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In vitro Evaluation the Effectiveness of New Fungicides for Control of *Phytium debarianum* in Tobacco Seedlings

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Abstract: *Tobacco seedlings protection from the damping off disease caused by Pythium debarianum, long time has been performed only with the active substance propamocarb. But today, many preparations with new active ingredients have been registered.*

The aim of these studies was to examine the efficacy of new fungicides and certain combinations, including commonly used ones. The investigations were performed by in vitro test, in 3 replications, including 6 fungicides and 4 combinations.

The standardly used Previcur N (0.15%) showed poorer results than some new or combination of preparations.

OrvegoTM (0.1%) did not give the expected results. However, when combined with contact fungicides Manfil 80 WP 0.25% and Enervin WG 0.2%, the effectiveness is 100%.

The Signum 33WG 0.1% fungicide showed 87.27% effectiveness. When combined with other preparations, the effectiveness is lower.

The best results in this studies (100% effiectiveness) were achieved by Quadris 25 SC (0.1%) as well as the contact fungicide Enervin WG 0.2%.

Fungicides, as well as high-efficient combinations in the control of P. debarianum will allow their application in the protection of tobacco seedlings from the damping off disease.

Keywords: *Phytium debarianum, active ingredient, radial development, effectiveness*

1. INTRODUCTION

Healthy and high-quality tobacco seedlings are the basis for successful tobacco production and obtaining a good yield and quality of tobacco. However, the production of such seedlings also means providing maximum protection in order to prevent diseases or achieve a minimum intensity of attack and reduce the damage caused by them to the lowest level.

The most destructive disease that occurs in the production of tobacco seedlings is the „damping off" disease. Its causing agent in most cases is the pathogenic fungus *Pythyum debarianum.* There is a complex of soil fungal pathogens in the tobacco seedlings production in which *Rhizoctonia solani* is the most accompained with *Pythyum* in this disease.

Pythium belongs to the family *Pythiaceae* in the order *Peronosporales* of the class *Oomycetes*-primitive fungi. They can reproduce sexually (in the form of oospores), as well as asexually. The *Pythiaceae* are considered opportunist parasites, preying on weakness and taking advantage of conditions which are not ideal for the plant (Morel Diffusion 2017).

Pythium can cause severe infection and damages. Symptoms of *Pythium* infection are variable and can include stem rot, chlorosis, wilting, loss of lower leaves, poor root development and root loss (Chase 2013).

Plants are stunted, wilt, and die. The cells of roots contain round, microscopic, thick-walled spores. The tissue becomes disorganised, loses its firmness and takes on a watery look. The disease may go so far as to make the plants literally dissolve and disappear from the surface of the growing

medium; so it is also referred to as seedling melt (post-apparition melt). There are withering or yellowing of a few leaves in the larger plants (Penn State Extension 2017; Morel Diffusion 2022).

It can occur at all developmental stages of the tobacco seedling, starting from the germination of the seed to the vigorous growth of the seedling. Sometimes it can appear immediately after sprouting, which makes further development of the seedling impossible. Damage is greatest at these stages of development. If *Pythium* infests a sedlings bed, large losses occur.

The fight against this disease is difficult because at first, there is no exact identification of the causing agent of the disease. Hence, in practice, fungicides are applied that are not appropriate, that is, they are not effective in suppressing the specific causing agent. Also, the number of preparations for control of *Pythium* in of tobacco seedlings is limited until now.

Propamocarb is selectively active against Oomycetes. The fungicide is xylem-mobile and can be used as drench, soil incorporation, dip or foliar spray (Kilian and Steiner 2003).

The active substance propamocarb has a dominant place in the suppression of this pathogen -in our area and tobacco seedlings, too. Previcur is a standard part of the protective program used by many commercial nurseries to prevent the damping-off caused by Pythium spp. (Bayer, 2022 a, b).

But, there are data on the activity of certain active ingredients and preparations, too.

Aliette Signature and Banol are effective fungicides for all *Pythium* diseases (*Pythium* Blight, *Pythium* Root Rot, *Pythium* Root Dysfunction). There are not resistance up to 20 years (Bayer 2022).

Over the past years, there are few new fungicides. The most effective product were those that include a.i. Etridiazole - effective to many Pythium species; a.i. Mefenoxam and the fungicides with phosphonates as active ingredient (a.i. mono and di-basic sodium, potassium and ammonium phosphites).

Strobilurins were found to be effective to *Phytium*, *Phytophthora*, as well as *Fusaium* and *Rhizoctonia* in ornamentals. As solution of *Pythium* control Chase (2013) lists etridiazole, mefenoxam, fluopicolide, phosphonates, strobilurins and *Trichoderma* sp.

There are some reports for the use of strobilurins against the damping off. For example, application of Quadris is recommended by the manufacturer (Syngenta, 2006).

Studies of the fungicides *in vitro* serve as a guide for their inclusion *in vivo*.

Mihajlovic et al (2013) investigated several active ingredients against *Pythium aphanidermatum* (propamocarb hydrochloride, fosetyl-Al, mancozeb, azoxystrobin, mefenoxan and tea tree oil). They also made a parallel of results between in vitro and greenhouse assays.

Prasad et al (2014) have investigated ten fungicides on *Pythium debarianum* (and *Rhizoctonia solani*) causing the damping off diseases in tomatto. There are active ingredients (and combinations) which shown 100 % inhibition of radial development of the *P. debarianum* - tebuconazole and trifloxystrobin, carbendazin and captan+hexaconazole. The other active ingredients also have shown a high degree of effectiveness.

Recently, there are more fungicides with new active ingredients on the market that are used in control of this disease.

The *in vitro* assay is the first and quickest way to screen the effectiveness of fungicides and their active ingredients. We can estimate the direct effect on the pathogen, free from environmental influences. This way of study provides significant knowledge of the mode of action and effectiveness of the fungicide / active ingredients before the application of a commercial product in the field and actually, they are guide for their inclusion *in vivo*.

Therefore, the aim of this study was to investigate the efficacy of several fungicides in control of this pathogenic fungus, based on the various, new active ingredients and some combinations by *in vitro* study.

The obtained results will make a great contribution to expanding the list of fungicides for control of the damping off disease, caused by *P. debarianum*.

2. MATERIAL AND METHODS

2.1. Poisoned Food Techniue

Pure culture of the fungus is isolated from infected tobacco seedlings by standard laboratory method on the medium potato dextrose agar (PDA).

The same medium - PDA was used for the tests, with the addition of fungicides. An overview of the tested fungicides is given in Table 1.

The medium with the fungicide was poured into petri boxes 110 mm in diameter, and then sown with a fragment of pure fungus culture (about 5 mm). Sown petri boxes with fungicide-free substrate served as a check.

The Petri boxes were incubated for 10 days at a temperature of 25 °C by daily measuring the diameter of the colony on both opposite sides of the Petrie box. The experiment was set 3 times. The results for the three replications as well as the average values are shown by tables.

Table1. *Tested fungicides*

Variant / Fungicide	Active ingredient /s	Concentaration (%)
Previcur® N	722 g/l propamocarb	0,15%
Signum 33 WG	267 g/kg boscalid +67g/kg pyraclostrobin	0,1%
Orvego™	300 g/l ametoctradin +225 g/l dimetomorph	0,1%
Enervin WG	120 g/kg ametoctradin +440 g/kg metiram	0,2%
Quadris 25 SC	250 g/l azoxystrobin	0,15%
Top-M 70%WP	70% thiophanate –methyl	0,1%
Signum 33 WG + Previcur® N	267 g/kg boscalid +67g/kg pyraclostrobin 700 g/l propamocarb	0,1% + 0,15%
Signum 33 WG + Orvego™	267 g/kg boscalid +67g/kg pyraclostrobin 300 g/l ametoctradin +225 g/l dimetomorph	0,1% + 0,1%
Orvego™ + Manfil 80WP	300 g/l ametoctradin +225 g/l dimetomorph 800 g/kg mancozeb	0,1% + 0,25%
Orvego™ + Enervin WG	300 g/l ametoctradin +225 g/l dimetomorph 120 g/kg ametoctradin +440 g/kg metiram	0,1% + 0,2%

2.2. Estimations

The effect of fungicides was monitored daily, in parallel with the development of control. *P. debarianum* has a very fast development and the maximum development of the air mycelium was achieved on the third day, Therefore, when calculating the efficiency, the value of the third day was taken.

The effectiveness of fungicides in control of *P. debarianum* is calculated according to the average value of the diameter of the colonies (in the appropriate variant, i.e. fungicide and the check) according to the formula of Singh et al (2015).

The development of the cultures in the variants is followed until the 10th day. The efficiency of this day is shown, in order to see the effect of the respective fungicides during the incubation period.

3. RESULTS AND DISSCUSION

3.1. Development of the Pathogenic Fungus *P. Debarianum*

In the first replication of the study, the fastest initial development was observed on a medium without fungicide, i.e. check. The colony continues to develop intensively and already on the third day the Petri dishes are completely filled. Among the variants (except for Top M as well as Previcur® N) no colony development was observed on the first day. There is at the second day with Orvego™ and the

combination Signum 33WG +Previcur ®N . In Quadris 25 SC the fungus started to develop after the trird day (Table 2).

Table2. *Development of the P. debarianum colony– 1st replication*

day / Variant	1	2	3	4	5	6	7	8	9	10
	Diameter in mm									
Previcur® N 0,15%	25,40	38,00	47,20	59,00	68,20	83,00	88,80	93,40	97,60	98,20
Signum 33 WG 0,1%	-	-	42,20	81,40	98,00	107,80	110,00	110,00	110,00	110,00
Orvego™ 0,1%	-	46,00	83,80	110,00	110,00	110,00	110,00	110,00	110,00	110,00
Enervin WG 0,2%	-	-	-	-	+-	+	5,00	5,00	5,00	5,00
Quadris 25 SC 0,15%	-	-	-	10,20	15,60	16,80	19,00	21,00	22,00	22,00
Top M 0,1%	35,60	64,40	102,20	110,00	110,00	110,00	110,00	110,00	110,00	110,00
Signum 33WG+ Previcur® N 0,1%+0,15%	-	12,00	53,60	76,80	95,00	104,40	107,40	110,00	110,00	110,00
Signum 33WG + Orvego™ 0,1%+0,1%	-	-	36,60	71,40	92,00	102,60	106,40	108,60	110,00	110,00
Orvego™ + Manfil 80WP 0,1%+0,25%	-	-	-	-	18,20	35,40	40,40	43,60	46,00	46,20
Orvego™ +Enervin WG 0,1%+0,2%	-	-	-	-	14,40	16,80	26,60	30,60	35,40	40,00
Check ø	32,20	101,00	110,00	110,00	110,00	110,00	110,00	110,00	110,00	110,00

In the variant Enervin WG (0,1%), an initial development was determined only on the sixth day and remained until the end of incubation.

The colony diameter in the variants, on the third day, reached a value from 36.60 mm in Signum 33WG + OrvegoTM to 102.20 mm in Top M.

But, the colony diameter in the variants with fungicide, at the end of incubation, reached a value from 22.00 mm in the Quadris 25 SC fungicide to 110.00 in Signum 33WG, OrvegoTM and Top-M 70%WP, as well as the combinations Signum 33 WG +Previcur® N and Signum 33 WG + OrvegoTM.

In the combinations OrvegoTM + Manfil 80WP and OrvegoTM + Enervin WG, the colony of the pathogen reached a diameter of 40.00 and 46.25 mm, respectively.

Although in Previcur® N the pathogen developed from the first day, however, the colony reached a smaller diameter compared to the fungicides in which no development was detected (98.20mm).

In the second replication, the development of the pathogen in the control starts at 33.10 mm and continues to develop intensively to the end of incubation (Ph. 1). As in the first replication, development of the pathogen started in the Petri dishes with the fungicides Previcur and Top M.

While the pathogen reaches full development on the third day, in some variants no such onset is observed at all, e.g. Signum33WG and Quadris 25 SC. In others, in the following days (combinations with OrvegoTM), while in Enervin WG, again on the sixth day.

Table3. *Development of the P. debarianum colony– 2nd replication*

day / Variant	1	2	3	4	5	6	7	8	9	10
	Diameter in mm									
Previcur® N 0,15%	25,40	59,66	106,33	110,00	110,00	110,00	110,00	110,00	110,00	110,00
Signum 33 WG 0,1%	-	-	-	7,00	31,00	56,00	77,20	82,00	91,60	96,40
Orvego™ 0,1%	-	40,4	62,80	85,40	88,00	94,40	98,60	100,40	103,00	106,60
Enervin WG 0,2%	-	-	-	-	+-	+	5,00	5,00	5,00	5,00
Quadris 25 SC 0,15%	-	-	-	6,40	7,00	14,40	34,20	41,00	46,40	50,60
Top M 0,1%	30,00	63,00	102,20	104,00	110,00	110,00	110,00	110,00	110,00	110,00
Signum 33WG+ Previcur® N 0,1%+0,15%	-	12,00	25,00	32,60	51,80	70,60	82,20	82,20	93,00	93,00
Signum 33WG + Orvego™ 0,1%+0,1%	-	-	29,25	50,75	66,75	83,50	90,77	97,00	10,00	104,50
Orvego™ + Manfil 80WP 0,1%+0,25%	-	-	-	-	17,60	33,40	41,40	47,80	52,20	57,40
Orvego™ +Enervin WG 0,1%+0,2%	-	-	-	-	9,60	16,00	25,20	31,20	35,60	40,40
Check ø	33,10	84,25	110,00	110,00	110,00	110,00	110,00	110,00	110,00	110,00

Table4. *Development of the P. debarianum colony– 3th replication*

day / Variant	1	2	3	4	5	6	7	8	9	10
	Diameter in mm									
Previcur® N 0,15%	18,00	25,60	30,20	37,80	45,60	51,00	59,00	64,80	73,80	81,80
Signum 33 WG 0,1%	-	-	-	-	-	26,40	38,40	55,00	61,20	75,80
Orvego™ 0,1%	-	41,40	67,80	100,60	110,00	110,00	110,00	110,00	110,00	110,00
Enervin WG 0,2%	-	-	-	-	+-	+	5,00	5,00	5,00	5,00
Quadris 25 SC 0,15%	-	-	-	7,40	10,80	15,60	19,20	21,60	24,60	30,20
Top M 0,1%	35,00	64,20	102,20	107,00	110,00	110,00	110,00	110,00	110,00	110,00
Signum 33WG+ Previcur® N 0,1%+0,15%	-	9,20	25,80	25,80	34,20	46,40	56,80	74,40	90,00	102,20
Signum 33WG + Orvego™ 0,1%+0,1%	-	44,00	69,80	101,60	109,00	110,00	110,00	110,00	110,00	110,00
Orvego™ + Manfil 80WP 0,1%+0,25%	-	-	-	-	16,80	35,80	39,80	44,60	48,20	51,60
Orvego™ +Enervin WG 0,1%+0,2%	-	-	-	-	13,60	16,60	21,80	28,40	35,40	42,00
Check ø	39,60	104,60	110,00	110,00	110,00	110,00	110,00	110,00	110,00	110,00

On the third day, the colony has the highest values in Top M and the standard preparation Previcur, and the smallest or more precisely, no development in Enervin WG, Signum 33 WG and Quadris 25 SC.

At the end of the incubation, the pathogen in the variants reached a diameter of 40.40 in Orvego™ +Enervin to 110.00 with Top M and the standard – Previcur, while in the other fungicides it has smaller values. In this replication it is noted that (except for Top M), the other fungicides showed a somewhat stronger reducing effect. These are Signum 33 WG and Orvego™ and their combinations (Table 3).

The results for the influence of the investigated fungicides on the development of the pathogen in the third repetition is the same as in the previously one, in relation to the initial development by days as well as separate preparations - on the third as well as on the 10th day (Table 4). The best results, again it was showed by Enervin WG 0.2%, Signum 33 WG 0.1% and Quadris 25 SC 0.15%, as well as combinations of Orvego™ 0.1% with the contact fungicides.

Analyzing the development of the pathogen as a mean value of the studies (Table 5), it can be concluded that the fungicide Top M showed the weakest effect, that is, the fungus reached the greatest radial development of the colony (Ph. 2). The values are almost similar to the control. It can be noticed already on the third day (when the control reaches the maximum development).

On the contrary, no radial development of the colony was ascertained in the substrate in which Enervin WG 0.2%, Quadris 25 SC 0.15%, and combinations of Orvego™ 0.1% with the contact fungicides. With Quadris, it reached a very low value by the end of the incubation and with Enervin WG 0.2% very small, insignificant development (Ph. 3 and 4).

At Signum 33 WG 0.1%, a development of only 14.00 mm has been determined, but still, by the end, it reaches not so small values (Ph. 5,6). Alone, it works better than in combination with other preparations.

At the end of the incubation, the smallest diameter was found in Orvego+ Enervin (40.80 mm) and Orvego™ +Manfil (51.73 mm), in contrast to the situation when only Orvego™ (Ph. 7.8) is used. But the Quadris 25 Sc fungicide also showed excellent results, where the diameter of the colony of the pathogen is 46.27 mm. In other preparations, the values are quite high.

The contact fungicide Enervin WG achieved the best results – in the three replications, a very small, insignificant development of the pathogen was found.

Table5. *Development of the P. debarianum colony– average value*

day / variant	1	2	3	4	5	6	7	8	9	10
	Diameter in mm									
Previcur® N 0,15%	22,93	41,09	61,24	68,93	74,60	81,33	85,93	89,40	93,80	96,67
Signum 33 WG 0,1%	-	-	14,00	29,47	43,00	63,40	75,20	82,33	87,60	94,07
Orvego™ 0,1%	-	42,60	71,47	98,67	102,67	104,80	106,20	106,80	107,67	108,87
Enervin WG 0,2%	-	-	-	-	+-	+	5,00	5,00	5,00	5,00
Quadris 25 SC 0,15%	-	-	-	8,00	11,13	15,60	24,13	31,20	36,67	46,27
Top M 0,1%	33,53	69,00	104,53	110,00	110,00	110,00	110,00	110,00	110,00	110,00
Signum 33WG+ Previcur® N 0,1%+0,15%	-	11,07	33,80	45,07	60,33	73,80	82,13	88,87	97,67	101,73
Signum 33WG + Orvego™ 0,1%+0,1%	-	14,67	45,22	74,58	89,25	98,70	102,39	105,20	106,67	108,17
Orvego™ + Manfil 80WP 0,1%+0,25%	-	-	-	-	17,53	34,87	40,53	45,33	48,80	51,73
Orvego™ + Enervin WG 0,1%+0,2%	-	-	-	-	12,53	16,47	24,53	30,07	35,47	40,80
Check ø	34,97	96,42	110,00	110,00	110,00	110,00	110,00	110,00	110,00	110,00

3.2. Efficacy of Fungicides in Control of the Pathogen *P. Debarianum*

Since the impact of the fungicides is determined depending on the maximum development of the check (in this case, on the third day), the efficacy of the fungicides according to the values of that day (theirs and the control) is a really relevant indicator. The graphic presentation of the efficacy of the fungicides on the third day is also given (Graph 1). However, the efficiency values calculated at the end of the incubation are also given (Table 6).

It can be concluded that the tested fungicides differ greatly in their effectiveness in control of *P. debarianum*.

According to the results, in the variant with the preparation Top M 0.1% there is almost no efficiency, which is also confirmed at the end of the incubation.

The active substance thiophanate-methyl has been proven in the suppression of *Rhizoctonia solani*, whose affiliation in the classification of phytopathogenic fungi is completely different than *P. debarianum* (Ivanović, 1992). It justifies such results for the efficacy of this fungicide, especially in *in vitro* conditions. Its inclusion in the selection of preparations comes from the fact that for a long time *R. solani* was considered as the causing agent of the damping off disease (due to the similarity of the symptoms in tobacco seedlings). However, these results confirm and impose the correct determination of the causing agent of the disease and hence, the target pathogen for the correct selection of the fungicide (Pataky, 1988).

Previcur 0.15% has little inhibition of mycelial growth. Propamocarb is selectively active against *Oomycetes* (Extoxnet 1997, Bayer Crop Science, 2009). The action of Propamocarb is related to cell membrane permeability, causing an efflux of cell compounds (Kilian and Steiner 2003).

But, Propamocarb although has little or no inhibition of mycelial growth nor any control of mycelial infection (in *Phytophthora nicotiana*), it consistently suppressed all other growth stages and reduced zoospore infection. These results have several significant practical implications (Wu et al 2020).

In other fungicides, the efficiency ranges from 35.03% for OrvegoTM (0.1%) to 87.27% for Signum 33 WG (0.1%).

Signum 33 WG (0.1%) has a good efficacy (87.27%). The efficacy of Signum may be due to the fact that the two active ingredients (pyraclostrobin and boscalid) have preventive and systemic activity and they are an excellent combination of two different biochemical modes of action on cellular respiration of fungi (Hauke et al., 2004).

Orvego™ (0.1%) did not show the expected results. Belonging to a new class of pyrimidylamines, a.i. ametoctradin is characterized as being a powerful respiratory inhibitor of complex III, cytochrome bc_1 at Q_O site, stigmatellin binding subsite. In *P. infestans*, it acts mainly on the formation, liberation, and motility of zoospores and the germination of cysts (Töfoli el al., 2016). These data explain the contradictory results of Orvego™ in natural infection, when it has very good effectiveness (Gveroska, 2018).

The fungicides Enervin WG (0.2%) and Quadris 25 SC (0.15%) as well as the combinations Orvego™ + Manfil 80WP (0.1%+0.25%) and Orvego™ +Enervin WG (0.1%+0, 2%) showed 100% efficiency in suppressing this pathogen.

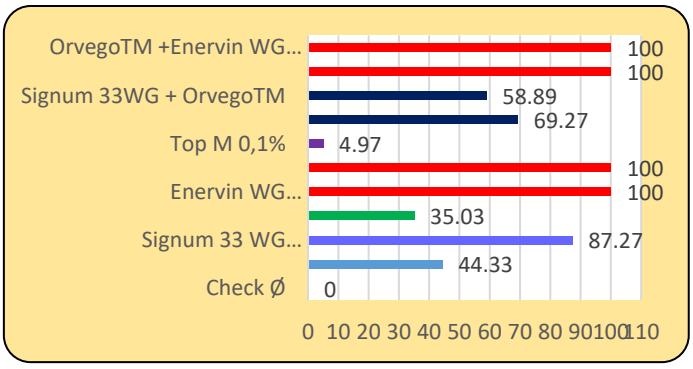
Enervin WG (0.2%) as a contact fungicide with two active ingredientsand has an excelent results. Only this fungicide did no lowered its efficacy during the incubation period. It rases the effectiveness of Orvego™ alone, too.

Ametoctradin with its associations (dimethomorph and metiram) represent a new alternative for the management of potato late blight, especially in the management of resistance (Töfoli el al., 2016). A.i. metiram is considered to has a multi-site, non-specific mode of action that disrupts many of the essential processes within the fungal cell (BASF, 2013).

Considering the mode of action of the active ingredients which are an integral parts of this preparation, the achieved results are justified and understandable.

Table6. *Efficacy of fungicides*

Variant (fungicide)	Diameter of the colony (mm) – 3 day	Efficacy of fungicide	Diameter of the colony (mm) – 10 day	Efficacy of fungicide
Previcur® N 0,15%	61,24	44,33	96,67	12,12
Signum 33 WG 0,1%	14,00	87,27	94,07	14,48
Orvego™ 0,1%	71,47	35,03	108,87	1,03
Enervin WG 0,2%	-	100,00	5,00	95,00
Quadris 25 SC 0,15%	-	100,00	46,27	57,94
Top M 0,1%	104,53	4,97	110,00	-
Signum 33WG+ Previcur® N 0,1%+0,15%	33,80	69,27	101,73	7,52
Signum 33WG + Orvego™ 0,1%+0,1%	45,22	58,89	108,17	1,66
Orvego™ + Manfil 80WP 0,1%+0,25%	-	100,00	51,73	59,97
Orvego™ +Enervin WG 0,1%+0,2%	-	100,00	40,80	62,91
Check ø	110,00	-	110,00	-



Graph1. *Efficacy of fungicides in control of Pythium debarianum*

Quadris 25 SC (azoxystrobin) has an excellent results. Only this fungicide has showed the initial pathogen development even up to the fourth day.

These results are confirmed by facts that azoxystrobin acts against respiration of fungi (Extension 2022). The mode of action of azoxystrobin is by choking out fungi via interrupting the activity of the electron transport chain. As a result, this prevents fungal spores from germinating and halts the growth process of the fungus (Solution, 2022).

Toxicity of Quadris in *in vitro* studies against *P. aphanidermatum* (in experiment with 5 fungicides and 1 biofungicide - propamocarb hydrochloride, fosetyl-Al, mancozeb, azoxystrobin, mefenoxan and tea tree oil) is confirmed by Mihajlović et al. (2013), too.

Quadris has effective protection of many crops against root diseases including tobacco (Poindexter and Wenzel, 2013; Schwartz and Gent, 2012; Gveroska, 2018). Application of Quadris against the damping off disease is also recommended by the manufacturer (Syngenta, 2006).

Combinations of contact fungicides with Orvego™ in this *in vitro* study had shown 100% efficiency. It is confirmed by previouous findings of high effectiveness (88,64%) achieved with treatment with Orvego TM + Manfil 80WP in control of damping off disease in tobacco seedlings in dual infection (Gveroska, 2018).

The good results in these investigations by combination of fungicides (active ingredients), fungicides with a wide range of activities or with preventive and systemic action are in accordance with the above

that combination of active ingredients with specific action against the pathogen / s contribute to the final result.

At the end of the incubation, the calculated value is quite reduced in the fungicides with lower efficiency, which is quite normal in the passage of time. But the situation has not changed much in terms of the ranking of the preparations. The most effective preparations have reduced their effectiveness twice or even several times. However, a very positive effect was observed at Quadris 25 SC – prevention of the initial development even up to the fourth day.

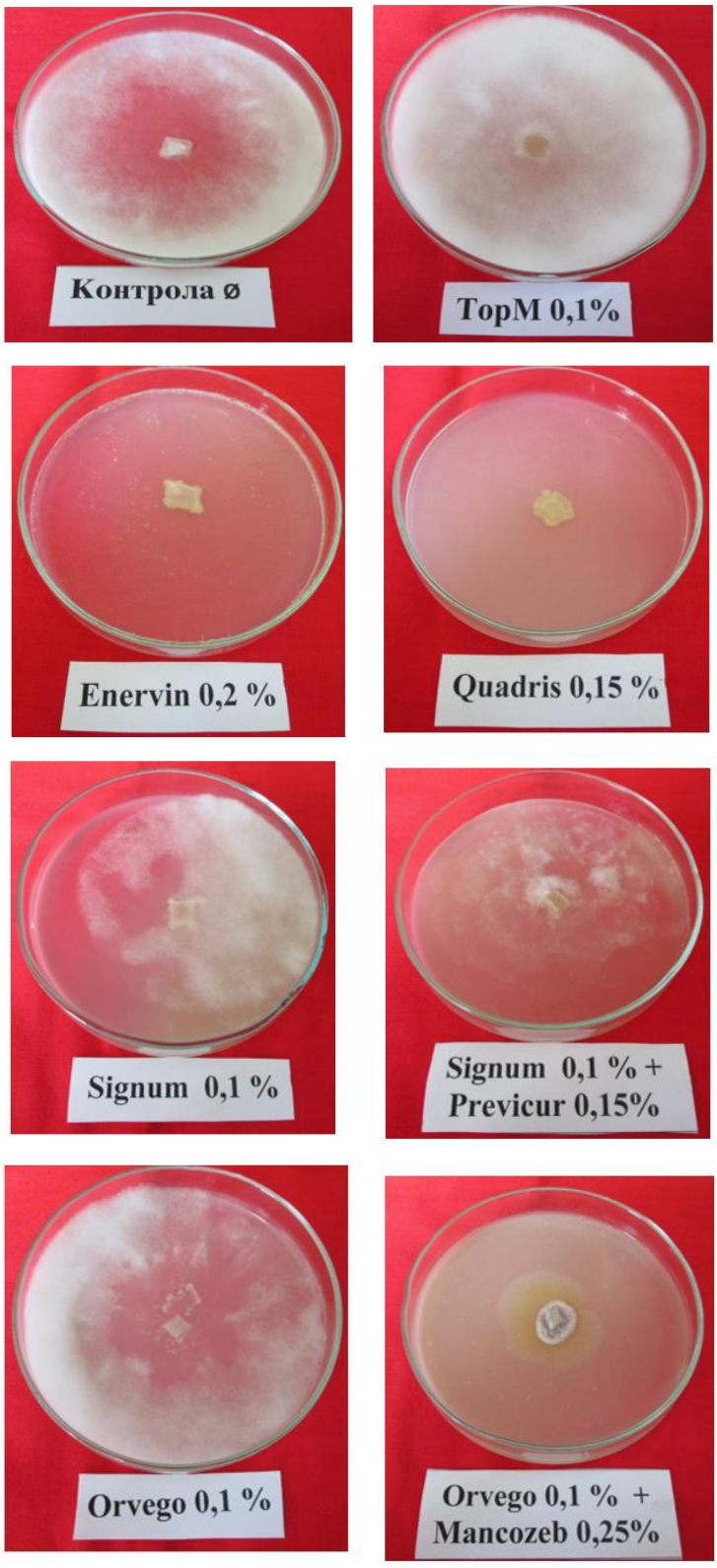


Photo1-8. *Development of Pythium debarianum colony in the variamts (on the seventh day)*

4. CONCLUSIONS

• The conducted researches contribute to increasing the number of active ingredients for the suppression of the pathogenic fungus P. *debarianum* in the Republic of Macedonia and provide an opportunity for practical application.

• The tested fungicides showed a very large difference in effectiveness in controlling the pathogenic fungus *P. debarianum.*

• The fungicide Top M 70 % WP (0.1%) has no efficacy in controlling this pathogen.

• The standard preparation Previcur® N (0.15%) showed weaker results compared to some new or combinations of preparations.

• Signum 33WG 0.1% fungicide showed 87.27% efficacy. When combined with other preparations, the efficiency is lower.

• Orvego™ (0.1%) did not show the expected results. But when combined with the contact fungicides Manfil 80 WP 0.25% and Enervin WG 0.2%, the efficiency is 100%.

• Quadris 25 SC (0.1%) has 100% efficiency.

• The contact fungicide Enervin WG 0.2% achieved the best results in this trial. Its efficiency is 100%.

• Fungicides i.e. active substances that achieved an excellent efficiency in contol of the pathogenic fungus *P. debarianum* have a good perspective in the protection against the damping off disease caused by this pathogen.

• Combinations of fungicides which have given excellent results also offer a good opportunity for their application in practice.

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