

**LABORATORY STUDIES ON THE EFFECT
OF STANDARDIZED *Artemisia absinthium* L. EXTRACT
ON *Nosema apis* INFECTION IN THE WORKER
*Apis mellifera***

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S u m m a r y

The aim of the present study was laboratory evaluation of the effect of feeding worker bees with sugar syrup containing *Artemisia absinthium* L. extract on the development of *N. apis* infection.

In this experiment, groups of caged bees (100 bees each) naturally infected with *N. apis* and groups of caged bees artificially infected with a dose of $21 \cdot 10^6$ spores/ml applied in 30 ml of 50% sugar syrup were fed for 17 days with 50% sugar syrup containing an addition of 5% and 10% *A. absinthium* L. extract. The control group of both bees were given 50% sugar syrup for 17 days also. At different intervals after inoculation (3, 10, 17 days), number of *N. apis* spores in homogenates of abdomens of worker bees from each group was determined by using Bürker haemocytometer.

It was found that *A. absinthium* L. standardized extract, after 17 days of treatment, significantly inhibited development of the *Nosema apis* in naturally and artificially infected worker bees. But we have found also a significantly greatest mortality of the treated worker bees in comparison with untreated ones.

Keywords: *Artemisia absinthium* L., extract, effect, honeybee, *Nosema apis*, infection.

INTRODUCTION

The honeybees in Polish apiaries, like those in many other countries are frequently infected with *Nosema apis* (Bailey and Ball 1991, Fries 1997). In temperate climate infections by *N. apis* are considered to be detrimental. *Nosema* disease causes significant losses in productive capacity, shortens bee life span and causes greater colony mortality in winter or early spring (Cantwell and Shimanuki 1969, Fries et.al. 1984, Wang and Moeller 1970).

The replacement of old and contaminated combs and disinfection measures are necessary but often treatment with antiprotozoal drugs is needed at least in

some areas (in temperate climate).

The veterinary drugs containing with the antibiotic fumagillin, which suppresses *N. apis* development, are the only effective for control of nosema disease (Katznelson and Jamiesson 1952, Furgala and Boch 1970, Liu 1973).

Council Regulation (EEC) 2377/90 laid down by a Community procedure for the establishment of MRLs for veterinary medicinal products in foodstuffs of animal origin. From January the 1st, 2000 on, the use of pharmacologically effective substances lacking in MRL is illegal. From this time, fumagillin is no available on the EU market because no MRL has been established for honey.

Furthermore, now, authorised veterinary medicinal products for the treatment of bacterial and protozoan honeybee diseases are not available on the market of EU countries (Mutinelli 2003).

Herbs and herb formulas have for a long time been used in human medicine. One from them is *Artemisia absinthium* L. Medicinal preparations are produced from leaves as Folium Absinthii or mingwort's herb - Herba Absinthii. *A. absinthium* L. contains many biologically active compounds: absinthinum, essential oils, flavonoids, organic acids, tannins and mineral salts and give evidence of disinfecting, diastolic, stimulating gastric juice secretion and antiparasitic therapeutic effect (Ożarowski 1987).

According to Council Regulation (EEC) No 2377/90 *A. absinthium* L. extract is included in Annex II listing substances which are not subject to MRL limits. These substances are considered to be safe when applied to all species of food-producing animals.

The aim of this study was to determine under laboratory conditions the influence

of standardized *A. absinthium* L. extract on the development of *Nosema apis* infection of naturally and artificially infected worker bees .

MATERIAL AND METHODS

The experiment was conducted in June and October 2003 and 2004. In the research we used Carniolan bees from the apiary of the Apiculture Division in Pulawy.

The bees were collected from brood nests of colonies infected with *Nosema apis* spores - group A, and non-infected - group B and confined in cages. The presence of *N. apis* spores in honeybee colonies was ascertained in prior routine check. Each cage was settled with ca 100 worker bees. All the cages were kept in an incubator at 34°C.

Inoculum preparation

The spores of *N. apis* were obtained from heavily infected bees by adding water to the dissected intestinal tracts, macerating in a blender, filtering through Whatman 4 filter paper and centrifuging

Table 1

The cumulative mortality rate of worker bees and consumption of pure sugar syrup and containing *Artemisia absinthium* extract

Groups and subgroups of bees		Average consumption of sugar food (ml/bee)	Average mortality rate of honey bees after 21 days (in %)
A artificially infected	A1 – Sugar syrup with 5% extract added	0.69 b	77.8 bcd
	A2 – Sugar syrup with 10% extract added	0.56 b	84.7 cd
	A3 – Pure sugar syrup (control)	0.71 b	57.1 b
B naturally infected	B1 – Sugar syrup with 5% extract added	0.67 b	64.4 bc
	B2 – Sugar syrup with 10% extract added	0.62 b	87.2 d
	B3 – Pure sugar syrup (control)	0.81 b	18.5 a

Differences between means in columns are significant when means are not marked with the same letter.

Table 2

Development of *Nosema apis* infection of worker bees fed with pure sugar syrup and with *Artemisia absinthium* extract

Groups and subgroups of bees		Mean number of <i>N. apis</i> spores x 10 ⁶ /bee			
		1 st day - the beginning of the experiment	3 rd days after artificial infection and before the beginning of treatment	after 10 days of extract treatment	after 17 days of extract treatment
A artificially infected	A1 – Sugar syrup with 5% extract added	0.0 a	9.8 a	66.0 c	187.0 c
	A2 – Sugar syrup with 10% extract added	0.0 a	13.7 a	68.1 c	138.5 c
	A3 – Pure sugar syrup (control)	0.0 a	6.9 a	90.0 cd	444.8 d
B naturally infected	B1 – Sugar syrup with 5% extract added	1.1 a	1.8 a	5.9 a	14.9 ab
	B2 – Sugar syrup with 10% extract added	1.1 a	2.7 a	2.3 a	0.08 a
	B3 – Pure sugar syrup (control)	1.1 a	1.5 a	12.7 ab	35.3 b

Differences between means in columns are significant when means are not marked with the same letter.

this filtrate. The precipitate was resuspended in water and a final concentration of spores was estimated by haemocytometer counts.

Inoculation with *N. apis* spores

For the first 3 days of the experiment each cage of bees from group A was infected with a dose of 21 10⁶spores/ml applied in 30 ml of 50% sucrose syrup. At the time bees from group B (naturally infected) were fed with pure sugar syrup.

Schedule of treatment

After the 3rd day cages of both groups were divided into 3 subgroups. For the next 17 days of the experiment the worker bees of A1 and B1 groups were fed ad libitum with 50% sugar syrup with an addition of 5% *A. absinthium* L. standardized

extract. The bees of A2 and B2 groups were fed with 50% sugar syrup with an addition of 10% *A. absinthium* L. standardized extract. The control groups – A3, B3 were fed with pure sugar syrup only.

Subgroups of bees	Treatment
A1 – artificially infected	Sugar syrup with 5% extract added
A2 - artificially infected	Sugar syrup with 10% extract added
A3 - artificially infected	Pure sugar syrup (control)
B1 – naturally infected	Sugar syrup with 5% extract added
B2 - naturally infected	Sugar syrup with 10% extract added
B3 - naturally infected	Pure sugar syrup (control)

On the following days of experiment 10 bees were collected from each cage in order to assess the degree of *N. apis* infection:

- **4th day** - 3 days after artificial infection of bees from group A and before the beginning of feeding with syrup containing extract;
- **14th day** - after 10 days of extract treatment;
- **21st day** - after 17 days of extract treatment - on the last day of experiment.

During the experiment bees' daily mortality rate and sugar consumption was assessed.

The experiments was carried out in 4 series, with 6 subgroups in each series and 5 cages as replication in a single subgroup. In total, 120 cages of experimental and control caged bees were used in this study.

Examination of bees for spores

The worker bees were examined individually. The abdomen of each bee was dissected and macerated in 1 ml of distilled water. A small drop of this solution was placed in Bürker haemocytometer (depth of layer 0.1 mm and small grid areas of 0.0025 mm²) and examined on a light microscope (multiplication 400) using phase contrast optics (Cantwell 1970). The number of spores was counted in 80 small squares. The total number of spores was calculated by equation:

$$\begin{aligned} &\text{number of spores per bee} = \\ &= (\text{total number of spores counted}) \\ &(4 \cdot 10^6) / \text{number of squares counted} \end{aligned}$$

The statistical analysis (Duncan test at $\alpha = 0.05$) was performed to test differences between artificially and naturally infected treated with 5 and 10% *A. absinthium* extract addition and untreated:

- mean number of spores per bee;
- mortality of worker bees;
- food consumption.

RESULTS AND DISCUSSION

The average consumption of the pure sugar syrup and syrup containing extract did not differ (tab. 1). During 17 days each worker bee consumed average from 0.5 to 0.8 ml of sugar food.

After 21 days of experiment, significant differences in the mortality of the workers was observed (Tab. 1). The percentage of dead bees in the all treatment subgroups was significantly higher than recorded in untreated (control) subgroups. The significant differences in the mortality of the bees between control subgroups (A3, B3) was observed also. It was lower in subgroups of the naturally infected workers.

The significant differences in the mean degree of Nosema infection occurred 14 days after artificial inoculation. At the time, in all artificially infected subgroups average number of spores/bee was higher than in all naturally infected ones. However there were no differences between treated and untreated workers. After 17 days of treatment the number of spores per bee significantly decreased in workers fed with both doses of extract in comparison to control subgroups.

It was found in the study, that extract addition did not reduce the consumption of syrup by bees but increased their mortality in comparison to "pure" sugar food.

In untreated subgroups A3, B3 the number of dead bees was 57% and 18% of the total number of the bees at the start of the experiment respectively.

The natural lifespan of the bees is about twice as long as those kept in cages in laboratory conditions (Muszyńska and Bornus 1981, 1983).

The initial of *N. apis* infection level also affected on the differences in the mortality between untreated bees from A3 and B3 subgroups.

There are some data which suggest that the level of infection of an individual bee

depend on the infecting dose (Fries 1988). Similar results were obtained in present study. It was found that the workers inoculated with large dose of *N. apis* ($21 \cdot 10^6$ spores/bee) had significant greater number of spores in comparison to bees naturally infected with low dose.

CONCLUSIONS

- Standardized *Artemisia absinthium* L. extract applied to bees naturally and artificially infected with *N. apis* spores inhibited *N. apis* infection.
- Antiparasitic activity of *Artemisia absinthium* L. extract depended both on its percentage content and the initial level of *N. apis* infection of the diseased bees.
- Increased mortality rate of the bees' may prove that standardized *Artemisia absinthium* L. extract harmful impact also on these insects.

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**BADANIA LABORATORYJNE WPŁYWU
STANDARYZOWANEGO WYCIĄGU Z BYLICY PIOŁUNU
(*Artemisia absinthium* L.) NA ZAKAŻENIE *Nosema apis*
PSZCZÓŁ ROBOTNIC *Apis mellifera* L.**

Pohorecka K.

S t r e s z c z e n i e

Celem pracy była ocena wpływu standaryzowanego wyciągu z piołunu (*Artemisia absinthium* L.) na rozwój zakażenia *N. apis* u pszczoł zakażonych sztucznie i naturalnie.

Badania prowadzono w warunkach laboratoryjnych w latach 2002-2003, w Oddziale Pszczelnictwa ISK w Puławach. Klateczki doświadczalne zasiedlano po około 100 sztuk pszczołami ulowymi, pobieranymi z części gniazdowej rodzin, w których w wyniku wstępnych badań laboratoryjnych nie stwierdzono obecności spor *N. apis* – **grupa A**, oraz z rodzin w, których stwierdzono obecność spor pierwotniaka *N. apis* – **grupa B**.

Klateczki z pszczołami przetrzymywane były w ciepłarkach, w temp 34°C.

W ciągu pierwszych trzech dni doświadczenia pszczoły z grupy A zostały zarażone sztucznie jednorazową dawką 21 10⁶/ml spor *N. apis* podaną pszczołom z każdej klateczki w 30 ml syropu 1:1 (woda:cukier). W tym czasie pszczoły z grupy B otrzymywały czysty syrop cukrowy. Po upływie 72 h ze wszystkich klateczek obu grup pobrano po 10 pszczoł w kierunku oceny stopnia zakażenia sporami *N. apis*. W tym dniu w obrębie grupy A i B utworzono po 3 podgrupy i rozpoczęto podawanie 50% syropu cukrowego dodatkiem wyciągu z piołunu. Pszczoły z podgrupy A1 i B1 otrzymywały syrop z 5% dodatkiem wyciągu, pszczoły z podgrupy A2 i B2 otrzymywały syrop z 10% dodatkiem wyciągu z piołunu. Kontrolę stanowiły pszczoły z podgrupy A3 i B3, które otrzymywały czysty syrop cukrowy. Okres podawania pokarmów cukrowych wynosił 17 dni.

Po upływie 10 dni od rozpoczęcia podawania pokarmów z dodatkiem wyciągu z piołunu oraz w dniu zakończenia doświadczenia ze wszystkich klateczek w obrębie utworzonych grup doświadczalnych pobierano ponownie po 10 pszczoł do badań laboratoryjnych w kierunku oceny stopnia ich porażenia sporami pierwotniaka *N. apis*.

Pszczoły badane były indywidualnie. Odwłoki pszczoł homogenizowano z wodą destylowaną w proporcji 1 odwłok / 1 ml wody. Po wymieszaniu homogenną zawiesinę przenoszono na szkiełko hemocytometru Bürkera. Spory liczono metodą Cantwella (1970).

Łącznie okres trwania doświadczenia wynosił 21 dni.

Podczas trwania doświadczenia oceniano również dobową śmiertelność pszczoł oraz ilość pobranego przez pszczoły pokarmu.

Ocenę istotności różnic pomiędzy średnią liczbą spor u pszczoł z grup doświadczalnych, ilością pobranego pokarmu przez nie pokarmu oraz śmiertelnością, wykonano przy pomocy testu Duncana przy poziomie ufności $\alpha = 0.05$.

Nie stwierdzono istotnych różnic w ilości pobieranych przez pszczoły pokarmów natomiast śmiertelność pszczoł karmionych syropem z dodatkiem wyciągu z piołunu była istotnie wyższa w porównaniu do pszczoł nie leczonych. Stwierdzono iż dodatek wyciągu redukował w sposób istotny poziom infekcji *N. apis* u pszczoł zakażonych sztucznie i naturalnie, po 17-to dniowym okresie jego podawania.

Słowa kluczowe: wyciąg z *Artemisia absinthium* L., wpływ, pszczoły robotnice, *N. apis*, zakażenie.