

# Control of pepper bacterial spot and tomato bacterial speck using bactericides in combination with a plant activator

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## SUMMARY

The efficacy of a formulated product of tea tree oil (TTO) and a standard copper based bactericide in combinations with acibenzolar-S-methyl (ASM) for control of bacterial spot of pepper and bacterial speck of tomato was evaluated under controlled conditions. Treatments with copper hydroxide+ASM at both application rates and TTO+ASM showed the best efficacy in control of bacterial spot disease in pepper plants (with respective efficacy values 83.5%, 83.7 % and 86.9%). Similar efficacy was achieved by treatment with copper hydroxide alone (82.2%). On the other hand, the efficacy of TTO, applied alone, was significantly lower at both rates (63.8% and 71.5%) in bacterial spot control in pepper. The same treatments (copper hydroxide +ASM at both application rates and TTO+ASM) were most efficient in suppressing the causal agent of bacterial speck disease in tomato (87%, 82.3% and 81.6%). The efficacy of treatment with the standard bactericide copper hydroxide, applied alone, was significantly lower compared to its combination with ASM (79.1%). Similarly to the bacterial spot experiment, TTO treatments alone showed lower, although satisfactory efficacy at both application rates (66.1% and 68.9%). The results of this study showed that combination of the bioactive compound - ASM with either the standard copper hydroxide bactericide or TTO significantly improved their efficacy in both bacterial spot and speck disease control, and thus implied that combining different biorational compounds, such as essential oils and bioactive products, with standard copper treatments and their inclusion into integrated management programs are of essential importance for the control of bacterial diseases of pepper and tomato.

**Keywords:** *Xanthomonas euvesicatoria*; *Pseudomonas syringae* pv. *tomato*; tea tree oil; acibenzolar-S-methyl; copper hydroxide

## INTRODUCTION

Bacterial spot is one of economically most important pepper diseases, which may also affect tomatoes. In pepper, the disease may be caused by *Xanthomonas euvesicatoria*, *Xanthomonas vesicatoria* or *Xanthomonas gardneri* species that belong to spot-causing xanthomonads (Jones et al., 2004). However, *X. euvesicatoria* strains have been identified as the most widespread in pepper fields worldwide (EPPO, 2018), and in Serbia (Obradovic et al., 2004; Gašić et al., 2011). Bacterial speck, caused by *Pseudomonas syringae* pv. *tomato*, is an important disease of tomato in economic terms (Louws et al., 2001; Milijašević et al., 2009). Both of these pathogens are commonly introduced by contaminated seed or may be autochthonous (Jones et al., 1998). Bacterial spot and speck diseases result in leaf lesions, defoliation, fruit lesions, and ultimately yield loss of marketable fruit (Louws et al., 2001; Jones et al., 2004).

Bacterial spot and speck management includes preventive measures (soil disinfection, use of healthy certified seeds and transplants, removal of plant debris and other cultural practices) and curative strategies comprising chemical treatments. Traditionally, these include multiple applications of copper compounds (alone or mixed with mancozeb) and antibiotics (streptomycin, kasugamycin) (Marco & Stall, 1983; Jones et al., 2012). However, the approach of disease management based entirely on the use of chemicals has encountered problems caused by bacterial strains resistant to copper compounds and antibiotics (Bender & Cooksey, 1986; Stall et al., 1986; Bender et al., 1990). In addition, implementation of chemical measures for controlling bacterial diseases is complex due to pathogen diversity, inability to find sources of resistance in host plants to the target pathogen, the ability of bacteria to rapidly reach high population densities and lack of effective chemical control (Jones et al., 2012).

In a continuous search for novel sources of biocides with broad-spectrum activities, plants and their derivatives have come up with effective alternatives or complements to synthetic chemical compounds, without showing secondary effects (Nazzaro et al., 2013). Some herb essential oils have demonstrated strong antimicrobial activity against plant bacterial pathogens (Soković & van Griensven, 2006; Kokoskova et al., 2011; Todorović et al., 2016). Tea tree oil (TTO) is an essential oil steam distilled from the Australian plant *Melaleuca alternifolia*. It consists mostly of cyclic monoterpenes exhibiting broad-spectrum antimicrobial activity which may be principally attributed to terpinen-4-ol (Markham, 1999; Carson et al., 2006). This natural essential oil has

been found to be an effective antiseptic, fungicide and bactericide, and has many safe and effective uses in the health and cosmetics industries, as well as in agriculture (Carson et al., 2006).

Meeting the goal of improving control of bacterially incited diseases, one strategy includes the use of bioactive products, known as “plant activators,” that induce systemic acquired resistance (SAR) in plants to limit pathogenesis of many plant pathogens, including bacteria (Louws et al., 2001). One such compound, acibenzolar-*S*-methyl (ASM) (Actigard 50WG, Bion 50WG, Syngenta, Basel, Switzerland), elicits plants to acquire preinfection biochemical processes that confer resistance to the same spectrum of pathogens as the biological elicitor. Numerous research studies have shown that ASM induces SAR in various hosts to a wide range of pathogens, including bacteria (Siegrist et al., 1997; Louws et al., 2001; Romero et al., 2001; Buonaurio et al., 2002; Obradovic et al., 2004). Likewise, earlier studies have shown that treatments with ASM alone or in combination with bacteriophages and harpin protein significantly reduced bacterial spot and speck of tomato (Louws et al., 2001; Obradovic et al., 2004).

The focus of this study was to evaluate the efficacy of a formulated product of tea tree oil and a standard copper-based bactericide in combinations with a plant activator (ASM) for control of bacterial spot of pepper and bacterial speck of tomato under controlled conditions.

## MATERIALS AND METHODS

### Test organisms, inoculum preparation and inoculation

The phytopathogenic bacteria used in the experiments were: *Xanthomonas euvesicatoria* strain NCPPB 2968 (National Collection of Plant Pathogenic Bacteria, UK) originating from pepper, and *P. syringae* pv. *tomato* strain PstBB-8 (culture collection of the Institute of Pesticides and Environmental Protection, Belgrade, Serbia) originating from tomato plants. The strains were stored in nutrient broth (NB) supplemented with 30% glycerol at -80°C. For inoculum preparation, the *X. euvesicatoria* strain was grown at 28°C for 24 h on YDC (yeast dextrose chalk) agar medium, while the *P. syringae* pv. *tomato* strain was sub-cultured on KBM (King's B medium) at 26°C for 24 h. Bacterial cells were suspended in sterile distilled water, and bacterial suspension was adjusted at 10<sup>8</sup> CFU ml<sup>-1</sup> using McFarland's scale and confirmed by dilution plating on NA medium (Klement et al., 1990).

Five-week-old pepper plants cv. 'Dukat' and four-week-old tomato plants cv. 'Saint Pierre' were transplanted into 10 cm × 5 cm pots filled with 400 ml sterile growth substrate (B medium course, Floragard, Germany) and used in experiments. Inoculum was applied by spraying with a hand sprayer and the inoculated plants were covered with plastic bags for 48 hours to obtain humid conditions.

### Efficacy of treatments and statistical analyses

Growth chamber experiments were conducted at the Institute of Pesticides and Environmental Protection. Timorex Gold (active ingredient 222.5 g/l tea tree oil, Stockton, Israel) and Kocide 2000 (a.i. copper-hydroxide 35% Cu, DuPont, Switzerland) applied alone or in combination with Actigard (a.i. acibenzolar-S-methyl 500 g kg<sup>-1</sup>, Syngenta, Switzerland) were tested for their efficacy in control of pepper bacterial spot and tomato bacterial speck disease. Plants inoculated with both pathogens and sprayed with tap-water were used as control. The experiment was conducted as a randomized complete block design, with 10 plants per treatment in four replications. The first application was performed one day before inoculation, except for the ASM combinations, which were applied seven days before inoculation. The following two applications were conducted at one-week intervals after inoculation. Disease severity was assessed seven days after the last treatment.

Ten randomly sampled leaves were collected from the upper plant parts from each individual treatment plot, and the percentage of leaves infected with each pathogen was estimated according to the following scale: 0 – no disease; 1 – 1-5% of leaf area affected; 2 – 5-10% of leaf area affected; 3 – 10-25% of leaf area affected 4 – 25-50% of leaf area affected; and 5 – >50% of leaf area affected.

Disease severity (DS) was evaluated using Townsend-Heuberger's formula (Townsend & Heuberger, 1943):

$$DS (\%) = \frac{\sum(nv)}{NV} \times 100$$

where

n – degree of infection according to the 5-grade scale, v – number of leaves per category, V – total number of leaves assessed, N – highest degree of infection.

Bactericide efficacy (EB) was calculated using Abbott's formula (Abbott, 1925):

$$EB (\%) = \frac{X - Y}{X} \times 100$$

where

X – disease severity in control, Y – disease severity in treated plots.

The effects of bactericide treatments on disease severity were analyzed for each trial using ANOVA, and the means were separated by Duncan's multiple range test.

## RESULTS AND DISCUSSION

In the assay conducted in the growth chamber, no phytotoxicity symptoms were observed as a result of applying treatments. However, statistically significant differences in disease severity were observed among treatments. Treatments with copper hydroxide +ASM at both application rates and TTO+ASM treatment showed the best efficacy in control pots of pepper plants with bacterial spot disease. The efficacy of treatment

**Table 1.** The efficacy of treatments in pepper bacterial spot control

Treatment	Application rate	Disease severity	Efficacy (%)
Tea tree oil (TTO)	1.0 l h <sup>-1</sup>	8.5±0.7 d	63.8
Tea tree oil (TTO)	1.5 l h <sup>-1</sup>	6.7±0.4 c	71.5
Tea tree oil (TTO)+ASM	1.0 l h <sup>-1</sup> + 20 g ha <sup>-1</sup>	5.4±0.2 b	77.0
Tea tree oil (TTO)+ASM	1.5 l h <sup>-1</sup> + 20 g ha <sup>-1</sup>	3.9±0.3 a	83.5
Copper-hydroxide+ASM	1.5 kg ha <sup>-1</sup> + 20 g ha <sup>-1</sup>	3.8±0.3 a	83.7
Copper-hydroxide+ASM	2.0 kg ha <sup>-1</sup> + 20 g ha <sup>-1</sup>	3.1±0.6 a	86.9
Copper-hydroxide	2.0 kg ha <sup>-1</sup>	4.2±0.5 ab	82.2
Control	-	23.5±2.1 e	-

\*Means marked with different letters are significantly ( $p < 0.05$ ) different according to Duncan's test.

with the standard copper hydroxide bactericide was not statistically different from those three treatments and treatment with the lower application rate of TTO+ASM. On the other hand, the efficacy of TTO applied alone was significantly lower at both rates. Notably, disease severity in all treatments tested in the study was significantly different from the untreated control. The efficacy of treatments in bacterial spot control is given in Table 1.

As for the control of bacterial speck disease in tomato, the most efficient treatment was the higher application rate of copper hydroxide +ASM. However, there was no significant difference between that treatment and copper hydroxide +ASM at lower rate and TTO+ASM at higher rate. Treatment with the standard bactericide copper hydroxide alone had a significantly lower effectiveness compared to its combination with ASM. Similarly to the bacterial spot experiment, TTO treatments alone (at both rates) showed satisfactory efficacy, although their efficacy was significantly lower compared to their combinations with ASM, as well as copper hydroxide alone and copper hydroxide +ASM (Table 2).

In our study, TTO formulated as Timorex Gold showed satisfactory efficacy (63.8% and 71.5%) at both application rates in bacterial spot control in pepper. The results were similar in the control of bacterial speck of tomatoes (66.1% and 68.9). Ciavareli Lukas et al. (2012) observed a reduction in the severity of bacterial spot disease at 0.1% concentration of several tested essential oils. The authors also reported an efficacy of TTO of about 60% against pepper bacterial spot. It may be attributed to multiple mechanisms of action of these natural compounds, i.e. the active compounds of the oils could act either directly on pathogens or induce host resistance through the production of phytoalexins, increased PRP activity, synthesis of structural compounds, or biochemical plant defense, resulting in disease reduction

(Carson et al., 2006). However, Carson et al. (1995) reported that the essential oil of tea tree can kill a variety of microorganisms (Gram-negative and Gram-positive bacteria, and yeasts) through alterations in cell membrane structures by disinfectant agents that actively denature proteins, leading to cytoplasm leakage, lyses, and cell death.

The results of this study showed that combining the bioactive compound ASM with either copper hydroxide as a standard bactericide or with TTO significantly improved their efficacy in both bacterial spot and speck disease control. This feature of ASM has been reported before. Field trials have shown that ASM increases resistance in bell pepper (Romero et al., 2001) and tomato (Louws et al., 2001) to bacterial infections. Bell pepper plants sprayed with the ASM showed resistance to subsequent infections with the bacterial spot agent *X. vesicatoria*. In those field trials, applications of ASM every two weeks, alone or in combination with copper, resulted in disease control similar to the standard treatment of copper plus maneb with variable yield, while weekly applications throughout the crop season had a negative impact on yield (Romero et al., 2001). Similar results were reported by Buonauro et al. (2002) after their growth chamber experiments, where ASM treatment protected pepper plants systemically and locally against *X. vesicatoria*. The efficacy of ASM was also assessed in open field conditions, where both leaves and fruit were protected from the disease (Buonauro et al., 2002). In addition, Louws et al. (2001) reported that the application of ASM reduced foliar disease severity in 14 of the 15 bacterial spot and all seven bacterial speck experiments in field trials. Also, disease control was similar or superior to what was achieved by using a standard copper bactericide program. Similarly, in our earlier trials, the efficacy of the standard copper-based bactericide was improved by its combination with ASM treatment. It is noteworthy that in some cases tomato yields have been

**Table 2.** The efficacy of treatments in tomato bacterial speck control

Treatment	Application rate	Disease severity	Efficacy (%)
Tea tree oil (TTO)	1.0 l h <sup>-1</sup>	5.4±0.6 c	66.1
Tea tree oil (TTO)	1.5 l h <sup>-1</sup>	4.9±1.0 c	68.9
Tea tree oil (TTO)+ASM	1.0 l h <sup>-1</sup> + 20 g ha <sup>-1</sup>	3.6±0.5 b	77.0
Tea tree oil (TTO)+ASM	1.5 l h <sup>-1</sup> + 20 g ha <sup>-1</sup>	2.9±0.4 ab	81.6
Copper-hydroxide+ASM	1.5 kg ha <sup>-1</sup> + 20 g ha <sup>-1</sup>	2.8±0.4 ab	82.3
Copper-hydroxide+ASM	2.0 kg ha <sup>-1</sup> + 20 g ha <sup>-1</sup>	2.1±0.3 a	87.0
Copper-hydroxide	2.0 kg ha <sup>-1</sup>	3.3±0.3 b	79.1
Control	-	15.8±1.5 d	-

(\*Means marked with different letters are significantly ( $p<0.05$ ) different according to Duncan's test).

affected when using a plant activator alone, but it seemed not to have negative effects in combination with standard bactericide/fungicide treatments against foliar diseases (Louws et al., 2001). Moreover, Louws et al., (2001) showed that *X. vesicatoria* population densities on greenhouse-grown tomato transplants were reduced by ASM treatment. Bacterial speck and spot population densities on leaves of field-grown plants were not dramatically affected. Similarly, Obradovic et al. (2004, 2005) reported that satisfactory control of bacterial spot of tomato was achieved by applying ASM, though the results were better in greenhouse experiments than in the field. Therefore, evaluation of the efficacy of treatments tested in growth chamber facilities in our study should be also conducted under open field conditions. Furthermore, it seems that combining different biorational compounds, such as essential oils or bioactive products, with standard copper treatments, and their inclusion in integrated management programs are of essential importance for controlling bacterial diseases of pepper and tomato in the context of a struggle with the problem of pathogen resistance and lack of effective chemical compounds.

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## Suzbijanje prouzrokača bakteriozne pegavosti paprike i bakteriozne pegavosti paradajza korišćenjem kombinacije baktericida i aktivatora otpornosti

### REZIME

Ispitivana je efikasnost formulisanog preparata na bazi ulja čajnog drveta (TTO) i standardnog baktericida na bazi bakra u kombinacijama sa acibenzolar-S-metilom (ASM) u suzbijanju prouzrokača bakteriozne pegavosti paprike i bakteriozne pegavosti paradajza u zaštićenom prostoru. U zaštiti paprike, tretmani na bazi kombinacije bakar hidroksid+ASM u obe količine primene kao i kombinacija TTO+ASM pri višoj količini primene, pokazali su najbolju efikasnost (83,5%, 83,7 % i 86,9%). Sličnu efikasnost pokazao je bakar hidroksid primenjen samostalno (82,2%). S druge strane, efikasnost preparata na bazi ulja čajnog drveta samostalno, bila je značajno niža u obe količine primene (63,8% i 71,5%). Isti tretmani (bakar hidroksid+ASM u obe količine primene kao i kombinacija TTO+ASM pri višoj količini primene) bili su najefikasniji i u suzbijanju prouzrokača bakteriozne pegavosti paradajza (87%, 82,3% i 81,6%). Efikasnost tretmana standardnim baktericidom, bakar hidroksidom, primenjenim samostalno bila je statistički značajno niža nego u kombinaciji sa ASM (79,1%). Slično prethodnom eksperimentu, preparat na bazi ulja čajnog drveta, samostalno primenjen, ispoljio je nižu, ali zadovoljavajuću efikasnost u obe količine primene (66,1% i 68,9%). Rezultati istraživanja pokazali su da kombinacija bioaktivnog jedinjenja – ASM, bilo sa standardnim baktericidom - bakar hidroksidom ili preparatom na bazi ulja čajnog drveta, značajno poboljšava njihovu efikasnost u suzbijanju prouzrokača bakteriozne pegavosti paprike i paradajza, što ukazuje na to da su kombinovanje bioracionalnih jedinjenja kao što su etarska ulja i bioaktivni produkti sa standardnim bakarnim jedinjenjima i uključivanje u programe integralne zaštite od ključne važnosti u suzbijanju prouzrokača bakterioza paprike i paradajza.

**Cljučne reči:** *Xanthomonas euvesicatoria*; *Pseudomonas syringae* pv. *tomato*; ulje čajnog drveta; acibenzolar-S-metil; bakar hidroksid