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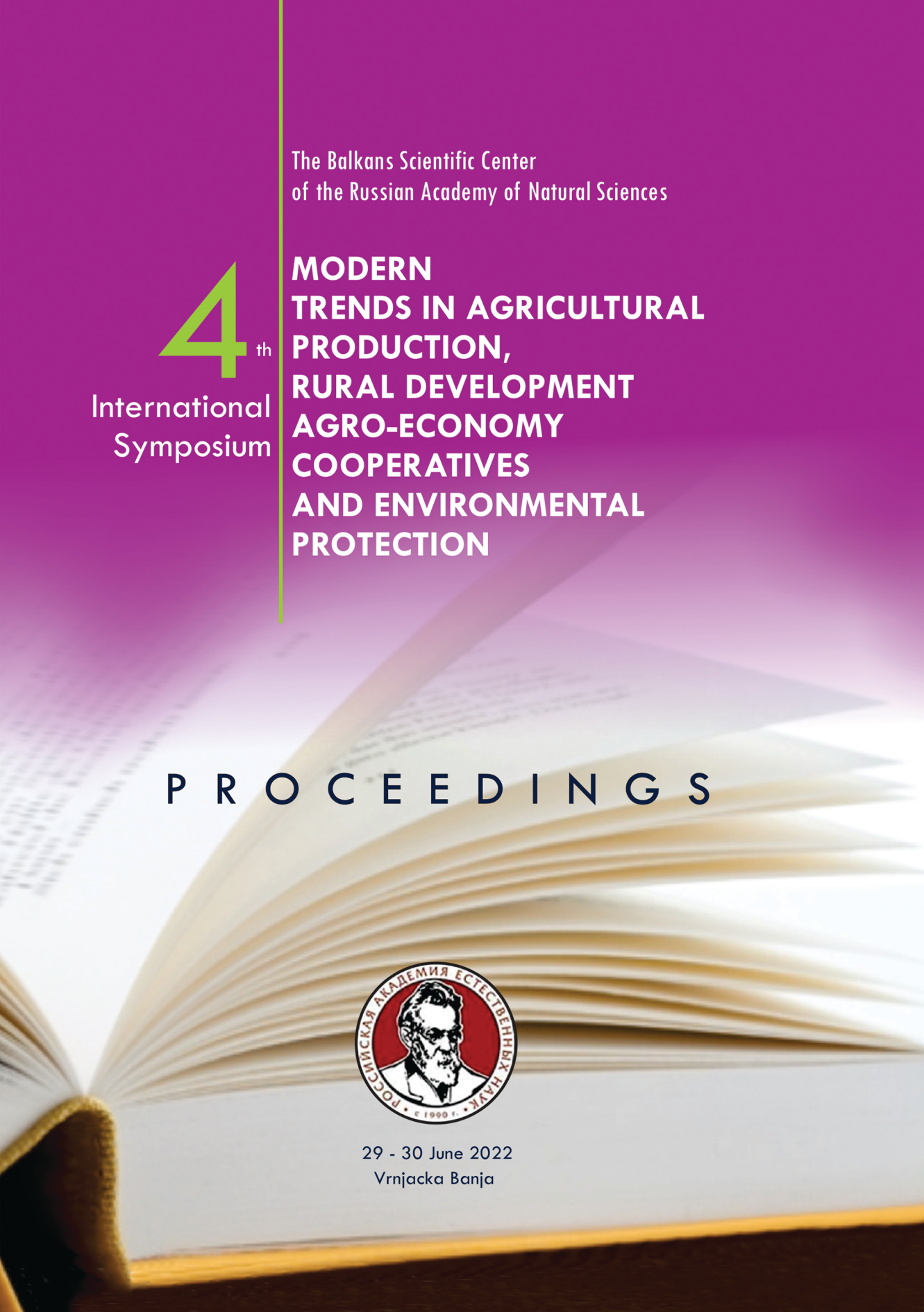
of the Russian Academy of Natural Sciences

4th International Symposium

MODERN TRENDS IN AGRICULTURAL PRODUCTION, RURAL DEVELOPMENT AGRO-ECONOMY COOPERATIVES AND ENVIRONMENTAL PROTECTION



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THE MYCOPOPULATION OF RADISH SEEDS

Slobodan Vlajić*, Jelica Gvozdanović - Varga, Vukašin Popović, Dragana Milosević, Gordana Tamindžić, Maja Ignjatov[1]

[1]Institut of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia

*Corresponding author: slobodan.vlajic@ifvcns.ns.ac.rs

Summary

Seeds are an important source of primary inoculum for the development of many diseases. Due to the very short vegetation period, controlling the causative agent of the disease on the radish is very difficult. Considering this fact, it is necessary to provide sufficient quantities of seeds of adequate quality and health. The aim of this study was to determine the mycopopulation of radish seeds during different years of production. Seeds used for testing were collected during the period between 2017-2021 from different varieties and localities. After conducting the study, the infection index ranged from 1 to 7%. Fungi of the following genus are represented on the seed in different intensities: Alternaria spp., Fusarium spp., Penicillium spp., Rhizopus spp. and the species Aspergillus niger.

***Keywords**: radish, seeds, fungi, mycopopulation*

Introduction

The radish (*Raphanus* spp.L.) is an annual vegetable from the Cruciferae family that has been used as food all over the world. Radishes are grown on all continents of the world. The first records of radish cultivation were inscriptions on the walls of the pyramids dating back to 2000 BC, but Herodotus (c. 484–424 BC) believed that the radish was grown as the main plant species in Egypt some 5,000 years ago (Becker, 1962) and was cultivated around the 13th century BC (Banga, 1976). In ancient times, it was assumed that the radish was grown as an oil plant (Curtis, 2008). This plant species was cultivated in Europe during the 15th or 16th century and was introduced to America during the 19th century. Many varieties and hybrids were developed in Asia, according to data it has been grown in China for 2450 years and in Japan for 1300 years (Kitamura 1958 loc. to Kaneko et al., 2007). In the diet, the thickened root is used as a fresh, fermented and dried product. Radishes can potentially be used to treat various diseases. It contains alkaloids and various compounds that act as calmodulin antagonists, growth inhibitors, antihypertensive agents and inhibitors of platelet aggregation (Shin et

al., 2015). Radishes are categorized into two groups based on root size: a group with a small root of short vegetation (one month) and a group with a large root of longer vegetation (three months) (Curtis, 2008).

The seed is a suitable substrate for the development of microorganisms that cause diseases, especially phytopathogenic fungi (Milošević et al., 2008). According to Petrović et al. (2010) due to the presence of pathogens, the seeds most often display a decrease in energy and germination, and even complete decay of the seedlings. The importance of pathogens and types of pathogens on radish seeds are cited by many authors. Noble and Richardson (1968) noted several fungal pathogens of edible radish seeds (*Raphanus sativus* L.). The aforementioned authors cite several species of *Alternaria* spp. and other fungi commonly found on the seeds of Cruciferous vegetable species. McLean (1947) and Noble and Richardson (1968) reported that seeds treated with 50°C warm water for 10 to 40 minutes successfully controlled *Alternaria raphani* from seeds.

The aim of the study was to determine the mycopopulation of radish seeds and the level of infection of seeds originating from different years, in order to determine the health status of the tested samples.

Material and methods

Seeds for analysis were collected in the phase of physiological maturity of the plant during the period 2017-2021. A total of 13 samples were collected from different localities (Tab. 1). Each sample consisted of 3 subsamples weighing 2 g. After the seeds were delivered to the laboratory, analysis was performed.

Tab.1: Seed sample data

Number	Code	Variety	Year of sampling	Locality
1.	R1/2017	Verica	2017	R. šančevi
2.	R2/2017	Saxa Treib	2017	Kucura
3.	R3/2017	Ledena sveća	2017	Kovilj
4.	R4/2018	Verica	2018	Kucura
5.	R5/2018	Saxa Treib	2018	Kucura
6.	R6/2018	Crna zimska	2018	Kulpin
7.	R7/2019	Saxa Treib	2019	Žabalj

8.	R8/2020	Ledena sveća	2020	Žabalj
9.	R9/2020	Saxa Treib	2020	R. šančevi
10.	10/2020	Verica	2020	Srbobran
11.	11/2020	Crna zimska	2020	Begeč
12.	12/2021	Verica	2021	Srbobran
13.	13/2021	Saxa Treib	2021	Kulpin

Seed incubation was performed on a PDA medium (potato-dextrose-agar) supplemented with 0.5 mg mL^{-1} streptomycin sulfate at a temperature of 25°C. In order to determine the presence of phytopathogenic fungi, a method developed by Vannacci and Gambogi (1980) and Punja et al. (2001) was used.

The seeds were surface disinfected in 2% sodium hypochlorite (NaClO) for 3 min, after which they were washed with sterilized distilled water. The experiment was set up in three replicates of 100 seeds placed in a Petri dish on PDA agar. The seeds were incubated at 25°C with 12h light and 12h darkness regime. The number of contaminated seeds was counted after 7 days according to the development of the colony. Contaminated seeds were detected after 5 and 8 days. The level of infection was estimated according to the following formula:

$$\text{Infection level (\%)} = \frac{\text{Total number of infected seed}}{\text{Total number of tested seed}} x\ 100$$

Identification of developed colonies: Incubated seeds were observed daily. The colonies that developed around the seeds were transferred to another Petri dish on a PDA substrate.

Determination of species of the genus *Fusarium* spp. was performed on the basis of macroscopic and microscopic properties on PDA and water-agar substrate (WA) according to the method of Nelson et al. (1983) and Burgess et al. (1994), while other genera were determined by the method of Champion (1997).

Results and Discussion

After the seed incubation period on PDA on the fifth day, the formation of colonies of fungi of different colors began around the seeds. The percentage of sporulation of fungi on radish seeds in 2017 ranged from 1 - 7%, in 2018 from 2 - 4%, in 2019, 5% in 2020 from 1 - 5% and in 2021, 2 - 3% (Tab. 2).

Based on the macroscopic characteristics of the colonies, isolates were formed in order to identify them according to the species level. After the development of colonies on the PDA substrate, the formation of a pale rose mycelium color was noted, indicating the species of the genus *Fusarium* spp., which was confirmed by determination.

The intensity of the infection with species of the mentioned genus is 1% in 2017 and 2% in 2020 (Table 2). Species of the genus *Fusarium* spp. are known to be pathogens of many plant species and conidia are widespread in the external environment. *Fusarium oxysporum* has been described in literature as a vascular pathogen of radishes originating from soil (Yu et al., 2019). However, very little information is available regarding the species of this genus that appears on radish seeds.

The genus *Alternaria* spp. was determined in the intensity of 1% in 2020 to 5% in 2019 (Tab. 2). Based on one year of research, Holtzhausen et al. (1978) reported the presence of *Alternaria brassicae, A. brassicicola, A. cheiranthi, A. raphani* and *Phoma lingam* on 18 samples of commercial Japanese radish seeds. Species of the genus *Alternaria* spp. are a group of destructive pathogens on fam. Cruciferae worldwide. They lead to reduced yield quantity and quality (Verma and Saharan, 1994) causing reduced photosynthetic potential, accelerated aging, premature pod bursting, and shriveled seeds (Shresta et al., 2000).

Fungi of the genus *Penicillum* spp. were determined in the intensity of 2% during 2017 in the variety Ledena sveća. Genus *Rhizopus* spp. was determined in an intensity from 2% in 2018 and 2021 to 7% in 2017. The appearance of black scattered mycelium indicated *Aspergilus niger*, which was confirmed by identification based on the morphology of the spores. The mentioned fungus was recorded during 2020 on the Saxa Treib radish with an infection intensity of 3% (Tab. 2).

Tab. 2: Level of infection in relation to varieties and years

Variety	**Code**	***Alternaria* spp.**	***Fusarium* spp.**	***Penicillium* spp.**	***Rhizopus* spp.**	***Aspergillus* spp.**
Verica	R1/2017	0	0	0	**7**	0
Saxa Treib	R2/2017	**2**	0	0	0	0

Ledena sveća	R3/2017	0	**1**	**2**	0	0
Verica	R4/2018	0	0	0	0	0
Saxa Treib	R5/2018	**3**	0	0	**4**	0
Crna zimska	R6/2018	**3**	0	0	**2**	0
Saxa Treib	R7/2019	**5**	0	0	0	0
Ledena sveća	R8/2020	0	0	0	0	0
Saxa Treib	R9/2020	**2**	**2**	0	**3**	**3**
Verica	R10/2020	**1**	0	0	**4**	0
Crna zimska	R11/2020	**2**	0	0	**5**	0
Verica	R12/2021	0	0	0	**3**	0
Saxa Treib	R13/2021	**3**	0	0	**2**	0

Conclusion

The analysis of 13 samples of radish seeds revealed the presence of fungi from the genus *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp. and *Aspergillus niger* in different percentages of infection. It is necessary to continue monitoring the mycopopulation of radish seeds, especially because of the established genera *Alternaria* spp. and *Fusarium* spp. due to the fact that certain species of this genus are significant pathogens in agricultural production.

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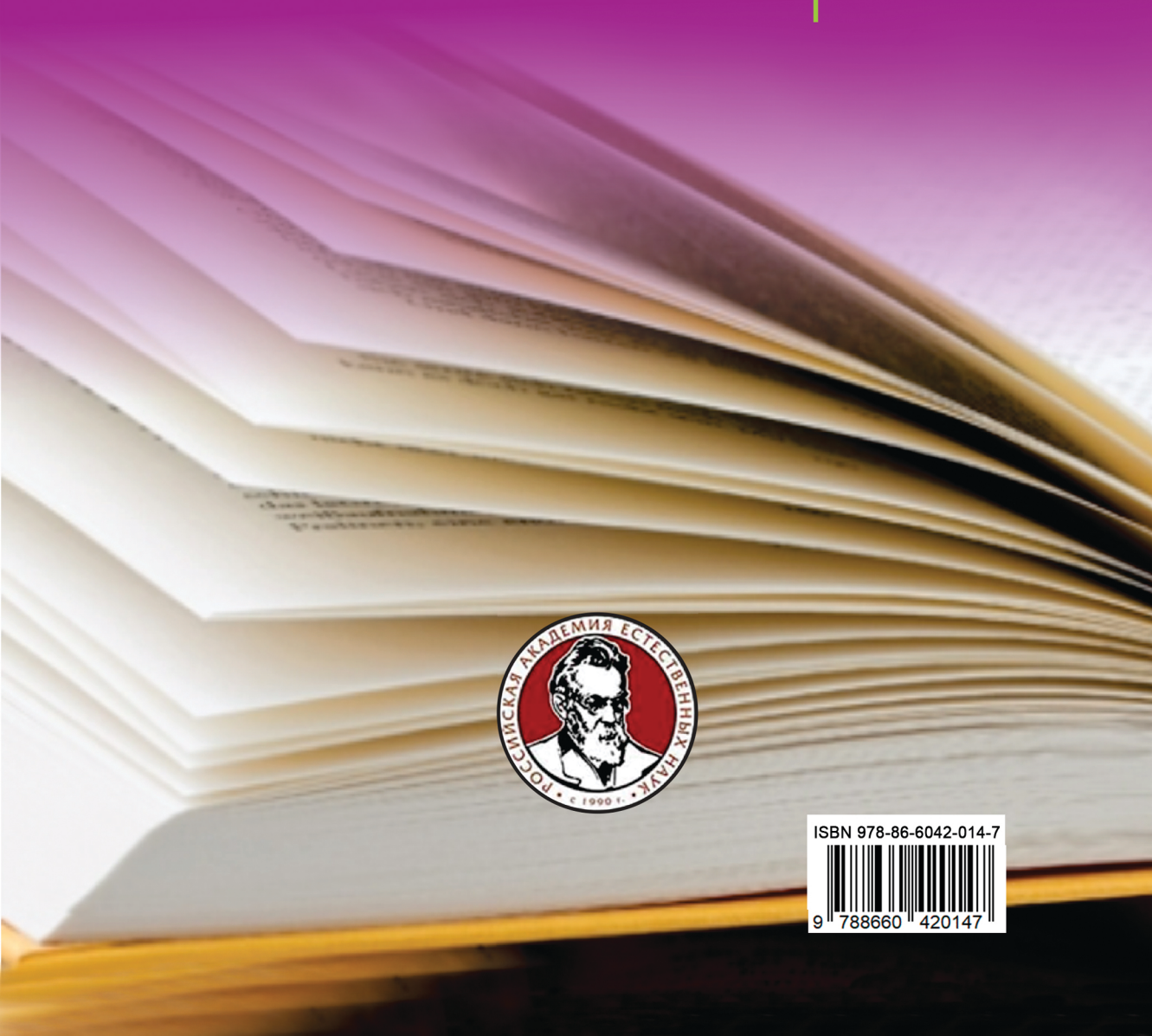

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