PRODUCTION OF POLYCLONAL ANTISERUM FOR LOCAL ISOLATE OF *Chilli Veinal Mottle Virus* (ChiVMV) IN *Capsicum annum* (CHILLI)

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Chilli Veinal Mottle Virus (ChiVMV) is the most prevalent virus in Chilli cultivations in Sri Lanka. Indexing of virus is mainly carried out using commercial kits. But those are of high cost. Locally developed polyclonal antiserum can be used to detect the local strain of ChiVMV efficiently and at a low cost using Enzyme Linked Immunosorbant Assay (ELISA) technique. In Sri Lanka, production of polyclonal antiserum for ChiVMV has not been recorded previously. Therefore, this study was carried out to produce polyclonal antiserum for ChiVMV. Purification of ChiVMV was carried out according to the previously developed protocol. New Zealand white female rabbit was used for the immunization process. Prior to immunization, pre- immune serum of the rabbit was withdrawn and tested by available indirect ELISA protocol to ensure that antibodies against ChiVMV is not present in the serum. Purified virus preparation was injected to the rabbit, one intraveinal (IV) followed by four intramuscular (IM) injections with Freund's Incomplete Adjuvant (FIA) at weekly intervals. Three bleeds were withdrawn at weekly intervals after third injection. An indirect ELISA procedure was carried out to determine the best bleed, best antiserum dilution, best extraction buffer and best conjugate dilution. As antiserum titer was low in the first bleed, second and third bleeds were used to optimize the protocol. Since both second and third bleed gave high absorbance values for healthy plant samples, antisera of both bleeds were cross-absorbed with healthy Chilli plant sap. Cross-absorbed serum of the second bleed was selected as the best antiserum for detection of ChiVMV. Of the three buffers tested, PBST+2%PVP+0.13%Na_2SO_3+0.2%EA was found to be the best extraction buffer to extract the virus. Best antiserum and conjugate dilutions were 1:100 and 1:200 respectively. Absorbance values at 405 nm wavelength taken one hour after substrate addition gave clear difference between healthy and infected plants. This locally produced polyclonal antiserum for ChiVMVcan be used to differentiate the ChiVMV infected plants from the non-infected plants.

Keywords: *Chilli, ChiVMV, Immunization, Indirect ELISA, Polyclonal antiserum*