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Оригинални научни рад

EFFICIENCY OF BIOLOGICAL PROTECTION IN NURSERY PRODUCTION

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Abstract: Chemicals are ubiquitous in our bodies and our global environment. One of the most compelling areas of potential innovation for sustainability is green chemistry. We studied the impact of alternative measures of protection on the occurrence of mass dieback in oak seedlings and forests in Central Serbia (caused by *Microsphaera alphitoides*) by applying various dosages of AQ-10 biofungicide. Examinations were conducted using standard OEPP methods PP1/69 (2) (1997). Its application will lead to the use and generation of inherently safer chemicals, the reduction of the risk of exposure to toxic chemicals, and the increasing availability of safer and healthier products. The best results in the suppression of oak powdery mildew were obtained by using sulphur SC in the concentration of 0.5%. Very satisfactory results were obtained when AQ-10 biofungicide was used in the highest dosage of application (70g/ha). The number of treatments was proven to have no significant impact on the increased efficiency of the biopreparation. In other words, it showed that besides the application dosage, the high efficiency of the biopreparation depends primarily on the proper timing of the application.

Keywords: Efficiency, AQ-10, Powdery Mildew, Oak

ЕФИКАСНОСТ БИОЛОШКЕ ЗАШТИТЕ У РАСАДНИЧКОЈ ПРОИЗВОДЊИ

Извод: Хемикалије су опште присутне у нашем глобалном окружењу. Једно од најважнијих подручја потенцијалних иновација одрживости је зелена хемија. Проучаван је утицај алтернативних мера заштите од појаве масовног обољевања садница храста и храстових шума у Централној Србији (узроковано дејством *Microsphaera alphitoides*) различитим дозама биофунгицида AQ-10. Испитивања су вршена стандардним OEPP методама PP1/69(2)(1997). Његова примена ће довести до употребе и стварања природно сигурнијих хемикалија, смањењем ризика од изложености токсичним хемикалијама и повећањем доступности сигурнијих, здравијих производа. Најбољи резултати у сузбијању храстове пепелнице постигнути су употребом сумпора SC у концентрацији од 0,5%, док су сасвим задовољавајући резултати добијени употребом биофунгицида AQ-10 у највећој дози примене (70g/ha). Доказано је да број третмана нема значајан утицај на повећану ефикасност биопрепарата, или другим речима, показао је да осим дозирања апликације, висока ефикасност биопрепарата зависи пре свега од правилног времена примене.

Кључне речи: ефикасност, AQ-10, пепелница, храст

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1. INTRODUCTION

Serbia is considered a medium-forested country. Of the total territory of Serbia, 29.1% is under forest. It means that the area under forest in Serbia is closer to the world's average forest cover of 30%, which is significantly lower than Europe's forest cover of 46%. The total area under forest in Serbia is 2,252,400 ha, while the most important oak species cover an area of 720,000 ha. Out of that, forests of *Q. cerris* L. stretch over 345,200 ha, *Q. petraea* (Matt.) Liebl. over 173,200 ha, *Q. frainetto* Ten. over 159,600 ha, *Q. robur* L. over 32,400 ha, and *Q. pubescens* Willd. over 10,400 ha (National Forest Inventory of the Republic of Serbia, 2009).

A massive die-back of oak forests in Serbia usually occurs after the primary defoliation by insects (gypsy moth, winter moth, tortrix roller, etc.) (Marovic, R. *et al*., 1997, Tabakovic - Tosic, M. *et al*., 2007). This defoliation is followed by the occurrence of powdery mildew on new, young foliage. It is caused by the activity of pathogenic fungus *Microsphaeraalphitoides* Griff. et Maubl. (1910) (Syn. *Erysiphe alphitoides*). Over 30 species of host plants of the *Quercus* genus show sensitivity to the agents of powdery mildew (Braun, U., Cook, RTA., 2012: Braun, U., Takamatsu S., 2000).

The need for the introduction of integrated protection of the environment and forests is imposed by one of the basic features of modern civilization – the environmental crisis. Rapid advancements in science and technology entail changes and disruption of the natural balance, which ultimately threatens the survival of some plant communities. However, the increasing risk raises the awareness of people that they are exposed to risk and, in that context, the value systems of society as a whole undergo a qualitative change that leads to the formation of environmental culture. This, in turn, produces a need to apply alternative measures of suppressing harmful organisms within the legislation related to the use of pesticides and prohibition of the use of some pesticides (Rajkovic, S. *et al*., 2010a; Rajkovic, S. *et al*., 2010b; Rajkovic, S. *et al*., 2010c; Stanivukovic, Z. *et al***.,** 2019; Radulovic, Z. *et al*., 2019a; Radulovic, Z. *et al*., 2019b).

In order to introduce the proper use of new disease-control preparations into any country, it is necessary to comply with certain relevant principles, requirements, and criteria that are primarily related to measures of risk assessment and mitigation as well as the list of hazardous and highly hazardous pesticides, with the possibility to apply alternative protection prescribed by the Council of Forest Management – Forest Stewardship Council (FSC). FSC certificates originate from Canada (1993), and there are now 15 bodies worldwide that assess the quality of forest management under this program. In the Republic of Serbia, certification according to FSC principles has so far included 10 forest estates (six in Central Serbia and the four in Vojvodina).

Nowadays, according to Djordjevic, S., 2008, there are 185 biopesticide preparations registered in the world, 72of which contain bacteria as active agents, 47 contain fungi, 40 entomopathogenic nematodes, 24 viruses and two has protozoa as active agents.

In the Republic of Serbia, no fungicides have been registered for the control of pathogens in forest ecosystems. It is therefore necessary to select eco-friendly fungicides complying with FSC policy on pesticide application to be registered for this region. This study deals with the control of oak powdery mildew in the Republic of Serbia by means of alternative protection measures. The protection implies the use of different doses of AQ-10 biofungicide. AQ-10 biofungicide (Ecogen Inc.) is a pelleted formulation of conidia of *Ampelomyces quisqualis* Ces. ex Schlechtend., a fungus that parasitizes powdery mildew colonies. The AQ-10 is intended for use as part of an integrated management program, and may thus be used in combination with compatible conventional chemical fungicides (Markovic, M., 2010).

2. MATERIALS AND METHODS

The tests were performed using the pathogenic fungus *Microsphaera alphitoides* Griff. et Maubl. that causes oak powdery mildew. Pedunculate oak was selected because this species is most susceptible to oak powdery mildew and nursery seedlings are particularly vulnerable.

The biofungicide used in the study was AQ-10. The treatment was applied before the symptom onset and later after the symptoms of infection had already been observed. AQ-10 biofungicide is completely harmless to humans and warm-blooded animals and has a very short half-life – only 24 hours, so it is safe to be used for the protection of crops used for human consumption.

Besides the biofungicide, Nu Film-17 and Nu Film-P polymers were tested in this study. Specific beta-pinene polymers are emulsions of concentrated formulations used as spray tank additives that can be applied in combination with insecticides and fungicides in the protection against pathogens and diseases. They are used in pest control programmes. When sprayed on plantations, they form a so-called film, encapsulate the pesticide, and thus protect it from various external influences, including rain and wind. Evaporation of the pesticide is thus minimized as the foliage is coated with the polymer film. The application of polymers thus extends the longevity of pesticides by 50-100%, with the usage of their lower doses. The chemical compound used in the study was sulphur. Production name: Sulphur SC. Active matter: Elementary sulphur 810.50g/l. Manufacturer: Galenika-Fitofarmacija a.d. Beograd-Zemun. Concentration: 0.5%.

The experiments were conducted following the instructions of PP 1/152 (2) (EPPO, 1997a) methods in a randomized block design. The experiments were set in four replications at Lazarevac site near Belgrade. The plot was (1 x 3 m), 25 m^2 in size, and contained 8 trees. The occurrence and development of the agent of powdery mildew were monitored from the onset of the disease in the control variant, particularly when the differences between the control variant and the variants treated with fungicides and biofungicides in combination with the polymer became obvious. The following doses of the preparation were applied: AQ-10 in doses of 30, 50, and 70 g/ha; Nu Film-17 in the dose of 1.0 l / ha; Nu Film-P in the dose of 1.5 l / ha; SC sulphur in the concentration of 0.5%. The assessment of the secondary infection of leaves was performed on 100 leaves in four replications per each variant. The assessment of each leaf was performed according to the following scale: 0 = no infection; 1 = very low infection; 2 = partial onset (individual spots of powdery mildew on leaves); 3 = moderate to severe infection (over one-half of the leaf is infected with powdery mildew); 4 = very severe infection (more than two-thirds of the leaf are infected, leaves curl up and fall off). The intensity of infection was determined using the methods PP 1/152 and PP 1/69 (1997a), (1997b). Phytotoxicity was monitored according to the method PP 1/135 (1997c). The data were analyzed using statistical methods – the intensity of infection using the method PP/181 (1997d), Townsend G.R. & Heuberger J. W. (1943) and efficiency according to Abott W. S.(1925). The differences in the intensity of infection were determined using the analysis of variance and LSD test.

3. RESULTS AND DISCUSSION

The test results obtained at the study locality, in the nursery near Lazarevac, are shown in Table 1.

Based on the results shown in Table 1, the highest efficiency was obtained in variants 10 and 6, i.e., the variant in which sulphur was used with the addition of Nu Film-17 polymer and the variant in which AQ-10 was used in the application dose of 70 g/ha with the addition of Nu Film-17 in the dose of 1.0 l/ha (the intensity of infection was 0.98% and 3.28%).

Slightly higher grades of infection, but still a high degree of efficiency were found in variant 11, in which only sulphur was used (1.61% intensity of infection). Good efficiency was achieved in variants 8 and 9 (6.75% and 4.25%) in which higher concentrations of AQ-10 (50 g and 70 g) were used with the addition of Nu Film-P in the dose 1.5 l/ha. Similar efficiency was achieved in variant 3 by using high concentrations of AQ-10 (70 g) without any additions – polymers (intensity of infection of 4.45%).

Table 1 The intensity of the attack of *M. alphitoides* on pedunculate oak and efficiency of fungicide, biofungicides, and polymers
Табела 1. Интензитет напада *M. alphitoides* на храсту лужњаку и ефикасност примене фунгицида, биофунгицида и полимера

No	Fungicide	Concentration/Dose (%), kg/ha	Infection (%)	Efficiency (%)
1.	AQ-10	30 g	10.60 a	61.21
2.	AQ-10	50 g	12.42 a	54.53

3.	AQ-10	70 g	4.45 a	83.71
4.	AQ-10 +Nu Film-17	30 g + 1.0 l/ha	9.13 a	66.61
5.	AQ-10 +Nu Film-17	50 g + 1.0 l/ha	10.55 a	61.39
6.	AQ-10 +Nu Film-17	70 g + 1.0 l/ha	3.28 a	88.01
7.	AQ-10 +Nu Film-P	30 g +1.5 l/ha	12.40 a	54.62
8.	AQ-10 +Nu Film-P	50 g +1.5 l/ha	6.75 a	75.30
9.	AQ-10 +Nu Film-P	70 g +1.5 l/ha	4.25 a	84.45
10.	Sulphur SC+Nu Film-17	0,5% + 1.0 l/ha	0.98 a	96.43
11.	Sulphur SC	0.5%	1.61 a	94.13
12.	Untreated	-	27.33 b	0.00
	lsd 005	10.34		
	lsd 001	14.61		

Somewhat lower efficiency was observed in variants 4 and 5, where AQ-10 was used in smaller concentrations (30 and 50 g) with the addition of Nu Film-17 polymer (intensities of infection of 9.13% and 10.55%). Lower efficiency was achieved in variants 1, 2, and 7 in which AQ-10 was used in smaller concentrations (30 and 50 g) without the addition of polymers and AQ-10 50 g with the addition of Nu Film-P (intensities of infection 10.60%, 12.42%, and 12.40%). In the control variant, in which no protection treatment was applied, the intensity of infection was 27.33%.

The statistical analysis of the obtained test results showed significant differences between all the tested variants and the control variants, as well as differences between variants 10 and 11, in which chemical treatment with or without the addition of polymers was used, compared to variants 2 and 7, in which lower doses of AQ-10 bioproduct were used with the addition of Nu Film-P polymer.

The analysis of variance of randomized block design showed that the difference between the mid-repetitions was statistically significant at the probability of 95%, since $F_0 > F_{0.05}$. A statistically significant difference was also found between the mid-treatments at the probability of 99%, since $F_0 > F_{0.01}$. When the comparative analysis was used to establish significant differences, three homogeneous groups were identified with statistically significant differences at 99%, which was consistent with the groups previously explained and processed in the analysis of variance.

An overview of meteorological data that had a direct impact on the intensity of the infection during the course of the experiment at the tested site is shown in Figure 1.

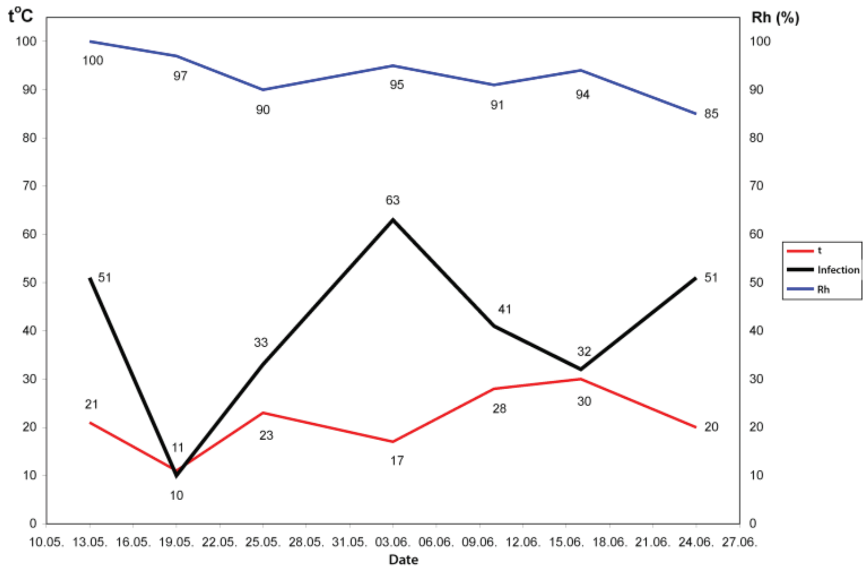

Figure 1 Infection of pedunculate oak seedlings with powdery mildew depending on outdoor air temperature and humidity
Слика 1. Интензитет инфекције храста лужњака пепелницом у зависности од спољне температуре и влажности ваздуха

In the reference literature, multiple authors from different climatic regions have confirmed and proved that the intensity of infection with powdery mildew on a variety of plant species is directly dependent on environmental conditions, primarily air temperature and humidity. The intensity of infection of pedunculate oak seedlings with powdery mildew in the nursery near Lazarevac was therefore examined in relation to air temperature and humidity, as shown in Figure 1, for the period from 13 May through 24 June.

The presented graph clearly shows that low and high air temperatures (11° and 30°C) had a direct impact on the reduction of the infection, which in this period amounted to 10% and 32% out of the total number of tested seedlings. When the air temperature was 11°C, the relative air humidity was extremely high amounting to 97%, which does not favour the development of the pathogen. This is probably the reason the infection was three times lower than at the temperature of 30°C when the air humidity was favourable for the pathogen and amounted to 94%. A very high rate of seedling infection was observed at temperatures of 17° to 21°C at the relative air humidity from 85% to 100% (the infection in this period amounted to 51% to 63% of all the tested seedlings).

Figure 1 clearly shows that the negative effect of one of these two factors (humidity and temperature) can be greatly mitigated by the positive effect of the other factor. Thus, the temperature of 21°C that is very favourable for the development of the pathogen directly reduced the negative impact of the maximum air humidity of 100% and led to a high degree of infection of seedlings of 51%, which was also registered under very good conditions of 85% of air humidity and temperature of 21°C. The effect of slightly lower temperature than the optimum one for the development of pathogen (17°C) was hence probably mitigated by the corresponding relative humidity of 94%, and therefore the infection of seedlings in that period was maximal and reached 63%. Temperatures of 30°C and above were fatal for the development of the pathogen. The growth of the fungus was also significantly higher at 20°C than at 25°C. High levels of relative humidity (80-90%) were favourable for the pathogen development in the short term, but longer exposure to these conditions led to a restricted infection. It should certainly be pointed out that the health condition of forests greatly depends on the implementation of preventive measures aimed at eliminating the factors that favour the development of diseases and providing forest trees with satisfactory levels of resistance and vitality.

Based on the research of the impact of weather conditions (environmental factors – air humidity and temperature) on the infection intensity on the territory of Serbia, we can safely say that the environmental conditions that favour intensive infections in this region occur from early to mid-July, i.e., the period when sunny intervals are prolonged, the outdoor temperature remains close to 20°C over extended periods of time, while the relative air humidity amounts to 80 to 85%. For these reasons, the health condition of oak trees in the tested localities needs to be monitored during that period and the appropriate control measures should be put in hand in case of severe infections. The measures should be in compliance with the relevant national legislation and FSC policy on application of chemical agents.

4. CONCLUSIONS

1. It was proven that forest protection is possible by applying alternatives to chemical measures that are harmful to human health and the environment. These alternatives are essential for the preservation and conservation of the environment;
2. The frequency of infection increases with the increase of relative humidity up to 90%, but the infection does not occur when the leaf surface is wet, or when it rains. Rain showers wash off the leaf inoculum and reduce the intensity of infection since the pathogen is epiphytic, while prolonged periods of high temperature favour the development of the pathogen;
3. The most favourable time for the outbreak of intense powdery mildew infection in Serbia is in early July, which is important from the aspect of protection since infections can be significantly reduced when AQ-10 is applied before the outbreak;
4. Besides the number of treatments, the timely application of the treatment is of utmost economic importance;
5. The best results in suppressing oak powdery mildew were obtained in the variants in which AQ-10 biofungicide was used in higher doses (50 and

70 g/ha) with the addition of the polymer film Nu Film-17 in doses of 1.0 and 1.5 l/ha;
6. The rationalization of use, as well as reduction of the treated area, is ensured through the appropriate method of application, timely implementation, and professional selection of non-toxic products.

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ЕФИКАСНОСТ БИОЛОШКЕ ЗАШТИТЕ У РАСАДНИЧКОЈ ПРОИЗВОДЊИ

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Резиме

Хемикалије су опште присутне у нашем глобалном окружењу. Једно од најважнијих подручја потенцијалних иновација одрживости је зелена хемија. Проучаван је утицај алтернативних мера заштите од појаве масовног обољевања садница храста и храстових шума у Централној Србији (узроковано дејством *Microsphaera alphitoides*) различитим дозама биофунгицида AQ-10. Испитивања су вршена стандардним OEPP методама PP1/69(2) (1997). Његова примена ће довести до употребе и стварања природно сигурнијих хемикалија, смањењем ризика од изложености токсичним хемикалијама и повећањем доступности сигурнијих, здравијих производа. Учесталост заразе расте са порастом релативне влажности и до 90%, али зараза се не јавља када је површина листа влажна, или када пада киша. Пљусковите кише испирају инокулум листа и смањују интензитет инфекције, јер је патоген епифитан, док продужени периоди високих температура погодују развоју патогена. Најповољнији период за појаву интензивних инфекција пепелницом у Србији је у првој половини јула, што је важно са аспекта заштите, с обзиром да се инфекције могу значајно смањити када се AQ-10 примени пре њеног избијања. Од пресудне важности поред броја третмана је и његова примена у одговарајуће време, што је са економске тачке гледишта веома значајно. Најбољи резултати у сузбијању храстове пепелнице постигнути су употребом сумпора SC у концентрацији од 0,5%, док су сасвим задовољавајући резултати добијени употребом биофунгицида AQ-10 у највећој дози примене (70g/ha). Доказано је да број третмана нема значајан утицај на повећану ефикасност биопрепарата, или другим речима, показао је да осим дозирања апликације, висока ефикасност биопрепарата зависи пре свега од правилног времена примене.