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Review paper
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INVASION PATHWAYS ALONG THE RIVERS IN SERBIA – THE EASTERN CORRIDOR OF *REYNOUTRIA* SPP.

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SUMMARY

Increasing levels of invasion worldwide have been the source of concern for scientists, due to significant costs and efforts required for managing them. The knowledge of invasion pathways, both those of initial introduction and subsequent spread, is of key importance, since further increase in the number of pathways and vectors of invasion is predicted for the 21st century. At regional scales habitat type has proven to be a reliable predictor of the level of invasion, as certain habitat types (i.e. frequently disturbed, under strong anthropogenic influence) is characterized by high invasion levels. Riparian habitats, as hotspots of alien species diversity and primary sources of their spread, represent some of the most important invasion corridors, where water acts as an effective dispersal mechanism. Some invasive plant species, like *Reynoutria* spp. show a strong tendency to invade riparian habitats. Preliminary findings of field surveys aimed to assess the level of riparian invasion by *Reynoutria* spp. in Serbia suggest that some river basins are significantly affected by the presence of these invasive species. Bearing in mind the principal means of their propagation, further spread of *Reynoutria* spp. along the rivers in Serbia is to be expected over the following years.

Key words: Invasion, invasion pathways, rivers, riparian habitats, *Reynoutria* spp.

INTRODUCTION

Concepts and definitions of invasion

Over the course of the last two centuries the level of anthropogenic introduction of alien species has increased, raising significant concerns worldwide (Pienimäki and Leppäkoski, 2004). Invasions as phenomena are often seen as a cause of great distress for agriculturists, conservation biologists and natural resource managers (Brown and Sax, 2004), as significant amounts of money and effort are needed to manage them (Vilà et al., 2010). Therefore, the issue of invasive species compiles biological, as well as social and ethical problems (Larson, 2007). When potential implications of biological invasions are observed, the importance of providing clear and objective definitions and models for managers and people in charge of native biodiversity protection becomes paramount (Colautti and MacIsaac, 2004).

The criteria defining the concept of invasive species are very disparate in available scientific literature, as many terms relevant to the field of invasion ecology (e.g. invasive, weed, noxious) represent qualities which are subjective and open to interpretation (see Richardson et al., 2000 and Colautti and MacIsaac, 2004 for examples). Due to subjectivity some species may be considered invasive in areas where they exhibit minimum impact, simply because they have been defined as such elsewhere.

The different terms used in invasion ecology are a result of human perception solely, rather than some real inherent ecological characteristics, thus confounding and complicating research of processes and patterns of invasion. Another problem with definitions is that many terms commonly used in invasion literature (e.g. adventive, alien, exotic) are used interchangeably, in defining the same concept, or inconsistently, in describing dissimilar phenomena. The variability of definitions has the potential to cloud theoretical issues (Colautti and MacIsaac, 2004) and impede further spread of scientific ideas and research efficiency (Colautti and Richardson, 2009). As a result of this, many authors demand greater objectivity in invasion biology (Brown and Sax, 2004, 2005; Colautti and MacIsaac, 2004; Colautti and Richardson, 2009).

The greatest level of ambiguity surrounds the term 'invasive', with a vast array of definitions existing in the literature (Richardson et al., 2000; Colautti and MacIsaac, 2004; Hulme et al., 2013). While Richardson et al. (2000) promote the biogeographical approach in defining terms like 'invasive', 'naturalized' and 'established' to reduce the existing confusion, Colautti and MacIsaac (2004) postulate that any proposal for a unified set of definitions is unlikely to succeed, unless the authors are willing to "forego their individual preferences", thus concluding that a successful invasion framework needs to be process-based and incorporate operational terms without any *a priori* meaning.

Richardson et al. (2000) in their paper propose a comprehensive model which describes invasion as a process in which nonindigenous species (NIS) pass through a series of invasion barriers. It is a model designed specifically for plant invasions. Building upon their model Colautti and MacIsaac (2004) have developed a framework, focusing on the stages of invasion, which further highlights the fact that invasions are in fact biogeographical, and not taxonomical, phenomena (Colautti and MacIsaac, 2004), which overlaps with Richardson et al. (2000) approach.

The problem with the overlapping and often interchangeable use of the terms 'invasive' and 'weed' has been a subject of many debates, and a number of authors have tried to make a clear distinction between these two terms (Ghersa, 2007). Rejmánek (2000) has made a distinction between weeds and invasive plants, by viewing weeds from an anthropomorphic perspective where weeds are "plants growing where they are not desired", and invasive plants from a biogeographical standpoint as "plants that have become locally established and spread to areas where they are not native". Ghersa (2009) points out that the main problem is that the term 'weed' has often been used with an anthropogenic connotation, providing very little insight into their biology, distribution and management practices.

In order to avoid ambiguity in scientific papers dealing with the term 'invasive species', Richardson et al. (2000) recommend the use of this term when describing "naturalized plants that produce reproducitve offspring, often in very large numbers, at considerable distances from parent plants, and thus have the potential to spread over a considerable area", and define weeds as "plants (not necessarily alien) that grow in sites where they are not wanted and which usually have detectable economic and environmental effects". For further definitions on terms 'alien', 'casual aliens', 'naturalized' and 'transformers' refer to Richardson et al. (2000).

Hulme et al. (2013) also include the connotations of impact in their definition of invasive species, saying that the term 'invasive' refers to "established alien organisms that are rapidly extending their range in the new region, usually causing significant harm to biological diversity, ecosystem functioning, socio-economic values, and/or human health in invaded regions".

INVASION PATHWAYS

Ricciardi and MacIsaac (2000) define invasion pathways as transportation pathways which enable long-distance dispersal of species towards specific regions. Terrestrial invasion corridors include important traffic routes (Ricciardi and MacIsaac, 2000), road verges, railway networks, rivers and ditches, where human factor is the main dispersal mechanism (Pyšek and Prach, 1994), while ballast waters are the most important dispersal mechanism of aquatic invasions.

Invasion corridors are affected by the intensity of vector traffic, and environmental conditions of both donor and recipient regions. It is necessary to identify corridors of invasion, linking donor and recipient regions, so that they can be incorporated into predictive models, as mass invasions may be a result of an intense propagule pressure of one or more invasion corridors into recipient systems (Ricciardi and MacIsaac, 2000).

The species' capacity for invasion is a result of a match between the species' ecophysiology and the environmental conditions of the area of its introduction, therefore enabling any given species to become invasive, at the right time and place, often with irreversible consequences (Pienimäki and Leppäkoski, 2004).

Studies mostly focus on recording pathways of initial introductions of NIS into a specific region, and rarely deal with their subsequent spread (Hulme et al., 2008). In order to develop preventive measures (e.g. screening and early warning systems, interception programmes and import regulations), the initial introduction is of key importance (Hulme, 2006). The spread of organisms through waterways can be a result of passive drifting, active dispersal or transport in ballast waters and on the hulls of ships (Galil et al., 2007), making it often difficult to distinguish the pathways of their initial introduction (Hulme et al., 2008).

Hulme et al. (2008) list three mechanisms as a result of which nonindigenous organisms may arrive into a new region: i) import of commodities, ii) arrival of transport vectors and iii) natural spread from neighbouring regions, where the species is also alien. Once introduced, alien species may spread further across the region as a result of natural dispersal. The rate of spread for terrestrial ecosystems is estimated to be 89 km/year (Pyšek and Hulme, 2005).

A dramatic increase in the number of vectors and pathways of invasion has been documented since the 19th century, from one (shipping) to five nowadays (shipping, fishing, aquaculture, accidental introduction and secondary spread, Karatayev et al., 2008). These authors predict a further increase in the number of vectors, during the 21st century, due to recreational activity, ornamental species and live food trade. Also, some of the potential vectors of NIS introductions are birds and semi-aquatic mammals, aquaculture, aquarium trade, fishing gear and unintentional release or escape of these species (Minchin and Gollasch, 2002). Estimating the risk of the introduction of NIS to aquatic environments is very difficult due to many factors which are uncertain and unknown. Nevertheless, the prospects are similar for most of the world's aquatic ecosystems – increase in the introduction of NIS, along with the intensification of their impact on native biodiversity (Pienimäki and Leppäkoski, 2004).

Transoceanic invasions

Plants, along with marine animals and other organisms have been transferred across the world's seas ever since the humans have started crossing them for exploration, colonization and trade. In contrast with the older transport vessels, which carried significant numbers of species on their hulls, nowadays most of the species are transported inside the vessels, in ballast waters (Carlton, 1999).

Ballast waters are waters taken intentionally by ships for greater stability, which are then carried in specifically dedicated ballast water tanks and empty cargo holds. Upon reaching their final destination the ships empty up to tens of thousands of tons of water to take up cargo (Carlton, 1999), leaving behind a number of various life stages of hundreds of plant and animal taxa (Carlton and Geller, 1993). In terms of transfer efficiency ballast waters have few, if any, parallels among transport mechanisms on land or at sea (Carlton, 1999), and represent major vectors of aquatic invasions worldwide (Carlton and Geller, 1993).

To predict potential invaders and in mapping hotspot areas for alien introductions, the origin of the ballast water and the route of the ship are crucial (Pienimäki and Leppäkoski, 2004). There is one rather astonishing estimate which says that modern vessels may carry from 3000 up to 10.000 species in their ballast waters, globally, per day (Carlton, 1999).

Rivers and canals as invasion corridors

Chytrý et al. (2009) stated that habitat type is the most effective predictor of the level of invasion at the regional scale, as same habitats are generally either strongly and frequently or weakly and rarely invaded by alien plants (Chytrý et al., 2008). Research of Chytrý et al. (2008) showed that frequently disturbed, human-influenced habitats, such as arable land, trampled and ruderal areas, as well as coastal, litoral and riverine habitats record typically high invasion levels. The European map on the level of invasion by neophytes anticipates highest levels of invasion in agricultural regions of central and eastern Europe, including the lower Danube valley, and along rivers and irrigated agricultural regions of the sub-Mediterranean zone (Chytrý et al., 2009).

It is to be expected that river catchments represent diversity hotspots and some of the most important natural corridors (Figure 1), especially in temperate areas (Naiman et al., 1993; Naiman and Décamps, 1997; Burkart, 2001). Many invasive plant species also show a preference for riparian zones, primarily in the early stages of invasion (Pyšek and Prach, 1994).

The spread of invasive species usually starts along watercourses, and inland areas are subsequently invaded (Burkart, 2001). The vegetative propagule pressure in riparian corridors is especially high, as water flow and floodwaters transport both buoyant and non-buoyant propagules from a variety of habitats located within the watershed downstream (Barrat-Segretain, 1996; Johansson et al., 1996), thus representing an important introduction vector. Therefore riparian zones, along with urban areas, represent significant hotspots for alien species and potential sources of their further spread (Pyšek et al., 1998).

Central European riparian zones have been subjected to human impact since the Neolithic age (Burkart, 2001). Some riparian forests have been almost completely cleared, and flooding dynamics have been dramatically altered by the construction of dams, dikes and locks, causing these habitats to become highly endangered ecosystems (Burkart, 2001 and references therein). Forest industry emissions, agricultural runoff, as well as the discharge of nutrients and other chemical pollutants into inland waters threaten the biological integrity of these systems (Pienimäki and Leppäkoski, 2004), thereby increasing the chances of invasion. Also, flooding is an important factor in the invasion of riparian habitats, since long periods of high waters have the potential to reduce the rate of survival and establishment of perennial terrestrial plant species, leaving the affected sites open for colonization (Burkart, 2001).

Complex network of inland waters of Europe comprises more than 28.000 km and connects 37 countries in Europe and beyond. Construction of canals has brought about the transfer of species between regions, and this impact was most evident with canals which connected two or more previously isolated biogeographical areas. Rivers connected through canals across narrow strips of land practically eliminate naturally existing barriers for the dispersal of organisms, thereby enabling them to spread both naturally and as a result of human transport, thus making all navigable waterways important invasion corridors (Galil et al., 2007).

There are four important invasion corridors which can be highlighted across Europe: i) "northern corridor", the largest, linking Black, Azov and Caspian Seas with the Baltic and White Seas; ii) "central corridor" connecting the regions of Black and Baltic Sea, via Dnieper river; iii) "southern corridor" connecting rivers Danube and Rhine, through the Main river; iv) "western corridor" linking the Mediterranean Sea with the North Sea, via the river Rhone and the Rhine-Rhone Canal (Galil et al., 2007).

Studies have shown that vectors of invasion are usually directed from a more diverse region, to a poorer one (Karatayev et al., 2008), in this sense, the Suez Canal represents a nearly unidirectional route for the biota of the Red Sea and the Indo-Pacific region to cross into the Mediterranean. As a result of this more than 500 species originating from the Red Sea basin have become established as far westward as the Adriatic Sea (Galil, 2000).

Further interconnection of European rivers and canals has enabled the invasion of native species from the basins of the Caspian and Black Seas into the Baltic and North Sea (Galil et al., 2007). The importance of canal construction in the transfer of NIS of Ponto-Caspian origin is clear from the fact that first exotic aquatic invertebrates in Belarus appeared after first interbasin canals have been constructed (Karatayev et al., 2008). Also, invasions of the Rhine River followed the opening of the Rhine-Danube-Main Canal in 1992, as a result of linking of the River Rhine, and its tributaries in western Europe, to the Black Sea (Ricciardi and MacIsaac, 2000). As a result of rising global temperatures, the number of introduced Ponto-Caspian, sub-tropical and tropical species of vascular plants has increased lately in the areas of the northern hemisphere, arriving via the Black Sea-Baltic Sea corridor (e.g. *Lemna gibba* L., *Vallisneria spiralis* (Tiger), *Phragmites altissimus* (Benth.) and *Typha laxmannii* (Lepech.)) (Pienimäki and Leppäkoski, 2004).

REYNOUTRIA SPP. AS RIPARIAN INVADERS

Certain invasive species have been proven to show a strong affinity towards riparian zones, and their spread is aided by rivers in a considerable measure, as water flow is their main mechanism of dispersal (Pyšek and Prach, 1994).

Among the most problematic invasive species, infamous for their tendency to invade riparian habitats, are Japanese Knotweed *s.l.* species: *Reynoutria japonica* Houtt. (syn. *Fallopia japonica* (Houtt.) Ronse Decr.), *Reynoutria sachalinensis* (F. Schmidt) Nakai (syn. *Fallopia sachalinensis* (F. Schmidt) Ronse Decr.), and their hybrid *Reynoutria* x *bohemica* Chrtek & Chrtková (syn. *Fallopia* x *bohemica* (Chrtek & Chrtková) J.P. Bailey). *Reynoutria japonica* has been categorized as one of the worlds 100 worst invasive alien species by the Global Invasive Species Programme (Lowe et al., 2000), and a highly invasive species in Serbia (Lazarević et al., 2012). The issue of the invasion of *Reynoutria* species has been extensively researched over the past two decades in Europe (Pyšek and Prach, 1994; Bailey et al., 1996; Bailey and Conolly, 2000; Bímová et al., 2003; Bímová et al., 2004; Mandák et al., 2004; Mandák et al., 2005; Bailey et al., 2009), and in Serbia (Glavendekić, 2008; Širka et al., 2013).

The species of *Reynoutria* have been introduced to Europe from the Far East (Japan, Korea, Taiwan, northern China) for ornamental purposes (Figure 5), in the 19th century, and have since escaped cultivation and become highly problematic across the continent. They show a distinctive competitive superiority over other ecologically similar native species, through a reduction in light availability and changes in soil environment (Pyšek and Prach, 1994; Barney et al., 2006) and can have detrimental effects on native riparian communities (Barney et al., 2006), (Figure 2).

Over the course of the summer/autumn period of 2013 an extensive field survey was conducted along the watercourses of Serbia. The survey was conducted to assess the level of plant invasion of these riparian areas and to determine the most important riparian invasion corridors, with *Reynoutria* species in focus. The field survey was carried out along the 500 m stretches of riverbanks, at regular intervals, according to RHS (Raven et al., 1997) river stretches. A signifficant number of tributaries in the water basins of Zapadna Morava, Drina, Danube, Velika and Južna Morava rivers was included in the survey. The mapping was done using the method of GPS positioning with a GPS Garmin eTrex 10 handheld GPS navigator. The data gathered was then included in an Excel database, and subsequently georeferenced using DIVA-GIS software.

The areas studied was scattered along the watercourses of Serbia, making it difficult to give an universal description of the study area. The rivers of Serbia belong to the drainage basins of the Black, Adriatic and Aegean Sea, where the Black Sea drainage basin is the largest one, and covers an area of 92% of the territory of Serbia. Of the rivers whose tributaries are most affected by the presence of *Reynoutria* spp. stands, Zapadna Morava and Drina rivers stand out.

Zapadna Morava is a river in Central Serbia which stretches far westward into the Dinaric mountains, originating east of Požega, from the Golijska Moravica and Đetinja headstreams. Along its course it passes through a number of valleys and gorges (Ovčar-Kablar gorge). Its river valley is of great economical importance, having a significant hydroelectric and irrigation potential and due to the developed industry of the cities it flows through (Užice, Požega, Čačak, Kraljevo, Kruševac). This river also stands out by the number of tributaries it receives along its course (85), the most imortant being Čemernica, Kamenica, Dičina, Ribnica, Rasina and Ibar, which is the largest one.

Drina is an international river, forming in part the border between Serbia and Bosnia and Herzegovina. It is the longest tributary of Sava, and the richest one in terms of its average water discharge. It is a fast river, which has carved several gorges along its length, but becomes a meandering river in its lower course, spilling into many arms and flows and creating a large floodplain in the Mačva region.

Preliminary findings (Figure 3) show that the rivers from the basins of Zapadna Morava and Drina stand out by the level of *Reynoutria* spp. invasion. Zapadna Morava river in itself shows a very high level of invasion, especially in the territory of the city Čačak, while its tributaries show signs of further spread of invasive *Reynoutria* spp. upstream. On the other hand, in the Drina river basin, the invasion seems to be spreading downstream towards the confluence of the Štira river into Drina. Other findings mostly show signs of initial stages of *Reynoutria* spp. propagule pressure, with a high potential for its further spread in the upcoming years, portraying Serbian watercourses as a potential eastern corridor of *Reynoutria* spp. invasion.

Dense stands (Figure 4) of *Reynoutria japonica* generally occupy large areas in habitats strongly influenced by man (Beerling et al., 1994), mainly in riparian and ruderal habitats (Bailey et al., 2009). It is a fast growing, strongly competitive species, often found in nutrient rich habitats, forming stands with a very dense canopy, beneath which not many species can survive (Beerling et al., 1994). When compared with *Reynoutria japonica*, *R. sachalinensis* is a less successful invader, however an increase in the spread of vegetative fragments by water has also been recorded for this species in the last several decades (Pyšek and Prach, 1994).

As asexual reproduction is the primary means of reproduction and colonization for invasive *Reynoutria* species in their introduced range, through the dispersal of rhizome and stem fragments (Barney et al., 2006), riparian habitats are primarilly affected due to the fact that their rhizome and stem fragments are transported mainly by water flow (Beerling et al., 1994).

Figure 1. River Čemernica as a potential invasion corridor (orig.).
Slika 1. Reka Čemernica kao potencijalni koridor invazije.

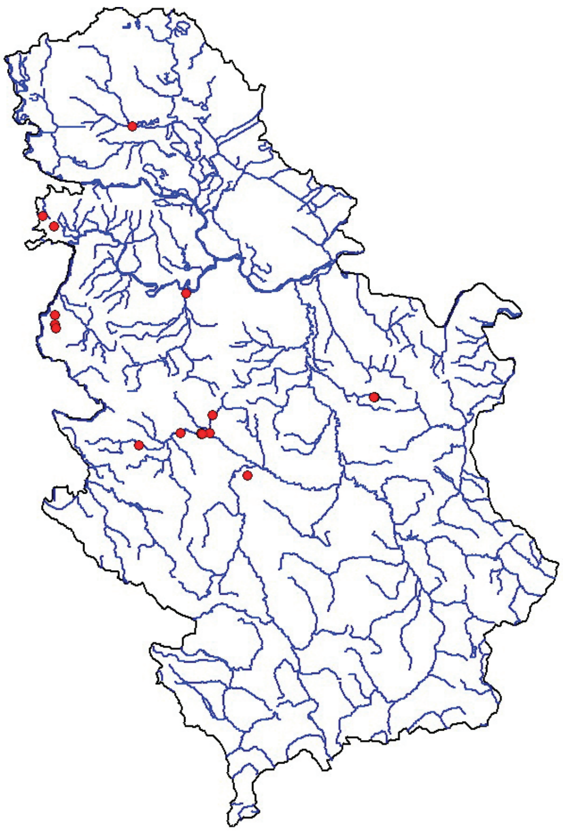

Figure 3. Potential corridors of *Reynoutria* spp. invasion in Serbia.
Slika 3. Potencijalni koridori invazije *Reynoutria* spp. u Srbiji.

Figure 2. *Reynoutria* spp. encroaching on riverbank's native vegetation (orig.).
Slika 2. *Reynoutria* spp. potiskuje nativnu vegetaciju rečne obale.

Figure 4. Large stand of *Reynoutria* spp. on the banks of Zapadna Morava (orig.).
Slika 4. Velika sastojina *Reynoutria* spp. na obali Zapadne Morave.

Figure 5. *Reynoutria* spp. inflorescence (orig.).
Slika 5. Cvast *Reynoutria* spp.

CONCLUSION

To successfully manage the invasive alien species problem, there is a need for preventive measures and the development of mechanisms which would minimise the risk of future introductions and attempt to reduce the uncertainty of existing risk assessments (Pienimäki and Leppäkoski, 2004).

A dramatically significant reduction of native species densities, and in certain cases almost complete removal of native species are two direct negative effects which invasive species may have on biodiversity. Even though their ecological role is often overlooked, their effects on ecosystems can be devastating (Karatayev et al., 2008). Even though Simberloff (2011) postulates that only a fraction of 10% of the total number of NIS have a noticeable effect on natural ecosystems, knowing that there are around 5789 naturalized alien plant species in Europe (Lambdon et al., 2008), the number of species potentially affecting natural ecosystems is still very significant (Hulme et al., 2013).

Since invasive species of the genus *Reynoutria* are known as some of the most troublesome alien invasive species in the world, and due to their well documented tendency to pose a serious threat to native biodiversity, especially in the riparian areas, *Reynoutria* spp. invasion should be a focal point of future invasive species research in Serbia. Up to this point, it has been documented that certain watercourses in our country show a significant level of Japanese Knotweed *s.l.* invasion, however only future results, along with the results of other researchers who have also been mapping the distribution of these species in Serbia will show us the magnitude of the *Reynoutria* spp. invasion. Since its dispersal is primarily linked with water flow, and its presence has already been confirmed in a number of river basins, further spread and intensification of its invasion are to be expected over the course of the years to come.

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REKE SRBIJE KAO PUTEVI INVAZIJE – ISTOČNI KORIDOR INVAZIJE *REYNOUTRIA* SPP.

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REZIME

Stepen invazija koji je u porastu širom sveta zabrinjava naučnike, zbog značajnih troškova i napora koji su neophodni u njihovom kontrolisanju. Poznavanje puteva invazije, kako početnog unosa tako i naknadnog širenja vrsta, od ključnog je značaja, jer je u 21. veku prognoziran dalji porast broja puteva i vektora invazije. Na regionalnom nivou tip staništa se pokazao kao pouzdani pokazatelj nivoa invazije, jer određene tipove staništa (često remećena, pod snažnim antropogenim uticajem) karakteriše visok nivo invazije. Riparijalna staništa, kao centri diverziteta alohtonih vrsta i primarni izvor njihovog širenja, predstavlju neke od najznačajnijih koridora invazije, u kojima voda deluje kao uspešan mehanizam disperzije. Neke invazivne vrste biljaka, poput *Reynoutria* spp. pokazuju snažnu tendenciju ka invaziji riparijalnih staništa. Preliminarni rezultati terenskih istraživanja koji su za cilj imali procenu nivoa invazije riparijalnih staništa vrstama roda *Reynoutria* u Srbiji ukazuju na to da su slivovi nekih reka pod značajnim uticajem prisustva ovih invazivnih vrsta. Imajući u vidu osnovni vid njihove propagacije, dalje širenje *Reynoutria* spp. duž reka u Srbiji može se očekivati tokom narednih godina.

Ključne reči: Invazija, putevi invazije, reke, riparijalna staništa, *Reynoutria* spp.

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ANTIBACTERIAL ACTIVITIES OF SOME *BACILLUS* SPP. AND *TRICHODERMA HARZIANUM* AGAINST PHYTOPATHOGENIC BACTERIA

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SUMMARY

Biological control is an innovative, cost effective and eco-friendly approach for control of many plant diseases. *Bacillus* sp. and *Trichoderma* sp. were known for its mycoparasitic and antagonistic mechanism in the control of wide range of phytopathogenic diseases in many types of crops. This research is based on the antibacterial activities of some *Bacillus* spp. and *Trichoderma harzianum* in relation to the 10 selected phytopathogenic bacteria in *in vitro* study.

The results indicated that among 52 strains of *Bacillus* spp. presented against phytopathogenic bacteria, only 6 of them showed inhibition but only against *Xanthomonas* genera. Other *Bacillus* spp. strains weren't effective against tested phytopathogenic bacteria. Results of testing of *T. harzianum* efficacy against the growth of selected phytopathogenic bacteria showed that this fungi could be effective to strains from genera *Pseudomonas* and *Xanthomonas* but for other tested genera was ineffective. In further work, all of this trial need to be supported by evaluation of antimicrobial activity in *in vivo*.

Key words: biocontrol, *Bacillus* spp., *Trichoderma harzianum*, *in vitro,* effects, phytopathogenic bacteria

INTRODUCTION

Many plant diseases are caused by phytopathogenic bacteria which greatly determine the quality of plant production (Agrios, 1998). The biological control of plant pathogenic bacteria is an alternative method to the application of chemicals, which may be accomplished through the destruction of existing inoculums, exclusion from the host, or the suppression or displacement of the pathogen after infection (Campbell, 1989). It offers an environmentally friendly approach to the management of plant disease and can be incorporated with cultural and physical control and limited chemical usage for an effective and integrated disease-management system (Monte, 2001; Mérillon and Ramawat, 2012). Biological control includes the use of beneficial microorganisms, such as specialized fungi and bacteria, to attack and control plant pathogens and the diseases they cause. Most of antagonists are mass-produced, commercialized, and perhaps patented (Cook and Baker, 1983). Common organisms among them include members of the bacterial genera *Pseudomonas* and *Bacillus*, and the fungal genus *Trichoderma*. They have different mechanisms of disease suppression, fungal antagonists depend mainly on physical contacts with their pathogen while, bacteria mainly use antibiotics as weapon for killing of the pathogens (Howell, 2003).

Bacteria of the genus *Bacillus* has showed antimicrobial activity against plant pathogenic microorganisms (Handelsman and Stabb, 1996; Compant et al., 2005; Živković et al., 2010; Popović et al., 2012a, 2012b; Ivanović et al., 2013). *Bacillus* spp. are natural inhabitants of the phyllosphere (Arias et al., 1999) and rhizosphere (Sessitsch et al., 2003; Berg et al., 2005). These bacteria are involved in the control of plant diseases through a variety of mechanisms of action, such as competition, systemic resistance induction and antibiotic production. The mechanism of antibiosis has been shown to be one of the most important (Tomashow and Weller, 1996). *Bacillus* spp. have the advantage of being already adapted to the environment where they can be applied as biological control. They have the characteristics of having high thermal tolerance, showing rapid growth in liquid culture, and readily form resistant spores. It is considered safe biological agents and their potential as biocontrol agents is considered to be high (Kim et al., 2003).

The fungus *Trichoderma harzianum* is an efficient biocontrol agent against a wide range of soilborne pathogens and has plant growth promote capacity (Chet, 1990; Rabeendran et al., 2000; Yedidia et al, 2001; Howell, 2003; Harman et al., 2004; Bal and Altintas, 2006). For a long time, *Trichoderma* species have been known as biological agents for control of plant diseases (Howell, 2003; Ranasingh et al., 2006). They interact with root, soil and leaf surroundings, and produce many components, which induce local or systemic plant resistance to abiotic stress. The main factor for ecological success of this genus is a combination of very active mycoparasitism mechanisms and an effective defensive strategy, induced in the plants (Rosado et al., 2007).

This study was performed to examine the antimicrobal activity of *Bacillus* spp. and *T. harzianum* against different plant pathogenic bacteria in *in vitro*.

MATERIALS AND METHODS

Phytopathogenic bacterial strains

From the several bacterial diseases that received high attention from research scientists because of their serious effects on many national economical crops, we have selected the following phytopathogenic bacteria showed in Table 1.

In vitro screening of *Bacillus* spp. for biocontrol activity

We used 52 native strains of *Bacillus* spp. isolated from phyllosphere (codes B1-B52) (Ivanović, unpublished data). The strains of *Bacillus* sp. were screened for inhibitory activity against 10 selected bacterial pathogens (Table 1) by following the modified well-diffusion assay (Harris et al., 1989). Petri dishes with appropriate solid medium were poured with 7 ml of soft LB medium, previously inoculated with 70 µl of the culture of indicator strain (10^6 CFU/ml). For agar well-diffusion assay, a well was made in the medium (ø 5 mm) using of sterile bottom parts of the 200 µl pipette tips. Well-diffusion assay was completed by adding tested substances into the well in the final volume of 50 µl. The sterile distilled water was served as a control. The plates were incubated overnight at optimal temperature

Table 1. Plant pathogenic bacteria tested for selection of antagonistic *Bacillus* spp. strains and *T. harzianum*.
Tabela 1. Fitopatogene bakterije korišćene za selekciju antagonističkih *Bacillus* spp. sojeva i *T. harzianum.*

Species	Collection	Code	Originated from	References
Pseudomonas syringae* pv. *syringae	Institute for Plant Protection and Environment, Serbia	IZB-26	Pear	(Ivanović, 2009)
Pseudomonas savastanoi* pv. *phaseolicola	Institute for Plant Protection and Environment, Serbia	TP11	Bean	(Popović, 2008)
Ralstonia solanacearum	La Collection Française de Bactéries Phytopathogènes, France	CFBP 3857	Potato	http://www-intranet.angers.inra.fr/cfbp/doc_pdf/catalogue.pdf
Xanthomonas axonopodis* pv. *phaseoli	Institute for Plant Protection and Environment, Serbia	TX11	Bean	(Popović, 2008)
Xanthomonas campestris* pv. *campestris	Institute for Plant Protection and Environment, Serbia	TKU1	Cabbage	(Popović et al., 2013)
Xanthomonas campestris* pv. *vesicatoria	Institute for Plant Protection and Environment, Serbia	TXv5	Tomato	(Popović et al., 2012)
Xanthomonas arboricola* pv. *juglandis	Institute for Plant Protection and Environment, Serbia	IZB-320	Walnut	(Ivanović et al., 2012)
Erwinia amylovora	Institute for Plant Protection and Environment, Serbia	TEad1	Quince	(Popović et al., 2012)
Brenneria nigrifluens	Institute for Plant Protection and Environment, Serbia	TOr1	Walnut	(Popović et al., 2013)
Clavibacter michiganensis* subsp. *sepedonicus	La Collection Française de Bactéries Phytopathogènes, France	CFBP 3561	Potato	http://www-intranet.angers.inra.fr/cfbp/doc_pdf/catalogue.pdf

for indicator strains, at 27°C. The result was obtained by measuring the zone of inhibition from the edge of the well and expressed in mm.

In vitro screening of *Trichoderma harzianum* for biocontrol activity

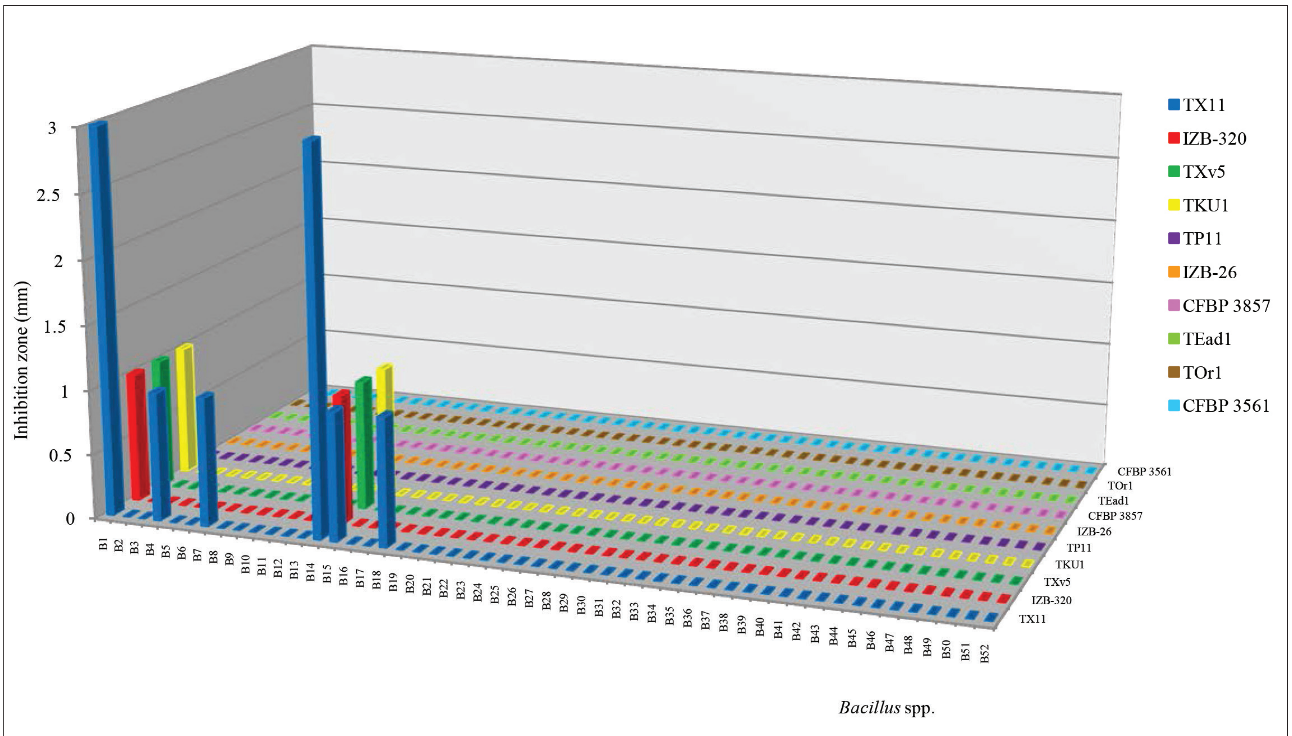
T. harzianum was evaluated for efficacy against the growth of 10 different bacterial pathogens (Table 1) by inhibition zone assay method (Raju, 2010). The antagonistic microorganism *T. harzianum* (DSM 63059), was obtained from the German Collection of Microorganisms and Cell Cultures.

A heavy suspensions (3 day old) of used bacterial strains multiplied in nutrient broth (per 20 ml for each strain) were mixed with Nutrient agar (1000 ml) contained in Erleyenmayer's flask. Fifteen to twenty ml of seeded medium was poured into the sterilized Petri dishes and allowed to solidify. Mycelial discs (ø 5 mm) taken from actively growing *T. harzianum* culture from Potato Dextrose Agar (PDA) were placed in the centre of Petri dishes containing the seeded medium. The inoculated Petri dishes were incubated at 27°C for 72 hours. Observations were recorded for the zone of inhibition produced by antagonistic microorganism around the growth of the pathogen.

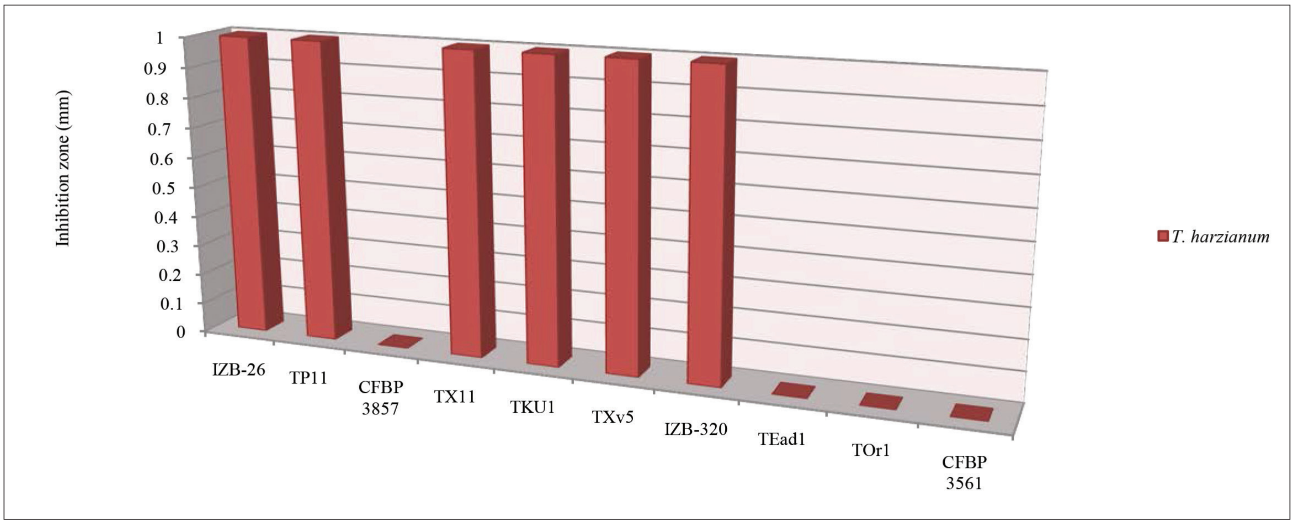
RESULTS

Study conducted on effect of *Bacillus* spp. on growth of 10 different phytopathogenic bacteria indicated that antagonism is showed only to *Xanthomonas* strains (Graph 1). Among tested *Bacillus* spp. strains only two of them (B1 and B14) were found significantly superior in inhibiting the growth of *Xanthomonas* sp. strains (ø inhibition zone 3 mm), while four of them (B4, B7, B15, B18) were less effective (ø inhibition zone 1 mm). Other *Bacillus* spp. strains were not showed antibacterial activities against tested phytopathogenic bacteria.

The results of *T. harzianum* effect against phytopathogenic bacteria showed that the growth of *Pseudomonas* and *Xanthomonas* genera was reduced (Graph 2). After three days, inhibition zones of 1 mm were recorded. *Trichoderma harzianum* was found ineffective to other phytopathogenic bacteria as it failed to inhibit the growth of tested strains.



Graph 1. Antibacterial effect of *Bacillus* spp. against tested phytopathogenic bacteria.
Grafikon 1. Baktericidno delovanje *Bacillus* spp. na testirane fitopatogene bakterije.



Graph 2. Antibacterial effect of *T. harzianum* against tested phytopathogenic bacteria.
Grafikon 2. Baktericidno delovanje *T. harzianum* na testirane fitopatogene bakterije.

DISCUSSION

In recent years, there has been substantial interest in the biological control of plant pathogens especially plant bacteria over the world (Cooksey and Moore, 1980; Volksch and May, 2001; Wilson et al., 2002; Krause et al., 2003). Under biological control, various antagonistic organisms have been identified, which fight against the pathogens by different mechanisms such as competition, lysis, antibiosis, siderophore production and hyper parasitism (Pal i McSpadden Gardener, 2006). Among the different antagonists tried as biocontrol agents, *Bacillus* spp. have been commonly used. Assis et al. (1996) tested the antagonism of 32 epiphytic *Bacillus* spp. isolated from cabbage, kale and radish and found among these isolates, 13 who reduced 100% the incidence of black rot in kale under greenhouse conditions. In field experiments, those 13 isolates reduced incidence in cabbage, ranging from 48-78% (Assis et al., 1997). Popović et al. (2012a) also studied antagonism activity of *Bacillus* soil isolates collected from the rhizosphere of different cultivated plants against *Xanthomonas campestris* pv. *campestris* from cabbage in *in vitro*. Growth inhibition given by Q18 and Q3 of *Bacillus* isolates showed significant value comparing to others. Tested *Bacillus* soil isolates were also used for their antagonistic activities against *Pseudomonas syringae* pv. *syringae* and among them two *Bacillus* (Q7 and Q13) formed inhibition zones (Popović et al., 2012b).

Berić et al. (2013) made screening of 203 natural isolates of *Bacillus* sp. for their antimicrobial activity against *Burkholderia glumae, B. cepacia, B. plantarii, Erwinia carotovora, Pseudomonas fuscovaginae, P. aeruginosa, Agrobacterium tumefaciens, Xanthomonas oryzae* pv. *oryzae*, *Ralstonia solanacearum,* as well as *Bacillus subtilis* 168 and *Escherichia coli.* Authors observed that 127 tested strains inhibit at least one sensitive strain, which illustrates their potential use as biocontrol agents.

Many species of *Bacillus* are known to suppress growth of several bacterial pathogens such as *Agrobacterium tumefaciens* (Ivanović et al., 2013), *Xanthomonas vesicatoria* (Al-Arabi, 2002; Filho et al, 2010), *Xanthomonas axonopodis* pv. *punicae* (Raju, 2010), *Xanthomonas campestris* pv. *campestris* (Monteiro et al., 2005), *Ralstonia solanacearum* (Seleim et al., 2011), *Pseudomonas savastanoi* pv. *savastanoi* (Krid et al., 2010), C*lavibacter michiganensis* subsp. *michiganensis* (Al-Arabi, 2002), *Xanthomonas arboricola* (Dimkić et al., 2013), *Pectobacterium carotovorum* (Dimkić et al., 2013).

Our preliminary screening performed with 10 strains of phytopathogenic bacteria used as indicator strains, showed that two *Bacillus* strain (B1 and B14) from the collection exhibited antagonistic activity against isolates from genera *Xanthomonas*. Therefore, these strains could be evaluated *in vivo* for further biological control potential. Strains from other genera were not showed sensitivity to tested *Bacillus* strains.

This research work also describes use of *T. harzianum* in control of plant pathogenic bacteria *in vitro*. It was found to be possible effective to strains from genera *Pseudomonas* and *Xanthomonas* but for other tested genera was ineffective. Trials should be supported by *in vivo* testing because of slow fungi growing comparing to fast growing of bacteria. Literature sources which are concerning on antimicrobal activity of *T. harzianum* on phytopathogenic bacteria are poor. Raju (2010) evaluated *T. harzianum* efficacy against the growth of *Xanthomonas axonopodis.* pv. *punicae* by inhibition zone assay method. It was found ineffective to inhibit the growth of *X. a.* pv. *punicae.*

T. harzianum is described as a biocontrol agent in control of soil-borne plant pathogens *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* (Mishra et al., 2011; Elad et al., 1980), *Pythium* sp. (Hadar et al., 1984), *Macrophomina phaseolina*, *Sclerotinia sclerotiorum* (Mishra et al., 2011), *Alternaria alternata* (Rocco and Perez, 2001; Monte, 2001), *Bipolaris oryzae* (Abdel-Fattah et al., 2007).

Evaluations of antimicrobial properties of selected *Bacillus* spp. and *T. harzianum* by *in vivo* studies are currently in progress.

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BAKTERICIDNO DELOVANJE NEKIH *BACILLUS* SPP. I *TRICHODERMA HARZIANUM* NA FITOPATOGENE BAKTERIJE

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REZIME

Biološka kontrola predstavlja inovativni, isplativ, ekološki pristup za suzbijanje mnogih biljnih bolesti. Vrste roda *Bacillus* i *Trichoderma* su poznati po svom mikoparazitskom i antagonističkom mehanizmu suzbijanja širokog spektra fitopatogenih prouzrokovača bolesti kod brojnih poljoprivrednih kultura. U ovom radu proučavano je baktericidno delovanje nekih vrsta roda *Bacillus*, kao i gljive *Trichoderma harzianum* na 10 odabranih fitopatogenih bakterija u *in vitro* uslovima.
Prema dobijenim rezultatima, od ukupno 52 testirana izolata iz roda *Bacillus*, samo 6 je inhibiralo porast fitopatogenih bakterija i to samo iz roda *Xanthomonas*. Rezultati ispitivanja efikasnosti gljive *T. harzianum* su ukazali na mogućnost delovanja ove gljive na bakterije iz roda *Pseudomonas* i *Xanthomonas*, ali ne i na druge rodove fitopatogenih bakterija testirane u ovom radu. Dalja istraživanja treba bazirati na proučavanjima antimikrobne aktivnosti testiranih agenasa prema fitopatogenim bakterijama u *in vivo* ogledima.

Ključne reči: biološka kontrola, *Bacillus* spp., *Trichoderma harzianum*, *in vitro*, efekat, fitopatogene bakterije

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UTICAJ PRISUSTVA ŽUTE CISTOLIKE NEMATODE *GLOBODERA ROSTOCHIENSIS* NA PRINOS RAZLIČITIH SORTI KROMPIRA

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REZIME

Tokom 2008. godine ispitivan je uticaj prisustva populacije žute krompirove cistolike nematode *Globodera rostochiensis* (ž-KCN) patotip Ro1 na prinos 15 različitih sorti krompira. Istraživanja su izvedena na lokalitetu Planina na Jagodnji kod Krupnja u zapadnoj Srbiji. U istraživanjima korišćene su sledeće osetljive sorte krompira prema ž-KCN Ro1: Desiree, Cleopatra, Riviera, Romano i Virgo, koje su ispoljile visok stepen neotpornosti (osetljivosti) prema Ro1 ž-KCN u ispitivanom likalitetu. Sorte deklarisane kao otporne prema Ro1 ž-KCN ispoljile su visok stepen otpornosti prema ovom patotipu ž-KCN u lokalitetu Planina (Agria, Arnova, Kuroda, Roko, Amorosa, Sante, Tomensa, Jelly, Naviga i Eldena). Najmanji prinos utvrđen je kod osetljivih sorti Romano (15,2 t ha^{-1}) i kod sorte Cleopatra 16,7 t ha^{-1}, dok je najveći prinos krtola krompira ustanovljen kod otpornih sorti Naviga 44,8 t ha^{-1} i Eldena 33,3 t ha^{-1}. Dobijeni rezultati ukazuju na praktični značaj gajenja otpornih sorti na zaraženom području u Mačvanskom okrugu u cilju postizanja većih prinosa krompira i iskorenjavanja karantinske nematode *G. rostochiensis*.

Ključne reči: *Globodera rostochiensis*, otpornost krompira, prinos, sorta

UVOD

Krompirove cistolike nematode (KCN) paredstavljaju realnost u Republici Srbiji, pošto su, počev od 2000. godine (*Globodera rostochiensis* Wollen, 1923), Behrens 1975), odnosno 2005. godine *Globodera pallida*, Stone 1973), utvrđene u više lokaliteta. KCN su karantinske vrste nematoda u većem broju zemalja širom sveta (Lehman, 2002). Gubici u prinosu na području Evrope prouzrokovani nematodama procenjuju se na oko 9 % proizvodnje krompira (Turner and Rowe, 2006). U ukupnoj proizvodnji hrane u Srbiji, krompir zauzima značajno mesto. Veliki ekonomski značaj krompira proizilazi iz činjenice da se ovaj usev gaji na 78.000 ha, sa prosečnim prinosom (u periodu 2003-2009) koji se kreće na nivou 11,5 t ha^{-1} (Statistički godišnjak Srbije, 2010). Navedeni prosečan prinos značajno zaostaje za prinosima krompira u Evropi i svetu od 37,0 do 55,0 t ha^{-1} (FAO, 2010). Komercijalna proizvodnja krompira odvija se na 50.000-60.000 hektara sa prosečnim prinosom od oko 15-25 t ha^{-1}, što još uvek ni približno ne zadovoljava standarde moderne poljoprivredne proizvodnje.

Prema našim istraživanjima *G. rostochiensis* utvrđena je 2000. godine u usevu krompira na Jagodnji, Tari (Ponikve), Javoru, Aljinovićima (kod Prijepolja) (Krnjaić et al., 2002, 2005, 2008.). Kasnijim ispitivanjima utvrđena su nova žarišta *G. rostochiensis*: u Moravičkom okrugu (Poljopromet), DPU Stranjanci, ZZ Milatovići i Voćar Dragačevo ITP Jagodnja) a u Mačvanskom okrugu (Pek komerca). Posle pet godina (2005. godine) konstatovana su još dva žarišta bele KCN *G. pallida* na Javoru DP Javor Kušići (u lokalitetima Šanac i Kladnica) i u dva lokaliteta mešana populacija *G. rostochiensis* i *G. pallida* DP Javor Kušići (lokalitet - Ograđenik) i DPK Stranjanci (lokalitet - Milatovići) (Krnjaić et al., 2005).

U strategiji suzbijanja i iskorenjivanja KCN izuzetno je značajno što ranije otkriti žarišta njihovog prisustva - pojedinačnog ili u mešanim populacijama, odrediti patotip svake od utvrđenih vrsta, uvoditi u plodored sorte krompira otporne prema utvrđenom patotipu KCN i trajno sprovoditi antinematodne mere uključujući i primenu sistemičnih nematocida, kao što su Aldicarb (Temik), Entoprophos (Mocap), Fosthiozat (Nematos), Oxamyl (Vydate), dok su tečni fumiganti od 1990. godine zabranjeni zbog toksičnosti i ostataka u zemljištu i vodi.

Ipak plodored, kao mera suzbijanja i iskorenjivanja KCN je od posebnog značaja. Najčešće se preporučivalo isključivanje gajenja krompira na površinama zaraženim sa KCN u trajanju od 3-7 godina. Prema novijim istraživanjnima vitalnost invazionog sadržaja *G. rostochiensis* u zemljištu, u odsustvu biljke hraniteljke, može se održati i do 20 godina (Pridannikov et al., 2006) što relativizuje primenu plodoreda, bez uvođenja u plodored otpornih sorti krompira i nematocida (Trudgill et al., 2003). Otpornost invazionog sadržaja cista (jaja, J2) prema faktorima sredine je izuzetno visoka. Pridannikov et al. (2002) utvrdili su da homogenat jaja *G. rostochiensis* zadržava sposobnost piljenja i nakon dvadest minutnog potapanja u ključalu vodu (100°C), kao i posle pet uzastopnih ciklusa hlađenja (smrzavanja) na -20°C i podizanja temperature na 22°C (Pridannikov et al., 2006).

Krnjaić i Poštić (2009) navode da plodored sa travno-leguminoznim smešama, bez obzira koliko trajao, ne obezbeđuje gašenje žarišta ž-KCN, jer se krtole krompira i u ovim uslovima reprodukuju, što omogućava održavanje ž-KCN. Isti autori preporučuju plodored sa okopavinskim kulturama (sve osim biljanih vrsta iz familije *Solanaceae*), zaostale krtole oranjem i drugim agrotehničkim merama izbacuju se na površinu ili u plići sloj zemljišta (5-10 cm dubine), koje bi tokom zime u periodu od 5-6 godina izmrzle, na ovaj način bi se prekinuo reproduktivni lanac KCN. Što se tiče izbora otpornih i tolerantnih sorti krompira prema ž-KCN mogućnosti su velike, posebno prema patotipu Ro1. Izbor otpornih sorti krompira prema jednom ili više tipova (3) bele krompirove cistolike nematode (b-KCN) je veoma ograničen i nepouzdan zbog velike agresivnosti ove vrste i brzine gubljenja otpornosti selekcionisanih sorti krompira prema ovoj vrsti.

Na primer u Engleskoj nije selekcionisana nijedna komercijalna sorta krompira otporna prema patotipovima b KCN (Martin et al., 2009). U SAD se koristi ograničen broj (16) sorti u proizvodnji krompira otpornih na Ro1 ž- KCN i sistem vrlo uspešno funkcioniše (Trugill et al., 2003). Površine zaražene sa b-KCN ili mešanim populacijama b-KCN i ž-KCN moraju biti podvrgnute dugogodišnjem plodoredu do konačnog gašenja žarišta zaraze. I u nas na površinama zaraženim sa ž-KCN - Ro 1 moguće je gajenje krompira setvom sorti otpornih na Ro 1 ž-KCN, kojih ima dosta (Krnjaić i Poštić, 2009; Poštić i sar., 2012, 2013).

Cilj ovog rada bio je da se ukaže na značaj gajenja otpornih sorti krompira prema ž-KCN *G. rostochiensis* patotipa Ro1, radi postizanja većih prinosa krompira, supresije i iskorenjavanja karantinske nematode *G. rostochiensis* patotip Ro1.

MATERIJAL I METODE

U cilju utvrđivanja uticaja populacije ž-KCN *G. rostochiensis* patotipa Ro1 na prinos različitih sorti krompira ogled je postavljen na zaraženoj parceli sa potesa Planina, KO Ljubovija na planini Jagodnja u Mačvanskom okrugu u zapadnoj Srbiji (759 m nv., 44˚ 19' 33«N, 19˚ 20' 33«E). Za izvođenje poljskog ogleda korišćeno je pet osetljivih sorti krompira prema ž-KCN *G. rostochiensis* patotipa Ro1 (Desiree, Cleopatra, Riviera, Romano i Virgo) i 10 otpornih sorti krompira na *G. r.* patotip Ro1 (Agria, Arnova, Kuroda, Roko, Amorosa, Sante, Tomensa, Jelly, Naviga i Eldena).

Zemljište na oglednom polju gde je postavljen mikro ogled pripadalo je tipu kiselih i smeđih podzolastih zemljišta. Prema sadržaju humusa (Tabela 1) u površinskom sloju od 3,40 %, zemljište je veoma dobro obezbeđeno. Sadržaj ukupnog azota iznosi 0,27 % spada u bogata zemljišta. Jako kisele je reakcije jer mu pH vrednost u H2O iznosi 4,35, a u nKCl 3,80. Zemljište je u orničnom sloju dobro obezbeđeno lako pristupačnim fosforom (19,96 mg/100 g zemljišta). Sadržaj lako pristupačnog K2O iznosi 36,04 mg/100 g zemljišta, što znači da je površinski sloj veoma dobro obezbeđen ovim elementom. Sadržaj lako rastvorljivog kalijuma nedovoljan je za postizanje visokih prinosa krompira, pa se njegov nedostatak mora nadoknaditi đubrenjem. Prema sadržaju karbonata spada u spada u slabo karbonatna zemljišta.

Meteorološki uslovi (Tabela 2) tokom vegetacionog perioda bili su povoljni, srednje mesečne temperature vazduha bile su u optimumu za proizvodnju krompira, kao i ukupna količina padavina od 538,7 mm je zadovoljila potrebe krompira za vodom.

Veličina elementarne parcele iznosila je 9 m^2, a obračunske parcele 2,1 m^2. Veličina oglednog polja iznosila je 135 m^2. Sadnja krtola je izvršena 04. 05. 2008. godine prema planu setve, po 40

Tabela 1. Osobine zemljišta na oglednom polju (Krupanj).
Table 1. Properties of soil at the experimental plot (Krupanj).

Dubina/ Depth (cm)	Tip zemljišta/ Tipe of Soil	CaCO3 %	pH u		Humus %	N %	mg/100g zemljišta/ Soil	
			H2O	nKCl			P2O5	K2O
0-30	**Smeđe podzolasto/ Brown podzolic**	0,69	4,35	3,80	3,40	0,27	19,96	36,04

Tabela 2. Meteorološki uslovi tokom vegetacije krompira 2008. godine.
Table 2. Metereological conditions during the potato growing season 2008 year.

Meseci/Month						
April/April	**Maj/May**	**Jun/Jun**	**Jul/July**	**Avgust/August**	**Septembar/September**	**Prosek/Average**
Temperatura vazduha/Air temperature (°C)						
10,8	15,2	18,8	19,2	19,6	13,5	16,2
Količina padavina/Amount precipitation (mm)						**Ukupno/Total**
52,5	54,2	179,3	112,7	21,7	118,3	538,7

krtola svake sorte (4 reda po 10 krtola) na međuredno rastojanje 70 cm i rastojanje između biljaka u redu od 30 cm. Agrotehničke mere koje su primenjene na oglednom polju spadaju u standardnu tehnologiju gajenja krompira. Pre sadnje u proleće i pred vađenje krtola u jesen 2008. godine sa svake elementarne parcele uzeti su pojedinačni uzorci zemljišta radi utvrđivanja prisustva, brojnosti i vitalnosti cista *G. rostochiensis*. U fazi intenzivnog nalivanja krtola po precvetavanju krompira, odnosno 01. 08. 2008. godine izvršeno je uzorkovanje po 10 biljaka od svake ispitivane sorte radi utvrđivanja prisustva cista na korenovom sistemu. Iz zone korenovog sistema uzorkovanih biljaka uzeti su uzorci zemljišta (0,5 kg) radi ispitivanja prisustva mužjaka u zoni korenovog sistema. Na kraju vegetacionog perioda utvrđivan je prinos krtola po svakoj ispitivanoj sorti i izračunat koeficijent varijacije (CV). (Statistica 8.0 Windows, Poljoprivredni fakultet, Novi Sad).

REZULTATI

Isptivanjem uzoraka zemljišta iz elementarnih parcela, uzetih neposredno pred sadnju sorti, utvrđena je podjednaka brojnost cista u svakoj parceli (prosečno 30 cista u 500 ml zemljišta) sa vitalnošću sadržaja od oko 50 %, što je predstavljalo dovoljan inokulacioni potencijal za zaražavanje ispitivanih sorti (Pi= 9 jaja i J2/1ml zemjišta). Pregledom korenovog sistema (Tabela 3) u fazi intenzivnog nalivanja krtola (01. 08. 2008. godine) kod svih ispitivanih osetljivih sorti (Desiree, Cleopatra, Rivijera, Romano i Virgo) utvrđeno je da su se masovno razvile ciste *G. rostochiensis*. Kod 10 otpornih sorti (Agria, Arnova, Kuroda, Roko, Amorosa, Sante, Tomensa, Jelly, Naviga i Eldena) na Ro 1 patotip *G. rostochiensis* nije utvrđeno prisustvo cista na korenovom sistemu kao ni mužjaka u zoni korenovog sistema (Tabela 3), što direktno ukazuje da su ove sorte ispoljile otpornost prema populaciji *G. rostochiensis* u lokalitetu Planina.

Analizom uzoraka zemljišta uzetih iz parcela nakon vađenja ispitivanih sorti krompira kod osetljivih sorti utvrđen je visok nivo novoformiranih cista (50 cista /500 ml zemlje) i gotovo potpuna ispražnjivost starih cista (Pf=30 jaja i J2/1 ml zemljišta). Kod svih ispitivanih osetljivih sorti krompira (Desiree, Cleopatra, Riviera, Romano i Virgo) konstatovan je manji ukupan prinos krtola, u odnosu na ukupan prinos ustanovljen kod otpornih sorti krompira (Agria, Arnova, Kuroda, Roko, Amorosa, Sante, Tomensa, Jelly, Naviga i Eldena). Najmanji prinos krtola utvrđen je kod osetljivih sorti Romano (15,2 t ha^{-1}) i kod sorte Cleopatra 16,7 t ha^{-1}, dok je najveći prinos krtola krompira ustanovljen kod otpornih sorti Naviga 44,8 t ha^{-1} i Eldena 33,3 t ha^{-1}.

Ispitivane sorte su pokazale visoku varijabilnost ukupnog prinosa (CV= 29,13) u uslovima prisustva *G. rostochiensis* na lokalitetu Planina (Tabela 4). Na parcelama kod svih otpornih sorti nakon njihovog vađenja nisu utvrđene novoformirane ciste, dok je vitalni sadržaj starih cista bio prepolovljen (50 % niži), u odnosu na nivo pre sadnje (Pf=4,5 jaja i J2/1 ml zemljišta). Kod osetljivih sorti krompira utvrđena je pozitivna (3,3) stopa rasta ž-KCN (Pf/Pi), dok je kod otpornih sorti ustanovljena negativna stopa rasta (Pf/Pi =0,5 jaja i J2/1 ml zemljišta).

Tabela 3. Raspored sorti krompira na parceli zaraženoj sa ž-KCN, pojava cista (c) na korenovom sistemu i mužjaka (♂) u zoni korenovog sistema u lokalitetu Planina 2008. godine.
Table 3. Distribution of the cultivars in experimental field infected with y-PCN, presence of the cysts on root system (c) and males (♂) in soil, locality Planina in 2008.

Desiree S	c=5; ♂=5		**Roko** R	c=0; ♂=0		**Jelly** R	c=0; ♂=0
Agria R	c=0; ♂=0		**Amorosa** R	c=0; ♂=0		**Romano** S	c=5; ♂=5
Arnova R	c=0; ♂=0		**Riviera** S	c=4; ♂=4		**Naviga** R	c=0; ♂=0
Cleopatra S	c=5; ♂=5		**Sante** R	c=0; ♂=0		**Eldena** R	c=0; ♂=0
Kuroda R	c=0; ♂=0		**Tomensa** R	c=0; ♂=0		**Virgo** S	c=5; ♂=5

S - osetljiva sorta (susceptible cultuvar), R - otporna sorta (resistant cultivar)

Legenda-Legend
Ro 1 = deklarisanost na određene patotipove KCN (01. 08. 2008.) - resistance on PCN
c = ciste na korenu od 0 do 5 na cm dužine korena - cysts on root system
♂ = mužjaci u zoni korenovog sistema od 0 do 5 u 100 ml zemljišta (01.08.2008.) - males in soil

Tabela 4. Ukupan prinos krtola (t ha^{-1}) u 2008. godini.
Table 4. Total yields of (t ha^{-1}) in 2008 years.

Sorta/ Cultivar	Osetljivost/ Susceptibility	Ukupan prinos/ Total yields (t ha^{-1})	Index (%)
Desiree	S	20,0	100
Cleopatra	S	16,7	83,5
Riviera	S	18,1	90,5
Romano	S	15,2	76,0
Virgo	S	20,5	102,5
Agria	R	27,6	138,0
Arnova	R	25,2	126,0
Kuroda	R	27,1	135,5
Roko	R	24,3	121,5
Amorosa	R	27,1	135,5
Sante	R	27,6	138,0
Tomensa	R	28,1	140,5
Jelly	R	32,4	162,0
Naviga	R	44,8	224,0
Eldena	R	33,3	166,0
		CV= 29,13 %	

S - osetljiva sorta (susceptible cultuvar), R - otporna sorta (resistant cultivar)

DISKUSIJA

Krompirove cistolike nematode, žuta *Globodera rostochiensis* (ž- KCN) i bela *G. pallida* (b-KCN), postale su vrlo ozbiljan problem u proizvodnji krompira u zemljama u kojima su KCN prisutne. U zavisnosti od stepena infekacije zemljišta sa KCN, gubici u proizvodnji se kreću u intervalu od 12-60% a ponekad su totalni. Ako se ima u vidu da je krompir po značaju u ishrani čovečanstva na četvrtom mestu u svetu, u slučaju daljeg širenja KCN bio bi ugrožen bilans proizvodnje hrane na lokalnim a potom i sve širim nivoima. Srbija spada u grupu zemalja u kojoj su KCN otkrivene tokom poslednjih 15 godina. Mere koje se za sada preduzimaju svode se na kontrolu uvoznih pošiljki semenskog krompira i kontrolu prisustva KCN na površinama na kojima se semenski krompir reprodukuje. Na taj način kontroliše se oko 1000 ha godišnje, što znači da kontroli izmiču značajne površine semenskog krompira.

Površine na kojima se proizvodi merkatilni krompir u Srbiji (oko 80 000 ha) ne podležu kontroli KCN, i pored toga što je nadležno telo EU ukazalo na potrebe postepenog uvođenja kontrole KCN na površinama na kojima se gaji merkatilni krompir. To je jedan od uslova za izvoz i plasman merkatilnog krompira u zemljama članicama EU i susednim zemljama. Zemlje u kojima su prisutne obe KCN, imaju mnogo teži i složeniji zadatak u sprečavanju njihovog širenja i suzbijanja. To su zapadno-evropske zemlje i neke zemlje u Južnoj i Centralnoj Americi. U zemljama u kojima je prisutna jedna od njih a to je najčešće *G. rostochiensis* (na pr. SAD i Kanada) problem se uspešno rešava. Na pr. u SAD utvrđen je prostor na kome je prisustna *G. Rostochiensis* patotip Ro 1 ž-KCN i strogo je regulisano koje se sorte mogu gajiti (samo sorte otporne na ovaj patotip). Na ovaj način u proizvodnim uslovima vrlo uspešno se kontroliše nivo populacija ž-KCN, dok se striktnim karantinskim merama sprečava unos *G. pallida* i uvoz sortimenta krompira neotpornog prema ž KCN (Ro 2-5).

U Engleskoj i Velsu bio je drugi slučaj. Već 30 godina vlada epidemija bele cistolike krompirove nematode (*G. pallida*), koja sve više potiskuje žutu (*G. rostochiensis*). Trudgil (2003) sa saradnicima to objašnjava činjenicom da je uvođenjem u proizvodnju (1966-71) vrlo komercijalne sorte krompira (Maris Piper) koja je bila otporna na *G. rostochiensis* a kasnije još dve sorte (Cara i Pentland Juvelin) tako da je 2001 godinne 52 % površina pod krompirom bilo zasejano sa ovim sortama otpornim prema ž-KCN. To je stvorilo prostor za ekspanziju bele krompirove cistolike nematode (b-KCN) koja je mnogo agresivnija. Sistem mera suzbijanja je vrlo složen pošto se radi o tri patotipa ove vrste i sa druge strane o ograničenom sortimentum komercijalnih sorti krompira u koje je ugrađen u jednu ili više gena otpornih prema Pa 1-3. Bilo da se radi o patotipovima za ž-KCN ili b KCN, otpornost sorti slabi pa čak i nestaje, tako da je neophodno krenuti od početka, selekcionisati nove otporne sorte prema KCN. Geni nosioci rezistencije krompira prema ž-KCN su H1, K1, Fa i Fb a prema b-KCN H2 i H3 (Phillips, 1994).

U proizvodnim uslovima poželjno je održavati inicijalni nivo populacije (Pi) na manje od Pi=2 jajeta i J2 u ml zemljišta (Brodie, 1996). Ako je Pi=0,1-1,0 jaja i J2 u 1 ml zemlje mogu se gajiti rezistentni kultivari svake treće godine itd.

Za Srbiju je olakšavajuća okolnost da se radi o ne tako davno introdukovanim KCN (verovatno pre 40 godina) i ne široko rasprostranjenim štetočinama krompira, koje se šire pasivnim putem (uglavnom semenskim krompirom). Iz ovoga se nameće potreba striktne kontrole uvoza, proizvodnje i prometa semenskog krompira i s druge strane kontrola prisustva KCN na površinama gde će se taj krompir gajiti, semenski ili merkatilni krompir. Površine namenjene za proizvodnju semenskog krompira ne smeju biti zaražene sa KCN. Površine namenjene za proizvodnju merkatilnog krompira takođe moraju biti pregledane na KCN , kako bi se u slučaju njihovog prisustva utvrditi o kom patotipu pripada. Na ovako ispitanim površinama mogle bi se gajiti sorte krompira koje su otporne prema toj KCN i određenom patotipu, ali u skladu sa nivoom inicijalne populacije (Pi) koja treba da bude ispod 0,2 jaja i J2 u ml zemljišta (Brodie, 1996).

U našim dosadašnjim ispitivanjima u području zapadne Srbije prisutna je uglavnom *G. rostochiensis* patotip Ro 1 prema kome je u svetu selekcionisan značajan broj visoko komercijalnih sorti krompira kojim se prisustvo ove KCN može vrlo uspešno kontrolisati. U ostalim lokalitetima neophodno je utvrditi da li se radi o jednoj vrsti i kojoj ili o mešanim populacijama. Potom je neophodno utvrditi patotip utvrđene KCN. U skladu sa ovim nalazima neophodno je propisati antinematodne mere i vršiti kontrolu njihovog sprovođenja sve do gašenja žarišta zaraza.

Gajenjem otpornih sorti krompira na lokalitetu Planina na Jagodnji zaraženom ž-KCN *G. rostochiensis* utvrđen je znatno veći ukupan prinos krtola, u odnosu na ustanovljen ukupan prinos krtola kod osetljivih sorti (Tabela 4), što se slaže sa rezultatima (Magnusson, 1984; Bačić, 2010). Na parcelama kod svih otpornih sorti nakon njihovog vađenja u jesen nisu utvrđene novoformirane ciste, dok je vitalni sadržaj starih cista bio prepolovljen (50 % niži), u odnosu na nivo pre sadnje. Ovakvi rezultati su slični sa rezultatima (Miroshnik, 1996; Zakabunina, 2000). Kod osetljivih sorti krompira utvrđena je pozitivna (3,3) stopa rasta ž-KCN (Pf/Pi), dok je kod otpornih sorti ustanovljena negativna stopa rasta (Pf/Pi =0,5 jaja i J2/1 ml zemljišta).

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EFFECT OF THE PRESENCE OF YELLOW CYST NEMATODE *GLOBODERA ROSTOCHIENSIS* ON YIELD DIFFERENT POTATO CULIVARS

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SUMMARY

The aim of this study was to determine the effect presence populations of yellow potato cyst nematode Globodera rostochiensis (Wollen, 1923; Behrens, 1975) on the yield 15 different varieties of potatoes, during 2008. Investigations were carried out in the locality Planina mountain Jagodnja, near Krupanj, West Serbia. The susceptible potato varieties on y-PCN pathotype Ro1: Desiree, Cleopatra, Riviera, Romano and Virgo, which was exhibited a high degree sensitivity to y-PCN pathotype Ro1 in the locality Planina. Potato cultivars: Agria, Arnova, Kuroda, Rocco, Amorosa, Sante, Tomensa, Jelly, Navigation and Eldena, which are declared as resistant on Ro 1 y-PCN, has been resistant-on root system we not faund female and cysts of y-PCN, and in soil male of y-PCN. The lowest yield was observed for susceptible varieties Romano (15.2 t ha^{-1}) and Cleopatra (16.7 t ha^{-1}), and the highest yield for resistant varieties Naviga (44.8 t ha^{-1}) and Eldena (33.3 t ha^{-1}). Obtained results indicate the practical importance of growing resistant varieties in the infected areas of Mačva District in order to achieve higher yields of potatoes and eradicate the quarantine nematode *G. rostochiensis.*

Key words: *Globodera rostochiensis*, potato resistance, yield, cultivars

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UTICAJ BAKAR-CITRATA NA PORAST KOLONIJA *VENTURIA INAEQUALIS*

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REZIME

Bakar-citrat je kompleksno jedinjenje bakra koje se odlikuje višim stepenom disocijacije u odnosu na druga bakarna jedinjenja koja su trenutno u primeni pa se može koristiti u nižim koncentracijama za suzbijanje biljnih patogena. Čađava pegavost lista i krastavost plodova (*Venturia inaequalis*) je jedna od ekonomski najštetnijih bolesti jabuke u svim regionima gde se ova voćna vrsta gaji. Uspešna proizvodnja jabuke nije moguća bez izvođenja intenzivnih hemijskih mera zaštite. Primena preparata na bazi bakra u zaštiti jabuke, pozicionirana je na sam početak vegetacije. Najranije zaraze jabuke su i najdestruktivnije, pa je efikasna zaštita jabuke u ovom periodu veoma važna. Cilj ovog rada je da se u *in vitro* uslovima ispita uticaj bakar-citrata u različitim koncentracijama primene na porast kolonija *Venturia inaequalis.* Dobijeni rezultati pokazuju da bakar citrat u znatno nižim koncentracijama primene 0,2 i 0,1% nego sandardni preparat, efikasno blokira porast micelije (kolonija) fitopatogene gljive *V. inaequalis*, što je rezultat znatno veće količine bakarnih jona koje ovo jedinjenje oslobađa.

Ključne reči: Čađava krastavost, bakar-citrat, porast kolonija, efikasnost

UVOD

Uspešna primena preparata na bazi bakra u suzbijanju prouzrokovača biljnih bolesti datira više od 100 godina unazad. Kao jedinjenja sa nespecifičnim mehanizmom delovanja još uvek se uspešno primenjuju sa nesmanjenom efikasnošću u suzbijanju biljnih patogena. S druge strane kao rezultat dugogodišnje primene bakarnih preparata, formiran je veliki depo bakra u zemljištu voćnjaka, koji izaziva negativne ekološke efekte kao i štene efekte na korisne zemljišne organizme i plodnost zemljišta (Georgopoluos et all. 2001, Van Zwieten et all. 2004). Čađava pegavost lista i krastavost plodova (*Venturia inaequalis* (Cooke) Winter) je jedna od ekonomski najšteštnijih bolesti jabuke u svim regionima gde se ova voćna vrsta gaji (Ivanović, 2001). Uspešna proizvodnja jabuke nije moguća bez izvođenja intenzivnih hemijskih mera zaštite. Primena preparata na bazi bakra u zaštiti jabuke, pozicionirana je na sam početak vegetacije do fenofaze roze pupoljka (BBCH 57). Najranije zaraze jabuke su i najdestruktivnije, pa je efikasna zaštita jabuke u ovom periodu veoma važna. Danas je u prometu veliki broj preparata na bazi bakra i to su uglavnom preparati na bazi bakar-hidroksida, bakar-oksida, bakar-oksihlorida i bakar-sulfata. Biološka aktivnost fuingicida, odnosno baktericida na bazi bakra meri se količinom slobodnih Cu^{2+} jona raspoloživih za usvajanje od strane gljiva ili bakterija, tako da biološka aktivnost fungicida ili baktericida raste sa povećanjem količine oslobođenih Cu^{2+} jona (Martinez, 2008). Bakar-citrat je kompleksno jedinjenje bakra koje se odlikuje višim stepenom disocijacije u odnosu na druga bakarna jedinjenja koja su trenutno u primeni pa se može koristiti u nižim koncentracijama za suzbijanje biljnih patogena (Popović i sar., 2012). To je veoma značajno i sa ekološkog aspekta, jer intenzivna primena bakra dovodi do njegove akumulacije u površinskim slojevima zemljišta, čime se narušava biološka aktivnost i plodnost zemljišta. S druge strane, bakar-citrat nema izraženo toksično delovanje na ribe, ptice, sisare i pčele, pa bi njegovo uvođenje u primenu bilo i u tom smislu prihvatljivo.

Cilj ovog rada je da se u *in vitro* uslovima ispita uticaj bakar-citrata u različitim koncentracijama primene na porast kolonija *Venturia inaequalis*, prouzrokovača čađave krastavosti jabuke, u poređenju sa preparatima na bazi bakar hidroksida koji se koriste u standardnoj zaštiti jabuke od ove fitopatogene gljive.

MATERIJAL I METODE RADA

Ogledi su izvedeni tokom 2012. godine u laboratorijama Instituta za zaštitu bilja i životnu sredinu. U in vitro uslovima ispitivana je efikasnost serije razređenja bakar-citrata sa početnom koncentracijom od 0,4%, zatim 0,2%, 0,1% i 0,01%. Za ispitivanja su korišćena 2 izolata gljive i to izolat poreklom sa voćnjaka iz lokaliteta Morović (M), u kome su sprovođene intenzivne mere zaštie i izolat sa izolovanih pojedinačnih stabala jabuke iz lokaliteta Neštin (N), gde nisu sprovođene mere zaštite. Vršena je monokonidijalna izolacija prema metodama Instituta za zaštitu bilja i životnu sredinu na KDA podlogu (Borić, 1987, Aleksić, 1996). Fragmenti micelije monokonidijalnih izolata, veličine 1 mm, uzimani su sa kolonija izolata i zasejavani u Petri kutije prečnika 5 cm na KDA hranjlivu podlogu, u koju je prethodno unesena odgovorajuća količina fungicida, a zatim inkubirani u termostatu na 20°C u uslovima tame. Praćen je porast kolonija izolata *V. inaequalis*, pri čemu je za konačan porast uzet prečnik izmeren 42-og dana od dana zasejavanja (Borić, 1985, Aleksić, 2005). Ogledi su izvedeni u pet ponavljanja. Kao standard za poređenje, korišćen je preparat na bazi bakar-hidroksida. Kontrolne varijante su zasejavane na KDA podlogu bez fungicida. Obrada podataka urađena je standardnim staističkim metodama. Značajnost razlika između varijanti urađena je analizom varijanse, a za međusobno poređenje korišćeni su Dankanov test (Dunkan, 1955) i LSD test.

REZULTATI I DISKUSIJA

U ispitivanjima su korišćena 2 izolata (M, i N) *V. inaequalis* poreklom iz dva lokaliteta sa područja Srbije (Morović i Neštin). Izolati iz lokaliteta Morović uzorkovani su iz komercijalnog zasada jabuke površine 100 ha, u kome se sprovode intenzivne mere zaštite dugi niz godina. U ovom voćnjaku se koristi čitava paleta fungicida registovana za suzbijanje prouzrokovača čađave krastavosti jabuke između ostalih i preparati na bazi bakra. Izolati iz lokaliteta Neštin su uzeti sa pojedinačnih stabala jabuke udaljenih od bilo kakvog komercijalnog zasada. Na tim stablima se ne sprovode mere zaštite protiv prouzrokovača čađave krastavosti, pa se ovi izolati mogu smatrati divljim izolatima.

Dobijeni rezultati su pokazali da nije bilo porasta kolonija gljive izolata M, na podlogama sa koncentracijama ispitivanog preparata od 0,4%, 0,2% i 0,1%, dok je na podlogama sa koncentracijom 0,01%, registrovan srednje jak porast kolonija ovog izolata (Tabela 1, Grafikon 1). Između ispitivanih varijanti i kontrolne varijante utvrđene su statistički značajne razlike. Statistički značajna razlika utvrđena je i između varijante sa najnižom koncentracijom ispitivanog preparata (0.01%) i ostalih varijanti preparata (0.1%, 0.2% i 0.4%).

Kod drugog ispitivanog Izolata (N), nije bilo porasta kolonija na podlogama sa koncentracijama ispitivanog preparata od 0,4% i 0,2%, dok je na podlogama sa koncentracijom 0,1 i 0,01%, registrovan srednje jak porast kolonija (Tabela 2, Grafikon 2). Između ispitivanih varijanti i kontrolne varijante utvrđene su statistički značajne razlike. Statistički značajna razlika utvrđena je i između varijanti sa nižom koncentracijom ispitivanog preparata (0.01% i 0.1%) i ostalih varijanti, sa višom koncentracijom preparata (0.2% i 0.4%).

U varijantama sa standardnim preparatom ($Cu(OH)_2$), kod ispitivanog Izolata M, porast kolonija registrovan je na podlogama sa svim koncentracijama ispitivanog preparata od 0,4% do 0,01%. Na podlogama sa koncentracijom 0,4 i 0,2% registrovan je slab porast kolonija, dok je na podlogama sa koncentracijama 0,1 i 0,01%, registrovan srednje jak porast kolonija (Tabela 3, Grafikon 3). Između ispitivanih varijanti i kontrolne varijante utvrđene su statistički značajne razlike, dok između varijanti sa različitim koncentracijama bakar hidroksida nije bilo staistički značajnih razlika.

U varijantama sa standardnim preparatom ($Cu(OH)_2$), kod ispitivanog Izolata N, porast kolonija registrovan je, takođe, na podlogama sa svim koncentracijama ispitivanog preparata od 0,4% do 0,01%. Na podlogama sa koncentracijom 0,4 i 0,2% registrovan je slab porast kolonija, dok je na podlogama sa koncentracijama 0,1 i 0,01%, registrovan jak porast kolonija (Tabela 4, Grafikon 4). Statistički značajne razlike utvrđene su između varijanti sa koncentracijama 0,4 i 0,2% i ostalih varijanti(0,1 i 0,01% i kontrola).

Registrovani porast kolonija na podlogama u koje je unesen bakar-citrat u koncentracijama 0,01% može se okarakterisati kao srednje jak, dok na podlogama sa koncentracijom 0,1 kod izolata M nije registrovan porast kolonija, a kod izolata N registrovan je srednje jak porast kolonija gljive. Kod varijanti sa standardnim preparatom (bakar-hidroksid), regi-

Tabela1. *V. inaequalis* - porast kolonija izolata M na podlozi sa bakar-citratom.
Table 1. *V. inaequalis* - colony growth of the M isolate on media with copper citrate.

Izolat M	**Ponavljanja**					**Ms***	
Konc. (%)	**A**	**B**	**C**	**D**	**E**		
0.01	++	++	+	++	+	++	**b**
0.1	-	-	-	-	-	-	**a**
0.2	-	-	-	-	-	-	**a**
0.4	-	-	-	-	-	-	**a**
Kontrola	+++	+++	+++	+++	+++	+++	**c**

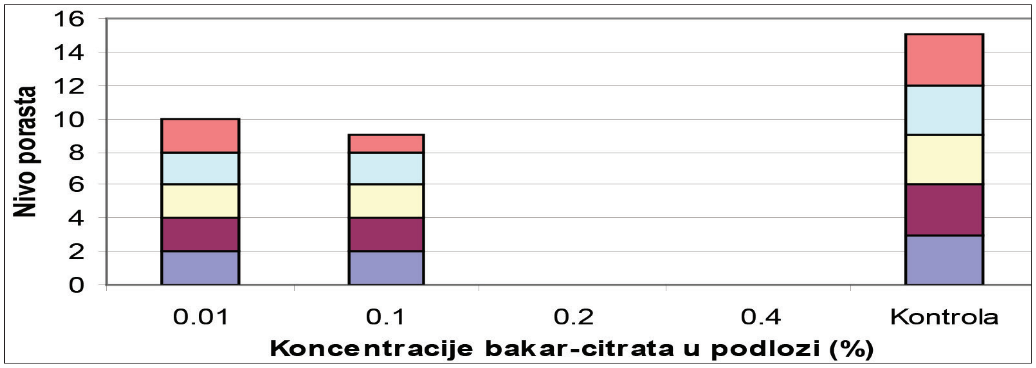
Napomena: $LSD_{0.01}$= 0.45; *Vrednosti obeležene istim slovima ne razlikuju se statistički značajno; Ms*- srednja vrednost;

Grafikon 1. *V. inaequalis* - porast kolonija izolata M na podlozi sa bakar-citratom.
Graph 1. *V. inaequalis* - colony growth of the M isolate on media with copper citrate.

Tabela 2. *V. inaequalis* - porast kolonija izolata N na podlozi sa bakar-citratom.
Table 2. *V. inaequalis* - colony growth of the N isolate on media with copper citrate.

Izolat N	**Ponavljanja**					Ms*	
Konc. (%)	A	B	C	D	E		
0.01	++	++	++	++	++	++	**b**
0.1	++	++	++	++	+	++	**b**
0.2	-	-	-	-	-	-	**a**
0.4	-	-	-	-	-	-	**a**
Kontrola	+++	+++	+++	+++	+++	+++	**c**

Napomena: $LSD_{0.01}$= 0.37; *Vrednosti obeležene istim slovima ne razlikuju se statistički značajno; Ms*- srednja vrednost;

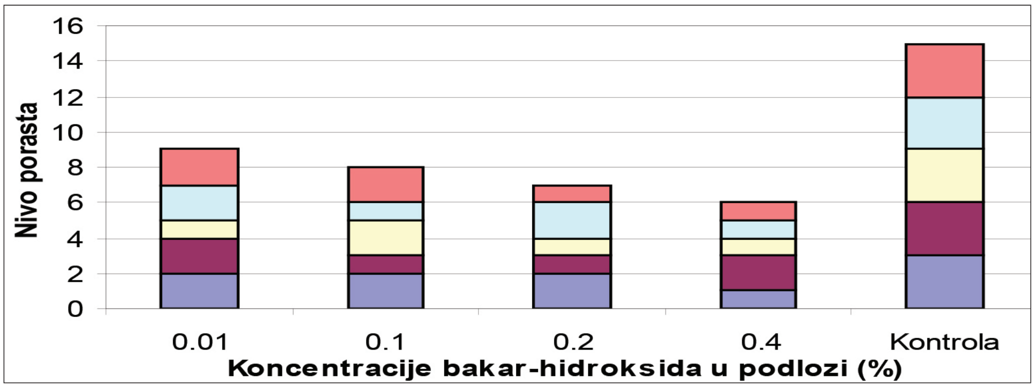


Grafikon 2. *V. inaequalis* - porast kolonija izolata N na podlozi sa bakar- citratom.
Graph 2. *V. inaequalis* - colony growth of the N isolate on media with copper citrate.

Tabela 3. *V. inaequalis* - porast kolonija izolata M na podlozi sa bakar-hidroksidom.
Table 3. *V.inaequalis* - colony growth of the M isolate on media with copper hydroxide.

Izolat M	Ponavljanja					Ms*	
Konc. (%)	A	B	C	D	E		
0.01	++	++	+	++	++	++	**a**
0.1	++	+	++	+	++	++	**a**
0.2	++	+	+	++	+	+	**a**
0.4	+	++	+	+	+	+	**a**
Kontrola	+++	+++	+++	+++	+++	+++	**b**

Napomena: $LSD_{0.01}$= 0.88; *Vrednosti obeležene istim slovima ne razlikuju se statistički značajno; Ms*- srednja vrednost;

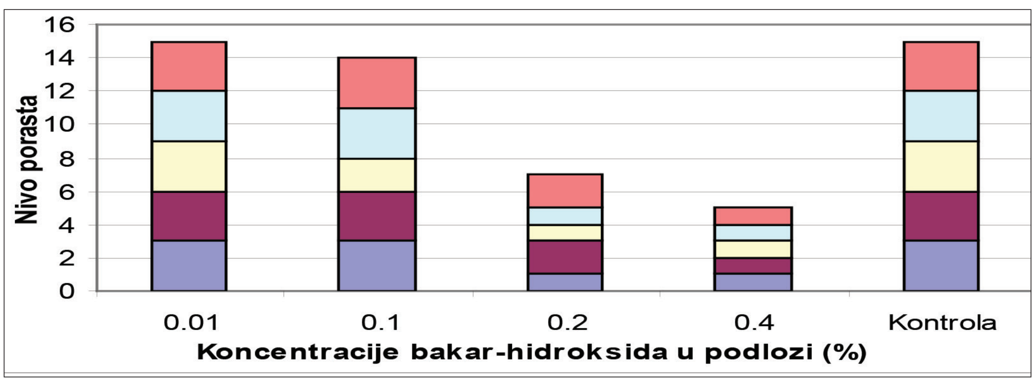


Grafikon 3. *V. inaequalis* - porast kolonija izolata M na podlozi sa bakar-hidroksidom.
Graph 3. *V. inaequalis* - colony growth of the M isolate on media with copper hydroxide.

Tabela 4. *V. inaequalis* - porast kolonija izolata N na podlozi sa bakar-hidroksidom.
Table 4. *V.inaequalis* - colony growth of the N isolate on media with copper hydroxide.

Izolat N	Ponavljanja					Ms*	
Konc. (%)	A	B	C	D	E		
0.01	+++	+++	+++	+++	+++	+++	**b**
0.1	+++	+++	++	+++	+++	+++	**b**
0.2	+	++	+	+	++	+	**a**
0.4	+	+	+	+	+	+	**a**
Kontrola	+++	+++	+++	+++	+++	+++	**b**

Napomena: $LSD_{0.01}$= 0.55; *Vrednosti obeležene istim slovima ne razlikuju se statistički značajno; Ms*- srednja vrednost.



Grafikon 4. *V. inaequalis* - porast klonija izolata N na podlozi sa bakar-hidroksidom.
Graph 4. *V. inaequalis* - colony growth of the N isolate on media with copper hydroxide.

strovan je slab porast kolonija na podlogama sa koncentracijom 0,2 i 0,4%, dok je na podlogama sa nižim koncentracijama bakar-hidroksida (0,1 i 0,01%) registrovan srednje jak porast kolonija izolata M i jak porast kolonija izolata N. Na podlogama bez fungicida (kontrolne varijante) registrovan je normalan (jak) porast kolonija gljive oba izolata. Iz dobijenih rezultata može se zaključiti da bakar citrat u znatno nižim koncentracijama primene 0,2 i 0,1% nego sandardni preparat, efikasno blokira porast micelije (kolonija) fitopatogene gljive *V. inaequalis*, što je rezultat znatno veće količine bakarnih jona koje ovo jedinjenje oslobađa. Standardni preparat bakar hidroksid nije inhibirao porast kolonija gljive ni u najvećoj primenjenoj koncentraciji od 0,4%. Ovi rezultati slažu se sa rezultatima koje su saopštili Popović i sar. (2012), koji su ispitujući uticaj bakar-citrata i više drugih fungicida na bazi bakra, utvrdili znatno viši stepen inhibicije porasta kolonija gljive *Monilinia laxa* koju je ostvario bakar-citrat u odnosu na druge ispitivane fungicide na bazi bakra u komercijalnoj primeni.

Balaž i sar. (2010) su u ispitivanjima mogućnosti suzbijanja *V. inaequalis* na jabuci (ajdared) ekološki prihvatljivim preparatima (neorganski fungicidi i kisela glina) tokom 2009. i 2010. godine utvrdili visoku efikasnost preparata na bazi bakra primenjenih u niskim koncentracijama (0,05%) u suzbijanju *V. inaequalis* na listovima (87,4-99,7%), dok je efikasnost bakarnih preparata na plodovima bila različta (48,5-90,2%). Autori navode da u sistemu organske proizvodnje, program zaštite jabuke od *V. inaequalis* tokom vegetacije treba dopuniti korišćenjem preparata na bazi bakra u niskoj koncentraciji.

Kurnik i sar. (2011) su, u cilju poređenja efikasnosti nekoliko formulacija bakra sa kontaktnim i sistemičnim delovanjem protiv prouzrokovača čađave krastavosti jabuke, obavili trogodišnje oglede u plantažnim zasadima jabuke. Testirane formulacije bile su na bazi bakar-kalcijum oksihlorida, bakar sulfata i kompleksa ili helata bakra sa aminokiselinama, peptidima, EDTA, urea, kaprilne i glukonske kiseline u cilju utvrđivanja da li će formulacije bakra sa sitemičnim delovanje omogućiti viši nivo biološke efikasnoisti u suzbijanju čađave krastavosti od tradicionalnih formulacija bakra sa kontaktnim delovanjem. Nisu utvrđene statistički značajne razlike u efikasnosti, mada je utvrđena neznatno viša efikasnost bakarnih formulacija sa sistemičnim delovanjem. Sve testirane formulacije bakra su pokazale malo niži nivo efikasnosti u suzbijanju čađave krastavosti nego konvencionalni fungicidi i preparati korišćeni u sistemu organske proizvodnje te kao takve mogu biti preporučene za suzbijanje proizrokovača čađave krastavosti jabuke.

Postojeći nivo primene bakarnih preparata mora biti značajno smanjen. Neke evropske zemlje su već odlučile da zabrane sve proizvode na bazi bakra, dok su druge odlučile da znatno smanje njihovo korišćenje. Značajna redukcija korišćenja bakarnih proizvoda može biti ostvarena delimičnom zamenom bakarnih preparata drugim aktivnim supstancama, smanjenjem broja aplikacija tokom godine ili smanjenjem količine primene bakra po jedinici površine (Jama and Lateur, 2007). Primena bakar-citrata kao kompleksnog jedinjenja bakra koje se odlikuje višim stepenom disocijacije u odnosu na druga bakarna jedinjenja koja su trenutno u primeni i mogućnost njegovog korišćenja u nižim koncentracijama za suzbijanje biljnih patogena je novi potencijalni prilog pomenutim nastojanjima.

ZAHVALNICA

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INFLUENCE OF COPPER CITRATE ON COLONY GROWTH OF *VENTURIA INAEQUALIS*

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SUMMARY

Copper citrate is a complex compound of copper, which is characterized by a higher degree of dissociation in relation to the other copper compounds that are presently in the use and can be used in lower concentrations for the control of plant pathogens. Apple scab (*Venturia inaequalis*) is on of the economically most important diseases of apples in all regions where this fruit species grown. A successful apple production is not possible without intensive chemical control. The use of copper-based products to protect apples, positioned at the start of the growing season. The earliest apple infections are the most destructive, and the effective protection of the apples in this period is very important. The aim of this study was to evaluate *in vitro* the influence of copper citrate at different concentrations applied to the growth of *V. inaequalis* colonies. The results show that copper citrate in significantly lower concentrations of application 0.2 and 0.1 % higher than the standard product, effectively inhibiting the growth of *V. inaequali* mycelium (colony), which is the result of significantly higher amounts of copper ions, that is released from compound.

Key words: apple scab, copper citrate, colony growth, efficacy

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ISPITIVANJE ZDRAVSTVENOG STANJA RAZLIČITIH GENOTIPOVA SEMENA LUCERKE

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REZIME

U ovom radu je ispitivano prisustvo fitopatogenih rodova gljiva na semenu tri različite sorte lucerke (K-22, NS-Banat i OS-88) sa po tri različite partije (lokaliteta) od svake sorte. Kod ispitivanih sorti identifikovani su sledeći rodovi gljiva: *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., *Mucor* spp. i sterilna micelija. Prisustvo identifikovanih rodova gljiva kretalo se u rasponu od 0% do 2%. Rod *Alternaria* spp. najmanju prosečnu vrednost imao je kod sorte lucerke K-22 (0.42%), dok je najveću prosečnu vrednost imao kod sorte OS-88 (0.58%), a takođe kod iste sorte konstatovano je najveće prisustvo od (1%) na lokalitetu Osijek I. Kod svih ispitivanih sorata lucerke utvrđen je visok koeficijent varijacije, što ukazuje na visoku varijabilnost unutar samih sorata za ovo svojstvo. Najveće prosečno prisustvo gljiva roda *Fusarium* spp. zabeleženo je kod sorte OS-88 (0.75%), a kod iste sorte je najveće prisustvo (1.25%) zabeleženo na lokalitetu Osijek II. Na proučavanim sortama lucerke ispitani su korelacioni odnosi između parametara kvaliteta semena i prisustva patogena na semenu različitih sorata i partija lucerke. Jake pozitivne korelacije zabeležene su između energije klijanja i ukupne klijavosti ($r=0,891^{***}$), kao i između mase 1000 semena i prisustva gljiva iz roda *Fusarium* spp. ($r=0,797^{**}$). Rezultati ovih ispitivanja ukazuju na zadovoljavajuće zdravstveno stanje svih sorata i partija semena lucerke a posebno u odnosu na ekonomski značajne fitopatogene gljive iz roda *Fusarium* spp.

Ključne reči: lucerka, seme, patogeni, zdravstveno stanje

UVOD

Plava lucerka (*Medicago sativa* L.) jedna je od najstarijih krmnih biljnih vrsta i bila je poznata još pre 8000 godina. Vodi poreklo sa teritorije današnjeg Irana i Arabije, mada se vrste roda *Medicago* mogu naći po celoj Aziji (Michaud et al., 1988). U svetu se gaji na površini od 33 miliona hektara, a u Srbiji je zastupljena na površini od oko 190.000 hektara (Štrbanović, 2010). Areal gajenja lucerke je na svim kontinentima u više od 80 zemalja, od umereno hladnog do tropskog pojasa. Široka geografska rasprostranjenost lucerke uslovljena je njenom velikom adaptabilnošću na različite klimatske i zemljišne uslove (Julier et al., 1995).

Lucerka je višegodišnja leguminozna stranooplodna biljka entomofilnog načina oprašivanja i prirodni je autotetraploid (2n=4n=32). Lucerka je, posle kukuruza, najvažnija krmna vrsta u našoj zemlji, zahvaljujući ne samo povoljnom hemijskom sastavu i visokom sadržaju proteina, već i visokim prinosima i veoma dobrim biološkim osobinama. U ishrani domaćih životinja može se koristiti kao zelena masa, seno ili konzervisana u kombinaciji sa drugim krmnim biljkama (Đorđević i Dinić, 2007).

Lucerku napada veliki broj prouzrokovača biljnih bolesti i biljne štetočine. Mikroorganizmi koji izazivaju trulež korena i uvenuća lucerke su glavni agensi koji izazivaju progresivnu pad produktivnosti lucerke. Mnogi patogeni koji izazivaju bolesti nadzemnog dela i korena lucerke se prenose zaraženim semenom, tako da seme može da predstavlja veoma opasan izvor zaraze. Razne patogene gljive (*Phytophthora* spp., *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp., *Colletotrichum* spp., *Verticillium* spp., *Sclerotinia* spp. itd.) izazivaju određene vrste truleži korena i stabljike lucerke, a mogu se javiti i nespecifični simptomi na biljkama kao što su niži porast, hloroza i uvenuće (Krnjaja i sar., 2011). Seme igra bitnu ulogu u proizvodnji zdravih useva, ali na sebi mogu da nose i neke destruktivne gljive koje mogu da izazovu trulež semena, smanjenje klijavosti i totalno uništenje klijanaca nakon klijanja semena (Abdul-Aziz A. et al., 2011). Najštetniji patogen na semenu lucerke je gljiva *Fusarium oxysporum* f. sp. *medicaginis* koja izaziva bolest uvenuća lucerke i prenosi se semenom (Štrbac i sar., 1996). Patogeni semena lucerke prouzrukuju štetu u manjoj ili većoj meri, a posledice su smanjen kvalitet i gubitak prinosa krme. Korišćenjem zdravstveno ispravnog semena za setvu lucerke, rasprostranjenost i širenje žarišta iz kojih bi se neke bolesti proširile i na većim površinama bilo bi sprečeno, što je posebno opasno ako se ima u vidu da je lucerke višegodišnja biljna vrsta (Krnjaja i Lević, 2005). Dakle, zbog opasnosti od širenja bolesti lucerke preko zaraženog semena, veoma je važno da se koristi kvalitetno i zdravstveno ispravno seme za setvu.

Istraživanja obuhvaćena ovim radom imaju za cilj utvrđivanje zdravstvenog stanja semena tri različite sorte lucerke i tri različite partije svake sorte i njegovog uticaja na parametre kvaliteta semena.

MATERIJAL I METODE

U cilju realizacije ovog ogleda istraživanje je obavljeno u akreditovanoj laboratoriji za ispitivanje kvaliteta semena i sadnog materijala Instituta za zaštitu bilja i životnu sredinu u Beogradu. Materijal koji se koristi u ovom istraživanju je seme tri različite sorte lucerke (K-22, NS-Banat i OS-88) sa po tri različite partije (lokaliteta) od svake sorte. Žetva semena obavljena je u avgustu mesecu 2013. godine, a seme svih partija ispitivanih sorata lucerke čuvano je u papirnim kesama i kontrolisanim uslovima temperature i relativne vlažnosti vazduha tj. u komori za skladištenje i čuvanje semena.

Ispitivanje zdravstvenog stanja semena lucerke utvrđeno je prema pravilniku o kvalitetu semena poljoprivrednog bilja „Službeni list SFRJ" br. 47/87 Republike Srbije. Radni uzorak predstavlja 4 x 100 semena od svake partije različitih sorata lucerke. Seme je dezinfikovano u 1% rastvoru natrijum-hipohlorita (NaOCl) u trajanju od 10 minuta a potom isprano sa destilovanom vodom i osušeno na filter papiru. Seme je postavljeno u petri šolje prečnika 90mm koje su predhodno sterilisane u autoklavu a za podlogu je korišćen filter papir natopljen destilovanom vodom do potpunog zasićenja. Uzorci su stavljeni u klijalište i inkubirani na t20^{o}C sedam dana sa naizmeničnim osvetljenjem, 12h u tami i 12h sa ultravioletnim osvetljenjem (UV) talasne dužine 360nm. Ispitivanje razvijenih kolonija gljiva oko semena izvršeno je na mikroskopu, a na osovu morfologije konidija rodovi gljiva identifikovani su prema (Nelson et al., 1983; Burgess et al., 1994; Watanabe, 1994). Rezultati ispitivanja zdravstvenog stanja semena iskazani su u % obolelog semena.

Parametri kvaliteta semena, energija klijanja, ukupna klijavost i masa 1000 semena takođe su utvrđeni prema navedenom pravilniku o kvalitetu semena poljoprivrednog bilja.

REZULTATI

Ispitivanjem energije klijanja i ukupne klijavosti kod različitih sorata i partija lucerke utvrđeno je da je u proseku najveću energiju klijanja (76%) i ukupnu klijavost (79%) imala sorta lucerke NS-Banat, a najveće vrednosti za energiju klijanja (84%) i ukupnu klijavost (86%) zabeležene su na lokalitetu Ada. U proseku najveću vrednost za masu 1000 semena imala je sorta lucerke OS-88 (2.08g), dok je najmanja prosečna vrednost zabeležena kod sorte lucerke K-22 (1.82g) (Tabela 1).

Na osnovu morfologije kolonija i njihovih konidija identifikovani su sledeći rodovi gljiva: *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., *Mucor* spp. i sterilna micelija. Prisustvo identifikovanih rodova gljiva kretalo se u rasponu od 0% do 2%. Rod *Alternaria* spp. najmanju prosečnu vrednost imao je kod sorte lucerke K-22 (0.42%), dok je najveću prosečnu vrednost imao kod sorte OS-88 (0.58%), a takođe kod iste sorte konstatovano je najveće prisustvo od (1%) na lokalitetu Osijek I. Kod svih ispitivanih sorata lucerke utvrđen je visok koeficijent varijacije, što ukazuje na visoku varijabilnost unutar samih sorata za ovo svojstvo. Najveći koeficijent varijacije utvrđen je kod sorte OS-88 (65.84%), kod sorte NS-Banat (48.31%), dok je kod sorte K-22 iznosio (34.37%). Ukupan koeficijent varijacije u okviru svih ispitivanih sorata lucerke iznosio je (49.55%). Najveće prosečno prisustvo roda *Fusarium* spp. zabeleženo je kod sorte OS-88 (0.75%), a kod iste sorte je najveće prisustvo (1.25%) zabeleženo na lokalitetu Osijek II. Najmanje prosečno prisustvo ove fitopatogene gljive konstatovano je kod sorte K-22 (0.33%), dok je kod sorte NS-Banat u proseku utvrđeno (0.48%). Visok koeficijent varijacije utvrđen je kod sorte OS-88 (23.57%), dok kod ostale dve sorte nije bio značajan, K-22 (5.83%)

i NS-Banat (7.37%). Ukupan koeficijent varijacije na nivou svih ispitivanih sorata bio je visok i iznosio je (42.66%). Prisustvo roda *Penicillium* spp. utvrđeno je samo kod sorte NS-Banat i to na lokalitetu Titel (0.5%), dok je prisustvo roda *Mucor* spp. takođe utvrđeno samo kod sorte NS-Banat na lokalitetu Rusko selo (2%). Sterilna micelija utvrđena je kod sorte K-22 na lokalitetu Osipaonica (0.5%) (Tabela 2).

Na proučavanim sortama lucerke ispitani su korelacioni odnosi između parametara kvaliteta semena i prisustva patogena na semenu različitih sorata i partija lucerke. Jake pozitivne korelacije zabeležene su između energije klijanja i ukupne klijavosti ($r = 0{,}891^{***}$), kao i između mase 1000 semena i prisustva gljive iz roda *Fusarium* spp. ($r = 0{,}797^{**}$), što se može videti i u prikazanim rezultatima gde su sorte sa većom masom 1000 semena imala i veći procenat zaraze sa fitopatogenim gljivama iz roda *Fusarium* spp. Tako u slučaju sorte OS-88 koja je imala najveću prosečnu masu 1000 semena (2.08g) u proseku imala prisustvo *Fusarium* spp. (0.75%), sorta NS-Banat imala je prosečnu masu 1000 semena (1.93g) i prosečno prisustvo *Fusarium* spp. (0.48%), dok je sorta K-22 imala najmanju prosečnu masu 1000 semena i prosečno prisustvo *Fusarium* spp. (0.33%). Za ostale praćene paremetre kvaliteta i prisustva patogena izračunate korelacije nisu bile statistički značajne (Tabela 3).

Tabela 1. Parametri kvaliteta semena.
Table 1. Seed quality parameters.

Sorta (Cultivar)	Lokalitet/partija Locality/lot	Energija klijanja(%) Germination energy(%)	Ukupna klijavost(%) Total germination(%)	Masa 1000 semena(g) Mass of 1000 seeds(g)
K-22	Ratari	70	74	1.80
	Osipaonica	49	53	1.83
	Aleksandrovo	45	52	1.83
prosek (average)		**55**	**60**	**1.82**
NS-Banat	Titel	72	75	2.03
	Rusko selo	72	75	2.03
	Ada	84	86	1.73
prosek (average)		**76**	**79**	**1.93**
OS-88	Osijek I	50	66	2.15
	Osijek II	69	73	2.13
	Istra	61	79	1.95
prosek (average)		**60**	**73**	**2.08**

Tabela 2. Izolacija fitopatogenih gljiva na semenu lucerke (%).
Table 2. Isolation phytopathogenic fungi on alfalfa seed (%).

Sorta (Cultivar)	Lokalitet/partija Locality/lot	Gljive (%) Fungus (%)				
		Alternaria spp.	*Fusarium* spp.	*Penicillium* spp.	*Mucor* spp.	sterilna micelija
K-22	Ratari	0.5	0	0	0	0
	Osipaonica	0.25	0.25	0	0	0.5
	Aleksandrovo	0.5	0.75	0	0	0
prosek (average)		**0.42**	**0.33**	**0**	**0**	**0.17**
CV (%)		**34.37**	**5.83**	-	-	-
NS-Banat	Titel	0.75	0.75	0.5	0	0
	Rusko selo	0.7	0.7	0	2	0
	Ada	0.25	0	0	0	0
prosek (average)		**0.57**	**0.48**	**0.17**	**0.67**	**0**
CV (%)		**48.31**	**7.37**	-	-	-
OS-88	Osijek I	1	1	0	0	0
	Osijek II	0.25	1.25	0	0	0
	Istra	0.5	0	0	0	0
prosek (average)		**0.58**	**0.75**	**0**	**0**	**0**
CV (%)		**65.84**	**23.57**	-	-	-
Ukupan CV (%) Total CV (%)		**49.55**	**42.66**	-	-	-

Tabela 3. Korelaciona međuzavisnost kvaliteta semena i prisustva patogena na semenu lucerke.
Table 3. Correlation interdependence of seed quality and the presence of pathogens on alfalfa seeds.

	Ukupna klijavost Total germination	Masa 1000 semena Mass of 1000 seeds	*Alternaria* spp.	*Fusarium* spp.	*Penicillium* spp.	*Mucor* spp.	Sterilna micelija Sterile mycelium
Energija klijanja Germination energy	0.891***	-0.117ns	-0.214ns	-0.243ns	0.241ns	0.241ns	-0.416ns
Ukupna klijavost Total germination		0.047ns	-0.013ns	-0.286ns	0.153ns	0.153ns	-0.57ns
Masa 1000 semena Mass of 1000 seeds			0.565ns	0.797**	0.217ns	0.217ns	-0.277ns
***Alternaria* spp.**				0.356ns	0.33ns	0.258ns	-0.395ns
***Fusarium* spp.**					0.181ns	0.141ns	-0.216ns
***Penicillium* spp.**						-0.125ns	-0.125ns
***Mucor* spp.**							-0.125ns

$p \leq 0{,}05^{*}$; $p \leq 0{,}01^{**}$; $p \leq 0{,}001^{***}$; ns-razlike nisu statistički značajne (differences are not statistically significant)

DISKUSIJA

Seme je veoma pogodan supstrat za održavanje i širenje patogenih prouzrokovača biljnih bolesti. Zdravstvena kontrola semena je neophodna kao preventivna mera u suzbijanju širenja ekonomski značajnih bolesti lucerke. U Srbiji ne postoji dovoljno podataka o zdravstvenom stanju semena lucerke u odnosu na prisustvo važnih patogenih gljiva. Ispitivanjem semena različite starosti na šest sorata lucerke identifikovano je sedam rodova gljiva, *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp., *Stemphylium* spp. i sterilna micelija sa prisustvom od 0 do 6,5% (Krnjaja i sar., 2011). Takođe, analizom zdravstvenog stanja semena šest domaćih sorti lucerke, u tretmanu gde je seme dezinfikovano natrijum-hipohloritom (NaOCl) ustanovljeno je prisustvo gljiva iz sledećih rodova: *Alternaria* spp. (0-5%), *Cladosporium* spp. (0-1%), *Fusarium* spp. (0-2%) and *Stemphylium* spp. (0-1%) (Krnjaja i sar., 2003). Analizom zdravstvenog stanja 10 domaćih sorti lucerke utvrđeno je prisustvo gljiva iz roda *Alternaria* spp. (3-48%), *Botrytis* spp. (0-10%) i *Fusarium* spp. (0- 8%) (Lukić i Purar, 1996).

Prema Pravilniku o zdravstvenom pregledu useva i objekata za proizvodnju semena, rasada i sadnog materijala i zdravstvenog pregleda semena, rasada i sadnog materijala dozvoljen je sledeći nivo infekcije semena lucerke (%): *Colletotrichum* spp. (1%), *Fusarium* spp. (2%), *Kabatiella caulivora* (2%), *Sclerotinia* spp. (0%), *Stemphylium* spp. (1%), *Verticillium albo atrum* (1%), virus mozaika lucerke (AMV) (0%), *Cuscuta* spp. (0%) (Službeni list SRJ, 1999).

Prema navedenom pravilniku i ispitivanja koja su sprovedena u našoj studiji možemo zaključiti da semena svih sorata i partija lucerke u odnosu na prisustvo fitopatogenih gljiva imaju zadovoljavajuće zdravstveno stanje, a najtolerantnija sorta na prisustvo navedenih patogena je K-22. Najmanju tolerantnost na prisustvo navedenih fitopatogenih gljiva pokazala je sorta OS-88 koja je imala najveći procenat prisustva gljive iz roda *Fusarium* spp. u odnosu na druge sorte i partije semena lucerke i to čak na dve lokaliteta, Osijek I (1%) i Osijek II (1.25%).

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HEALTH TESTING DIFFERENT GENOTYPES ALFALFA SEEDS

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SUMMARY

In this study we investigated the presence of plant pathogenic genera of fungi on seeds of three different cultivars of alfalfa (K-22, NS-Banat and OS-88) with three different lots (locality) of each cultivars. When tested cultivars were identified following genera of fungi: *Alternaria* spp ., *Fusarium* spp., *Penicillium* spp., *Mucor* spp. and sterile mycelium. The presence of the identified genera of fungi ranged from 0% to 2%. Genus *Alternaria* spp. lowest average value was in alfalfa cultivars K-22 (0.42%), while the highest average value of a cultivar OS-88 (0.58 %), and also at the same cultivar was noted by the presence of one (1%) at the locality of Osijek I. In all the cultivars of alfalfa, a high coefficient of variation, indicating a high variability within the three cultivars for this trait. The highest average attendance of the genus *Fusarium* spp. was observed in cultivar OS-88 (0.75%), and in the same cultivar is the largest presence (1.25%) were recorded at the locality of Osijek II. For the studied alfalfa cultivars were examined correlations between parameters of seed quality and the presence of pathogens on seeds of different cultivars of alfalfa and lots. Strong positive correlations were observed between germination energy and total germination ($r=0.891^{***}$), and between mass of 1000 seeds and the presence of *Fusarium* spp. ($r=0.797^{**}$). The results of these tests indicate satisfactory state of health of all cultivars and alfalfa seed lots and in particular in relation to the economically important phytopathogenic fungi of the genus *Fusarium* spp.

Key words: alfalfa, seed, pathogens, health condition

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UPUTSTVO AUTORIMA

Časopis “Zaštita bilja“ objavljuje naučne radove, pregledne radove i prethodna saopštenja iz oblasti zaštite bilja. Radovi se štampaju na srpskom ili engleskom jeziku. Uz radove na engleskom jeziku štampa se i rezime na srpskom jeziku. Rukopis (1)otkucan sa duplim proredom sadrži: zaglavlje, naslov, imena autora i adrese, rezime, ključne reči, tekst rada (sa poglavljima: uvod, materijal i metode, rezultati, diskusija, zahvalnica, literatura i rezime sa ključnim rečima), tabele i grafikone, fotografije i crteže.

ZAGLAVLJE – u gornjem, desnom uglu upisuje se kategorizacija rada. **NASLOV** – pisan velikim slovima (bold) treba da bude kratak, jasan, bez skraćenica. Ne navoditi istovremeno ime vrste na srpskom i na latinskom jeziku. **APSTRAKT** – treba da sadrži najviše 200 reči teksta. **KLJUČNE REČI** – treba navesti do 6 ključnih reči. **TEKST** – treba da sadrži poglavlja: UVOD, MATERIJAL I METODE, REZULTATI, DISKUSIJA, LITERATURA i REZIME (na srpskom i engleskom jeziku istog sadržaja) sa ključnim rečima. LITERATURA se navodi na posebnoj stranici, po abecednom redu. Npr. Arsenijević, M., Draganić M., Knežević Tatjana (1996): Vrste nekadašnjeg roda *Helminthosporium* utvrđene u Jugoslaviji (1992-1995). Zaštita bilja, 216: 93-119. Citat iz knjige navesti prema primeru: Dhingra O. D., Sinclair, J. B. (1955): Basic Plant Pathology Methods. CCR Press Inc., Baco Raton, pp. 355-360. U tekstu, na kraju citata, navesti autore na sledeći način: (Matijević 1994; Stojanović i Borić, 1990; Manojlović i sar., 1998). REZIME sa ključnim rečima treba da je na engleskom i srpskom i daje se na posebnim stranicama na kraju teksta i treba da sadrži ime autora i naziv ustanove.

Naslov poglavlja u radu (prvi nivo naslova) pisati centrirano, velikim slovima, boldovano.

Podnaslov (drugi nivo naslova) pisati centrirano, prvo slovo veliko, ostala slova mala, boldovano, sa jednim redom razmaka od teksta na koji se odnosi.

Podnaslov (treći nivo naslova) pisati od početka reda, prvo slovo veliko, ostala slova mala. Pisati zakošeno (italik), sa jednim redom razmaka od teksta na koji se odnosi.

Podnaslov (četvrti nivo naslova) pisati na početku reda zakošenim slovima (italik), s tim da je sastavni deo teksta na početku reda i od njega odvojen crticom.

TABELE I GRAFIKONI – Tabele i grafikoni se daju na posebnim stranicama. U rukopisu označiti mesto za tabele i grafikone. Naslove tabela i grafikona obavezno dati prvo na srpskom, pa na engleskom jeziku, a ako je rukopis na engleskom, onda prvo navesti engleski, pa srpski tekst naslova.

FOTOGRAFIJE I CRTEŽI – Fotografije i crteži treba da su kontrasni i oštri. Na poleđini

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A manuscript, double-spaced printed, contains: chapter, title, the name of an author and addresses, abstract, key words, text (including the chapters: introduction, material and methods, results, discussion, acknowledgements, references and summary followed by key words), tables and graphs, photographs and drawings.

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