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RESEARCH ARTICLE

New and dangerous bacterial disease of oleander *Pseudomonas savastanoi* pv. *Nerii* in greenhouses of Ukraine

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Abstract

Object: Study of symptoms and etiology of oleander tuberculosis causative agents and determination of causes of their appearance in conditions of the closed ground of the greenhouses of Ukraine.

Methods: Samples for research were selected in greenhouses of Botanical Garden named after acad. A. V. Fomin from different kinds and forms of oleander (*Nerium oleander* L.). The microbiological and phytopathological analysis was carried out after K. I. Beltyukova. Biolog GN MicroPlate system was used to identify isolated bacterial strains. Calculations for bacteria identification in relation to genus, species and other taxonomic units are based on the similarity index.

Results: On oleander plants, the greatest disease progress shows itself on the branches of the second year of development. Up to 5 bacterial nodes of different sizes can be formed on one branch. Galls can be formed on the leaves both on the upper (62.1%) and lower (37.9%) sides. Bacteria actively spread with water from branches and leaves, especially when spraying plants from a hose. The infection degree of different varieties of oleander was not the same. Such varieties and forms as of *Nerium oleander* "Album", *Nerium oleander* "Album Plenum" suffer from bacteriosis the most.

Conclusions: For the first time in Ukraine tuberculosis *Pseudomonas savastanoi* pv. nerii was detected on oleander (*Nerium oleander* L.). The infection degree of different varieties of oleander was not the same. *Nerium oleander* "Album", *Nerium oleander* "Album Plenum", are the most severely leased, *Nerium oleander* "Flavescens", *Nerium oleander* "Lacteum" are leased to the lesser extent. The spread of the disease is favored by some methods of this group of plants growing: pruning plants, branches, and leaves bruise..

Keywords: Oleander, Bacterial node, *Pseudomonas savastanoi* pv. nerii

Introduction

The famous plant is the oleander (*Nerium oleander* L.) of various forms and varieties in conditions of closed ground and especially of the greenhouses of Ukraine. Spread of the evergreen poisonous shrub with branching stems is primarily caused by the presence of bright inflorescences of different colors at the ends of shoots, which are capable of long and active blossoming under bright sunlight.

Despite the phytoncidal abilities of this plant, the species is often leased by pathogens, among which the bacterium *Pseudomonas savastanoi* pv. nerii, belonging to the family Pseudomonadaceae, has been especially

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dangerous in recent years (Iacobellis et al. 2001).

Pathogens of bacterial etiology are widespread in nature and affect many plant species in the crop. Because of high plasticity and rapid adaptation of the bacteriosis pathogens, they become resistant to many chemical (e.g. streptomycin) and biological drugs (e.g. antagonist bacteria-*Pantoea agglomerans*, strain P10C or *Bacillus subtilis*, strain BsBD 170) (Bokshan & Sadlyak 2015).
That is why the determination of the causes of galls appearance on various parts of oleander, determination of bacteria species that cause them in conditions of the closed ground of the greenhouses of Ukraine is very urgent.

Materials and Method

We observed the development of *Pseudomonas savastanoi* pv. nerii on varieties and forms of oleander (*Nerium oleander* L.) in the greenhouses of Botanical Garden named after acad. A.V. Fomin in 2015-2017. Samples were taken to identify the causative agent of the disease. The microbiological and phytopathological analysis was carried out after K.I. Beltyukova (Beltyukova et al. 1968). Isolation of pathogens from leased samples of oleander plants was performed on the verge of affected and healthy tissues. Small pieces of the leased tissue were washed with non-sterile water for 10-15 minutes and with sterile water- for about 5 minutes. Further, it was grated to a homogeneous state in a sterile porcelain mortar and plated with a microbiological loop in a Petri dish on the surface of potato agar (PA). Morphology of the colonies was described on the 3rd-4th day with their growth on PA. Morphology of vegetative cells and spores was studied with the help of an electron microscope (Beltyukova et al. 1968).

Biology GN MicroPlate system was used to identify isolated bacterial strains. Calculations for bacteria identification in relation to genus, species and other taxonomic units are based on the similarity index (Beltyukova et al. 1968).

Results

On oleander plants, the greatest disease progress shows itself on the branches of the second year of development (Fig. 1a and 1b). Up to 5 bacterial nodes of different sizes can be formed on one branch. Galls can be formed on the leaves both on the upper (62.1 %) and lower (37.9 %) sides (Fig. 1a and 1c). Manifestations of the disease are observed on reproductive organs (inflorescence) in April-June. Bacteria *Pseudomonas savastanoi* pv. nerii were isolated and identified as a result of laboratory studies *Nerium oleander* "Album", *Nerium oleander* "Album Plenum", are the most severely leased by bacteriosis, *Nerium oleander* "Flavescens", *Nerium oleander* "Lacteum" are leased to the lesser extent.

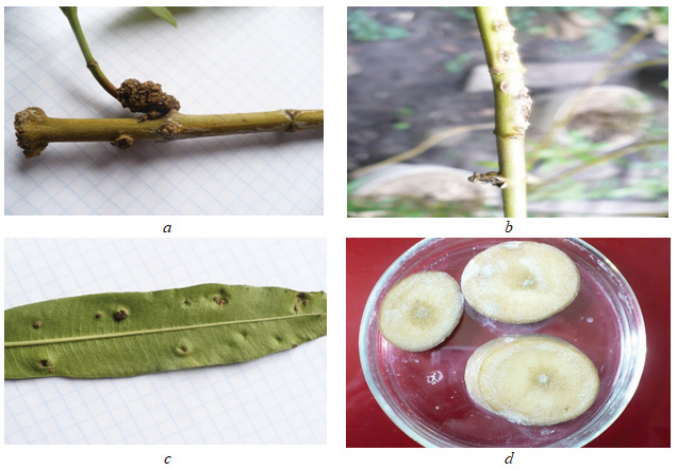

Figure 1. Symptoms and microbiology of *Pseudomonas savastanoi* pv. nerii on *Nerium oleander* L.: **a**-parenchymal nodes on the stem; **b**-arenchymal nodes on the leaf area; **c**-primary and secondary parenchymal nodes; **d**-*Pseudomonas savastanoi* colonies; (original photos).

Bacteria actively spread with water from branches and leaves, especially when spraying plants from a hose. The spread of the disease is favored by certain features of cultivation of this group of plants, in particular, because they are taken out of the greenhouses outdoors in summer and are back in autumn. It is also known that the obligatory method of oleander formation is a regular pruning of plants, which also favors penetration of bacteria into open wounds, especially when disinfection of secateurs is ignored and with use of tree-pruning paste. Mentioned above is also due to the breaking of branches and leaves, which in turn contributes to the penetration of infection.

It is found with our research, conducted in the greenhouses of Botanical Garden named after A. V. Fomin, that oleander plants are leased with four species of arthropods: two-spotted spider mite (*Tetranychus urticae* Koch.), oleander aphid (*Aphisnerii* Boyerde Fons.), oleander scale (*Pseudococcus longispinus* Targ.) and mealybug hirsute (*Pseudococcus longispinus* Targ.) (Schaad et al. 2001). Taking into account that these types of insects' damage plants due to the presence of a piercing-sucking apparatus, the influence of the above-mentioned species of phytophagous pests on the spread of the causative agent of oleander tuberculosis seems to be quite real, that requires special and profound study.

It should be noted that next to oleanders affected by tuberculosis, olive (*Olea europea* L.) grows, but bacteriosis lesion is not yet detected on this plant species. Based on the idea of persistence emergence in representatives of parasitic forms of microorganisms, it should be noted that they are constantly forced to solve diverse tasks (change peculiarities of their development).

Thus, parasitic forms of microorganisms can die together with the infected organism, as the result of the host's protective factors influence. In its turn, high

virulence of the microorganism can cause the death of the infected plant before the formation of reproductive individuals of the pathogen itself. That is why, pathogenic microorganisms, on the one hand, should strive to create forms that are resistant to the protective factors of the infected organism, and on the other hand-to create forms with reduced virulence (Frolov 2001).

Considering the bacteriosis of *Pseudomonas savastanoi* pv. fraxini, *Pseudomonas savastanoi* pv. nerii, *Pseudomonas savastanoi* pv. oeae from the point of view of their persistence occurrence, it should be noted that *Pseudomonas savastanoi* pv. nerii differs from the introduced bacteriosis by the ability to affect reproductive organs (inflorescence) of the host plant. This may indicate that the co-evolution of the given parasite and the host plant is in the process of becoming.

By reducing the reproductive capacity of the host plant, the parasite narrows its potential possibilities for further spread. Proceeding from the idea of persistence emergence in parasitic forms of microorganisms, the revealed differences indicate the incompleteness of this process in this species.

Discussion

We should note that *Pseudomonas savastanoi* pv. nerii. the disease is widely spread in Italy, Spain, France, South Africa, Australia, the USA, on the Black Sea coast of the Caucasus: Sukhumi-Sochi and it causes tuberculosis of plants of *Nerium* L. genus (Chumayevskaya 1956; Krushhvili 1960; Golenko 1966; Wilson & Magie 1963).

All parts of plants are leased with tuberculosis. Swellings are formed on the branches and leaves and the leased inflorescences unusually increase in size. The disease was first described in Italy. The causative agent of this disease was recognized as an independent species and it was named Ps. *tonelliana* (Fer.) Krass. (Golenko 1966). Based on experiments with artificial infection and serological studies of Ps. *tonelliana* isolated from tuberculosis leased oleander and of *P. savastanoi* (E. F. Smith) strains Stevens from olives a close serological relationship is revealed (Wilson & Magie 1963). According to modern data, the causative agent of oleander cancer is considered one of the forms of the main species.

Pseudomonas savastanoi (E. F. Smith) Stevens: *P. savastanoi* pv. oeae, *P. savastanoi* pv. fraxini, *P. savastanoi* pv. nerii, *P. savastanoi* pv. phaseolicola.

Bacteria of this species are sticks 0.4-0.8 × 1.2-3.0 mc or 0.4-0.5 × 1.2-1.7 mc (Sutic & Dowson 1963) in size, located singly, in pairs or in chains, non-sporinferious, gram-negative, the colonies are transparent on the agar, they are white, round, with flat edges, they are flat and grow slowly, they form brown pigment on potato (Golenko 1966; Ponyak & Yakovleva 1979).

Conclusion

We note that *Pseudomonas savastanoi* pv. nerii. tuberculosis was for the first time discovered in Ukraine in the greenhouses of Botanical Garden named afrer A. V. Fomin in 2015 on the purchased oleander (*Neriumo leander* L.). In this connection, it can be assumed that the disease was introduced together with the introductory species of the plant. Infection degree of different oleander varieties was not the same. *Nerium oleander* "Album", *Nerium oleander* "Album Plenum", were the most severely leased, *Nerium oleander* "Flavescens", *Nerium oleander* "Lacteum" were leased to the lesser extent.

The spread of the disease is favored by some methods of this group of plants growing: pruning plants, branches and leaves bruise.

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