In vitro Evaluation of the Effectiveness of Some New Fungicides in the Control of *Rhizoctonia solani* in Tobacco Seedlings

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Abstract— *The damping off disease of tobacco seedlings caused by the pathogen Rhizoctonia solani causes a huge damage. The limited numbers of products are used in protection from the disease, but lately new fungicides appeared on the market.*

The aim of these investigations was to determine the effectiveness of new fungicides in control of this pathogen, compared with commonly used. The tests were carried out in conditions in vitro, with 8 fungicides. Two fungicides were evaluated in 2 or 3 concentrations.

All tested fungicides showed extremely high effectiveness in the control of this pathogenic fungus –the percentage of inhibition of radial growth ranges from 80, 45 to 100%.

The preparate Orvego (300 g / l ametoctradin + 225 g / l dimetomorph) is exception –it showed 48.05%.

The best effectiveness showed contact fungicides Manfil 80WP (800 g/kg mancozeb) and Enervin WG (120 g/kg ametotradin +440 g/kg metiram) as well as systemic Signum 33 WG (267 g/kg boscalid +67g/kg pyraclostrobin) and Quadris 25 SC (250 g/l azoxystrobin)with100%inhibition of pathogen's development.

Fungicides with such high effectiveness in control of R.solani will ensure their use in protection of tobacco seedling from the damping off disease.

Keywords— active ingredient, disease, pathogen, radial development, reduction.

I. INTRODUCTION

Healthy and high-quality tobacco seedling is the basis for successful tobacco production and for obtaining good yield and quality. But, production of such seedling must be protected to a maximum in order to prevent diseases or to achieve minimum intensity of attack and minimum damage.

The most destructive disease that attacks tobacco seedling is the damping off disease. It can occur in all stages of seedling growth, starting from seed germination to rapid growth. Sometimes it appears immediately after germination and prevents further development of the seedling.

The causing agent of the disease in most cases (besides *Pythyum debarianum*) is the pathogenic fungus *Rhizoctonia solani (Kühn*). The symptoms of disease begin with occurrence of small watery spot at the base of the stem which begins to spread. The tissue becomes necrotic and the movement of water and nutrients is disabled. The plant falls down on the surface of the seedbed, as if it was cut. Infection spreads rapidly, infected patches in the seedbed increase and if protective measures are not taken, they occupy larger area. The damage is evident, especially in the early stages of growth.

The soil pathogen is difficult to control because *Rhizoctonia solani* is common in various soil types and has many hosts. The fungus causes serious damage to many important field and horticultural crops (Nunez, 2005). Also, the application of chemicals is more difficult because the number of preparations for protection of tobacco seedling is limited and inappropriate fungicides are used in practice, which are not efficient in the control of the causing agent.

Several researchers studied the effectiveness of various active ingredients for control of this pathogen. According to Csinos and Stephenson (1998), the active ingredients flutolanil, iprodione, fluazinam and tebuconazole were effective in preventing the infection in tobacco seedbeds.

Fungicides that contain PCNB (Terrachlor), Iprodione (Rovral) or Azzoystrobin (Quadris) are effective against *R. solani* (Koenning, 2007), as well as Difenokonazol (Dahmen and Staub, 1992). According to Boogert and Luttikholt (2004), pencycuron and flutalonil are specific active ingredients for *Rhizoctonia* control. Azoxystrobin and trifoxystrobin are also reported as highly effective.

Mocioni et al. (2003) reported that tebuconazole applied in 3 different formulations is also highly effective against *R. solani*. This was also confirmed by Breneman and Murphy (1991).

Singh et al. (2015), in field trials as well as *in vitro*, obtained the best results with carbendazym 25% + flusilazole 12.5% SE (NS) compared to the other combination (trade name) of these active ingredients, as well as kresoxime-methyl 44.3% and validamycine 3% L.

Among 14 fungicides with different active ingredients tested in vitro against *R. solani*, Metalaxyl, Mancozeb, Tricyclazole, Thiophanate methyl and Carbendizim + Mancozeb (all at concentration of 0.1%) were most effective in suppressing the development of the fungus (Srinivas et al., 2014). Sriraj et al. (2014) tested several fungicides with active ingredients azoxystrobin, tebuconazole, tridemorph, fosetyl-aluminum, trifloxystrobin, fenamidone + mancozeb, carbedazim, tebuconazole + trifloxystrobin and reportedthat inhibition percentage increased with higher concentrations, but it differed depending on the active ingredient.

Anjana and Kumar (2008) studied the efficacy of six different active ingredients - carbendazim, mancozeb, hexaconazole, propiconazole, copper oxychloryde and thiram forcontrol of *R. solani* under *in vitro* conditions in soybean. They found that all fungicides show variable inhibition of radial growth of the pathogen depending on the concentration, but propiconazole, hexaconazole and carbendazim reached the highest efficacy at all concentrations.

Rajput et al. (2016) tested 16 fungicides (contact, systemic and combinations)- and one antibiotic and found high inhibition effect in fungicides of each group and combination, including the antibiotic Validamycin.

Rajendra prasad et al. (2017) conducted extensive *in vitro* studies on the efficacy of various fungicides and combinations, as well as biocontrol agents - fungal and bacterial, from which useful recommendations can be obtained for both chemical and biological method of pathogen control.

Recently, many new fungicides and active ingredients for control of the pathogen have appeared on the market. The most popular against *R. solani* in soybean are the strobilurins pyraclostrobin and trifloxystrobin (Mueller, 2014), but Signum fungicide - combination of pyraclostrobin and boscalid - is more and more recommended. It has preventive and systemic activity for use against certain diseasesin various crops (BASF, 2008). Such a combination provides a wide range of activities and reduces the risk of resistance to various pathogens (Hauke et al., 2004). The lack of data on the use of Signum and its application in tobacco urged us to include this fungicide in our trials.

Azoxystrobin (Quadris) is a fungicide with a wide range of protection from plant diseases (Syngenta, 2006). It is also recommended in the control of target spot on tobacco caused by *R. solani* AG-3 (Bertrand, 2012). According to LaMondia (2012), the treatment of tobacco seedling with Quadris should be a key measure for control of *R. solani*. Despite the promising results for the last three active ingredients, there are scant data on the inhibitory effect of mycelial growth of R. solani.

The aim of this study was to investigate the efficacy of several fungicides for control of pathogenic fungus *R. solani* under in vitro conditions. The obtained results will make a great contribution to expand the list of fungicides for the control of this pathogen. Application of highly efficient fungicide against the cause of the disease will provide high protection on one hand and lower costs of the protection products, on the other.

II. MATERIALS AND METHODS

A pure fungus culture was isolated from an infected tobacco seedling by a standard laboratory method, on potato-dextrose agar (PDA) as nutrient media.

A ten-day old culture was used for *in vitro* studies on the effects of fungicides. Investigations were carried out with 8 fungicides. In the selection of chemicals, other contact and systemic fungicides were included besides the standard Top M (Table 1), at usually recommended concentrations. In order to estimate their effect at lower or higher concentration, two fungicides were applied in 2 and 3 concentrations.

For this investigation, the nutrient media was the same as that used for isolation - PDA. After preparation, sterilization and cooling to a moderate temperature, fungicides were added with gentle stirring. The medium with the fungicide was poured in Petri dish (110 mm diam.) and then fragment of pure culture of the fungus (5 mm) was seeded on the fungicide medium. Petri dishes seeded with nutrient medium not containing fungicide were used as a check.

Petri dishes were incubated for 10 days at a temperature of 25^0C and each day diameter of the colony was measured on the opposite sides of the Petri dish. The results for each replication and also their mean values are presented in a table. The results on fungicides effectiveness in the control of *R. solani* are calculated from the mean value of the colony diameter, according to the formula of Singh et al. (2015):

$$I = \frac{C - T}{C} x100$$

where I = percentage inhibition of mycelia growth

C = average colony / fungal growth diameter of the check

T = average colony / fungal growth diameter of the treatment

TABLE 1
INVESTIGATED FUNGICIDES

Fungicide	Active ingredient	Concentration (%)
Top-M 70%WP	70% thiophanate –methyl	0,1%
Manfil 80WP	800 g/kg mancozeb	0,25%
Enervin WG	120 g/kg ametoctradin + 440 g/kg metiram	0,2%
Orvego™	300 g/l ametoctradin + 225 g/l dimetomorph	0,1%
Signum 33 WG	267 g/kg boscalid + 67g/kg pyraclostrobin	0,1% and 0,15%
Quadris 25 SC	250 g/l azoxystrobin	0,1% , 0,15% and 0,2%
Folicur EW-250	tebuconazole 250 g/*l*	0,1%
Agrizole 250 EC	difenoconazole 250 g/*l*	0,05%

III. RESULTS AND DISCUSSION

The cause of the damping off disease is the pathogenic fungus *Thanatephorus cucumeris* (Frank), which belongs to the class *Hymenomycetes,* order *Tulasnellales*, family *Ceratobasidiaceae*, and genus *Thanatephorus.*

Its anamorphic form is known as *Rhizoctonia solani* (Kühn) (Ivanović, 1992).

The colony of *Rhizoctonia solani* whitish, creamy to light ochre in color. Each cell is binuclear to polynuclear. Mycelium is branching at right angle. It does not form organs for vegetative propagation. It forms basidiospores only in natural conditions, at high temperature and humidity (Ceresini, 1999).

InPDA medium and at25^0C it develops rapidly, radially, forming concentric circles and filling the whole Petri dish. The pure culture is the check in these trials (Fig. 1).

FIG. 1. R. *SOLANI*- PURE CULTURE

The fungus develops smoothly from the first day only in the check, and at the end of the test it reached a diameter of 107.20 mm (Table 2). In the presence of fungicide, however, its development starts earliest in the variant with Orvego - on the 5th day, in Manfil 80 WP on the 7th day and in Agrizole 250-EC on the 8th day.

The final value of the colony diameter in variant with fungicide ranges from 8 mm in Quadris 25 SC - 0.1% (which is actually an attempt for colony development in the last days of incubation - Fig 5) to 41.00 mm in Orvego. This is not the case with the other two concentrations of this preparation. In the other fungicides treatments no fungus development was observed during the investigation.

In the second replication, development of the fungus begins earlier in all variants and reaches a larger diameter (Table 3). The check variant fills the Petri dish completely, while in the presence of the Orvego fungicide more than half (i.e. 62.40 mm) of the total diameter is occupied. In this replication too, with the lowest concentration of Quadris 25 SC - 0.1% the fungus reached a diameter of 20.80mm. Similar diameters were obtained with both the standard fungicide Top M and the Agrizole 250-EC (Fig 3 and 4). No fungus development was observed at concentrations of 0.15 and 0.2% Quadris 25 SC.

The fungus has not developed in the presence of contact fungicides Manfil 80 WP and Enervin WG (Fig 2 and 3). The same is true for the systemic fungicides Folicur EW -250 (Fig 4), Quadris 25 SC (0.15 and 0.2%) and Signum 33WG (in both concentrations) (Fig 6).

TABLE 2
DEVELOPMENT OF *RHIZOCTONIA SOLANI* COLONY – 1ST REPLICATION

Day / Variant (fungicide)	1	2	3	4	5	6	7	8	9	10
	Diameter in mm									
Top M 0,1%	-	-	-	-	-	-	8,32	14,40	15,40	15,40
Manfil 80 WP0,25%	-	-	-	-	-	-	-	-	-	-
Manfil 80 WP0,25%	-	-	-	-	-	-	-	-	-	-
Enervin WG0,2%	-	-	-	-	-	-	-	-	-	-
Orvego 0,1%	-	-	-	-	9,60	17,00	28,73	34,00	37,00	41,00
Signum 33WG 0,1%	-	-	-	-	-	-	-	-	-	-
Signum 33WG 0,15%	-	-	-	-	-	-	-	-	-	-
Quadris 25 SC 0,1%	-	-	-	-	-	-	-	+	++	8,00
Quadris 25 SC 0,15%	-	-	-	-	-	-	-	-	-	-
Quadris 25 SC 0,2%	-	-	-	-	-	-	-	-	-	-
Folicur EW -250 01%	-	-	-	-	-	-	-	-	-	-
Agrizole 250-EC 0,05%	-	-	-	-	-	-	-	7,60	10,60	11,60
Check ø	8,00	24,00	46,00	68,80	80,10	91,60	102,00	104,00	106,80	107,20

FIG. 2. DEVELOPMENT OF *R. SOLANI* ON MEDIUM WITH A.I. MANCOZEB (FUNGICIDE MANFIL 80WP)

FIG. 3. THE EFFECT OF ENERVIN WG ON DEVELOPMENT OF *R. SOLANI*

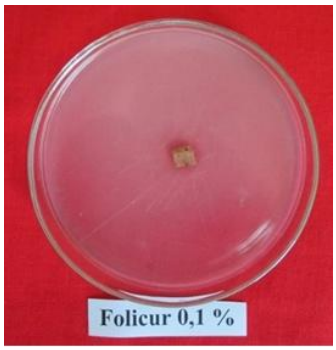


FIG. 4. THE EFFECT OF FOLICUR EW-250 ON DEVELOPMENT OF *R. SOLANI*

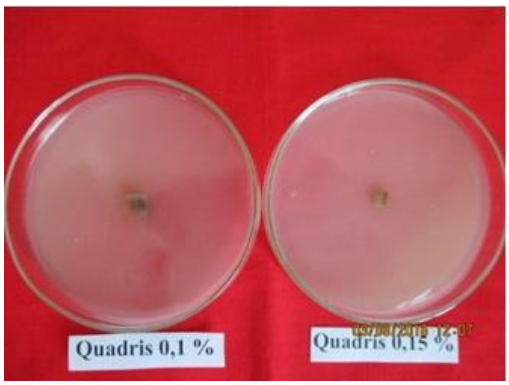


FIG. 5. THE EFFECT OF QUADRIS 25 SC ON THE DEVELOPMENT OF *R. SOLANI*.

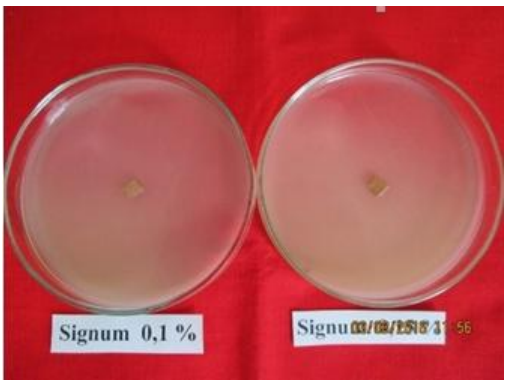


FIG. 6. THE EFFECT OF SIGNUM 33 WG ON THE DEVELOPMENT OF *R. SOLANI*

TABLE 3
DEVELOPMENT OF *RHIZOCTONIA SOLANI* COLONY – 2ND REPLICATION

day / Variant (fungicide)	1	2	3	4	5	6	7	8	9	10
	Diameter in mm									
Top M 0,1%	-	13,40	15,80	16,68	18,13	19,00	19,60	20,20	20,20	21,00
Manfil 80 WP 0,25%	-	-	-	-	-	-	-	-	-	-
Enervin WG 0,2%	-	-	-	-	-	-	-	-	-	-
Orvego 0,1%	-	-	8,40	16,80	22,61	30,80	37,00	48,30	54,40	62,40
Signum 33WG 0,1%	-	-	-	-	-	-	-	-	-	-
Signum 33WG 0,15%	-	-	-	-	-	-	-	-	-	-
Quadris 25 SC 0,1%	-	-	-	-	-	+	12,00	16,40	19,40	20,80
Quadris 25 SC 0,15%	-	-	-	-	-	-	-	-	-	-
Quadris 25 SC 0,2%	-	-	-	-	-	-	-	-	-	-
Folicur EW -250 01%	-	-	-	-	-	-	-	-	-	-
Agrizole 250-EC 0,05%	-	-	-	-	-	7,60	15,20	17,60	22,40	26,00
Check ø	10,80	26,40	47,00	62,90	74,80	92,00	102,60	105,30	110,00	110,00

In the third replication, the situation with pathogen development was the same as in the previous two cases. In the presence of fungicide, it develops rapidly with Orvego and Top M (on the 3rd and 4thday), but with application of Top M the lowest diameter was obtained among all tested fungicides. Also, the values obtained with Agrizole 250 EC and Quadris 25 SC - 0.1% was triple less than with Orvego.

FIG. 7. THE EFFECT OF ORVEGO™ ON THE DEVELOPMENT OF *R. SOLANI*

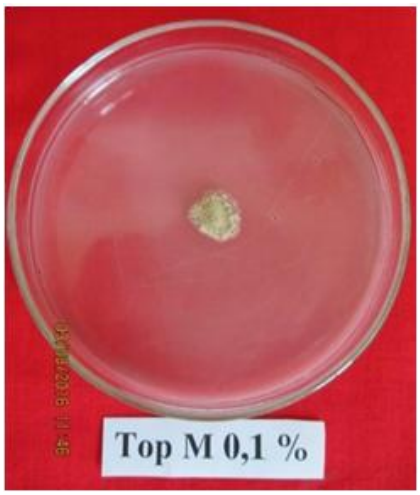


FIG. 8. THE EFFECT OF TOP-M 70%WP ON THE DEVELOPMENT OF *R. SOLANI*

FIG. 9. THE EFFECT OF AGRIZOLE 250 EC ON THE DEVELOPMENT OF *R. SOLANI*

FIG. 10. THE EFFECT OF VARIOUS FUNGICIDES

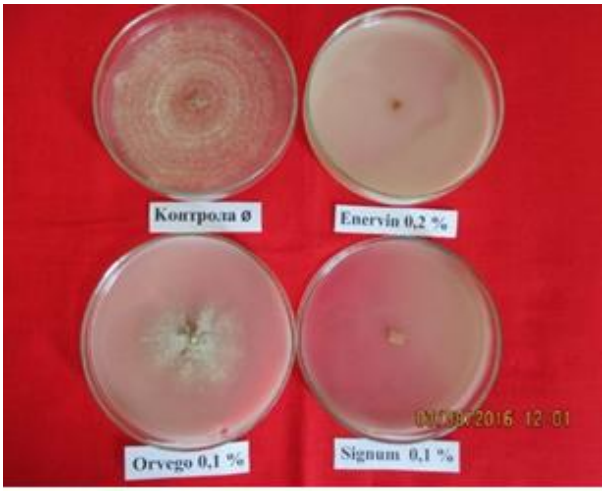


FIG.11. DIFFERENCES IN COLONY DEVELOPMENT WITH VARIOUS FUNGICIDES

TABLE 4
DEVELOPMENT OF *RHIZOCTONIA SOLANI* COLONY – 3RD REPLICATION

day / Variant (fungicide)	1	2	3	4	5	6	7	8	9	10
	Diameter in mm									
Top M 0,1%	-	-	+	8,20	8,43	8,80	9,00	9,20	9,80	14,00
Manfil 80 WP 0,25%	-	-	-	-	-	-	-	-	-	-
Enervin WG 0,2%	-	-	-	-	-	-	-	-	-	-
Orvego 0,1%	-	-	15,80	20,00	29,61	35,80	41,80	51,40	58,30	62,40
Signum 33WG 0,1%	-	-	-	-	-	-	-	-	-	-
Signum 33WG 0,15%	-	-	-	-	-	-	-	-	-	-
Quadris 25 SC 0,1%	-	-	-	-	-	-	-	9,00	15,40	21,40
Quadris 25 SC 0,15%	-	-	-	-	-	-	-	-	-	-
Quadris 25 SC 0,2%	-	-	-	-	-	-	-	-	-	-
Folicur EW -250 01%	-	-	-	-	-	-	-	-	-	-
Agrizole 250-EC 0,05%	-	-	-	-	-	8,60	11,20	16,80	20,20	24,80
Check ø	6,20	9,60	20,40	32,80	49,80	63,70	72,00	86,40	99,60	102,00

TABLE 5
DEVELOPMENT OF THE COLONY OF *RHIZOCTONIA SOLANI* – AVERAGE VALUE

day / Variant (fungicide)	1	2	3	4	5	6	7	8	9	10
	Diameter in mm									
Top M 0,1%	-	+	++	8,29	8,85	9,27	12,31	14,60	15,13	16,80
Manfil 80 WP 0,25%	-	-	-	-	-	-	-	-	-	-
Enervin WG 0,2%	-	-	-	-	-	-	-	-	-	-
Orvego 0,1%	-	-	8,06	12,27	20,61	27,87	35,84	44,63	49,90	55,27
Signum 33WG 0,1%	-	-	-	-	-	-	-	-	-	-
Signum 33WG 0,15%	-	-	-	-	-	-	-	-	-	-
Quadris 25 SC 0,1%	-	-	-	-	-	-	+	9,13	13,07	16,73
Quadris 25 SC 0,15%	-	-	-	-	-	-	-	-	-	-
Quadris 25 SC 0,2%	-	-	-	-	-	-	-	-	-	-
Folicur EW -250 01%	-	-	-	-	-	-	-	-	-	-
Agrizole 250-EC 0,05%	-	-	-	-	-	+	8,80	14,00	17,73	20,80
Check ø	8,33	20,00	38,00	54,83	68,23	82,43	92,20	98,57	105,47	106,40

The mean value for pathogen development in all investigated variants (Table 5) shows that the fungicide Orvego™ has the poorest effect, i.e. the fungus reached the highest colony diameter - 55.27 mm (Figure 7). At the end of the investigations, the fungus reached the poorest radial development (colony diameter - 16.80mm) with the fungicide Top M (0.1%) (Figure 8), followed by Agrizole 250 EC (0.05%) - 20.80mm (Figure 9). With Quadris 25 SC, only at concentration of 0.1%, poor development was observed - 16.73mm (Figures 5, 10).

The effectiveness of some fungicides on colony development is presented in Figures 10 and 11

Table 6 presents data oncolonies diameter (mean value of the replications) on the last day of investigation and the calculated effectiveness of the fungicides.

TABLE 6
IN VITRO EVALUATION OF THE EFFECTIVENESS OF FUNGICIDES IN CONTROL OF *RHIZOCTONIA SOLANI*

Variant (fungicide)	Diameter of colony (mm)	Effectiveness of fungicide
Top M 0,1%	16,80	84,21
Manfil 80 WP 0,25%	-	100
Enervin WG 0,2%	-	100
Orvego 0,1%	55,27	48,05
Signum 33WG 0,1%	-	100
Signum 33WG 0,15%	-	100
Quadris 25 SC 0,1%	16,73	84,27
Quadris 25 SC 0,15%	-	100
Quadris 25 SC 0,2%	-	100
Folicur EW -250 01%	-	100
Agrizole 250-EC 0,05%	20,8	80,45
Check ø	106,40	-

The most pronounced colony development was recorded in variants treated with Orvego 0.1%. Subsequently, this is the lowest ranked fungicide, with the lowest effectiveness in pathogen control - 48.05%. Such results can be explained by the mode of activity and the target class of pathogenic fungi. Dimethomorph has a strongly expressed activity to disable the development of cell walls, resulting in a death of fungus cells at all stages of development (Zenith Crop Science, 2017). Ametoctradin is active ingredient with strong inhibitory effect on mitochondrial respiration in complex III (cytohrome bc1) of the class of Oomycetes (Minnesota Department of Agriculture, 2012), but not of the class to which the investigated pathogen belongs.

The treatment with Agrizole 250 EC (0.05%), according to the final estimates, reached 80.45% effectiveness, although some fungus development was noticed in the last days of investigation. These results, although somewhat lower, are consistent with those of Rajput et al. (2016), in which difenoconazole at concentration of 0.05% achieved 94.1% effectiveness and average value of 95.2%.

Although in treatment with the standard fungicide Top M (0.1%) the pathogen develops continuously, its final diameter is only 16.80 mm, which denotes an effectiveness of 84.21%. The effectiveness of the active ingredient Thiophanate methyl in the control of *R. solani* under *in vitro* conditions was also studied by Srinivas et al. (2014). Rajput et al. (2016) reported that Thiophanate methyl at a concentration of 0.1% achieved 100% effectiveness and the mean value of tested concentrations was 95.2%.

No development of *R. solani* on nutrient medium i.e. 100% effectiveness was achieved with the fungicides Manfil 80 WP 0.25% and Enervin WG 0.2%. Our results on the effectiveness of Mancozeb are contrary with those of Anjana and Kumar (2008). It should be mentioned, however, that the concentration they used was 2.5 times lower than ours, which certainly affected the obtained results. Still, even with such concentration, they achieved 67.67% effectiveness.

Results of our investigations are almost identical to those of Rajendra prasad et al. (2017), in which Mancozeb (0.3%) showed 98.11% effectiveness in the control of *R. Solani*. At twice lower concentration, its effectiveness was 88.47%.

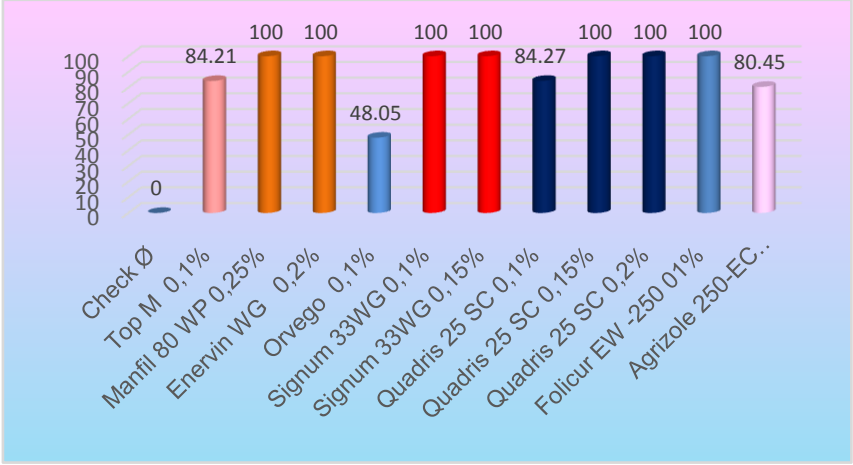
The effectiveness of contact fungicides in the control of *R. solani* was confirmed by Rajput et al. (2016). They reported excellent results with Enervin WG 0.2%. Rajendra prasad et al. (2017) reported 93.3% effectiveness of the active ingredient metiram (in combination with pyraclostrobin) at recommended concentration of 0.2%.

No fungus development was observed in treatment with systemic fungicides Signum 33WG 0.1% and 0.15%, Quadris 25 SC 0.15% and 0.2% and Folicur EW -250 01%. They all reached 100% effectiveness in control of this pathogenic fungus. According to Hauke et al. (2004), Signum is a combination of two active ingredients with two different biochemical modes of activity in cellular respiration of the fungus. This is the reason for such a high inhibitory effect on pathogen development.

The high effectiveness of Quadris 25 SC confirm the research of Sriraj et al. (2014), who reported 100% inhibition of mycelial growth of this pathogen even at much lower concentrations (50 ppm) of azoxystrobin.

Breneman and Murphy (1991) reported that tebuconazole inhibited the mycelial growth much more than pentachloronitrobenzene (PCNB), which is reflected in more than 100 times lower ED50 value compared to the standard. The 100% effectiveness of the active ingredient tebuconazole in inhibition of the radial development obtained in our investigation confirms the above results.

The effectiveness of fungicides in the control of pathogenic fungus *R. solani* obtained in this investigation is presented in Graph 1.



GRAPH 1. THE EFFECTIVENESS OF FUNGICIDES ON *RHIZOCTONIA SOLANI*

IV. CONCLUSION

- The tested fungicides showed good effectiveness in the control of pathogenic fungus *R. solani*, ranging from 80.45% to 100%, with the exception of OrvegoTM (300 g/l ametoctradin +225 g / l dimetomorph) at a concentration of 0.1% , which showed very poor results (48.05% effectiveness).
- The lowest effectiveness of the fungicides tested was about 80%, which is a good result in the control of the pathogen and which justifies their use of these preparations against damping off disease.
- The effectiveness of the most frequently used fungicide Top M 70% WP (0.1%) is 84.21% and that of Agrizole 250 EC (difenoconazole 250g/l) 0.05% is 80.45%.
- Quadris 25 SC showed a lower inhibitory effect only at a concentration of 0.1% and achieved 84.27% effectiveness.
- Contact fungicides Manfil 80 WP 0.25% and Enervin WG 0.2% inhibited the fungus development with 100% effectiveness.
- 100% effectiveness in the control of this pathogenic fungus was also achieved with systemic fungicides Signum 33WG 0.1% and 0.15%, Quadris 25 SC 0.15% and 0.2% and Folicur EW-250 01%.
- Fungicides, i.e. active ingredients that achieved 100% effectiveness make contribution to the list of active ingredients for control of pathogenic fungus *R. Solani* in the Republic of Macedonia.

- Fungicides that achieved 100% effectiveness should be included in protection of tobacco seedling from damping off disease caused by this pathogen.

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