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EFFECT OF ELECTROMAGNETIC WAVES ON SUSCEPTIBILITY OF FUNGI OF THE GENUS *CANDIDA* TO MICONAZOLE

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ABSTRACT. Increasing use of elctromagnetic fields (EF) in the treatment of various diseases may have potential impacts on fungi – possible aetiological factors of concomitant mycoses. The aim of this study was to investigate the effects of EF on miconazole susceptibility among fungi of the genus *Candida* showing confirmed pathogenicity in humans. Fifteen *Candida* strains obtained from patients were used and their susceptibility to miconazole was determined by diffusion in agar gel. Antifungal activity of miconazole was assessed as minimal inhibitory concentration (MIC) which was calculated for *Candida* strains not exposed to EF (control) and EF-exposed (3 experiments). In the majority of cases the susceptibility of *Candida* species to miconazole decreased (higher MICs) after the first week of EF exposure, regardless of the EF's parameters. This was followed by an increase in susceptibility (lower MICs) after the second week of exposure to EF of 2 mT intensity and frequency 3 Hz (experiment I) and an EF of 9 mT intensity and 12.5 Hz frequency (experiment II) relative to control. An increase in susceptibility (lower MICs) was observed in the second week of exposure, whatever the parameters of the EF. The application of low intensity, low frequency EF for a period of at least two weeks may be beneficial in the treatment of mycoses caused by pathogenic fungi of *Candida* genus.

Key words: *Candida*, electromagnetic waves, miconazole, susceptibility.

In previous years, alternating magnetic fields of very low frequency have been used mainly in physiotherapy (Adey 1981, 1993; Kirschvink 1992). As a non-invasive method, magnetotherapy is becoming increasingly popular in various areas of medical speciality (Sieroń et al. 1994, Antonow et al. 1997, Valberg et al. 1997). The results of experimental studies have suggested potential benefits of electromagnetic fields (EF) in the treatment of mycoses. This has prompted research on the effects of EF in relation to fungal pathogens (Budak et al. 1996, 1997, 1998).

Increasing use of EF in the treatment of various diseases may have potential impacts on fungi – possible aetiological factors of concomitant mycoses. Gaining insight into the mechanisms of these effects may have significant importance in the understanding of certain aspects of the host-fungus system and in establishing guidelines for effective antifungal therapy (Howlett and Sguier 1980, Kurnatowska 1995).

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The aim of this study was to investigate the effects of EF on miconazole susceptibility among fungi of the genus *Candida* showing confirmed pathogenicity in humans.

MATERIALS AND METHODS

For the purpose of the present study, we used 15 *Candida* strains obtained from a variety of clinical isolates in patients registering at the Centre for Treatment of Parasitic Diseases and Mycoses, Medical University of Łódź.

The susceptibility of *Candida* strains to miconazole was determined by diffusion in agar gel. 0.5 ml of liquid 24 h culture on Sabouraud medium was transferred into 5 ml of liquid Sabouraud broth. From this dilution, 1 ml (10^6 fungal cells) inoculum was transferred onto a Petri dish of 10 cm diameter, containing 30 ml of 3% Sabouraud agar at pH 5.6. The suspension was then evenly distributed on the surface of the dish using a crooked glass tube. After 1 h thermostatic incubation at 37° C, wells of 10 mm diameter were formed in the medium using a sterile rotating drill. The wells were numbered and 100 µl of subsequent dilutions of miconazole were added using an automatic pipette. The following test dilutions of miconazole in 10% DMSO (dimethyl sulfoxide) were used: 10; 5; 2.5; 1.25; 0.612; and 0.306 mg/ml. The plates were then incubated for 24 h at 37° C in a thermostat, then the diameter of growth inhibition zone (in mm) was measured for each well.

Antifungal activity of miconazole was assessed as minimal inhibitory concentration (MIC), calculated by Kadłubowski (1971) method from semi-log linear regression curve:

$$\log MIC = \overline{\log_1 C} + \frac{\overline{\log_2 C} - \overline{\log_1 C}}{\overline{N}_2 - \overline{N}_1} (10 - \overline{N}_1)$$

$\overline{N}_1$ and $\overline{N}_2$ – arithmetic means for two groups of growth inhibition zone diameters, $\overline{\log_1 C}$ and $\overline{\log_2 C}$ – arithmetic means for log values of respective miconazole concentrations.

The susceptibility of *Candida* strains to miconazole was assessed in:

– *Candida* strains not exposed to EF (control)

– EF-exposed *Candida* strains.

Three experimental models were used:

* experiment I: the fungi were exposed to EF of 2 mT intensity and frequency 3 Hz during 15 min for 14 days; susceptibility was evaluated on day 7 and 14;

** experiment II: the fungi were exposed to EF of 9 mT intensity and frequency 12.5 Hz during 15 min for 14 days; susceptibility was evaluated on day 7 and 14;

*** experiment III: the fungi were exposed to EF of 2 mT intensity and frequency 25 Hz during 15 min for 14 days; susceptibility was evaluated on day 7 and 14;

Statistic evaluation was performed using Statistica 4.0, with Wilcoxon sequence of pairs test.

RESULTS

At test concentrations, miconazole was shown to inhibit the growth of all 15 *Candida* strains used in the present study. The growth inhibition zone diameters in the case of non-exposure to EF ranged from 10.5 to 23.0 mm; for most strains (2/3), the effective inhibitory concentration being 0.612 μg/ml. Miconazole at the concentration of 1.25 μg/ml inhibited the growth of all test strains, and mean growth inhibition zone diameters ranged from 12.0 to 14.4 mm. MICs of miconazole, obtained from activity curves, ranged between 1.05 μg/ml (strain no. 8) and 8.84 μg/ml (strain no. 12), with mean value of 3.62 μg/ml. For most strains (11 out of 15), MICs of miconazole ranged from 2.21 to 3.92 μg/ml.

The EF-exposed *Candida* strains – regardless of the experimental treatment – retained their sensitivity to miconazole, as evidenced by growth inhibition in the presence of the antifungal agent.

Table 1. MICs (*g/ml) of miconazole after the first (a) and the second (b) week of electromagnetic field exposure in *Candida* sp.

Strain no.	Control	Exp. I a	Exp. I b	Exp. II a	Exp. II b	Exp. III a	Exp. III b
46	3.92	12.8	1.84	5.29	1.39	5.16	1.33
45	3.61	6.01	3.25	6.56	2.65	1.83	7.87
25	2.82	2.62	2.15	5.22	4.00	4.96	4.7
12	8.84	8.84	4.97	2.18	2.75	2.61	3.68
34	3.86	8.84	2.35	6.17	3.62	3.31	5.32
10	3.29	12.7	5.63	13.3	5.25	7.82	4.42
7	3.19	3.83	2.63	5.29	3.39	8.01	4.77
13	4.48	12.7	2.33	2.65	1.56	3.59	6.12
8	1.05	1.22	2.18	6.69	1.49	1.99	2.87
2	3.28	12.8	6.02	6.05	3.26	4.74	2.88
39	6.12	7.36	1.56	2.94	1.81	7.49	2.44
31	2.89	1.81	5.45	4.64	1.44	8.18	3.46
47	2.36	12.7	3.07	3.15	1.43	2.21	4.79
35	2.21	3.12	2.18	5.44	2.58	4.04	2.59
49	2.32	3.93	2.96	2.97	1.65	1.52	2.78

Growth inhibition zone diameters for miconazole in experiment I reached up to 20.5 mm after the first week, and 26 mm after the second week. Only in 3 cases (20%) an inhibition zone of 11 mm was obtained with 0.612 μg/ml miconazole, whereas 0.306 μg/ml miconazole inhibited the growth of all the strains after the second week in 12 cases (80%). MICs of miconazole ranged from 1.22 to 12.8 μg/ml (mean 7.42 μg/ml) after the first week and 1.56-6.02 μg/ml (mean 3.24 μg/ml) after

the second week, as shown in Table 1. In the majority of cases (2/3) MICs of miconazole obtained following EF exposure were higher after the first week, but after the second week, there was a decline in MICs as compared to controls in more than 50% of cases (8 out of 15). It should be noted that in 13 out of 15 cases, MICs were lower in the second as compared to the first week; it may indicate increased susceptibility of test strains to the antifungal agent in question.

Miconazole-induced growth inhibition zone diameters in fungi following EF exposure in experiment II reached 22.5 mm after the first week, and 25 mm after the second week. An inhibition zone of more than 10 mm for 0.612 µg/ml miconazole was obtained in all (100%) strains after the first week and nearly all (14/15) strains after the second week. 0.306 µg/ml miconazole inhibited fungal growth in 4 cases (26.7%) after the first week and in 5 cases (33.3%) after the second week of EF exposure. MICs calculated from the given formula ranged from 2.18 to 13.3 µg/ml (mean 5.34 µg/ml) after the first week, and from 1.39 to 5.25 µg/ml (mean 2.55 µg/ml) after the second week, as shown in Table 1. According to these results, in the majority of cases (12/15) MICs of miconazole were higher after the first week of EF exposure, whereas in the second week there was a decrease in MICs relative to control in 60% (9 out of 15) of cases. It should be stressed that in 14 strains MICs were lower after the second week as compared with the first week.

Growth inhibition zone diameters for miconazol in EF-exposed fungi in experiment III reached up to 20.5 mm after both the first and the second week. According to these results, an inhibition zone of more than 10 mm after the first week was obtained only in 4 cases (27%) for 0.612 µg/ml miconazole; after the second week, this concentration of miconazole failed to suppress the growth in all of the investigated strains. 0.306 µg/ml miconazole was ineffective in inhibiting fungal growth both in the first and the second week. MICs calculated from the given formula ranged from 1.58 to 8.18 µg/ml (mean 4.50 µg/ml) after the first week, and from 1.33 to 6.12 µg/ml (mean 3.96 µg/ml) after the second week, as shown in Table 1. In over 50% of cases (8/15) following EF exposure, MICs of miconazole were higher after the first week, as well as the second week, with a decline in MICs relative to control in the majority (11 out of 15) of cases. In over 50% of cases MICs were lower after the second week as compared with the first week.

Scheme 1. MIC distribution in relation to experimental treatments and study period

	Experiment					
	I		II		III	
	a ↑ b ↓	a ↓ b ↑	a ↑ b ↓	a ↓ b ↑	a ↑ b ↓	a ↓ b ↑
Strain no.	46	31	8	10	31	45
Figure no.	1	2	3	4	5	6

Activity curves for miconazole in selected *Candida* strains which best illustrate the pattern of MIC variation after the first (a) and the second (b) week for all three experiments of EF exposure are shown in Scheme 1 and Figs. 1-6.

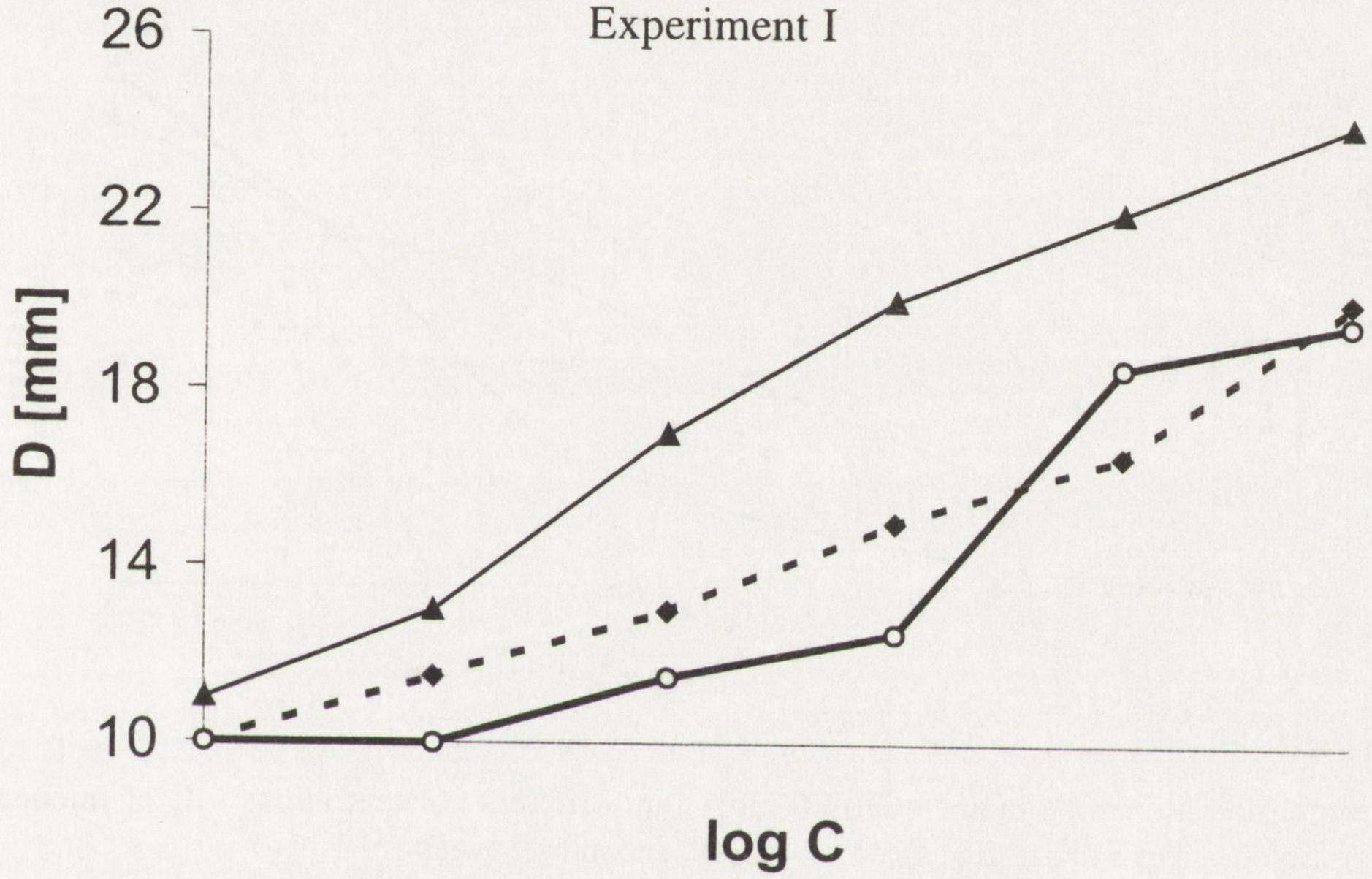

Fig.1. Growth inhibition zone diameters – D (mm) after different concentrations – C of miconazole *Candida* strain no 46 exposure to EF after first (a) and second (b) week

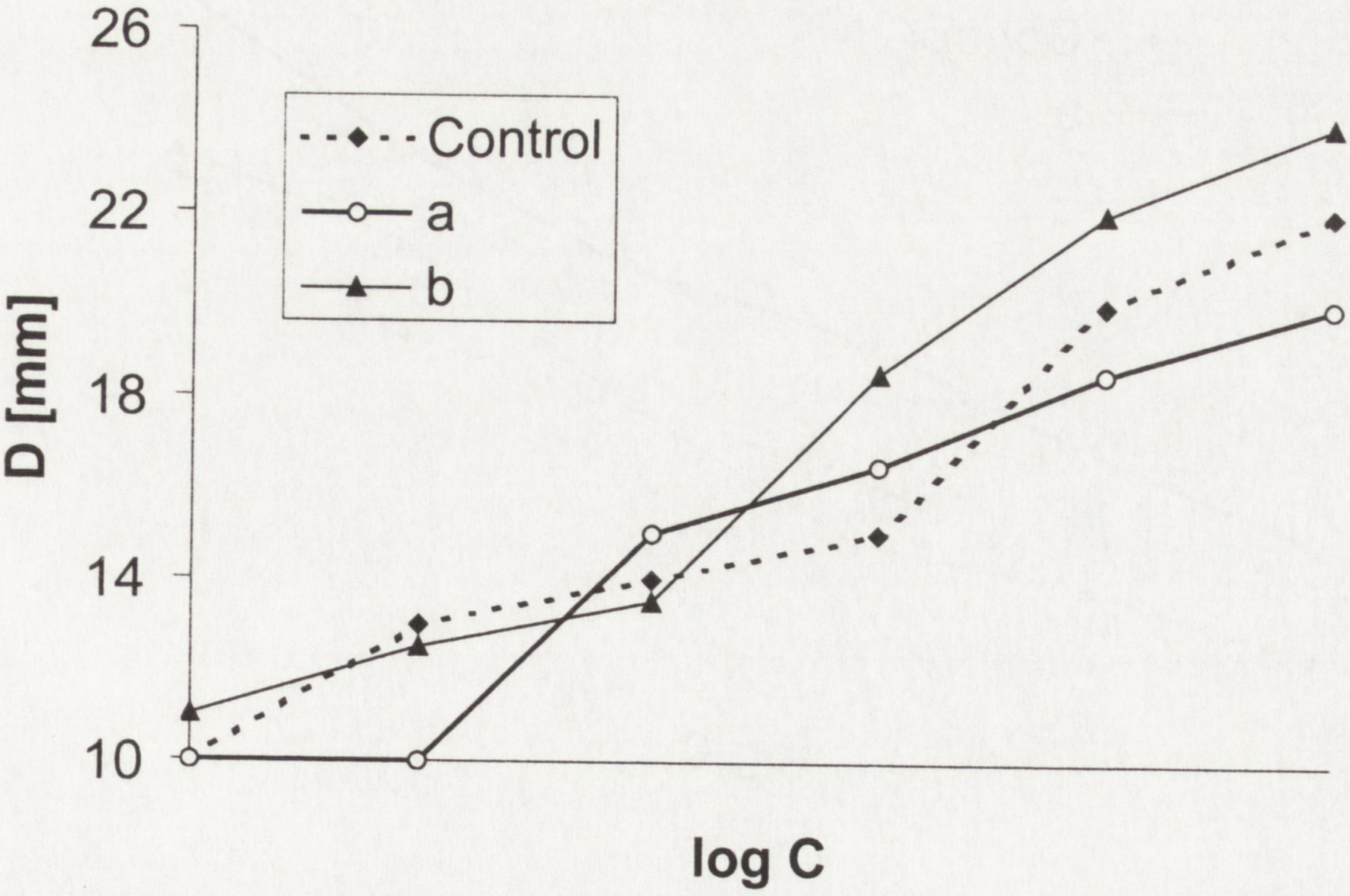

Fig.2. Growth inhibition zone diameters – D (mm) after different concentrations – C of miconazole *Candida* strain no 31 exposure to EF after first (a) and second (b) week

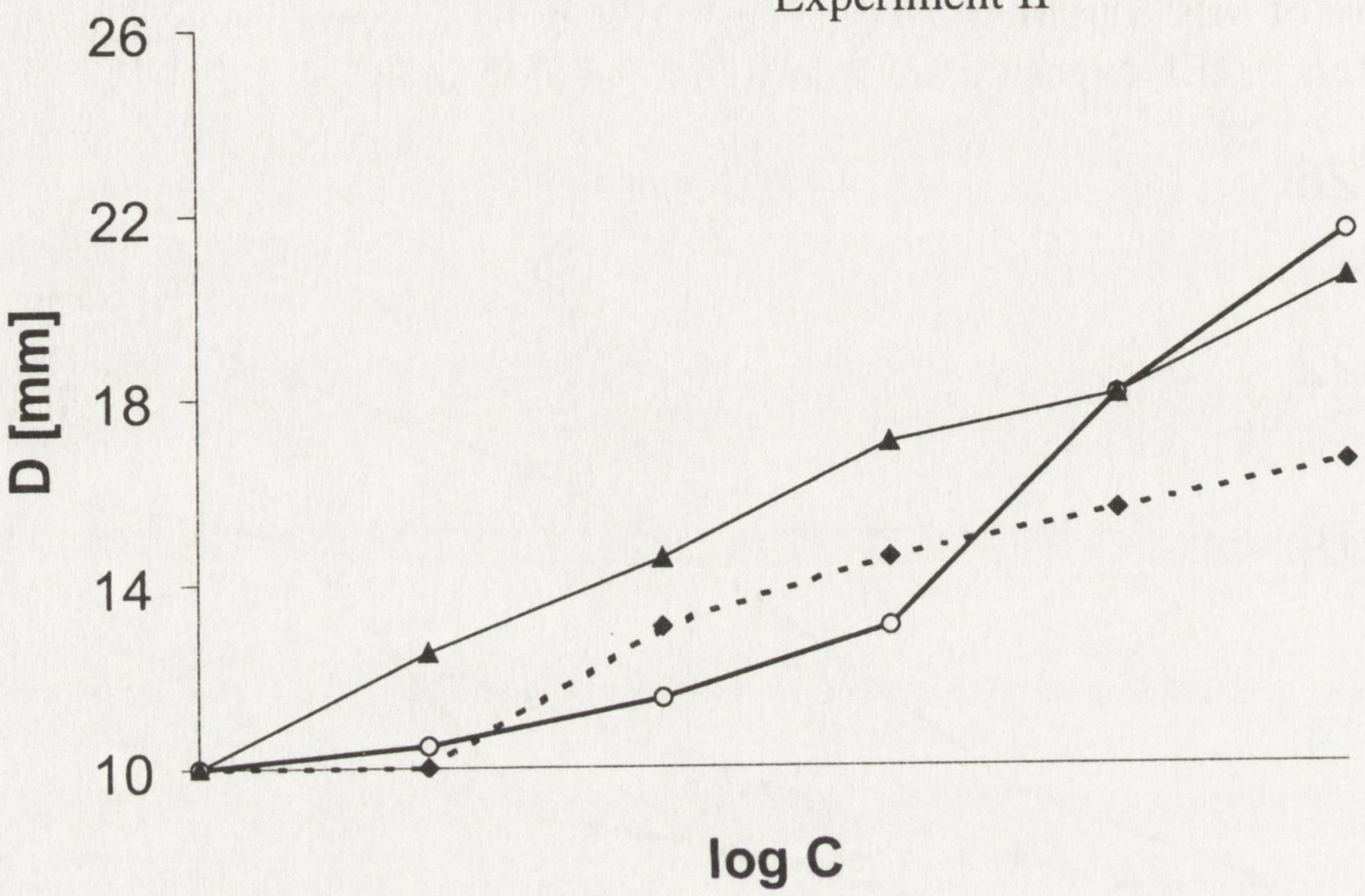

Fig.3. Growth inhibition zone diameters – D (mm) after different concentrations – C of miconazole *Candida* strain no 8 exposure to EF after first (a) and second (b) week

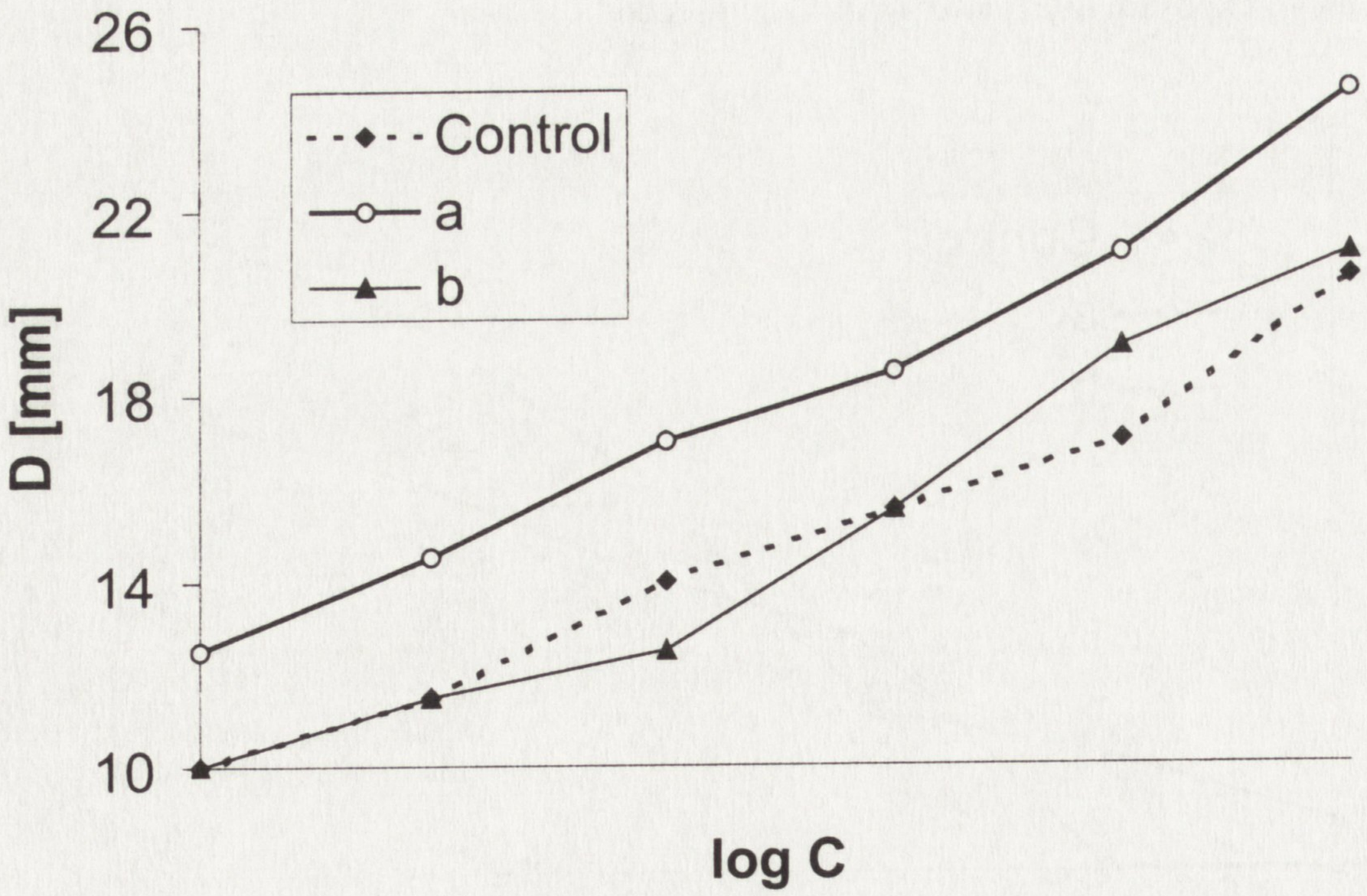

Fig.4. Growth inhibition zone diameters – D (mm) after different concentrations – C of miconazole *Candida* strain no 10 exposure to EF after first (a) and second (b) week

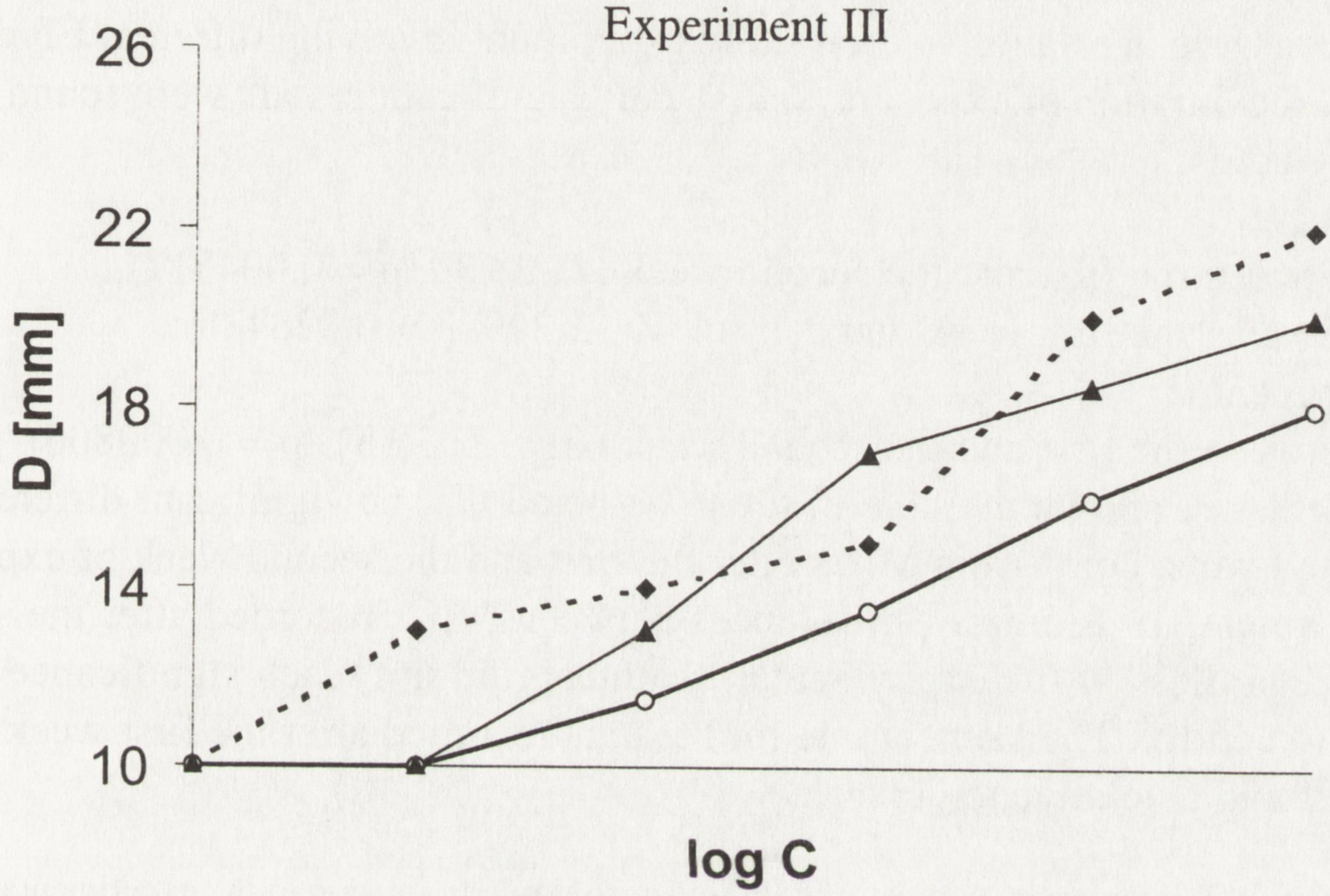

Fig.5. Growth inhibition zone diameters – D (mm) after different concentrations – C of miconazole *Candida* strain n0 31 exposure to EF after first (a) and second (b) week

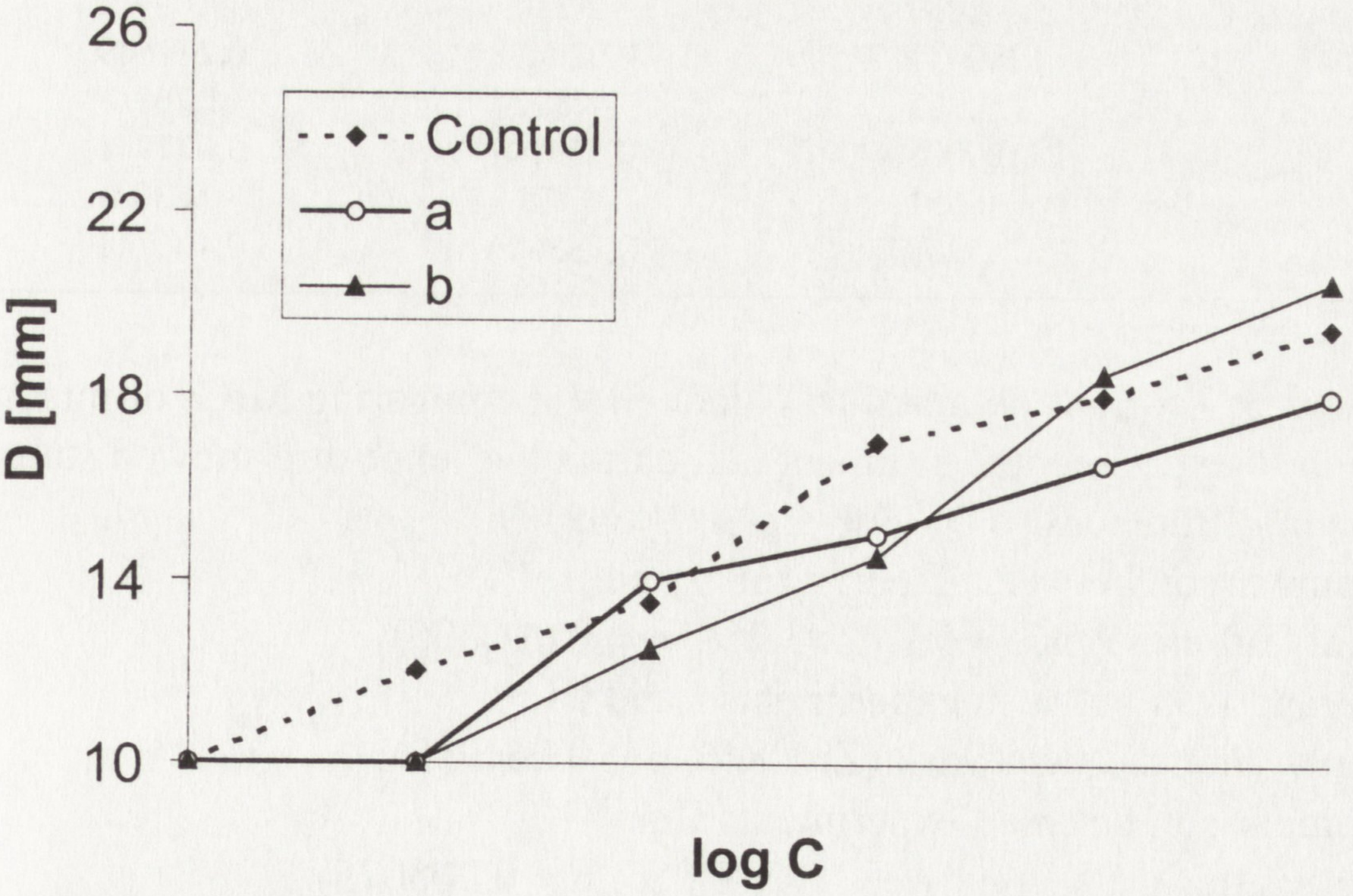

Fig.6. Growth inhibition zone diameters – D (mm) after different concentrations – C of miconazole *Candida* strain no 45 exposure to EF after first (a) and second (b) week

Using Wilcoxon rank test for the assessment of differences while comparing MICs of miconazole in relation to each study experiment involving different EF parameters and the duration of exposure, statistically significant results were found in the following cases:

* experiment I:

α between the first and the second week (Z: 2.839, p = 0.004517),

α between the first week and control (Z: -2.220, p = 0.026438);

** experiment II:

α between the first and the second week only (Z: 3.351, p = 0.000806).

These results are shown in Table 2. It can be noted that no significant differences were found when comparing MICs after the first and the second week of exposure in experiment III. Similarly, differences between MICs recorded after the second week, regardless of the experimental treatment, did not reach significance in comparison to control. The same is true for the data recorded after the first week in experiments I and II relative to control.

Table 2. Z value in Wilcoxon test comparing MICs of miconazole in relation to the experimental treatment and duration of EF exposure (a – first week, b – second week) against control (k)

Experiment	Comparison a – b p-value	Comparison a – k p-value	Comparison b – k p-value
I	2.839	-2.220	-1.099
	0.004517*	0.026438*	0.271649
II	3.351	-1.887	-1.763
	0.000806*	0.059107	0.077941
III	0.651	-0.871	-0.830
	0.525847	0.383686	0.406741

*statistically significant difference

The analysis of Z values obtained in Wilcoxon test comparing MICs of miconazole in relation to experimental treatment and duration of exposure showed statistically significant differences ($0.000806 \leq p \leq 0.064919$):

❑ comparison between experiments I and II:

→ only after the first week (Z: 3.351, p = 0.000806);

❑ comparison between experiments I and III:

→ only after the first week (Z: 1.846, p = 0.064919);

❑ comparison between experiments II and III:

→ only after the second week (Z: -3.085, p = 0.008075).

Details are shown in Table 3. No statistically significant differences were found when comparing MICs of miconazole after the second week of EF exposure in strains under study in experiments I and II, as well as in strains under study in experiments I and III. Similarly, no statistically significant difference was detected when

comparing MICs of miconazole after the first week of EF exposure in strains in experiments II and III.

Table 3. Z value in Wilcoxon test in the comparison of MICs of miconazole in relation to experimental treatments and duration of EF exposure

Comparison between experiments	First week Z-value p-value	Second week Z-value p-value
I vs II	3.351 0.000806*	1.590 0.111779
I vs III	1.846 0.064919*	1.590 0.255997
II vs III	1.193 0.232988	-3.085 0.008075*

*statistically significant difference

DISCUSSION

Apart from the determination of the aetiologic factor responsible for infection, antifungal chemotherapy rationale is based on the microorganism's susceptibility to a given drug (Pawlik 1980, Zaremba 1994, Kurnatowska 1995). In vitro activity of antifungal chemotherapeutic agents, as in the case of antibacterials, can be assessed by a variety of methods, also quantitative ones. One of these methods is to determine, for a given compound, the minimal concentration which is effective in suppressing fungal growth (MIC – minimal inhibitory concentration). High in vitro activity of a compound in relation to a given fungal strain corresponds to low MIC (Kadłubowski 1971).

The impact of EF on bacteria and fungi is ambiguous. Some investigators (Riabtseva and Kuzminski 1981, White et al. 1982, Kozakov and Krasilnikov 1986, Ramon et al. 1987, Schwartz et al. 1989, Kirschvink and Kobayashi 1992) reported inhibitory effects on bacterial growth in *Shigella*, *Escherichia*, and *Mycobacterium* species, while others found that EF stimulated the growth of *Bacillus* (Ramon et al. 1987) or failed to produce any effect at all on the test organisms (Moore 1979). Studies investigating the influence of magnetic field on pathogenic fungi of the species *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum canis*, and *Scopulariopsis brevicaulis* have also proved non-conclusive (White et al. 1982; Budak et al. 1996, 1997, 1998; Valberg et al. 1997). According to Budak et al. (1997, 1998) magnetic field suppresses the growth of dermatophytes, the mycostatic effect being directly proportional to the duration of exposure and to the frequency and intensity of the field; the most susceptible microorganism proved to be *Trichophyton mentagrophytes*, and *T. rubrum* was the least susceptible.

A study conducted by Mamos (1999) found that exposure to resonating magnetic fields affects a number of measurable morphometric parameters in fungi of the genus *Candida*, as well as their physiology, with an overall negative effect on the biological activity of these microorganisms.

Our own study indicates that independently of the duration of exposure, an alternating EF produces a variety of effects in fungal cells, as reflected by changes in miconazole susceptibility of *Candida* strains. MICs were calculated in order to evaluate the efficacy of miconazole in *Candida* strains exposed to electromagnetic field.

The duration of exposure seems to be an important factor, independently of the field's parameters, in determining the impact of an electromagnetic field on fungal cells to a sufficient degree in order to modify their biology as reflected by changes in their miconazole susceptibility.

CONCLUSIONS

(1) In the majority of cases the susceptibility of *Candida* species to miconazole decreased (higher MICs) after the first week of EF exposure, regardless of the EF's parameters. This was followed by an increase in susceptibility (lower MICs) after the second week of exposure to EF of 2 mT intensity and frequency 3 Hz (experiment I) and an EF of 9 mT intensity and 12.5 Hz frequency (experiment II) relative to control. An increase in susceptibility (lower MICs) was observed in the second week of exposure, whatever the parameters of the EF.

(2) The application of low intensity, low frequency electromagnetic field for a period of at least two weeks may be beneficial in the treatment of mycoses caused by pathogenic fungi of the genus *Candida*.

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