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Review paper

Methods for management of soilborne plant pathogens

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SUMMARY

Soilborne pathogens cause significant economic losses in agricultural production all over the world. These species can survive for many years in the absence of a host plant by forming persistent structures such as microsclerotia, sclerotia, chlamydospores or oospores. Consequently, soilborne diseases are particularly difficult to predict, detect, diagnose and successfully control. Over the past 30 years, a fumigant, methyl bromide, has been widely used for their control in many crops. In 1992, methyl bromide was listed as an ozone-depleting substance under the Montreal Protocol – an international treaty to protect the ozone layer. During the phaseout of methyl bromide, problems generated in agricultural production made it clear that dependence on a single method or a single chemical should be avoided. The objective of this review paper was to summarize the current knowledge about different methods of soilborne disease control including: crop rotation, steam soil disinfection, soil amendments, hydroponics and soilless growing systems, soil solarization, grafting, biological control and use of natural compounds, and chemical control. Positive and negative aspects of all available methods were reviewed. Benefits, achieved by simultaneous application of several methods based on different mechanisms of actions, are discussed.

Keywords: Soilborne diseases; Methyl bromyde alternatives; Management; Soil disinfestation

INTRODUCTION

Soilborne pathogens cause significant economic losses in crop production. They affect crops from their initial stages in nurseries until harvest. Plant diseases caused by *Rhizoctonia* spp., *Fusarium* spp., *Verticillium* spp., *Sclerotinia* spp., *Pythium* spp., and *Phytophthora* spp. affect a number of important crops, including wheat, cotton, vegetables and temperate fruits. Economic losses caused by Fusarium wilt of column stock pathogens are estimated at 50-75% of the attainable yield for many crops (Lewis & Papavizas, 1991). The symptoms of soilborne disease, caused by different pathogens, are very similar. They include root rot, root blackening, wilt, yellowing, stunting or seedling damping-off, bark cracking and twig or branch dieback. Soilborne species can survive for many years in the absence of a host plant by forming resistant structures such as: microsclerotia,

sclerotia, chlamydospore or oospores. Consequently, these pathogens are particularly difficult to predict, detect, diagnose and control (Åström & Gerhardson, 1988).

Fumigants have always been a major tool for soil disinfestation. However, certain fumigants are associated with harmful side effects, such as health hazards, environmental pollution, and even potential atmospheric ozone depletion. Historically, over the past 30 years, the fumigant methyl bromide has been widely used to control soilborne diseases and pests in fruit and vegetable production. In 1992, methyl bromide was listed as an ozone-depleting substance under the Montreal Protocol – an international treaty to protect the ozone layer (Bell, 1996).

During the phaseout of methyl bromide, problems generated in agricultural productions made it clear that dependence on a single method for soilborne disease and pest management should be avoided (Katan, 1999, 2000). To overcome problems with soilborne diseases, different approaches have been tested in the past. However, none of the approaches completely eliminates pathogens from soil. In addition, their effects are not immediately efficient, like the effects of conventional fungicides that are the most common method to control soilborne diseases. Using fungicides against pathogens can help to control diseases. However, their frequent and indiscriminate use may also lead to atmospheric pollution and development of fungicide resistance (Christopher et al., 2010).

Due to environmental concerns, researchers have focused on finding alternatives to chemical pesticides for suppression of soilborne plant pathogens and plant parasitic nematodes (Larkin et al., 1998). In this context, alternative approaches including crop rotation, use of soil amendment, solarisation, biofumigation, biological soil disinfestations, grafting, and application of biocontrol agents or organic amendments, such as composts, are of considerable interest among scientists and agricultural producers (Kirkegaard et al., 2000; Ryckeboer, 2001; Bailey & Lazarovits, 2003; Louws et al., 2010).

The objective of this review paper was to summarize current knowledge about different methods of soilborne disease control which includes: crop rotation, steam soil disinfection, soil amendments, hydroponics and soilless growing systems, soil solarization, grafting, biological control and natural compounds, and chemical control.

CROP ROTATION

Crop rotation, in general, provides numerous benefits to crop production. It has been associated with enhanced soil fertility, increased soil tilth and aggregate stability, improved soil water management, and reduced erosion (Ball et al., 2005). Crop rotation is without a doubt a valuable method for plant disease management. However, it is insufficiently effective in reducing diseases caused by soilborne pathogens that have a wide host range or produce long-living survival structures, such as sclerotia, oospores or chlamidospores (Umaerus et al., 1989).

Small grains, especially barley, are highly recommended to improve organic matter content and reduce problems from pink root and Fusarium basal rot of onion (Schwartz, 2004). Oat-potato, annual ryegrass-potato or clover-potato crop sequences have been found to reduce both *Rhizoctonia solani* inoculum levels in soil and suppress subsequent disease development in a potato crop (Johnston et al., 1994). Canola and rapeseed were the most consistent and effective rotation crops for reducing the soilborne diseases commonly observed in those fields, including Rhizoctonia canker, black scurf, and common scab, with overall reductions ranging from 18 to 38 %. Green bean and soybean rotations had no positive benefits related to any of the soilborne potato diseases, and disease development was comparable with continuous potato. Barley and clover, on the other hand, demonstrated reductions in Rhizoctonia canker and black scurf through the first couple of rotation cycles, after which these diseases increased to levels comparable with continuous potato (Larkin et al., 2010).

Full-season rotation crops (barley, ryegrass, canola, and rapeseed) in 2 and 3-year rotations with potato have substantially reduced Rhizoctonia-caused diseases of potato (15-50%) (Larkin et al., 2012). Wiggins and Kinkel (2005) found that cropping sequences involving potato had a profound effect on soil microbial community. However, in their study, crop rotation was ineffective in controlling potato scab disease. Alfalfa-potato, corn-potato and potato-potato crop sequences were also ineffective against Vericillium wilt. Furthermore, the severity of Verticillium wilt in winter rapeseed grown in crop rotation system (winter rapeseed, winter wheat, field peas, spring wheat and winter rapeseed) was not found to decrease compared to a 5-year monoculture (Cwalina-Ambroziak et al., 2016). Additionally, wheat grown in four different long-term crop rotations including winter wheat, maize, sugar beet and oil radish as pre-crops, had no positive effect on suppression of *Fusarium* spp. The results of that study indicated that sugar beet and maize sawn as pre-crops of wheat may increase the risk of *Fusarium* infestation to a much greater extent than wheat grown after wheat, which did not have such a critical effect on disease incidence (Tillmann et al., 2016).

STEAM SOIL DISINFECTION

Steam was first applied in 1888 in Germany. It was the primary method of soil sterilization in the greenhouse industry prior to the emergence of soil fumigants (Baker & Olsen, 1960). Soil steaming is a sound alternative to methyl bromide for controlling soilborne diseases and pests in protected production systems. Steam disinfection represents an effective method that does not leave any harmful residues in soil, nor generate toxic volatile compaunds in the environment (Runia, 2000). Furthermore, no waiting period is required for planting. Therefore, steamed soils can be used for cultivation as soon as they cool, unlike chemically treated soils that require a relatively long aeration period (Slusarski et al., 2012a).

Steam disinfection involves injecting or diffusing hot water vapour into the soil with the aid of a boiler and conductors. Soil treatment by steaming at 80-100°C for half an hour effectively controls most soilborne pathogens, pests and weeds (Braun & Supkoff, 1994). The method seems to be acceptable for greenhouse production of ornamental plants, grown directly in the soil or substrates (Labrada, 2008). It is also applied pre-planting in small areas for the production of vegetables and other minor crops (Tateya, 2001). Soil disinfection by steaming has effectively controlled *R. solani* on tobacco; the pathogen was eliminated by steaming trays for 30 min at 80°C (Gutierrez et al., 1997). However, Minuto et al. (2005) reported that nearly 3 hours of steaming were needed for elimination of *F. oxysporum* and *R. solani* from a sandy-loam soil at a depth of 16 cm. On the other hand, stem was not effective against Fusarium wilt of column stock in UK. According to O'Neill et al. (2005), steam treatment reduced the viability of *F. oxysporum* f.sp. *mathioli* by 97 to 41 %.

The use of stream is very expensive and only practical and cost-effective under greenhouse conditions. It requires high initial investments and consumes a high quantity of energy (Gullino & Lodovica, 1992). Soil steaming is non-selective, so it leaves a biological "vacuum" suitable for fast re-infestation by plant pathogens (Mus & Huygen, 1992).

SOIL AMENDMENTS

Plants and plant products, such as organic amendments, crop residues, compost, green manures, fish and blood meal, biochar, chitosan-based products, etc. can result in significant reduction levels of soilborne disease incidence (Bailey & Lazarovits, 2003; Garbeva et al., 2004).

Organic amendments

Many herb plants contain essential oils, including terpenes, as well as phenols, alcohols, organic acids and other compounds with potentially biocidal activity (Paret et al., 2010). However, literature data on the use of herbs as organic amendments for control of soilborne pathogens are rather rare (Kirkegaard et al., 2000; El-Sharouny, 2015). There are some reports of their successful application in the United States, Indonesia, United Kingdom, Israel and Italy (Mazzola et al., 2001; Yulianti et al., 2006; Larkin & Griffin, 2007; Klein et al., 2007; Colla et al., 2012).

Crops of the family *Brassicaceae* (cabbage, broccoli, kale, turnip, radish, canola, cauliflower, rapeseed and various mustards) contain substances that have been associated with reductions in soilborne pathogens and pests. *Brassica* crops produce sulfur compounds called glucosinolates that release biologically active products during enzymatic hydrolysis. Isothiocyanates, the main metabolites of the glucosinolates hydrolysis are vapour compounds that are toxic to many soil organisms. They have been successfully used to reduce populations of soilborne pathogens in a process referred to as biofumigation (Larkin & Honeycutt, 2006; Larkin & Griffin, 2007). Thus, soil amendment with *Brassica napus* seed meal reduced the incidence of apple root infection by *Rhizoctonia* spp. (Mazzola et al., 2001; El-Sharouny, 2015). Xiao et al. (1998) observed that broccoli residues flail-mowed and allowed to dry on the soil surface for several days prior to incorporation were effective in reducing soil populations of *Verticillium dahliae* in cauliflower fields.

Plants belonging to the *Alliaceae* family, such as garlic, onion, and leek also contain molecules with either a direct or indirect effect on pests and pathogens. Crushed *Allium* spp. plants produce thiosulfinates and related compounds such as disulfides, which have the same spectrum of pesticide activity as methyl bromide (Auger et al., 2004). Therefore, *Allium* tissues or extracts having biocidal activities against fungal pathogens could be used in agriculture as a part of Integrated Pest Management (Arnault et al., 2013).

Although organic amendments may be useful for the management of soilborne diseases, they are not widely implemented due to concerns about potential side-effects (non-selective activity, cost effectiveness and scale practicality) (Yulianti et al., 2006; Klein et al., 2007; Colla et al., 2012).

Compost and animal manure

Manure and compost are organic sources of nutrients that have been shown to increase soil organic matter and improve soil quality (Wright, 1998). Besides its nutrient components, compost contains high amounts of beneficial organisms that prevent and help controlling soilborne diseases. It has multiple mechanisms of disease suppression: increased plant vigor caused by nutrient availability, presence of large populations of beneficial microorganisms, and increased drainage (Mehta et al., 2014). Compost-inhabiting microorganisms produce plant growth hormones and chemical compounds (e.g. siderophores, tannins, phenols) which are antagonistic to various soilborne pathogens. Compost provides effective control of soilborne pathogens such as *Phoma* spp. and *Pythium* spp. The use of composted softwood and hardwood barks gave reproducible control of *Pythium ultimum* in lettuce, *R. solani* in cucumber, radish, and bedding plants under greenhouse conditions (Lagunas-Solar et al., 1993; Stephens & Stebbins, 1985).

Animal manure is used for soil fertilization. It is also an important component of substrates for container media, used to improve soil properties, plant health and yield. Decomposition of animal manure leads to ammonia (NH3) release (Stapleton et al., 2000). Ammonia has been widely reported to adversely affect the survival or germination of certain soilborne fungi and nematodes (Rodríguez-Kábana, 1986).

Poultry manure, that contains a high percentage of nitrogen and other elements (e.g. potassium and phosphorus) and cattle manure, widely available in commercial quantities, seems to be an appropriate material to be used in combination with soil solarization. Conn and Lazarovits (1999) showed that the application of fresh chicken manure could be highly effective in reducing the incidence of potato scab, Verticillium wilt and parasitic nematodes. The reduction in potato scab was also achieved by swine manure. Numerous studies have confirmed that solarization of soil amended with chicken manure, chicken litter or plant residues was highly effective in controlling soilborne pathogens. Furthermore, this combination was more effective than single treatment alone (Gamliel et al., 2000; Stevens et al., 2003).

Biochar

Over the last 10 years, the use of biochar as an organic soil amendment for sequestering carbon and enhancing soil fertility has received a considerable research attention. Biochar is a form of charcoal produced from the heating of biomass-derived feedstocks under oxygen-limited conditions via pyrolysis (Lehmann, 2007). The original concept envisioned that the solid co-product, biochar, would not be used for its energy value but would be added to soil to improve its fertility (Lehmann, 2007). Among its many reported benefits, biochar has been reported to significantly improve soil tilth, nutrient availability to plants, plant productivity, water-holding capacity, limit the bioavailability of heavy metals and toxins, alter soil microbial diversity and population dynamics and reduce nutrient leaching loss, which in turn can reduce fertilizer needs, as well as contamination of the environment (Lehmann et al., 2011; Spokas et al., 2012; Eyles et al., 2015). In addition to these effects, it was recently discovered that amending soil with biochar can affect the progress of plant diseases caused by both foliar and soilborne pathogens (Graber et al., 2014).

In regard to soilborne pathogens, biochar's suppressive capability has been reported for the following species: *F. oxysporum* f. sp. *asparagi*, *F. oxysporum* f. sp. *radicis-lycopersici*, *Fusarium proliferatum*, *Pythium aphanidermatum*, *Phytophthora cactorum, Phytophthora cinnamomi*, and *R. solani* (Matsubara et al., 2002; Elmer & Pignatello, 2011; Postma et al., 2013; Jaiswal et al., 2014).

Soil amendment with chitosan

Chitosan is a naturally-occurring compound and one of the most promising bioactive oligosaccharides that have potentials in agriculture with regard to controlling plant diseases. It is a safe and cheap biopolymer produced from chitin, the major constituent of arthropod exoskeleton and fungi cell walls, and the second renewable carbon source after lignocellulosic biomass (Kurita, 2006). Chitosan oligomers have attracted attention because of their unique biological properties including their inhibitory effect on the growth of various pathogenic fungi and their ability to be potent elicitors of plant defense reactions (Ragab et al., 2001; Nawar, 2005). Chitin and chitosan amendments are able to effectively reduce soilborne diseases. The antifungal activity of chitosan has been observed against different species including: *Penicillium digitatum*, *Macrophomina phaseolina*, *Fusarium solani*, *Fusarium fujikuroi*, *F. oxysporum* f.sp. *lycopersici*, *F. oxysporum* f. sp. *cubense*, *Phomopsis asparagi*, *Colletotrichum gloeosporioides*, *Rhizopus stolonifer*, *Sclerotium rolfsii* and *R. solani* (Ali et al., 2010; Al-Hetar et al., 2011; Oliveira Junior et al., 2012; Bhattacharya, 2013; Tien et al., 2014; Kim et al., 2016).

HYDROPONICS AND SOILLESS GROWING SYSTEMS

Plant production in hydroponics and soilless culture is an effective and environmentally friendly alternative to replace methyl bromide for soilborne pest control (Vallance et al., 2011). These systems have become popular over the last 20 years all over the world for growing high-value crops in glasshouses (Savvas, 2012). Growing crops in inert media, which has become a common practice in western Europe, significantly reduces problems with soilborne diseases. Nowadays, hydroponics is applied in limited planting areas but its use is expected to reach 30-40 percent of greenhouse production in the forthcoming years. Greenhouse area on artificial substrates increases around 10-20 ha every year (Neshev, 2008).

This technology is highly productive, provides good quality produce, improved phytosanitary control, and is easy to implement (Gruda, 2009). On the other side, this production system has also some disadvantages, such as high initial investments, difficult fumigation of the used substrate, and difficulties in recycling the nutritious solutions of the substrates. It is important to emphasize that soilless culture is no guarantee for pest-and-disease-free plant cultivation. Among the pathogenic microorganisms frequently detected in hydroponic cultures, those producing zoospores, such as *Pythium* spp. and *Phytophthora* spp., are particularly well-adapted to this cultivation system (Favrin et al., 1988; Rafin & Tirilly, 1995).

SOIL SOLARIZATION

Soil solarization is a non-chemical approach for soil disinfestation and one of the most promising methods to control soilborne pathogens. It is performed by placing polyethylene sheets over soil surface after sufficient irrigation. Soilborne pathogens, pests and weeds become inactivated by high temperature and excessive moisture during the hot season in July and August (Al-Karaghouli & Al-Kayssi, 2001). Under suitable climatic conditions, solarization can successfully control a wide range of soilborne diseases and pests (DeVay, 1991). Since soil solarization is a climate-dependent measure, it should therefore be adapted to specific regions and seasons only (Katan, 1999). Its effective application in moderate climate areas in most parts of Serbia is not expected.

The mode of action of solarization is complex, involving direct thermal destruction of propagules, shifts in microbial populations and activity, and changes in the physical and chemical properties of soil. Solar heating involves the use of heat as a lethal agent for pest control through the use of clear plastic film to trap solar radiation and accumulate heat (Katan, 2000). It was reported that 5 days of solar heating were sufficient to eliminate 100% of *V. dahliae* sclerotia at a depth of 5 cm, while only a slight killing was observed at a depth of 25 cm. However, after 8 additional days of solarisation, complete killing of the sclerotia was obtained at the 25 cm depth (Katan et al., 1976). Similarly, after 19 days of solarisation, mortality rates of *Sclerotium rolfsii* sclerotia at 5 and 20 cm depths were 100% and 25%, respectively, while, the respective 100% and 80% sclerotia mortality rates were observed after 21 additional days (Elad et al., 1980).

GRAFTING

Grafting of vegetable cultivars on rootstocks of less susceptible genotypes is an important strategy for managing soilborne pathogens and pests. Grafting is mostly used in fruit and nut production, but also in high-value vegetables. The primary intent of its application in vegetable production was to manage soilborne pathogens (Kubota, 2006). Nowadays, this technology is a common practice in Japan, Korea, and several European countries (Sugiyama et al., 2006; Louws et al., 2010). Official data about the use of grafing in vegetable production in Serbia are scarce or not available. Based on information supplied by farmers, the use of grafted seedlings in commercial vegetable production in Serbia is negligable (Mihajlović et al., 2016).

Grafting is commonly used to manage Verticillium wilt in tomato and eggplant and, less commonly, in cucurbit production systems. *Fusarium* pathogens have been the target of successful grafting practices in multiple crops due to available rootstock resistance genes and the biology of the pathogen. In solanaceous crops, host resistance commonly occurs in the form of major gene resistance. Thus, an excellent control of many tomato soilborne pathogens, particularly of *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *radicis-lycopersici*, *P. lycopersici* and *Meloidogyne* spp., has been achieved by using resistant rootstocks. In addition to the control of soilborne pathogens, tomato grafting has also many other purposes, such as growth promotion and yield increase, low temperature tolerance, growth period extension and fruit quality (Louws et al., 2010).

The intermittent nature of many Pythium diseases and efficacy of other management options have not created a priority to seek rootstocks resistant to specific Pythium-caused problems. In one report, *P. debaryanum* caused wilt of watermelon grafted on bottle gourd but squash rootstock offered protection (Tominaga et al., 1983).

Phytophthora crown and root rot is the most common disease of pepper that could be mitigated by grafting. Most sweet pepper rootstocks are susceptible, although breeding programs are advancing effective resistance. In an evaluation of 5 potential rootstocks of pepper, one rootstock offered commercially acceptable levels of *P. capsici* control combined with production benefits in a series of on-farm research trials. It also had modest tolerance to *Meloidogyne incognita* and limited efficacy against a complex of wilt pathogens (Morra et al., 2007). More recently, another programme developed pepper rootstock lines that offered a high level of tolerance to *P. capsici* (Gisbert et al., 2010), and Chili pepper rootstocks, compatible with sweet pepper and tolerant to *P. capsici*, *R. solanacearum*, flooding and high soil temperatures (Palada & Wu, 2008).

BIOLOGICAL CONTROL AND NATURAL COMPOUNDS

In an attempt to reduce the use of pesticides, there is an increasing interest in introducing biological agents and putting to use plant compounds as natural commercial products for managing soilborne pathogens (Cook, 1993).

Antagonistic microorganisms

Numerous studies have shown suppression of disease incidence in different crops after supplementing soils with fungal or bacterial antagonists. Biocontrol of soilborne diseases, as an alternative to synthetic pesticides, is particularly complex because such diseases occur in dynamic environments at the interface of root and soil known as the rhizosphere. A rhizosphere is defined as the region surrounding a root that is affected by it. It is important to point out that bioagents can reduce harmful effects of some pathogens below a certain threshold with no substantial changes in the soil microbiological balance, something that does not occur when chemicals are applied (Neshev, 2008). Various mechanisms are involved in the biological control of fungal pathogens. These mechanisms include: the production of secondary metabolites (antibiotics, siderophores, hydrolytic enzymes, volatile extracellular metabolites, hydrogen cyanide), parasitism, competition for nutrients, promotion of plant growth and, finally, induced resistance within the plants (Moeinzadeh et al., 2010).

Biological control agents, as living organisms, are much more sensitive to various external conditions than synthetic pesticides. On the other side, soil is a complex ecosystem in which many factors, such as soil structure, pH and moisture can limit the success of introduced biocontrol agents. Therefore, many of these biological control agents can be effective but require specific environmental conditions to flourish (Cook & Baker, 1983).

Numerous studies have indicated that several established biocontrol agents, including strains from the genera *Bacillus, Pseudomonas, Sphingomonas, Stenotrophomonas* and *Serratia,* can suppress vascular or soilborne fungal pathogens (Ramette et al., 2003; Berg et al., 2000; Mavrodi et al., 2012; Mihajlović et al., 2012a; Mihajlović et al., 2012b; Mihajlović et al., 2013a; Mihajlović et al., 2013b; Bhattacharjee & Dey, 2014). Fungi belonging to the genus *Trichoderma* and bacteria such as *Pseudomonas*.spp, or *Bacillus subtilis,* are the most promising biocontrol agents (Bhattacharjee & Dey, 2014). On the one hand, they stimulate plant growth, while on the other they eliminate plant pathogens by their unique antimicrobial activities, including the production of antibiotics and toxins to compete with pathogenic organisms (Mukry et al., 2010).

B. subtilis, *Trichoderma harzianum*, and *T. virens* have been reported as biocontrol agents against soilborne potato diseases (Minuto et al., 2000; Brewer & Larkin, 2005). *Trichoderma* spp. are well-studied, efficient mycoparasites that perform best in moist, slightly acidic soils (Cook & Baker, 1983). In a strawberry trial, the amendment of *Trichoderma* sp. to a soil treated with ozone gas decreased Verticillium wilt in the first year, but this was not repeated the following year. *Trichoderma harzianum* and *T. koningii* are effectively used for seed treatment against *R. solani*, *Pythium* spp., and *Sclerotia rolfsii* (Labrada, 2008). *Coniothyrium minitans* and *Sporidesmium sclerotivorum* were effective in controlling Sclerotinia diseases in lettuce (Jones et al., 2004). *Pseudomonas fluorescens* is a commonly used biocontrol agent against soilborne diseases. The bacterium is a soil-dwelling antagonist that has several modes of action against pathogenic microorganisms. It favors moist soils with high organic matter and is compatible with mulching (Cook & Baker, 1983). Root pretreatment of olive plants with some isolates of *P. fluorescens* Mig. during nursery propagation can help in the biocontrol of *V. dahliae* (Mercado-Blanco et al., 2004). *Bacillus* and *Pseudomonas* species are very beneficial and effective in the biocontrol of Fusarium wilt of chickpea (Abed et al., 2016).

One possible approach to improving biological control may be the application of combinations of biocontrol agents (Duffy & Weller, 1995). By combining microorganisms, multiple antifungal traits can be achieved and one may assume that at least one biocontrol mechanism will be functional under the conditions faced by the released biocontrol agents. Moreover, combinations of biocontrol strains are expected to result in a higher level of protection (Dunne et al, 1998), stabilization of biocontrol efficacy (Guetsky et al., 2002), and suppressiveness of multiple plant diseases (Jetiyanon & Kloepper, 2002). It has been demonstrated that natural suppressiveness of the Châteaurenard soil in France against Fusarium wilt was based on various mechanisms involving several microbial populations acting alone or together to limit the activity of the pathogen (Alabouvette et al., 1998). Yet, the use of biological control agents against soilborne pathogens cannot fully replace methyl bromide fumigation, they could rather function together in an integrated pest management strategy (Akrami et al., 2011).

Essential oils

Plant extracts and especially the volatile essential oils from medicinal plants, have been reported to possess antimicrobial activity against a variety of plant pathogens and pests (Kalemba & Kunicka, 2003; Burt, 2004; Soylu et al. 2006; Soylu et al. 2007; Tanovic et al., 2012; Hrustić et al., 2012; Tanović et al., 2013a; Tanović et al., 2013b; Tanović et al., 2014). Very few studies, however, have focused on the antifungal activities of essential oils against soilborne pathogens (Soylu et al. 2005; Mihajlović et al., 2011; Mihajlović et al., 2014; Türkölmez & Soylu, 2014). Essential oils and their components are gaining in interest because of their relatively safe status, their wide acceptance by consumers and their exploitation for potential multi-purpose use (Jobling, 2000).

Oregano, fennel and laurel oils demonstrated antimicrobial activity against soilborne fungi of bean under laboratory conditions (Türkölmez & Soylu, 2014). In addition, cinnamon, thyme, basil, and fenchel essential oils showed fungicidal effects on *Pythium* sp., *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *pisi*, *Verticillium albo-atrum*, and *Rhizoctonia* sp (Tanović et al., 2004). Mycelial growth of *Fusarium* spp. was inhibited by 5 µl of lemongrass, cumin, fennel, cinnamon and cassia essential oils when tested in a filter paper diffusion assay (Pawar & Thaker, 2007). Antimicrobial activity of essential oils against *Verticillium* sp. has rarely been evaluated. In a study by Soković & van Griensven (2006), where ten different essential oils were tested against *V. fungicola*, only oregano, thyme and spearmint oils demonstrated high *in vitro* activity at ≥1.0, 2.0 and 5.0 µl/ml, respectively. *F. oxysporum* and *V. dahliae* were reported to be inhibited by essential oils derived from oregano and spearmint. Strong activity was also recorded for lavender oil when tested against *F. oxysporum* at the dosage of 60 µl oil (Kadoglidou et al., 2011).

Although there have been numerous reports on antifungal activities of essential oils under *in vitro* conditions, efficacy data about antifungal activity of the essential oils towards soilborne fungal pathogens *in vivo* are limited. A recent study by McMaster et al. (2013) showed that thyme, origanum and clove bud essential oils had excellent *in vitro* antimicrobial activity when mixed with media against mycelial inoculum of several soilborne pathogens isolated from vegetable crops *(Pythium irregulare*, *P. sulcatum*, *P. aphanidermatum*, *F. oxysporum*, *Sclerotinia minor*, *R. solani). In vivo* tests confirmed that fennel and oregano oils at concentrations of 1.5 and 2.0 µl/ml air, respectively, resulted in reduced viability of sclerotia of *S. sclerotiorum* (Soylu et al., 2007).

Chemical control

Nonchemical alternatives against soilborne pathogens are not sufficiently effective to meet current growers' needs and will be turning to one or more of the known chemical alternatives to methyl bromide for fumigation of soil (Labrada, 2008). Among alternative chemicals, only dazomet has been registered as a broad-spectrum soil fumigant in most countries, including Serbia. It is used for soilborne pest and disease control in greenhouses and substrates, as well as in open fields (Spasić, 2016).

Chemical control of soilborne pathogens in vegetable crops is highly demanding because of the complexity of soil environment and biological differences among pathogens. In addition, only a limited number of registered products is available. Thus, no product has been registered in Serbia for the control of *Verticillium* spp. Fungicides registered for the control of *Fusarium* species (prothioconazole and tebuconazole, prochloraz, fludioxonil, guazatine, captan, thiram, mancozeb, thiophanate-methyl, thiabendazole, and iprodione) are mainly intended for prevention of Fusarium head blight (FHB) of wheat or Fusarium root rot in sugar beet and oilseed rape (carboxin + thiram). Boscalid and carbendazim have been registered for suppression of *S. sclerotiorum* in sunflower crops, and procymidone for the suppression of white mold in rapeseed (Spasić, 2016).

Products that effectively reduce soilborne pathogens of some crops by soil and plant applications are fungicides in the dicarboximide, benzimidazole and triazole chemical groups (Budge & Whipps, 2001; Matheron & Porchas, 2004; Mihajlović et al., 2010; Rekanović et al., 2010; Rekanović et al., 2011; Rekanović et al., 2012; Vatchev & Maneva, 2012). Strobilurins, especially azoxystrobin, were very effective in reducing the severity of Verticillium wilt in eggplant under field and greenhouse conditions (Bubici et al., 2006). In addition, azoxystrobine fungicides are widely used to control *R. solani* (Windels & Brantner, 2005; Sundravadana et al., 2007). An effective disease measure against vascular pathogens could also be the application of imidazoles, benzimidazoles and triazoles either to soil or to plants (Kurt et al., 2003; Everts et al., 2014). Fungicides based on cyprodinil and fludioxonil are recomended against *S. sclerotiorum* (Marín, et al., 2003; Benigni & Bompeix, 2010). Propamocarb-hydrochloride, fosetyl-Al, metalaxyl and azoxystrobin are fungicides that are commonly used to control *Pythium* spp. and *Phytophthora* spp. on pepper (Rekanović et al., 2011; Mihajlović et al., 2013a).

However, it is very important to emphasize that long-term use of pesticides has a negative influence on microbial growth and activity, leading to reduced soil fertility and productivity (Wang et al., 2006). Inactivation of nitrogen-fixing and phosphorus-solubilizing microorganisms is observed in pesticide-contaminated soils (Kyei-Boahen et al., 2001). Similarly, many studies have shown that pesticides reduce the activities of soil enzymes that are key indicators of soil health (Antonious, 2003). The applied pesticides may also affect many biochemical reactions, such as mineralization of organic matter, nitrification, denitrification, ammonification, redox reactions, methanogenesis, etc. All these consequences of prolonged pesticide use make this measure environmentally unfriendly and unsustainable in the extreme.

COMBINED METHODS OF CONTROL

There is an increasing tendency in crop protection to integrate different methods of control. Combining methods of control is at the heart of integrated pest management, and may result in either additive or synergistic effect. The expected benefit of this strategy is improved and sustainable control of pests and diseases. The goal of IPM methods is to employ measures that are more efficient, healthier, and more environmentally friendly in the long run, and to reduce the amount of pesticides used (Katan, 1999).

The use of biocontrol agents in conjunction with fungicides may be one of the strategies for control of some soilborne diseases. Thus, combination of *Bacillus megaterium* with carbendazim has provided an effective control of Fusarium crown and root rot of tomato (Omar et al., 2006). Steam disinfection can be used in combination with other control methods, e.g. soil amendments and biological control agents such as *Trichoderma* spp., which have been shown to increase disease control and horticultural productivity (Slusarski et al., 2012b). In addiition, the use of organic amendments to improve soil properties, plant health and yield has expanded in recent years. Organic amendments in combination with soil heating or solarization have the potential to improve crop health (Klein et al., 2007; Gamliel & Katan, 2009). Likewise, heating of soils covered with plastic film and amended with appropriate organic material activates a chain reaction of chemical and microbial degradation, generating antimicrobial compounds. Thus, Ramirez-Villapudua and Munnecke (1988) reported that solar heating of soil amended with cabbage residues eliminated *F. oxysporum* f. sp. *conglutinans* in closed containers.

CONCLUSION

The phaseout of methyl bromide for soil fumigation under the Montreal Protocol indicates to the world that pesticides that damage the environment will no longer be tolerated in agriculture. This has been a major turning point for science, as it provoked a massive research effort to find a suitable replacement. Nowadays, there is no single solution for the many problems posed by growers. Non-chemical options, such as soil solarization, crop rotation, biological control, soil amendments or steaming, may be considered too risky and/or uneconomical when used alone. However, all of these, used in combination, are viable as a part of IPM strategy, even though they do not completely eliminate pathogens from the soil. Initial results obtained by combining different methods for the control of soilborne diseases imply a necessity to continue research in this area in order to insure long-lasting sustainibility of crop protection.

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Mogućnosti suzbijanja patogena iz zemljišta

REZIME

Fitopatogene gljive i pseudogljive iz zemljišta, predstavljaju značajan problem u poljoprivrednoj proizvodnji širom sveta. Zahvaljujući perzistentnosti tvorevina za preživljavanje (mikrosklerocije, sklerocije, hlamidospore ili oospore), ovi patogeni opstaju u zemljištu godinama i bez prisustva biljke domaćina. Preparati na bazi metil bromida činili su okosnicu njihovog suzbijanja preko 30 godina. Međutim, Montrealskim protokolom iz 1992. godine, metil bromid je označen kao supstanca koja ima štetan uticaj na ozonski omotač, što je dovelo do njegovog postepenog povlačenja iz upotrebe. Nakon ukidanja metil bromida postalo je jasno da ne postoji jedna efikasna mera za suzbijanje ovih patogena, kao i da je za održivo suzbijanje neophodno primeniti kompleks mera. U ovom radu sistematizovana su najnovija saznanja o merama koje se koriste za suzbijanje patogena iz zemljišta, a koje obuhvataju: primenu plodoreda, sterilizaciju zemljišta vodenom parom, đubrenje zemljišta zelenišnim i organskim đubrivima, hidroponički sistem gajenja povrća, solarizaciju zemljišta, korišćenje supstanci prirodnog porekla, korišćenje kalemljenih biljaka i hemijske mere suzbijanja. Istaknuti su pozitivni i negativni aspekti svake pojedinačne mere, kao i prednosti istovremene primene kompleksa mera zasnovanih na različitim mehanizmima.

Keywords: Patogeni iz zemljišta; Alternative metil bromidu; Suzbijanje; Dezinfekcija zemljišta