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Electromagnetic Fields and Colony Collapse Disorder of the Honeybee

***Abstract**. The abrupt disappearance of the bees that pollinate crops is a significant subject of recent study. One theory points to the development of telecommunications technology and an increasing number of electromagnetic field sources as a possible cause of the phenomenon.This paper presents the results of preliminary studies of honeybee exposure to extremely low frequency electromagnetic field (50 Hz; 1 mT, 7 mT)*

***Streszczenie.** Nagłe znikanie pszczół, które zapylają uprawy, jest jednym z istotnych tematów ostatnich badań. Jedna z teorii wskazuje na rozwój telekomunikacji i zwiększenie liczby źródeł pola elektromagnetycznego jako możliwą przyczynę tego zjawiska. Artykuł prezentuje wyniki wstępnych badań nad ekspozycję pszczół miodnych na pole elektromagnetyczne o wyjątkowo niskiej częstotliwości (50 Hz; 1 mT 7 mT) (**Pola elektromagnetyczne i zespół masowego ginięcia pszczoły miodnej**).*

Keywords: electromagnetic fields, honeybee, colony collapse disorder, behaviour change
Słowa kluczowe: pola elektromagnetyczne, pszczoła miodna, zespół masowego ginięcia pszczoły miodnej, zmiany w zachowaniu

Introduction

Honey bees (Apis mellifera L.) are the most important pollinators of many agricultural crops worldwide. The abrupt decline in pollinator abundance and diversity is not only a conservation issue but also a threat to crop pollination. This problem is one of the most popular among recently conducted studies. Theories involving mites, pesticides, global warming, and genetically modified crops, have been proposed, but all have drawbacks. Another possible cause of that phenomenon is the development of telecommunications technology and the increasing number of electromagnetic field sources [1-4].

It is assumed the electromagnetic fields (EMFs) interfere with bees' navigation systems, preventing the home-loving species from finding their way back to their hives. Colony Collapse Disorder (CCD) occurs when a hive's inhabitants suddenly disappear, leaving only queens, eggs and a few immature workers. The vanished bees are never found but are thought to die singly, far from home [2].

As with many other eusocial animals, honeybees have a fascinating ability to sense the Earth magnetic field and use it for the spatial orientation. The presence of organized magnetic nanoparticles in bee bodies is well documented and indicated as a possible magnetic detector [5-14]. Magnetoreception is applied by bees, during their waggle dances [15]. This kind of communicative dance is performed by bees in the hive’s interior, in complete darkness, to inform other workers about potential food sources. In swarming colonies, dances provide information regarding the new nest location, and when to move in. The honeybees have a sensitivity to the Earth’s magnetic field poles and lines, and they use the information about the location and orientation of the hive entrance relative to the direction of the EMF force lines. Many experimental data confirm a spatial orientation loss and behavioural disturbances in the honeybee colonies, whose location and orientation to the Earth’s magnetic field direction and poles were modified [2, 9].

Besides natural EMFs, an important factor in the environment is man-made EMFs, which are stronger than those of the natural origin by many orders of magnitude. In this study, we focused on extremely low frequency (ELF) EMFs, whose principal man-made sources are high-voltage transmission lines and all devices containing the current carrying wires, including the equipment and appliances used in the industry, and at home.

Recent studies have shown that chronic exposure to ELF EMFs act as a major stress factor in animals [16, 17]. Our previous study also indicates the potential impact of ELF-EMFs on insects leading to physiological and behavioural changes as well as increased levels of stress proteins [18, 19].

Several pieces of research have shown that the bees' behaviour changes near power lines. The honey bee hives exposed to high voltage transmission lines showed increased motor activity, abnormal propolisation, reduced weight gain of hives, queen loss, impaired production of queen cells, a decrease in sealed brood chambers, and poor winter survival. These are all effects that could be caused by the poor acquisition of food sources and metabolic inefficiencies. Cognitive behaviour and flight, therefore, are not the only types of behaviours modified by EMFs that lead to colony level stress. The results suggest that ELF-EMFs may represent a prominent environmental stressor for honey bees, with the potential impact on their orientation and motor abilities, which could, in turn, reduce their ability to pollinate crops [1- 4].

The aim of our study was to determine the influence of acute exposure to 50 Hz ELF-EMFs at levels of 1 mT, and 7 mT, found within 1 m of the overhead power lines, on the forager honeybee locomotor activity and their survival.

Material and Methods

Animals

Experiments were performed on worker honeybees (*Apis mellifera carnica* F.). Only the oldest bees in the colony, performing their tasks outside the hive (guard and foraging bees) and manifesting circadian rhythm of locomotor activity [20], were collected from hives of the apiary located near Toruń, in Central Poland. Insects walking at the hive entrance were caught in July 2017. The studied honeybee groups were kept in the isolated room (under the temperature of 30°C) in plastic boxes covered with glass lids, under LD 12:12 photoperiod conditions. Altogether, 99 worker honeybees were divided into 22 groups and six experimental series.

EMF Exposure

ELF-EMFs (with a dominant magnetic component) were generated using a 19 cm (inner diameter) coil (Elektronika i Elektromedycyna Sp. J.; Poland). The coil and variac power supply produced homogeneous (8%), sine-wave alternating electromagnetic fields at 50 Hz and with intensities ranging from 0.1 to 8 mT. Magnetic flux densities were measured with a Magnetometer (Model GM2, Alphalab Inc., USA) A detailed description of the apparatus and the distribution of the magnetic field was provided in a previous report [21, 22]. Animals were exposed for 24 hr to control conditions (sham coils) or ELF-EMFs. Exposures to 1 and 7 mT ELF-EMFs (series E1, E7) were analysed. Sham-exposed groups (series C1, C7 respectively) were kept within sham coils providing the same experimental conditions but without an ELF-EMF. Bees from the control series (C00, C01) were not subjected to any experimental procedure. The animals' survival and locomotor activity were studied during the next day after the ELF-EMF/sham exposure.

Estimation of the Locomotor Activity

The locomotor activity of worker honeybees was recorded in infra-red radiation using a video camera (Sony Handycam HDR-SR5E). The data were digitized in video files at 15 s sequences by computer by the Pinnacle Studio ver.11.0.0.5082 (Pinacle Systems Inc.) software. During the visual inspection of the consecutive video recordings, each insect was given a value of 1 when was active (walking, running, rotating, or changing its posture) or 0 when was motionless. Next, the percentage of moving animals versus all that were living at a given time was calculated. The inspection results were averaged either over two 24-hour periods for light and darkness periods.

Estimation of Worker Honeybee Survival

The survivability analysis took two days. In control groups (C00, C01), the number of living animals on the exposure day, and a day after the exposure was compared. Bees were counted every 2 hours, i.e., after 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 hours of the experiment.

During the first day of the experiment, honeybee groups of experimental series C1, E1, C7 and E7 were located in experimental set-ups and were either exposed to an EMF or they were not. In these experimental series, like in the control, the survivability index was determined every 2 hours (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 hours of the observations) and referred to number of living insects at the start of the post-exposure period (in both cases - hour 0).

Statistical Analysis

All values are reported as means ± standard error of the means (±SE) and were analysed using non-parametric Mann–Whitney U test with significance level set at P<0.05. Statistical analyses were carried out using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Results of the honeybee worker locomotor activity are shown either as 24-hour averages (Fig.1A) or as averages for light and darkness periods (Fig.1B). The effect of the ELF-EMF exposure on the honeybee locomotor activity was insignificant. There were no significant differences between mean values from 24-hours of the locomotor activity for all the experimental groups. We observe a slight decrease in activity of honeybee exposed to 7 mT and the increase in the 1 mT- exposed group. We also do not notice any significant impact of exposure to ELF-EMF by analyzing the activity in periods of light / darkness (Fig. 1B). The increased value of activity after 1mT exposure during the dark period is an exception. This value is statistically different in comparison to the corresponding control group. Significant differences during this experimental series appear mainly between groups of non-treated animals (C00, C01) and those taking part in the experiment, control groups (C1, C2). Figure 1B shows how the activity of bees is significantly varied during the light and dark phase. The difference between these periods is statistically significant (p<0,0001).

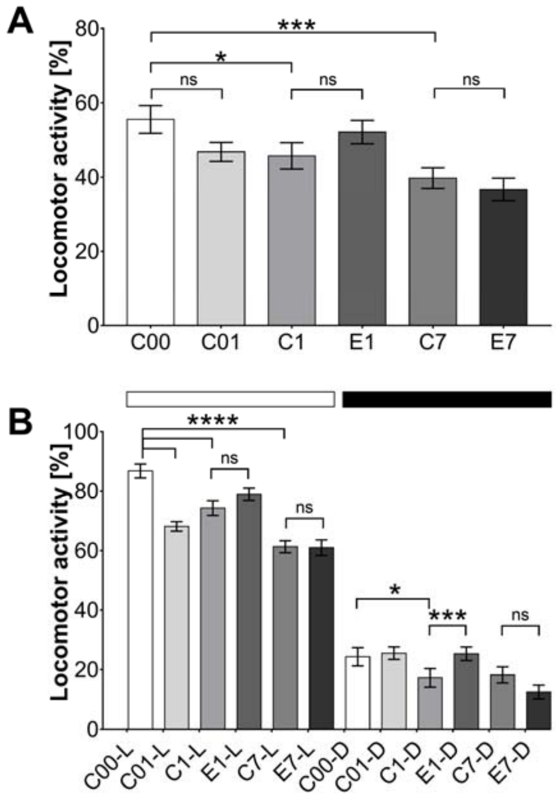

Fig. 1. Effects of ELF-EMF exposure on locomotor activity. Results are shown for exposure to 1 mT (E1) and 7 mT (E7) compared to control animals: non-treated, animals (C00, C01) and sham-exposed groups (C1 and C7, respectively). Values are expressed as means (± SE) for 24h (**A**) and for 12h of periods of light (L-white bar) and darkness (D- black bar) (**B**). Asterisks indicate the significant difference (****p<0.0001, ***p<0.001, *p<0.05, respectively).

The ELF-EMF exposure has no significant effect on the honeybee worker's survival. In groups non-treated (C00), on the first day of the experiment (during the sham/ELF-EMF exposure of other groups), no survivability reduction was observed. Similarly, there was no decrease in survival at that time in groups exposed, before the ELF-EMF application. We do not register any significant changes in mortality by analyzing changes that have occurred within 24 hours after sham/ELF-EMF exposure (Figure 2A). The numbers of live bees counted every 2 hours for 24 hours after the sham/ELF-EMF exposure, shown in figure 2B, indicate potential trends. The increase in mortality seems to be higher in the case of groups exposed to 7 mT compared to the groups kept at 1 mT. And just as there is no difference in the decreasing of survivability between the 1 mT- exposed group and the corresponding control group, we can see a clear difference between the mortality of the insects exposed to 7 mT compared to the corresponding control group. The greatest reduction in survival was observed during the second day of the experiment at the 22nd hour point in the group exposed to the 7mT and the non-treated insect group C02. All differences between the individual mean values of the survivability within the groups and between the experimental groups were insignificant.

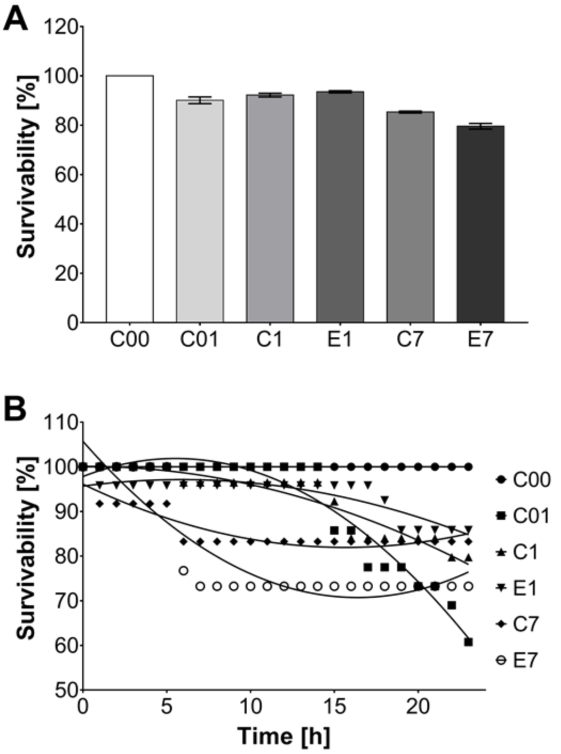

Fig. 2. Effects of ELF-EMF exposure on survivability. Results are shown for exposure to 1 mT (E1) and 7 mT (E7) compared to control animals: non-treated, animals (C00, C01) and sham-exposed groups (C1 and C7, respectively). Values are expressed as means (± SE) for 24h (**A**) and for 2h (**B**). Differences are not a statistically significant.

Discussion

Our experiments were aimed to determine the effect of the ELF-EMF exposure on survivability and locomotor activity of the honeybee workers. We suspected that it could induce serious disturbances in their physiology and behaviour. It has been proven that bees have the ability to detect EMFs. There are a number of literature reports indicating the modification of insect behaviour under the influence of magnetic fields [2-4, 23 ,24]. Nevertheless, in our preliminary experiments, we found only insignificant impact of the ELF-EMF on the observed parameters. Therefore, it is necessary to reanalyze the used experimental protocol before the proceeding to the next set of observations.

The bees used in our experiments were naturally the oldest in the colony. Hence, their survival period might have been shortened in principle, which led to the extreme shortening of the experiment duration and, perhaps, the after-exposure period (the longest 100%-survivability was held until the 40th hour of observations, in the control series). Very low survival in bee control groups could override the effect of ELF-EMF exposure on honeybee survival.

The exposure procedure itself could also affect both locomotor activity and the survivability of the examined bees. Considering the 100% survival rate of non-treated insects in a room with quite dim light (LD12:12) and constant ambient temperature (of about 30 °C), the high mortality of bees of the experimental groups could be caused by the constant ambient temperature, which did not change according to the daily changes. The coils increase its temperature during the generating of an EMF, therefore every EMF-exposed group has a corresponding control group kept in exactly the same experimental conditions but without an EMF presence. Our observations were conducted in optimal temperature conditions for bees (about 30 °C). This value is exactly in the middle of the range of temperature usually selected by the honeybee workers in the thermal gradient (28-33 °C) [25-27]. Maybe the fact that the bees were deprived of choosing different temperatures acted as a strong stress factor.

The observation of the bees after ELF-EMF exposure was intentional, so the experimental chambers with the insects were moved from the sham/ELF-EMF exposure room to the observation room. Although we tried to provide and keep stable conditions, the honeybees could be exposed to small but sudden temperature change and open field stress.

Our observations indicate that for home-loving animals such as the honeybee is, this type of stress, acting under artificial conditions, is deadly. Not only the experimental procedures but also the presence of the experimenter was a stress factor manifested with increasing wings movements of bees.

Another crucial stress factor for bees was their isolation from the colony. Moreover, it seems necessary to preserve a proper and unchanging orientation of the boxes with bee groups to face toward the Earth's magnetic field force lines and poles. All of this should be taken into account when conducting research on bees, that seem to be very sensitive to procedures, which are used in these experiments. Hence, in our future experiments, we will try to minimize the impact of these factors.

In previous studies, Wyszkowska et al. [16, 18, 19] reported the action of ELF-EMF exposure as a stress factor. Exposure to EMF (50 Hz, 7 mT) evidently enhanced the motor activity of insects which is probably connected with the increased releasing of octopamine, a multi-potent neuroactive amine associated with general arousal in insects [28]. The mean value locomotor activity in bees exposed to ELF-EMFs at 7 mT is lower than in exposed to 1 mT analyzed in the 24h period. Although, this difference is not statistically significant it might indicate a dose-dependent influence of EMF exposure similar to that observed in locusts [17]. Leaving aside the discussed factors above that could significantly affect bees, by concealing the ELF-EMF exposure effect it seems that the all-day increased activity (during sham/ELF-EMF exposure) might be exhaustive for the studied bees leading to the decrease, which was observed in our experiment. A similar result was also obtained in experiments on desert locusts. The walking ability, as well as kick force, decreased after ELF-EMF exposure (24h, 7 mT). It might be explained by muscular fatigue [16, 18].

The obtained results do not allow us to draw unambiguous conclusions about the impact of ELF-EMFs, but they do force us to reanalyze the experimental protocol for future experiments on potential connections between increasing EMF exposure and the Colony Collapse Disorder of the honeybee.

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