Serological and morphological diagnosis of potato virus X (PVX(

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ABSTRACT

After conducting an ELISA test on potato samples using antisera (PVX, PVY, PLRV), it was found that the potato samples were infected with potato virus X. The ELISA test using the mentioned antisera gave the highest wavelength absorption results for virus X, which amounted to (1,260) compared to the control, which gave (0,180). The transmission routes of the virus were identified, and we noticed through research that the virus is transmitted, mechanically and by insects via the Aphid Myzus persicae, where this insect gave the highest rate of 99% compared to the bean aphid Aphis faba and the oleander aphid Aphis nerii (50% and 30%), respectively. There was also a clear decrease in the weight of the infected green mass and the infected root mass, as the fresh weight and the weight of each were taken into account, and we noticed the effect of the virus on the internal contents of the plant.

Keywords: Virus, Anti-Virus , ELISA,potatoes (Solanum tuberosum L,(

.1 INTRODUCTION

The Solanaceae family includes potatoes (Solanum tuberosum L.). The most significant member of this family and one of Potato crop is considered the most in demand in most countries of the world. In 2008, the United Nations stated that potato (Solanum tuberosum L.) cultivation would be a staple crop in the future, as the crop contains large quantities of proteins and carbohydrates, especially in some countries suffering from food shortages [23]. The People's Republic of China is one of the world's largest potato producers, producing more than a quarter of the world's production and about 30% of the potato crop [24.[

Approximately 409,535 feddans (172,005 hectares) were used to cultivate potatoes In 2014 in the Arab Republic of Egypt, the Food and Agriculture Organization of the United Nations estimated that, [25]. A total of 4,611,065 tons of potatoes were produced, with an average yield of 11.26 tons per acre (26.81 tons per ha). Currently, one of the biggest exporters of potatoes worldwide is Egypt. Exports to international markets like Russia, England, Western European nations, and some Arab countries totaled roughly 679 thousand tons in 2014 [25]. PVX virus is classified as a member of the genus Potex virus within the Alphaflexiviridae family. PVX virus is one of the oldest plant viruses, with the earliest scientific references published in 1938 [1]. Virus X is one of 48 species in the genus Potox, which causes numerous and severe diseases [27]. The pathogenicity of virus X results in a Mild mosaic disease in eggplant plants, including but not limited to potato, tomato, and tobacco. PVX is prevalent across various regions due to worldwide trade and commerce, thus making it one of the most commonly dispersed viruses. According to [2].The transmission of PVX commonly

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occurs via pollen or true seeds, as well as through the use of contaminated farming equipment. Additionally, transmission may occur through direct contact between foliage or roots of healthy and infected plants [26]. The act of transmission by chewing insects has been documented; however, no particular chomping insect is deemed as a discriminatory vector for Potato Virus X (PVX). Previously, PVX has been the subject of significant investigation in the field of potato seed production . However, By working on seed potato certification programs and incorporating disease resistance genes into potato breeding, efforts have been made to effectively reduce the economic consequences associated with PVX [3]. The aim of this research is to detect viral diseases affecting the treated crop, including PVX virus, and to detect them through antisera for detection by indirect ELISA test.

.2 MATERIALS AND METHOD

.2.1 Isolation

Samples of potato leaves showing signs were collected from a farm located in the Babylon Governorate. . included mild mosaic, mottling and maintained on Nicotiana glutinosa and Nicotiana tabacum and Nicotiana benthamiana plants by mechanical transmission [4].The inoculated plants were kept in greenhouses. Ten days after

inoculation, mild mosaic symptoms appeared on Nicotiana glutinosa and chlorotic ring spots on Chenopodium amaranticolor. The inoculum was generated through the utilization of infected potato leaf tissues that were finely macerated using a mortar and pestle. This process required the addition of 0.1 M infection solution at pH 7. During the cultivation of healthy Nicotiana glutinosa plants , a dusting of 600 mesh carborundum was applied to their leaves. Subsequently, a freshly prepared inoculum was administered to the plants via the forefinger method. Following the inoculation, the plants were immediately subjected to a tap water rinse and subsequently placed in a controlled greenhouse environment guarded against insect exposure for the purposes of symptom evolution observation.The virus was cultured in Nicotiana glutinosa plants to facilitate its propagation and to enable its use as a viral source for subsequent research investigations [7.[

.2.2 Identification

Identification of one isolate, which caused normal symptoms of PVX, was carried out by studying host range, symptomatology, mode of transmission and serological tests.

.2.3 Diagnostic hosts

In this study, plant hosts identified for potato virus X were used, diagnostic hosts including Nicotiana glutinosa, Chenopodium amaranticolor, N. repanada chlorotic, and Gomphrena globosa, were employed based on the characteristic symptoms they typically exhibit for PVX, as previously documented by [4,5], and [6]. After inoculating plant hosts with the potato virusX, we follow the viral symptoms for four weeks until symptoms appear

.2.4 Source of antisera:

Antibodies to Potato mosaic virus, potato leaf rurl virus, potato virus yellow

,provided by Bioreba (Switzerland) were used

.2.5 Indirect ELISA:

In brief, the indirect ELISA method is an established method for most plant viral diseases, as described by [7,8]. Infected leaves were crushed in a grinding solution (0.05 M carbonate, pH 9.6) at a ratio of 1:10 (w/v) using a mortar and pestle, and then filtered using double-layered gauze. Anti-virus antibodies were placed inside the holes of the plate by adding 100 µl of each sample to the bottom. The plates were incubated overnight at 4°C in a refrigerator. The plates were washed three times by immersing the wells in phosphate-buffered saline and Tween 20 (PBST) for 5 minutes. To minimize nonspecific interactions, antivirals requiring cross-adsorption were diluted

1:500with healthy tissue extract 1:20 (w/v) in serum buffer (PBS) Tween 20 (PBST)

containing 2% soluble polyvinylpyrrolidone (PVP) and 0.2% bovine albumin (BSA) was added to each well and incubated for 45 minutes at 37°C. The resulting impurities were removed by centrifugation for 10 minutes at 5,000 rpm. 100 µL of diluted antiserum sample was added to each well, and the plate was then incubated at 37°C for 2 hours or at 4°C overnight, then washed as before. Goat anti-rabbit gamma globulin, conjugated to alkaline phosphatase (Whole Molecule, Sigma-Aldrich, St. Louis, MO, USA), was diluted 1:1000 in serum buffer, 100 µl was added to each well, incubated for 1 h at 37°C, and then washed. 100 µl of the enzyme-substrate, 1mg/ml para- nitrophenyl phosphate (Sigma Aldrich, Saint Louis, Mo, USA) in 10% diethanolamine buffer, pH 9.8, were added to each well and incubated at room temperature for 30 min. The enzyme activity was stopped by adding 50µl of 3M NaOH.The ELISA values, measured by Sunrise ELISA reader, were expressed as absorbance at 405 nm. Absorbance values of at least double of healthy control considered positive. Wells lacking antigen (coating buffer only) were included as blanks In summary, the indirect ELISA assay method is a stable step with most plant viral diseases as Known by [8].extracts from infected and healthy potato plants were diluted with Coating Buffer (0.05 M Carbonate, pH 9.6) to 1:10. Antibodies were added by adding 100 µL into each well of an ELISA plate and incubated for 3 h at 37 °C. or overnight at 4 °C. Then the ELISA plate was washed three times by immersing the pits of the plate with washing buffer solutions for 3 minutes each.Antisera, which requires cross adsorption, has been diluted at a ratio of 1: 250, according to the manufacturer's instructions, to the antiserum. 100 µL of diluted antibody was added to each well of the ELISA plate, after which the plates were incubated at 37 °C for 2 h or at 4 °C overnight, then washed as before.Goat anti-rabbit gamma globulin conjugated to alkaline phosphatase was diluted 1:1,500 in serum buffer, and 100 µl was added to each hole, followed by an hour incubation at 37 °C, and then washed as before.100 µl of enzyme substrate, 1mg/mL paranitrophenyl phosphate in 10% diethanolamine solution, pH 9.8 was added to each well of the ELISA dish and incubated at room temperature (25°C) for approximately 30 min. Enzyme activity was stopped by adding 50 µL of 3 M NaOH. The ELISA values measured with an ELISA reader at 405 nm were expressed and absorbance values at least twice that of the healthy control were considered positive.

.2.6 Mode of Transmission:

.2.6.1 Mechanical of Transmission

It has been shown that PVX is easily transmitted to plants through mechanical means, including mechanical industrial infection or through contact of virus-contaminated tools with uncontaminated plants.It is not transmitted through seeds or pollen [4,5]. Mechanical transmission is often transmitted to all parts of the plant with a healthy person Plant, and PVX is also transmitted by insects [9.[

.2.6.2 Aphid transmission:

Mode of transmission Virus by Aphis faba, Aphis nerii, and Aphids (Myzus persicae) insects, starved for 1 hour in petri dishes before allowing 7–10 minutes of tentacles on infected Nicotiana tabacum leaves. This aphid transmission method was used to spread potato X virus. Each healthy N. glutinosa plant received ten aphids, which were then inoculated for 10 minutes before being sprayed with an insecticide to kill them. The symptoms of pollination of the plants were tracked for 20 to 25 days while they were placed in the greenhouse.

.3 Chemical Analysis 3.1.Moisture

In summary, [10], reported that PVX infection had no effect on moisture content

.3.2Dry and fresh weight of the shoots

In this experiment, we demonstrate the effect of PVX on the vegetative system. Plants are cut from the soil surface and weighed before drying. They are then placed in paper bags (paper envelopes), sealed,the plants were

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placed in an oven at 180°C to dry with hot air for 72 hours., then the samples are taken out of the oven and the dry weight is taken compared to the control. We will notice that there is a clear change in the dry weight and fresh weight in the presence of the virus compared to the control [18.[

.3.3Dry and fresh weight of the roots

In this experiment, we demonstrate the effect of the PVX virus on the root system. The root system was weighed before and after drying, placed in paper bags, then it was placed in the oven at 180 degrees Celsius for 72 hours. After that, the dry weight was measured , and the changes were observed. The difference between fresh and dried roots in the presence of the virus was clear and tangible compared to the control.

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.4 RESULTS

.4.1 Diagnosed families

Diagnosed host interaction after potato virus X infection. The virus induced mosaic on N. glutinosa (A), mosaic and blasters on N. repanada chlorotic (B), and systemic spread on Chenopodium amaranticolor (C), and mosaic and on Gomphrena globosa (D

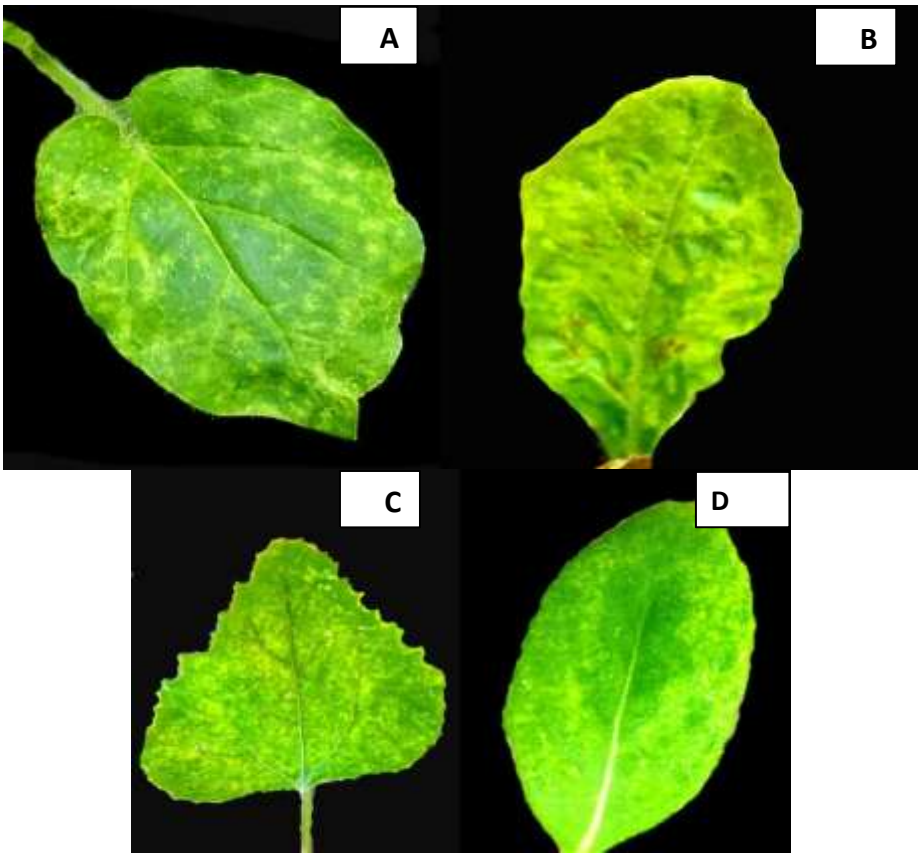

Fig. (1). Explanation of the systemic symptoms of potato virus X

PVX symptoms include mosaic on N. glutinosa (A), and N. repanada (B), systemic spread on Chenopodium amaranticolor (C), and mosaic on Gomphrena globosa (D). PVX was identified through the familial run of the virus where systemic symptoms were found Featured on Nicotiana glutinosa, Chenopodium amaranticolor, and also hosts

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were obtained with symptoms similar to those of the previous hosts: N. repanada chlorotic, and Gomphrena globosa, [19], and [9], [21.[

.4.2 Serological reaction

The anti-PVX antiserum reacted with the virus isolated from the plant when used at a dilution of 1:250 in an indirect ELISA test. We did not observe cross-reactivity between the anti-PLRV and anti-PVY antiserums with the virus isolated from the plant.

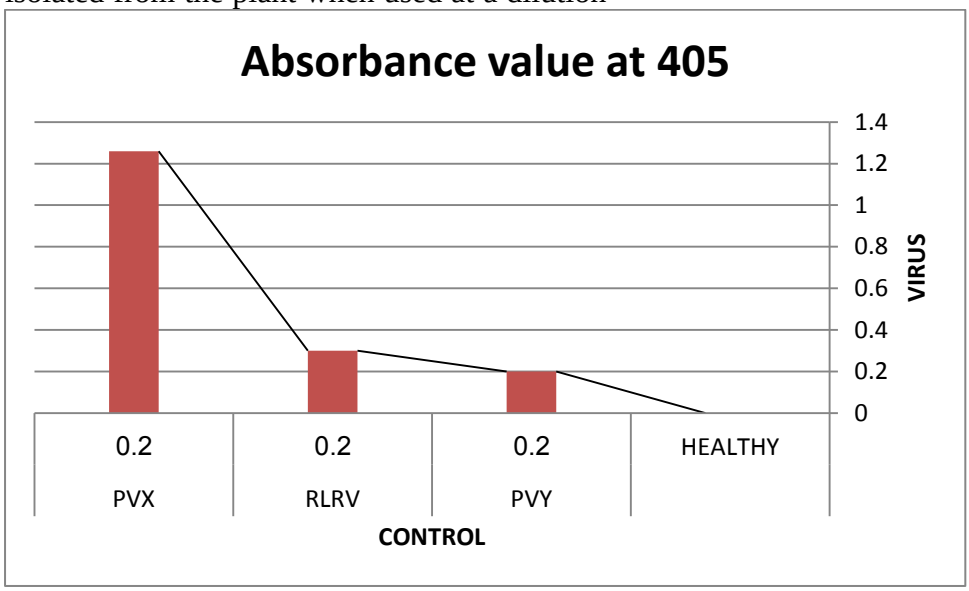

Fig (2). Indirect ELISA reaction of PVX, PLRV, and PVY antisera against contaminated potato leaf extract.

From the table shown, we notice that three types of antiserum (PVX, PVY, PLRV) were used for the potato isolate, and the type of isolate was identified after it was detected by the ELISA test. We notice that the PVX antiserum gave a higher result (1.260) compared to the control (0.180), as for the primer. PVY and PRLV gave a lower result (0.2, 0.3) compared to the control as well. This means that the plant isolate is infected with the X virus. The PVX virus isolate was detected using an ELISA test, which was used successfully. A positive reaction was obtained. Many researchers have used this serological ELISA test To detect PVX, [11, 12, 13, 14, 15,[
.[22]

.4.3 Aphid transmission

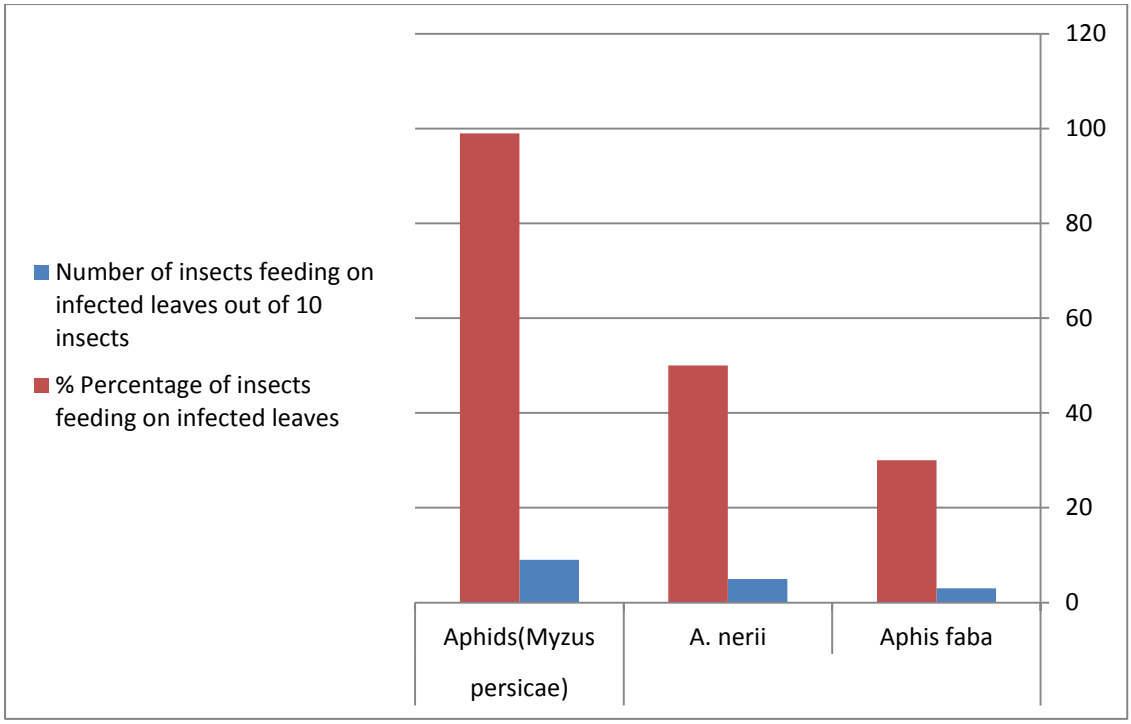

Fig (3). Transmission of PVX by aphid species

In Fig No. (3), The virus was found to be transmitted by insect vectors, including Myzus persicae, A. faba, and A. nerii, using ten aphids that were starved for one hour and then fed on infected leaves. The results showed that Myzus persicae recorded the highest virus transmission rate, reaching 99%, compared to oleander aphids, which recorded 50%, followed by bean aphids ,
which recorded the lowest transmission rate, at 30%.[16.[

.4.4 Dry and fresh weight of the shoots

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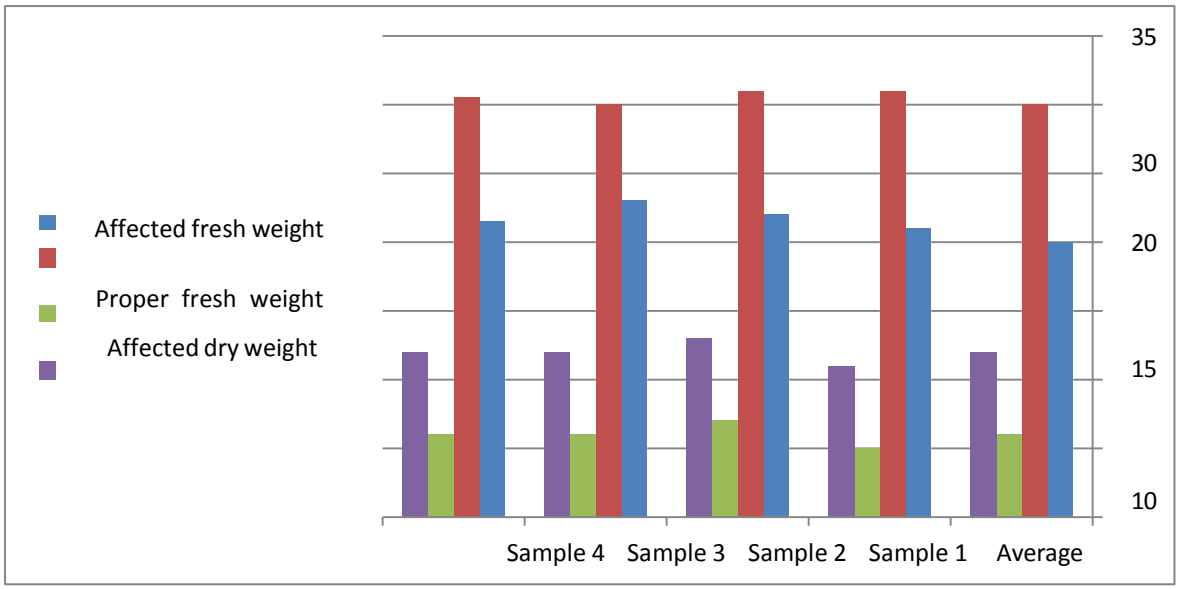

Fig (4). A diagram showing the variation in the weight of the virus infected green group before and after drying compared to the control.

In Fig No. (4), it was shown from the results obtained that infection with the PVX virus directly affected the dry weight and wet weight of the shoots compared to the control. We note that the average rate of infected wet shoots was 21.5g, while the average rate of healthy wet shoots was 30.5g. This means that potato virus X had a clear effect on plant contents, We also note that the average rate of the infected dry shoot was 6 g compared to the healthy dry shoot, which was 12g. [17,18] , We notice the damage of the X virus on the size of potatoes [10.[

.4.5 Measurement of root mass weight before and after drying

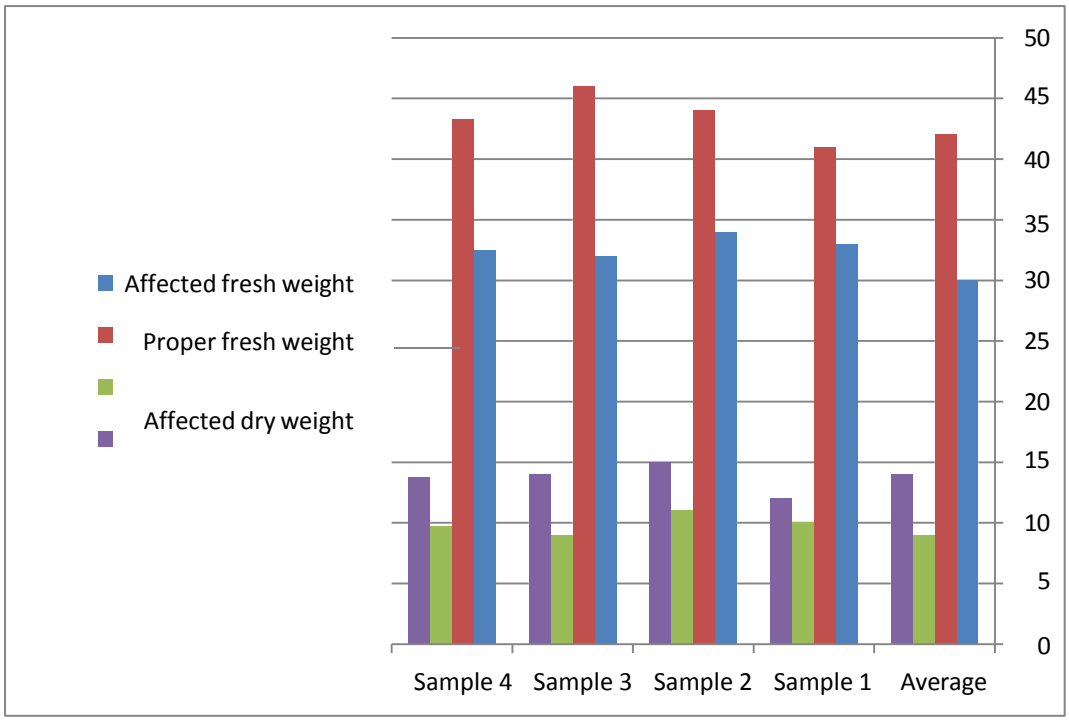

Fig (5). A diagram showing the variation in the weight of the virus-infected root system before and after drying.

In Fig No. (5), The results obtained after infection with PVX virus showed a clear variation in weight before and after drying.We note that the average average of the infected fresh root system was 32.25g, compared to the healthy fresh weight, which was 43.25gThis means that after a period of time, the virus spreads within the plant and affects the internal contents of the plant. We also notice a difference in the weight of the root before and after drying, compared to the control. The table showed that the average infected dry root mass was 9.75g and the average healthy dry root weight was 13.75g [18]. Dry matter production depends on the rate of photosynthesis per unit green leaf surface, the rate of respiration, and the size and longevity of green foliage. In addition, dry matter content also depends on plant density, start time and tuber maturity [20.[

Conclusions:

The study concluded that infection with the X virus had a clear impact on potato yields, including deformed leaves and tubers, as well as reduced shoot and root weight. This indicates that the virus clearly affected the plant's internal components.

Recommendations:

We recommend using certified tubers from a reliable source that are free of viral diseases. We also recommend that any plant in the field be disposed of immediately if a viral infection appears on it. If insects appear in the field, they must be eliminated, as they are a major cause of the rapid transmission of viral diseases.

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REFERENCES

. (1)Loughnane, J. B., & Murphy, P. A. (1938). Mode of dissemination of potato virus X. Nature, 141(3559), 120-121.

.(2) Bercks, R. (1949). Potato virus X. Nature, 163, 502–503.

.(3) Bragard, C., Dehnen- Schmutz, K., Gonthier, P., Jacques, M. A., Jaques Miret, J. A., ... & Di Serio, F. (2020). Pest categorisation of potato virus S (non- EU isolates). EFSA Journal, 18(1), e05855.

.(4) Bercks, R. (1970). Potato virus XCMI/AAB Descriptions of Plant Viruses No. 4. WM. Culross and Son Ltd. Coupar Angus, Perthshire, Scotland, UK.

.(5) Purcifull, D. E., & Edwardson, J. R. (1981). Potexviruses. Handbook of plant virus infections and comparative diagnosis, 627.

.(6) Jones, R. A. C., Fribourg, C. E., & Slack, S. A. (1982). Potato virus and virus-like diseases.

.(7) Karrar,A.H.(2021). Induced systemic resistance to legume virus diseases by plant growth-promoting rhizobacteria. Ph. Thesis. Fac. Agric. (Saba - Basha) Alexandria University . 17-18pp.

.(8) Fegla, G. I., El-Samra, I. A., Noaman, K. A., & Younes, H. A. (1997). Host range, transmission and serology of an isolate of tomato yellow leaf curl virus from tomato of plastic houses in northern Egypt. Proceeding of the first Scientific Con of Agric Sc Fac of Agric Assiut, 1, 549-568.

.(9) Salazar, L. F. (1996). Potato viruses and their control. International Potato Center.

.(10)Yousef, A. A. (1992). Studies on some viral diseases of potato. M.Sc. Thesis, Fac. of Agric., El-Mansoura Univ., Egypt.

.(11)Bobkova, A. F., Natsvlishvili, N. M., Sorokina, N. V., Egorov, A. M., & Atabekov, I. G. (1983). Diagnosis of potato virus X in plants and tubers with the help of immuno-enzyme analysis (ELISA test.(

.(12)Goth, R. W., & Webb, R. E. (1985). Detection and distribution of latent viruses in the potato cultivar Atlantic. Plant Disease 69: 851-853.

.(13)Koenig, R., & Torrance, L. (1986). Antigenic analysis of potato virus X by means of monoclonal antibodies. Journal of General Virology, 67(10), 2145-2151.

.(14)Lizarrage, C., and Fernandez-Northcote, E. N. (1989). Detection of potato viruses X and Y in sap extracts by a modified indirect enzyme linked immunosiorbent assay on nitrocellulose membranes (NCM ELISA). Plant Dis. 73(1): 11-14.

.(15) Kavanagh, T., Goulden, M., Santa Cruz, S., Chapman, S., Barker, I., & Baulcombe, D. (1992). Molecular analysis of a resistance-breaking strain of potato virus X. Virology, 189(2), 609-617.

.(16) Zaied, M. A. (2013). Studies on some viruses infecting potato. Ph. Thesis. Fac. Agric.(Saba-Basha) Alexandria University.

.(17) Yamaguchi, A. (1960). Increased respiration of leaves bearing necrotic local virus lesions. Virology, 10(3), 287-293.

.(18) Ouf, M. F., Omar, R. A., and Rushdi, M. (1969). ABiochemical study on virus diseased potato tubers. Bulletin of Science and Technology (AssiutUniv.) 12: 1969. Part II.

.(19) Abdel-Salam, A. M., Ashoub, A. M. A., El-Din, T. M. N., & Ibrahim, I. A. M. (1994). Detection of double stranded RNAs of potato virus X (PVX) in potatoes as an indication of virus infection.

.(20) Reestman, A. J. (1972). Incidence of infection in commercial crops and consequent losses. Viruses of Potatoes and Seed -Potato Production. De Bokx, J. (ed) pp. 152-157. Pudoc. Wageningen Center for Agricultural Publishing and Documentation.

.(21) Younes, H. A. A. (1995). Studies on certain virus diseases affecting some vegetable crops under greenhouse conditions.

.(22) Abd El-Aziz, M. H. (2000). Detection of certain plant viruses. Ms. C (Doctoral

dissertation, Thesis. Fac. Of Agric.(Saba–Basha) Alex. Univ. Egypt.

.(23) Dreyer, H., (2017). Towards Sustainable Potato Production: Partnering to Support 469 Family Farmers in Africa. POTATO RESEARCH 60, 237–238. 470 https://doi.org/10.1007/s11540-018-9354-7.

.(24) Statistia, (2023). Potato production worldwide from 2002 to 2021. 580 https://www.statista.com/statistics/382174/global-potato-production./

.

.(25) FAO, (2018). FAOSTA: FAO Statistical Databases. Food and Agriculture 474 Organization of the United Nations. http://www.fao.org/faostat/zh/#data/QC.

.(26) Halterman, D., Charkowski, A., & Verchot, J. (2012). Potato, viruses, and seed certification in the USA to provide healthy propagated tubers. Pest Technology, 6(1), 1-14.

.(27) Martelli, G. P. (2014). Directory of virus and virus-like diseases of the grapevine and their agents