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RESULTS OF *IN VITRO* INVESTIGATIONS OF SOME NEW PESTICIDES UPON THE DEVELOPMENT OF SOIL BORNE PHYTOPATHOGENIC FUNGI

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ABSTRACT

Soil borne pathogenic fungi *Pythium debaryanum, Rhizoctonia solani* and *Phytophthora parasitica var. nicotianae* are important problem which causes serious damage in tobacco seedling production. The aim of the investigation was to evaluate the effectiveness of some new fungicides in the control of these pathogens. The paper presents the results obtained with the use of chemicals Orvego, Enervin and Signum, while Previcur and Top M. served as a standard. Investigations were performed during 2013 at *in vitro* conditions, in the phytopathological laboratory of Tobacco Institute – Prilep. Recommended rates of chemicals were added to the nutrition media infested with culture of the investigated pathogenic fungi and incubated for a period of ten days. The highest effectiveness of 100 % for all three pathogenic fungi was achieved by the chemical Enervin. Orvego showed 100 % effectiveness against the pathogen *P. parasitica var. nicotianae*, and the same effectiveness was achieved with Signumagainst *R. solani*. The new fungicides showed higher effectiveness than the standard products in control of the pathogenic fungi.

Key words: pathogens, *P. debaryanum, R. solani, P. parasitica var. nicotianae*, fungicides

РЕЗУЛТАТИ ОД ИСПИТУВАЊЕТО НА ПОНОВИ ФУНГИЦИДИ ВРЗ РАЗВОЈОТ НА НЕКОИ ПОЧВЕНИ ФИТОПАТОГЕНИ ГАБИ ВО IN VITRO УСЛОВИ

Во расадопроизводството кај тутунот посебен проблем претставуваат почвените фитопатогени габи *Pythium debaryanum*, *Rhizoctonia solani* и *Phytophthora parasitica var. nicotianae*, кои му нанесуваат огромни штети на тутунскиот расад. Заради тоа, целта на ова испитување беше да се провери ефикасноста на некои понови фунгициди за сузбивање на овие патогени. Во трудот се изнесени резултатите од испитувањето на препаратите Orvego, Enervinи Signum, а како стандардни се земенипрепаратите Previcuri Top M. Испитувањата се извршени во in vitro услови на хранлива подлога КДА во текот на 2013 година во фитопатолошката лабораторија на Научниот институт за тутун-Прилеп. Предвидената количина на препарат е додадена во хранливата подлога на која беше засеана култура од испитуваните патогени габи и е инкубирана за време од десет дена. Највисока ефикасност од 100% кај сите три патогени габи беше постигната со препаратот Enervin. Препаратот Orvego покажа 100% ефикасност спрема патогенот *P. parasitica var. nicotianae*, а со фунгицидот Signum исто таква ефикасност беше постигната спрема патогенот *R. solani*. Стандардните препарати покажаа добро фунгистатично дејство спрема патогените габи. Новите испитувани фунгициди покажаа повисока ефикасност во однос на стандардните препарати во сузбивањето на овие патогени габи.

Клучни зборови: патогени, *P. debaryanum*,*R.solani*, *P.parasitica var. nicotianae*, фунгициди

INTRODUCTION

Tobacco seedlings are frequently attacked by many pathogenic soil borne fungi that cause the damping-off disease. Due to favorable temperature and humidity

conditions in seedbeds which also favor the development of phytopathogenic fungi, the damages on tobacco can often reach over 50%. Symptoms that appear in seedbeds are similar and it is very difficult to visually determine the causing agent of the disease. The most common agents that attack tobacco seedlings are *Pythium debaryanum*, also known as *Pythium ultimum* - one of the main agents of damping-off disease in vegetable crops (Ivanović, 1992), *Rhizoctonia solani, Phytophthora parasitica var. nicotianae, Thielaviopsis basicola, Botrytis* sp., *Fusarium sp*. etc. Infestation is manifested through necrotization of seedlings root system and lower part of the stalk. Although the symptoms are similar, they are caused by different pathogens and therefore special attention should be paid to the choice of chemicals. Products that are used to control one causing agent will often not be effective against the other.Thus, before application of *fungicide* it is *essential*to *determine*the*cause*of the symptoms. Investigations were conducted with a number of standard chemicals offered by manufacturers. Taskoski (2001, 2005, 2009) obtained good results with propamocarb, metalaxyl and kaptan based chemicals in the control of *P. debaryanum*, with metalaxyl in the control of *P. parasitica var. nicotianae* and with thiophanate methyl in the control of *R. solani*. According to literature data (Ivanović, 1992), good protection in field conditions was achieved by application of fungicides based on chlorthalonil, thiram, kaptan, metalaxyl and promocarb. Some of the known fungicides, however, showed poor performance in practice. For that reason, our investigations include some newer products for seedlings protection from soil borne pathogens. The purpose of investigations was to estimate the effect of new fungicides on development of most frequently represented pathogens that cause serious damage to tobacco seedlings.

MATERIALS AND METHOD

In vitro investigations were made in phytopathological laboratory of the Scientific Tobacco Institute - Prilep. Tobacco seedlings were infested with pure culture of *P. debaryanum, R. solan i*and *P. parasitica var. nicotianae* – phytopathogenic fungi that cause damping off disease. The growth media used was potato dextrose agar (PDA). Culture of the pathogenic fungi was isolated from infested tobacco plants grown in Petri dishes using standard laboratory methods. The investigation included three new fungicides and two standard fungicides which have already been used in tobacco seedlings protection (Table 1).

Table 1.Investigated fungicides

Fungicide	Active ingredient	Concentration %
Orvego	Ametoctradin 300g/l + Dimethomorph 225g/l	0,1%
Enervin WG	Ametoctradin 120g/kg+ Metiram 440 g/kg	0,2%
Signum WG	Boscalid 267 g/kg + Pyraclostrobin 67 g/kg	0,1%
Previcur 607SL	Propamocarb 70%	0,25%
Top M 70WP	Thiophanate methyl 70%	0,1%

After autoclaving, different concentrations of fungicides were added to the media cooled at certain temperature. While the media was still warm it was placed in 90 mm Petri dishes sown with 3x3 mm fragment of the fungus culture and then incubated in a thermostat at 25°C for ten days.
Three tests were performed for each pathogenic fungus, with five rerplicates for each variant (chemical). Growth of the fungus colony in variants treated with fungicides was compared with the control, i.e. with the untreated fungus colony.
The readings were performed in a period of 10 days, with regular measuring of radial growth of the colonies. Average values from the five replicates were taken as end value for each variant. The percentage of the tested fungicides was calculated by the formula of Mudri (2000) and Siameto (2010):

Effectiveness % = (a - b / a) x 100,

where:
a = radial growth of the pathogen in the control
b = radial growth of the pathogen in the presence of fungicide

RESULTS AND DISCUSSION

Results of the experiments are presented in table, through the average values obtained from the five replicates.

Table 2 shows the results for daily growth of the pathogenic fungus *P. debaryanum*, obtained in the first experiment.

Table 2. Colony growth of the fungus *P. debaryanum*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	12	40	45	45	45	45	45	45	45	45
Orvego0,1%	3	10	25	30	35	40	45	45	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	3	12	22	40	42	45	45	45	45	45
Previcur0,25%	5	7	15	15	17	18	20	20	20	20
Top M 0,1%	5	28	45	45	45	45	45	45	45	45

24 hours after incubation, the radial growth of fungus colony in the control was 12 mm. Due to the fungus rapid development, the maximum increase of 45 mm was reached on the third day, which means that the Petri dish was full. Unlike control, somewhat slower growth was observed in variants treated with fungicides. Thus, in media treated with Orvego 0.1%, radial growth of the colony ranged from 3 mm after 24 hours to 45 mm on the seventh day of incubation. Similar results were obtained with Signum 0, 1 %. The highest fungal growth was recorded on the media treated with Top M 0,1% (5mm after 24 hours, and the maximum 45 mm on the third day). The lowest growth was registered with Previcur 0, 25 % (5 mm on the first day and 20 mm by the end of observation). Only in the media treated with Enervin 0, 2 % no colony growth of the pathogen P. debaryanum was observed.
Development of *R. solani* from the first experiment is shown in Table 3. This pathogenic fungus showed good growth, with the maximum of 45mm achieved on the fifth day of incubation. Somewhat lower growth was observed in the fungus grown on media treated with Orvego 0, 1 % and Previcur 0, 25 %. With both fungicides,

radial growth of 45 mm was measured on the seventh day of incubation. The lowest growth was measured on media treated with Top M 0,1% (3 mm on the second day, and only 15 mm by the end of observation). No mycelial growth of the fungus was observed in media treated with the fungicides Enervin 0, 2 % and Signum 0, 1%.

Table 3. Colony growth of the fungus *R. solani*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	2	12	30	40	45	45	45	45	45	45
Orvego0,1%	-	5	15	20	30	40	45	45	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	-	-	-	-	-	-	-	-	-
Previcur0,25%	-	5	20	30	35	40	45	45	45	45
Top M 0,1%	-	3	10	10	10	10	12	15	15	15

In the first experiment, pathogenic fungus *P. parasitica var. nicotianae* reached 30 mm by the end of observation in the control (Table 4). Somewhat poorer growth (20mm) was measured in the variant treated with Signum 0, 1%, and the lowest growth was observed with the fungicides Top M 0,1% (13 mm) and Previcur 0, 25% (15mm). No fungal growth was recorded in media treated with Orvego 0, 1% and Enervin 0, 2%.

Table 4. Colony growth of the fungus *P.parasitica var. nicotianae*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	2	8	10	15	18	20	25	25	30	30
Orvego0,1%	-	-	-	-	-	-	-	-	-	-
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	2	5	8	11	13	15	17	20	20
Previcur0,25%	-	4	6	8	10	12	12	15	15	15
Top M 0,1%	-	-	2	3	5	6	9	10	10	13

Results of investigations on the three pathogenic fungi obtained in the second experiment are presented in Tables 5, 6 and 7. In the second experiment, the fungus *P. debaryanum* showed rapid growth as in the first one. After 24 hours of incubation, the growth of the control was 10 mm, and the maximum radial growth of 45 mm was reached on the third day (Table 5). No major differences were observed in development of the colony grown in media treated with Orvego 0,1 %, Signum 0,1 % and Top M 0,1%. In all these treatments the fungus developed gradually and by the end of observation the colony growth reached 45 mm. Somewhat poorer growth was observed in the colony grown in media treated with Previcur 0,25 %, while absence of fungal growth was observed in media treated with Enervin 0,2 %.

Table 5.Colony growth of the fungus *P. debaryanum*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	10	32	45	45	45	45	45	45	45	45
Orvego0,1%	5	15	30	45	45	45	45	45	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	5	16	25	30	40	45	45	45	45
Previcur0,25%	5	10	12	15	16	18	20	25	30	36
Top M 0,1%	5	25	45	45	45	45	45	45	45	45

Pathogenic fungus *R. solani* which served as control had a successful growth in the second experiment (Table 6). The first day mycelial growth measured 2 mm and radial growth of 45 mm was measured on the sixth day. A similar growth was measured with variants treated with Previcur 0,25% and Orvego 0,1%, while the poorest growth (13 mm) was obtained with Top M 0,1%. No fungal growth was recorded in media treated with Enervin 0,2% and Signum 0,1.

Table 6.Colony growth of the fungus *R. solani*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	2	8	14	22	35	45	45	45	45	45
Orvego0,1%	2	8	15	25	30	35	45	45	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	-	-	-	-	-	-	-	-	-
Previcur0,25%	-	3	10	21	29	40	45	45	45	45
Top M 0,1%	-	2	5	7	8	10	10	10	12	13

Results on the development of pathogenic fungus *P. parasitica var. nicotianae* in the second experiment are presented in Table 7.

Table 7.Colony growth of the fungus *P. parasitica var. nicotianae*

Variant	Colony growth inmm bydays									
	1	2	3	4	5	6	7	8	9	10
Control	-	2	4	6	10	15	18	20	20	25
Orvego0,1%	-	-	-	-	-	-	-	-	-	-
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	-	2	3	5	8	10	10	10	10
Previcur0,25%	-	1	3	4	5	8	10	10	10	11
Top M 0,1%	-	-	-	-	3	6	10	10	12	15

By the end of observation, radial growth of the fungus reached 25 mm in the control variant, which was the highest growth achieved. In treatments with Signum 0, 1

%, Previcur 0, 25 % and Top M 0,1%, the growth was 10 mm, 11 mm and 15mm, respectively. In this experiment too, no fungal growth was recorded in media treated with the fungicides Orvego 0, 1 % and Enervin 0,2%.

Results of investigations on pathogenic fungi *P. debaryanum, R. solani and P. parasitica var. nicotianae* obtained in the third experiment are presented in Tables 8, 9 and 10.

Data on growth of *P. debaryanum* are presented in Table 8. Due to the rapid growth of this fungus, 15 mm radial growth of the colony was measured after 24 hours, and the second day the Petri dish was full, i.e. radial growth was 45 mm. In media treated with Enervin 0, 2 % no mycelia growth was recorded until the last day of observation. Poor colony growth of 30 mm was observed in the variant treated with Previcur 0, 25 % on the tenth observation day. In variants treated with Orvego 0, 1 %, Signum 0,1% and Top M 0,1%, the growth of the colony started from the first day, to reach radial growth of 45 mm by the end of observation (Table 8).

Table 8. Colony growth of the fungus *P. debaryanum*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	15	45	45	45	45	45	45	45	45	45
Orvego0,1%	-	8	20	25	30	40	45	45	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	5	20	40	45	45	45	45	45	45	45
Previcur0,25%	5	8	11	16	18	20	22	25	27	30
Top M 0,1%	5	27	45	45	45	45	45	45	45	45

Mycelial growth of the phytopathogenic fungus *R. solani* was observed in the control from the first day, and the last day of observation it measured 45 mm (Table 9).

Media treated with Enervin 0, 2 % and Signum 0, 1 % showed no mycelial growth to the last day of observation. The poorest growth of 18 mm was measured in the variant treated with Top M 0,1%, while in the variants treated with Orvego 0,1% and Previcur 0, 25 % the mycelial growth started from the first or second day, and on the tenth day radial growth of 45mm was measured.

Table 9.Colony growth of the fungus *R. solani*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	2	10	25	35	38	40	40	45	45	45
Orvego0,1%	2	8	15	25	30	37	40	43	45	45
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	-	-	-	-	-	-	-	-	-
Previcur0,25%	-	4	12	22	30	38	45	45	45	45
Top M 0,1%	-	2	8	12	15	15	15	17	17	18

Colony growth of *P. parasitica var. nicotianae* is presented in Table 10. In the control variant, mycelial growth was observed on the second observation day,

but since this pathogenic fungus has poorer growth, only 20 mm were measured on the tenth day. No fungal growth was observed in media treated with Orvego 0,1 % and Enervin 0,2 % and the poorest growth of only 5 mm was recorded in fungicide treatments with Signum 0,1 %. In media treated with Previcur 0,25 % and Top M 0,1%, just like in the first and second experiment, there were no significant differences in colony growth of the fungus and it reached 12 mm and 13 mm, respectively.

Table 10.Colony growth of the fungus *P. parasitica var. nicotianae*

Variant	Colony growth in mm by days									
	1	2	3	4	5	6	7	8	9	10
Control	-	3	6	8	8	9	9	12	20	20
Orvego0,1%	-	-	-	-	-	-	-	-	-	-
Enervin0,2%	-	-	-	-	-	-	-	-	-	-
Signum0,1%	-	-	2	4	4	5	5	5	5	5
Previcur0,25%	-	3	5	7	10	11	12	12	12	12
Top M 0,1%	-	-	-	2	5	7	9	10	12	13

According to the results in the above tables, none of the three pathogenic fungi showed occurrence and growth of mycelia in media treated with Enervin 0,2 %. Also, there was no occurrence and growth of *R. solani* and P. *parasitica var. nicotianae*in media treated with Signum 0,1 %, and Orvego 0, 1 %, respectively.

The effectiveness of tested chemicals in the control of soil borne pathogenic fungi *P. debaryanum, R. solanii P. parasitica var. nicotianae*, i.e. their fungicidal and fungistatic activity in the I, II and III experiment is presented in Table 11.

Table 11.The effectiveness of tested fungicides, in %

Variant	*P. debaryanum*				*R.solani*				*P.parasitica var. nicotianae*			
	I	II	III	$\bar{x}$	I	II	III	$\bar{x}$	I	II	III	$\bar{x}$
Control	-	-	-	-	-	-	-	-	-	-	-	-
Orvego0,1%	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	100	100	100	100
Enervin0,2%	100	100	100	100	100	100	100	100	100	100	100	100
Signum0,1%	0,00	0,00	0,00	0,00	100	100	100	100	33,33	60,00	75,00	56,11
Previcur0,25%	55,55	20,00	33,33	36,29	0,00	0,00	0,00	0,00	50,00	56,00	40,00	48,66
Top M 0,1%	0,00	0,00	0,00	0,00	66,66	71,11	60,00	65,92	56,66	40,00	35,00	43,88

The highest effectiveness in the control of above soil borne phytopathogenic fungi during *in vitro* investigations was obtained with the fungicide Enervin applied in a concentration of 0.2 %. In all three experiments its effectiveness was 100 %, i.e. no occurrence and growth of these pathogenic fungi was recorded. High fungicidal effect (100 %) against pathogenic fungus *P. parasitica var. nicotianae* in all three experiments was obtained with the chemical Orvego in concentration of 0.1 %. The chemical Signum in concentration of 0.1 % showed 100% effectiveness in the control of pathogenic fungus *R. solani* and high fungistatic effect 75,00% in the control of *P . parasitica var. nicotianae* (in 3de replication, or 56,11% in average).

The standard chemicals confirmed their fungistatic effect against the investigated pathogens. By application of Previcur 0,25%, 55,55% effectiveness was obtained

in *P. debaryanum* in the Ist replication and 56,00% in *P. parasitica var. nicotianae* in the IInd replication.The obtained average values were 36,29% and 48,66% in *P. debaryanum* and *P. parasitica var. nicotianae* respectively. Similar results with 53.10 % to 69.46 % effectiveness were reported by Taskoski (2009), in his in vitro investigations of this fungicide in the control of *P. debaryanum*. The chemical Top M applied in concentration of 0.1 % showed high effectiveness (60,00 -71,11%) in the control of *R. solani* in the IIIrd and IIndreplication, with average value of 65,92%. This product also showed good fungistatic effect against *P. parasitica var. nicotianae* (35,00-56,66%, i.e.43,88% in average). Taskoski (2001) reported high effectiveness of chemicals with a.i. thiophanate methyl in the control of *R. solani* both at *in vitro* and in natural conditions of seedlings growing.

CONCLUSION

In our investigations, soil borne phytopathogenic fungi *P. debaryanum, R. solani and P. parasitica var. nicotianae* were successfully grown on potato dextrose agar (PDA) and in some of them maximum colony growth was obtained three days after incubation. The investigated chemicals showed big differences in colony growth, depending on the pathogenic fungus. Some fungicides showed high fungicidal effect against one pathogen and fungistatic effect against another.

Of the fungicides investigated, 100 % effectiveness was obtained with Enervin 0.2 % against all three pathogenic fungi and with Orvego 0.1 % against *P. parasitica var. nicotianae.*Signum 0.1% showed 100% effectiveness against *R. solani* and certain fungistatic effect against *P. parasitica var. nicotianae*.

Of the standard chemicals, fungistatic effect was confirmed with Previcur 0.25 % against pathogenic fungi *P. debaryanum*and *P. parasitica var. nicotianae*, and with Top M 0.1 % against *R. solani* and *P. parasitica var. nicotianae*.

Due to their high fungicidal effect on growth and development of *P. debaryanum, R. solani and P. parasitica var. nicotianae*, the chemicals Enervin, Orvego and Signum can find practical application in future, in protection of tobacco seedlings from these disease causing agents.

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