

## APPLICATION OF SOME NEW FUNGICIDES IN THE CONTROL OF *RHIZOCTONIA SOLANI* KÜHN IN TOBACCO SEEDLINGS

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### ABSTRACT

Tobacco plants in seedbeds are often attacked by the soil phytopathogenic fungus *Rhizoctonia solani*, the causing agent of damping off disease. Investigations were made in biological laboratory of the Scientific Tobacco Institute - Prilep with tobacco variety NS72 in order to determine the effectiveness of some newer fungicides in the control of this pathogen. Seedlings were sown in 0,3 m<sup>2</sup> plastic trays in naturally infested soil and in soil inoculated with fungal culture before sowing. The following fungicides were used in investigation: Top M 0,1% (70% thiophanate methyl), Orvego 0,1% (ametoctradin 300 g/l + dimethomorph 225 g/l), Enervin 0,2% (ametoctradin 120 g/kg + metiram 440 g/kg) and Signum 0,1% (boscalid 267 g/kg + pyraklostrobin 67 g/kg). Seedlings were treated twice, by watering with 1 l/m<sup>2</sup> of the solution. The first watering was done in the 4-leaf stage and the second in the stage of rapid growth. The coefficient of fungicide effectiveness was calculated according to Abbott's formula. In seedlings treated with Signum no symptoms of the disease were observed, while in the check variant the infection rate was 75.00 to 100.00%. Due to its high effectiveness, the Signum fungicide can be used in practice, in protection of tobacco seedlings from this pathogen.

**Key words:** tobacco seedlings, *R. solani*, disease, fungicides, effectiveness

## ПРИМЕНА НА НЕКОИ НОВИ ФУНГИЦИДИ ЗА КОНТРОЛА НА *RHIZOCTONIA SOLANI* KÜHN ВО ТУТУНСКИОТ РАСАД

При производството на расад, често во леите се појавува и почвената фитопатогена габа *Rhizoctonia solani*, причинител на болеста сечење. За да се заштити расадот од ова заболување, направивме испитување на понови препарати, со цел да се провери нивната ефикасност во сузбивањето на овој патоген. Испитувањето е изведено во биолошката лабораторија на Научниот институт за тутун-Прилеп на расад од сортата НС72. Расадот е одгледуван во пластични корита со површина од 0,3 m<sup>2</sup> на природно инфицирана почва и почва инокулирана со култура од габата каде инокулот беше додаден пред сеидба на расадот. За испитување беа користени стандардниот препарат Top M 0,1% (70% thiophanate methyl) и препаратите Orvego 0,1% (ametoctradin 300 g/l+dimethomorph 225 g/l), Enervin 0,2% (ametoctradin 120 g/kg+metiram 440 g/kg) и Signum 0,1% (boscalid 267 g/kg+pyraklostrobin 67 g/kg). Расадот е третиран два пати со полевање со по 1 l раствор на m<sup>2</sup>. Првото полевање е направено во фаза вкрстување на расадот, а второто во буен пораст. Коефициентот на ефикасност на фунгицидите е пресметан според формулата на Abbott. Кај третираниот расад со фунгицидот Signum немаше појава на заболување, додека кај контролата заразата изнесуваше од

75,00-100,00%. Заради високата ефикасност што ја покажа овој препарат, истиот може да најде и поширока практична примена во заштитата на тутунскиот расад од овој почвен патоген.

**Клучни зборови:** тутунски расад, *R. solani*, болест, препарати, ефикасност

## INTRODUCTION

Tobacco production is a complex and specific process. Tobacco growth and development, yield and quality depend on the climate and soil conditions and the applied cultural practices. During the production of seedlings for tobacco, vegetable and ornamental plants, the sprouts and young plants can be seriously damaged. The most common cause of this phenomenon, despite *Pythium debaryanum*, is the soil phytopathogenic fungus *Rhizoctonia solani*. The ground part of the stem is covered with water-soaked spots, the tissue decays and the infected plant eventually dies. The disease spreads in larger or smaller patches and it is necessary to apply protective agro-technical measures as an integral part of the production. For prevention of disease outbreak, it is recommended to apply optimum spacing among plants in the seedbeds, moderate irrigation, regular ventilation and chemical protection. In North Carolina - where 95% of tobacco seedlings are grown in greenhouses (hydroponic float trays) - one of the most common diseases is damping off, caused by the fungus *R. solani* (Gutierrez et al., 2001). According to Kenneth et al. (2011), float trays in which tobacco seedlings are grown are ideal place for development of this pathogen.

In our country, too, damping off disease caused by *Rhizoctonia solani* (teleomorph, *Thanatephorus cucumeris*) is economically important disease. This is plant pathogenic fungus with a wide host range and worldwide distribution. It belongs to the class Basidiomycetes - fungi that do not produce asexual (conidia) spores (Annonimus 2016 a,b). It exists in the soil as mycelium and produces sclerotia on plant residues, but occasionally it can also produce sexual spores - basidiospores. The fungus *Thanatephorus*

*cucumeris* (teleomorph stage) was earlier described under the name *Rhizoctonia solani* and it is an anamorph stage (Pejcinovski et al., 2009). According to Gonzalez et al. (2011), the fungi can persist in soil for years as mycelium and they also survive by producing sclerotia, which makes it difficult to control them with conventional fungicides. The most successful results can be achieved by integrated protection, which is a combination of fungicide application and agro-technical measures.

Shew and Ridge (2016) confirmed that fungicides containing active ingredients azoxystrobin and mancozeb show high effectiveness in protection of tobacco seedlings from this pathogen. According to La-Mondia (2012), in vitro tests showed that azoxystrobin inhibited mycelium growth. Initially, it reached 98.8% but the results obtained in biological laboratory show that fungicides with this active ingredient can significantly reduce the pathogen infection. Seedlings treated with Quadris based on azoxystrobin have a more developed root system, greener color and lower rate of infection compared to the untreated seedlings. High effectiveness in the control of this pathogen was achieved in the tests made with a.i. thiophanate methyl and with fungicides from the strobilurin group -Quadris 25SC and Stroby WG (Taskoski et al., 2001, 2015 a).

Signum, a new product of the BASF company, showed high effectiveness in the control of gray mold (*Botrytis cinerea*) on strawberries (Hauke, 2004) and lettuce (Callens, 2005). Taskoski (2015) reported high effectiveness of this fungicide in the control of *Pythium debaryanum* in tobacco seedlings.

The aim of this study was to examine the

effectiveness of some newer fungicides, including Signum, in protection of tobacco

seedlings from the soil phytopathogenic fungus *R. solani*.

## MATERIAL AND METHODS

Investigations with tobacco seedlings of NS 72 variety were performed in 2014 and 2015 at the biological laboratory of Tobacco Institute - Prilep in two treatments with three replications. Seedlings were planted in 0,3 m<sup>2</sup> plastic trays on 5.5.2014 and 7.5.2015. In the first trial, they were planted in soil inoculated with pure culture of the fungus, while in the second trial we used naturally infested soil, without additional artificial inoculation. Culture of the fungus grown on nutrient medium potato dextrose agar was used as inoculum in a thermostat at 25° C for a period of 10 days. The inoculum

for one tray was prepared from mycelial colony in two Petri dishes, blended in 200 ml distilled water and added to the soil before sowing the seedlings. Two treatments with fungicides were made during seedlings growth - in the 4th leaf stage and in the stage of rapid growth. Seedlings were treated with 1 l fungicide solution/m<sup>2</sup> and check variants were poured only with pure water. Four fungicides were included in the investigation. Their active ingredient and concentration are presented in Table 1. Regular agro-technical measures were applied during the cultivation of seedlings.

**Table 1. Investigated fungicides**

Fungicide	Active ingredient	Concentration %
Orvego	300 g/l ametoctradin + 225 g/l dimethomorph	0,1
Enervin WG	120 g/kg ametoctradin + 440 g/kg metiram	0,2
Signum WG	267 g/kg boscalid + 67 g/kg pyraclostrobin	0,1
Top M 70 WP	70% thiophanate methyl	0,1

Health condition of seedlings was estimated according to the number of infected plants. Two assessments were made during the growing season - the first one ten days after the second treatment and the second - ten days after the first assessment. Spots where seedlings were infected were measured and

the empty area and average intensity of disease attack were estimated by processing the obtained data. According to the disease intensity in the second assessment, coefficient of fungicides effectiveness was calculated by the Abbott's formula (1925).

## RESULTS AND DISCUSSION

Results of the two-year investigations on fungicide effectiveness in protection of tobacco seedlings from the soil pathogen *R. solani* are presented in tables. In trials performed during 2014 with seedlings planted in inoculated soil, the disease intensity in

the check variant ranged from 83.33% in the first assessment to 100.00% in the second (Table 2). In variants treated with fungicides, the disease intensity varied, reaching 50.00 - 65.30% with Orvego 0,1% , 45,00% with Top M 0,1% and 25.00 - 41.20 % with

Enervin 0,2%. Only the seedlings treated with Signum 0,1% applied alone or in com-

bination with other fungicides showed no symptoms of disease.

**Table 2. Disease intensity in seedlings grown in soil inoculated with fungal culture of *R. solani* in 2014**

Variant	Infected area, %	
	I assessment	II assessment
Check	83,33	100,00
Orvego 0,1%	50,00	65,30
Enervin 0,2%	25,00	41,20
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,2%+Signum 0,1%	0,00	0,00
Top M 0,1%	45,00	45,00

According to the results, disease intensity of seedlings planted on naturally infested soil in 2014 in the check variant ranged from 80.00% in the first and 90.00% in the second assessment (Table 3). The highest rate of infection in the first assessment was recorded with Orvego 0,1% - 27,40%, Enervin 0,2% - 13,00% and Top M 0,1%

- 2,00%. Higher rate of infection was recorded in the second assessment (40.00% with Orvego 0,1%, 30,50% with Enervin 0,2% and 16.40% with Top M 0,1%). In this case again, no symptoms of disease were observed in variants treated with Signum 0,1%.

**Table 3. Disease intensity in seedlings grown in naturally infested soil, 2014**

Variant	Infected area, %	
	I assessment	II assessment
Check	80,00	90,00
Orvego 0,1%	27,40	40,00
Enervin 0,2%	13,00	30,50
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,2%+Signum 0,1%	0,00	0,00
Top M 0,1%	2,00	16,40

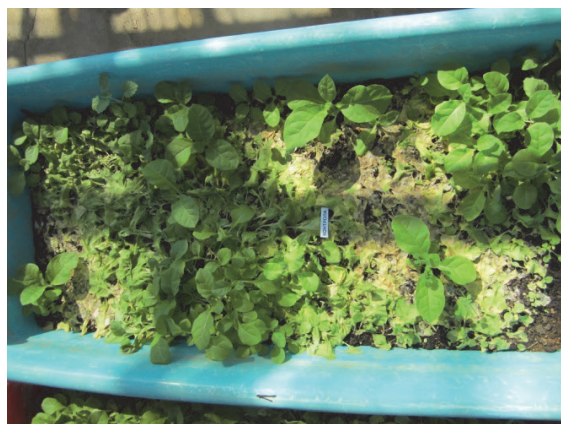
In 2014, in soil inoculated with fungal culture, the highest fungicide effectiveness (100.00%) was obtained with Signum 0,1% in all three variants (Table 4). The other fungicides tested showed lower effectiveness, reaching 58.80% with Enervin 0,2%, 55,00% with Top M 0,1% and 34.70% with Orvego 0,1%. Similar effectiveness was

achieved in seedlings grown in naturally infested soil. The lowest effectiveness was obtained with Orvego 0,1% (55.55%) and Enervin 0,2% (66,11%), and somewhat higher effectiveness (81.77%) was obtained with Top M 0,1%. No symptoms of disease were recorded in seedlings treated with Signum 0,1%.

**Table 4. The effectiveness of investigated fungicides in 2014**

Variant	Effectiveness, %	
	Seedlings grown in inoculated soil	Seedlings grown in naturally infested soil
Check	-	-
Orvego 0,1%	34,70	55,55
Enervin 0,2%	58,80	66,11
Signum 0,1%	100,00	100,00
Orvego 0,1%+Signum 0,1%	100,00	100,00
Enervin 0,2%+Signum 0,1%	100,00	100,00
Top M 0,1%	55,00	81,77

In 2015, disease intensity was variable. In soil inoculated with pathogenic fungus, the highest intensity was achieved in the check (Fig. 1), reaching 86.00% in the first assessment and 100.00% in the second (Table 5). Lower disease intensity (60.00 - 80.00%) was achieved in the variant with Orvego 0,1% (Fig. 2). With Enervin 0,2% it ranged from 2.70% in the first assessment to 30.00% in the second assessment (Fig. 3).



**Fig. 1. *R. Solani* – infected seedlings in the check variant**



**Fig. 2. Tobacco seedlings treated with Orvego 0,1%**

**Table 5. Disease intensity in seedlings grown in soil inoculated with fungal culture of *R. solani* in 2015**

Variant	Infected area %	
	I assessment	II assessment
Check	86,00	100,00
Orvego 0,1%	60,00	80,00
Enervin 0,2%	2,70	30,00



Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,2%+Signum 0,1%	0,00	0,00
Top M 0,1%	0,00	7,50



Fig. 3. Seedlings treated with Enervin 0,2%



Fig. 4. Seedlings treated with Top M 0,1%



Fig. 5. Seedlings treated with Signum 0,1%

In naturally infested soil, the disease intensity in the check variant ranged from 30.00% in the first assessment to 75.00% in the second (Table 6). Somewhat lower intensity was observed in the variant with Orvego 0,1% (26.00% in the first and 43.40%

in the second assessment). The lowest disease intensity of 3.00% in both assessments was recorded with Enervin 0,2%. As in the previous trial, no symptoms of disease were recorded in seedlings treated with Signum 0,1% and Top M 0,1%.

**Table 6. Disease intensity in seedlings grown in naturally infested soil, 2015**

Variant	Infected area %	
	I assessment	II assessment
Check	30,00	75,00
Orvego 0,1%	26,00	43,40
Enervin 0,2%	3,00	3,00
Signum 0,1%	0,00	0,00

Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,2%+Signum 0,1%	0,00	0,00
Top M 0,1%	0,00	0,00

According to the results (Table 7), 100.00% effectiveness in protection of tobacco seedlings from this pathogen in 2015 was achieved with Signum 0,1%, applied alone or in combination with other fungicides. In both variants (seedlings grown in soil inoculated with fungal culture and seed-

lings grown in naturally infested soil), no symptoms of disease were recorded. This fungicide provided maximum effectiveness in protection of tobacco seedlings from the pathogen *Pythium debaryanum*, too (Tashkoski, 2015).

**Table 7. The effectiveness of investigated fungicides in 2015**

Variant	Effectiveness, %	
	Seedlings grown in inoculated soil	Seedlings grown in naturally infested soil
Check	-	-
Orvego 0,1%	20,00	42,13
Enervin 0,2%	70,00	96,00
Signum 0,1%	100,00	100,00
Orvego 0,1%+Signum 0,1%	100,00	100,00
Enervin 0,2%+Signum 0,1%	100,00	100,00
Top M 0,1%	92,50	100,00

High effectiveness was achieved with Top M 0,1%, reaching 92.50% in seedlings grown in soil inoculated with the pathogen and 100.00% in seedlings grown in naturally infested soil. Effectiveness of 70.00 - 96.00% was achieved with Enervin 0,2% and the lowest effectiveness of 20.00 - 42.13% was obtained with Orvego 0,1%. Enervin is a fungicide which achieved 100% effectiveness in the control of the causing agent of damping off disease Pyth-

ium spp. in tomato and pepper (Rusevski et al., 2012) and 98.83% effectiveness against the same pathogen in tobacco seedlings (Tashkoski, 2015).

Due to their high effectiveness, the application of Signum and Enervin fungicides will provide control of the soil pathogen *R. solani* - causing agent of damping off disease on tobacco seedlings.

## CONCLUSION

The disease caused by the soil-borne pathogen *R. solani* is a serious problem in production of tobacco seedlings. Severe losses caused by this disease can be reduced by applying appropriate agro-technical and chemical measures. For this aim, the effectiveness of some newer fungicides was studied during 2014 and 2015. This effectiveness was achieved at extreme-

ly high disease intensity in 2014 which reached 100.00% in the check variant (soil inoculated with culture of the fungus) and 90.00% in the trial with naturally infested soil.

Effectiveness of 55.00% was obtained by application of the standard fungicide Top M 0,1% in soil inoculated with fungal culture and 81.77% in naturally infested soil. The

lowest effectiveness (34.70% and 55.55%, respectively) was obtained by the Orvego 0,1% fungicide. In 2015, disease intensity in the check variant was 100.00% in the soil inoculated with fungus and 75.00% in the trial with naturally infested soil.

Application of the above fungicides reduces the infection of tobacco seedlings by this pathogen. The effectiveness achieved with the standard fungicide Top M 0,1% was 92.50% in seedlings grown in inoculated soil and 100.00% in seedlings grown in naturally infested soil. Again, the lowest effectiveness in both variants was obtained with Orvego 0,1% ( 20,00% and 42.13%,

respectively). The effectiveness achieved by Enervin 0,2% was 70,00% in seedlings grown in inoculated soil and 96.00% in seedlings grown in naturally infested soil.

In both years and both trials, the highest effectiveness in protection of tobacco seedlings from *R. solani* was achieved by the fungicide Signum 0,1%, used alone or in combination with other fungicides. Seedlings treated with this fungicide showed no symptoms of disease.

Due to its high effectiveness, it can be recommended for practical application in protection of tobacco seedlings from this soil-borne pathogen.

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