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RESEARCH ARTICLE

Fungicide application and residues in control of *Blumeriella jaapii (Rehm)* Arx in sweet cherry

Vladimir Božić[1], Slavica Vuković[2], Mila Grahovac[2], Sanja Lazić[2], Goran Aleksić[3], Dragana Šunjka[2]

[1]CI "Plant protection", Toplicki partizanski odred 151, Niš, Serbia, [2]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovica 8, Novi Sad, Serbia, [3]Institute of Plant Protection and Environment, Teodora Drajzera 9, Belgrade, Serbia

ABSTRACT

Fungicides are significant disease control tool in increasing agricultural production, however, their intensive application has led to environmental problems including health hazards. To minimize harmful effects of the fungicide application in sweet cherry orchards, it is necessary to use them in accordance with the good agricultural practice and to monitor presence of their residues. Cherry leaf spot caused by *Blumeriella jaapii* is a significant sweet cherry disease which control is heavily dependent on fungicide treatments. In this study, effects of fungicide treatments against *B. jaapii* and fungicide residues remaining in sweet cherry fruits after the treatments were evaluated, and the causal agent of cherry leaf spot was confirmed on cherry leaves from untreated control plots using conventional phytopathological techniques (isolation on nutrient media and morphological traits of developed fungal colonies). The trial was set up at two localities in south Serbia (District of Niš), in sweet cherry orchards, according to EPPO methods. Fungicides tested against *B. jaapii* were based on dodine (650 g a.i./kg) WP formulation, at concentration of 0.1% and mancozeb (800 g a.i./kg) WP formulation, at concentration of 0.25%. During the trial, two evaluations were carried out. Fungicide efficacy was determined according to Abbott. The obtained data were statistically processed by ANOVA and LSD test. In both sweet cherry orchards high efficacy in control *B. jaapii* was registered in case of dodine application efficacy of 96.3-98.9%, while mancozeb showed slightly lower efficacy of 91.0-95.6%. The results of the dissipation dynamic suggest that the dodine dissipation curves followed the first-order kinetic ($Ct = 6.23^{e-0.09}$, with $R^2 = 0.986$) and its half-life in sweet cherry fruits was 7.7 days. The final residues in sweet cherry fruits were below the MRL (3 mg/kg) 21 days after the application. The results indicate that sweet cherry fruits can be safely consumed after dodine based fungicides applications at the recommended rate.

Keywords: *Blumeriella jaapii*; Efficacy; Fungicides; Isolation and identification; Sweet cherry; Residues

INTRODUCTION

Sweet cherry is a very profitable crop due to highly valued fruits on the market (Sredojević, 2011). The export classifies cherries as a perspective fruit crop in the Republic of Serbia. Areas under sweet cherries are increasing from year to year, taking into account that in 2010 there was 3.655 ha under cherries, while in 2017 the area increased to 4.613 ha (RZS, 2018). The assortment of cherries is quite outdated in our country, but new plantations with new varieties are establishing, which results in increased yields, higher fruit quality, and especially the attractiveness of the fruits (Milatović et al., 2013). Cherry production is endangered by numerous pathogens, but cherry leaf spot caused by the fungus *Blumeriella jaapii* (Rehm) Arx. is one of the most significant diseases. This pathogen attacks all varieties of cherries (Schuster, 2004). In Europe, this disease appeared in the Netherlands in the middle of the twentieth century and it has, so far, spread to the whole Europe. Cherry leaf spot is present in all regions where cherries are grown (Gelvonauskiene et al., 2004; Todorović et al., 2009; Pfeiffer, 2010; Pedersen et al., 2012). Under agro-ecological conditions of Serbia, and especially in areas with high humidity, pathogens that cause leaf diseases are constantly present in the production of this fruit crop (Iličić et al., 2019). Under humid conditions, defoliation occurs in mid-summer, in the second part of the vegetation period (Holb et al., 2010), which negatively affects the acclimatization of buds or their resistance to low temperatures, and reduces the possibility of overwintering buds and fruiting in the following year (McManus et al., 2007). The pathogen overwinters in fallen leaves, in the spring apothecia develops from which ascospores are released, after the appearance of favorable conditions. The most favorable conditions for the release of ascospores are humid weather lasting 5-6 hours at temperatures of 16 - 21°C. The release of ascospores can also occur at lower or higher temperatures with longer periods of

***Corresponding author:**
Slavica Vuković, University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovica 8, Novi Sad, Serbia. **E-mail:** vukovic@polj.uns.ac.rs

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humidification (Elis, 2008).Therefore, as one of the most common pathogens of cherries, it is a significant obstacle in maintaining the health of orchards and achieving high and quality yields. Due to the above-mentioned, the application of fungicides is an inevitable part of the production process. For the control of *B. japii* in sweet cherry, PPPs based on fungicides dodine and mancozeb are registered in the Republic of Serbia. The dissipation rate represents one of the most important parameters for assessing the fate of pesticide residues (Li et al., 2016). Different factors such as plant species, pesticide chemical structure, type of formulation, application method, climate, and photo degradation affect the dissipation of pesticides after the application (Lazić et al., 2018). Therefore, it is necessary to conduct dissipation studies of pesticides under different agroecological conditions. Results obtained in the dissipation study could be used for the estimation of the time required for keeping the residues below MRLs (Ambrus and Lantos, 2002).

Apart from their benefits in terms of leaf spot control, the side effects of fungicides are a well-known problem. One of the most significant disadvantages is the presence of fungicides residues in fruits above the maximum residue level, and the risk to consumer health. In order to minimize the harmful consequences of the application of the fungicide in sweet cherry orchards on human health, it is necessary to apply them in accordance with the good agricultural practice and to monitor their residues in cherry fruits. However, there is a still lack of information about the dissipation and behaviour of some pesticides in agricultural products.

In this study, the effects of fungicides dodine and mancozeb for the control of *Blumeriella jaapii* and its residues remaining in sweet cherry fruits after the treatments were evaluated. The causal agent of cherry leaf spot was confirmed on cherry leaves from untreated control plots using conventional phytopathological techniques.

MATERIAL AND METHODS

Blumeriella jaapii (Cylindrosporium padi) isolation and identification

Leaf samples of sweet and sour cherries expressing symptoms of cherry leaf spot were collected from untreated, control plots at both localities at the first evaluation date, and transported to the laboratory. Most of the collected samples had typical purple to brownish spots on the upper side of the leaf. On the lower side of the leaf area small, brown to white spots were observed developing into white or yellowish spore cushions. In some cases, the necrotic spots fell out and the shot-hole symptom was observed. Also some leaves turned completely yellow and premature shedding was observed.

The leaf samples with typical spots were used for isolation. Isolation was performed following partially modified method given by Wharton et al. (2003). Conidia produced on the lower side of the leaf were observed under the microscope and used as morphological trait for the identification, as well as colonies formed on nutrient media after isolation.

Pathogenicity test

Young, healthy cherry leaves were collected and injured by carborundum powder and placed in Petri dishes on filter paper soaked with 1% solution of sucrose (Schuster and Tobutt, 2004). Subsequently, the leaves were sprayed with the spore suspension. Spore suspension was prepared from conidia of actively growing cultures of the obtained isolates, suspended in sterile water, and set to the final concentration of 1×10^6 conidia/ml. Uninoculated, injured cherry leaves were soaked in the same manner and used as a control.

Field trial

The trial was set at localities Mršelj (locality 1) and Donje Brijanje (locality 2) (District of Niš, south Serbia) in sweet cherry orchards (variety Sunburst and Carmen) (Fig. 1). The trial was carried out according to the standard EPPO method PP 1/30(2) (2004), according to the completely randomized block design PP 1/152(4) (2012). Orchard in Mršelj is 5 years old, and in Donje Brijanje 4 years, growing shape is improved pyramid, with distance of 4×3 m.

The following products were applied: dodine (650 g a.i./kg of product) WP formulation, at concentration of 0.1% and mancozeb (800 g a.i./kg of product) WP formulation, at concentration of 0.25%. The product based on dodine was applied in the post flowering phase (BBCH 69) and in the fruiting phase (BBCH 75), and mancozeb after the appearance of the first leaves

Fig 1. Sweet cherry orchard (locality Mršelj) (orig.).

(BBCH 11) and in the post flowering phase (BBCH 69). The trial was carried out in four replications. Foliar treatments were carried out by backpack sprayer with consumption of 1000 l/ha of water. In previous years in the mentioned orchards, presence of heavy leaf spot infections was observed. During the trial, other plant protection products were not used, and irrigation was not performed. During the trial, two evaluations of effects were conducted. In the first evaluation in BBCH 81 phenophase, after the occurrence of the first symptoms in control variant plots, the occurrence and development of the disease was monitored on 10 leaves on 10 marked branches per repetition, and the percentage of the leaf area with disease symptoms, i.e. disease intensity (%) was determined. The second evaluation was performed at BBCH 85 phenophase, 4 weeks after the first, and the percentage of defoliation on marked branches was recorded according to the scale: 1- without defoliation 2 – up to 25% defoliation and 3 – more than 25% defoliation. Results were presented according to the mean values of disease intensity, the efficacy of the studied fungicides (E%) according to Abbot (Wentzel, 1963) and significance of differences (LSD5%).

Dodine residue determination

After the application of dodine based fungicide at the recommended rate, sweet cherry fruit samples were randomly collected from various branches within the experimental plot, with the aim of obtaining the representative samples. For the analysis of dodine residues, sweet cherry samples were collected two hours after the application (dried deposit), and 7, 14, 21 and 23 days later. Fruit samples were collected into plastic bags, delivered to the laboratory and stored (-20 °C) until the analysis. Moreover, untreated fruits samples, used as blank samples for the validation of the method, were also collected. Dodine certified analytical standard (97%) was obtained from Dr. Ehrenstorfer GmbH (Germany), acetonitrile (HPLC purity) was from J.T. Baker and ammonium acetate was purchased from Fisher Chemical.

The analytical standard was dissolved in acetonitrile and ultrasonically homogenized. The QuEChERS based method (Anastassiades et al., 2003) (Fig. 2) employed within the pre-analytical step was combined with high-performance liquid chromatography (Agilent Technologies, USA). The analysis was performed using a Zorbax Eclipse XDB-C18 (50 mm × 4.6 mm, 1.8 µm) column, with a 25 µl injection. The column temperature was constant at 20 ◦C. A mobile phase was acetonitrile and water Mobile phase was composed of HPLC-grade water containing 5 mM ammonium acetate and acetonitrile, with a flow rate of 0.55 ml/min.

RESULTS AND DISCUSSION

Blumeriella jaapii (Cylindrosporium padi) identification

Conidia formed after 24 h incubation of leaf fragments in wet conditions on the lower side of the leaf. The conidia formed abundantly in milky white matrix (Fig. 3).

Under the microscope (Fig. 4), the observed conidia were hyaline, long and slender, curved or flexuous, continuous

Fig 2. (orig.). Dodine residue determination (orig.).

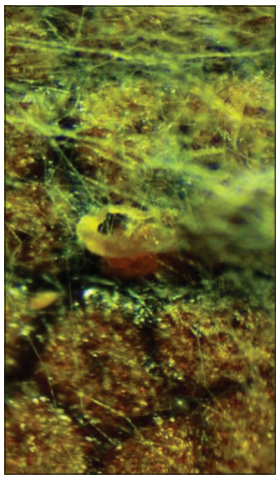


Fig 3. Milky white matrix containing conidia on the lower side of the leaf (orig.).

or septate with 1–3 septa, which correspond with the description of conidia of *Cylindrosporium padi* described by Higgins (1914).

The colonies were observed on nutrient medium after 14 days of incubation. The colonies were whitish brown in color with small, black, hard ball-like fruiting bodies – stromata (Fig. 5). This description is in agreement with authentic descriptions given by Higgins (1914), as well as by Khan et al. (2016) and Williamson and Bernard (1988) for the fungus identified as *Cylindrosporium padi* (Lib.) P. Karst. Ex Sacc.the anamorph of *Blumeriella jaapii* (Rehm) Arx. (Jones 1995). Sexual state of the fungus was not observed either in field, during the trial or in culture.

Pathogenicity test

Pathogenicity tests resulted in small circular red-brown spots on inoculated leaves with well-defined borders characteristic of *Cylindrosporium padi* leaf lesions within 14 days. No spots were observed on uninoculated, control cherry leaves.

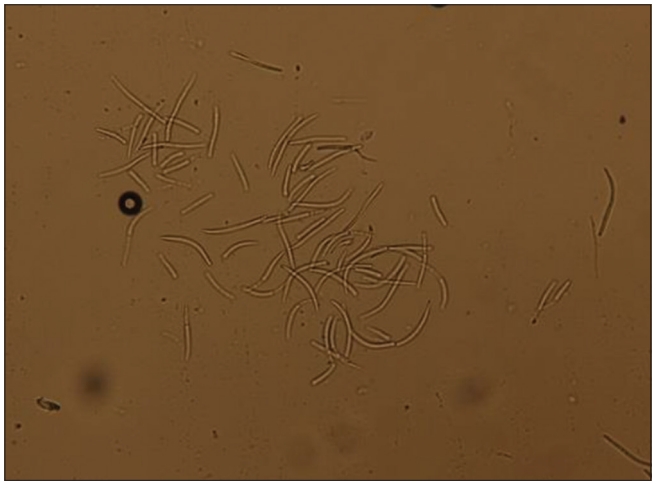


Fig 4. *Cylindrosporium padi* conidia formed on the lower side of cherry leaf after incubation in wet chamber for 24h (orig.).

Fig 5. *Cylindrosporium padi* colony on cherry leaf agar medium 14 days after incubation at 23 ±1°C (orig.).

Morphological traits of isolates obtained from sweet cherry leaves expressing leaf spot symtoms, originating from untreated plots from trials at both localities, as well as proven pathogenicity confirmed that causal agent of the monitored disease in the trials was *Cylindrosporium padi* (*Blumeriella jaapii*).

Field trial

Results of the study (Table 1) suggest that in the first evaluation of disease intensity (%) of cherry leaf spot in fungicide treated variants disease intensity was at significantly ($F=188.49^{**}$, $p<0.01$) lower level (0.35-0.75%) compared to the untreated control (9.47%). Results obtained at locality 2 showed that significantly ($F=91.37^{**}$; $p<0.01$) lower intensity of cherry leaf spot disease in fungicide treated variants, compared to control. The highest efficacy in control of *B. jaapii* in both of the monitored sweet cherry orchards in the first evaluation was recorded for the fungicide based on dodine, with 96.3% at locality 1 and 98.9% at locality 2. Fungicide based on mancozeb also showed high efficacy of 91.0% at locality 1 and 95.6% at locality 2 (Tables 1 and 2).

In the second evaluation, fungicide effects are presented over the medium values of defoliation percentage, using a scale of 1 to 3, as prescribed by the OEPP method. All of the applied fungicides provided efficient protection of cherry leaves. In variants in which fungicides were applied, no premature defoliation was recorded, or the percentage of defoliation was low, while in control variants over 25% of leaves fell at both localities (Tables 1 and 2).

Studies of Perić (2005), demonstrate that the product based on dodine shows high efficacy (93.8 – 95.9%) in control of the mentioned pathogen, which is in accordance with the results obtained in this study. In the Republic of Serbia, for control of sweet cherry leaf spot four products on the basis of dodine and mancozeb are registered and available on the market (Petrović and Sekulić, 2020). Factors that contribute to the occurrence and spread of this pathogen are latently infected planting material, new cultivars, rootstocks, new technology of sweet cherry production as well as the epiphytic nature of the pathogen (Iličić et al., 2018).

The PHI of both applied fungicides is 21 days. Dodine is a local-systemic and contact fungicide with protective and curative action, while mancozeb is a contact fungicide, with protective action.

Dissipation dynamic of dodine in sweet cherry fruits

Validation of the method for the analysis of the residues of dodine fungicide in sweet cherry fruit samples was

Table 1: Efficacy fungicides in control of *B. jaapii* in sweet cherry fruits at locality 1

Fungicide treatment	first assessment	Disease intensity $\bar{x}$ (%)	Efficacy (%)	second assessment	Percentage of defoliation $\bar{x}$ (scale 1-3)
Dodine	81 BBCH	0.35 a	96.3	85 BBCH	1.075 a
Mancozeb		0.75 a	91.0		1.20 a
Control		9.47 b	/		2.3 b
LSD (0.05)		0.85		0.22	

x̄– average number; F=188.49**; p<0.01 F=84.93**; p<0.01

Table 2: Efficacy fungicides in control of *B. jaapii* in sweet cherry fruits at locality 2

Fungicide	First assessment	Disease intensity $\bar{x}$ (%)	Efficacy (%)	second assessment	Percentage of defoliation $\bar{x}$ (scale 1-3)
Dodine	81 BBCH	0.10 a	98.9	85 BBCH	1.05 a
Mancozeb		0.43 a	95.6		1.17 a
Control		9.53 b	/		2.5 b
LSD (0.05)		1.35		0.28	

x̄– average number; F=91.37**; p<0.01 F=50.24**; p<0.01

performed through the linearity (0.999), precision (RSD=12.32%), recovery (104.3-112.1%), and limit of quantification (0.1 mg/kg). Obtained results completely fulfill SANTE/12682/2019 criteria.

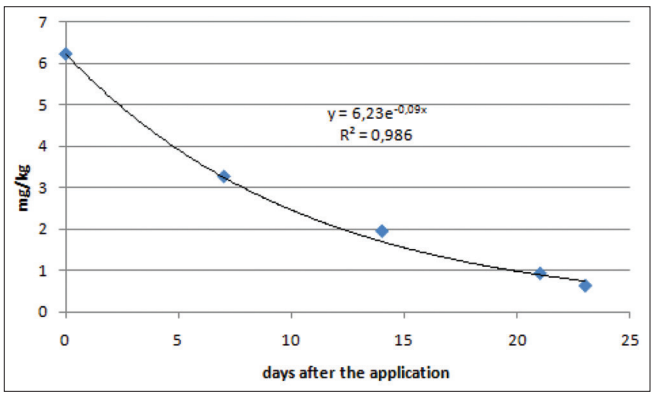
The validated method was applied for the analysis of sweet cherry fruit samples, after the dodine application at the concentration of 0.1%. The determination of dodine residues in sweet cherry fruits was performed using the matrix-match calibration curve.

The average value of the initial deposit of dodine in sweet cherry fruit samples was 6.23 mg/kg (Graph. 1). Seven days after fungicide application, residue level decreased (3.27 mg/kg) by a loss of 47.51%. Further analysis showed gradual decrease in the content of dodine in sweet cherries, and at the end of the pre-harvest interval (21. day) average residues level were 0.93 mg/kg, far below the MRL (3 mg/kg) prescribed in the Republic of Serbia and in the European Union.

In the cases were the temporal variation in pesticide concentration within or on the matrix was reported (Lewis and Tzilivakis, 2017), half-lives were determined via first-order kinetics using $C_t=C_0e^{-kt}$, $DT_{50}=\ln 2/k$, where C_t represents the concentration of pesticide at time (t), C_0 represents the initial concentration and k is the pesticide dissipation rate constant.

The dissipation regressive equation was $C_t=6.23e^{-0.09}$, with $R^2=0.986$. According to the results obtained in this study, the DT_{50} of dodine in sweet cherry fruits is 7.7 days.

The available data show that there is a lack of research on dodine behaviour in sweet cherries. Dasika et al. (2012) tested an efficient and effective analytical method to screen fate of dodine based fungicide in fruits using liquid



Graph 1. Dissipation of dodine in sweet cherry fruit samples.

chromatography tandem mass spectrometry (LC-MS/MS). They concluded that residual levels for dodine were below the maximum allowed values (5 mg/kg) and there were no significant levels of dodine present in apple fruits. High persistency of dodine was documented in experiments conducted by Ticha et al. (2008). Dodine was the only one of detected fungicides found after 5 months in apple fruits. Pesticide residues were below 0.07 mg/kg that is below maximum residue limit values. Bursić et al. (2018) tested a total of 42 sour cherry samples that were collected and analysed for pesticide residues by LC-MS/MS. The study showed that 42.86% of the analysed samples contained pesticide residues. In percentage terms, it seems to be high, but most of the detections were below the recommended MRL values. Results for dodine based fungicide residues in sour cherry fruits were between 0.013-0.439 mg/kg, which is also below MRL values.

CONCLUSION

Conducted isolation from the leaf samples collected in control plots and examination of morphological and

pathogenic properties of the obtained isolates undoubtedly confirm that the occurred changes observed on the cherry leaves were caused by phytopathogenic fungi *Cylindrosporium padi* (*B. jaapii*), at both localities where field trials were performed. Based on the conducted trials and the obtained results it can be concluded that the studied fungicides showed high efficacy in control of *B. jaapii* and provided adequate protection of sweet cherry orchards at localities of Nišava District. The highest efficacy of 96.3-98.9% was recorded for dodine based product, while efficacy of 91-95.6% was recorded for the product based on mancozeb. Moreover, it is important to stay up to date with innovative IPM approaches in order to minimize pesticide application and to increase biodiversity. In this study method for the determination of dodine residues in sweet cheery fruits was validated and applied for the evaluation of the dissipation dynamic. The results (DT_{50}=7.7 days) indicated that sweet cherry fruits could be safely consumed after the application of dodine based fungicides at the recommended rate. Moreover, the suggested PHI of 21 days could be considered to be shorter under agroecological conditions of Serbia.

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Authors' contributions

Conceptualization, Vuković S. and Božić V.; efficacy analysis Vuković S. and Božić V.; phytopathological analysis Grahovac M. and Aleksić G.; residue analysis Šunjka D. and Lazić S.; writing – original draft preparation, Vukovic S. and Bozic V.; writing – review and editing, Grahovac M. and Šunjka D.

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