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DETERMINATION OF GROWTH AND SPORE-CYST DEVELOPMENT BY *ASCOSPHAERA APIS* ON DIFFERENT GROWTH MEDIA

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Summary

The spore-cyst fungus *Ascosphaera apis* has been spread all over the world causing the chalkbrood of honeybee larvae, therefore it is important to examine of the disease by application of culturing. Samples of mummies were collected and cultured on different semisynthetic agar media supplemented with different concentrations of monosaccharides and disaccharides. Using potato dextrose agar the growth of *A. apis* colonies proved to be faster than using traditional Sabouraud solid media. The growth and the spore-cyst development could be determined most characteristically when sugars were added in concentration of 7% (glucose) and 20% (sucrose).

Keywords: *Ascopshaera apis*, chalkbrood, spore-cyst development.

INTRODUCTION

Chalkbrood is a common and economically very harmful infectious disease of the honey bee (*Apis mellifera* L.). It is caused by the spore-cyst fungus *Ascosphaera apis*. This fungus produces closed dark spherical fruiting body and belongs to the class of *Plectomycetes* and to the order *Ascosphaerales* (Eriksson and Hawksworth, 1985).

MATERIALS AND METHODS

The laboratory diagnosis and the epidemiological examination of chalkbrood require microbiological culturing of the pathogen. Our purpose was to establish a more reliable and efficient microbiological culturing method than the current technics based on using traditional Sabouraud-glucose agar.

Samples of mummies were collected in Hungarian apiaries. *A. apis* spore suspensions were injected onto potato dextrose agar (PA) or Sabouraud solid medium (S) with different concentration of glucose, lactose, sucrose. They were held at 26°C. Streptomycin sulfate was added to each medium to inhibit bacterial growth. The growth of *A. apis* was determined in cm on the 6^{th}, 7^{th}, 8^{th}, 11^{th} day of incubation.

RESULTS

The *A. apis* growth was best facilitated by potato agar. On PA medium supplemented with 7% glucose a rate of growth x=6,9 cm (CV%=6,5) was determined on the 7th day (table 1.). The spore-cyst development was considerable on the 11th day (x=9,0 cm; CV%=0).

Table 1.

Ascosphaera apis growth (cm) on the 7 day
Wzrost *Ascosphaera apis* 7 dnia (cm)

Repeat Powtórzenie	SG 4%	SG 6%	SG 8%	SG 10%	SG 15%	SG 20%	PG 5%	PG 6%	PG 7%	PG 8%	PG 9%	PG 10%	PG 15%
1	0	0	3,7	5 x	5 x	3,3 x	4,6	5,7 xx	6,5 xx	7,2 xx	7,1 xx	4,6	5,8 xx
2	0	0	1,4	0	3,8 x	0	6,3 xx	5,2 xx	6,7 xx	6,2 xx	7,1 xx	6,6 xx	6,3 xx
3	0	3	0,6	0	4,1 x	0	6,7 xx	5,4 xx	7,2 xx	6,3 xx	6,6 xx	6,8 xx	6,8 xx
4	0	1,9	0	0	0	0	5,6 xx	6,8 xx	7,5 xx	4,7	6,8 xx	5,8 xx	6,4 xx
Min.	0	0	0	0	0	0	4,6	5,2	6,5	4,7	6,6	4,6	5,8
Max.	0	3	3,7	5	5	3,3	6,7	6,8	7,5	7,2	7,1	6,8	6,8
An average Średnio	0	1,225	1,425	1,25	3,225	0,825	5,8	5,775	6,975	6,1	6,9	5,95	6,325
Deviation Odchylenie	0	1,484	1,621	2,5	2,2096	1,65	0,92	0,714	0,457	1,036	0,245	0,998	0,411
CV%		121,1	113,8	200	68,516	200	15,86	12,36	6,557	16,98	3,55	16,78	6,503

The growth of *A. apis* in Sabouraud was first measurable only on the 11th day. It was best facilitated by 15% glucose (x=7,6 cm; CV%=35,9). However, the spore-cyst development was observable only by a few stains (table 2.).

Table 2.

Ascosphaera apis growth (cm) on the 11th day
Wzrost *Ascospaera apis* (cm) 11 dnia

Repeat Powtórzenie	SG 4%	SG 6%	SG 8%	SG 10%	SG 15%	SG 20%	PG 5%	PG 6%	PG 7%
1	8 x	8,7 x	9 x	9 x	9 x	9 x	9 xx	9 xx	9 xx
2	6,9	6,4	8,8 x	0	8,9 x	0	9 xx	9 xx	9 xx
3	5,2	6,1	7,6	0	9 x	0	9 xx	9 xx	9 xx
4	5,2	9	1,4	5,8	3,5	1,3	9 xx	9 xx	9 xx
Min.	5,2	6,1	1,4	0	3,5	0	9	9	9
Max.	8	9	9	9	9	9	9	9	9
An average Średnio	6,325	7,55	6,7	3,7	7,6	2,575	9	9	9
Deviation Odchylenie	1,374	1,511	3,587	4,4677	2,7337	4,327	0	0	0
CV%	21,73	20,01	53,54	120,75	35,97	168,04	0	0	0

S=Sabouraud; P=Potato - ziemniak; G=glucose - glukoza
x=weak spore-cyst development - słaby rozwój spor
xx=marked spore-cyst development - wyraźny rozwój spor

The fungus growth was x=8,8 cm (CV%=3,9) on PA with 20% lactose on the 11th day (table 3.).

Table 3.

Ascosphaera apis growth (cm) on the 11th day
Wzrost *Ascosphaera apis* (cm) 11 dnia

Repeat Powtórzenie	P 0%		PL 5%		PL 10%		PL 15%		PL 20%		PS 5%		PS 10%		PS 15%		PS 20%	
1	9	x	0		9	xx	8,9		9		9		9	xx	0		9	xx
2	5,4		8		5,1		8,1	xx	9		9	x	9		9		9	xx
3	0		7,3		0		0		9		7,6		9	xx	9	xx	9	
4	6,1		0		0		0		8,3		0		8,9	xx	9	xx	9	
Min.	0		0		0		0		8,3		0		8,9		0		9	
Max.	9		8		9		8,9		9		9		9		9		9	
An average Średnio	5,125		3,825		3,525		4,25		8,825		6,4		8,975		6,75		9	
Deviation Odchylenie	3,755		4,426		4,371		4,9183		0,35		4,3174		0,05		4,5		0	
CV%	73,27		115,7		124		115,73		3,966		67,459		0,557		66,67		0	

Its spore-cyst building was slender. On PA with 20% sucrose the growth was x=7,4 cm (CV%=13,9) on the 8th day. The spore-cyst development was clearly observable on the 11th day (table 4.).

Table 4.

Ascosphaera apis growth (cm) on the 8th day
Wzrost Ascosphaera apis (cm) 8 dnia

Repeat Powtórzenie	P 0%		PL 5%		PL 10%		PL 15%		PL 20%		PS 5%		PS 10%		PS 15%		PS 20%	
1	5,1		0		4,1		0,8		1,9		1,8		7,3	xx	0		9	xx
2	0		1		0		0		2,8		8	x	0,7		5,1		6,8	xx
3	0		0		0		0		2,8		0		7,8	xx	6,7	x	6,8	
4	0		0		0		0		0		0		8,8		7,4	xx	7,3	
Min.	0		0		0		0		0		0		0,7		0		6,8	
Max.	5,1		1		4,1		0,8		2,8		8		8,8		7,4		9	
An average Średnio	1,275		0,25		1,025		0,2		1,875		2,45		6,15		4,8		7,475	
Deviation Odchylenie	2,55		0,5		2,05		0,4		1,32		3,7961		3,686		3,342		1,044	
CV%	200		200		200		200		70,402		154,94		59,94		69,62		13,96	

L=lactose - laktoza; S=sacharose - sacharoza; P=Potato - ziemniak
x=weak spore-cyst development - saby rozwój spor
xx=marked spore-cyst development - wyraźny rozwój spor

CONCLUSIONS

1. When applying Sabouraud agar for culturing of *A. apis* our data suggest higher concentration of glucose than the 4% used traditionally.
2. Furthermore, our data also point out that potato agar as culture medium allows a more reliable and efficient detection of *A. apis*.

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OKREŚLENIE WZROSTU I ROZWOJU SPOR *ASCOSPHAERA APIS* NA RÓŻNYCH PODŁOŻACH WZROSTU

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Streszczenie

Grzyb *Ascosphaera apis* jest powszechnie rozpowszechniony w świecie i wywołuje grzybicę wapienną czerwiu. Próbki mumii były grupowane oraz wysiewane na różnych półsyntetycznych podłożach agarowych z dodatkiem monocukrów i wielocukrów o różnej koncentracji. Używając agaru z dodatkiem dekstrozy ziemniaczanej stwierdzono szybszy wzrost kolonii *A. apis* w porównaniu do ich wzrostu na tradycyjnym podłożu Sabourauda. Wzrost i rozwój spor grzyba był najbardziej charakterystyczny, kiedy agar był wzbogacany 7% glukozą i 20% sacharozą.

Słowa kluczowe: *Ascopshaera apis*, grzybica wapienna, spory, rozwój.