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Original scientific paper

INVESTIGATION ON THE EFFECTIVENESS OF SOME FUNGICIDES IN THE CONTROL OF *RHIZOCTONIA SOLANI* IN CONDITIONS OF ARTIFICIAL INOCULATION

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ABSTRACT

Investigations of biological effect of fungicides in the control of pathogenic fungus *Rhizoctonia solani* - causing agent of the damping off disease on tobacco seedlings were carried out in biological laboratory at Scientific Tobacco Institute-Prilep in 2009 and 2010. The aim was to study the effectiveness of fungicides that have already been used in the production of tobacco seedlings, and some other fungicides, i.e. active ingredients. The results will contribute to possible expanding of the list of products aimed for protection of tobacco seedlings from this disease.

Artificial inoculation was made with suspension of pure culture of the pathogen and products with appropriate concentrations were applied through watering. The poorest results were obtained with the fungicide Previcur (0,15%). Products with active ingredient chlorothalonil showed an average effectiveness of 78.27%. The best results were achieved with Top M (0,1%) and Quadris (0,2%) with 87.85% and 94.36% average effectiveness in both years respectively.

Key words: *Rhizoctonia solani*, fungicide, active ingredient, effectiveness

ИСПИТУВАЊЕ НА ЕФЕКТИТЕ ОД НЕКОИ ФУНГИЦИДИ ЗА КОНТРОЛА НА *RHIZOCTONIA SOLANI* ВО УСЛОВИ НА ВЕШТАЧКА ИНОКОЛУАЦИЈА

Испитувања на биолошкото дејство на фунгицидите за сузбивање на патогената габа *Rhizoctonia solani* – предизвикувач на болеста сечење на тутунскиот расад беа извршени во Биолошката лабораторија при Научниот институт за тутун-Прилеп во 2009 и 2010 год. Тие имаа цел да се испита ефикасноста на фунгицидите кои веќе се употребуваат во производството на тутунски расад, но и некои други фунгициди, односно активни материи. Резултатите би придонеле за можно проширување на листата на препарати за заштита на тутунскиот расад од сечењето.

Истражувањата беа извршени со вештачка инокулација со суспензија од чиста култура од патогенот, а препаратите со соодветната концентрација беа аплицирани со полевање. Најслаби резултати имаше фунгицидот Previcur (0,15%). Препаратите со активна материја chlorothalonil покажаа просечна ефикасност 78,27%. Најдобри резултати покажаа препаратите Top M (0,1%) со 87,85% и Quadris (0,2%) со 94,36% просечна ефикасност во двете години.

Клучни зборови: *Rhizoctonia solani,* фунгицид, активна материја, ефикасност

INTRODUCTION

Tobacco seedlings production suffers from serious damage caused by damping off disease each year. It occurs in all stages of seedling development and can be manifested as pre-emergence damping off, post-emergence damping or as rot pruning (Numez, 2005). Bearing in mind the importance of seedlings for successful tobacco production, the seriousness of the damage becomes more obvious.

The most common cause of this disease in our conditions is the pathogenic fungus *Rhizoctonia solani*. It is a widespread pathogen known for its harmful effects on many important agricultural and horticultural crops (Grosh, 2003) including beans, peas, soybeans, black tomato, cucumber, corn, alfalfa, and many others (Uchida, 2012). Damages that *R. solani* causes to yield are proportional to agricultural land and can reach up to 50% (Wallwork, 2000, loc cit. Hollaway, 2008).

The application of preventive measures is of great importance in plant protection. These measures include crop rotation, optimum seed quantities, proper use of fertilizers in accordance with the growing stage, moderate irrigation, aeration, etc. (Uchida, 2012). Protection programs should be based on prevention of pathogen infection and maintenance of vigorous plants (Pataky, 1988).

Despite this, the fight against this pathogen is difficult. According to Nunez (2005), *R. solani* is a soilborne pathogen which lives in many types of soils. It persists for many years as sclerotia or as mycelium on oganic substances, in various environmental conditions (Pataky, 1988; Grosh, 2003). Sclerorotia have an ability to float and to exist in water and thereby they present a primary inoculum (Ceresini, 1999).

Hollaway (2008) reported that the control of this pathogen is difficult because the fungus has a wide circle of hosts, i.e. limited possibilities for crop rotation, and there are not resistant varieties because the fungus can live and develop even in the absence of living plant organism – it has a "saprophytic ability." Because of all this, it can not be eliminated, but it can be controlled to a level that will not cause economic losses.

Due to previous statements, the application of chemical products in protection of tobacco from this disease is necessary.

A number of active ingredients have been reported for the control of this pathogen. Frisina (1988) made investigations with benomyl, iprodione, chlorothalonil and benodanil. Mueller (1996) recommended the use of pentachloronitrobenzene (PCNB). From there investigations of ED50 of many active ingredients Csinos and Stephenson (1998) recommended flutolanil, iprodione, fluazinam and tebuconazole to prevent the spreading of infection in tobacco seedbeds.

According to Mocioni et al. (2003), azoxystrobin and trifoxystrobin, as well as tebuconazole applied in three different formulations showed significant effectiveness in the control of *Rhizoctonia*.

Fungicides that contain PCNB (Terrachlor), Iprodione (Rovral) or Azoxystrobin (Quadris) are effective against *Rhizoctonia solani* (Koenning, 2007). Wong (2008) gives an extensive list of active ingredients and preparations for control of *R. solani* in lawns. Fludioxinil, maneb, penthiopyrad, thiophanate-methyl, PCNB and azoxystrobin, under different trade names, are recommended by Schwartz and Gent (2012) in potato.

The number of products for protection of tobacco seedlings is limited and in certain cases unsuitable fungicides are applied which are not effective in the control of specific causing agent. Therefore, the purpose of this research was to study the effectiveness of some fungicides in the control of causing agent of damping off disease - the pathogenic fungus *R.solani* in conditions of artificial inoculation.

MATERIAL AND METHODS

Investigations were carried out in Biological laboratory of Tobacco Institute-Prilep, in 2009 and 2010. For this purpose, seeds of P 23 tobacco variety were sown in 8 pots for each variant in both years. The seedlings were grown in traditional way.

Pathogenic fungus *R. solani* was isolated from infected tobacco seedlings and grown on

potato dextrose agar (PDA). 15-day pure culture of the fungus was used for inoculation.
Artificial inoculation of the seedling was carried out prior to the stage of intensive growth and fungicide treatment was applied the following day. The check variant was only inoculated.

Fungicides were applied by watering with 3 l/m2 of the suspension with appropriate concentration. The disease development was followed every day and several calculations were made on the intensity of disease attack through estimation of the percentage of infected area, starting few days to 10-15 days after inoculation. For assessment of the effectiveness, the last estimation was taken into consideration. The method of Abbott was used to estimate the effectiveness of the products.

The selection of products was made according to world literature and our personal investigations, including the experiences from the production and protection of tobacco seedlings in Tobacco Institute-Prilep. The list of investigated fungicides is presented in Table 1.

Table 1. Investigated fungicides

Fungicide	Active ingredient	a.i. content	Concentration %
Top - M 70% WP	Thiophanate- methyl	70%	0.1
Pilarić 75% WP	Chlorthalonil	75%	0.2
Bravo 500 SC	Chlorthalonil	515 g/l	0.2
Quadris 25 SC	Azoksistrobin	250 g/l	0.1
Previcur- N	Propamocarb	70 g/dm^3	0.15

RESULTS AND DISCUSSION

Damping off disease emerges on the stem at the ground line, where the tissue necrotizes and dies, making the further development of plant impossible. Infected plants fall down on soil surface as if "cut off". Sometimes, in conditions of higher seedbed moisture, a whitish mold can be observed on the seedlings. The disease also spreads on adjacent plants, resulting in infected infected patches which coalesce and cover large areas of the bed. For this reason, the major part of the seedbed is devastated and the seedlings production is reduced (Fig. 1).

Fig. 1. Symptoms of damping off disease on tobacco seedbeds

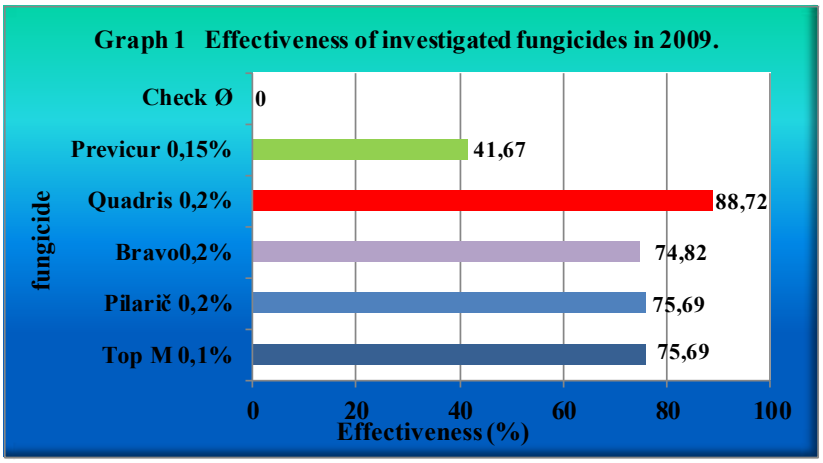
In artificial inoculation with the pathogen in 2009, the percentage of infected area in the check variant ranged from 37.75 to 72.00% (Table 2). The lowest intensity of attack was recorded with application of Quadris 0.2% and Top M 0.1% and the highest intensity with Previcur 0.15%, The latter showed the highest percentage of infected area compared to other products investigated.

With further analysis, the intensity of the disease increases. The highest percentage of infected area in the next two analyses was observed with application of Previcur 0.15% and the lowest with Quadris 0.2%. With Top M 0.1% the percentage of infected area significantly increases, compared to the first analysis. Similar is the case with Pilarić 0.2%, while with Bravo 0.2% it has been held in almost the same level.

According to the intensity of attack, the lowest effectiveness (41.67%) was obtained with Previcur 0.15% and the highest with Quadris 0.2% (88.72%). Other products showed similar effectiveness, ranging about 74 - 75% (Table 2, Graph 1).

Table 2. Effectiveness of fungicides investigated in 2009

Fungicide	infected area %			Effectiveness %
	Date of estimation			
	16.06	20.06	25.06	-
Top M 0.1%	3.29	12.50	17.50	75.69
Pilarič 0.2%	6.32	6.25	17.50	75.69
Bravo 0.2%	16.25	17.50	18.13	74.82
Quadris 0.2%	3.13	5.63	8,13	88.72
Previcur 0.15%	22.50	25.63	42.00	41.67
Контрола Ø	37.75	50.00	72.00	-



In 2010 lower intensity of the disease attack was manifested both in the check (21.88 - 55.63%) and in the variants treated with fungicides (3.13% with Bravo 0.2% and 4.38% with Pilarić 0.2%). In application of Top M 0.1% and Quadris 0.2% no occurrence of the disease was observed. The highest intensity of the attack appears with Previcur 0.15% (Table 3).

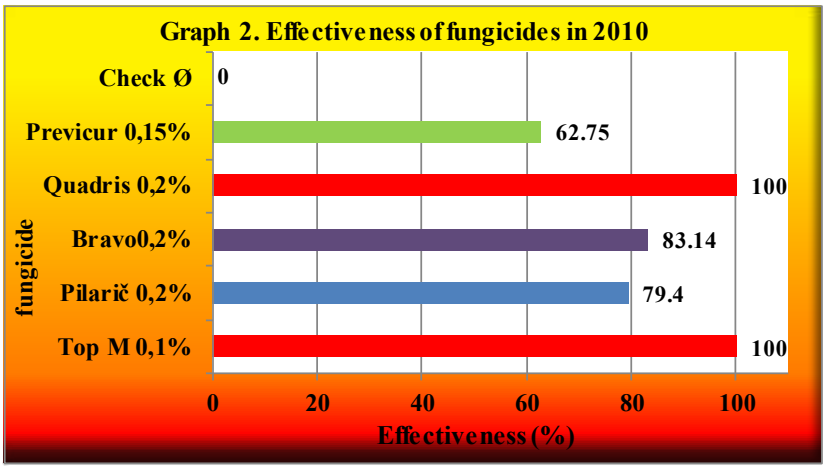
In the following analysis, again, there is no presence of the disease in plants treated with the fungicides M Top 0.1% and Quadris 0.2%. With application of Pilarić 0.2% and Bravo 0.2%, a more significant increase of the infected area was observed in the last analysis. It certainly had an impact on their effectiveness, which ranged 79.40% and 83.14%, respectively.

Top M 0.1% and Quadris 0.2% showed 100% effectiveness in the control of the pathogen *R. solani* (Table 3, Graph 2).

Table3. Effectiveness of fungicides investigated in 2010

Fungicide	infected area %			Effectiveness %
	Date of estimation			
	21.05	25.05	31.05	
Top M 0.1%	-	+-	-	100.00
Pilarič 0.2%	4.38	6.25	11.46	79.40
Bravo 0.2%	3.13	3.22	9.38	83.14
Quadris 0.2%	-	-	-	100.00
Previcur 0.15%	10.63	13.06	20.63	62.75
Контрола Ø	21.88	26.94	55.63	-

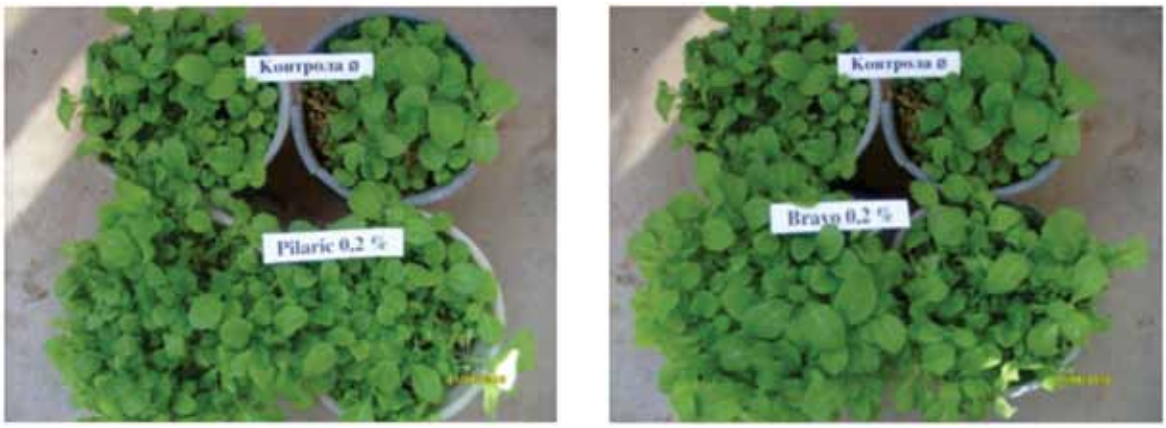
- no occurrence of disease
+- insignificant occurrence



The lowest effectiveness, just as in 2009, was determined with Previcur 0.15% (Graph 2). These results are justified because these product is characterized by a specific activity toward several fungi of the class Oomycetes (Extoxnet 1997, Bayer Crop Science, 2009). In practice it is used in control of the pathogen *Pythium debarianum*, which place in the classification of phytopathogenic fungi is completely different than that of *R. solani*. For a long time it has been considered as the only causing agent of the disease, due to the resemblance of symptoms in tobacco seedlings. For this reason it was included in the list of selected products. Results of these investigations imposed the need for correct determination of the causing agent prior to the use of chemicals in disease control (Pataky, 1988).

The problem with the presence of two pathogens (in this case *Pythium ultimum* and *R. solani*) is also emphasized in investigations of other authors. Most of the fungicides give efficient control of one pathogen only and therefore great attention should be paid to the choice of most appropriate product (Mueller, 1996).

Figure 2. The effectiveness of Previcur (0.15%)



Figures 3, 4. Effectiveness of the products with active ingredient chlorothalonil

The products Pilarić and Bravo (a.i. chlorothalonil) showed 74.82% to 83.14% effectiveness in both years (Figures 3 and 4). In 2009 their effectiveness was the same with that of M Top, and in 2010 it was lower. Compared to Quadris, their effectiveness is lower in both years. Also in the investigations of Frisina (1988) on pure cultures of *R. solani* (with mean value of ED50 for the investigated isolates) the degree of inhibition of chlorothalonil was 40-65%. This active ingredient is recommended for control of *R. solani* by spraying the seedlings of horticultural plants prior to or after transplanting (UC Pest Management Guidelines, 2009).

The effectiveness of Top M in 2010 was 100% (Figure 5). In 2009 it was lower, just as that of other products, taking into consideration the higher intensity of attack in the check variety. Compared to Quadris, its effectiveness in the same year was lower, which is in accordance with investigations on sugar beet carried out by William et al. (2004), according to which thiophanate-methyl was less effective than strobilurins.

Nevertheless, the mean value of the effectiveness in both years was 87.85%. Therefore, this product provides good protection from damping off disease and is applied in seedlings production in Tobacco Institute-Prilep. Products with active ingredient thiophanate-methyl are recommended for control of *R. solani* in a number of programs for protection of various crops (Wong 2008, UC Pest Management Guidelines 2009, Schwartz and Gent 2012).

Figure 5. The effectiveness of Top M (0.1%)

In both years, the product Quadris gave excellent results in the control of *R. solani* (Figure 6). According to *in vitro* investigations of Gveroska (2008) on a number of fungicides against the pathogen *R. solani*, Quadris had shown no reducing effect, which is contrary to the results obtained with application of another isolate. Blazier and Conway (2004) reported that isolates of a different, and even of the same anastomosis group are characterized by different susceptibility to azoxystrobin.

In vitro activity of azoxystrobin is not so effective as in field conditions and in biological laboratory. Mycelial growth on PDA can be only 30% at 1000 mg / ml a.i. *R. solani* belongs to the fungi that are using the alternative oxidase enzyme (AOX). It functions well in *in vitro* conditions, but not in the tissues treated with strobilurins. Additionally, the antioxidants of the host-plant as flavones may interfere with the alternative way *in vivo*, which affects the reduction of the intensity of attack. It means that this fungus is more susceptible to strobilurins in natural conditions. In that way, the effectiveness of this active ingredient *in vivo* is higher. Azoxystrobin (Quadris) is a registered product for protection of tobacco from blue mold disease, but it can be also used in treatments of ready-to-transplant tobacco seedlings to protect them from root rot caused by *R. solani* (La Mondia, 2012).

The application of strobilurins during inoculation or half dose in inoculation and the other half two weeks later, provides the most effective protection. In our investigation, too, fungicides were applied the next day after inoculation and the product with a.i. azoxystrobin (Quadris) gave excellent results. Even with a non-optimal period of application, azoxystrobin showed higher effectiveness compared to other strobilurins (William et al., 2004).

Figure 6. The effectiveness of Quadris (0.1%)

CONCLUSIONS

- The lowest effectiveness of fungicides in both years of investigation (2009, 2010) was recorded with Previcur (0.15%).
- Effectiveness of the product Pilarić (0.2%) was 75.69%, i.e. 79.40% and that of Bravo (0.2%) - 74.82% and 83.14% in both years.
- The average value of effectiveness of the products with active ingredient chlorothalonil was 78.27%.
- The standard product Top M (0.1%) in 2009 reached 75.69% effectiveness and in 2010 - 100%. The mean value of effectiveness in both years equaled 87.85%.
- The effectiveness of Quadris (0.2%) was 88.72% in 2009 and 100% in 2010. The average effectiveness was 94.36%.
- The highest effectiveness was obtained with the products Quadris (0.2%) and Top M (0.1%).
- Recommended products the control of damping off disease on tobacco seedlings caused by the pathogen *R. solani* are those which showed highest effectiveness in conditions of artificial inoculation.
- Investigations should be continued in natural conditions of seedlings production.

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