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THE MOST COMMON DISEASES OF ORNAMENTAL PLANTS IN THE ENTITY OF REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA)

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ABSTRACT

During five year period (2011-2015) on territory of Republic of Srpska the continuous monitoring was carried out on different ornamental flowers for the presence of plant diseases. For laboratory analysis following species *Alyssum* sp., *Aster* sp., *Bacopa* sp., *Begonia* sp., *Bellis perennis*, *Calceolaria* sp., *Chrysanthemum* sp., *Cyclamen* sp., *Dahlia* sp., *Dianthus* sp., *Fuchsia* sp., *Gazania* sp., *Gloxinia* sp., *Lobelia* sp., *Myosotis* sp., *Pelargonium* sp., *Petunia* sp., *Petuniaxhybrida*, *Phlox* sp., *Plectranthus* sp., *Portulaca grandiflora*, *Primula* sp., *Ranunculus* sp., *Rusmarinus officinalis*, *Salvia* sp., *Sansevaria* sp., *Tagetes* sp., *Impatiens* sp., *Verbena* sp. and *Viola* sp. were taken. Determination of causal pathogens was carried out on the basis of studying their morphological, pathogenic, biochemical and physiological, serological and molecular characteristics. The most common causal pathogens were *Alternaria* sp., *Botrytis* sp., *Golvonomyces* sp., *Pestalotia* sp., *Phomopsis* sp., *Peronospora* sp., *Phytophtora* sp., *Puccinia* sp. and *Septoria* sp. Also, in much less extent, bacterial and viruses diseases were present. The most common bacterial diseases appear to be genera *Pseudomonas* and *Erwinia*, while the most frequent plant viruses were *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV).

Keywords: *ornamental plants, common diseases, Republic of Srpska.*

INTRODUCTION

In recent years production of ornamental plants in the area of Republic of Srpska (RS) is in a significant increase. However, in this production together with modernization and increasing the production of ornamental plants appeared certain problems. Different types of flowers are often hosts of many plant parasites (fungi, bacteria and viruses), which often lead to their complete extinction, in a stronger occurrence intensity. Therefore an extremely important part of disease control is proper identification of causal pathogen, which was the main goal of this study.

MATERIAL AND METHODS

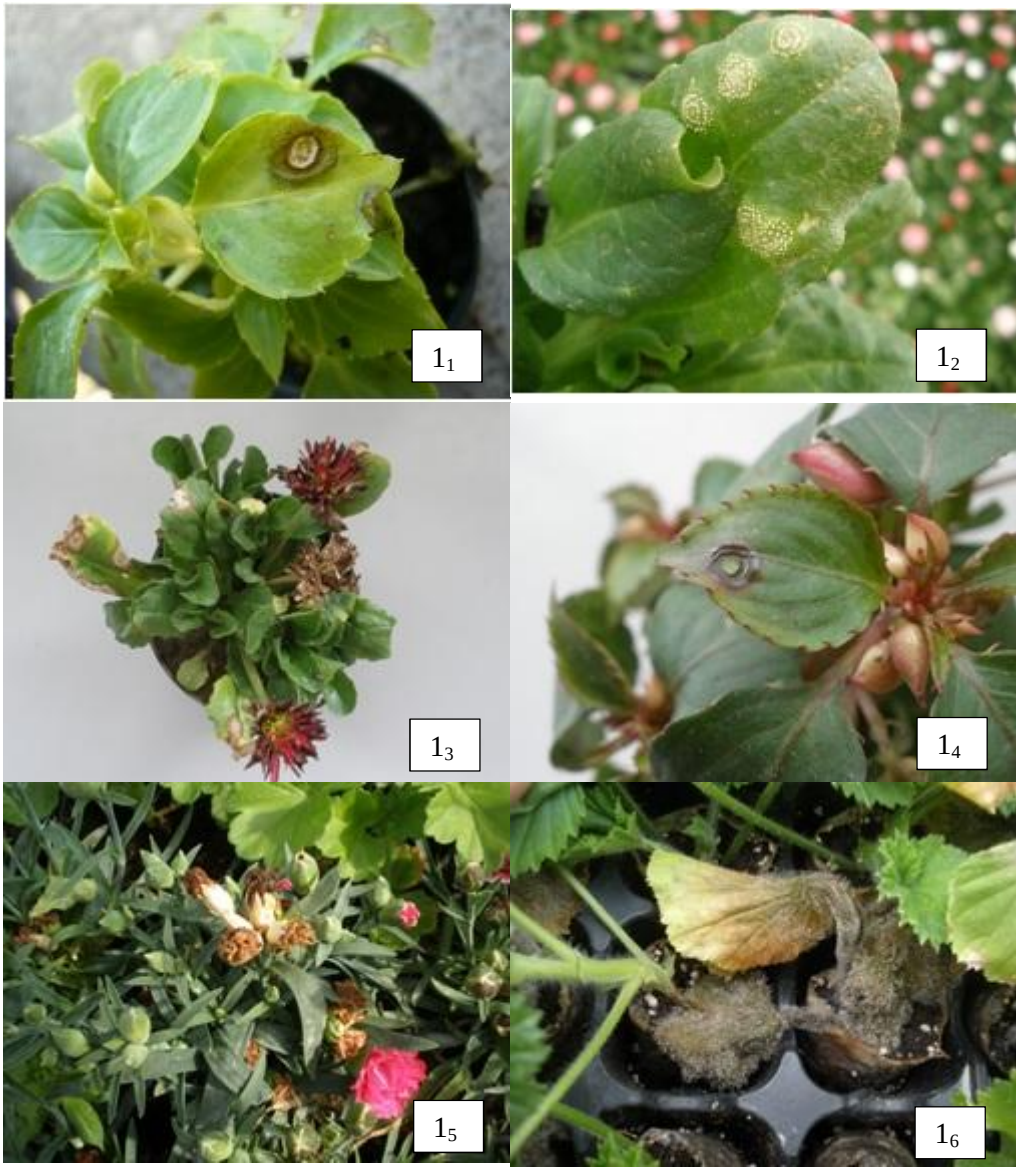
During five year period (2011-2015) on territory of Republic of Srpska the continuous monitoring was carried out on different ornamental flowers for the presence of plant diseases. For laboratory analysis following species *Alyssum* sp., *Aster* sp., *Bacopa* sp., *Begonia* sp., *Bellis perennis*, *Calceolaria* sp., *Chrysanthemum* sp., *Cyclamen* sp., *Dahlia* sp., *Dianthus* sp., *Fuchsia* sp., *Gazania* sp., *Gloxinia* sp., *Lobelia* sp., *Myosotis* sp., *Pelargonium* sp., *Petunia* sp., *Petuniaxhybrida*, *Phlox* sp., *Plectranthus* sp., *Portulaca grandiflora*, *Primula* sp., *Ranunculus* sp., *Rusmarinus officinalis*, *Salvia* sp., *Sansevaria* sp., *Tagetes* sp., *Impatiens* sp., *Verbena* sp. and *Viola* sp. were sampled. Plant material with visible symptoms (photo $1_{1\text{-}16}$) was collected and grouped according suspicion to fungal, bacterial or virus diseases.

A first group of ornamental plants was cut into fragments, 5 mm long, and surface sterilised with 5% sodium hypochloride (ACE) for 30 sec. Then fragments were washed under tap water and placed into disinfected paper to remove water from their surface. After surface disinfection plant fragments (5 per dish) were placed on PDA (Difco, pH 6.5) and incubated at 25°C. Identification of chosen fungal isolates was carried out after 14 to 21 days of incubation based on morphological and cultural characteristics, as well as on pathogenicity test.

Selected tissue of second group of ornamental plants was washed under tap water and placed into disinfected paper to remove water from its surface, and then air dried on room temperature. Small fragments of tissue were cut, surface sterilised with 96% ethanol and then crushed with sterile water. The suspension was placed on mesopeptonagar in Petri dishes (Arsenijević, 1992, 1997). After 48 hours of development in the incubator at a temperature of 26°C, single colonies of developed bacteria were placed on mesopeptonagar. In this way, pure cultures were obtained by a large number of isolates. Vitality of isolated strains was maintained with frequent growing on media at intervals of 20-30 days. For better preservation of culture 24 hours old pure culture were sealed with paraffin oil and kept in a refrigerator at a temperature of 4°C. Chosen bacterial isolates were investigated for pathogenicity in inoculation test and identification was performed based on morphological and biochemical - physiological characteristics.

Third group of symptomatic plants were analysed by serological method, DAS-ELISA test to detect the presence of phytopatogenic viruses using double-antibody sandwich (DAS)-ELISA kit (AG Bioreba, Reinach, Switzerland). The procedure was performed according to the manufacturer's instructions. ELISA (enzyme-linked immunosorbent assay) method is based on the reaction of the antibody–antigen and has been used for the diagnosis of the presence and amounts of specific molecules in the mixed sample. Primary antibody (specific for the protein being tested) was adsorbed onto a solid substrate in the wells of the ELISA plate, after which the known amount of sample was added in the same wells. In the case of the presence of the corresponding virus in the plant material, the entire antigen in the sample binds to the antibody. Another specific enzyme–labelled antibody has been added to the second place in the test protein. The enzyme causes

a colour change in the presence of a substrate reagent, with different intensity depending on the concentration of virus in the test sample. Reading the intensity of wells coloration on ELISA plates was carried out on the MULTISCAN FS instrument (Thermo Scientific, Germany). This instrument registers the transience of the light beam, wave length 405 nm through wells of ELISA plates. The reaction was considered positive if the absorption of light at 405 nm was at least twice as high compared with the absorption of the corresponding control (commercially available negative and positive control).



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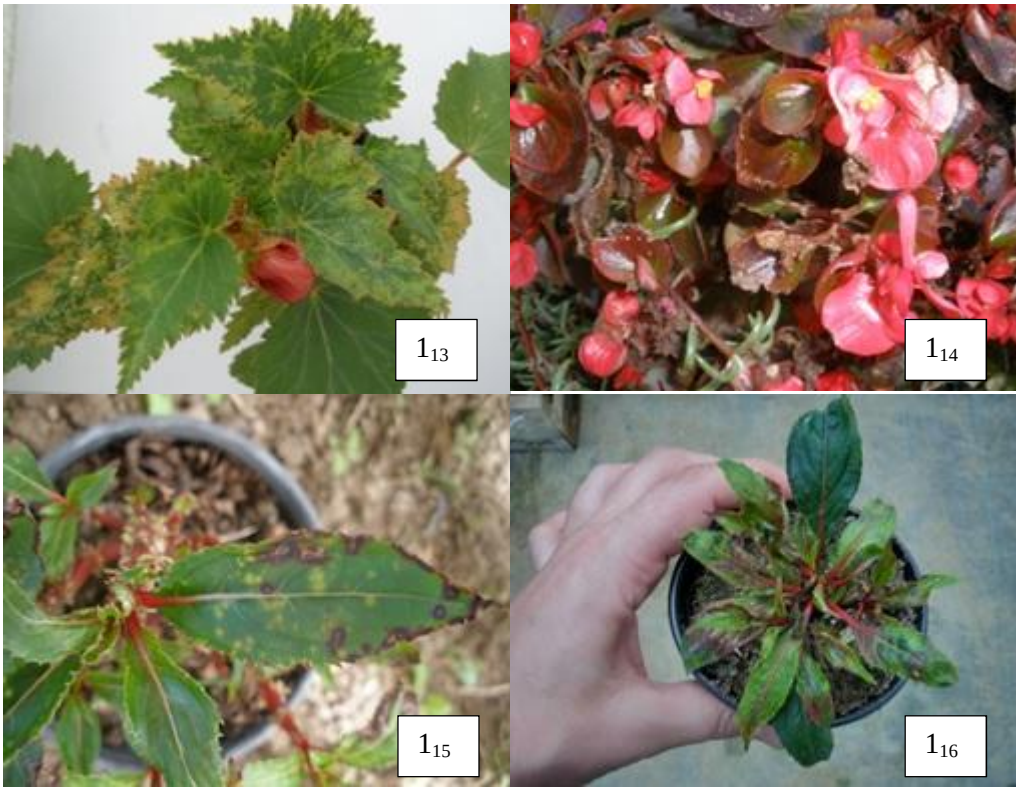

Photo $1_{1\text{-}16}$. Plant material with visible symptoms of different ornamental plants collected and grouped according suspicion to fungal, bacterial or virus diseases.

RESULTS AND DISCUSSION

According to the conducted research during five years period (2011-2015) on territory of Republic of Srpska, studied pathogenic, morphological and biochemical-physiological characteristics, as well as serological and molecular methods of chosen disease simptomatic ornamental plants the most common causes of diseases appear to be phytopatogenic fungi and viruses, while in much less extent bacterial diseases seem to be present in ornamental plants.

Chosen fungal isolates were investigated for pathogenicity in inoculation test on ornamental host plants. Morphological characteristics were studied under the light microscope magnification of 40x, while cultural characteristics of tested isolates, appearance, structure, color, mycelial growth and fructification were also studied. According to the identification of chosen fungal isolates based on morphological and cultural characteristics and pathogenicity in inoculation test, five year monitoring showed that the most common phytopatogenic fungi on ornamental plants were species from the following genera: *Alternaria, Botrytis*, *Golvonomyces*, *Pestalotia*, *Phomopsis*, *Peronospora, Phytophtora*, *Puccinia* and *Septoria* (photo $2_{1\text{-}8}$).

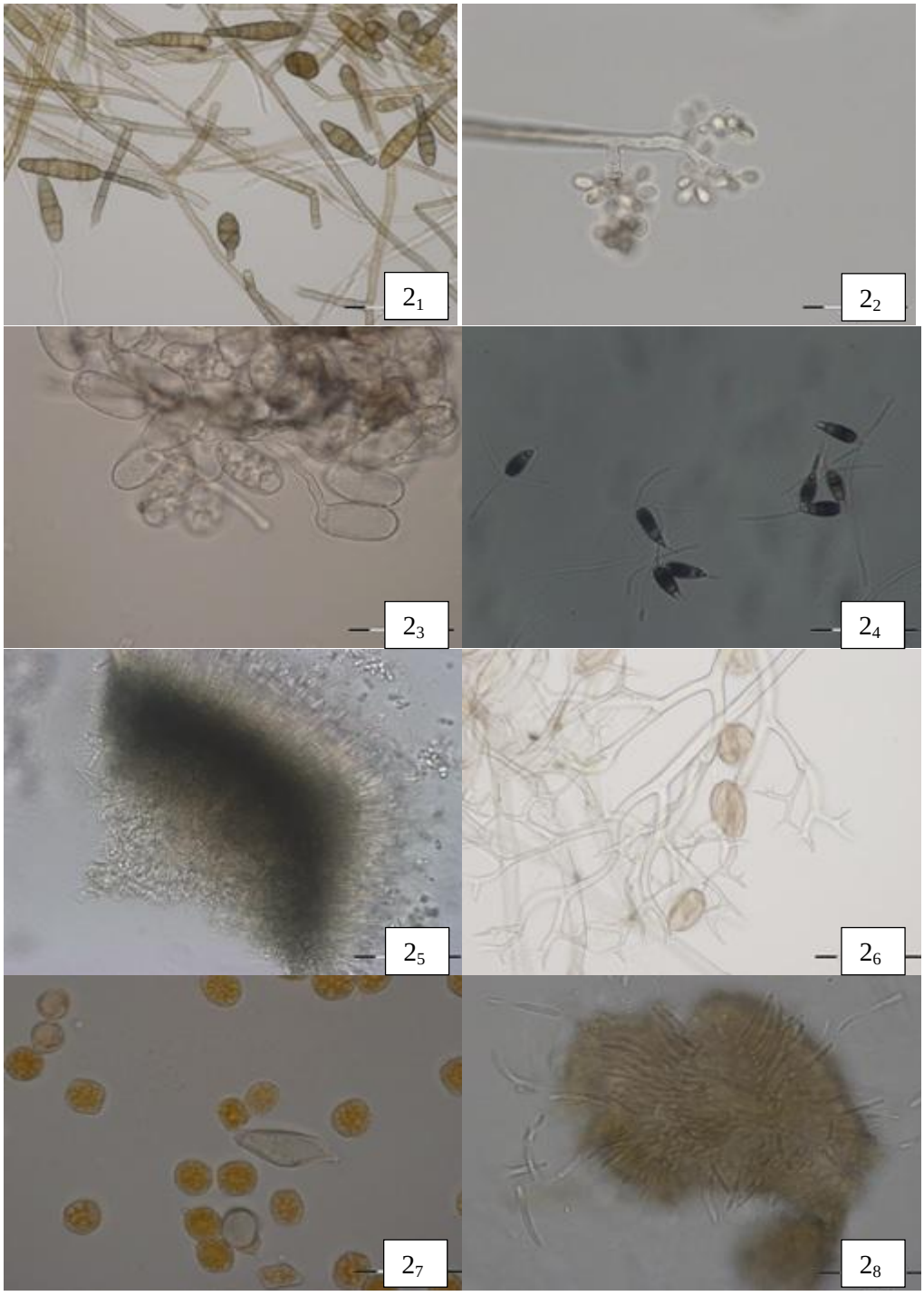

Photo 2_{1-8}. The most common phytopatogenic fungi were species from the following genera: *Alternaria*, *Botrytis*, *Golvonomyces*, *Pestalotia*, *Phomopsis*, *Peronospora*, *Puccinia* and *Septoria*.

Bacterial isolates were investigated for pathogenicity in inoculation test on ornamental host plants. Morphological characteristics of chosen bacterial isolates were studied under light microscope, while Gram staining is confirmed with 3% KOH (Arsenijević *et* Jovanović, 1995). Cultural characteristics, development on mesopepton media and King B (fluorescence), as well as development on Levan and YDC media were studied. Among a biochemical and physiological characteristics creation of catalase, oxidase and arginine-dihydrolase and O/F test glucose metabolism were studied (Fahy and Persley, 1983; Lelliott and Stead, 1987; Arsenijević, 1992, 1997). According to the studied pathogenic, morphological and biochemical-physiological characteristics of chosen bacterial isolates the most common causes of bacterial diseases in ornamental plants appear to be *Pseudomonas* sp. (Photo 3_{1-2}) and *Erwinia* sp.

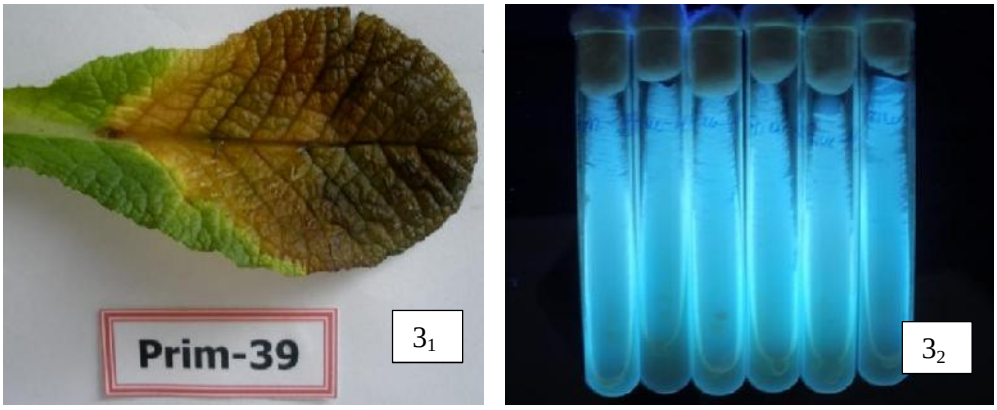

Photo3_{1-2}. Symptoms on the leaves primrose (artificial inoculation) and creation of a fluorescent pigment on King B medium

According to serological test, DAS–ELISA, of third group of symptomatic plants it is confirmed the presence of *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV). TSWV was confirmed in genera *Begonia, Impatiens* and *Gloxinia,* while INSV was confirmed only in *Begonia* and *Impatiens* genera.

CONCLUSION

The most important fungi on ornamental plants confirmed during five year monitoring (2011-2015) on territory of Republic of Srpska are species from the following genera: *Alternaria, Botrytis, Golvonomyces, Pestalotia, Phomopsis, Peronospora, Phytophtora, Puccinia* and *Septoria.* Some species of ornamental plants like *Chrysanthemum* sp. and *Primula* sp. were confirmed as hosts of *Erwinia* spp., which on them can cause a mushy, brown, smelly, soft rot or leaf spots. These bacteria can cause symptoms on a wide range of different ornamental plants (Daughtrey *et al*., 1995; Moorman, 2016). Among *Pseudomonas* species according to Trkulja *et al*. (2012), *Pseudomonas marginalis* (Brown) Stevensis reported as causal agent of bacterial spot and soft rot of primrose leaves and flowers. It is

polyphagous bacteria and can cause damp rot on lettuce as well as on a number of other hosts, such as Chinese cabbage (Choi *et* Han, 1989), garlic (Choi *et* Han, 1990a), ginger (Choi *et* Han, 1990b), broccoli (Canaday *et al*., 1991), rice (Cottyn *et al*., 1996) and onions (Kim, 2002). During the five year monitoring (2011-2015) by serological tests confirmed the presence of two viruses - *Tomato spotted wilt virus* and *Impatiens necrotic spot virus* in ornamental plants on territory of Republic of Srpska (Trkulja *et al.*, 2013a,b).

Considering research results it is necessary to take control measures for pathogens of ornamental flowers, in order to prevent the spread of the pathogens on numerous other hosts which are grown indoor and outdoor in the Lijevce field, Posavina and Semberia.

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