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Diagnostic specificity, sensitivity and cross reactivity of In house established indirect ELISA for detection of Weligama Coconut Leaf Wilt Disease associated Phytoplasma

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Weligama Coconut Leaf Wilt Disease (WCLWD) is a phytoplasma associated disease of coconut palms (*Cocos nucifera* L.), confined to the Southern Province of Sri Lanka. Lack of an efficient approach for control of the disease has compelled the Coconut Research Institute, Sri Lanka to destroy to date, nearly 100,000 coconut palms in the Matara District. Phytoplasma disease diagnosis is hampered due to the inability of *in vitro* cultivation, low concentration, erratic distribution and seasonal fluctuation in the host plant. Hence, a sensitive, specific and rapid diagnostic test for WCLWD is an imperative need for routine detection.

Previously reported in-house established indirect ELISA using WCLWD phytoplasma specific polyclonal serum, was validated using 374 coconut palms including 182 disease positive and 192 disease negative palms. Infected palms and apparently healthy palms were collected from the Matara and Kegalle Districts respectively. Sugarcane White Leaf Disease (SCWLD) (N = 28 samples), Bermuda Grass White Leaf Disease (BGWLD) (N = 10), and Areconut Yellow Leaf Disease (AYLD) (N = 22) were used as controls to determine the cross reactivity of the assay. Out of the 182 PCR positive palms, 12 screened as negative and, 21 PCR negative, palms scored positive by the established ELISA. The receiver operating characteristic (ROC) curve analysis revealed that the area under the curve is 0.904, indicating high accuracy in distinguishing healthy from infected palms. At ROC optimized cut-off of 0.286, the sensitivity and the specificity of the test were 90.01% and 88%, respectively. The false positivity, false negativity and the overall efficiency of the test were 15.14%, 6.04% and 86%, respectively. The predictive value of a positive test was found to be 93.25%, whereas that of a negative test was 81.04%.None of the SCWLD samples scored positive in the test. Conversely the ELISA showed a considerable degree of cross reactivity with BGWLD (70%) and AYLD (68.18%). Pairwise comparisons using Mann-Whitney U Test following Kruskal-Wallis test; ($p = 0.001$), revealed significant differences between positive WCLWD and AYLD ($p = 0.016$) /BGWLD ($p = 0.001$).

In conclusion, the validation of the indirect ELISA for diagnosis of WCLWD showed a high sensitivity and somewhat limited specificity. Nevertheless, the high degree of cross reactivity with phylogenetically related phytoplasma strains showed the importance of the development of diagnostic monoclonal antibodies against WCLWD associated phytoplasma.
Keywords: Coconut, indirect ELISA, phytoplasma, polyclonal antiserum

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