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THE EFFECT OF ABIOTIC FACTORS ON THE *IN VITRO* DEVELOPMENT OF *MONILINIA FRUCTIGENA* FUNGUS (ADERH. & RUHL.) HONEY, ISOLATED FROM THE APPLE

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Abstract

Monilinia fructigena (Aderh. & Ruhl.) Honey, the pathogenic agent of the apple rot, is common in apple orchards every year, causing important harvest losses that could continue in storage. In vitro studies have been carried on the impact of temperature, light and culture medium on the development of Monilinia fructigena fungus, isolated from apple fruits from 'Topaz', 'Florina', 'Goldrush', 'Idared' and 'Generos'. The minimum pathogen development threshold was +4°C and the optimal range was between 24-28°C. At higher 36°C temperatures, development has significantly decreased. The pathogenic agent developed well in light and continuous darkness (24 h). Fungal isolates preferred oat and potato-dextrose-agar culture media to form well-developed colonies.

Key words*: fungus, varieties, apple, abiotic factors.*

INTRODUCTION

Monilinia fructigena (Aderh. & Ruhl.) is the pathogenic agent of the apple brown rot or Monilinia (Agrios, 2005). This is one of the most spread apple diseases which could lead to significant losses in the orchards and storehouses (Byrde & Willettts, 1977). Generally, apple varieties are affected by *Monilinia fructigena* fungus and its severity is associated to the environment, the presence of damages caused by biotic and abiotic factors (Holb, 2004), the age of wounds affecting the fungus incubation period (X. M. Xu & J. D. Robinson, 2000).

Controlling the disease by applying treatments and cultural hygiene of orchards contributes to the significant reduction of brown rot (Helmann, 1998; Chițulescu & Cristea, 2017). Research has also been carried out on the control of *Monilinia* spp. pathogens by using the antifungal effect of some plant bio – complexes (Cristea et al., 2017; Cristea et al., 2017). Knowing the biological parameters of pathogens under laboratory conditions may support studies on the evolution of pathogen attack in field conditions, attack prediction and treatment provenience.

MATERIALS AND METHODS

The biological material used consisted of apple fruits from storage conditions attacked by *Monilinia fructigena*. Harvested isolates were used from five apple varieties: 'Topaz', 'Florina', 'Goldrush', 'Idared' and 'Generos'. Isolation of the pathogen was carried out after storage sampling on the potato-dextrose-agar culture medium, divided into 90 mm diameter Petri dishes. Isolates were identified based on spore morphology then pricked out and stored in pure cultures at the thermostat, at 22°C. Isolated cultures were used after a 15 days culture.

The effect of temperature, light and culture media on the development of the *Monilinia fructigena* pathogen was studied. Observations were made on the development of the fungus by measuring the average diameter of the colonies at 3, 6, 9 days after inoculation. Each variant was placed in three repetition ratios. Observations were made on the biological development thresholds of the fungus *in vitro*.

RESULTS AND DISCUSSIONS

Abiotic factors play an important role in the development of *Monilinia fructigena* fungus.

Laboratory studies were carried out on the effect of abiotic factors on the temperature, light, culture medium on the development of seed pathogens whose development continues in storage conditions, as well (Mardare et al., 2015; Dudoiu et al., 2015; Dudoiu et al., 2016). The data shows the fungus evolution at different temperature values (Table 1).

Table 1. Effect of temperature on the *in vitro* development of *Monilinia fructigena* fungus

Temp. °C	Colony diameter (mm)														
	3 days					6 days					9 days				
Isolated symbol M	TO	GE	GO	ID	FL	TO	GE	GO	ID	FL	TO	GE	GO	ID	FL
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	1	0	0	1	0	1	1	2	3	3	4
6	0	0	0	0	1	0	0	1	0	2	2	2	4	4	5
8	1	1	1	1	2	3	1	2	2	2	5	7	8	6	10
10	1	1	1	1	2	4	2	3	2	3	8	10	18	10	15
12	2	2	1	2	3	6	2	8	3	4	10	22	30	25	29
14	3	3	2	3	3	10	3	15	9	8	25	30	70	35	45
16	4	5	3	6	7	18	18	20	14	17	45	40	80	55	80
18	5	7	6	10	15	27	30	45	20	29	63	65	85	59	75
20	7	20	9	11	25	43	55	70	26	55	85	85	90	65	75
22	7	22	23	20	30	43	50	70	40	55	85	60	88	68	80
24	8	15	24	22	30	38	55	65	45	48	80	70	90	70	90
26	7	12	15	23	28	36	50	60	48	43	78	65	90	65	85
28	6	10	12	25	28	30	45	48	49	42	73	60	70	75	85
30	5	9	10	25	27	30	42	40	40	39	72	60	62	60	80
32	5	8	7	20	25	28	40	25	35	32	68	59	45	55	70
34	4	6	5	16	23	27	18	20	30	30	65	40	38	50	62
36	2	2	2	14	22	10	14	18	25	21	50	42	39	40	45
38	2	2	1	8	8	8	9	10	12	18	40	38	20	18	20
40	0	0	1	2	5	2	3	5	7	6	18	15	10	12	15

TO - 'Topaz', GE - 'Generos', GO - 'Goldrush', ID - 'Idared', FL - 'Florina'

Analyzing the data on temperature effect on *Monilinia fructigena* fungus development, it was found that the fungus developed at 4^0C after 9 days of incubation, forming 4 mm diameter colonies in the case of 'Florina' isolate.

The optimal developmental threshold of the fungus ranged between 24-28^{0}C (Figures 1 and 2).

The fungus formed sporulated colonies with an an average diameter ranging from 70-90 mm to the studied isolates.

The development rate of the colony was slower after 28^0C, and after 34^0C the fungus was no longer frustrated.

At temperatures higher than 36^0C the fungal development was significantly reduced (Table 1).

Figure 1. *Monilinia fructigena* (MGO) - 24^{0}C - PDA (3 days)

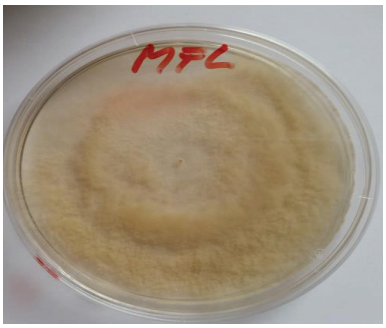

Figure 2. *Monilinia fructigena* (MFL) - 24^{0}C - PDA (9 days)

In order to analyze the effect of the culture medium on the development of *Monilinia fructigena* colonies isolated from the studied varieties, studies on the culture natural media were considered: prune agar, rice extract agar (REA), oat flakes agar, semi-dextrose agar (PDA) semi-synthetic media, 2% agarized water medium.

The data show that the fungus preferred the PDA semi-synthetic medium and the natural medium with oatmeal flakes (Table 2). *Monilinia fructigena* fungus grew best on PDA and oatmeal media (Figure 3).

Monilinia fructigena ('Florina') recorded a rapid growth rate reaching 90 mm after 9 days of observation (Figure 4).

Table 2. The effect of culture medium on the development of *Monilinia fructigena*

Monilinia fructigena		Diameter (mm)/days														
Varieties	Isolated medium	PDA			Prune-agar			REA			Oat flakes agar			2% agarized water		
		3	6	9	3	6	9	3	6	9	3	6	9	3	6	9
'Topaz'	MTO	7	40	85	6	28	40	0	0	0	20	60	80	6	7	9
'Florina'	MFL	25	70	90	12	40	65	20	45	70	18	75	90	16	60	75
'Goldrush'	MGO	9	55	75	0	0	0	0	25	40	18	65	83	0	0	0
'Idared'	MID	11	26	65	8	16	30	10	42	70	10	50	85	8	32	65
'Generos'	MGE	20	55	85	0	0	0	10	47	63	10	65	80	6	44	70

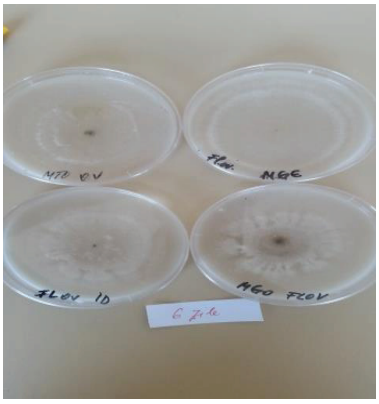

Figure 3. *Monilinia fructigena* - oatmeal flakes medium (6 days)

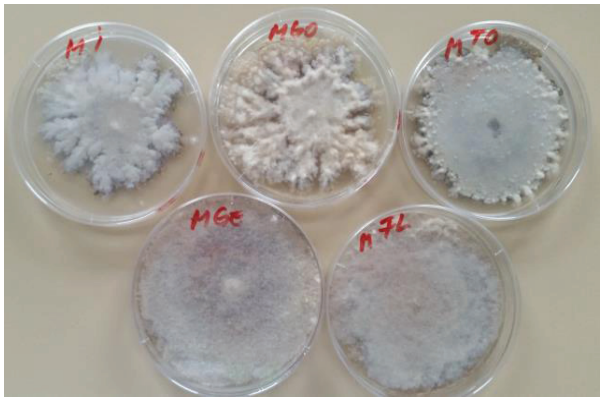

Figure 4. *Monilinia fructigena* - PDA medium (9 days)

Effect of light on the fungus development has also been studied in the following variances: continuous light (24 h), continuous dark (24 h), light/dark alternation (12 h/12 h and 16 h light/8 h dark) (Table 3).

The data exhibited *Monilinia fructigena* fungus preference to continuous light and dark whereupon the highest values of the colony diameter and abundant sporulated were registered (Table 3) (Figures 5 and 6).

Also, for the alternant variance 12 h light/12 h dark, colonies developed between 75 mm in diameter at MFL and 90 mm in MGO. *Monilinia fructigena*, isolated from the 'Idared' variety, developed more slowly, forming colonies with a diameter of 60-65 mm in the complete light and dark variances, as well as the light/dark alternation.

In 16 hours of light/darkness alternation, *Monilinia fructigena* fungus colonies developed more slowly, reaching 78 mm in diameter in the case of the 'Florina' isolate.

Table 3. Effect of light on *Monilinia fructigena* development

Monilinia fructigena		Diameter (mm)/days - continuous light 24 h		
Varieties	Isolated symbol	3 days	6 days	9 days
'Topaz'	MTO	30	55	85
'Florina'	MFL	45	60	90
Goldrusk	MGO	50	70	90
'Idared'	MID	35	40	65
'Generos'	MGE	37	65	90
Monilinia fructigena		Diameter (mm)/days - dark - 24 h		
Varieties	Isolated symbol	3 days	6 days	9 days
'Topaz'	MTO	32	60	80
'Florina'	MFL	50	90	90
Goldrusk	MGO	55	90	90
'Idared'	MID	15	30	60
'Generos'	MGE	35	89	90
Monilinia fructigena		Diameter (mm)/days - 12h light/12h dark		
Varieties	Isolated symbol	3 days	6 days	9 days
'Topaz'	MTO	7	40	85
'Florina'	MFL	25	55	75
Goldrusk	MGO	9	70	90
'Idared'	MID	11	26	65
'Generos'	MGE	20	55	85
Monilinia fructigena		Diameter (mm)/days - 16h light/8 h dark		
Varieties	Isolated symbol	3 days	6 days	9 days
'Topaz'	MTO	8	30	42
'Florina'	MFL	30	56	78
Goldrusk	MGO	15	35	60
'Idared'	MID	10	28	40
'Generos'	MGE	12	38	43

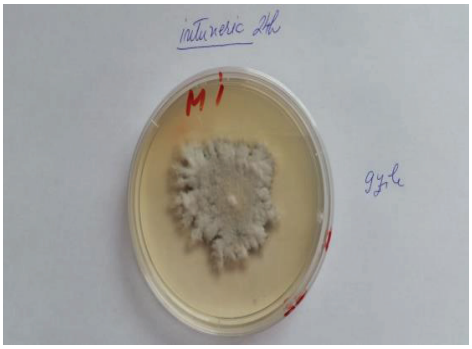

Figure 5. *Monilinia fructigena* (MID) - 24h dark (9 days)

Figure 6. *Monilinia fructigena* (MFL) - 24h dark (9 days)

CONCLUSIONS

The following conclusions can be drawn from the present research: *Monilinia fructigena* fungus (Aderh. & Ruhl.) Honey develops well at temperatures of 22-28^{0}C, with a minimum development threshold starting at 4^{0}C; the pathogen prefers semisynthetic and natural oat flakes media, forming well developed colonies in continuous total light and dark.

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