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Enilconazol tolerance of bee brood, adult bees and queens

Željka Matašin[1]*, and Ljerka Zeba[2]

[1]*Department for Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*State Veterinary Administration, Ministry of Agriculture and Forestry Republic of Croatia, Zagreb, Croatia*

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ABSTRACT

The incorrect or excessive use of antibiotics and of acaricides against bee diseases, as well as various stressors, including queen rearing, can increase the incidence of chalk-brood. A promising drug against this disease could be Enilconazol. Its influence on bees, bee brood and queens was therefore tested for the first time under laboratory and apiary conditions. Amounts of 200, 400, and 1000 mg of Enilconazol were dissolved in 96% ethyl alcohol, added to 50 ml of sugar solution (1:1, w/v) and fed to experimental groups. Bees accepted the experimental feeds well. Observation of bees and bee brood during 30 days in the laboratory glass hives with one frame (apisarium) showed no difference between treated and control groups fed with plain sugar solution. The influence of Enilconazol on quality of queens was examined in AZ type pavilion hives with several especially prepared and divided frames. It was established that during 25 days there was no difference between treated and control colonies in acceptance and vitality of queens and in development of bee brood

Key words: Enilconazol, chalk-brood, bee brood, queens

Introduction

Old textbooks on honeybee diseases (TOMAŠEC, 1955; BAILEY, 1963) and on beekeeping (GROUT, 1949, BELČIĆ et. al., 1968) denoted fungus diseases

* Contact address:
Dr. •eljka Matašin, Department for Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia. Phone: +385 1 2390 150; Fax: +385 1 2390 152; E-mail: sdjuro@vef.hr

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(chalk-brood and stone-brood) as "not serious", "rare" or "of little economic importance". Causative organisms of these diseases - *Ascosphaera apis* and *Aspergillus* sp. - are wide spread in nature and can be found even in healthy bee colonies. Only the weakness of larvae caused by poor nourishment, exposure to low temperatures in small colonies, or infection by other bee diseases encouraged fungal infections.

Subsequently, TABER et al. (1975) reported the spread of chalk-brood disease all over the U.S.A. and KORDOS (1977) found that it caused great damage in Hungary. In Croatia, the first case of chalk-brood was diagnosed in 1976 and of stone-brood in 1982 (Register of the Central Bee Disease Laboratory, Zagreb). ČANAĐIJA (1978) informed about problems with chalk-brood in his apiary, while SULIMANOVIĆ and MATAŠIN (1983) observed the spread of chalk- and the stone-brood. These developments led TABER et al. (1975), SULIMANOVIĆ (1982; 1986), HEATH (1985), BAILEY and BALL (1991), as well as SULIMANOVIĆ et al. (1995) to attribute the new epizootiological situation to the excessive use of antibiotics and/or of acaricides against Varroa disease. The young larvae are often sensitive to doses of acaricides that are harmless for adult bees because most of them have some insecticidal activity. Measurements of a detoxifying enzyme in bees and bee larvae by ZEBA (1994) proved the more frequent chalk-brood occurrence to be linked with improper and prolonged usage of acaricides containing fluvalinate as an active substance

Professional beekeeping requires the annual or biannual change of queens (SULIMANOVIĆ et. al., 1993). Recently, several beekeepers in Croatia became professional queen breeders. Queen rearing is very stressful for bee colonies and chalk-brood therefore often appears during this process. A drug for prevention of this disease in bee colonies and in mating nuclei would therefore be desirable. Enilconazol is a promising drug against chalk-brood but there are no data about its influence on the quality of queens. Experiments were therefore carried out in the laboratory and at the apiary to examine the influence of Enilconazol on the vitality and egg laying activity of queens as well as on the quality and behaviour of bees and bee brood.

Materials and methods

Enilconazol-sulphate used in experiments was a yellowish, partly cristallized powder. Each of the selected quantities (200, 400 or 1000 mg)

was dissolved in 2 ml of 96 % ethyl alcohol and added to sugar solution, prepared at a w/v ratio of 1:1 (1 kg of sugar + 1.000 ml of water).

The glass observation hives (apisariums) used for testing the influence of Enilconazol on bees and bee brood had one frame of freshly-built worker comb, populated with young worker bees and a young mated queen. Communication with the environment was provided through a plastic tube. Fifty ml of sugar solution with 0 (controls), 4, 8 or 20 mg of Enilconazol per ml was given to each of three bee colonies for each dose on day one of the experiment. Hives were observed daily for the presence of a queen and her egg laying activity, for the number and behaviour of worker bees as well as for the development of larvae and pupas. The experiment was terminated after 30 days.

The influence of Enilconazol on queens was also examined in bee colonies for keeping young mated queens. The experiment was performed in AZ-type of pavilion hives. Thin lattices (1 cm) divided two frames in each hive into 16 equal parts. Each part received a piece of comb with honey, pollen and some cells with larvae and pupae, as well as a young queen from the mating hive. Plastic, transparent queen excluders covered booth sides of the frame. On one side, the excluder was divided into 16 parts, making it possible to open each part separately. Frames with these boxes were placed into the hive between frames with honey. The experimental bee colonies were fed as previously described (50 ml of sugar solution with 0, 4, 8 or 20 mg of Enilconazol per ml). They were observed for 25 days, every third day.

Results

The doses of 4, 8 or 20 mg/ml of Enilconazol in 50 ml of sugar solution were acceptable for bees. Results of testing the influence of Enilconazol on the bees and bee brood are presented in Tables 1 and 2. There were no significant differences between experimental and control colonies.

There was no statistical difference among experimental and control groups in queen acceptance tested in the apiary (Table 3). The eggs were present n boxes where queens were accepted from the first day in all experimental and in control colonies. Also, there were no differences in

Table 1. The presence of queens, bees, eggs, larvas and pupas in all experimental observation hives (apisarium) (Enilconazol 200, 400 or 1000 mg/50 ml sugar solution)

	Days of experiment												
Presence of	0	1	2	3	4	5	6	7	8	9	10	20	30
Queen	+	+	+	+	+	+	+	+	+	+	+	+	+
Bees	+	+	+	+	+	+	+	+	+	+	+	+	+
Eggs	-	-	-	+	+	+	+	+	+	+	+	+	+
Larvae	-	-	-	-	-	+	+	+	+	+	+	+	+
Pupae	-	-	-	-	-	-	-	-	-	-	+	+	+

Table 2. The presence of queens, bees, eggs, larvas and pupas in control observation hives (apisarium) (sugar solution, only).

	Days of experiment												
Presence of	0	1	2	3	4	5	6	7	8	9	10	20	30
Queen	+	+	+	+	+	+	+	+	+	+	+	+	+
Bees	+	+	+	+	+	+	+	+	+	+	+	+	+
Eggs	-	-	-	+	+	+	+	+	+	+	+	+	+
Larvae	-	-	-	-	-	+	+	+	+	+	+	+	+
Pupae	-	-	-	-	-	-	-	-	-	-	+	+	+

vitality of queens and development of the brood during 25 days of observation.

Discussion and conclusions

Enilconazol is an Imidazol derivate with a bad taste, soluble in oil and organic solvents, but very poorly in water. It inhibits ergosterol biosynthesis in fungus and yeast cell membranes with consequent irreversible damage of organelles (SAKAR and SAKAR, 1999). Fungistatic, fungicidal (higher

Table 1. Specification of myomorphus mammals examined by renoculture and microscopic agglutination acording to the trapping area with corresponding results

Table 3. The acceptance of queens

		Control				Enilconazol mg/ml			
Hive	Frame			4		8		20	
		N°	%	N°	%	N°	%	N°	%
1	1	5/16	31,25	6/16	37,50	7/16	43,75	7/16	43,75
	2	6/16	37,50	7/16	43,75	7/16	43,75	6/16	37,50
2	1	5/11	45,45	6/13	46,15	7/14	50,00	6/13	46,15
	2	6/11	54,54	7/13	53,85	5/14	35,71	5/13	38,46
3	1	6/16	37,50	5/15	33,33	5/16	31,25	5/16	31,25
	2	6/16	37,50	5/15	33,33	6/16	37,50	6/16	37,50
1+2+3	1+2	34/86	39,53	36/88	40,91	37/92	40,22	35/90	38,88

concentrations) and sporocidal activity affects many fungi, yeasts and gram-positive bacteria of veterinary medical importance, including *Aspergillus* spp. and *Ascosphaera* spp. MIC for *Aspergillus fumigatus in vitro* is 1 mg/ml.

In the laboratory and apiary trials, Enilconazol in feed was consumed and safe for bee colonies. All three tested doses (4, 8 or 20 mg/ml) gave similar results. We noticed no changes in the behaviour of worker bees and queens or any abnormality in bee brood development. Other possible routes of Enilconazol treatment (i.e. by spraying or by fume) would directly expose the queen to the drug and may be more likely to cause chronic toxicity in this longest-living member of the bee colony. Further experiments are therefore needed to obtain data on chronic toxicity of oral and other ways of colony treatment with Enilconazol.

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References

BAILEY, L. (1963): Infections diseases of the honey bee. Lannd Books. London.

BAILEY, L., B. V. BALL (1991): Honey Bee Pathology. Academic Press. London.

BELČIĆ, J., J. KATALINIĆ, D. LOC, S. LONČAREVIĆ, L. PERADIN, F. ŠIMIĆ, I. TOMAŠEC (1968): Pčelarstvo. Nakladni zavod Znanje. Zagreb.

ČANAĐIJA, J. (1978): Na našim pčelinjacima vapnenasto leglo. Pčela 97, 246.

GROUT, R. A. (1949). The hive and the honey bee. Hamilton.

HEATH, L. A. F. (1985): Occurrence and distribution of chalk brood disease of honeybees. Bee Wld. 66, 9-15.

KORDOS, L. (1978): O liječenju vapnenastog legla. Pčela 97, 211.

SAKAR, D., T. SAKAR (1999): Remedia veterinaria Croatica. Argos, Zagreb.

SULIMANOVIĆ, Đ. (1982): Širi li se vapnenasto leglo? Pčela 101, 73-76.

SULIMANOVIĆ, Đ. (1986): Vapnenasto leglo. Pčela 105, 33-36.

SULIMANOVIĆ, Đ., Ž. MATAŠIN (1983): Vapnenasto i kamenito leglo. IX. Znanstvena konferencija "Veterinarska medicina i biotehnika", Zagreb, 11. i 12.10.1982. Vet. arhiv 53 (Suppl.), 69-70.

SULIMANOVIĆ, Đ., LJ. ZEBA, J. MARKOVIĆ (1993): Temelji suvremenog pčelarenja. PIP. Zagreb.

SULIMANOVIĆ, Đ., LJ. ZEBA, J. MARKOVIĆ (1995): Prepoznavanje i suzbijanje pčelinjih bolesti. PIP. Zagreb.

TABER, S., R. SACRETT, J. MILLS (1975): A possible control of chalkbrood disease. Am. Bee J. 115, 20.

TOMAŠEC, I. (1955): Bolesti pčela. Poljoprivredni nakladni zavod. Zagreb.

ZEBA, LJ. (1994): Povezanost varooze i vapnenastog legla. PIP. Zagreb.

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SAŽETAK

Pogrešna ili prekomjerna uporaba antibiotika i akaricida u suzbijanju pčelinjih bolesti, kao i različiti stresori, uključujući uzgoj pčelinjih matica, mogu povećati učestalost vapnenastog legla. Enilkonazol bi mogao biti lijek od izbora za liječenje te bolesti. Njegov utjecaj na pčele, pčelinje lego i matice istražen je stoga po prvi puta u laboratorijskim uvjetima i na pčelinjaku. Količine od 200, 400 i 1.000 mg enilkonazola otopljene su u 96 %-nom etanolu, dodane u 50 ml šećernog sirupa (1:1, masa/volumen) i pohranjene pokusnim skupinama. Promatranja pčela i pčelinjeg legla u laboratorijskim košnicama s jednim okvirom (apisarij) tijekom 30 dana nisu pokazala razlike između tretiranih i kontrolne skupine hranjene samo šećernim sirupom. Utjecaj enilkonazola na kvalitetu matica istražen je u AŽ košnicama s nekoliko posebno pripremljenih i podijeljenih okvira. Utvrđeno je da između tretiranih i kontrolnih zajednica tijekom 25 dana nije bilo razlike u prihvaćanju i vitalnosti matica, kao ni u razvoju pčelinjeg legla.

Ključne riječi: enilkonazol, vapnenasto leglo, pčelinje leglo, matica