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APPLICATION OF THE SIGNUM FUNGICIDE IN CONTROL OF *PYTHIUM DEBARYANUM* HESSE ON TOBACCO SEEDLINGS

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ABSTRACT

Favorable microclimate conditions in seedbeds contribute to the outbreak of damping off disease on tobacco seedlings. The aim of this paper was to estimate the effectiveness of the fungicide Signum in control of *Pythium debaryanum*, the disease causing agent. Investigations were made during 2013 and 2014 in the biological laboratory of the Scientific Tobacco Institute -Prilep. Seedlings of the variety NS 72 were sown in 0,3 m^2 plastic trays. Standard fungicide Proplant 722 SL 0,25% (722 g/l propamocarb-hydrochlorid) and the preparations Orvego 0,1% (ametoctradin 300 + dimethomorph 225), Enervin 0,2% (ametoctradin 120 + metiram 440) and Signum 0,1% (boscalid 267 + pyraklostrobin 67) were used in the investigations. Two waterings were applied on seedlings with 1 l/m^2 suspension prepared from the fungicides. The first watering was applied prior to the 4th-leaf stage and the second one 15 days after. The coefficient of fungicide effectiveness was calculated by applying the formula of Abbott (1925). In both years of investigation, Signum 0,1% showed the highest effectiveness in protection of the seedlings. No infected plants were observed during the growing season in variants where this fungicide was applied.

Keywords: tobacco seedlings, disease, intensity, fungicides, effectiveness

ПРИМЕНА НА ПРЕПАРАТОТ SIGNUM ВО ЗАШТИТАТА НА ТУТУНСКИОТ РАСАД ОД ПАТОГЕНОТ *PYTHIUM DEBARYANUM* HESSE

Поволните микроклиматски услови во леите допринесуваат за честа појава на болеста сечење односно "топење" на расадот. Целта на испитувањето беше да се провери ефикасноста на фунгицидот Signum за сузбивање на патогенот *Pythium debaryanum* причинител на ова заболување. Испитувањата се изведени во текот на 2013 и 2014 година во биолошката лабораторија на Научниот институт за тутун. Тутунскиот расад од сортата НС72 беше одгледуван во пластични корита со површина од 0,3 m^2. За испитување беа користени стандардниот препарат Proplant 722 SL 0,25% (722 g/l propamocarb-hydrochlorid) 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 со полевање со суспензија подготвена од препаратите. Направени се две полевања на расадот со по 1 l раствор на m^2. Првото полевање е направено во фаза вкрстување на расадот, додека второто полевање е извршено после 15 дена од првото. Коефициентот на ефикасност на фунгицидите е пресметан според формулата на Abbott

(1925). Во двете испитувани години препаратот Signum 0,1% покажа највисока ефикасност во заштитата на тутунскиот расад. Во сите варијанти каде што беше применет овој препарат, во текот на вегетацијата немаше забележано појава на заразени растенија.

Клучни зборови: тутунски расад, болест, интензитет, фунгициди, ефикасност

INTRODUCTION

In the Republic of Macedonia, tobacco seedlings are traditionally grown in cold seedbeds covered with polyethylene, while in most of the large-leaf tobacco producing countries they are grown in hydroponic nutrient solution - float system (Boić et al., 1999). The period of seedling production consists of the following stages: seed germination, emergence, 4th leaf stage, root formation and the period for transplanting, when seedlings are highly susceptible to diseases. For successful production of tobacco it is necessary to produce healthy and high quality seedlings and to provide suitable preventive measures.

Tobacco is a host to many plant parasites. The diseases caused by these parasites can deteriorate the whole tobacco production, if appropriate measures are not taken. Protection of tobacco, particularly of seedlings, is very important but delicate question. Some of the seedling diseases are the following: black root rot (caused by *Thielaviopsis basicola*), root burn (*Olpidium brasicae*), root rot (*Rhizoctonia solani*), damping off (*Pythium debaryanum*), etc. According to literature data (Boić et al., 1999), the most common diseases on tobacco seedlings in America are root rot (*Rhizoctonia solani*), sclerotinia (*Sclerotinia sclerotiorum*) and black leg (*Erwinia carotovora*). Until recently it was considered that disease of tobacco seedlings in seedbeds was caused by the phytopathogenic fungus *R. solani*. Last few years, however, it has been determined in several tobacco producing regions that the causing agent of damping off disease is *P. debaryanum*. Common characteristic of fungi diseases in seedlings is that they occur in densely planted beds, with excessive moisture in the seedbed, insufficient aeration and unfavorable climate conditions (cloudy and rainy weather).

The genus Pythium consists of more than 100 species (Ćosić et al., 2006), the most important of which are *P. debaryanum*, *P. irregulare*, *P. ultimum*, *P. splendens*, *P. aphanidermatum* etc. It is optional parasite which lives in soil rich in organic matter and infects a large range of hosts, including vegetables, ornamental plants, fruit and forest species (Pejcinovski et al., 2009), but it especially attacks sugar beet and tobacco (Ćosić et al., 2006). The most susceptible are young plants at the stage of germination and emergence while the older plants are not endangered (Ćosić et al., 2006). Species of the genus Pythium regularly appear all around the world, making a huge damage. They often cause complete destruction of germ while in emerged plants they necrotise the root and the stem base, as a result of which the plants collapse and are covered with mycelium (Jurković et al., 2009). The plants die quickly, and at higher humidity and temperature they are "melting", due to which the disease is also known as melting of seedlings. The disease spreads in the seedbed in a form of circles, forming smaller or larger empty patches, depending on the intensity of attack. Similar symptoms, however, can be caused by other fungal species, including *Fusarium spp*., *R. solani*, *Phytophthora parasitica var. nicotianae,* etc.

All external factors that negatively affect the seedlings germination favor the development of disease causing agents: heavy and poorly drained soils, acidic soils, low temperatures after germination, high hu-

midity and higher temperatures in the rapid growth stage, poor aeration, etc. One of the common errors in the production of tobacco seedlings is excessive irrigation (Radunović, 7). In such conditions, plants have poorly developed shallow-rooted system and, due to favorable conditions, the seedlings are infected by pathogenic fungi from the surface layer of the soil. Similar problems in the production of seedlings in the vegetable crops, in addition to recommended preventive measures and cultural practices (Balaž, 1), led to the use of chemicals, i.e. fungicides Previcur 607 SL and Previcur Energy (Radunović, 7). High effectiveness in the control of this pathogen in tobacco was also achieved by formulations with active ingredient etridiazol (Seebold, 2008; Kenneth, 2011).

Signum is a new fungicide developed by BASF, applied in recent years against fungal diseases. According to Hauke (2004), it showed high effectiveness in the control of *Botrytis cinerea* in strawberries and gave good protection against some species of powdery mildew and Phytophthora. Good results with application of this fungicide against *Botrytis cinerea* were also observed in lettuce Callens (2005).

The aim of this study was to estimate the effectiveness of some new fungicides, including Signum, in protection of tobacco seedlings from the phytopathogenic fungus *P. debaryanum*, the causing agent of damping off disease.

MATERIAL AND METHODS

Investigations were conducted in the biological laboratory of the Scientific Tobacco Institute-Prilep in 2013 and 2014. Seedlings of the variety NS72 were planted in 0.3 m^2 polystyrene trays on 7.5.2013 and 5.5.2014, in two trials with three replications. Seedlings in the first trial were grown on soil inoculated with pure culture of the fungus and in the second trial they were grown in naturally infected soil without additional artificial inoculation. Fungal culture was grown on potato dextrose agar in a thermostat at 25^{0}C for 10 days.

The inoculum for the 0.3 m^2 tray was prepared from mycelial colony in two Petri dishes and mixed in 200 ml distilled water. The suspension was added in the tray before sowing the seedlings. Two treatments with fungicides were made during seedlings growth, the first one in the stage of 4th leaf on 20.5.2013 and 19.5.2014, and the second one in the stage of rapid growth on 4.6.2013 and 3.6.2014. The seedlings were treated with 1 l fungicide solution/m^2 and the check variants were poured only with pure water.

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	267 g/kg boscalid + 67 g/kg pyraklostrobin	0,1
Proplant 722 SL	722 g/l propamocarb hydrochlorid	0,25

Two assessments were made during the growing season, the first one on 12.6.2013 and the second on 18.6.2013, i.e. on 16.6.2014 and 23.6.2014. After the inspection of seedlings health condition, the infected area was measured in the sites where infection occurred. The measurement data were used to evaluate the percentage of empty area, i.e. the disease intensity. According to the disease intensity measured in the second assessment, the coefficient of fungicide effectiveness was calculated by the Abbott's (1925) formula.

RESULTS AND DISCUSSION

Results of the investigation on fungicides effectiveness in the control of *P. debaryanum* on tobacco seedlings are presented in tables. Data obtained in 2013, from the trials with seedlings grown in soil inoculated with culture of the pathogenic fungus, are presented in Table 2. In the check variant, the infection ranged from 73.00% in the first assessment to 100.00% in the second assessment (Fig. 1). The infection in seedlings treated with Proplant 0,25% reached 94.00% and in treatment with Orvego 0,1% it ranged from 57.00% in the first assessment to 80.00% in the second assessment (Fig. 2). The lowest rate of infection (3.00%) was evaluated in the variant treated with Enervin 0,2% (Fig. 3). In the variant treated with Signum 0,1%, alone or in combination with other fungicides, no incidence of infection was observed (Fig. 4). This fungicide showed high effectiveness in the control of *P. debaryanum* on tobacco seedlings.

Table 2. Disease intensity in seedlings grown in soil inoculated with culture of the fungus P. debaryanum in 2013

Variant	Infected area %	
	I Assessment	II Assessment
Check	73,00	100,00
Orvego 0,1%	57,00	80,00
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 0,25%+Signum 0,1%	0,00	0,00
Proplant 0,25%	94,00	94,00

Fig. 1. P. debaryanum – Infected seedlings in the check variant

Fig. 2. Seedlings treated with Orvego 0,1%

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

Fig. 4. Seedlings treated with Signum 0,1%

Similar results were obtained with seedlings grown on naturally infected soil (Table 3). In the first assessment, the infected area in the check variant was 51.00%. In the second assessment the disease affected larger area and the infection increased to 86.00%. As in the previous case, treatment with Orvego 0,1% did not give satisfactory results and the disease intensity ranged from 47.00% in the first assessment to 60.00% in the second.

Table 3. Intensity of the disease in seedlings grown on naturally infected soil in 2013

Variant	Infected area %	
	I Assessment	II Assessment
Check	51,00	86,00
Orvego 0,1%	47,00	60,00
Enervin 0,2%	1,00	1,00
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,25%+Signum 0,1%	0,00	0,00
Proplant 0,25%	20,00	53,00

Somewhat better results were obtained with Proplant fungicide used at a concentration of 0.25%, with disease intensity ranging from 20.00% in the first assessment to 53.00% in the second. Once again, the best results were obtained with Enervin 0,2%, with only 1.00% infected area, and Signum 0,1% in all variants, with no outbreak of disease.

Data on the occurrence and intensity of the disease caused by *P. debaryanum* received in the second assessment were used to calculate the effectiveness of the applied fungicides. According to the results given in Table 4, the highest effectiveness (100.00%) in the control of this pathogen both in seedlings grown in soil inoculated with pure culture of the fungus and in seedlings grown in naturally infected soil was obtained with Signum 0 1%, where no occurrence of infection in tobacco seedling was recorded. With Enervin 0,2%, the percentage of infected plants was insignificant and its effectiveness ranged 97.00% and 98.83%, respectively. The lowest effectiveness was obtained with Orvego 0,1% (20,00%, i.e. 30.23%) and Proplant 0,25% (6.00% in seedlings grown in inoculated soil and 38.37% in naturally infected soil).

Table 4. The effectiveness of fungicides tested in 2013

Variant	Effectiveness %	
	Seedlings grown on inoculated soil	Seedlings grown on naturally infected soil
Check	-	-
Orvego 0,1%	20,00	30,23
Enervin 0,2%	97,00	98,83
Signum 0,1%	100,00	100,00
Orvego 0,1%+Signum 0,1%	100,00	100,00
Enervin 0,25%+Signum 0,1%	100,00	100,00
Proplant 0,25%	6,00	38,37

In the investigations of 2014, seedlings grown in soil inoculated with fungal culture showed high disease infection in the check variant, with intensity ranging from 80.00 to 90.00% (Table 5). No major difference in seedlings damage was observed in the treatment with Orvego 0,1%, where the intensity of disease was 65.00% and 75.00%, respectively. Similar results were obtained with Enervin 0,2% and Proplant 0,25%. In the first assessment the percentage of infected area was 19.70% and 15.50%, while in the second assessment the infection was more severe, with disease intensity reaching 40,00% in Enervin 0,2% and 34.67% in Proplant 0,25%.

Table 5. Disease intensity in seedlings grown in soil inoculated with culture of the fungus *P. debaryanum* in 2014

Variant	Infected area %	
	I Assessment	II Assessment
Check	80,00	90,00
Orvego 0,1%	65,00	75,00
Enervin 0,2%	19,70	40,00
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,25%+Signum 0,1%	0,00	0,00
Proplant 0,25%	15,50	34,67

On the other side, no outbreak of disease was observed in variants treated with Signum 0,1 alone or in combination with other agents.
In the trial where seedlings were grown in naturally infected soil, no major differences in infection rate were observed compared to previously obtained results. The intensity of disease in the check ranged from 60.00% in the first assessment to 85.00% in the second (Table 6).

Table 6. Disease intensity in seedlings grown on naturally infected soil in 2014

Variant	Infected area %	
	I Assessment	II Assessment
Check	60,00	85,00
Orvego 0,1%	55,00	68,00
Enervin 0,2%	20,00	20,00
Signum 0,1%	0,00	0,00
Orvego 0,1%+Signum 0,1%	0,00	0,00
Enervin 0,25%+Signum 0,1%	0,00	0,00
Proplant 0,25%	5,00	18,00

In the variant treated with Orvego 0,1%, 55.00% infected area was measured in the first assessment and 68.00% in the second. In this case, too, good results were obtained in treatments with Enervin 0,2% and Proplant 0,25%, with disease intensity of 20.00% and 18.00%, respectively. Only the seedlings treated with Signum 0,1% in all variants showed no symptoms of disease.

Table 7 shows the results on product effectiveness in seedling protection from the pathogen *P. debaryanum* during 2014. In variants where seedlings were grown in soil inoculated with fungus culture, the poorest attack (16.66%) was recorded with Orvego 0,1%. Good results were obtained with preparations Enervin 0,2% (55,55%) and Proplant 0,25%, which achieved an effectiveness of 61.47%. The highest effectiveness of 100.00% was obtained with Signum 0,1% in all three combinations, with no symptoms of disease on treated seedlings.

Table 7. The effectiveness of investigated fungicides in 2014

Variant	Effectiveness %	
	Seedlings grown in inoculated soil	Seedlings grown in naturally infected soil
Контрола	-	-
Orvego 0,1%	16,66	20,00
Enervin 0,2%	55,55	76,47
Signum 0,1%	100,00	100,00
Orvego 0,1%+Signum 0,1%	100,00	100,00
Enervin 0,25%+Signum 0,1%	100,00	100,00
Proplant 0,25%	61,47	78,82

Similar results were obtained with seedlings grown in naturally infected soil: 20,00% effectiveness was obtained with Orvego 0,1%, and somewhat higher effectiveness was achieved with preparations Enervin 0,2% and Proplant 0,25% (76,47% and 78.82%, respectively). Only the seedlings treated with Signum 0,1% showed no symptoms of disease. High effectiveness with this fungicide was achieved in protection of tobacco seedlings in field conditions (Tashkoski et al., 2014), and according to literature data, high effectiveness against *Botrytis cinerea* was observed in strawberries and lettuce (Hauke, 2004; Callens, 2005). According to Gutierrez (2012), the best results in protection of tobacco seedlings from this pathogen were achieved by

application of the strobilurin fungicides, with active ingredient azoxystrobin.
This confirms the results obtained with the product Signum 0,1%, which active ingredient is pyraklostrobin, belonging to the group of strobilurins.

CONCLUSION

-The results obtained indicate that damping off disease occurs with almost the same intensity in both years of investigation. Thus, in 2013 the disease intensity in the check variant ranged from 86.00% in seedlings grown in naturally infected soil to 100.00% in soil inoculated with fungus culture. In 2014 similar results were obtained, with infection in the check ranging from 85.00% to 90.00%. Unlike this, in the varieties treated with fungicides in both years of investigation, infection of tobacco seedlings was much lower.
-The lowest effectiveness in 2013 in both variants was observed with Orvego 0.1%, with 20.00% effectiveness and 30.23%. Similar effectiveness was achieved with Proplant 0,25%, ranging from 6.00% in seedlings grown in inoculated soil to 38.37% in seedlings grown in naturally infected soil. Effectiveness of 97.00% and 98,83% was achieved with Enervin 0,2% in both variants.
-In 2014, the lowest effectiveness of about 20.00% was obtained with Orvego 0,1% in seedlings grown in naturally infected soil. The fungicides Proplant 0,25% and Enervin 0,2% gave approximately the same results and they did not provide complete protection of seedlings.
-The highest effectiveness in seedlings protection in both years and in both variants was achieved by the fungicide Signum 0,1%, applied separately or in combination with other fungicides. Wherever this preparation was applied during the growing season, no symptoms of disease were observed on seedlings, they were healthy and had a good development. The high effectiveness recorded during the investigation gives this fungicide an opportunity to find practical application in protection of tobacco seedlings from the pathogen *P. debaryanum*.

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