Proceedings of the 7th Congress on Plant Protection

Доклады 7-ого Конгресса по защите растений

Plant Protection Society of Serbia
Общество по защите растений Сербии

International Organization for Biological Control
-East Palearctic Regional Section (IOBC-EPRS)
-West Palearctic Regional Section (IOBC-WPRS)

Международная организация по биологической борьбе
- Восточно палеарктическая региональная секция (МОББ-ВПРС)
- Западно палеарктическая региональная секция (МОББ-ЗПРС)

Editors/Редакторы

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„Integrated Plant Protection – a Knowledge-Based Step towards Sustainable Agriculture, Forestry and Landscape Architecture“

(November 24-28, 2014, Zlatibor, Serbia)

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„Интегрированная защита растений - научно обоснованный шаг к устойчивому развитию сельского хозяйства, лесоводства и пейзажной архитектуры“

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PREFACE

The Plant Protection Society of Serbia (PPSS) and two regional sections of the International Organization for Biological and Integrated Control (IOBC-EPRS and IOBC-WPRS), on the occasion of the 60th anniversary of the PPSS organized VII Congress on Plant Protection with a motto: *"Integrated Plant Protection – a Knowledge-Based Step towards Sustainable Agriculture, Forestry and Landscape Architecture"* (November 24-28, 2014, Zlatibor, Serbia). The Congress enabled exchange of up-to-date scientific and technical information on plant protection in Agriculture, Forestry and Landscaping among researchers, teachers, experts in extension and public services and the business community, and promoted international cooperation. The Congress focused on basic knowledge and management practices established in plant protection, as well as on the development of alternative and innovative approaches. In addition, biological control as an important tool for the control of the harmful organisms with a minimal risk for ecosystems was discussed. A total of 209 contributions was presented - 8 keynote presentations, 28 oral presentations and 173 poster presentations - prepared by 467 authors from 26 countries. The Congress Proceedings comprise 65 contributions - 5 keynote presentations and 60 oral and poster presentations in six sessions, prepared by the authors from 18 countries (Algeria, Austria, Bosnia-Herzegovina, France, Georgia, Hungary, Italy, Kazakhstan, Montenegro, Poland, Russia, Rwanda, Serbia, Slovenia, Switzerland, Turkey, Uganda, USA). All contributions were reviewed by members of the Scientific Committee and other reviewers selected and invited by the editors of this publication.

Belgrade, November 2015 Editors

ПРЕДИСЛОВИЕ

Общество по защите растений Сербии (ОЗРС), Международная организация по биологической борьбе с вредными животными и растениями - Восточно палеарктическая региональная секция (МОББ-ВПРС) и Международная организациая по биологической борьбе и интегрированной системе защиты растений - Западно-палеарктическая региональная секция (МОББ-ЗПРС), по поводу 60-летия ОЗРС организировали VII Конгресс по защите растений, под девизом: *"Интегрированная защита растений - научно обоснованный шаг к устойчивому развитию сельского хозяйства, лесоводства и пейзажной архитектуры"* (24-28 ноября 2014 года, Златибор, Сербия). Цель Конгресса была обеспечение континуитета взаимообмена научно-техническими информациями, отвечающими современным требованиям защиты растений в сельском хозяйстве, лесоводстве и пейзажной архитектуре, которые представляют интерес для ученых, исследователей, преподавателей, экспертов-советников в области сельского хозяйства, лесоводства и пейзажной архитектуры, специалистов государственных и коммунальных служб, деловых кругов и средств массовой информации. Целью Конгресса является и продолжение содействия развитию и популяризации международного сотрудничества. Конгресс был концентрирован на основные знания и практический менаджмент в защите растений, а также на развитие алтернативних и новых подходов. Биологическая защита каторая представляет значительный способ для безопасной борьбы с вредними организмими была тоже рассмотривана. На конгрессе представлено 209 презентаций - 8 докладов по приглашению, 28 устных и 173 постер презентаций - которые подготовило 467 авторов из 26 стран. Сборник имеет 65 докладов - 5 докладов по приглашению и 60 устных и постер презентаций, распределенных в шести секциях. Авторы докладов приехали из 18 стран (Алжир, Австрия, Босния-Герцеговина, Франция, Грузия, Венгрия, Италия, Казахстан, Черногория, Польша, Россия, Руанда, Сербия, Словения, Швейцария, Турция, Уганда, США). Рецензенты всех опубликованных докладов в сборнике – члены Научного совета и другие рецензенты, выбранные редакторам этого издания.

Белград, Ноября 2015 Редакторы

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INTEGRATION OF BIOLOGICAL AND CHEMICAL METHODS IN CONTROL OF PEPPER BACTERIAL SPOT

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ABSTRACT

Bacterial spot, caused by *Xanthomonas euvesicatoria*, is widely spread disease of pepper in Serbia. When weather conditions are favorable for disease development, pepper producers do not have adequate resources to control this pathogen. Copper based bactericides registered in our country are not effective enough. However, effective protection could be achieved only by integrating positive effects of different protection methods. In order to develop sustainable and integrated control strategy for this disease, we investigated various combinations of biological control agents and chemicals. Intensity of the disease ranged from 31 to 59% on untreated control plants. All integrated treatments were effective against *X. euvesicatoria* and significantly reduced disease severity in all experiments, compared to untreated control. The most efficient treatment was integration of acibenzolar-S-methyl, copper hydroxide and bacteriophages, reducing disease severity 97-99%. This combination may be an effective new tool for pepper growers to manage bacterial spot.

Keywords: *Xanthomonas euvesicatoria*, copper compounds, acibenzolar-S-methyl, antagonist, bacteriophage

INTRODUCTION

Bacterial spot, caused by *Xanthomonas euvesicatoria* (Jones et al., 2004) is the most important pepper disease in Serbia. The intensity of infection and economic losses depend on the cultivar susceptibility, applied protection measures and weather conditions. Routine disease management practices, such as use of good quality seed, crop rotation, growth of less susceptible cultivars and application of copper compounds, have failed to provide satisfactory disease control, especially when weather conditions favored the spread of the pathogen. Application of copper compounds alone proved less effective than in combination with ethylene-bis-dithiocarbamates (EBDC) fungicides (Marco et al., 1983). However, due to the frequent application, copper tolerant or resistant strains of *X. euvesicatoria* were reported (Marco et al., 1983; Adaskaveg et al., 1985). The use of antibiotics, especially streptomycin, for bacterial diseases control has begun in the 50's. Successful control of *X. euvesicatoria* in tomato and pepper crops did not last long due to streptomycin resistant bacteria populations observed in the early sixties (Stall and Thayer 1962). Streptomycin-resistant strains spread rapidly and became widely distributed (Argentina, Brazil, California, Florida, Georgia, Ohio, Pennsylvania and Taiwan), forcing plant pathologists to search for other solutions (Obradović et al., 2004).

According to the recent literature data, biological agents as bacteriophages and some new alternative

methods such as resistance inducers and harpin protein represent new strategies in control of *X. euvesicatoria* in tomato crops (Obradović et al., 2004, 2005; Jones et al., 2007). Treatments of acibenzolar-S-methyl in combination with bacteriophages, or bacteriophages and harpin protein, significantly reduced bacterial spot of tomato in the fields of Florida (Obradović at al., 2004).

The aim of our research was to study efficacy of various combinations of biological control agents and chemicals in order to develop sustainable and integrated control strategy for bacterial spot control.

MATERIAL AND METHOD

The experiments have been conducted on the experimental field of the Institute of Vegetable Crops in Smederevska Palanka. In order to develop sustainable and integrated control strategy for pepper bacterial spot, we investigated various combinations of biological control agents: bacteriophages (strain КΦ-1(Gašić at al., 2011), conc. $2.3x10^{1o}$ PFU/ml) and *Bacillus subtilis* (strain AAac, conc. 10^8 CFU/ml and Serenade AgraQuest, Inc conc. 0.4%), systemic acquired resistance inducer acibenzolar-S-methyl (ASM, Bion 50WG, Syngenta crop protection; 0.003%) and copper hydroxide (Kocide 2000, DuPont, 0.19%) (Table 1). Copper hydroxide was applied as a standard treatment one day before inoculation and then once a week. *Bacillus subtilis* treatment was applied one day before inoculation and then once a week. ASM was applied 9 and 4 days prior to inoculation and after that at 14 day intervals. The total number of three aforementioned treatments was six. Nonformulated bacteriophages were applied immediately prior to inoculation followed by twice a week at dusk, with a total of 12 treatments. Pepper plants (cv. Early California Wonder) were artificially inoculated 9 days after transplanting. Inoculation was done by spraying water suspension of *X. euvesicatoria* strain KFB 13 (sensitive to copper compounds) (conc. 10^8 CFU/ml) using hand-held mister. Concentration of bacteria in the suspension was adjusted to 10^8 CFU/ml using McFarland's scale and confirmed by a serial dilution plating method (Klement et al., 1990). Noninoculated and inoculated tap water-treated plants were used as a control. The experiment was repeated two times. Each treatment consisted of four replications and the experiment was designed as a complete randomized block system. Percentage of the leaf surface covered with necrotic spots was evaluated by using the Horsfall-Barratt (HB) rating scale, 7 days after application of the last treatment (Horsfall-Barratt, 1945). Data were analyzed by applying one-way ANOVA and Duncan's multiple range test.

RESULTS AND DISCUSSION

Results of two field experiments showed that all integrated treatments were effective against *X. euvesicatoria* and significantly reduced disease severity in all experiments, compared to untreated control (Table 1). Intensity of the disease ranged from 31 to 59% on untreated control plants. The most efficient treatment was integration of acibenzolar-S-methyl, copper hydroxide and bacteriophages, reducing disease

Table 1. Efficacy of integration of biological and chemical methods in control of pepper bacterial spot.

Treatments	Concentration	Experiment 1		Experiment 2	
		Mean *	Efficacy %	Mean*	Efficacy %
Bion 50 WG + Kocide 2000 + Bacteriophage	0.003% + 0.19% + $2.3x10^{10}$ PFU/ml	1.463 E	97.5	0.293 B	99.0
Bion 50 WG + Kocide 2000	0.003% + 0.19%	2.925 DE	95.0	1.463 B	95.3
Kocide 2000 + Bacteriophage	0.19% + $2.3x10^{10}$ PFU/m	3.511 DE	94.0	2.633 B	91.5
Kocide 2000 + Bacteriophage + Serenade	0.19% + $2.3x10^{10}$ PFU/m + 0.4%	5.268 CDE	91.1	1.755 B	94.3
Kocide 2000	0.19%	6.439 CDE	89.1	4.095 B	86.8
Kocide 2000 + Serenade	0.19 + 0.4%	8.198 CDE	86.1	4.389 B	85.9
Bion 50 WG + Serenade + Bacteriophage	0.003% + 0.4% + $2.3x10^{10}$ PFU/ml	9.370 CDE	84.2	2.633 B	91.5
Bion 50 WG + Bacteriophage	0.003% + $2.3x10^{10}$ PFU/ml	11.714 CD	80.2	2.048 B	93.4
Bion 50 WG + Antagonist (Strain AAac)	0.003% + 10^8 CFU/ml	13.474 BC	77.3	4.096 B	86.8
Bion 50 WG + Serenade	0.003% + 0.4%	20.506 B	65.4	2.340 B	92.5
Untreated inoculated control	-	59.375 A	-	31.250 A	-

*Means followed by different letters within a column are significantly different according to Duncan's multiple range test, P = 0.05 level.

severity 97-99%. However, in experiment 2 there was no statistically significant difference in efficacy of standard treatment (copper hydroxide) and various integration of biological control agents: bacteriophages *and Bacillus subtilis* strains and ASM. ASM alone was not effective for controlling bacterial spot in the field as was observed in greenhouse and climatic chamber conditions (Šević at al., 2011, 2011a, 2012). Biweekly applications of ASM and applications of bacteriophages twice a week at dusk significantly reduced the disease severity (80-93%). In these experiments we used non-formulated bacteriophages. Balogh et al. (2003) reported that formulation of bacteriophages with skim milk and sucrose contributed to greater stability on leaf surfaces and therefore better efficiency. In the present study copper and antibiotics-sensitive strain of the pathogen was used, favoring more effective disease control with standard copper hydroxide (86-89%). However, these integrated treatments may be relatively more effective compared to the standard when copper resistant *X. euvesicatoria* strains are predominant in natural epidemics. In this study we demonstrated that some alternative methods (bacteriophages, ASM) could serve as a new promising tool for pepper producers to control bacterial spot.

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