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EFFECTS OF ELECTROMAGNETIC FIELDS AND THEIR SHIELDING ON THE QUALITY OF CARROT (*Daucus carota* L.) SEEDS

WPLYW PÓL ELEKTROMAGNETYCZNYCH ORAZ ICH EKROWANIA NA JAKOŚĆ NASION MARCHWI (*Daucus carota* L.)

Abstract: The aim of the study was to determine the effect of electromagnetic fields and their shielding on carrot seed quality. Three sectors were separated on the device emitting electromagnetic fields: "E" - sector emitting electromagnetic radiation with the predominance of the electrical component, "EM" - sector emitting electromagnetic radiation without domination of its components and "M" - sector with a predominance of magnetic component. Fields generated by the device were also shielded with ADR TEX screen, based on a nanocomposite in which the electric component of the electromagnetic radiation is absorbed by water dispersed in a dielectric matrix in various ways. The composites exhibit high dielectric absorption and shield electric fields within the frequency range from ~100 MHz to ~100 kHz. Seed germination and vigour were evaluated at 20 °C in darkness. Mycological analysis was performed using a deep-freeze blotter test. Exposure of seeds to radiation with the predominance of the electrical component and electromagnetic radiation without domination of its components combined with shielding of electromagnetic fields with ADR TEX (*E*+ADR TEX and *EM*+ADR TEX) increased seed germination energy and germination capacity compared to these treatments without shielding and control. The percentage of abnormal diseased seedlings in treatments with shielding of electromagnetic fields with ADR TEX (*E*+ADR TEX, *EM*+ADR TEX and *M*+ADR TEX) was significantly lower than in the treatments without shielding and in control. None of the treatments affected seed vigour. Generally, exposure of seeds to electromagnetic radiation did not influence the incidence of fungi.

Keywords: electromagnetic field, ADR TEX shielding, seed germination, vigour, fungi

Introduction

Nowadays, searching for new non-chemical methods of plant protection against pathogens, including seed treatments, is a matter of great importance in agriculture. A lot of attention has been paid to physical methods. They include i.a. exposure of seeds to static magnetic field, electromagnetic field, gamma radiation, X-rays, UV-radiation, microwave

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and electron paramagnetic resonance [1]. These methods are environmentally friendly and safe for the applicator.

Electromagnetic fields are produced by permanent magnets, electrical appliances, power lines, electric wiring and also by natural sources such as the Sun [2]. The electromagnetic field consists of two interrelated components - electric and magnetic. Dannehl [3] reported that both magnetic and electric fields are the physical factors that improve seed quality. The positive impacts of these fields include better seed germination, seedling growth and higher yields. It has been found that seedlings obtained from treated seeds are more resistant to unfavourable environmental conditions [4]. However, Das and Bhattacharya [2] showed that high electric field had a negative effect on the growth of gram (*Cicer arietinum* L.) roots.

In general, there is lack of data concerning the impact of low frequency electromagnetic fields on seed quality. Jedlicka et al. [5] found that 50 Hz electromagnetic fields with the induction of 20, 40 and 60 mT and the exposure time of 20 minutes a day had a stimulatory effect on seed germination, plant growth and size of tomato fruits. Hasan et al. [6] conducted in laboratory a simulation of magnetic field of 400 kV high voltage transmission line. Authors evaluated the growth of maize plants which were inserted far away from the magnetic field and plants exposed to magnetic fields of 0.045 mT and 0.084 mT. Magnetic field had a negative influence on the maize growth. Different results were obtained by Bhattacharya and Barman [7] who studied the impact of high voltage lines on the growth of mustard. Authors found that the plant growth and pod numbers per plant were higher in exposed plants compared to non-exposed ones.

Rochalska et al. [8] claimed that pre-sowing stimulation with 16 Hz alternating magnetic field with induction 5 mT had a positive influence on wheat seed germination. On the other hand, Balakhnina et al. [9] did not observe the effect of exposure of wheat seeds to magnetic field strength of 30 mT and 50 Hz frequency for 30 s on their germination and seedling growth processes, but they found that plant antioxidant potential under soil hypoxia was increased.

Vashisth et al. [10] reported that exposure of tomato seeds to 100 mT static magnetic field for 30 min resulted in the enhancement of seed germination and vigour and in the increase in seedling length and dry weight. Similar results obtained Hozayn et al. [11] in case of onion seeds exposed to 0.06 T magnetic field for 30 min. The treatment improved seed germination percentage, germination rate, speed germination index and seedling growth parameters.

Grzesik et al. [12] showed the additive role of pulsed radio frequency (PRF) in breaking dormancy of apple seeds cv. Ligol. The exposure of seeds for 1 h to PRF (25 V, 4 Hz, 20 ms) during their stratification increased the percentage and dynamics of seed germination as well as dynamics of seedling growth.

There is lack of data to explain the effects of electromagnetic fields on pathogenic fungi colonizing seeds.

The aim of the study was to determine the effect of electromagnetic fields and their shielding on germination and vigour of carrot seeds and their infestation with fungi.

Material and methods

Material

Carrot (*Daucus carota* L.) seeds cv. Amsterdam were used in the experiment. They were subjected to electromagnetic fields emitted by a specially designed device (EM emitter) as shown in Figure 1.

The device emitting electromagnetic fields is composed of 3 spiral coils, constructed using a glass-epoxy laminate coated with copper (20 μm), which is used for printed circuits. The coils are placed directly behind the upper wall of the housing which is made of plastic and connected by electrical wires. The wiring diagram is shown in Figure 1, the electrical wires are separated from the coils at a distance of about 5 cm. The distance between the coils is 15 cm. The electricity receiver is a 57 W halogen bulb, which was placed in the lamp outside the casing of the device, within a few meters distance to minimize the influence of thermal radiation of the bulb on the tested object. A black shade on the lamp protected against light exiting to the outside and its possible influence on the experiment's objects.

Three sectors were separated on the device. Thus different types of electromagnetic fields were produced. They were marked as "E" - a sector emitting electromagnetic radiation with the predominance of the electrical component, "EM" - a sector emitting electromagnetic radiation without domination of its components and "M" - a sector with a predominance of magnetic component. The method for creating the predominance of electric (E) and magnetic field (M) is shown in the diagram (Fig. 1). When the bulb is lit, between the bulb and zero (neutral) there is a predominance of magnetic field (M), between the lit bulb and the phase (live) there is electric and magnetic, where the antenna is connected only to the phase (live) then there is a predominance of electric field (E).

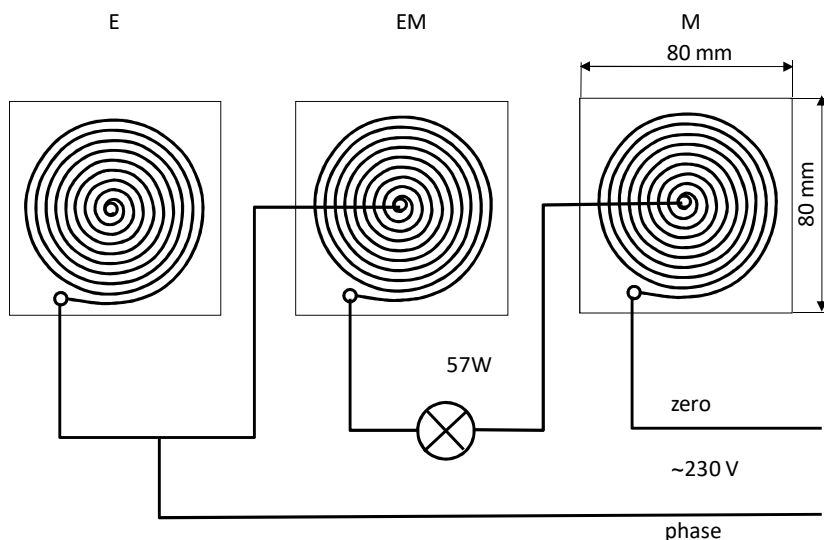


Fig. 1. Schematic diagram of the equipment used in the application of electromagnetic fields (EMF): E - electromagnetic field with the predominance of the electrical component, M - electromagnetic field with a predominance of magnetic component, EM - electromagnetic field without domination of its components

Petri dishes with seeds were placed individually in the mentioned sectors, emitting differential electromagnetic fields. Specific values of field components generated by the device are shown in Tables 1.

Table 1

Values of the intensity of electromagnetic fields measured 13 mm above the field generating device. Measurements were made using Maschek E-100. The field generating device was loaded with 57 W receiver

Components	Sector E	Sector EM	Sector M
$SE [V \cdot m^{-1}]$	2290	2330	136
$SM [\mu T]$	0.073	4.880	4.710

SE - electrical component, *SM* - magnetic component, Sector E - a sector emitting electromagnetic field with the predominance of the electrical component, Sector EM - a sector emitting electromagnetic field without domination of its components, Sector M - a sector emitting electromagnetic field with a predominance of magnetic component

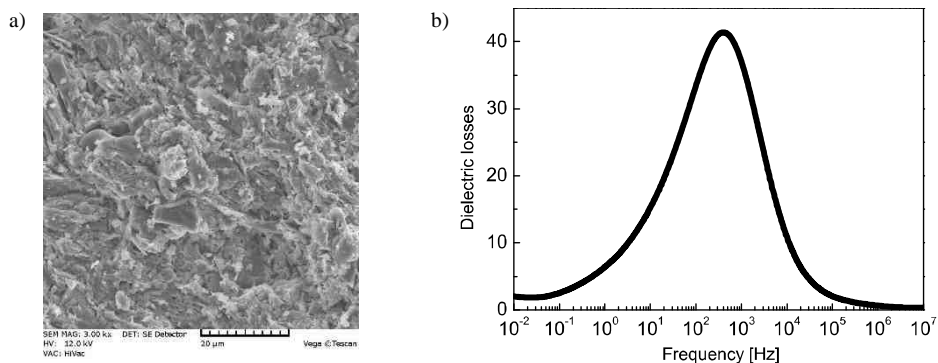


Fig. 2. a) Scanning electron microscopy image of a fracture of a ceramic matrix (pressure, $p = 20$ MPa, temperature $T_{\text{cal}} = 1100$ °C); b) Frequency dependence of dielectric loss, $\tan \delta$ of the composite shield at 16 °C and relative humidity of 60 %

For comparison, the quality of carrot seeds in sectors E, EM and M of the field generating device, after its accurate wrapping with shielding material ADR TEX was evaluated. ADR TEX has been developed and patented by Wosinski [13, 14], a co-author of this paper. He has designed an advanced technology based on a nanocomposite in which the electric component of the electromagnetic radiation is absorbed by water dispersed in a dielectric matrix in various ways. As the frequencies of the dielectric absorption bands of water are determined by its state of aggregation (Fig. 2), an ice-like behaviour of water has been engineered by nanopore confinement. The advanced dielectric composite consists of a polymer or ceramic matrix in which water is randomly dispersed in various ways. The composite exhibits high dielectric absorption in the low-frequency range and does not need grounding. The shielding ability of the composite can be tailored to various applications by selecting an appropriate microstructure for the dielectric matrix (varying the pore sizes by processing conditions or choosing characteristic properties) and by loading with aqueous solutions of various hydrated salts and modifiers. The composites exhibit high dielectric absorption and shield electric fields within the frequency range from ~100 mHz to ~100 kHz. The electric field strength can be decreased by about two orders of

magnitude, and the harmful effects of the electric component of electromagnetic fields on the living organisms can be lowered considerably.

Figure 2a shows an example of the topography of a ceramic matrix. The frequency dependence of dielectric absorption of the matrix impregnated with the aqueous solution containing $MgCl_2$ and modifiers is presented in Figure 2b.

An example of the shielding efficiency of such a flexible EMF screen, composed of an active part consisting of a composite with a dielectric matrix made from a selected fabric inserted between two protective layers, is shown in Figure 2. Comparing the field distributions shown in Figure 3a) and 3b) one can observe that the electric field shielding is highly efficient.

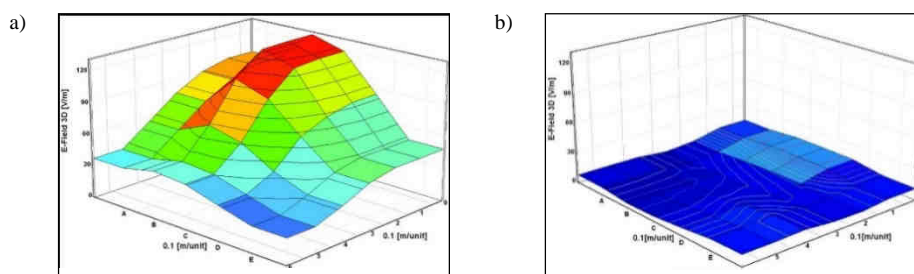


Fig. 3. a) Spatial distribution of electric field over an electric source without shielding; b) electric field distribution over the same electric source after shielding with a flexible dielectric screening

Specific values of field components generated by the device after shielding with ADR TEX screen, in particular sectors are shown in Table 2.

Table 2
Values of the intensity of electromagnetic fields measured 13 mm above the field generating device after shielding with ADR TEX. Measurements were made using Maschek E-100. The field generating device was loaded with 57 W receiver

Components	Sector E	Sector EM	Sector M
SE [$V \cdot m^{-1}$]	215	207	185
SM [μT]	0.141	5.110	5.140

For explanations see Table 1

Methods

Seed germination was tested according to ISTA Rules [15], in each treatment 300 seeds (six 50 seed replicates) were evaluated. Seeds were placed in Petri dishes (50 seeds per dish), on 6 layers of blotter paper moistened with distilled water. Petri dishes with seeds were incubated at 20 °C in darkness. They were placed directly on the top of the device emitting electromagnetic fields. Germination energy was evaluated after 7 days of incubation, whereas germination capacity, the percentages of abnormal diseased seedlings, abnormal deformed seedlings, fresh seeds and dead seeds were determined after 14 days. Additionally the percentage of germinating seeds (G_{max}) was calculated on the base of seed vigour test.

Seed vigour evaluation was also conducted in six 50 seed replicates. The test conditions were the same like in the previous test. Germinating seeds were counted daily and removed from the plate, until no new germs appeared. Seeds with the radicle at least 1 mm long were considered as germinating. Based on obtained results the speed and uniformity of germination i.e. T_{10} - time to 10 % of G_{max} , T_{75} - time to 75 % of G_{max} , MGT - mean germination time, U_{75-25} - time between 25 and 75 % of G_{max} were calculated according to Joosen et al. [16].

Mycological analysis was performed using a deep-freeze blotter test. In each treatment 200 seeds (four replicates of 50 seeds) were evaluated. They were placed in 9 cm diameter Petri dishes on six layers of blotter moistened with distilled water, 20 seeds per dish. The seeds were incubated for three days at 20 °C in darkness, next frozen at -20 °C for 24 h, and then incubated at 20 °C under 12 h alternating cycles of NUV (Near Ultraviolet) light and darkness. During incubation Petri dishes with seeds were placed on the top of the device emitting electromagnetic fields. Fungi were identified on the base of their growth and sporulation using a stereomicroscope and a compound microscope [17-19]. The percentages of seeds infested with individual fungi were determined.

The results obtained were evaluated by one-way analysis of variance followed by Duncan's multiple range test, at a level $\alpha = 0.05$. Parameters characterising seed vigour were calculated using Germinator software [16].

Results

Untreated seeds were characterized by low germination capacity and high percentages of abnormal diseased seedlings and dead seeds. They were infested to a large extent with fungi, especially *Alternaria alternata* (Fr.) Keissler, *Alternaria radicina* Meier, Drechsler & E.D. Eddy and *Fusarium* spp. Moreover, *Alternaria dauci* (J.G. Kuhn) J.W. Groves & Skolko, *Drechslera* spp., *Epicoccum nigrum* (Link) and *Stemphylium botryosum* Wallr. were detected (Table 3).

Table 3
Effects of electromagnetic fields on the seed infestation with fungi (the percentage of infested seeds)

Treatments	<i>Alternaria alternata</i>	<i>Alternaria dauci</i>	<i>Alternaria radicina</i>	<i>Drechslera</i> spp.	<i>Epicoccum nigrum</i>	<i>Fusarium</i> spp.	<i>Stemphylium botryosum</i>
C	98.5 a	8.0 a	72.0 b	0 a	21.5 c	58.5 a	1.0 a
E	99.0 a	8.5 a	77.5 b	3.5 b	14.0 bc	62.0 a	2.5 a
E+ADR TEX	98.0 a	10.0 a	82.0 b	2.0 b	12.0 bc	67.0 a	4.0 a
EM	99.5 a	8.5 a	74.5 b	1.0 ab	15.5 bc	64.0 a	2.0 a
EM+ADR TEX	99.0 a	8.0 a	79.5 b	1.5 ab	9.0 ab	65.0 a	1.5 a
M	96.0 a	9.0 a	60.0 a	3.5 b	16.5 bc	64.0 a	1.0 a
M+ADR TEX	95.5 a	7.5 a	77.5 b	3.5 b	5.0 a	63.0 a	4.0 a

Means in columns followed by the same letters are not significantly different at $\alpha = 0.05$ level according to Duncan's test. C - untreated seeds, E - electromagnetic radiation with the predominance of the electrical component, E+ADR TEX - electromagnetic field with the predominance of the electrical component shielded with ADR TEX screen, EM - electromagnetic field without domination of its components, EM+ADR TEX - electromagnetic field without domination of its components shielded with ADR TEX screen, M - electromagnetic field with a predominance of magnetic component, M+ADR TEX - electromagnetic field with a predominance of magnetic component shielded with ADR TEX screen

The percentage of germinating seeds (G_{\max}) in *E*+ADR TEX and *M*+ADR TEX treatments was significantly lower than in control. Exposure to electromagnetic radiation with the predominance of the electrical component and electromagnetic radiation without domination of its components combined with the shielding of electromagnetic fields by means of ADR TEX screen (*E*+ADR TEX and *EM*+ADR TEX) resulted in an increase in seed germination energy and germination capacity compared to these treatments without shielding and control. The improvement of germination capacity was observed also in case of seeds subjected to electromagnetic radiation with the predominance of the electrical component (*E*). All treatments in which shielding was applied i.e. *E*+ADR TEX, *EM*+ADR TEX and *M*+ADR TEX decreased the percentage of abnormal diseased seedlings compared to control. At the same time the number of these seedlings in treatments with shielding of electromagnetic fields with ADR TEX screen was significantly lower than in the treatments without shielding. The highest seed germination energy and germination capacity and the lowest percentage of abnormal diseased seedlings were found in *E*+ADR TEX treatment. Exposure of seeds to electromagnetic radiation did not affect the percentages of abnormal deformed seedlings and dead seeds. Only *M*+ADR TEX treatment increased the percentage of fresh seeds compared to control (Table 4).

Table 4

Effects of electromagnetic fields on seed germination

Treatments	G_{\max} [%]	Germination energy [%]	Germination capacity [%]	Abnormal diseased seedlings [%]	Abnormal deformed seedlings [%]	Fresh seeds [%]	Dead seeds [%]
C	82.2 b	43.3 b	45.0 a	34.3 c	0 a	2.3 ab	18.3 a
<i>E</i>	80.7 b	50.7 a-c	54.7 bc	31.0 c	1.0 a	1.0 a	12.3 a
<i>E</i> +ADR TEX	72.3 a	61.7 d	63.7 d	13.3 a	0 a	2.7 ab	20.3 a
<i>EM</i>	85.0 b	46.0 ab	48.7 ab	30.0 c	0 a	1.0 a	17.7 a
<i>EM</i> +ADR TEX	82.3 b	57.7 cd	59.7 cd	21.3 b	0.3 a	2.3 ab	16.3 a
<i>M</i>	78.7 ab	47.0 ab	47.3 ab	30.3 c	1.0 a	4.0 bc	17.3 a
<i>M</i> +ADR TEX	70.3 a	53.0 bc	53.3 a-c	19.3 b	0.3 a	7.7 c	19.3 a

Means in columns followed by the same letters are not significantly different at $\alpha = 0.05$ level according to Duncan's test. G_{\max} - the percentage of germinating seeds. For other explanations see Table 3

Table 5

Effects of electromagnetic fields on seed vigour

Treatments	Speed of germination [days]			Uniformity of germination [days]
	T_{10}	T_{75}	<i>MGT</i>	U_{75-25}
C	1.97 a	3.67 a	3.20 a	1.30 a
<i>E</i>	2.06 a	3.69 a	3.23 a	1.24 a
<i>E</i> +ADR TEX	2.05 a	3.49 a	3.10 a	1.10 a
<i>EM</i>	2.06 a	3.47 a	3.13 a	1.09 a
<i>EM</i> +ADR TEX	1.94 a	3.54 a	3.12 a	1.24 a
<i>M</i>	1.97 a	3.51 a	3.13 a	1.20 a
<i>M</i> +ADR TEX	1.93 a	3.48 a	4.55 a	1.20 a

Means in columns followed by the same letters are not significantly different at $\alpha = 0.05$ level according to Duncan's test. T_{10} - time to 10 % of G_{\max} , T_{75} - time to 75 % of G_{\max} , *MGT* - mean germination time, U_{75-25} - time between 25 and 75 % of G_{\max} . For other explanations see Table 3

None of the treatments affected seed vigour i.e. speed (T_{10} , T_{75} , MGT) and uniformity (U_{75-25}) of germination (Table 4). Exposure of seeds to electromagnetic radiation did not influence the incidence of *A. alternata*, *A. dauci*, *Fusarium* spp. and *S. botryosum*. Seed infestation with *A. radicina* decreased only after exposure of seeds to field with a predominance of magnetic component, M , compared to control. The percentage of seeds infested with *E. nigrum* was reduced after $EM+ADR$ TEX and $M+ADR$ TEX treatments. On the other hand, seeds exposed to electromagnetic radiation with the predominance of the electrical component and to electromagnetic field with a predominance of magnetic component with and without shielding (E , $E+ADR$ TEX, M , $M+ADR$ TEX) were infested with *Drechslera* spp. to a higher degree than untreated seeds (Table 5).

Discussion

It has been found in the present investigation that exposure of carrot seeds to electromagnetic radiation with the predominance of the electrical component and electromagnetic radiation without domination of its components combined with shielding of electromagnetic fields with ADR TEX screen ($E+ADR$ TEX and $EM+ADR$ TEX) increased seed germination energy and germination capacity compared to these treatments without shielding and to untreated seeds. Shielding decreased the electric field strength in sectors E and EM of the device emitting electromagnetic fields over ten times, respectively from 2290 to 215 $V \cdot m^{-1}$ and from 2330 to 207 $V \cdot m^{-1}$. Hence, harmful effects of the electric component on seeds were lowered considerably. Das and Bhattacharya [2] reported that the application of electric field of 150 $kV \cdot m^{-1}$ on gram seeds for 20 min decreased the root length by 14 % compared to control. With the increase of electric field strength the root growth was reduced further. Dorna et al. [20] investigated the effect of a permanent magnetic field together with shielding of an alternating electric field with ADR-4 (Advanced Dielectric Radiation Trap) on seed germination of two carrot cultivars Nantejska and Perfekcja. They found that ADR-4 improved germination capacity of seeds in both cultivars compared to untreated seeds. Racuciu and Creanga [21] observed an acceleration of the plant growth and root development as well as stimulation of protein synthesis after *Cucurbita pepo* seeds' exposure to low frequency electromagnetic field.

All treatments in which shielding was applied i.e. $E+ADR$ TEX, $EM+ADR$ TEX and $M+ADR$ TEX decreased the percentage of abnormal diseased seedlings compared to control and the treatments without shielding. Dorna et al. [20] also observed the reduction in the percentage of diseased seedlings in two carrot cultivars and the decrease in the percentage of dead seeds in cultivar Nantejska after shielding of an alternating electric field with ADR-4. It has been found in our study that the use of ADR TEX screen increased the magnetic field strength in all sectors to some extent. Anyway, the values of magnetic field induction were very low and ranged from 0.141 to 5.140 μT . Weak magnetic field does not cause thermal effects, it has too low energy to raise the temperature of the object. However, non-thermal effects can stimulate in cells protein biosynthesis of "stress response" similar to response to heat or other physical stresses [22]. Shabrangi et al. [23] found in *Zea mays* L., after pre-sowing stimulation of seeds with extremely low frequency electromagnetic fields, an increase in activity of stress enzymes: superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in root and shoot tissues. The decrease in the number of diseased seedlings may indicate activation of "stress response" in seedlings after their exposure to electromagnetic fields what probably provided some protection against

infection caused by pathogenic fungi. Shabrangi et al. [24] observed a positive effect of electromagnetic field on maize seed germination. Germination of seeds treated with 3 and 10 mT for 4 h was higher by 33.3 and 21 % respectively, in comparison with control. Authors observed also the increase of protein content in 14 days seedlings after pre-sowing treatment with 3 mT. They suggested that protein accumulation may be related to the synthesis of stress tolerance proteins and alleviation of stress conditions. Krolicka et al. [25] presume that ADR-4 through its influence on water in transformed callus culture of *Ammi majus* L. stimulated the plant's defence system that resulted in an increase of coumarin and furanocoumarin production.

Generally, exposure of seeds to electromagnetic radiation did not influence the incidence of fungi. Examined carrot seeds were infested to a large extent with fungi. From among pathogens or potential pathogens *Alternaria dauci*, *A. radicina* and *Fusarium* spp. were detected. The percentages of seeds infested with *A. radicina* and *Fusarium* spp. in control were 72.0 and 58.5 %, respectively. Such severe infection probably resulted in a high infection of inner tissues. In this case a control of pathogens is very difficult. Infection by *A. radicina* was reduced to some extent only after exposure of seeds to field with a predominance of magnetic component (*M*). It may indicate a stronger effect of the magnetic field than the electric one on the development of this fungus. According to Afzal et al. [26] static or alternating magnetic fields may have positive impacts on seed germination, a plant growth and yield and they can also reduce negative effects of fungi infection. However, the influence of alternating magnetic field depends on its intensity, time of exposure and plant species. Out of positive effects Grabowska et al. [27] mentioned an acceleration of seed germination, an increase in yield and chlorophyll content, whereas negative impacts may include a slowdown of seed germination, deterioration of seed quality, decrease of dynamic and static module elasticity, a yield decrease, as well as lowering the survivability in field conditions.

In the present experiment the applied low frequency electromagnetic fields without and with shielding with ADR TEX screen did not influence carrot seed vigour. So far much research has been devoted to investigating the effect of magnetic fields on the speed of seed germination. The intensity of the applied magnetic fields and the time of seed exposure varied greatly. In general, magnetic fields speeded up seed germination. Dorna et al. [20] reported that carrot seeds of cv. Perfekcja exposed to magnetic field germinated faster than untreated seeds, whereas in case of cv. Nantejska a delay in seed germination was noted. The authors did not observe either the effect of ADR-4 on the speed and uniformity of carrot seed germination. Zardzewialy et al. [28] found that sugar beet seeds exposed to magnetic field induction of 40 mT and 50 Hz frequency for 60 s germinated significantly faster than control seeds. According to Podlesna et al. [29] seeds stimulated with magnetic field imbibe and germinate faster than untreated ones because of more advanced enzyme activity. Seedlings grown from stimulated seeds produce longer sprouts and roots and often have better vigour. Pietruszewski et al. [22] reported that the mechanism of influence of electromagnetic fields on plants is still unknown and positive effect on the percentage of seed germination, germination rate and the growth speed is temporary and impermanent.

Conclusions

1. Exposure of seeds to electromagnetic radiation with the predominance of the electrical component and electromagnetic radiation without domination of its components

- combined with shielding of electromagnetic fields with ADR TEX screen (*E*+ADR TEX and *EM*+ADR TEX) increased seed germination energy and germination capacity compared to these treatments without shielding and control.
2. All treatments in which shielding was applied i.e. *E*+ADR TEX, *EM*+ADR TEX and *M*+ADR TEX decreased the percentage of abnormal diseased seedlings compared to the treatments without shielding and control.
 3. None of the treatments affected seed vigour.
 4. Generally, exposure of seeds to electromagnetic radiation did not influence the incidence of fungi.

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