

Synergistic Interaction Between Paired Combinations of Natural Antimicrobials in Vitro Screening Against Poultry-Borne Pathogens

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Results and Discussion

1. The comprehensive analysis showed that the order of the inhibitory effects of the EO on pathogens was: Thymol > Oregano > Carvacrol > Trans-cinnamaldehyde > Eugenol > Geraniol > Citral > Linalool > Mugwort. **Thymol had the strongest inhibitory effect on common pathogens, and showed a good antibacterial effect.**

2. The combined interaction of plant essential oils (carvacrol, eugenol, trans-cinnamaldehyde, Oregano and thymol) was evaluated by **CI model**, and the synergistic effect of the combination of carvacrol and thymol was found, and the combination had better inhibition effect on common pathogens in vitro.

◆ The combination of “**Thymol + Carvacrol**” was selected for comprehensive analysis to study the effect of EO on microbial resistance in broilers.

Abstract

Natural antimicrobials are **promising candidates** for the successful **control of poultry-borne bacteria**, carrying potent antimicrobial activity against a wide range of multidrug-resistant pathogens. Individual activities of carvacrol, eugenol, trans-cinnamaldehyde, oregano, and thymol, along with the combined activity of paired compounds, were examined using **broth microdilution** and **checkerboard techniques**. The characteristic interactions between the compounds were calculated using an improved method, based on **combination index (CI) values**. Our results indicated that **thymol** was most effective, exhibiting the lowest **minimum inhibitory concentration (MIC)** value against *Salmonella pullorum*, *Escherichia coli*, and *Klebsiella pneumoniae*, establishing the order of antimicrobial efficacy as: thymol > oregano > carvacrol > trans-cinnamaldehyde > eugenol > geraniol > citral > linalool > mugwort. In the interaction study, the **paired combination** of **carvacrol and thymol** showed **synergistic effects** and was highly effective in reducing the antibiotic resistance of all the evaluated pathogens. Notably, all **CI values** were **< 1.0** in evaluations of *Salmonella pullorum*, indicating the absence of antagonism between eugenol and thymol (or oregano). Moreover, the improved evaluation method of this study provides a **precise** and **extensive** means to assess the synergistic effects of natural antimicrobials.

Introduction

The World Health Organization (WHO) pointed out that **antibiotic resistance has become a hot key issue in the global clinical and food fields**. Poultry farming is an important link area in the emergence and spread of antibiotic resistance. The control of bacterial resistance and its spread in poultry is a key issue to be urgently solved at present. **Plant essential oils (EO)** are potential substitutes because of the **rich resources** and **extensive pharmacological** effects. However, there is no systematic report on the effect of plant essential oils on the resistance in poultry. Ten common plant essential oils were selected.

Methods

- Bacterial strains (Positive control: *Escherichia coli* BNCC 336435, *Klebsiella pneumoniae* BNCC 102997, and *Salmonella pullorum* BNCC 19945; Sample: *Escherichia coli* 8G4 and *Klebsiella pneumoniae* 208G28)
- trans-cinnamaldehyde** (99% purity), **eugenol** (99% purity), **carvacrol** (98% purity), **thymol** (99% purity), **geraniol** (99% purity), **linalool** (99% purity), **citral** (99% purity), **oregano** (98% purity), **mugwort** (98% purity)
- The interaction of several plant essential oils with common pathogenic bacteria in chicken was studied using the **CI model**. following equation was applied:

$$(CI)_x = \sum_{j=1}^n \frac{(D)_j}{(D_x)_j} =$$

$$\sum_{i=1}^n \frac{(D_x)_{1-n} \left\{ \frac{[D]_j}{\sum_1^n [D]} \right\}}{(D_m)_j \left\{ \frac{(f_{ax})_j}{1 - (f_{ax})_j} \right\}^{1/m_j}}$$

- CI < 0.5 indicates **highly synergistic** outcome;
- CI < 1 indicates **synergistic effect**;
- CI = 1 indicates **additive outcome**;
- CI > 1 indicates **antagonistic effect**

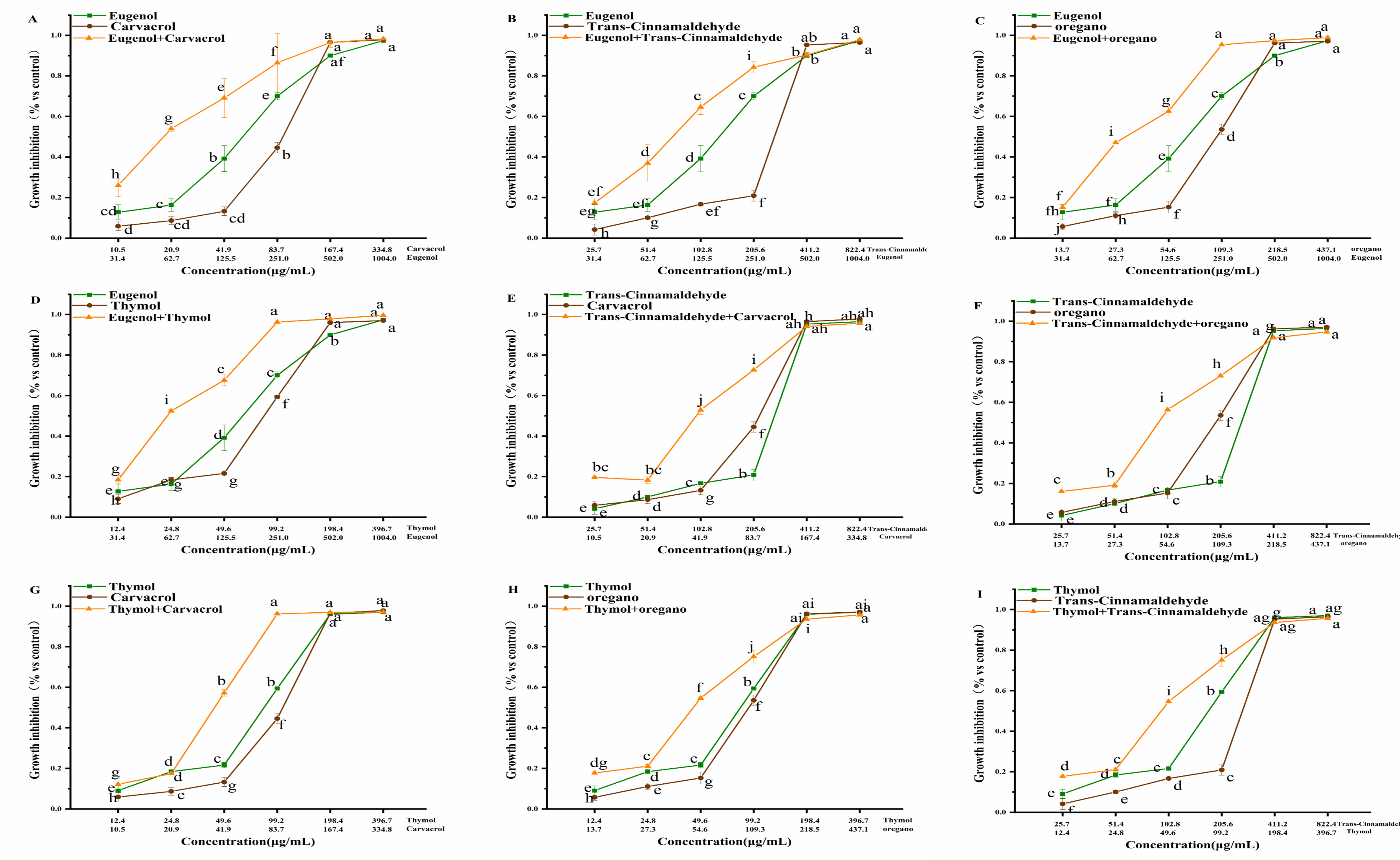


Fig 1. Effect of single and combined of natural antimicrobials on the growth inhibition rate of *Salmonella pullorum* BNCC 19945

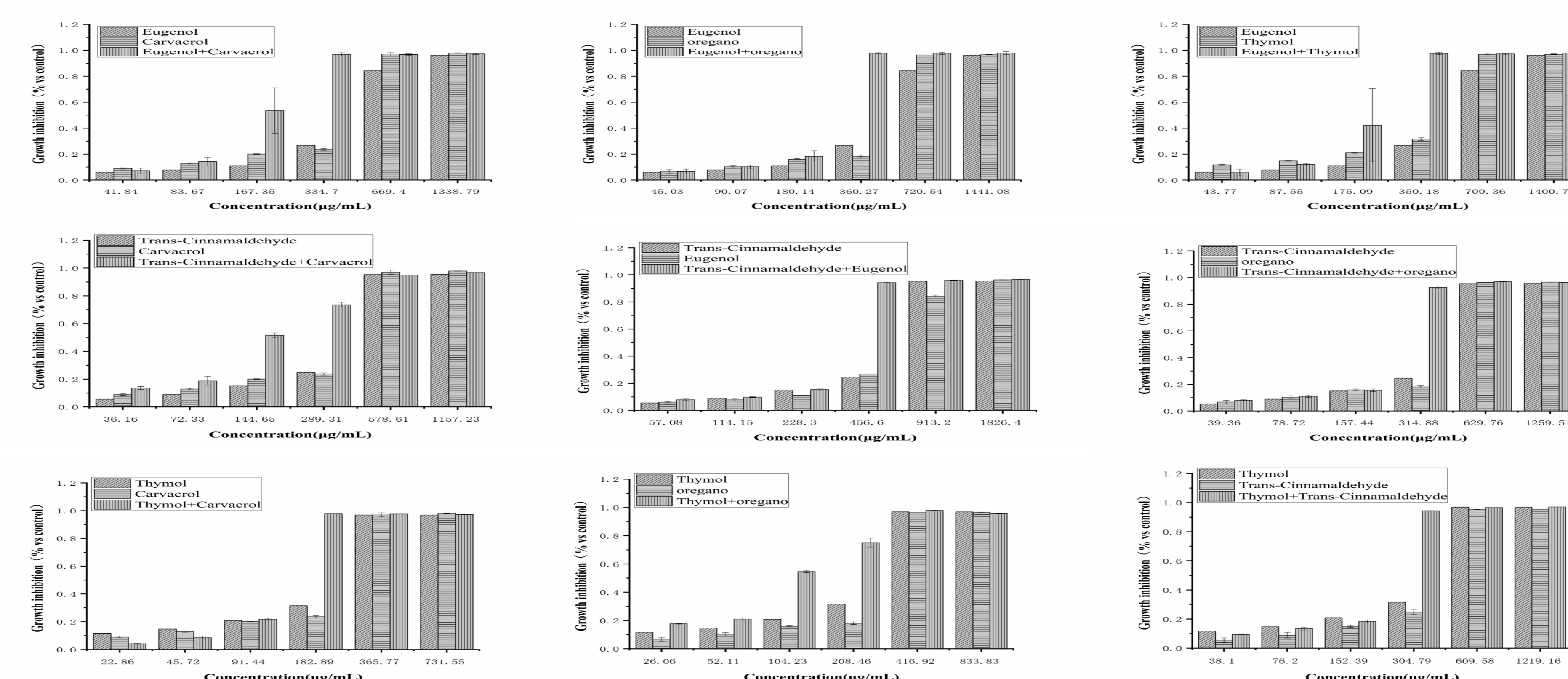


Fig 2. Effect of single and combined of natural antimicrobials on the growth inhibition rate of *Klebsiella pneumoniae* BNCC 102997.

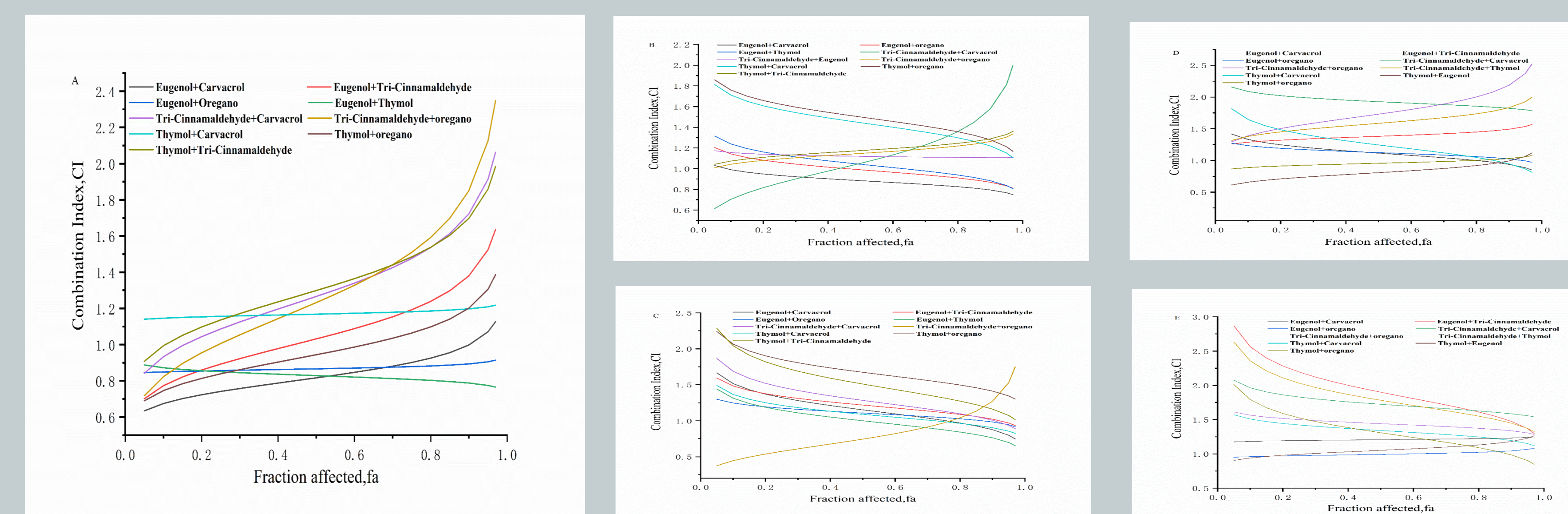


Figure 3. Evaluation of combined effects of binary plant essential oils combinations based on CI model.