E. coli*, **B:** *S. typhimurium.*

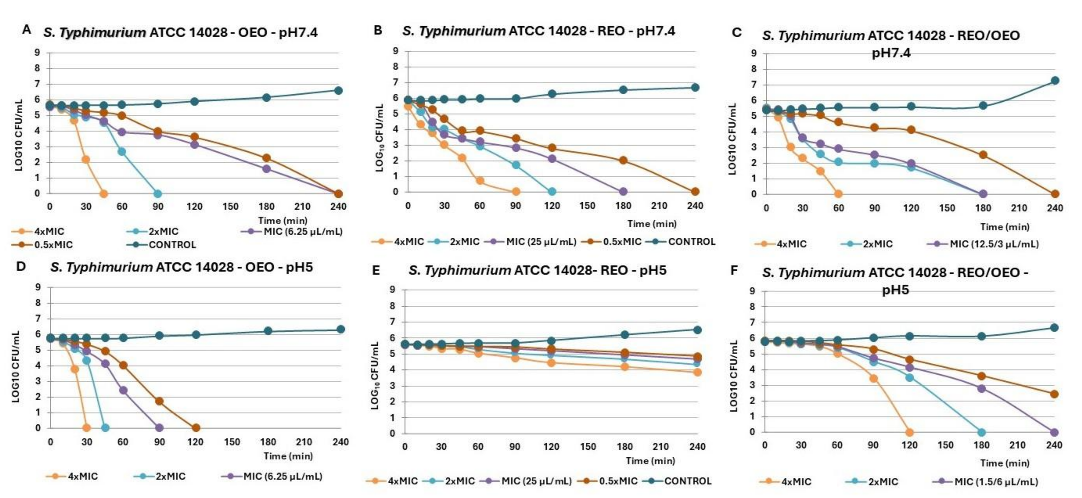


Figure 2. Time–kill curves showing the antibacterial activity of OEO (A,D), REO (B,E), and the OEO + REO combination (C,F) against *S. Typhimurium* strains under pH 7.4 (A-C) and pH 5.0 (D-F).

CONCLUSIONS

- ✓ Oregano and rosemary EOs showed rapid *in vitro* antibacterial activity against reference strains and poultry field isolates of *E. coli* and *S.Typhimurium*. Their combination enhanced bacterial killing and allowed bactericidal or eradication effects to be reached under selected experimental conditions.
- ✓ These findings support the further evaluation of OEO + REO combinations as promising non-antibiotic alternatives for poultry production systems. However, additional studies are required to confirm their efficacy, safety, optimal formulation, and *in vivo* performance.
- ✓ Within a One Health approach, plant-derived antimicrobial alternatives may contribute to reducing reliance on conventional antibiotics, limiting antimicrobial selection pressure, and supporting animal health, food safety, and sustainable production.

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