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Detection of Asymptomatic Candidatus Liberibacter asiaticus-Infected Citrus using an Electronic Nose
Anthoni Sulthan Harahap, Prof. Dr. Ir. Siti Subandiyah, M.Agr.Sc.
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DETECTION OF ASYMPTOMATIC *Candidatus* Liberibacter asiaticus-INFECTED CITRUS USING AN ELECTRONIC NOSE

Anthoni Sulthan Harahap
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Abstract

Huanglongbing (HLB) is one of the most destructive citrus diseases worldwide. This study used a portable, laboratory-built electronic nose (e-nose) equipped with 10 metal-oxide semiconductor (MOS) sensors for detection of asymptomatic *Candidatus* Liberibacter asiaticus (*C*Las) infection in Purworejo Siamese citrus leaves. A total of 454 leaf samples were confirmed by polymerase chain reaction (PCR) to establish the healthy sample group and the asymptomatic *C*Las-infected groups. The study first identified the most suitable sample preparation method, then used the e-nose to convert volatile organic compound (VOC) interactions into electrical signals for pre-processing, feature extraction, and machine-learning classification. Model optimisation were conducted using windowing and hyperparameter tuning. The e-nose performance was corroborated using headspace gas chromatography–mass spectrometry (HS-GC/MS) analysis of VOC profiles, followed by chemometric analysis. The optimal sample preparation method was whole leaves with heating. The best e-nose performance The best e-nose performance was obtained with a 3 windowing quadratic discriminant analysis (QDA) model, achieving 81.99±4.24% cross-validation accuracy (CV) and 81.00% external validation accuracy. The best e-nose performance under hyperparameter tuning was obtained with a stratified five-fold cross-validated extra trees model achieved 84.57% training accuracy (95% confidence interval [CI]: 80.98–88.15%), test accuracy 79.00%, specificity 67.00%, and sensitivity 91.00%. HS-GC/MS confirmed differences in VO,C composition between the two groups of sample. Although principal component analysis (PCA) showed substantial within-group variability, supervised analysis identified three terpenoid putative biomarkers: 1-methyl-4-(1-methylethylidene)-cyclohexene, (+)-4-carene, and β-myrcene. The optimal 10-compound panel achieved an area under the curve (AUC) of 0.96 (95% CI: 0.23–1.00) and 88% accuracy using Monte Carlo cross-validation partial least squares-discriminant analysis (MCCV PLS-DA).

Keywords
Huanglongbing; e-nose; asymptomatic infection; volatile organic compounds; headspace gas chromatography–mass spectrometry

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DETEKSI JERUK TERINFEKSI Candidatus Liberibacter asiaticus *ASIMPTOMATIK MENGGUNAKAN HIDUNG ELEKTRONIK*

Anthoni Sulthan Harahap
19/450326/SPN/00651

Intisari

Huanglongbing (HLB) merupakan penyakit jeruk yang paling merusak di seluruh dunia. Penelitian ini menggunakan hidung elektronik portabel dengan 10 sensor metal-oxide semiconductor (MOS) untuk infeksi Candidatus Liberibacter asiaticus (*C*Las) *yang asimptomatik pada daun jeruk Siam Purworejo. Sebanyak 454 sampel daun dikonfirmasi menggunakan* polymerase chain reaction (PCR) *untuk menentukan kelompok sampel sehat dan kelompok terinfeksi C*Las *asimptomatik. Penelitian diawali dengan menentukan teknik preparasi sampel yang paling optimal, dilanjutkan penggunaan hidung elektronik dengan optimasi windowing dan penyesuaian hiperparameter untuk menguji kemampuan hidung elektronik dalam mendeteksi infeksi C*Las *asimptomatik. Kinerja hidung elektronik divalidasi dengan analisis* headspace gas chromatography–mass spectrometry (HS-GC/MS) *terhadap profil komponen organik volatil (KOV) yang dilanjutkan dengan analisis kemometrik untuk mengetahui kandidat biomarker. Preparasi sampel optimal menggunakan daun utuh dengan proses pemanasan. Kinerja terbaik e-nose dengan optimasi windowing diperoleh pada model* quadratic discriminant analysis (QDA) *tiga* window*, dengan akurasi* cross-validation (CV) 81,99 ± 4,24% *dan validasi eksternal* 81,00%. *Kinerja terbaik hidung elektronik dengan optimasi hyperparameter tuning diperoleh pada model extra trees dengan akurasi latihan* 84,57% (CI: 95% [CI]: 80,98–88,15%), *akurasi uji* 79.00%, *spesifisitas* 67.00%, *dan sensitivitas* 91.00%. *Analisis* HS-GC/MS *mengonfirmasi perbedaan komposisi KOV antara kedua kelompok sampel. Meskipun* principal component analysis (PCA) *menunjukkan keragaman didalam grup yang substansial*, *analisis tersupervisi* mengi*dentifikasi tiga putatif biomarker terpenoid yaitu 1-methyl-4-(1-methylethylidene)-cyclohexene, (+)-4-carene, dan β-myrcene. Panel optimal yang terdiri atas 10 senyawa menghasilkan AUC sebesar 0,96 (CI 95%: 0,23–1,00) dan akurasi 88% menggunakan MCCV PLS-DA.*

Kata kunci
Huanglongbing; hidung elektronik; infeksi asimptomatik; komponen volatil organik; headspace gas chromatography–mass spectrometry