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MOLECULAR AND IMMUNOLOGICAL DETECTION OF KENCHU VIRUS INFECTING SILKWORM (*Bombyxmori* L)

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ABSTRACT

In India, silk textile is economically important industry and Karnataka alone accounts for 63% of the country's silk production. The silk industry is affected by many fungal and viral major diseases and thus there is decrease in the silk production. In this study the early detection of the viral disease caused by Kenchu virus to silkworm (BmKV) was studied by molecular and immunological methods. The Kenchu virus isolate was collected from Vijayapura, Karnataka, India and ultrapurified using sucrose gradient centrifugation. The DNA was isolated from ultrpurified virus using standard protocol. The PCR detection of BmKV was done using DNV1 and DNV2 gene specific primers where, the isolate of Kenchu virus collected from Vijayapura, Karnataka, India amplified ~700 bp band specific to DNV2 primer and belongs to Japanese or Yamanashi isolate and DNV1 primer did not amplify. The antibodies were raised against the purified virus and immunologically Dot-ELISA and plate-ELISA were standardized to check the specificity of antibodies raised against the BmKV.The titer 1:50 of antigen, 1:16000 of primary antibody and 1:1000 of secondary antibody dilutions was found optimum for detection of the virus with BCIP/NBT as substrate for Dot-ELISA and for plate ELISA 1:100 dilution of crude and ultrapurified antigen and 1:1000 dilution of both primary antibody (antisera raised against BmKV) and secondary antibody was optimum with $OD_{405\ nm}$readings of1.041 and 1.03 respectively. This study thus helps in development of immunodiagnostic kit for Kenchu disease detection and help farmers to avid heavy losses of silkworm production.

KEYWORDS: Bombyxmori, Kenchu Virus, PCR, Dot-ELISA, Plate ELISA