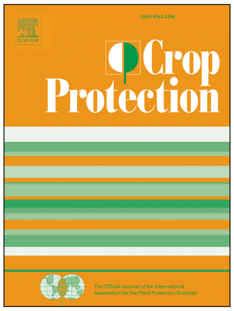

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INTEGRATION OF BIOLOGICAL AND CONVENTIONAL TREATMENTS IN CONTROL OF PEPPER BACTERIAL SPOT

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ABSTRACT

Bacterial spot caused by *Xanthomonas euvesicatoria* is one of the most devastating pepper diseases in Serbia. Questionable seed quality, climatic conditions, and frequent irrigation during summer favour the disease occurrence and spread. The available management practices do not provide adequate disease control. Therefore, development of alternative and more sustainable disease management strategies is needed. Integration of classical and biological treatments could be an effective, environmentally safe option for reducing pepper bacterial spot severity. In order to develop an efficient integrated disease management program, we studied efficacy of biocontrol agents (bacteriophage strain KΦ1 and two strains of *Bacillus subtilis* AAac and QST 713), systemic acquired resistance (SAR) inducer (acibenzolar-S-methyl - ASM), a commercial microbial fertilizer (Slavol), copper based compounds (copper hydroxide and copper oxychloride) in combination with or without mancozeb, and antibiotics (streptomycin sulphate and kasugamycin). They were applied as single treatments in two separate field experiments. Based on the single treatment efficacy, various combinations of the treatments were chosen for further testing in three separate field experiments. Additionally, we evaluated potential negative effect of ASM on pepper growth and yield in the growth chamber experiment. All the tested single treatments significantly reduced disease severity compared to the inoculated control (IC), except microbiological fertilizer and the antagonistic strain AAac. Integration of copper hydroxide, ASM and bacteriophages was the most efficient treatment, reducing the disease intensity by 96-98%. The results indicated that this combination may be an adequate alternative program for control of pepper bacterial spot.

Keywords: *Xanthomonas euvesicatoria*; copper compounds; antibiotics; resistance inducers; antagonists; bacteriophages; disease management

INTRODUCTION

Bacterial spot is one of the widespread and economically most important pepper diseases worldwide. The disease may be caused by *Xanthomonas euvesicatoria*, *Xanthomonas vesicatoria*, and *Xanthomonas gardneri* species that belong to spot-causing xanthomonads (Jones et al., 2000; 2004; Obradović et al., 2004). However, *X. euvesicatoria* strains are identified as the most widespread in pepper fields (EPPO, 2018). *Xanthomonas perforans*, a related species causing bacterial spot of tomato, has not been reported as a pepper pathogen.

Bacterial spot, caused by *X. euvesicatoria* has been a major limiting factor of pepper production in Serbia, due to endemic nature of the pathogen, favourable climatic conditions, questionable seed quality and limited control practices (Obradović et al., 1999; 2000; 2001). Based on differential reactions of 11 pepper genotypes, four physiological races of the pathogen (P1, P3, P7, P8) have been identified so far, with P8 being most widespread (Ignjatov et al., 2012). Currently, there are no commercially available pepper cultivars resistant to the pathogen races present in Serbia (Obradović et al., 2004; Ignjatov et al., 2012).

Pepper bacterial spot management practices include preventive and curative strategies. Cultural practices, such as disinfection of soil and substrates in seedlings production, planting of healthy certified seeds and transplants, maintenance of optimum temperature and water regime in protected areas, removal of plant residues, implementation of appropriate agro-technical measures and cultivation of less sensitive varieties, are important for disease prevention. Unfortunately, they are often omitted or fail to provide satisfactory control, especially when weather conditions favour spread of the pathogen, resulting in severe epidemics. New races of the *X. euvesicatoria* , antibiotics and copper resistance development, make the disease control even more difficult (Marco and Stall, 1983; Adaskaveg and Hine, 1985; Ritchie and Dittapongpitch, 1991).

The most common disease control is still based on preventative application of copper bactericides, alone or in combination with ethylene-bis-dithiocarbamate (EBDC) fungicides and antibiotics (Marco and Stall, 1983; Sherf and MacNab, 1986; Vallad et al., 2010).

Roberts et al. (2008) and Fayette et al. (2012) reported suppression of bacterial spot on tomato plants with the use of various combinations of famoxadone, famoxadone plus cymoxanil, mancozeb and copper. However, the overuse of copper compounds led to appearance of copper resistance in *X. euvesicatoria* populations (Marco and Stall, 1983; Adaskaveg and Hine, 1985; Ritchie and Dittapongpitch, 1991; Mirik et al., 2007; Ignjatov et al., 2010). There have been studies showing toxicological problems associated with EBDC use and cancerogenic properties of their metabolites (Janjić, 2005). Moreover, residues of these pesticides have been reported on treated vegetables (Gullino et al., 2010). Therefore, after development of new active substances, the use of EBDC in plant protection might be reduced or forbidden in the future (Gullino et al., 2010; Janjić, 2005).

Antibiotics, especially streptomycin, have been successfully used for many years in control of tomato and pepper bacterial spot, until streptomycin-resistant bacterial populations emerged and became widely distributed (Stall and Thayer, 1962). Development of resistance to kasugamycin in *Xanthomonas* spp. is also possible due to similar mode of action with streptomycin (Woodcock et al., 1991). (). Although the use of antibiotics in plant protection is restricted in most EU countries, as well as in Serbia, variation in bacterial population sensitivity to kasugamycin (50 μg ml^{-1}) has been observed among *X. euvesicatoria* strains isolated from pepper in Serbia (Obradović and Ivanović, 2007; Ignjatov et al., 2010). Limited efficacy of chemical treatments, as well as adverse negative environmental effects, stimulated plant pathologists to search for more suitable disease management solutions (Stall et al., 1986; Ritchie and Dittapongpitch, 1991; Obradović et al., 2004a).

There were several attempts of using biological agents in control of pepper and tomato bacterial spot (Jones and Stall, 1998; Ji et al., 2006; Mirik et al., 2008; Abbasi and Weselowski, 2015). Bacteriophages, viruses that infect bacteria, have been recently studied as a promising natural antimicrobial agents in different pathosystems, including pepper and tomato spot-causing xanthomonads (Jones et al., 2007; Buttimer et al., 2017). *Xanthomonas euvesicatoria* specific bacteriophage KΦ1, isolated from rhizosphere of pepper plants in Serbia (Gašić et al., 2011), showed significant efficacy in control of pepper bacterial spot in greenhouse conditions (Gašić et al., 2018). Moreover, combination of *X. vesicatoria* specific bacteriophages and acibenzolar-S-methyl (ASM), that activates systemic acquired resistance (SAR) in plants, was presented as a new alternative approach in control of tomato bacterial spot (Obradović et al., 2004a; 2005; Jones et al., 2007). Treatments with ASM in combination with bacteriophages, or bacteriophages and harpin protein, significantly reduced bacterial spot of tomato (Obradović et al., 2004a). Although ASM showed high potential in control of bacterial spot of tomato and pepper, some studies indicated it can negatively affect yield. Low yield is a limiting factor for use ASM to control the disease (Louws et al., 2001; Romero et al., 2001; Abbasi et al., 2002). In order to achieve disease control without affecting yield, it is necessary to determine the concentration, time of application, and number of treatments of ASM.

In this work, we explored the benefits of different strategies that could be considered as part of an integrated management of pepper bacterial spot in Serbia. Under field conditions we studied the efficacy of bactericides that are traditionally used in practice, as well as substances not registered for pepper bacterial spot control in Serbia, several biocontrol agents, and the integration of different biological agents and resistance inducers. Incorporation of novel alternate methods into the existing crop protection programme may provide more effective, durable and sustainable disease control.

MATERIALS AND METHODS

Growth chamber experiment

Pepper plant development in response to different concentrations of ASM

***Experiment* 1.** This experiment was conducted in a growth chamber at the Institute of Vegetable Crops, Smederevska Palanka, Serbia. Pepper plants cv. Early California Wonder grown in 10-cm (510 ml) pots containing soilless medium (Klasmann Substrate TS2;

Klasmann-Deilmann GmbH), at 3-4 leaf stage, were used in the experiment. To evaluate the response of pepper plants to ASM, drench and foliar treatments were applied using three active ingredient concentrations: 0.0015, 0.0025 and 0.0035%. For soil drench, 50 ml of the respective ASM solution was applied per each pot. Foliar treatments were applied by spraying leaves of each plant using a hand-held sprayer until run-off (approximately 15 ml of the ASM suspension per plant). The treatments were applied twice by the model that has been the most effective in previous experiments (Šević et al., 2016). Initial ASM treatment was applied ten days after transplanting pepper seedlings from the polystyrene containers into the pots, followed by the second treatment five days after the first one. Tap-water treated plants were used as controls. After the treatments, pepper plants were kept in the growth chamber with an alternating regime of 15 h day^{-1} of daylight and 9 h day^{-1} of darkness. The experiment was designed as a complete randomized system with five replications. Experimental units were represented by five plants per replicate. The results were recorded 10 days after the second treatment and 7 days later by measuring the height of the above soil part, as well as the total weight of the fresh plant tissue including the root system.

Field Experiments

The pathogen, inoculum preparation and inoculation.

A copper-sensitive strain of *X. euvesicatoria*, KFB 13 (Obradović et al., 2004) was used for inoculation of pepper plants. The strain was stored in Nutrient Broth (NB) supplemented with 30% glycerol at -80°C and sub-cultured on Nutrient Agar (NA) plates incubated at 28°C during experiments. Inoculum was prepared from 24 h old culture suspended in sterile distilled water. Concentration of bacteria was adjusted to 10^8 CFU ml^{-1} using McFarland´s scale and confirmed by a serial dilution plating (Klement et al., 1990).

Pepper plants (*Capsicum annuum* L.) cv. Early California Wonder were used in all experiments. Plants, at the five-leaf stage, were spray-inoculated using hand-held mister until run-off (approximately 15 ml of bacterial suspension per plant).

Efficacy of different treatments in control of pepper bacterial spot and their influence on the pepper yield

Experiment 2. Field experiments were conducted at the Institute of Vegetable Crops, Smederevska Palanka, Serbia, during the summer of 2011. Previous greenhouse and growth chamber experiments have shown that application of chemical pesticides, systemic resistance inducer and different biocontrol agents, provided significant control of *X. euvesicatoria* infection (Šević et al., 2016). The most efficient treatments in the controlled conditions were selected and evaluated for the efficacy and integration potential under the field conditions. Copper based compounds, streptomycin and kasugamycin, ASM, two strains of *Bacillus subtilis* (QST 713 - Serenade®, and AAac strain), bacteriophage strain KΦ1 (Gašić et al., 2011) and commercial microbial fertilizer (Slavol®), were tested for their efficacy in control of pepper bacterial spot. The tap-water treatment was used as a negative control (Table 1). Pepper plants were grown in 104 cells (R=3.5 cm) float containers in a greenhouse for 7 to 8 weeks. During the last week of May plants were transplanted into the field as single rows. The experiment was designed as a randomized complete block design, with 12 treatments in four replications, and repeated twice (test 1, test 2) Each plot consisted of a single row of 25 plants. Rows were spaced 70 cm.

Pepper plants were artificially inoculated by spraying bacterial suspension 9 days after transplanting. All treatments were applied one day before inoculation and then once a week, except for ASM and bacteriophages. ASM was applied 9 and 4 days prior inoculation and after that at biweekly intervals, up to six treatments in total. Non-formulated bacteriophages were applied immediately prior to inoculation and then twice a week at dusk, with a total of 12 treatments. Pepper plants were harvested one time and the total yield was measured for each treatment. During experiment, pepper plots were irrigated by overhead sprinklers which created favourable conditions for the disease development.

Table 1

Experiment 3. To study the most efficient integrated strategy for controlling pepper bacterial spot, we tested different combinations of the bacteriophage (strain KΦ1), *B. subtilis* (strain AAac and QST 713), ASM (Bion 50WG®) and copper hydroxide (Kocide 2000®) treatments. The experiments were conducted during the seasons of 2012 and 2013. Inoculations were performed as described in the previous experiments. Copper hydroxide was applied as a standard treatment one day before inoculation and then once a week. All treatments were applied in a similar manner as described above in the experiment 2. When integrated, biocontrol agents were applied at least three days after (*B. subtilis*) or before (bacteriophage strain KΦ1) copper hydroxide application. Non-inoculated and tap water-treated plants were used as controls. Each treatment consisted of four replications and the experiment was designed as a complete randomized block system repeated three times (test 1, test 2, test 3).

Pepper yield measurements

In all experiments, fruits from 10 pepper plants, avoiding plants at the beginning and the end of the rows, were harvested manually during the last week of August or first week of September, at the biological maturity of pepper fruits. The fruits from each plot were weighed to determine total fruit yield per treatment.

Disease severity assessment

Pepper bacterial spot severity was evaluated by estimating percentage of the leaf surface covered with necrotic spots using the Horsfall-Barratt (HB) rating scale (Horsfall and Barratt, 1945). All plants in the field plots were rated for foliar disease severity three times (28 July, 26 August and 15 September, 2011). Area under the disease progress curve (AUDPC) values were calculated using the formula $\Sigma[([x_i + x_{i-1}]/2)(t_i - t_{i-1})]$, where x_i is the rating at each evaluation time and $(t_i - t_{i-1})$ is the time between evaluations (Shaner and Finney, 1977). AUDPC values of all treatments were compared with the AUDPC for the inoculated control plot, and efficacy of the treatments was expressed as percentage of the disease reduction.

Statistical analysis

Experimental data were analysed using IBM SPSS statistical software version 20 (IBM SPSS Statistics 20, 2012). Analysis of variance (ANOVA) was performed, and when P values indicated significant difference ($P \leq 0.05$), means were compared by Duncan's multiple range test.

RESULTS

Growth chamber experiment

Pepper plants development in response to different concentrations of ASM

Experiment 1. When used in indicated concentrations, ASM did not produce any negative effect, such as chlorosis, spotting or necrosis, on pepper leaves. However, all three concentrations of ASM significantly reduced the plant growth. The height of the plants and the total weight of the fresh plant tissue were significantly affected as compared to the untreated control (Table 2). Treated plants showed a height declining trend along the time after the treatments. In the second measurement, 17 days after the last application, the plant height was reduced to a greater extent (Table 2). The level of reduction in the plant growth corresponded to the applied concentration of ASM. The lowest reduction (24%) was caused by the lowest concentration of ASM (0.0015%) regardless of the type of application. And consequently, the highest reduction of the plant height (38%) was observed when the highest concentration of ASM (0.0035 %) was applied by spraying.

It was found that all three concentrations of ASM, applied either by spraying or drenching, significantly affected the weight of pepper plants, compared to the untreated control (Table 2). However, the smallest negative impact (20%) on the total weight of the pepper plants was observed in the ASM spraying treatment using the lowest concentration (0.0015%). Considering the disease control effectiveness of this treatment as well as the lowest negative effect on the growth of the treated pepper plants, this ASM concentration was chosen for the subsequent experiments.

Table 2.

Field experiments

Efficacy of different treatments in control of pepper bacterial spot and their influence on the pepper yield

Experiment 2. According to the AUDPC results in test 1 and 2, the highest disease severity was recorded in plots treated with the antagonistic strain AAac and microbial fertilizer (Slavol). The level of efficacy of these treatments was not significantly different from the IC (Table 3). Copper compounds and streptomycin treatments showed the highest efficacy by controlling the disease from 79 to 90%. The next group of treatments with statistically significant efficacy included ASM, kasugamycin and bacteriophage KΦ1. As compared to aforementioned treatments, *B. subtilis* QST 713 was less effective but still significantly different from IC (Table 3).

Consequently, the yield harvested from the plots treated with the least effective treatments (microbial fertilizer and antagonistic strain AAac) was significantly lower than in the rest of the plots (Table 3). The highest yield was harvested from the plots treated with streptomycin and bacteriophages. However, in the test 2 there was no statistical difference in total yield between these two treatments in spite of the differences in the disease control efficacy.

Experiment 3. According to the AUDPC results from the field experiments (test 1, 2 and 3), all the integrated treatments significantly reduced disease severity as compared to the IC (Table 4). The most effective was the treatment combination of copper hydroxide + ASM + bacteriophages, showing efficacy of 96-98%. This treatment combination provided better protection than the copper hydroxide standard treatment in all three tests (Table 4). Treatments combination of copper hydroxide + bacteriophages, copper hydroxide + *B. subtilis* QST 713, and ASM + bacteriophages + *B. subtilis* QST 713, showed statistically the same level of efficacy as compared to copper hydroxide standard treatment in all tests. The integrated application of copper hydroxide + ASM and copper hydroxide + bacteriophages + *B. subtilis* QST 713, showed high level of efficacy but was not always statistically different from copper hydroxide. Although the integration of ASM + bacteriophages and ASM + *B. subtilis* QST 713 significantly reduced disease severity compared to the IC, the level of control achieved by these treatment combinations was significantly lower compared to copper hydroxide standard in test 1 (Table 4).

All integrated treatments provided significantly higher yield compared to the IC (Table 4). However, there were no significant differences between the treatments in all three tests regarding the total yield. The only exception was recorded in the test 1 where copper hydroxide + ASM + bacteriophages had significantly higher yield thanASM + phage KΦ1 and ASM + *B. subtilis* QST 713.

Table 3.

Table 4.

DISCUSSION

Bacterial spot has been limiting pepper production in Serbia, especially when weather conditions favour the disease development. The disease management is a challenge due to limited efficacy of commonly used control strategies relying mostly on copper bactericides. Reduced copper sensitivity among *X. euvesicatoria* strains, as well as concerns about the environmental impact of copper residues, contributed to the increased interests in developing more effective control strategies that will facilitate economically and environmentally sustainable pepper production. Recent studies indicated that application of antagonistic microorganisms, plant growth promoting rhizobacteria (PGPR), bacteriophages and plant resistance activators could contribute to better control of bacterial spot (Louws et al., 2001; Romero et al., 2001; Abbasi et al., 2002a; 2002b; 2003; Al-Dahmani et al., 2003; Obradovic et al., 2004a, Wen et al., 2007; 2009). However, some experiments showed that single treatments could not provide satisfactory control, indicating that integration of their effects including cultural practices could be a way to the solution.

To evaluate the effect of foliar sprays of copper compounds alone or in combination with mancozeb, as well as antibiotics, ASM, two biocontrol agents and the microbial fertilizer on pepper bacterial spot, field experiments were conducted. In order to achieve more sustainable and efficient control, we applied various combinations of these treatments trying to optimize their benefits and develop stable disease management. We have demonstrated that the aplication of ASM, bacteriophages and copper compounds provided significant reduction of bacterial spot severity compared to the IC. ASM was the most effective treatment in controlling bacterial spot in the greenhouse and growth chamber experiments (Šević et al., 2016). However, in the field experiments, ASM applied alone did not show the same efficacy. Similar observation in controlling bacterial spot of tomato was reported previously (Obradović et al., 2004a; Huang et al., 2012).

For maximum efficiency of the ASM treatment, the concentration and frequency time between the applications should be carefully adjusted as overexploitation of the plant defence mechanisms can lead to metabolic overload, delay and the decrease in productivity. In addition to numerous advantages reported by many authors, the use of ASM can adversely affect the yield of pepper (Louws et al., 2001; Romero et al., 2001). In the growth chamber experiments, spraying of ASM in concentration of 0.0015% had the lowest negative impact on the plant growth and the fresh plant tissue. When this concentration was applied in the field experiments, it effectively controlled the disease intensity and caused minimal negative effect on pepper growth and yield. Biweekly application of ASM did not reduce the yield of pepper (Table 3 and 4), nor phytotoxicity was observed.

Recently reported method for controlling bacterial spot of tomato was the application of bacteriophages, viruses that infect bacteria (Balogh et al., 2003; Obradović et al., 2004a). Bacteriophages possess a number of advantages over the chemical pesticides; they are natural components of the biosphere, self-replicating and self-limiting, non-toxic to the eukaryotic cells, highly specific, eliminating only target bacteria (Jones et al., 2007). Moreover, they can be integrated with other pesticides and biocontrol agents. However, they may be sensitive to some environmental factors such as UV light or desiccation, which delimits the efficacy of phage treatment. In our previous studies we reported that bacteriophage KΦ1 treatment was more effective in controlling pepper bacterial spot in the growth chamber (78-85%) than in the greenhouse conditions (38%) (Šević et al., 2016). Possible explanation for this variation could be limited survival of bacteriophages in the greenhouse conditions and use of non-formulated suspension (Šević et al., 2016). Based on this study field experiment results (Table 3), non-formulated phages applied twice a week at dusk reduced the disease severity and therefore could be recommended for the management of bacterial spot in the field. Applications of ASM in concentration 0,0015% in 14 days interval and applications of bacteriophages twice a week at dusk significantly reduced the bacterial spot symptoms (Table 3) demonstrating that bacteriophages and ASM can be integrated and used as an effective strategy for controlling bacterial spot in pepper greenhouse and field production. In our trials, copper compounds, applied alone or in combination with mancozeb, reduced the disease severity compared to the IC. Although it was reported earlier that addition of maneb or mancozeb fungicides to the copper bactericides enhance their efficacy (Marco and Stall, 1983; Sherf and MacNab, 1986; Pernezny et al., 2008), this was not confirmed in our experiments. The combination of copper with mancozeb did not affect the treatment efficacy (Table 3). Therefore, it could be excluded from the bacterial spot disease management practice in a future, which would reduce the risk of EBDC residue accumulation (Gullino et al., 2010;

Janjić, 2005). Due to the frequent use of copper compounds, copper tolerant or resistant strains of *X. euvesicatoria* were already reported (Marco and Stall, 1983; Adaskaveg and Hine, 1985; Ritchie and Dittapongpitch, 1991; Mirik et al., 2007). Use of copper- and antibiotic-sensitive strain of *X. euvesicatoria* favoured the disease control with copper bactericides and antibiotics. However, integration with biocontrol agents and plant resistance inducers (ASM) would reduce the population pressure and risk of *X. euvesicatoria* resistance development to these bactericides (Šević et al., 2016). Based on the three year field experiments, the combination of copper-hydroxide, ASM and bacteriophages provided the best results in the disease control, and could be considered an effective alternative strategy in control of pepper bacterial spot. Additionally, the combination of ASM and bacteriophages might contribute to significant reduction of copper sprays in bacterial spot management.

Microbial fertilizer (Slavol) and antagonistic strain AAac did not provide satisfactory control of pepper bacterial spot in the field experiments. Although *B. subtilis* strain AAac exhibited strong competitive ability to *X. euvesicatoria* strain *in vitro* (unpublished data), the field experiments showed limited activity and low competitive ability of this strain *in vivo* conditions.

During the four-year study, the best control of pepper bacterial spot was obtained by integrating copper hydroxide, ASM and bacteriophages (Table 4). Different mode of action of these treatments confronted the pathogen more efficiently and provided sustainable disease management. Similar model was used in Florida in tomato bacterial spot control when host specific phage strains (AgriPhage^{TM}) were applied with other alternative or standard treatments, resulting in improved disease control (Obradovic et al., 2004a).

This study outlines the possibility of an efficient control of pepper bacterial spot, even in conditions of high inoculum pressure. The strategy is based on timely and integrated application of a combination of natural agents such as bacteriophages, the plant resistance activator (ASM) and the conventional copper-based bactericides. By using natural enemies and plant defense mechanisms, the application of chemical substances could be reduced, which makes this integrated approach a more efficient alternative, cost effective and safe to the crop and environment.

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TABLES

Table 1. Products applied in this study.

Commercial name	Active ingredient	Manufacturer
Bion 50 WG®	Acibenzolar-S-methyl (ASM) 500g kg^{-1}	Syngenta International AG Switzerland
Kocide 2000®	Copper-hydroxide 538g kg^{-1}	DuPont International Operations S.a.r.l Geneva - Switzerland
Cuprozin 35WP®	Copper-oxychloride 350g kg^{-1}	Galenika fitofarmacija, Serbia
Mankogal 80®	Mancozeb 800g kg^{-1}	Galenika fitofarmacija , Serbia
Streptomycin P®	Streptomycin sulphate 1000g kg^{-1}	NCP, Serbia
Kasumin 2L®	Kasugamycin 20g kg^{-1}	Sumitomo Chemicals Corporation, Japan
Bacteriophage	Strain KΦ1, conc. 10^{10} PFU/ml	/
Serenade®	*Bacillus subtilis* strain QST 713 5×10^{8} CFU/g	AgraQuest, Inc, Davis, CA, USA
Antagonist *Bacillus subtilis*	Strain AAac, conc. 10^{8} CFU/ml	/
Slavol®	Microbiological fertilizer	Agrounik, Serbia

Table 2. ***Experiment 1.*** Height and weight of pepper plants cv. Early California Wonder in response to different concentrations of acibenzolar-S-methyl

Concentration of ASM treatment	The mean height of pepper plants (mm)		The mean weight of pepper plants (g)
	[a]First measurement	[b]Second measurement	
Control	139 a[e]	170 a	20.80 a
0.0015 d[c]	96 b	129 b	16.64 b
0.0015 s[d]	94 b	126 bc	15.20 c
0.0025 s	92 b	116 cd	15.18 c
0.0025 d	86 b	110 d	15.08 c
0.0035 d	84 b	107 d	12.02 d
0.0035 s	82 b	106 d	11.74 d

[a] First measurement was performed 10 days after the second application

[b] Second measurement was performed 7 days after the first measurement

d [c] - ASM applied by drenching, s[d] - ASM applied by spraying, a[e] - The mean height and weight of plants marked with the same letter does not differ at the significance level of 0.05

Table 3. ***Experiment 2.*** Efficacy of applied treatments in control of pepper bacterial spot in the field during summer 2011.

			Field experiment					
Treatments	Rate a.i. (ha^{-1})	Timing[cd]	Test 1			Test 2		
			AUDPC [a]	Efficacy (%)	Yield (t/ha)	AUDPC	Efficacy (%)	Yield(t/ha)
Copper-hydroxide + mancozeb	1.02 + 1.44 kg	1	83.4 d	90.9	14.4 cd	57.3 e	87.7	8.9 a
Streptomycin	0.2 kg	1	89.2 d	90.3	16.7 a	65.8 e	85.8	10.6 a
Copper-hydroxide	1.02 kg	1	112.0 d	87.8	15.4 bc	69.0 e	85.1	10.2 a
Copper-oxychloride	1.23 kg	1	129.0 d	86.0	14.6 cd	97.7 d	79.0	8.7 a
Copper-oxychloride + mancozeb	1.23 + 1.44 kg	1	129.6 d	85.9	15.2 cd	74.9 de	83.9	9.2 a
ASM	0.015 kg	2	187.6 c	79.6	14.9 cd	169.4 c	63.5	9.3 a
Kasugamycin	0.04 kg	1	195.4 c	78.7	14.3 cd	194.8 c	58.1	8.7 a
Bacteriophage KΦ1	10^{9} PFU/ml	3	203.9 c	77.8	16.5 ab	175.2 c	62.3	10.4 a
Bacillus subtilis QST 713	2×10^{6} CFU/ml	1	472.0 b	48.6	14.0 d	268.4 b	42.2	8.5 a
Bacillus subtilis AAac	10^{8} CFU/ml	1	897.5 a	2.3	9.4 e	448.6 a	3.4	5.3 b
Microbiological fertilizer	20 l	1	939.7 a	0	9.5 e	453.9 a	2.3	5.8 b
Inoculated control [b]	-	None	918.6 a	-	9.1 e	464.4 a	-	5.9 b

[a] Area under the disease progress curve (AUDPC). Means followed by different letters within a column are significantly different according to Duncan's multiple range test, P = 0.05 level.

[b] Control was sprayed with water only.

[c] Indicates applied respectively in concentration and timing indicated for particular treatment.

[d] Application timing: 1 = once prior inoculation, after that weekly; 2 = two applications before inoculation, then at biweekly intervals; and 3 = once prior inoculation, then twice a week at dusk.

Table 4. ***Experiment 3.*** Efficacy of integration of biological and conventional treatments in control of pepper bacterial spot during summer 2012 and 2013.

Treatments	Field experiment								
	Test 1			Test 2			Test 3		
	AUDPC[a]	Efficacy %	Yield (t/ha)	AUDPC	Efficacy %	Yield (t/ha)	AUDPC	Efficacy %	Yield (t/ha)
Copper-hydroxide	237.6 de	91.2	8.9 ab	180.2 bc	86.7	9.3 a	256.5 bc	88.3	10.3 a
Copper-hydroxide + ASM + Bacteriophage KΦ1	54.4 f	98.0	11.2 a	49.1 d	96.4	10.0 a	45.9 d	97.9	10.8 a
Copper-hydroxide +ASM	121.1 ef	95.5	10.5 ab	67.3 d	95.1	10.2 a	163.5 cd	92.6	10.3 a
Copper-hydroxide + Bacteriophage KΦ1	131.6 ef	95.1	10.6 ab	87.8 cd	93.5	10.5 a	149.8 cd	93.2	9.4 a
Copper-hydroxide + phage KΦ1 + *Bacillus subtilis* QST 713	208.8 de	92.2	10.4 ab	74.9 d	94.5	9.7 a	174.4 cd	92.1	9.5 a
Copper-hydroxide + *Bacillus subtilis* QST 713	330.2 cd	87.7	9.7 ab	193.1 b	85.8	9.1 a	316.7 bc	85.6	10.4 a
ASM + phage KΦ1 + *Bacillus subtilis* QST 713	345.4 cd	87.2	10.2 ab	93.0 cd	93.2	9.5 a	299.7 bc	86.4	9.4 a
ASM + phage KΦ1	386.5 c	85.6	8.2 b	100.6 bcd	92.6	9.8 a	344.2 bc	84.3	9.9 a
ASM + *Bacillus subtilis* QST 713	745.2 b	72.3	7.9 b	103.0 bcd	92.4	9.5 a	453.3 b	79.4	9.7 a
Inoculated control[b]	2690.6 a	-	4.4 c	1359.4 a	-	4.1 b	2196.9 a	-	5.4 b

[a] Area under the disease progress curve (AUDPC). Means followed by different letters within a column are significantly different according to Duncan's multiple range test, $P = 0.05$ level.

[b] Control was sprayed with water only.

Highlights

- Bacterial spot is one of the economically most important pepper diseases worldwide
- The currently available bactericides fail to provide satisfactory disease control
- Integration of copper hydroxide+ASM+bacteriophages was the most effective treatment