Occurrence and Prevalence of Pepper Pathogens in the Vojvodina

A paprika kórokozóinak előfordulása és prevalenciája a Vajdaságban

Ferenc Bagi[1*], Slobodan Vlajić[2], Tatjana Popović Milovanović[3], Nemanja Pavković[1], Đina Konstantin[1] and Renata Iličić[1]*

[1]*University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia; ferenc.bagi@polj.edu.rs; nemanja.pavkovic@polj.edu.rs; djina.konstantin@polj.edu.rs; renatailicic@gmail.com;*

[2]*Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Maksima Gorkog 30, 21000 Novi Sad, Serbia; slobodan.vlajic@ifvcns.ns.ac.rs;*

[3]*Institute for Plant Protection and Environment, Teodora Drajzera 9, 11040 Belgrade, Serbia; tanjaizbis@gmail.com*

**Correspondence: ferenc.bagi@polj.edu.rs;renatailicic@gmail.com*

Abstract*:* One of the key challenges in pepper production in the Vojvodina Province is the presence of pathogens that cause serious diseases and epidemics, leading to reduced and compromised yields. This study aimed to identify fungal and bacterial pathogens under intensive production conditions. During a two-year period, 2023–2024, a total of 35 pepper samples showing symptoms of diseases, were collected from several locations and examined using classical and molecular methods. The results showed that *Alternara solani* was the most common fungal pathogen, followed by *Colletotrichum* spp., *Botrytis cinerea, Macrophomina phaseolina*, and *Fusarium* spp. (*subglutinans* and *oxysporum*). Among the bacterial pathogens, *Xanthomonas euvesicatoria* was found in all seven examined pepper fields. Additionally, *Pectobacterium* spp. and '*Ca*. Phytoplasma solani' were also detected. These findings provide practical insights that can support the development of effective control measures for pathogens affecting pepper production.

Keywords: *pepper, diseases, detection, identification*

Összefoglalás*:* A vajdasági paprika-termelés egyik legfontosabb kihívása a kórokozók jelenléte, amelyek súlyos betegségeket és járványokat okoznak, ezáltal csökkentve és veszélyeztetve a terméshozamot. A jelen tanulmány célja a gombás és bakteriális kórokozók azonosítása intenzív termelési körülmények között. A 2023–2024-es két év során összesen 35, betegség tüneteket mutató paprika mintát gyűjtöttünk több helyszínről, majd klasszikus és molekuláris módszerekkel vizsgáltunk. Az eredmények azt mutatták, hogy az *Alternaria solani* volt a leggyakoribb gombakórokozó, ezt követték a *Colletotrichum* spp., a *Botrytis cinerea*, a *Macrophomina phaseolina* és a *Fusarium* spp. (*subglutinans* és *oxysporum*). A bakteriális kórokozók közül a *Xanthomonas euvesicatoria* mind a hét vizsgált paprikatáblában jelen volt. Emellett *Pectobacterium* spp. és a '*Ca.* Phytoplasma solani'-t is kimutattuk. Ezek az eredmények gyakorlati betekintést nyújtanak, és hozzájárulhatnak a paprikatermelést érintő kórokozók elleni hatékony védekezési módszerek kidolgozásához.

Kulcsszavak: *paprika, betegségek, kimutatás, azonosítás*

1 Introduction

Pepper (*Capsicum annuum* L.) is widely cultivated worldwide and includes many diverse varieties (Tripodi et al. 2021**;** Živković et al., 2023). In Serbia, most pepper production takes place in the southern part of the country, which accounts for an average of 107,408 tons, or 76.58% of total output. Within this region, the Šumadija and West Serbia region produces about 55,789 tons (39.78%), while the South and East Serbia region contributes around 51,619 tons (36.80%). Northern Serbia produces the remaining 32,842 tons (23.42%), with the Belgrade region averaging 3,664 tons (2.61%) and Vojvodina 29,178 tons (20.81%). Among all regions, Vojvodina achieves the highest average yield at 13.9 tons per hectare (Kljajić et al., 2025).

One of the major challenges affecting pepper production in the Balkan countries is the widespread presence of pathogens that cause severe diseases and epidemics, leading to significant yield losses. Among the most destructive and widely distributed pepper pathogens are *Phytophthora capsici*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, *Fusarium* spp., *Phomopsis capsici*, *Colletotrichum* spp., *Pseudomonas syringae* pv. *syringae*, *Xanthomonas* spp. (*X. euvesicatoria, vesicatoria, performans, gardneri*), *Pectobacterium* spp., *Pepper mild mottle virus* (PMMV), *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Alfalfa mosaic virus* (AMV), and *Potato virus Y* (PVY) (Rodeva et al., 2012; Vučurović et al., 2015; Milošević et al., 2018; Babić et al., 2023; Živković et al., 2023; Popović Milovanović et al., 2025). In Serbia, two phytoplasma diseases, stolbur (‘*Candidatus* Phytoplasma solani’) and aster yellows (‘*Candidatus* Phytoplasma asteris’) have been reported in pepper crops (Duduk and Duduk, 2013).

Disease development in pepper is strongly influenced by weather conditions, including average maximum and minimum temperatures, relative humidity, and rainfall. When control measures are insufficient and conditions favor leaf, fruit–diseases development, infections can intensify and lead to premature defoliation. One of the key strategies for managing and preventing plant pathogens is seed testing and certification, along with producing seed in areas free of pathogens or where conditions limit their development (Živković et al., 2023). Proper detection and identification also play an important role in controlling pathogens in pepper production. The object of this study was to update current knowledge on the most economically significant and emerging *C. annuum* pathogens in the Vojvodina region. Comprehensive knowledge of these pathogens is crucial for the effective disease management in sustainable agriculture production.

2. Materials and Methods

Symptom observation and sample collection. Between 2023 and 2024, the health status of pepper crops was monitored at several locations in the Vojvodina province (Despotovo, Kać, Kovilj, Rimski šančevi, Pivnice, Srbobran, Bački Petrovac). Diseased pepper plants (type babura and kapija) – including 35 samples of leaves, stems, roots and fruits showing various symptoms–were collected during the spring and summer months. The disease incidence was calculated by using the following formula: Disease incidence (%) = Number of infected plants / Total number of plants assessed x 100, collected for all localities and expressed as the average values.

Isolation and pathogen identification. Diseased samples were first rinsed under tap water, dried on sterile filter paper, and surface disinfected with 70% alcohol before being dried again. Samples that showed presence of fungal or bacterial disease were then subjected to isolation on appropriate media: Potato Dextrose Agar (PDA) for fungi and Nutrient Sucrose Agar (NSA) and Yeast Dextrose CaCO3 Agar (YDC) for bacteria. Small fragments taken from the boundary between healthy and diseased tissue were placed on PDA for fungal isolation. For bacterial isolation, tissue fragments were macerated in sterile distilled water (SDW), left for 20 minutes, and then plated on NSA and YDC medium. Petri dishes were incubated at 25 ± 1 °C for 3 to 14 days. Total DNA was extracted from samples or pure cultures grown on NSA, YDC or PDA medium, using a genomic DNA isolation kit (DNeasy Plant Mini Kit, Qiagen, Germany) according to the manufacturer's instructions. The obtained DNA was stored at −20 ºC until use. To identify the pathogens involved in pepper diseases, different methods including classical and molecular tools for fungi, bacteria (including phytoplasma) identification were used (White et al., 1990; Grifoni et al., 1995; Schaad et al., 2001; Agrios, 2005; Koenraadt et al., 2009; EPPO, 2016).

Table 1 Methods used for the identification of pathogens associated with pepper diseases

Pathogen	Method of identification	Reference
Alternaria	Colony and conidia morphology; PCR confirmation	*White et al. (1990); Agrios (2005)*
Colletotrichum	Colony, conidia and acervuli morphology	*Stojšin et al. (2008)*
Fusarium	Colony and conidia morphology; PCR confirmation	*White et al. (1990); Agrios (2005)*
Botrytis	Colony and conidia morphology; PCR confirmation	*White et al. (1990); Agrios (2005)*
Macrophomina	Colony morphology; microsclerotia	*Stojšin et al. (2008)*
Xanthomonas	Colony morphology; PCR confirmation	*Schaad et al. (2001); Koenraadt et al. (2009)*
Pectobacterium	Colony morphology; PCR confirmation	*Grifoni et al. (1995); Schaad et al. (2001);*
Ca. Phytoplasma	Real-Time PCR confirmation	*EPPO (2016)*

3 Results and Discussion

This study describes the main fungi, bacteria and phytoplasma affecting pepper production in Vojvodina, with the aim of identifying which pathogens are most frequent and potentially harmful under local agro–ecological conditions. Using several pathogen–identification methods (Table 1), our analysis indicates that the pathogen structure in pepper is highly complex. The pathogens listed below appear each year, though their intensity varies with agro–ecological conditions and the cultivated pepper assortment. The following section summarizes the findings from the period 2023–2024.

Diseases caused by fungi. Among the fungal pathogens found in diseased pepper samples, *Alternaria solani* was the most common, causing both leaf spots and fruit rot (Figure 1a). This pathogen can infect pepper plants at almost any stage of growth and spreads gradually. It enters through leaves, roots, or wounds on plant tissues (Attia et al., 2025), allowing it to cause damage throughout the entire season. In addition, several *Colletotrichum* species and *Botrytis cinerea* were identified as major causes of pepper fruit rot in the field (Figure 1b, 1c). Their ability to spread and overwinter has contributed to notable reductions in pepper yield. Isolates of *Fusarium* (including *F. subglutinans* and *F. oxysporum*) and *Macrophomina phaseolina* were isolated from the roots of infected pepper plants, after the flowering stage. *F. subglutinans* was also found in both internal and external fruit rot. In some cases, these pathogens appeared together as mixed infections. All identified pathogens are generally polyphagous, infecting a wide range of plant species worldwide (Van Poucke et al., 2012; Anila Younas et al., 2016; Frans et al., 2017; Cerkauskas, 2017; Engalycheva et al., 2024), including pepper, which adds to the challenges of effective disease management. Fusarium wilt typically begins with slight vein clearing on young leaves, followed by drooping of older leaves. Affected plants become stunted, lower leaves turn yellow, wilting and defoliation occur, and eventually the whole plant dies. Average disease incidence of pepper pathogens across all surveyed locations is presented in Figure 2.

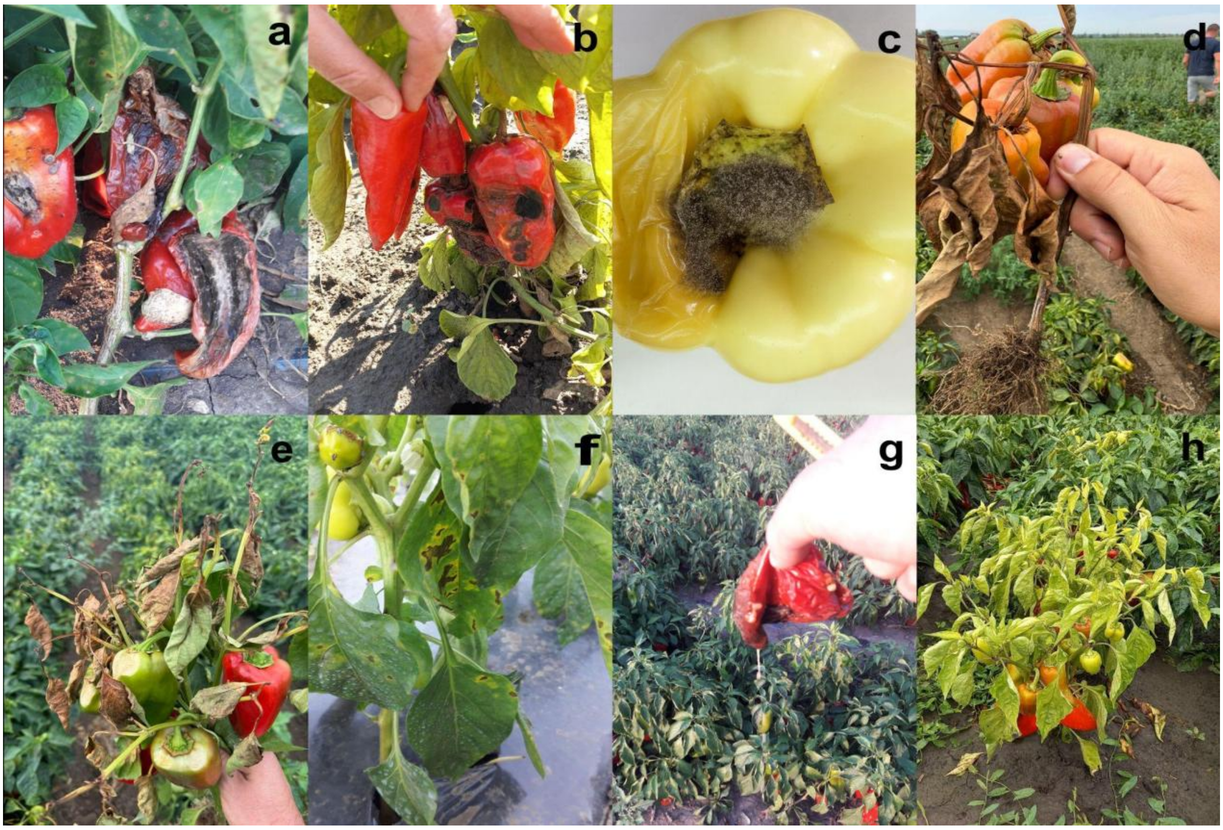

***Figure 1** Symptoms on pepper plants: a) A. solani; b) Colletotrichum spp.; c) B. cinerea; d) M. phaseolina; e) Fusarium spp.; f) X. euvesicatoria; g) Pectobacterium spp.; h) 'Ca. P. solani'.*

Diseases caused by bacteria. The predominant bacterial pathogen affecting pepper production was *Xanthomonas euvesicatoria*, causing bacterial spots on both leaves and fruits (Figure 1f). This pathogen was detected at all surveyed locations, during the 2-4 leaf stage of development, with disease incidence varying mainly according to agroecological conditions and pepper cultivars. The results obtained were expected given that *X. euvesicatoria* is one of the most common pepper diseases in Serbia (Vlajić et al., 2017; Popović Milovanović et al., 2025). Bacterial spot occurs widely in both open-field and greenhouse pepper production, causing extensive necrotic lesions on leaves, stems, and fruits, along with defoliation and notable reductions in yield and fruit quality. Under favorable conditions, *X. euvesicatoria* often becomes the dominant species, driving epidemic development and increasing disease severity in susceptible pepper cultivars (Živković et al., 2023). Seedborne transmission further supports its spread, especially in production systems that rely on undeclared or non-certified seed. Furthermore, based on the occurrence of bacterial diseases, presence of *Pectobacterium* spp. was determined as the causative agent of fruit soft rot in the pepper samples. *Pectobacterium* species cause soft rot in plant tissues and were detected in ripe pepper fruits, leading to pronounced parenchyma softening. Typical symptoms include water-soaked lesions and a characteristic foul odor resulting from cell wall degradation (Van Gijsegem et al., 2021; Ma et al., 2007; Popović Milovanović et al., 2023). Under high humidity and in the presence of mechanical injuries, these bacteria can trigger significant losses, especially during storage and postharvest handling. In our survey, the pathogen was observed mainly on red-ripening pepper fruits harvested in the autumn.

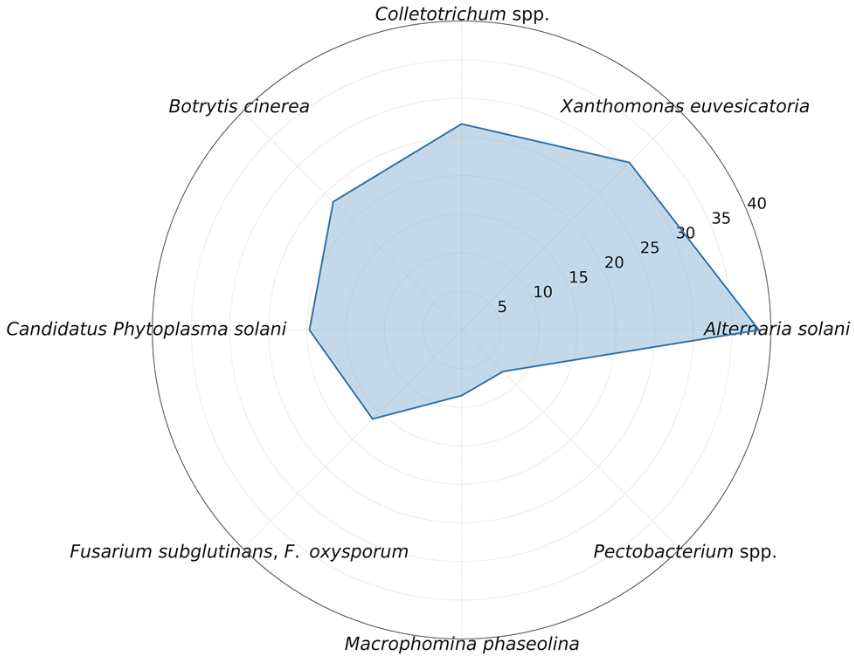

Figure 2 *Average disease incidence of pepper pathogens across surveyed locations in the Vojvodina Province.*

Additionally, based on the results, the presence of '*Candidatus* Phytoplasma solani' (Stolbur phytoplasma) was confirmed in the pepper samples after flowering stage resembling the symptoms of yellowing, chlorosis and stunting of the leaves with sporadic necrosis (Figure 1h). With the development of the disease regardless of soil moisture and intensity of insolation diseased plants continue rapidly to lose turgor. In recent years, phytoplasmas have increasingly affected not only pepper production but other vegetables as well in Serbia (Mitrović et al., 2013, 2016; Popović et al., 2021; Iličić et al., 2022). As the use of insecticides for controling leafhoppers in vegetables is very limited and generally ineffective, protection should focused on removing the dual host-plants, simultaneously hosting '*Ca*. P. solani' and vector, is a precondition for the reduction of the disease incidence (Popović et al., 2021). Although no detection of virus diseases in pepper was carried out during this research, they are common under field conditions and of considerable economic importance. Nevertheless, their presence in the surveyed production areas cannot be ruled out, as certain symptoms may visually resemble those of abiotic disorders or infections caused by other pathogens.

Managing diseases in pepper production is challenging because the detected pathogens are highly variable, survive well in complex environments, persist in plants as latent infections, and remain in the soil for extended periods. Although chemical pesticides have traditionally been used, their effectiveness often declines over time, they can be costly, and they may pose risks to human health and the environment. These limitations highlight the need for continuous monitoring of pathogen presence in pepper fields, as well as the integration of preventive measures such as the use of healthy planting material, crop rotation and sanitation, and growing resistant cultivars. Strengthening monitoring and implementing integrated disease management strategies is therefore essential for reducing disease pressure and maintaining sustainable pepper production.

5 Conclusions

In conclusion, our results showed that the pathogens affecting pepper production present complex, involving various fungi, bacteria, phytoplasmas, and visually observed—but not laboratory-confirmed—viruses that threaten both production and yield. As many of these pathogens are seed-borne, the findings highlight the importance of using pathogen-free seeds as a primary control measure, supported by precise pathogen detection and effective control management.

Acknowledgements

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia [contract numbers 451-03-136/2025-03/200117, 451-03-136/2025-03; 451-03-136/2025-03/200010].

References

Agrios, G. N. 2005. *Plant pathology.* Elsevier.

Anila Younas, A. Y., Khajista Jabeen, K. J., Sumera Iqbal, S. I. and Sumera Javed, S. J. 2016. Effect of Macrophomina phaseolina on germination, growth and physiology of Capsicum frutescens L. *Pakistan Journal of Phytopathology.* **28** (2) 207–211.

Attia, M. S., Moustafa, M. H., Hashem, A. H., Elsayed, S. M., Aloufi, A. S., Abdelaleem, I. M. I., Alshahed, K. A., Ismail, A. S., Ibrahim, A. M., Abdel-Maksoud, M. A., Alamri, A., Ebaid, H., Kiani, B. H., Bakkali, H. E. and Abdelaziz, A. M. 2025. Bioprotective potential of biosynthesized copper oxide nanoparticles and copper phosphite against Alternaria-solani-induced leaf spot in pepper plants. *Antonie van Leeuwenhoek.* **118** (10) 1–16. https://doi.org/10.1007/s10482-025-02143-3

Babić, G., Trkulja, V., Budakov, D., Iličić, R., Mališević, R. and Bagi, F. 2023. Ekonomski značajni virusi paprike utvrđeni na području Republike Srpske. *Biljni Lekar.* **51** (3) 488–502. https://doi.org/10.5937/BiljLek2303488B

Cerkauskas, R. F. 2017. Etiology and management of Fusarium crown and root rot (Fusarium oxysporum) on greenhouse pepper in Ontario, Canada. *Canadian journal of plant pathology.* **39** (2) 121–132. https://doi.org/10.1080/07060661.2017.1321044

Duduk, N. and Duduk, B. 2013. Some important viruses and phytoplasmas of pepper in Serbia. *Plant Doctor.* **41** (3) 328–340.

Engalycheva, I., Kozar, E., Frolova, S., Vetrova, S., Tikhonova, T., Dzhos, E., Engalychev, M., Chizhik, V., Martynov, V., Shingaliev, A., Dudnikova, K., Dudnikov, M. and Kostanchuk, Y. 2024. Fusarium species causing pepper wilt in Russia: molecular identification and pathogenicity. *Microorganisms.* **12** (2) 343. https://doi.org/10.3390/microorganisms12020343

EPPO. 2016. PM 7/079 (2) Grapevine flavescence dorée phytoplasma. *EPPO Bulletin.* **46** (1) 78–93. https://doi.org/10.1111/epp.12280

Frans, M., Aerts, R., Van Laethem, S. and Ceusters, J. 2017. Environmental effects on growth and sporulation of Fusarium spp. causing internal fruit rot in bell pepper. *European Journal of Plant Pathology.* **149** (4) 875–883. https://doi.org/10.1007/s10658-017-1235-4

Grifoni, A., Bazzicalupo, M., Di Serio, C., Fancelli, S. and Fani, R. 1995. Identification of Azospirillum strains by restriction fragment length polymorphism of the 16S rDNA and the histidine operon. *FEMS Microbiological Letters.* **127** (1-2) 85–91.

Iličić, R., Barać, G., Marković, S., Jelušić, A., Stanković, S. and Popović, T. 2022. New data on presence of *Candidatus* Phytoplasma solani on potato in Vojvodina Province. FEMS Conference on Microbiology, June 30 - July 2, Belgrade, Serbia, Abstract Book, 916.

Kljajić, N., Vuković, P. and Nastić, L. 2025. Production and economic analysis of pepper in the Republic of Serbia. *International Review.* **1-2** 203–213. https://doi.org/10.5937/intrev2501203K

Koenraadt, H., van Betteray, B., Germain, R., Hiddink, G., Jones, J. B., Oosterhof, J., Rijlaarsdam, A., Roorda, P. and Woudt, B. 2009. Development of specific primers for the molecular detection of bacterial spot of pepper and tomato. *Acta Horticulturae.* **808** 99–102. https://doi.org/10.17660/ActaHortic.2009.808.13

Ma, B., Hibbing, M. E., Kim, H. S., Reedy, R. M., Yedidia, I., Breuer, J., Breuer, J., Glasner, J. D., Perna, N. T., Kelman, A. and Charkowski, A. O. 2007. Host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya*. *Phytopathology.* **97** 1150–1163. https://doi.org/10.1094/PHYTO-97-9-1150

Milošević, D., Ignjatov, M., Stanković, I., Nikolić, Z., Gvozdanović-Varga, J. and Krstić, B. 2018. Occurrence and diversity of viruses infecting pepper in Serbia. *Acta Agriculturae Serbica.* **23** (46) 141–155. https://doi.org/10.5937/AASer1846141M

Mitrović, J., Pavlović, S. and Duduk, B. 2013. Survey and multigene characterization of stolbur phytoplasmas on various plant species in Serbia. *Phytopathologia Mediterranea.* **52** (3) 434–441. https://doi.org/10.14601/Phytopathol_Mediterr-11681

Mitrović, M., Jakovljević, M., Jović, J., Krstić, O., Kosovac, A., Trivellone, V., Jermini, M., Toševski, I. and Cvrković, T. 2016. 'Candidatus Phytoplasma solani' genotypes associated with potato stolbur in Serbia and the role of Hyalesthes obsoletus and Reptalus panzeri (hemiptera, cixiidae) as natural vectors. *European Journal of Plant Pathology.* **144** (3) 619–630. https://doi.org/10.1007/s10658-015-0800-y

Popović Milovanović, T., Iličić, R. Jelušić, A., Mitrović, P., Trkulja, V., Milovanović, P. and Zečević, K. 2023. Proučavanje prouzrokovača vlažne truleži paprike i mrkve u Vojvodini. XVII Simpozijum o zaštiti bilja, Zlatibor, 27-30. novembar, pp. 48. (in Serbian).

Popović Milovanović, T., Jelušić, A., Iličić, R., Trkulja, N., Damnjanović, J., Adžić, S. and Živković, I. 2025. Biocontrol potential of indigenous pepper seed *Bacillus* strains against *Xanthomonas euvesicatoria*. *Pest Management Science*. **81** (8) 4875–4885. https://doi.org/10.1002/ps.8843

Popović T., Mitrović P. and Kosovac A. 2021. Molecular characterization of '*Candidatus* Phytoplasma solani' in Celery: Case Study in Futog. *Ratarstvo i Povrtarstvo.* 58 (2) 66–71. https://doi.org/10.5937/ratpov58-33227

Rodeva, R., Kostova, D., Chavdarov, P., Mijatovic, M., Merkuri, J., Cara, M., Pasev, G., Stoyanova, Z., Karov, I., Mitrev, S., Kovacevik, B., Goudoudaki, S. and Manoussopoulos, I. 2012. Pepper Diseases in Balkan Region. *Acta Horticulture.* **960** 365–370. https://doi.org/10.17660/ActaHortic.2012.960.53

Schaad, N.W., Jones, J.B. and Chun, W. 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3rd Edition, *American Phytopathological Society Press*, St. Paul.

Stojšin, V., Bagi, F., Balaž, F. 2008. *Praktikum iz Fitopatologije, Mikoze i pseudomikoze ratarskih i povrtarskih biljaka.* Faculty of Agriculture, Novi Sad (in Serbian).

Tripodi, P., Rabanus-Wallace, M. T., Barchi, L., Kale, S., Esposito, S., Acquadro, A., Schafleitner, R., van Zonneveld, M., Prohens, J. and Bombarely, A. 2021. Genomics and breeding of *Capsicum* species: Progress and prospects. *Horticulture Research.* **8** 1–17. https://doi.org/10.1038/s41438-021-00565-5

Van Gijsegem, F., van der Wolf, J. M. and Toth, I. K. (Eds.). 2021. Plant Diseases Caused by *Dickeya* and *Pectobacterium* Species. *Springer.* https://doi.org/10.1007/978-3-030-61459-1

Van Poucke, K., Monbaliu, S., Munaut, F., Heungens, K., De Saeger, S. and Van Hove, F. 2012. Genetic diversity and mycotoxin production of Fusarium lactis species complex isolates from sweet pepper. *International Journal of Food Microbiology.* **153** 28–37. https://doi.org/10.1016/j.ijfoodmicro.2011.10.011

Vlajić, S., Iličić, R., Maširević, S., Feldeždi, M. and Jošič, D. 2017. Appearance of Xanthomonas euvesicatoria on pepper in Vojvodina during 2016. In Proceedings of the 69th International Symposium on Crop Protection (p. 159). Ghent, Belgium: University of Ghent, Faculty of Bioscience Engineering.

Vučurović, A., Stanković, I., Vučurović, I., Bulajić, A. and Krstić, B. 2015. Presence and distribution of pepper viruses in Serbia. *Pesticidi i fitomedicina.* 30 (1) 33–43. https://doi.org/10.2298/PIF1501033V

White, T. J., Bruns, T., Lee, S. J. W. T. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications.* **18** (1) 315–322.

Živković, I., Iličić, R., Barać, G., Damnjanović, J., Cvikić, D., Trkulja, N. and Milovanović, T. P. 2023. Influence of Xanthomonas euvesicatoria on quality parameters of pepper seed from Serbia. *Pesticides and Phytomedicine/Pesticidi i fitomedicina.* **38** (1) 1–9. https://doi.org/10.2298/PIF2301001Z