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SPREAD OF PLUM POX VIRUS (PPV) IN PLUM BASED ON GEOGRAPHICAL POSITION AND VARIETIES IN ALBANIA

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Abstract

Sharka is the most severe viral disease of Prunoids in Europe and the Mediterranean because of the widespread and the economic damage it causes. The study was conducted in 2016-2017 in Kukës, Tropoja, Dibër, Pogradec, Korçë in the varieties of plum: Tropojane, Dente 707, Shuker, Sugar, Black magic, Stanley, Agen, Black amber, Black Star, Klaudia, Friat. According to the ELISA test the percentage of infection from PPV resulted in: Korçë 56.29%, Pogradec 44.8%, Dibër 36.6%, Tropojë 28.42% and Kukës 0%. While according to varieties: Tropojane (57%), Dente 707 (56.6%), Stanley (37.6%), Blac amber (33.3%), Sugar (30%), Black magic (27.5%), Agen (24.4%) Claudia (0%) and Friar (0%).

Keywords: Sharka; Plum Pox Virus; Prunoids; varieties; Elisa test

Introduction

Our place for good climatic-territorial conditions to present the growth of a large number of fruit tree species. The production of fruits and mainly the rise of new blocks of fruit trees show great importance of their values, and one of them is plum as a laxative fruit and a microelement source (iron and magnesium). Therefore, this study was undertaken to see the spread of Plum Pox Virus (PPV) at country level and in the plum cultivars. The main studies in Albania have been carried out in stone cultures, having often plum pox as a main reference, which is a quarantine disease with particular economic importance caused by the Plum pox virus (Myrta 2015). Plum pox, also known as sharka, is the most devastating viral disease of stone fruit from the genus Prunus. (Cabi 2017). Sharka is the most severe viral disease of Prunoids in Europe and the Mediterranean because of the widespread and the economic damage it causes (Mërkuri 2007). Plum pox virus (PPV), a member of the genus Potyvirus, family Potyviridae, is a positive-sense single-stranded RNA virus (James 2013). First discovered in Bulgaria, PPV is now a day's present in most of continental Europe (with an endemic status in many central and southern European countries) and has progressively spread to many countries on other continents (García et al. 2014). The plum pox virus does not infect humans or animals (Celetti et al. 2016). Sharka is considered as one of the most serious threats to plum culture in Europe mainly because of the symptoms in the important varieties: grooving and pitting of the fruits, necrosis of the fruit flesh and a premature dropping of affected fruits (van Oosten 1973). In Europe, PPV is considered a plant pest against which a permanent campaign is

established (Mora-Aguilera et al. 1999). This virus reduces fruit yield and quality. It also shortens the productive lifespan of orchards and can render stone fruit trees useless for fruit production (CU 2008). The pest is of great economic importance especially in some southern and central European countries, causing considerable losses in production of primarily plum, apricot and peach. The extent of losses varies between countries of different climates, depending on cultivars and virus strains (Németh 1994). Plum pox virus infects plants in the genus Prunus (Rosaceae). Plum pox virus is a serious pathogen of stone fruit trees (apricots, cherries, nectarines, peaches, and plums) in Europe. Other hosts include almond and ornamental Prunus species (MSU 2010). The virus is spread to new areas by moving uncertified infected plant material through budding, grafting and transplanting, and by migrating aphids. (Bulatovic-Danilovich and Shane 2006). All Prunus species can be infected by pox virus (PPV), although the presence and intensity of symptoms vary according to species and cultivar, virus strain and sanitary status of the host (Teshale 2014). Although the spirea aphid, Aphis spiraecola (Patch), was a less efficient vector than M. persicae it is perhaps more important for the spread of PPV due to its greater abundance and occurrence earlier in the season when peach trees are thought to be more susceptible to infection (Lowery et al. 2015). Infected plant parts can be transported long distances, bypassing natural barriers such as mountain ranges, forests and oceans (Celetti et al. 2016). PPV is on the A2 list of the European Plant and Mediterranean Plant Protection Organization (EPPO), which includes those diseases and injuries that are present in the European region. The causal agent, Plum pox virus (PPV) is easily transmitted by many aphid species in a non-persistent manner, by manmade grafting (nursery trade), and has a very wide host range among Prunus species. Infected plants may not show symptoms for several months and symptoms are often transient in appearance (Cabi 2017). PPV spreads over long distances by uncontrolled movement of plant material, and many species of aphid transmit the virus locally in a nonpersistent manner (García et al. 2014). Plum pox develops slowly inside the tree, usually affecting only one or two branches at first but spreading through the tree as the virus multiplies over a period of several years (DPI 2012). Diagnostic symptoms occur mainly on leaves and fruits. Leaf symptoms include vein yellowing or light green to yellow rings. Foliar symptoms may develop during the cooler temperatures of spring and fall but fade during the host summer months (CU 2008). The introduction of PPV to a new country or region is usually through propagative materials and the subsequent distribution of contaminated materials. The secondary spread can be rapid and results from aphid transmission (Levy et al. 2000). PPV is the first viral disease described in our country finding the first hearth in the Durres district and later in Korçë. This virus can be developed in plants, where at the same time they are also affected by other viruses such as mosaic plum (Kaltani and Çelo 1982). PPV can be diagnosed by visual examination, particularly, during the period of active growth (Krishna et al. 2011). The PPV does not kill the plant, but if infected plants are not removed, they will serve as inocula to spread the virus to healthy plants (Herrera 2013). The economic damage comes from the premature fall of unripe fruits, the fruits infected by this virus have low sugar content which affects the plumbing disinfection industry (Pakashtica 2016). The intensity of the symptoms is determined by some factors such as cultivation sensitivity, viral strain pathogenicity, climatic conditions and cultural services (Ibrahimllari and Hasani 1998). Four strains of PPV have been identified in the world.

These strains are: PPV-M, PPV-EA, PPV-C and PPV-D (Rosenbaun and Hansen 2006). Studies for this disease are made and continue to be made in our country, because the territory of Albania is contaminated with such infections, which have been ascertained from Peshkopia up to the coastal zone (Isufi 2003). (Németh 1994) has reviewed the economic importance of PPV in Europe. Crop losses reported from various central and eastern European countries exceeded 75%. Complete crop losses have been reported in susceptible cultivars of plum (PPSMEA 2011). The losses caused by PPV in each affected country depend on many factors, including the susceptibility of the grown species and cultivars, stain presence and the control measures applied (I. Kamenova and Milusheva 2014). The results from a wide-scale outbreak of PPV could lead to a decrease in stone fruit exports and higher prices for domestic consumers (USDA 2008).

2. Materials and methods

2.1. Location and varieties

This study was conducted during 2016-2017 in the northeastern and southeastern parts of Albania and mainly in the Kukës, Tropojë, Dibër, Pogradec and Korçë regions. In these regions dominates the cultivation of plum because of the continental climber and the appropriate soil conditions. In this study were examined plum varieties such as: Tropojane, Dente 707, Shuker, Sugar, Black magic, Stanley, Agen, Black amber, Black star, Klaudia and Friat. In total there were 495 champions where: for the Kukës region took 50 champions, Tropojë 95 champions, Dibër 90 champions, Pogradec 125 champions and Korçë 135 champions. Below are some pictures from the places where the study was conducted.

2.2. Sampling

Appropriate sample selection is critical for serological. The samples collected in the study regions were brought to the viral laboratory and divided according to varieties. They consisted from flowers, young shoots and small fruit. The samples are stored in the refrigerator for no more than 7 days before the process.

2.3. Preparation of the sample for testing

Approximately 1 g of plant material is weighed, cut into small pieces and placed in plastic bag for processing. Approximately 20 volumes of extraction buffer are added and the sample are homogenized in plastic bags using the Homex 6 machine (Bioreba). The composition of the extraction buffer is: phosphate-buffered saline (PBS) pH 7.2–7.4 supplemented with 2% Polyvinylpyrrolidone (PVP-10) and 0.2% sodium diethyl dithiocarbamate (DIECA).

2.4. Buffers

Phosphate-Buffered Saline (PBS) pH 7.2–7.4.
Carbonate buffer pH 9.6.
Washing buffer (PBS, pH 7.2–7.4 with 0.05% Tween 20). Substrate buffer for alkaline phosphatase.

2.5. Serological tests

DAS-ELISA (Double Antibody Sandwich) EPPO protocol.

Set the solution of antibody

Prepare an appropriate dilution of rabbit-PPV polyclonal immunoglobulins in carbonate buffer pH 9.6. Add 100 µL to each well. Incubate at 37 °C for 4 h. Wash the wells three times with PBS-Tween (washing buffer).

Preparation and aplication of samples

Add 100 µL per well of the plant extract (see sample preparation). Use two wells of the plate for each sample or positive controls and at least two wells for negative controls. Incubate at 4 °C for 16 h. Wash as before.

Preparation and aplication of conjugatit

Add antimouse immunoglobulins conjugated with alkaline phosphatase: prepare an appropriate dilution of antimouse immunoglobulins conjugated with alkaline phosphatase in PBS plus 0.5% BSA. Add 100 µL to each well. Incubate at 37 °C for 4 h. Wash as before.

Preparation and aplication of the substrate

Prepare 1 mg/mL alkaline phosphatase solution (pnitrophenylphosphate) in substrate buffer (Appendix 1). Add 100 µL to each well. Incubate at room temperature and read at 405 nm after 30 and 60 min. The ELISA test is negative if the absorbance of the sample is less than three times the absorbance of the healthy control. The ELISA test is positive if the absorbance of the sample is equal or greater than three times the absorbance of the healthy control.

3. Results and discussion

3.1. Assessment of the infection

The leaves are tested using the Enzyme-Linked Immuno Sorbent Assay (ELISA) test. Serological analyses of samples confirmed the presence of PPV in 192 samples from 495 samples collected. From the examinations performed, the presence of PPV was in 36.16 % in the samples analysed. The infected samples showed a positive serological reaction and proved that 192 samples were infected with plum pox virus.

3.2. Distribution of viral infection PPV in the main regions of Albania

The champions were taken in 5 regions of Albania where plums dominate because of continental and terrestrial climate. From the sample taken in the study the largest infection resulted in Korçë region with 56.29%, continuing with Pogradec 44.8% and then with Dibër 36.6%, Tropojë 28.42%. The samples taken in the district of Kukës did not result with infection from plum pox virus. The percentage of infection in the studied regions is presented in the graphic below (figure 1).

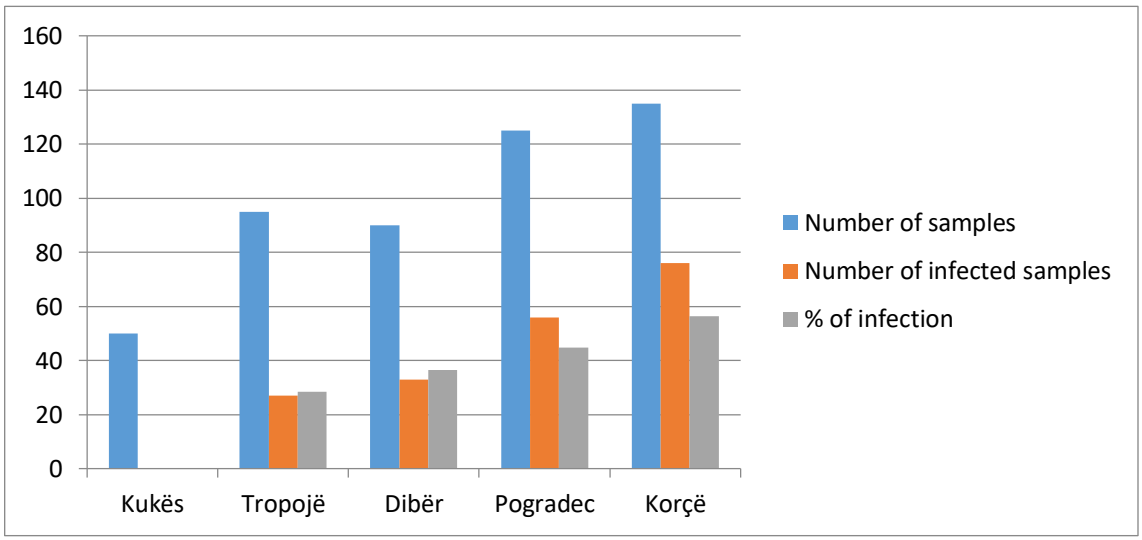


Figure 1. Distribution of PPV in main regions of Albania for 2016-2017 year.

3.2. Distribution of Plum pox virus after varieties

From plum varieties tested in laboratory, the highest percentage of infection resulted in Tropojane varieties (57%) followed by Dente 707 (56.6%) and continues the Stanley (37.6%), Blac amber (33.3%), Sugar (30%), Black magic (27.5%) and Agen (24.4%). While 2 varieties Klaudia and Friar resulted without infection from PPV (plum pox virus).

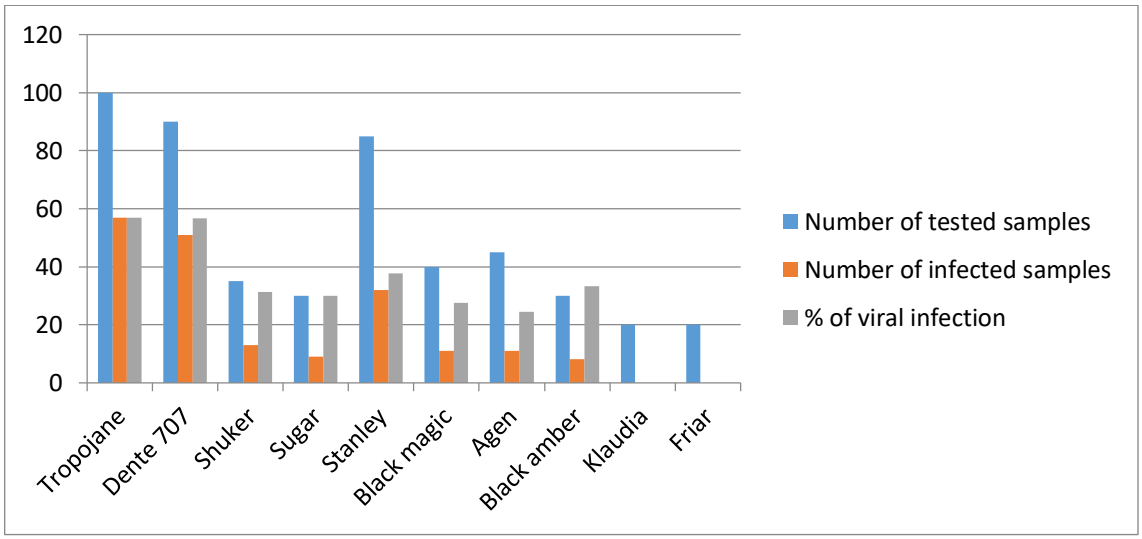


Figure 2. Distribution of Plum pox virus after varieties for 2016-2017 year.

As a result of the serological test, out of 495 tested samples, 303 resulted healthy (61.2%) while 192 were infected (38.8%).

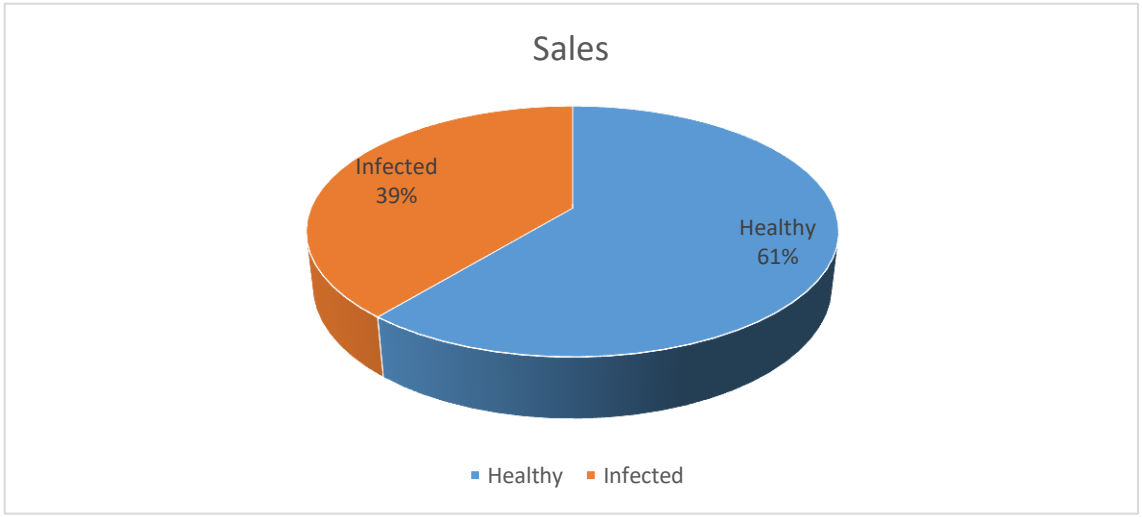
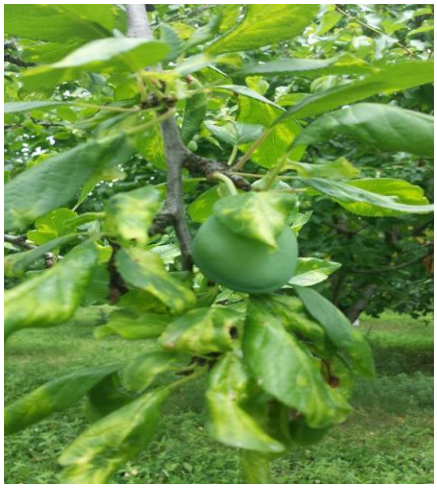


Figure 3. Plum Pox Virus spread in percentage.

Figure 4. Symptoms of PPV in tropojane plum. Figure 5. Symptoms of PPV in Stanley plum.



Discussion

This study presents an assessment of the health condition of the plum culture in relation to the PPV pathogenesis in the main regions of Albania where plums are planted. The results showed that Sharka disease is widespread almost in all cultivation areas of this culture, in the Diber district (this is cited in the literature "Virusi dhe Bima" (Merkuri 2007) largest spread of PPV is in areas near the city which are planted with more vegetables and vectors serve for the spread of this disease. Comparing the results from northeast with the southeastern part of Albania shows that plum culture is less affected because in the southeastern area there are also dried and abandoned blocks from this disease. Analyzing the plum cultivars on the presence of the PPV, result that this disease is more widespread in the European cultivars, which we have mentioned above, and this corresponds to the writing "Bazat e virusologjise bimore" (Myrta 2015) where it is said that: The disease is widespread in Albania and Kosovo, more in european plumage where the infection in the old blocks reaches up to 100% of the trees and less in peaches and apricots, pointing out that losses from sharka in european plumage have a considerable cost.

Conclusions

In the northeastern area and mainly in Dibër and Tropojë, PPV is widespread in the municipalities around the city, where many vegetables are planted and vectors serve for to spread of this infection.

The above results show that in the northeastern area the spread of infection is greater in Dibër continuing with Tropojë region. While in the Kukës region there was no infection from the laboratory tests performed on taken samples.

In Dibër region, PPV infection has mostly affected Tropojane variety, even though there are pulled out large plum surface affected by this disease.

In Tropojë region, PPV infection has affected some lowland villages, although many infected surfaces with plum from PPV are pulled and replaced with certified young shoots and cleaned by PPV.

The Kukës region did not resulted with PPV infection, but sporadic manifestations of this virus occurred and measures were taken to prevent its spread.

While in Pogradec and Korçë, PPV infection resulted to be higher in %, because here the infection in some of the old plum blocks reaches 100%.

As far as varieties are concerned, the largest infection has resulted in the Tropojane variety followed by Dente 707, Stanley, Black amber, Shuker, Blac magic and Agen. While Klaudia and Friar varieties were not affected by PPV infection.

However, as in all viruses transmitted by aphids, this virus is very difficult to control, so there are some intervention possibilities:

Continually monitoring of this disease reduces the degree of affection in prune trees from plum pox virus.

Agricultural quarantine makes it possible to eliminate plum pox virus from the beginning because later the measures taken to limit them do not have the proper effect.

Detection and elimination of infection sources. Chemical control of vector insects, etc.

This disease should be seen with priority as it may otherwise appear on a larger scale.

Acknowledgment

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