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OBTAINING AND DETERMINING THE PURITY OF A PURE PREPARATION FOR THE "POTATOX X" VIRUS USING HELPHILTRATION METHOD

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Abstract
This in the article , KXV 's clean drug separate get for gel filtration method selected , gel chromatographic from TSK 75 gel in the column was used . The simplest, most convenient and most common method of detection in laboratory conditions - spectrophotometry method - was used to assess the concentration of viruses. The degree of purity is determined depending on the absorption of UV light of the purified viral drug using the gel chromatography method. The concentration of the purified virus was measured in a spectrophotometer of the brand "METASH-5100" at a wavelength of 220-310 nm [1]. The E 260nm 0.1% 1cm of KXV is 2.9, and the 260/280 ratio is 1.2.

Keywords: KXV, TSK 75 gel, gel filtration, Chromatographic column, elution, phosphate buffer, ELISA, PCR.

Introduction

Selection of plants infected with the virus is the main step in the preparation of a pure preparation of KXV. This important process requires a careful examination of the potato plants, identifying the obvious signs of KXV infection: mosaic, leaf wilting, leaf band breakage and necrosis. Field expeditions are carefully organized to identify plants showing symptoms that indicate viral infection. These suspect samples are then subjected to rigorous diagnostic testing using methods such as enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) to confirm the presence of KXV. Once a sample has been determined to be infected with KXV, they are subjected to a careful visual inspection, cross-checked with the descriptions of the relevant KXV symptoms. Detailed documentation of the location, date, and history of specific symptoms of each suspected KXV-infected plant facilitates careful follow-up [11]. Plants showing clear and consistent signs of KXV infection are selected for further investigation, while plants with mixed infections or with ambiguous symptoms are immediately discarded. This careful selection scheme is important in ensuring the purity and reliability of subsequent virus preparations, ensuring that they meet the strict standards required for research, diagnostics and other work in plant virology.
Clean phytoviruses drugs get is carried out in several stages. First, biological purification of the virus is carried out, that is, this virus is purified from other viruses and biological organisms visible under a microscope with the help of indicator plants (but sometimes inside the necrotic cells of the host plant, sometimes in the cell of the host plant will be). In this work, indicator (identifier), differentiator (separator) and collector (systemically infected with the virus) plants are used for

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each virus, which is used in the practice of virology with clear characteristics **[2].**

Gel filtration using the method, the column was filled with TSK 75 gel . Chromatographic the bottom before pouring the gel into the column to the part filter paper placed and gently washed off the gel with to the column and precipitated to a volume of 155 ml and first in 500 ml of distilled water and then in 400 ml of 0.02 M, pH-7.4 phosphate clipboard with was washed The top of the prepared gel was removed using a phosphate buffer pipette on filter paper, and 2 ml aliquots of cleaned viral example put the virus separate take the process went The speed of elution from the gel was 11 seconds, and the volume was 20 ml/h. The purity of the virus in the fractions extracted from the gel was checked using several methods [3].

Determining the dry mass concentration of viruses requires an accurate measurement method and very pure virus. When assessing the concentration of viruses, the content of nucleic acid (RNA or DNA) in the preparation is often determined. In addition to being highly specific, this method requires pure viral nucleic acid, free of other nucleic acids from the cell. The simplest, convenient and most common method of determination in laboratory conditions is the spectrophotometry method. In this case, the viral sample is determined based on its ability to absorb light. The degree of purity is determined depending on the absorption of UV-light of the viral drug purified by the gel chromatography method. The absorption index of RNA and DNA changes at a wavelength of 260-280 nm. The maximum absorption index of DNA or RNA of the purified virus is 260 nm. The concentration of the purified virus was measured in a spectrophotometer of the brand "METASH-5100" at a wavelength of 220-310 nm [7]. Usually, for a virus suspension with a concentration of 10 mg/ml ($E_{260nm;\ 1cm}^{1\%}$) or 1 mg/ml ($E_{260nm;\ 1cm}^{0.1\%}$), the value of light absorption at 260 nm is determined in a cuvette 1 cm long. $E_{260nm}^{0.1\%}$ 1cm ≈of KXV is 2.9, and the ratio 260/280 is 1.2 [5].

Obtaining a biologically pure preparation of viruses is the basis for the next steps in the study of the virus. Obtaining a clean drug requires several stages of biological and physical chemical purification methods . Isolation, biological purification and breeding of viruses involves the purification of this virus from other viruses by passing it through specific necrotic and systemically diseased plants. After it was determined that the symptoms of the disease detected by the indicator plant were characteristic of KXV, the plant *D.stramonium* L was used as a collector of this virus. *D. stramonium* An inoculum was prepared from the leaf of the plant *L.* in a porcelain dish with 0.02 M, 10 mM trilon-B, 1 mM tris-HCl, pH 7.4 phosphate buffer. and *G. globosa* plants were mechanically infected and the resulting necrosis was cut off and inoculum was prepared using buffer and the above process was repeated. The process of biological cleaning (mononecrosis) was carried out in three stages.

The next stage of biological purification of the virus is a physico-chemical method, initially the viral sample is pH 7.4; 10mM trilon-B, 0.02M phosphate buffer containing 1mM tris-HCl was homogenized in a homogenizer for 10 minutes, and after filtration of viral sap through a four-layer gauze, it was centrifuged at 5000 rpm to clear the viral sap from leaf cell components. After centrifugation for 25 min, the supernatant (ChUS) was obtained.

To precipitate pigments and similar high molecular weight substances in the supernatant, chloroform (1/10) was added, shaken vigorously for 20 minutes, and centrifuged at 5500 rpm for 30 minutes, and then the resulting supernatant from the centrifuge tube The upper layer of the 3-layer suspension, i.e. the viral layer, was carefully removed into a separate clean container. Care

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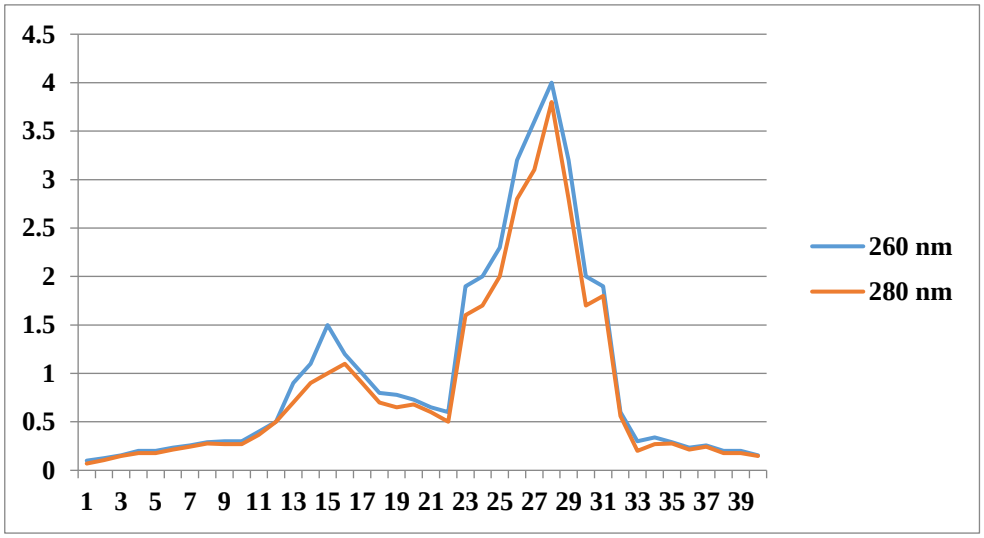
should be taken not to interfere with the chloroform when removing the viral layer. In order to isolate and concentrate the virus from the obtained viral suspension, a saturated solution of ammonium sulfate (40%) was added to the viral sample in a volume of 1:0.5 ml and kept overnight in a refrigerator (+4°C). After that, the suspension was centrifuged at 5800 rpm/30 min, the ChUS was discarded, and the precipitate was dissolved in 0.02M phosphate buffer (pH 7.4).

The viral suspension was dialyzed 3-4 times using distilled water 90-100 times the volume of the sample to remove excess sulfate salts. Dialysis in the product excess ions 3000 ay/ 5 min during centrifuge by doing take thrown away This is a virus partially cleaned drug from him pure virus preparation get for and the supernatant is next stage TSK 75 gel with is filled kalonka using gel chromatography to do through of the virus cleaned drug received

2 ml of the partially purified viral sample was loaded onto a TSK 75 gel and 4 ml fractions were collected. The rate of separation of eluents was 20 ml/hour. Fractions separated by TSK 75 gel were measured using a spectrophotometer (METASH-5100) at a wavelength of 260/280 nm and the point of separation of viruses in fractions was determined. Pic-1.



Pic-1. Purity level of fractions separated by TSK 75 of KXV. Plate 2.3×80 cm ^{3}, viral sample 2.0 cm ^{3}, volume of pure virus 40 cm ^{3}, elution rate 20 cm ^{3}/h

As shown in the graph, when a partially purified preparation of the virus was passed through the TSK 75 gel and the fractions were measured by spectrophotometry at 260 nm and 280 nm wavelengths, the virus and cell components were separated in separate fractions, the 12-20th fraction contained the virus, and the 21-35th fraction cell components can be seen to separate.

In general, the requirements for a pure preparation of the virus purified using the TSK 75 gel were checked based on the following criteria:

1. The infectivity of viral fractions was tested by mechanical inoculation of Gomphrena globosa and a positive result was obtained;
2. When the purified virus preparation was analyzed by spectrophotometry, the absorption index was at 260 nm, the absorption spectrum of the virus at the wavelength of 220 nm-310 nm of UV light was found to have a peak at 260 nm, and the ratio of 260/280 was 1.2 it was confirmed that 5-6% is characteristic of nucleic acid containing viruses, i.e. KXV;

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3. When the viral preparation was examined by immunological methods, a single precipitation line characteristic of the virus was formed. Obtaining a pure preparation of the virus is the basis for the next steps in the diagnosis of the virus. The higher its level of purity, the more accurate the diagnostic analysis.

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