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THE EFFICACY OF *BACILLUS SUBTILIS* (EHRENBERG) COHN AGAINST CHALKBROOD OF HONEYBEE

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Summary

Ascosphaera apis *(Maassen ex Claussen) Olive & Spiltoir, the causal agent of chalkbrood disease in honeybees, now spread to Southern part of Turkey. The possible control measure of disease was investigated by testing the culture filtrate of* Bacillus subtilis *(Ehrenberg) Cohn against* A. apis *in laboratory conditions. The efficacy of culture filtrate was compared to those of boric acid at 0.5% and cycloheximide at 0.0025%. There was a strong correlation between increasing dosage of culture filtrate of* B. subtilis *and an inhibition of mycelial growth of fungus. The culture filtrate of* B. subtilis *was as effective as boric acid at 0.5%. The efficacy of* Bacillus subtilis *culture filtrate should be tested in field conditions for future application.*

Key words: Bacillus subtilis, Ascosphaera apis, *chalkbrood, honeybee.*

Introduction

Ascosphaera apis (Maassen ex Claussen) Olive & Spiltoir is a fungal pathogen which causes chalkbrood disease in honeybees (*Apis mellifera*). Chalkbrood generally occur in the spring of the year, affecting larvae of honeybees, and fungus germinate in the larval gut transforming them into a white chalk like mummy (THOMAS and LUCE, 1972). It has been demonstrated that disease can be transmitted by transfer of queens, workers, or by feeding contaminated pollen to colonies (KOENIG et al., 1987). Since fungal spores can be remain infective for up to 15 years chalkbrood became an important disease of the honeybees.

In 1913, chalkbrood was first described from infected honeybees in Germany (CLAUSSEN, 1921). Since that time there have been several reports of chalkbrood from various species of solitary bees in Europe (MELVILLE and DADE, 1944; CLOUT, 1956) and in the United States (BAKER and TORCHIO, 1968). In 1988, chalkbrood was first reported in Northern Turkey, however, causal organism was not identified (TUTKUN, 1989).

Extensive screening of potential chemicals has been undertaken to identify materials which may be used to attract. *A apis* (STEPHEN et al., 1981). The use of heat sterilization or dipping of newly-emerged adults in sodium hypochloride (WATERS, 1980) or in a free iodine (STEPHEN, 1982) decontaminate the body surface but may result in severe bee losses. An alkylamine, N-N dimethydodecanamine (IPL-12, showed promise for effective control of chalkbrood (HERBERT et al., 1987). MENAPACE and HALE (1984) tested sorbic acid and griseofulvin for preventing chalkbrood infections. Unfortunately, neither sorbic acid nor griseofulvin controlled chalkbrood. LIU (1991) reported that Benlate and other immidazole compounds were effective at a very low concentration and were not toxic to honeybees. New alternative control technologies of chalkbrood disease that are perceived as safe by the people and have negligible risk to human

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health and the environment need to be developed.

The objectives of this study were to test the efficacy of culture filtrate of *B. subtilis* against *A. apis* isolated from diseased larvae of honeybee and to compare the efficacy of culture filtrate of *B. subtilis* to those of boric acid and cycloheximide against mycelial growth of *A. apis*.

Material and Methods

Ascosphaera apis was isolated from mumified larvae in Southwestern Turkey and identified earlier (BASIM and GÜREL, unpublished data). *Bacillus subtilis*, an effective antagonist *in vitro* (BASIM, 1990; BASIM et al., 1991; BASIM et al., 1998) was used against *A. apis*.

Bacillus subtilis cells were grown in medium containing 20 g of dextrose, 5 g of DL-glutamic acid, 1.02 g of $MgSO_4$, 10 g of K_2HPO_4, and 0.5 g of KCl. Approximately 10^6 cells/ml were added to 100 ml of the medium and incubated at 30°C for 72 h in continuous shaker. The suspension of cells was filtrated through 0.2 µm membrane filter. Different solutions of culture filtrate (1, 5, 10, 15, 20, 25 ml/30 ml) were added to PDA plates and pathogen was transferred to plates and incubated at 29°C. At twenty four hours intervals the growth of mycelium was measured. Each test was conducted in the presence of control (without any treatment), cycloheximide at 0.0025% and boric acid at 0.5%.

Results and discussion

In this study the efficacy of *Bacillus subtilis* against *Ascosphaera apis*, causal agent of chalkbrood disease was investigated. The culture filtrate of *Bacillus subtilis* at different dilutions were tested. Also, known effects of boric acid at 0.5% and cycloheximide at 0.0025% were compared to the effect of *B. subtilis* on the fungus (Table 1).

Table 1

Effects of culture filtrate of *Bacillus subtilis*, Boric acid (0.5%) and Cycloheximide (0.0025%) against *Ascosphaera apis*

Doses of culture filtrate (ml/30 ml)	Growth of mycelium (%) Time (day)						
	1	2	3	4	5	6	7
1	3.67	20.05	36.88	49.14	62.08	73.88	88.09
5	0.00	3.78	5.96	8.25	12.71	21.53	29.21
10	0.00	0.00	0.00	4.35	6.41	10.19	12.37
15	0.00	0.00	0.00	2.86	3.67	5.04	5.50
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boric acid (0.5%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cycloheximide (0.0025%)	0.00	0.00	8.59	23.71	35.85	48.57	63.57
Control	11.57	29.44	47.31	60.48	73.08	84.42	100.00

There is a significant correlation between declining growth of mycelium and increasing concentration of the culture filtrate during 7 days incubation (Table 1 and Figure 1-2). The mycelial growth on control plate was 100% at the end of 7 days. Mycelial growth of the pathogen was inhibited at the

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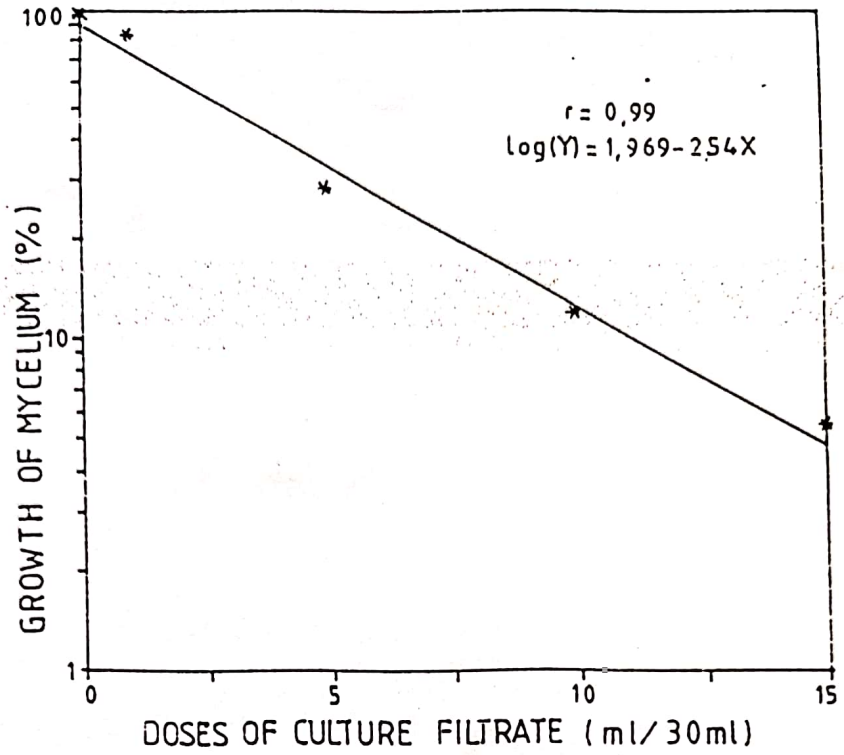


Fig. 1 – The linear relation between doses of culture filtrate and percent inhibition of mycelium of Ascosphaera apis

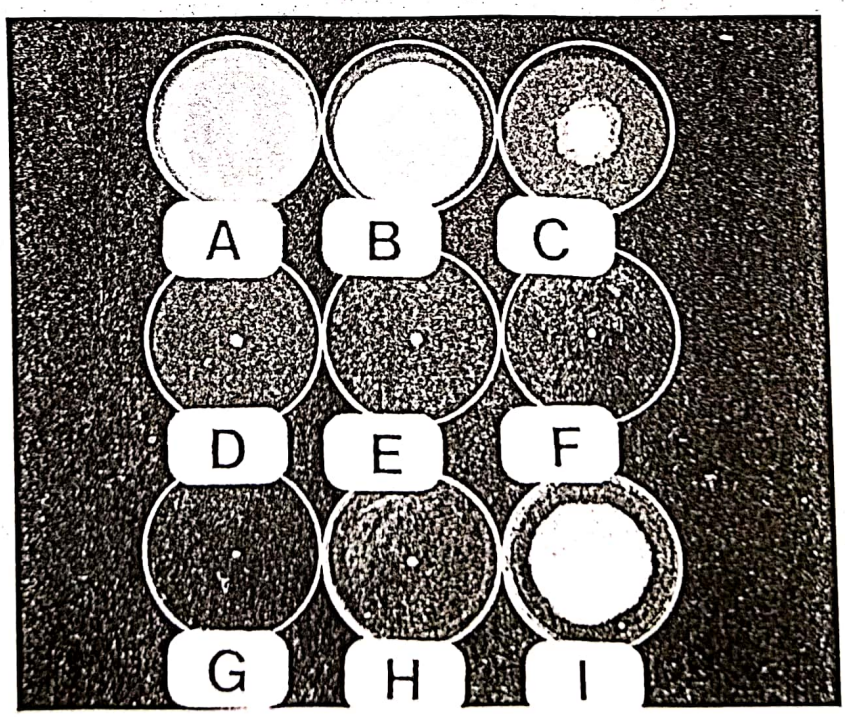


Fig. 2 – Effect of the culture filtrate of Bacillus subtilis *against* Ascosphaera apis
A. Control, B: 1/30 ml, C: 5/30 ml, D: 10/30 ml, E: 15/30 ml, F: 20/30 ml, G: 25/30 ml, H: Boric acid, I: Cycloheximide

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concentration of 5/30 ml of culture filtrate. The concentrations of 10/30 ml and 15/30 ml have greatly reduced the mycelial growth, but not completely at the end of the 7 days (Table 1 and Figure 1). The concentration of 23.2 ml/30 ml of the culture filtrate has been found to be an effective dosage of the culture filtrate that completely inhibited the mycelial growth even at the end of the 7 days (Figure 2). Boric acid was effective at 0.5%. However, cycloheximide at 0.0025% was not as effective as boric acid and the culture filtrate against mycelial growth of the pathogen fungus.

In this study the different concentrations of culture filtrates of *B. subtilis* have significantly reduced the mycelial growth of *A. apis*. The antagonistic feature of the culture filtrate obtained from *B. subtilis* may come from the antibiotics such as bacillicin and phengymycin produced by *B. subtilis* (PUSEY and WILSON, 1984).

For future application, the efficacy of *B. subtilis* should be tested in field conditions. This may help to consider *B. subtilis* as a one of the potential and saver alternative control measures of chalkbrood disease in future.

REFERENCES

- BASIM, H. (1990) – Bazi *Bacillus subtilis* izolatlarinin önemli bitki patojeni funguslara karşi *in vitro* koşullarda antagonistik etkilerinin araştirilmasi. Yüksek Lisans Tezi, Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Bölümü, 74 sayfa
- BASIM, H.; M.T. MOMOL; O. YEĞEN (1991) – *Bacillus subtilis* ile elma ve armutta hasat sonrasi görülen *Gloeosporium sp.* ve *Botrytis cinerea*' nin biyolojik kontrolü. VI. Türkiye Fitopatoloji Kongresi, 7-11 Ekim 1991, Izmir, Türkiye Fitopatoloji Derneği Yayinlari, **6**: 223-228
- BASIM, H.; E. HACIOĞLU; O. YEĞEN, (1998) – The potential efficiency of culture filtrate of *Bacillus subtilis* AB-27 against *Botrytis cinerae* and *Gloeosporium sp.* on Golden Delicious and Ankara pear. Turk. J. Phytopathology **27**: 27-37
- BAKER, G.M.; P.F. TORCHIO (1968) – New records of *Ascosphaera apis* from North America. Mycologia **60**: 189-190
- CLOUT, G.A. (1956) – Chalkbrood and hunchback flies. *Bee Craft* **38**: 135
- HERBERT, E.W.; D.J. CHITWOOD; H. SHIMANUKI (1987) – Chalkbrood research at Beltsville **127** (7): 488-491
- KOENIG, J.P.; G.M. BOUSH; Jr. E.H. ERICKSON (1987) – Isolation of the chalkbrood pathogen, *Ascosphaera apis*, from honey bee (*Apis mellifera*) surfaces, pollen loads, and a water source. *Apicultural Research* **127**: 581-583
- LIU, T.P. (1987) – Fine structure of the sporocysts of *Ascosphaera apis* during development as revealed by the scaning electron microscope. *Mycopathologia* **100**: 155-158
- MELVILLE, R.; H.A. DADE (1944) – Chalkbrood attacking a wild bee. *Nature* **153**: 112
- MENAPACE, D.M.; W.T. WILSON (1976) – The spread of chalkbrood in the North American honeybee, *Apis mellifera*. *Amer. Bee. Jour.* **116**: 570-573
- PUSEY, P.L.; C.L. WILSON (1984) – Postharvest biological control of stone fruit brown rot by *B. subtilis*. *Plant Dis.* **68**: 753-756
- STEPHEN, W.P.; J.D. VANDERBERG; B.L. FICHTER (1981) – Etiology and epizootiology of chalkbrood in the leafcutting bee, *Megachile rotundata* with notes on *Ascosphaera apis*. Station Bulletion of Agricultural Experiment Station Oregon State University, Corvalis **653**: 1-7
- STEPHEN, W.P. (1982) – Chalkbrood control in the leafcutting bee. Proceedings of First International Symposium Alfalfa Leafcutting bee Manage: 98-107
- THOMAS, G.M.; A. LUCE (1972) – An epizootic of chalkbrood, *Ascosphaera apis* (Maasen ex Claussen) Olive and Spiltoir, in honeybee, *Apis mellifera* L. in California. *Am. Bee. J.* **112**(3): 88-90
- TUTKUN, E. (1989) – Bal arisi larvalarinda ölüme neden olan kireç hastaliği ariciliğimizi tehdit ediyor. *Teknik Aricilik* 25-26: 10-13
- WATERS, N.D. (1980) – Basics of leafcutting bee management. Proceedings 11th A. Interstate Alfalfa Seed growers School, University of Idaho Extension Service Boise: 4-7

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