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ETHIOLOGICAL STUDIES OF ROOT ROT OF PARSNIP (*PASTINACA SATIVA* L.) IN VOJVODINA

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ABSTRACT

In the past few years, the symptoms of parsnip root rot (Pastinaca sativa L.) have been more intense in production. This had a significant impact on the decrease in the yield as well as the market value of the roots. Symptoms of rot are only noticed during harvest. Continued development of the pathogen has been reported in storage rooms, especially if the root is stored under uncontrolled conditions of temperature and humidity. The aim of the study was the etiological study of root rot in the open field and in storage rooms. Total of 56 root samples were collected in the period 2016 – 2019 years. Isolation was performed by standard of phytopathology procedure on PDA medium for fungi, MPA and CVP for bacteria. After growth, a collection of isolates was formed, which was included in the pathogenicity test. For the isolates that showed pathogenicity, the identification was performed, the fungi were identified on the basis of morphological characteristics, while the bacteria were studied for biochemical - physiological characteristics. Of the 42 fungus and bacterial isolates collected, 37 caused similar symptoms of rot, on artificially inoculated parsnip root, which was considered a pathogenic trait. Fusarium spp. was isolated from the symptoms of dry rot on the leaf rosette crossing, while Pectobacterium carotovorum subp. was isolated from the wet rot. The symptom of rot, followed by the white mycelium of the fungus, was preliminary indicated by the species Sclerotinia sclerotiorum, which was confirmed by isolation *and identification. The dominant species in storage rooms which provide root rot were Botrytis cinerea and Sclerotinia sclerotiorum. They were followed by Rhisopus spp. and Penicillium spp. species, but they have not been proven pathogenic and have been considered saprophytes. Root rot caused damage during harvest, as insects too, which allowed pathogens and saprophytes to penetrate.*

Keywords: *Pastinaca sativa, root rot, fungi, bacteria*

INTRODUCTION

Parsnip (*Pastinaca sativa* L.) belongs to the group of root vegetables, which are grown on light-humus land in the territory of Vojvodina province. It belongs to the family *Apiaceae*, and is used as fresh vegetable, cooked and in the form of dry spices (Rawson et al., 2010; Koidis et al., 2012; Castro et al., 2013).Species originates from Europe and Western Asia (Rubatzky et al., 1999). Parsnip root contain a high content of dietary fiber, according to some studies of 4.7 - 4.9% (Southgate, 2001). It contains vitamins C, B1, B2, B6 and minerals that include potassium, phosphorus, calcium, and iron (Ilić and Sunić, 2015).

Pathogens that parasitized parsnips are important factors in reducing the quality and yield of roots. In the agro ecological conditions of Vojvodina province, various pathogens often appear on the leaves and roots. Root pathogens are usually noticed only during extraction, i.e. the appearance of symptoms is recorded, which is often in the form of rot. In recent years, however, the appearance of parsnip root rot has been observed more often, which can significantly reduce the yield of the parsnip roots during storage (Gavrilović et al., 2007). Storing parsnip roots at low temperatures (< 1 ° C) leads to starch degradation which increases root sweetness (Bufler and Horneburg, 2013) and delays the beggining of fungal growth (Edelenbos et al., 2020). However, lower temperatures do not inhibit bacterial infection (Bartz and Wei, 2003). The roots then can be stored for 2 to 6 months at 0 ° C with 95 - 100% RH (Israelsson, 2000).

According to Machowicz - Stefaniak and Zalewska (2009) the species that often parasitize parsnips are *Alternaria alternata*, *A. raphani*, *Fusarium oxysporum, F. equiseti, F. solani and Stemphylium botryosum* and especially the species *Itersonilia pastinacae* which causes black cancer. Symptoms of rot on plant parts, that are rich in carbohydrates can be caused by bacteria of the genera *Pectobacterium* and *Pseudomonas* (Arsenijević, 1997). According to Gavrilović et al. (2007) in agro ecological conditions of Serbia rot of celery and parsnips is caused by the bacterium *Pseudomonas marginalis*.

The objective of this study was to investigate the etiology of parsnip root rot from collected samples, originates from the open field and in storage rooms in the period 2016 - 2019 years.

MATERIALS AND METHODS

Pathogen isolation

In the period 2016-2019 years, 56 samples with rot symptoms were collected. Pathogen isolation was performed using a standard phytopathological procedure on potato dextrose agar (PDA) media for fungi, MPA and CVP (crystal violet pectate medium) for bacteria. After isolation and obtaining pure cultures, the isolates were maintained on an oblique PDA and MPA substrate at temperature of 4 ° C.

Fungal identification was performed based on the classical macroscopic and microscopic characteristics, while bacteria were identified by using biochemical - physiological characteristics to species levels according to protocols by Schaad et al. (2001).

Pathogenicity test

The pathogenicity test of all isolates included in the our research, was performed by using inoculation of five parnsip roots. Prior to inoculation, the root was thoroughly examined and sterilized with 4% NaClO on surface and then washed for 5 min in sterile distilled water. The root was injured with a scalpel in the form of a depression, into a suspension of fungi or bacteria were inserted.

Suspension of fungi conidia were prepared from 10 day old cultures grown on at 24 °C PDA in the dark. Sterile distilled water was poured into the developed colonies and the conidia were separated from the medium with a glass rod, and the concentration of the obtained suspension was adjusted with a hemocytometer to 2 x 10^3 conidia / ml. The bacterial suspension was made from 48 h cultures grown on an oblique MPA medium. After development of colony, sterile distilled water was poured into the tubes to make a suspension of 10^8 CFU / mL. As a negative control, were used roots in which sterile distilled water was poured.

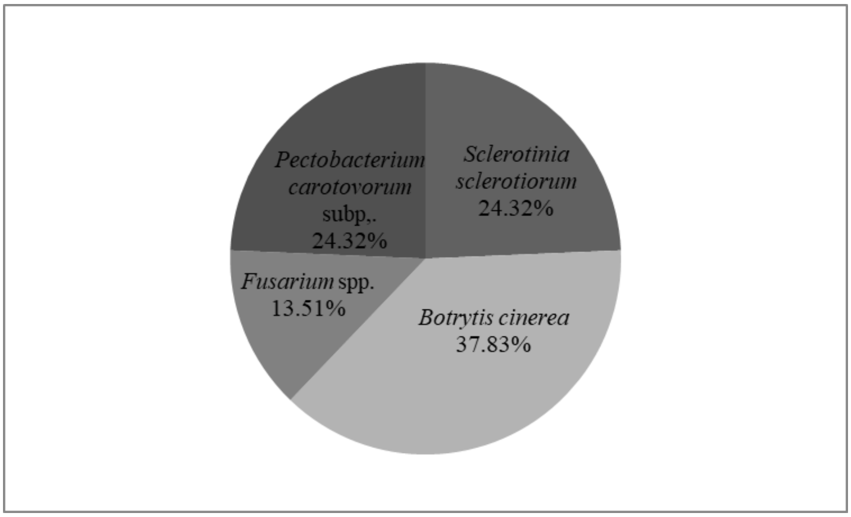
In order to provide high humidity conditions, the plants were covered with plastic bags after inoculation, which were removed after two days. The occurrence of symptoms was monitored daily up to 14 days after inoculation. The test was considered positive if the reproduced symptoms occurred under conditions of spontaneous infection of plants.

RESULTS AND DISCUSSION

Symptoms of root decay has been showed in all locality of research such as crops and warehouse. The symptoms has been manifested in the form of dry or wet rot with or without sporulation per tissue area. An unpleasant odor was often present in the warehouse. By cutting the roots vertically, the interior was often disorganized, some plants has showed red color. During the monitoring, 56 samples were collected, from which 42 isolates were obtained by isolation and pathogenicity was checked.

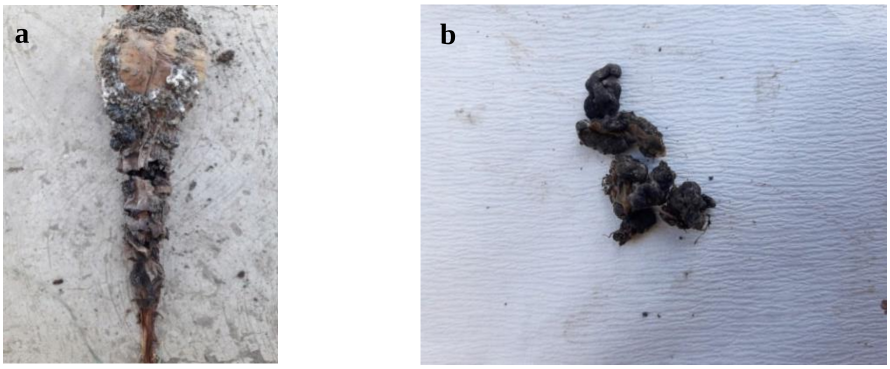
By examining the pathogenicity of 42 isolates, 37 reproduced symptoms on inoculated roots, which occurred under conditions of spontaneous plant infection. Of the number of all isolates that showed pathogenicity, 32 isolates were fungi and five isolates belonged to bacteria (Fig.1). Fungal isolates showed symptoms of decay after 5 days, while the intensive rot tissue observed after 8 days. Bacterial isolates caused symptoms of rot after only 24 hours, and after three days the symptoms were clearly visible. During the experiments symptoms of root rot has not been visible in control roots with sterile distilled water.

Fig. 1: Percentage of representation pathogens



On 9 samples, originating from the plots, an abundant appearance of white mycelium was noted, within which black sclerotia were formed, 5 - 10 mm in diameter. (Fig. 2). Diseased tissue quickly soft and rot covers the whole root. By isolating this type of symptoms, a multicellular well-developed mycelium was obtained. After the formation of the mycelium, the anastomosis of the hyphae results in sclerotia, which are initially soft consistency and later turn black. Based on these characteristics in accordance with Balaž et al. (2010) this pathogen was preliminarily identified as *Sclerotinia sclerotiorum.*

Fig. 2: a - Occurrence of sclerotia at the root of parsnip; b – sclerotia



Similar symptoms with 14 samples, but with the appearance of grayish mycelium, were separated into a special group. The multicellular mycelium with long conidiophores, which are upright and branched at the apex, was determined by isolation. Conidia were unicellular and branched. The fungus also formed small sclerotia, which indicates the species *Botrytis cinerea*. This is a worldwide pathogen that parasitizes many plant species (Ellis, 1971). And losses can reach up to 50% (CAB, 2004).

Samples with symptoms of dry rot and brown spots were analyzed as particular samples. Isolation obtained air mycelium, white to red in color. After sporulation on a PDA substrate with carnation leaves, the appearance of macroconidia was determined by microscopy. Macroconidia are septate with 2 - 6 transverse septa. Based on the determination keys (Leslie and Summerell, 2006), this fungus belongs to the genus *Fusarium* spp., More detailed determination must be performed to determine the species. In warehouses, parts of the examinated root sample were covered with blue or black mycelium, which preliminarily indicated that this fungi belongs to the genus *Rhisopus* spp. and *Penicillium* spp.

Wet-looking brown spots could be seen at the root. On the vertical section of the roots, a disorganization of the wet-looking tissue (wet rot) was noticed, which is accompanied by the appearance of an unpleasant odor. In some plants, which have completely decay, was observed the appereance of bacterial exudate. By isolating the bacterium on an MPA substrate, round, grayish-white, smooth colonies were obtained., while they formed depressions on the CVP substrate which indicate the pectinolytic activity.By determining the biochemical - physiological characteristics of 5 isolates, it was found that the bacterium grows at 36 ° C, gram negative, produces catalase, oxidase is negative, reduces nitrates, produces β - galacturonase and H_2S from sodium thiosulfate, rots on potato slices, which indicates it belongs to the group of *P. carotovorum* (Schaad et al., 2001; Balaž et al., 2010) (Tab. 1).

Tab. 1: Bacteriological properties of investigated strains originated from parsnip

Tast	**Strains**					
	P1b	**P2b**	**P3b**	**P4b**	**P5b**	**Ec[a]**
Grown at 36 °C	+[b]	+	+	+	+	+
Gram stain	-[c]	-	-	-	-	-
Catalase	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+
β - galakturonase	+	+	+	+	+	+
Hydrogen sulphur H_2S	+	+	+	+	+	+

[a] Ec check strain of *Erwinia carotovora* originting from cabbage; [b] positive result; [c] negative result

CONCLUSIONS

In recent years, parsnip root rot is increasingly emerging as a significant problem in production. Studing the etiology, 37 isolates were obtained, among which fungi were dominate: 14 isolates of *Botrytis cinerea*, 9 isolates of *Sclerotinia sclerotiorum* and *Fusarium* spp. The bacterium *P. carotovorum* was isolated from the symptoms of wet rot. In warehouses as saprophytes, *Rhizopus* spp. and *Penicillium* spp. were dominant.

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