

EXISTENCE OF *CERCOSPORA BETICOLA* ISOLATES RESISTANT TO BENZIMIDAZOLE AND TRIAZOLE FUNGICIDES IN NATURAL POPULATIONS

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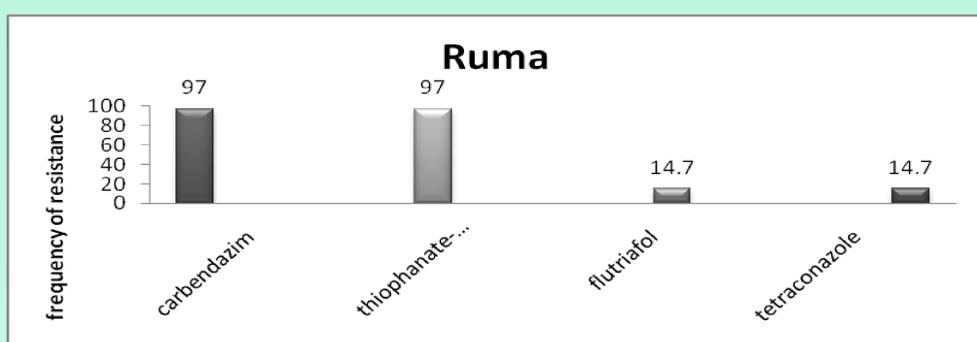
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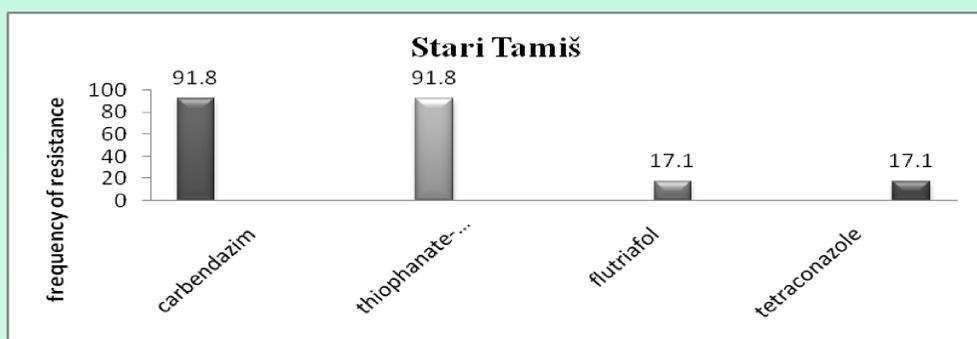
Sugar beet leaf spot caused by *Cercospora beticola* Sacc. is the most important foliar disease of sugar beet worldwide (Weiland and Koch, 2004). In the absence of control measures *C. beticola* could cause significant yield losses up to 50% (Shane and Teng, 1992). For the management of *C. beticola* in Serbia benzimidazole fungicides were used a four decades ago, while triazoles were used somewhat less. To investigate the existence of *C. beticola* populations resistant to benzimidazole and triazole fungicides in natural populations, we compared a frequency of resistant populations in the natural populations with populations treated with these fungicides. Collection of sugar beet leaves with sporulated lesion of cercospora leaf spot disease was conducted during 2009 at localities Ruma and Stari Tamiš, in order to collect isolates which were intensively treated with fungicides in the past few decades. On the other hand, from the field of beet root which have newer been treated with fungicides, leaves with symptoms of cercospora leaf spot were collected to isolate natural populations of *C. beticola*.

Isolates were tested at the discriminatory concentrations (DC) which for carbendazim was 1 mg/l according to Karaoglanidis et al., (2003) while for thiophanate-methyl was 5 mg/l as determined by Weiland and Halloin (2001). According to previously conducted research, appropriate discriminatory concentration was 1 mg/l for both fungicides flutriafol and tetraconazole (Georgopoulos, 1987; Karaoglanidis et al., 2003). Mycelial plugs (5 mm in diameter) were taken from the edge of 14-day-old colonies of each isolate and placed on the centre of PDA plates amended with fungicides in order to discriminate resistant from sensitive isolates. Control Petri dishes were not amended with fungicides. After incubating the plates at 25°C for seven days in the dark, the radial growth (colony diameter) of each isolate was measured and the diameter of the original mycelial plug (5mm) was subtracted from these measurements. For each plate, the average colony diameter (measured in two, perpendicular directions) was used to calculate relative growth (RG). The RG was calculated by formula: $RG = (\text{average colony diameter at DC} / \text{average colony diameter in control}) \times 100$ (Karaoglanidis et al., 2003). Isolates of *C. beticola* were classified as resistant if the RG measured using the discriminatory concentration was $\geq 50\%$ compared with the control (Russell 2004).

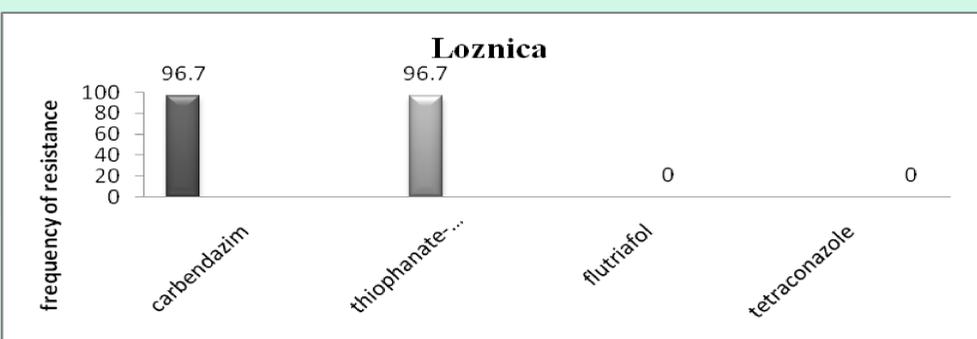
From 34 isolates originated from locality Ruma, only one was sensitive to benzimidazole fungicides i.e. carbendazim and thiophanate-methyl, while the remained 33 were resistant. Relative growth of resistant isolates was very intense compared to a control treatment and exceeded 90%. Frequency of resistance to benzimidazole fungicides at locality Ruma was very high 97%. Five out of 37 *C. beticola* isolates from locality Ruma were resistant to triazole fungicides flutriafol and tetraconazole. Frequency of resistant isolates was 14.7% (Graph. 1). At locality Stari Tamiš where benzimidazole and triazole fungicides were applied, a frequency of resistant isolates to benzimidazole fungicides was very high (91.8%). From total of 37 isolates, 34 had exhibited a low sensitivity to benzimidazole fungicides. At discriminatory concentration of triazole fungicides, six out of 37 isolates were resistant and had a relative growth up to 80% compared to control. Frequency of resistance to triazole fungicides at locality Stari Tamiš was 17.1% (Graph. 2). Isolates of *C. beticola* obtained from locality Loznica exhibited a very high level of resistance frequency to benzimidazole fungicides. From the total of 31 isolates, 30 had a low sensitivity to carbendazim and thiophanate-methyl. On the contrary there was no isolates of *C. beticola* resistant to triazole fungicides (Graph. 3).



Graph. 1. Frequency of *C. beticola* isolates resistant to benzimidazole and triazole fungicides at locality Ruma.



Graph. 2. Frequency of *C. beticola* isolates resistant to benzimidazole and triazole fungicides at locality Stari Tamiš.



Graph. 3. Frequency of *C. beticola* isolates resistant to benzimidazole and triazole fungicides at locality Loznica.

Resistance to benzimidazole fungicides in populations of *C. beticola* for the first time was recorded in 1973 in Greece after two years of their application (Georgopoulos and Dovas, 1973) and later in the USA as well (Ruppel et al., 1974). In Serbia, benzimidazole resistance was detected for the first time by Marić et al. (1976), whereafter the benzimidazole fungicides were used for once a year. Despite their lower application in the field of sugar beet, a high frequency of resistant *C. beticola* populations was detected (Trkulja et al., 2010).

In this study we detected a high frequency of resistance *C. beticola* isolates at localities Ruma and Stari Tamiš, where benzimidazole fungicides were used intensively in the past. Conversely frequency of resistant populations was surprisingly high at locality Loznica in the field of root beet where benzimidazole fungicides have newer been used. Dovas et al. (1976) in their study detected a low but constant level of resistant *C. beticola* isolates in the field where benzimidazole fungicides have newer been used. Nevertheless this research has provided no evidence that the frequency of benzimidazole-resistant strains declines after fungicides were discontinued. However, Karaoglanidis et al. (2003) detected very low frequency of resistant *C. beticola* at locality where benzimidazole use was discontinued after 1973. Due to polygenic control of resistance to triazole fungicides, development of resistance is slow and quantitative.

This research revealed presence of resistant isolates in populations Ruma and Stari Tamiš, while in locality Loznica resistant isolates have not been found. This research is in correlation with the fact that isolates resistant to triazole fungicides have a high fitness penalty. Karaoglanidis et al. (2002) suggested that the frequency of resistant isolates to triazole fungicides was significantly lower during the first sampling and after the end of the spraying period of the previous year, indicating that resistant strains either cannot compete well with the sensitive strains in the absence of DMI treatments or that they cannot survive well during overwinter. Moretti et al. (2003) detected low fitness and virulence isolates of *C. beticola* resistant to triazole fungicides compared to sensitive populations.