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THE EFFECTIVENESS OF FUNGICIDES IN THE CONTROL OF ALTERNARIA ALTERNATA DEPENDING ON THEIR IMPACT ON PATHOGEN BIOLOGY

Biljana Gveroska

University "St. Kliment Ohridski" - Bitola
Scientific Tobacco Institute-Prilep, Kicevski pat bb, 7500 Prilep
e-mail: gveroska@t-home.mk

ABSTRACT

Investigations were carried out to study the effectiveness of some fungicides in the control of pathogenic fungus Alternaria alternata in laboratory conditions. Their impact on conidial germination and growth in solid and in liquid media was studied in this paper.

The highest reducing effect on conidia germination was obtained by application of fungicides Folicur EW-250, Score 250-EC and Acrobat MZ – with only 2.97%, 11.60% and 15.19% germinated conidia. The poorest results were obtained with Dithane M-45 and Antracol WP-70.

Fungal growth in solid and liquid media was prevented by application of Folicur EW-250. With application of Score 250 EC it reached only 8.30 mm and with Baycor WP 25 -14.70 mm

However, high yield of dry biomass was obtained with Baycor WP 25 (140.00 mg), while with Dithane M-45 it was 20.00 mg.

According to the investigations, the effectiveness of fungicides varies in different environmental conditions, depending on its impact on the biology of the pathogen.

Fungicides Folicur EW-250 (0.1%), Score 250-EC (0.05%) and Acrobat MZ (0.25%) showed the best results in control of the pathogen.

Keywords: Alternaria alternata, fungicide, inhibition, biology

ЕФИКАНОСТ НА ФУНГИЦИДИТЕ ЗА СУЗБИВАЊЕ НА *ALTERNARIA ALTERNATA* ВО ЗАВИСНОСТ ОД НИВНОТО ВЛИЈАНИЕ ВРЗ БИОЛОГИЈАТА НА ПАТОГЕНОТ

Истражувањата беа извршени со цел да се испита ефикасноста на фунгицидите за сузбивање на патогената габа Alternaria alternata во лабораториски услови. Беше испитувано влијанието врз 'ртењето на конидиите како и развојот врз цврста и течна подлога.

Најголем редуцирачки ефект врз 'ртењето на конидиите покажаа фунгицидите Folicur EW-250, Score 250-EC и Acrobat MZ со само 2.97%, 11.60 % и 15.19% из'ртени конидии. Најслаби резултати имаа Dithane M-45 и Antracol WP-70.

Габата не се развиваше на цврста и течна подлога со препаратот Folicur EW-250. Кај Score 250-EC достигна само 8.30 mm а кај Baycor WP 25 -14.70 mm

Но, приносот на сувата биомаса кај Baycor WP 25 е голем (140.00 mg), а кај Dithane M-45 тој изнесува 20.00 mg.

Според истражувањата, ефикасноста на фунгицидите варира во разни еколошки услови, во зависност од влијанието врз биологијата на патогенот.

Фунгицидите Folicur EW-250 (0.1%), Score 250-EC (0.05%) и Acrobat MZ (0.25%) покажаа најдобри резултати во сузбивањето на патогенот.

Клучни зборови: Alternaria alternata, фунгицид, инхибиција, биологија.

INTRODUCTION

Brown spot is one of the fungal diseases in the Republic of Macedonia which appears each growing season in all tobacco types. Its presence in the oriental tobacco is particularly harmful because it occurs at the end of the season, on the top leaves which have the best quality. According to Rotem (1994), in diseases caused by fungi of the genus Alternaria, the yield may be reduced through: a) reduction of photosynthetic activity and leaf production, without direct infection, b) direct attack by the pathogen, c) reduction of tobacco quality, d) combination of all the activities.

The causing agent of brown spot disease on tobacco - Alternaria alternata reduces the total economic effect of tobacco production through a combination of all mechanisms that affect tobacco yield and quality. It also has an impact on the smoking properties of tobacco raw. As severity of the disease increases, the "good" taste is reduced in favor of the "bad" taste (Lucas, 1975, loc cit Rotem, 1994). Also, the creation of AT toxin by this fungus in tobacco and its persistence in the raw material affects the smokers' health.

The disease is becoming even more important because the intensive way of tobacco production disables the proper performance of agro-technical operations, i.e. the application of preventive measures for protection. The outbreak of the disease is especially affected by the undue harvest of tobacco leaves and excessive irrigation. Because of the above reasons, the application of chemical protection is unavoidable. Therefore, continuous investigations are made on the effectiveness of some active ingredients and fungicides in the control of this pathogenic fungus.

In investigations of Shaeik and Taha (1984), Dithane M 45 applied in concentration of 0.25% appeared to be effective in control of the disease. Nagarajan (2000) recommended the application of Mancozeb, Difenoconazol and Propiconazol for this purpose.

In investigations of Colturato et al. (2009) on the effect of some active ingredients and their combinations on disease intensity and yield increase, all fungicides included in investigation showed a positive effect on reducing the intensity, but trifloxystrobin + propiconazole appeared to be the most effective in increasing the yield.

Fungicides effectiveness in control of A. alternata has been investigated in vitro. Bozukov (2002) studied the biological effect of 15 fungicides in order to determine the most appropriate preparations for tobacco protection from the brown spot disease.

Survilience and Dambrauskiene (2006) reported that active ingredients showed significant inhibitory effect on the

development of several species of Alternaria. Issiakhem and Bouznad (2010) found such an effect of Difenoconazole and Chlorthalonil on A. solani and A. alternata. Inhibitory effect on the growth of A. alternata was found in five contact and five systemic fungicides (Chandhary and Patel, 2010).

Mahatabi et al. (2001), in their investigations in vitro and in vivo, found several active ingredients that can be applied in protection of tobacco from brown spot disease. Sometimes, however, certain ambiguities arise between the results obtained in laboratory and in field conditions (Zellner et al., 2011).

Research of biological effect of fungicides in the control of certain pathogen begins with investigation of their impact on its biology as a basic principle for effective protection. Therefore, the aim of this study was to examine the impact of several fungicides on the biology of this pathogenic fungus.

MATERIAL AND METHOD

The selection of fungicides for this investigation was made in accordance with our intention to include higher number of active ingredients known for their protection of tobacco, as well as other substances which proved to be efficient in the control of Alternaria. Some ambiguities in fungicides investigations in field conditions also imposed the need for this research.

The list of systemic and contact fungicides applied on tobacco in recommended concentrations is presented in Table 1.

Table 1. Investigated fungicides

Fungicide	Active ingredient	a.i. content	Concentration, %
Dithane M-45 (WP)	Mancozeb	80 %	0.25
Acrobat MZ (WP)	Dimetomorf +Mancozeb	(9 + 60)%	0.25
Ridomil MZ 72 (WP)	Metalaksil +Mancozeb	(8 +64) %	0.3
Antracol WP –70	Propineb	70 %	0.2
Score 250 -EC	Difenokonazol	250 g/l	0.05
Euparen multi WP 50	Tolyfluanid	50%	0.25
Baycor WP 25	Bitertranol	25 %	0.25
Folicur EW- 250	Tebuconazol	250g/dm^3	0.1
Poliram DF (WG)	Metiram	80 %	0.2

Fungicides effect on conidia germination

Conidia from infected leaves, previously kept in Petri dish on moistened filter paper for 24 hours were used as material for investigation. Water solutions of the fungicides were prepared in appropriate concentrations. The percentage of germinated conidia was estimated by the method of Ko et al. (1975).

Conidia were transferred from lesions with sterile bacteriological needle into the drops of prepared fungicide solutions. They were placed in Van-Tieghem chambers and incubated in a thermostat at 28^0C. The percentage of germinated conidia was estimated after 4-5 hours. The moment when the length of initial hypha was equal to the width was taken as a criterion for germinated conidium.

Five microscopic preparations of each chemical were observed, with 10 visual fields. Conidia suspension in a drop of distilled water was used as a check. The trial was replicated three times and the results present the mean value of the investigations.

The percentage of inhibition of conidia germination was estimated by the Ogbebor and Adekunle formula (2005).

Fungicides effect on pathogen development in solid nutrient media with addition of fungicide

Pure culture of *A. alternata* was used in the trial, obtained by conidia transfer from fresh infected plant material on potato dextrose agar.

The same media was used for monitoring the fungus development in a presence of fungicide. It was prepared in a usual way and fungicides were added after sterilization and cooling to $40\text{-}50^0$C. The media with appropriate concentration of the fungicide was spread on 110 mm Petri dishes.

3-5 mm^2 fragments of the pure culture (7-10 days old, incubated at 25^oC) were transferred in media with fungicide. The Petri dishes were incubated at the same temperature. The trial was set up in three replications; with every replication five Petri dishes were sown for each variant. The development of the fungus was followed and colony diameter was measured each day, in two opposite directions at right angle. The results given for the 3rd, 5th, 10th and 15th day represent the mean value of the replications. A colony grown in solid media without addition of fungicide was used as a check.

Percentage of reduction of pathogen development in solid and liquid media was estimated according to the formula of Shovan et al. (2008).

Fungicides effect on pathogen development on liquid nutrient media with addition of fungicide

For these investigations, liquid potato dextrose agar was prepared and fungicides were added after sterilization and cooling. 20ml of the media with fungicide was dispensed in Petri dishes and then sowing was performed with fragments of the mycelium using a sterile needle. Five Petri dishes were sown for each variant, and the trial was set up in three replications. In this case, too, the media without fungicide was used as a check. Incubation lasted 15 days at 25^0 C, and it

was followed by separation (filtration) of the fungus from the liquid media and drying of the filtrate at 25-28^{0}C up to 3 consecutive measurements with constant value, according to the method of Sarić (1986). The mean value of all replications shows the yield of dry biomass in mg.

RESULTS AND DISCUSSION

All investigated fungicides showed a reducing effect on conidial germination of *A. alternata*. Some fungicides make their impact by inhibiting the development of germ tube, appressorium formation and mycelial growth (Obanor et al., 2005).

The lowest percentage of germinated conidia was determined with Folicur EW-250, only 2.97%, but also in fungicides Score 250 EC and Acrobat MZ - 11.60% and 15:19, respectively. The highest percentage of germinated conidia was determined with fungicides Dithane M-45 and Antracol WP-70 (Table 2).

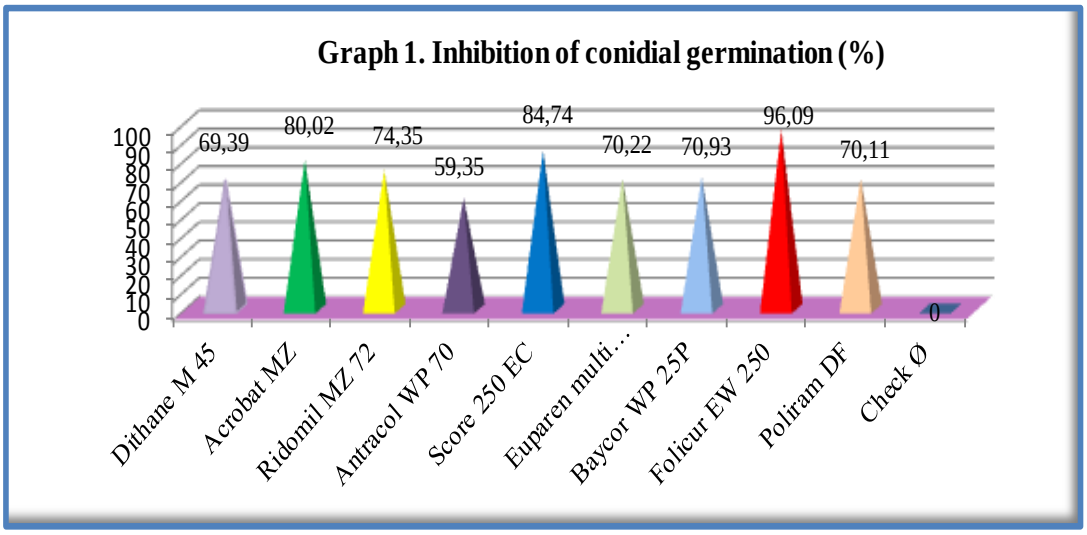
According to the above data, the lowest percentage of inhibition was obtained with the fungicide Antracol WP-70 - 59.35%. The highest percentage of inhibition of conidia germination was obtained with the fungicide Folicur EW-250 - 96.09%, but also with Score 250-EC and Acrobat MZ (Figure 1).

Table 2. The effect of fungicides on conidia germination

Fungicide	Dithane M-45	Acrobat MZ	Ridomil MZ 72	Antracol WP -70	Score 250 EC	Euparen multi WP 50	Baycor WP25	Folicur EW 250	Poliram DF	Check Ø
Concentration, %	0.25	0.25	0.3	0.2	0.05	0.25	0.25	0.1	0.2	-
Germinated conidia, %	23.27	15.19	19.50	**3.90**	11.60	22.64	22.10	**2.97**	22.72	76.02
Inhibition of conidia germination, %	69.39	80.02	74.35	**59.35**	84.74	70.22	70.93	**96.09**	70.11	-

Similar results were reported by Issiakhem and Bouznad (2010), who also noted that Difenoconazole is effective in conidial germination of *A. alternata* and *A. solani*.

Fungicides differentiated their effectiveness in specified concentration. Those with higher inhibitory activity on germination, even at lower concentrations when the effect on spore germination is slightly reduced, still have an influence which can be seen through the fact that the length of germ tube is significantly reduced (Obanor et al., 2005).



With respect to the development of pathogenic fungus in solid nutrient media with the addition of fungicide, it can be noted that in the initial days of incubation no colony growth was observed with Score 250 EC, as well as with Folicur EW 250, and with the latter it does not appear throughout the entire incubation period (Table 3).

The products start to differentiate with respect to their effect about the fifth day, but in Antracol WP-70, the good development that was observed at the beginning continues up to the end, when the colony size is the largest - 70.90mm. In Ridomil MZ 72, Dithane M-45 and Poliram DF, the colony diameters range from 41.73 to 54.98 mm. These results are similar to those reported by Zellner et al. (2011), in which Poliram WG appeared to have almost no effect on *A. alternata*.

According Chandhary and Patel (2010), Mancozeb shows good results in the inhibition of fungus growth, which is not the case in this type of investigations.

The largest diameter of the colony was observed in the media with Antracol WP-70, i.e. this chemical showed the lowest percentage of inhibition of colony growth - 33.92% (Table 3, Figure 2).

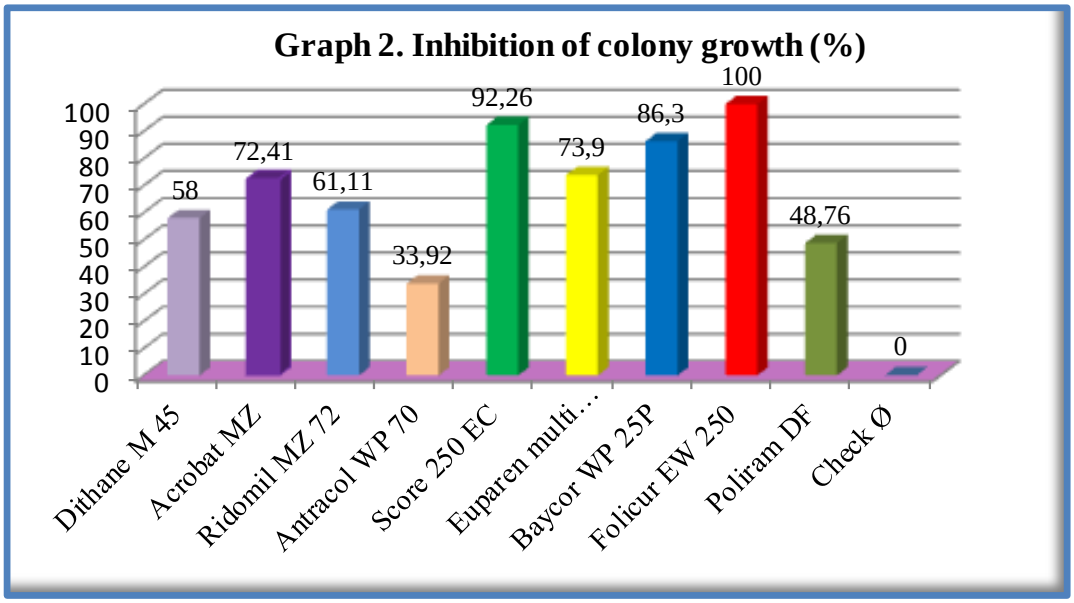
Table 3. The effect of fungicides on colony growth in solid nutrient media

Fungicide	Concentration%	Diameter of the colony (mm)				Inhibition of colony growth %
		3rd day	5th day	10 th day	15 thday	
Dithane M-45	0.25	6.16	8.33	26.50	45.07	58.00
Acrobat MZ	0.25	6.00	8.40	17.70	29.60	72.41
Ridomil MZ 72	0.3	6.00	8.75	24.15	41.73	61.11
Antracol WP-70	0.2	8.50	21.50	42.40	**70.90**	**33.92**
Score 250 EC	0.05	0.00	6.00	6.80	**8.30**	**92.26**
Euparen multi WP 50	0,25	9.30	12.50	21.40	28.00	73.90
BaycorWP25	0.25	6.00	6.00	10.40	14.70	86.30
Folicur EW 250	0.1	0.00	0.00	0.00	**0.00**	**100.00**
Poliram DF	0.2	6.60	10.60	32.23	54.98	48.76
Check Ø	-	27.20	44.02	85.59	107.30	-

Ridomil MZ 72 did not show a significant percentage of inhibition of colony growth (Graph 2, Figure 1). In investigations of Batta (2001), the formulation metalaxyl + mancozeb showed significant curative effect in the disease caused by *A. alternata*. This chemical is also used in the control of other pathogens in tobacco. However, *A. alternata* is less invasive parasite in the complex of fungi that attack tobacco and it is even regarded as secondary parasite (Rotem, 1994). Therefore, certain chemicals may affect other pathogens, which causes an error in determination of the intensity of attack of *A. alternata*. Such explanations are also given by Zellner et al. (2011), for differences in fungicides effect appearing in field and laboratory investigations.

Of the investigated chemicals, only Folicur EW 250 showed 100% inhibition of colony growth (Graph 2, Figure 1 and 2). These results are in accordance with investigations on biological effect of 15 fungicides made by Bozukov (2002), according to which the fungicide effect was recognized in only five chemicals, and the others showed fungistatic and inhibitory effect.

In vitro investigations made by Survilience and Dambrauskiene (2006) revealed that all investigated fungicides have shown satisfactory inhibition of *Alternaria spp*. colonies, reducing them in average 94 to 25% after the 21st day. Folicur EW 250 (Tebuconazol), however, differed by its inhibitory activity which persisted at 71% to 62% even after the 21st day.



Score 250 EC have also achieved high percentage in inhibition of colony growth - 92.26% (Graph 2, Figure 2). These results are in agreement with those of Issiakhem and Bouznad (2010), according to which Difenoconazole is efficient in inhibition of colonial growth of *A. solani* and *A. alternata* and has better effectiveness than the active substance Chlorthalonil.

According to Dahmen and Staub (1992), Difenoconazole shows high effectiveness against the the Ascomycetes, Basidiomycetes and Deuteromycetes classes of fungi.

According to Batta (2001), Difenoconazole together with Cyprodinil + Flodioxonil are the most efficient preventive fungicides in the control of this pathogen in fruits.

In liquid nutrient media with Folicur EW 250, no yield of dry biomass was observed, confirming 100% inhibitory effect of the fungicide in these trials, too. With Acrobat MZ and Score 250 EC it was only 10 mg, or both have shown

95.24% inhibition. These three products showed the best results in the inhibition of dry biomass yield (Table 4, Graph 3).

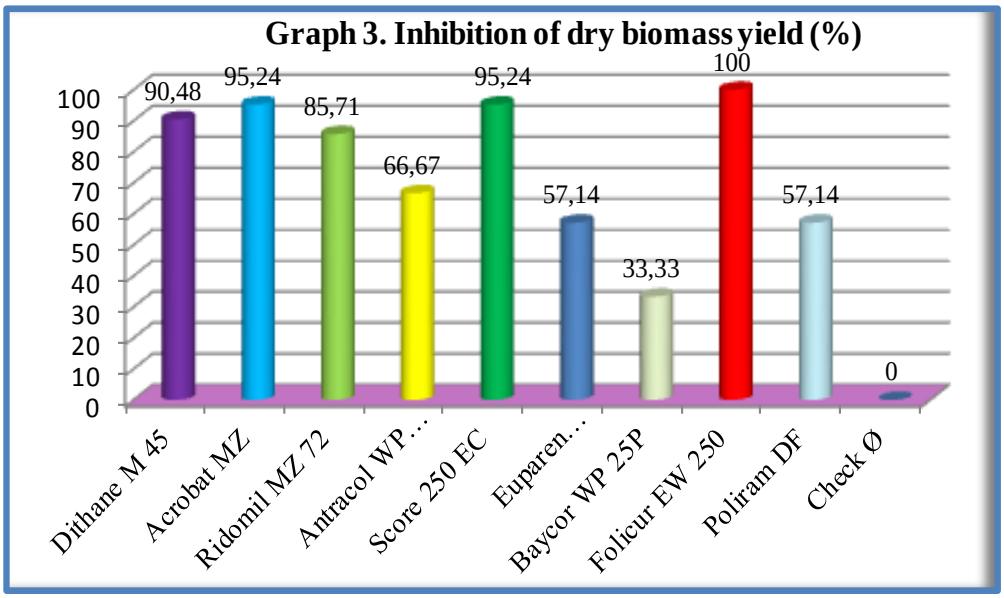
Baycor WP25 showed high efficiency in inhibition of pathogen growth. However, the inhibition of dry biomass yield in liquid nutrient media was only 33.33%, which is the lowest value (Table 3 and 4, Graph 3). Despite the modest results of this product (Table 2) and the small diameter, the colony had a specific look, i.e. it was very thick, in white color, with pronounced aerial growth (Figure 2).

The lowest effectiveness in these trials, besiden Baycor WP 25, were observed with Antracol WP 70, Euparen multi WP 50 and Poliram DF (Graph 3).

With Dithane M-45 fungicide, which showed low inhibitory effect on colony growth, the dry biomass yield was 20mg. It means that in this case the fungicide showed good inhibitory effect, i.e. 90.48% inhibition of dry biomass yield. Similar to this, Chandhary and Patel (2010) reported that percentage of inhibition with mancozeb was 97.80%.

Table 4. The effect of fungicides on dry biomass yield

Fungicide	Dithane M-45	Acrobat MZ	Ridomil MZ 72	Antracol WP -70	Score 250-EC	Euparen multi WP 50	Baycor WP 25	Folicur EW-250	Poliram DF	Check Ø
Concentration, %	0.25	0.25	0.3	0.2	0.05	0.25	0.25	0.1	0.2	
Dry biomass, mg	20.00	10.00	30.00	70.00	10.00	90.00	140.00	**0.00**	**90,00**	210.00
Inhibition of dry biomass yield %	90.48	95.24	85.71	66.67	95.24	57.14	33.33	**100.00**	**57.14**	



During investigations, some of the fungicides showed better results in inhibition of colony growth than in conidial germination. The ineffectiveness of certain preparation in protection from conidial germination is due to the fact that it causes higher inhibitory effect on the development of hyphae than on spore germination (Obanor et al., 2005).

Some fungicides (e.g. Acrobat MZ and Dithane M-45), had higher inhibitory impact in liquid than in solid media. Our

results are in accordance with findings of Ko et al. (1976), according to which the agar affects the diffusion of fungicides, i.e. it binds to a fungicide, thereby reducing its activity.

Among the investigated fungicides, the following were distinguished by their inhibitory effect on the biology of *A.alternata*: Folicur EW-250 (0.1%) (Tebuconazol), Score 250-EC (0.05%) (Difenoconazole) and Acrobat MZ (0.25%) (Dimetomorf +Mancozeb).

In vitro and in vivo investigations of several active ingredients have shown that Propiconazol, Tebuconazol and Mancozeb in concentration 0.1, 0.1 and 0.2%, respectively, can provide good protection from brown spot disease on tobacco (Mahtabi et al., 2001).

Figure 1. The effect of some fungicides on fungus growth in solid nutrient media

Figure 2. The effect of: (down, left to right) Baycor WP 25, Score 250-EC and Folicur EW 250

CONCLUSIONS

All fungicides investigated had an inhibitory effect on pathogenic fungus A. alternata.

• The best results in the inhibition of conidial germination were obtained with fungicide Folicur EW-250 (0.1%). Good results were also obtained with Score 250-EC (0.05%) and Acrobat MZ (0.25%).

• Folicur EW-250 (0.1%) showed the highest inhibitory effect on pathogen growth. The fungus does not grow in the presence of this product, neither in solid nor in liquid media.

• The lowest yield of dry biomass was obtained with the fungicide Acrobat MZ (0.25%), which showed also good results in solid media.

• Fungicides Baycor WP 25 (0.25%) and Dithane M-45 (0.25%) showed contradictory results in investigations in solid and in liquid media.

• Fuingicides Antracol WP-70 (0.2%) and Poliram EW-250 (0.2%) showed the poorest inhibitory results.
• Fungicides Folicur EW-250 (0.1%), Score 250-EC (0.05%) and Acrobat MZ (0.25%) had the best results in these trials. They can be used in protection of tobacco from brown disease.

*S*According to the results, the effectiveness of fungicides to a particular pathogen varies in different environmental conditions, depending on their impact on biological properties.

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