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NEW FUNGICIDE OPPORTUNITIES IN THE CONTROL OF Rhizoctonia solani IN TOBACCO SEEDLINGS

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ABSTRACT

Damping off is the most destructive disease on tobacco seedlings. Effectiveness of some fungicides against this disease was tested in seedbeds and in biological laboratory.
The aim of investigations was to test the fungicide already used in seedlings protection along with some other fungicides - active ingredients and to determine their effectiveness when applied in higher and lower concentrations. The obtained results may lead to expanding the list of products used in protection of tobacco seedlings from damping off disease.
Signum 33 WG and Quadris 25 SC fungicides showed higher effectiveness in the control of pathogenic fungus Rhizoctonia solani compared to the standard product Top M (0.1%). Fungicides Signum 33 WG at concentrations of 0.1% and 0.15% and Quadris 25 SC at concentrations of 0.15% and 0.2% offer new opportunities in protection of tobacco seedlings from damping off disease.

Keywords: Rhizoctonia solani, fungicide, active ingredient, effectiveness

НОВИ ФУНГИЦИДНИ МОЖНОСТИ ЗА СУЗБИВАЊЕ НА Rhizoctonia solani КАЈ ТУТУНСКИОТ РАСАД

Болеста сечење е најдеструктивна болест на тутунскиот расад. Испитувањата за ефикасноста на некои фунгициди за заштита од оваа болест беа извршени во леи и вобиолошка лабораторија.
Тие имаа цел да се испита ефикасноста на фунгицидот кој веќе се употребува во производството на тутунски расад, но и на некои други фунгициди, односно активни материи, и да се утврди концентрацијата во која новите препарати покажуваат најголема ефикасност, односно каква е таа во случај на намалена концентрација. Резултатите би придонесле за можно проширување на листата на препарати за заштита на тутунскиот расад од сечењето.
Фунгицидите Signum 33 WG и Quadris 25 SC покажаа повисока ефикасност во сузбивањето на патогената габа Rhizoctonia solani од стандардниот препарат Top M (0,1%). Signum 33 WG во концентрации 0,1% и 0,15% и Quadris 25 SC во концентрации 0,15% и 0,2% претставуваат нови фунгицидни можности за заштита на тутунскиот расад од болеста сечење.

Клучни зборови: Rhizoctonia solani, фунгицид, концентација, ефикасност

INTRODUCTION

Production of healthy and good quality tobacco largely depends on successful seedling production and on providing enough quantities of healthy planting material to enable timely and complete seeding of the areas. However, the main problem in production of tobacco seedling is the outbreak of damping off disease. In most cases, pathogenic fungus Rhizoctonia solani is the causing agent of the disease.

According to Gutierez et al. (2001), the fungus can cause two types of diseases: target spot and stem rot (sore shin or damping off). The latter is usually seen in the early stages of seedlings development. The author described the symptoms of damping off disease.

According to Ceresini (1999), some diseases caused by Rhizoctonia in beans, sugar beet and tobacco occur as a result of infection from basidiospores. However, sclerotia and/or mycelium present in the soil or in host-plant tissue are primary inoculum. R. solani persists for many years as mycelium in organic matter or as sclerotia in soils, in variable environmental conditions. The sclerotia can also survive in water (Pataky, 1988; Grosh, 2003).

R. solani is a widespread pathogen which causes serious damage to many agricultural and horticultural plants (Grosh, 2003). The reduction of yield is proportional to agricultural areas and can reach up to 50% (Wallwork, 2000, loc cit. Hollaway, 2008).

Destructive effects caused by this fungus are the reason why the control of this pathogen is so important (SMBSC, 2012).

Protection programmess should be primarily based on preventing pathogen invasion (Pataky, 1988). Uchida (2012) emphasized the importance of such measures, and similar statement was made by Miller and Miller (2009), with special emphasis on seed treatment with fungicide.

Saprophytic ability, however, and wide range of host plants, along with the limited opportunity for crop rotation greatly obstruct the disease control (Hollaway, 2008). Still, efforts are made to maintain the loss at minimum level. The application of chemicals is still the most acceptable protective measure.

Azoxystrobin, trifoxystrobin, and tebuconazole are effective active ingredients against Rhizoctonia (Mocioni et al., 2003). According to Koenning (2007), fungicides containing PCNB (Terrachlor), Iprodione (Rovral) or Azoxystrobin (Quadris) are effective in the control of R.solani. Recommended products for control of this pathogen in potatoes are fludioxinil, maneb, penthiopyrad, thiophanate-methyl, PCNB and azoxystrobin, with their trade names and modes of application (Schwartz and Gent, 2012).

Azoxystrobin (Quadris) is recommended against root rot caused by R.solani (Bredehoert, 2012). Compared to penthiopyrad, Azoxystrobin applied in protection of sugar beet proved efficient in all variants of treatment. Even in severe attacks of Rhizoctonia, the mortality of plants in treatments with azoxystrobin was significantly lower (Poindexter and Wenzel, 2013).

Out of the wide range of products with various trade names and active ingredients for control of R. solani in soybean seedlings, the most recommended fungicides are strobilurins pyraclostrobin and trifloxystrobin (Mueller, 2014). In protection of beans, the recomended fungicide (besides Quadris) is Signum, in which pyraclostrobin is combined with boscalid (Annonimus, 2015). This combination enables a wide range of activities and reduced risk of resistance to different target pathogens (Hauke et al. , 2004).

Signum is a fungicide with preventive and systemic action against many diseases in a variety of crops (BASF, 2008).

Despite the great number of active ingredients, the number of fungicides for control

of this pathogenic fungus in our conditions is limited. The most commonly used fungicide in protection from the disease is Top M is (Gveroska, 2012).

Some active ingredients are effective in tobacco protection from the pathogen. Csinos and Stephenson (1999) recommend flutolanil, iprodione, fluazinam and tebuconazole to prevent the spread of infection in seedbeds.

Bertrand (2012) recommends azoxystrobin (Quadris F) in the control of target spot on tobacco caused by R.solani AG-3. Application of Quadris against this disease is also recommended by the manufacturer (Syngenta, 2006).

According to LaMondia (2012), seedlings treatment with azoxystrobin (Quadris) may be the key measure in protection from sore shin caused by R.solani. The author made in vitro and in vivo tests and concluded that the effectiveness of the product at in vitro conditions is not as good as in field and in biological laboratory. These data focused the investigations on the use of fungicides in conditions of natural infection by the pathogen.

The aim of our investigations was to study the effectiveness of some new fungicides and the concentrations in which they will be effective in various conditions of infection. This can create new fungicide opportunities in protection of tobacco seedlings from damping off disease.

MATERIALS AND METHODS

Investigations were conducted in Scientific Tobacco Institute - Prilep in seedbeds and in biological laboratory.

- Tobacco seedbeds

Sowing was carried out on 21.4.2015, in 10 m^2 seedbeds, using a rate 0,67 g/m^2, in traditional way. Seedlings were fertilized once with 10 g/m^2 ammonium saltpeter, with no other fungcicide treatments.

The trial was set up with three replicates, in randomized block system. The area of each variant was 2,5 m^2. First treatment was made on 15.5.2015 and the second one in 27.5.2015. The assessment of the infected area was made on 8.6.2015, after which the the effectiveness of tested fungicides was evaluated.

- Biological laboratory

The trial was set up two times with 3 replicates at 0,1 m^2. Tobacco seed was sown at a rate of 0,5 g/m^2 and seedlings were grown in traditional way.

The first sowing was carried out on 2.6.2015. Treatment of seedlings was made on 23.6.2015 and assessment of disease infection (% of infested area) on 6.7.2015.

The second sowing was carried out on 11.9.2015, seedbeds were treated on 28.09.2015 and assessment of disease infection was made on 9.10.2015.

The fungicides were applied in appropriate concentrations, using 200 ml water.

Fungicides for both investigations were chosen according to the target pathogen, literature data and data from the investigations and experience in production and protection of tobacco seedlings in the Scientific Tobacco Institute-Prilep. In most cases, the causing agent of damping off disease was the pathogenic fungus Rhizoctonia solani.

After estimation of the disease infection, artificial isolation of the pathogen was made on nutrient medium potato dextrose agar and it was found that damping off was caused by this pathogen.

After assessment of the effectiveness of above fungicides in seedbeds, we tested their effectiveness in higher and lower concentrations. Therefore, two concentrations of the products, along with the standard fungicide Top M, were tested in biological laboratory,

Results on tested fungicides and concentrations in both investigations are given in Table 1.

Table 1. Investigated fungicides

Fungicide	Active ingredient	A.i. content	Seedbeds	Biolaboratory
			Concentration (%)	
Top - M 70% WP	Thiophanate- methyl	70%	0,1	0,1
Signum® 33 WG	Boscalid + pyraclostrobin	26,7% +6,7%	0,1	0,1 0,15
Quadris 25 SC	Azoksistrobin	250 g/l	0,2	0,15 0,2

Seedlings development and disease severity were monitored daily. The observed difference in growth between the two trials was due to the period of sowing. The fungicides were applied before the stage of rapid growth. Assessment of disease was made 7-12 days after infection. Fungicides effectiveness was estimated by the method of Abbott, based on the mean value of the percentage of infected area in the three replicates and the check. Figure 1 is a graphic representation of the mean value of fungicides effectiveness in both trials.

RESULTS AND DISCUSSION

Specific symptom of the disease is that seedlings immediately rot and fall over the soil surface.

The first symptom is the appearance of small watery lesions on the stalk close to the ground, which quickly become brown and hollow. In favorable temperature and humidity conditions the lesions expand, turn brown and inhibit further growth of the plant. Symptoms like these were also described by Gutierrez et al. (2001).

Sometimes, when moisture in the seedbed is higher, a whitish mold can be observed on seedlings. The infected area increases, making patches which coalesce to form large necrotic areas, destroying most of the seedbed. Such symptoms on tobacco seedlings were also observed in the biological laboratory (Ph. 1 and 2).

Ph. 1. Symptoms of damping off disease in tobacco seedbeds

Ph. 2. Symptoms of damping off disease in pots

Although it is not possible to achieve complete control of Rhizoctonia, severity of the disease can be reduced in the early stages of vegetation (Miller and Miller, 2009). By treatment with the investigated fungicides, the infected area in tobacco seedbeds is reduced, i.e. it is significantly smaller compared to the check and to the area treated with the standard fungicide (Table 2). It can be noted that the infected area in all

variants is lower in the second replicate. The lowest effectiveness was achieved with the standard fungicide Top M (0,1%) and the highest with Quadris 25 SC (0,2%), but high effectiveness in protection of tobacco seedlings from damping off disease was also obtained with the fungicide Signum® 33 WG (0,1%) (Table 2).

The manufacturer recommends application of Signum in protection of beans immediately after the first signs of disease. For optimal control, spraying must be repeated at 3-4 week intervals, depending on the disease development (BASF, 2008). Hence, our results on the effectiveness of this product in seedbeds, where two treatments of seedlings were applied, are in accordance with these recommendations.

Post-planting application of Signum in lettuce also provides good protection against three pathogens, including R. solani (AHDB, 2011).

Table 2. Effectiveness of fungicides in the seedbeds

Fungicide (concentration)	Infected area %			Average value	Effectiveness %
	Replicates				
	I	II	III		
Top M (0,1%)	2,23	0,91	2,51	1,88	65,82
Signum® 33 WG (0,1%)	1,27	0,22	1,77	1,09	80,18
Quadris 25 SC (0,2%)	0,56	0,06	0,89	0,50	90,91
Check Ø	6,53	1,38	8,59	5,50	-

Tests conducted in biological laboratory revealed that the intensity of damping off attack was higher compared to that in seedbeds, which is certainly a result of the time period and the conditions in the lab. In the first trial, damping off attacked the untreated seedlings (check variant) with an average percentage of 41.66% of planted area. In seedlings treated with fungicides, damping off disease was observed only in variant with Top M 0,1% (Ph. 3). Seedlings treated with Signum 33 WG and Quadris 25 SC showed no symptoms of disease. Such condition was observed with both concentrations. In the variant with Quadris, for example, no outbreak of damping off was observed even in lower concentration (Table 3). Thus, reducing the concentration of this product seems justified. Brantner and Windels (2011) confirmed that in conditions of less severe attack of damping off disease, the benefit from the use of Quadris in any concentration is more than enough to pay off its application.

Top M (01%), achieved 78.00% effectiveness, compared to Signum (0,1 and 0,15%) and Quadris (0,15 and 0,2%) which achieved an effectiveness of 100% (Ph. 4, 5 and 6).

Table 3. The effectiveness of fungicides in biolaboratory (I trial)

Fungicide (concentration)	Infected area %			Average value	Effectiveness %
	Replicates				
	I	II	III		
Top M (0,1%)	10,00	10,00	8,33	9,44	78,00
Signum® 33 WG (0,1%)	-	-	-	-	100,00
Signum® 33 WG (0,15)	-	-	-	-	100,00
Quadris 25 SC (0,15%)	-	-	-	-	100,00
Quadris 25 SC (0,2%)	-	-	-	-	100,00
Check Ø	43,33	25,00	56,66	41,66	-

- no symptoms of disease

Ph. 3 Infected area in the check variant - Fungicides Top M and Signum 33WG

Ph.4 Effectiveness of investigated fungicides

Ph. 5. Infected area - fungicide Signum (0,1 and 0,15%)

Ph. 6. Infected area – fungicide Quadris (0,15 and 0,2%)

In the second trial, the intensity of disease attack in the check (mean value 10.33%) was lower compared to the first trial. However, symptoms of disease were found in almost all variants and replicates.

The calculated effectiveness of the fungicides is in compliance with the infected area. It was the highest (3.15%) in Top M (0,1%), i.e. the lowest effectiveness was achieved with this preparate. Similar value (79.00%) was achieved with Signum (0,1%), while the higher concentration of this fungicide increased the effectiveness (Table 4, Ph 7).

Table 4. The effectiveness of fungicides in biolaboratory (II trial)

Fungicide (concentration)	Infected area %			Average value	Effectiveness %
	Replicates				
	I	II	III		
Top M (0,1%)	3,59	3,06	2,82	3,15	70,00
Signum® 33 WG (0,1%)	-	4,35	2,23	2,19	79,00
Signum® 33 WG (0,15)	2,47	1,29	0,53	1,43	87,00
Quadris 25 SC (0,15%)	-	-	1,53	0,51	96,00
Quadris 25 SC (0,2%)	-	0,26	0,11	0,12	99,00
Check Ø	5,00	17,66	8,33	10,33	-

- no symptoms of diseas

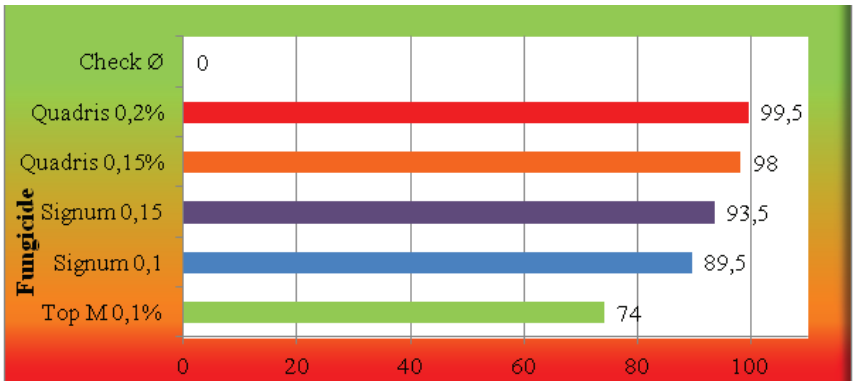
Ph.7. Infected area in variants with Top M and Signum 33 WG (0,1%)

Ph. 8. Infected area in the variant with Quadris 25 Sc (0,15%)

Ph. 9. Infected area in the variant with Quadris 25 SC (0,2%)

The lowest percentage of infected area (0.12%) was obtained with Quadris (0,2%). However, even with reduced concentration of this product (0.15%), the percentage of infected area was considerably low (Table 4, Ph 8). The highest effectiveness was achieved in the variants with Quadris 25 SC (0,2%) - 99,00% and Quadris (0,15%) - 96,00% (Ph. 8 and 9).

Fig. 1. The effectiveness of fungicides (average value of two trials)



The mean value of fungicide effectiveness in both trials confirmed the factual situation (Figure 1), i.e. the lowest effectiveness in control of the pathogen R. solani was obtained with the fungicide Top M (0,1%). Similar results were reported in investigations of Gveroska (2012).

The highest effectiveness was obtained with Quadris 25 SC (0,2%) - 99,5%, but it should be noted that even with lower concentration (0.15%), the fungicide achieved 98.00% effectiveness. Hence, the treatment of tobacco seedlings with azoxystrobin (Quadris) could be a key measure in the control of R.solani (LaMondia, 2012). The high effectiveness of Quadris is due to the direct effect of azoxystrobin in the soil inoculum and the mutual effective combination with the plant tissue. The obtained results are certainly due to the timely treatment of tobacco seedlings, monitoring the conditions for disease outbreak and the optimum amount of water. This was confirmed by Khan (2015), who reported that Quadris application should begin prior to disease development and not as a curative treatment, preferably in the stage of cotyledone (4-6 leaf stage), especially when conditions for infection are favorable. It should be applied in enough water for thorough coverage (Syngenta, 2006).

Signum® 33 WG fungicide in concentrations of 0,1% and 0,15% also provided high effectiveness against damping off disesase - 89.50% and 93.50%. This effectiveness may be due to the fact that both active ingredients are an excellent combination of two different biochemical modes of action in the fungal cell respiration (Hauke et al., 2004).

According to AHDB (2011), fungicides application at less than full label rate, in the case of lower risk of infection, should be justified by well-grounded facts. In this sense, our results on the effectiveness of investigated fungicides in concentration that differs from the recommended, confirms the possibility of their application from both practical and economic aspects.

CONCLUSIONS

Standard fungicide in the control of the pathogen R. solani Top M (0.1%) showed the lowest effectiveness both in seedbeds and in biological laboratory.

- In seedbed trials, 80.18% effectiveness was achieved with Signum 33 WG (0,1%) and 90,91% effectiveness with Quadris 25 SC (0,2%).
- The effectiveness of investigated fungicides in biological laboratory was lower in the second trial.
- The effectiveness of the fungicide Signum 33 WG (0,1%) was 100% in the first trial and 79% in the second. With increased concentration (0.15%), effectiveness of 100% was achieved in the first and 87.00% in the second trial.
- Quadris 25 SC achieved very high effectiveness in the control of this pathogen, reaching 100% and 99.00% at 0.2% concentration in the first and second trial, respectively. Even at reduced concentration of 0.15%, the fungicide had 100.00% and 96.00% effectiveness in the first and second trial, respectively.
- The fungicides Signum 33 WG and Quadris 25 SC showed higher effectiveness in the control of pathogenic fungus R. solani compared to the standard fungicide Top M.

- Signum 33 WG at concentrations of 0.1 and 0.15% and Quadris 25 SC at concentrations of 0.15% and 0.2% offer new fungicide opportunities in protection of tobacco seedlings from damping off disease.

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