

## Effect of extremely high frequency electromagnetic fields on the microbiological community in rhizosphere of plants

A.A. Ratushnyak<sup>1</sup>, M.G. Andreeva<sup>1</sup>, O.V. Morozova<sup>1</sup>, G.A. Morozov<sup>2</sup>, and M.V. Trushin<sup>3,4\*</sup>

<sup>1</sup>Institute for Ecology of Natural Systems, Tatarstan Academy of Sciences, Kazan, Russia

<sup>2</sup>Kazan State Technical University, Kazan, Russia

<sup>3</sup>Department of Genetics, Kazan State University, Kazan, Russia

<sup>4</sup>Kazan Institute of Biochemistry and Biophysics, Lobachevskiy 2/31, P.O. Box 30, Kazan 420111, Russia

Received January 16, 2007; accepted February 13, 2008

**A b s t r a c t.** Electromagnetic fields (EMF) are widely used to stimulate germination of seeds, improve their quality and speed up the growth of plants. This research was aimed at investigation of the influence of EMF and extensively used seed disinfectant (thiram) on the content of rhizosphere microflora of *Pinus sylvestris* seedlings. For this purpose, pine seeds were treated with EMF (alone or in combination with thiram), and the rhizosphere microflora was analysed. Various growth media were used to classify bacteria originated from pine rhizosphere. We found that EMF treatment resulted in proliferation of agronomically useful microorganisms including nitrogen-fixing ones. The suggested approach allows improving the microbiological content of soils and to avoid the use of a big amount of mineral fertilizers.

**K e y w o r d s:** electromagnetic fields, rhizosphere microflora, presowing treatment

### INTRODUCTION

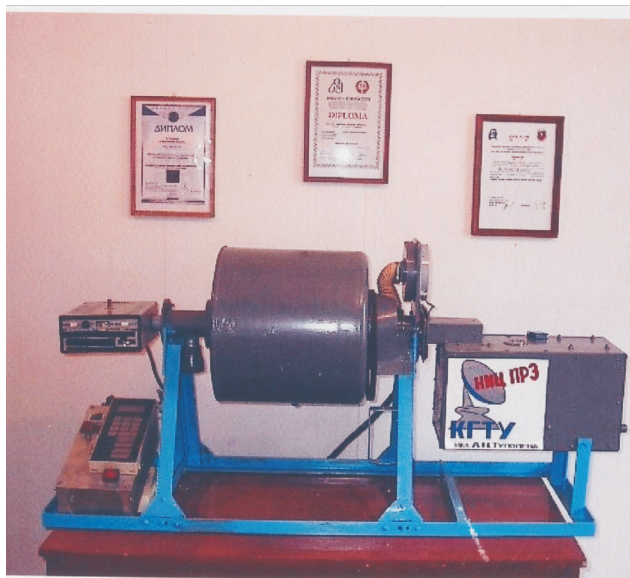
The enhancement of microbiological nitrogen fixation, improvement of structural organization of soil microflora, and inhibition of phytopathological fungi are the main tasks of modern forestry and agronomy (Pretty, 2007; Hazell and Wood, 2007). Generally, various herbicides and insecticides, seed disinfectants and mineral fertilizers are used to solve these tasks. Soil enrichment with some bacteria of *Azotobacter* genus is also performed to fight deleterious fungal species (Gilligan, 2007). However, colonization of plant rhizosphere with beneficial bacteria is occasionally not permanent (Parlevliet, 2002). Sometimes, the artificially introduced microorganisms fall out from biocenological activity and transform into inactive condition.

The aim of the present work was to investigate the influence of electromagnetic fields (EMF) of extremely high frequencies (EHF) and of a widely used seed disinfectant (thiram) on the content of rhizosphere microflora of pine seedlings.

### MATERIALS AND METHODS

For this study, conditioned and nonconforming pine (*Pinus sylvestris*) seeds were provided by forest nursery 'Suburban'. Control seeds were not influenced by any external factor. Other seeds were treated by electromagnetic fields (EMF) alone, thiram (aapirol,  $[(\text{CH}_3)_2\text{NC}(\text{S})\text{S}]_2$ ) alone, or by both EMF and thiram. Presowing treatment of pine seeds was performed using EMF EHF device (Fig. 1) with the following characteristics: frequency – 30-60 GHz, intensity -  $10^{-16}$ – $10^{-10}$  W cm<sup>-2</sup>, time of exposure – 5-15 min (Morozov *et al.*, 2007). To analyse rhizosphere microflora of pine seedlings, soil near the seedling radicles was sampled for a month. For this purpose, pine seedlings (5-8 cm in height) with adjacent soil were withdrawn using special metallic cylinders; this approach allowed us to preserve the natural structure of biocenosis. Soil samples (5 g) were extracted from the cylinders (at depth of 1 and 5 cm) and diluted tenfold with water. Diluted samples (soil suspension) were inoculated on the following selective microbiological media:

\*Corresponding author's e-mail: mtrushin@mail.ru



**Fig. 1.** Physical configuration of EMF EHF device.

1. Peptone agar ( $\text{g l}^{-1}$ : peptone – 100,  $\text{KH}_2\text{PO}_4$  – 5, glucose – 0.3,  $\text{MgSO}_4$  – 0.1) – this medium is used to detect ammonifiers;

2. Amylum-ammoniac agar ( $\text{g l}^{-1}$ :  $(\text{NH}_4)_2\text{SO}_4$  – 2,  $\text{K}_2\text{HPO}_4$  – 1,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  – 1, NaCl – 1,  $\text{CaCO}_3$  – 3, amyllum – 10, agar – 20) – this medium is used to detect actinomycetes and bacteria using mineral nitrogen;

3. Ashbi medium ( $\text{g l}^{-1}$ :  $\text{K}_2\text{HPO}_4$  – 0.2,  $\text{MgSO}_4$  – 0.2, NaCl – 0.2,  $\text{KH}_2\text{PO}_4$  – 0.1,  $\text{CaCO}_3$  – 5, mannitol – 20, agar – 20) – this medium is used to detect nitrogen-fixing microorganisms;

4. Giltai medium, composed of two solutions: A: ( $\text{g l}^{-1}$ )  $\text{KNO}_3$  – 2, asparagine – 1,  $\text{H}_2\text{O}$  – 250 ml; and B: sodium citrate – 2.5,  $\text{MgSO}_4$  – 2,  $\text{KH}_2\text{PO}_4$  – 2,  $\text{CaCl}_2$  – 0.2,  $\text{H}_2\text{O}$  – 500 ml. Solution A and B should then be mixed and bromine thymol blue ( $2 \text{ ml l}^{-1}$ , 1.6% alcohol tincture) should be added to the final variant of Giltai medium: this indicator colours it in green colour. Giltai medium is used to detect denitrifiers;

5. Peptone broth (the same content as peptone agar but without agar). This medium is used to detect ammonifiers.

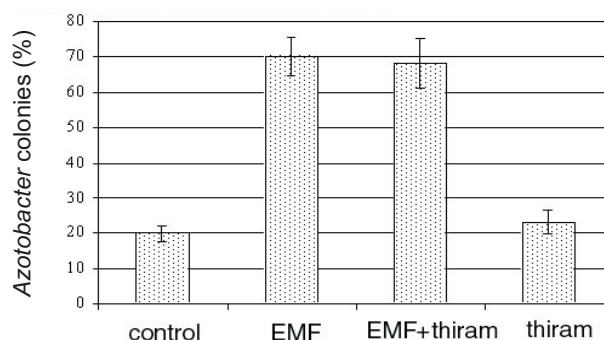
Paired Student test (*t*-test) was used for statistical analysis. A *p* value of  $<0.05$  was considered to indicate significance. Data are presented as mean  $\pm$  standard deviation.

## RESULTS

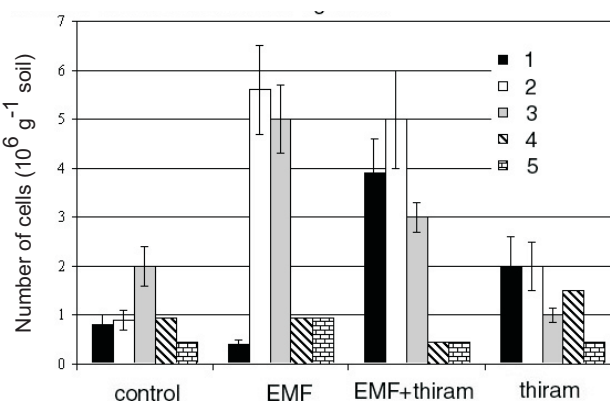
We found 3-fold increase in the amount of bacteria of *Azotobacter* genus due to the action of EMF and thiram on pine seeds (Fig. 2). We did not detect any difference when thiram alone was used to treat pine seeds.

EMF and EMF with thiram treatment resulted in 5-fold increase of nitrogen-fixing microorganisms and in 2.5-fold increase of bacteria using mineral nitrogen (EMF alone). At the same time, we detected 2-fold decrease in the amount of denitrifiers (EMF with thiram treatment). Other findings are presented in Fig. 3.

The action of EMF and EMF with thiram has similar properties: it results in increase of the number of agronomically useful micro-organisms. On the other hand, EMF treatment (without the use of thiram) resulted in growth of ammonifiers and bacteria using mineral nitrogen: the same effect was not detected when we used combined effect of EMF and thiram. It is clear from our results that bacteria using mineral nitrogen act as antagonistic to heterotrophs (Fig. 3): this fact is confirmed by a high coefficient of organic mineralization. Thus, the development of useful micro-organisms (oligonitrophyles and ammonifiers, bacteria using mineral nitrogen) acts in favour of nitrogen enrichment of soils available to plants.



**Fig. 2.** Alteration in the amount of *Azotobacter* bacteria colonies due to various presowing treatments.



**Fig. 3.** Influence of various treatments on the number of different microorganisms in the rhizosphere of pine seeds; 1 – heterotrophs, 2 – oligonitrophyles, 3 – bacteria using mineral nitrogen, 4 – denitrifiers, 5 – ammonifiers.

## DISCUSSION

In recent decades, EMF presowing treatment of seeds became very popular in agricultural sector (Berzhanskaya *et al.*, 1993; Pandita *et al.*, 2007; Soriano-Martín *et al.*, 2005). These treatments allow usually to avoid or to decrease the probability of infectious diseases caused by *Corinobacterium*, *Sclerotinian*, *Fusarium oxysporum*, *Botritis cinerea*, and some other species (Reddy *et al.*, 1998; von Hoersten and Luecke, 2001). Additionally, EMF may stimulate germination of seeds, improve their quality and speed up the growth of plants (Alexander and Doijode, 1995; Pietruszewski, 1999; Pietruszewski *et al.*, 2007; Wójcik, 1995). However, there are no studies devoted to EMF-mediated microbiological alterations of soils (in particular, in the rhizosphere area) due to presowing treatment of seeds. The first results are presented in this report.

Concerning the mechanisms of the observed phenomena, a few remarks should be made. There is no doubt that all living organisms are sensitive to EMF (Presman, 1970). Really, shielding from the Earth's fields results in a wide range of patterning defects and physiological alterations in plants (Brown and Chow, 1973). Evolving at constant influence of EMF, plants as other living organisms developed a special pattern of response to the stimuli. EMF of EHF (millimeter waves ( $\lambda = 1-10$  mm),  $f = 300-30$  GHz; these waves lie between the microwave band and the optical (infrared) band) have a variety of biological effects. EMF EHF is absorbed by water and aqueous solutions of organic and inorganic substances (Betskii and Kislov, 1988). Needless to say that this can particularly result in alteration of seed-connected microbial activity (Tambiev *et al.*, 2002). It is very likely that treatment with EMF EHF is able to stimulate positive changes (in relations to microbiocenosis) on the surface of treated seeds. This, in turn, appears to promote the beneficial conditions for growth of nitrogen-fixing microorganisms in the rhizosphere of pine seedlings. Further studies are required to investigate the mechanisms of the reported phenomena.

## CONCLUSIONS

1. Extremely high frequency electromagnetic fields positively influence the rhizosphere microflora.
2. The effect seems universal: the similar results were obtained with wheat seeds (unpublished results).
3. The used approach used appears to be safe and inexpensive, and allows avoiding seed dressing with potentially dangerous disinfectants.

## REFERENCES

- Alexander M.P. and Doijode S.D., 1995.** Electromagnetic field, a novel tool to increase germination and seedling vigour of conserved onion (*Allium cepa* L.) and rice (*Oryza sativa* L.) seeds with low viability. Plant Genetic Res. Newsletter, 104, 1-5.
- Berzhanskaya L.Yu., Beloplotova O.Yu., and Berzhanskii V.N., 1993.** Effect of electromagnetic radiation on higher plants (in Russian). Millimetrovye Volny v Biologii i Meditsine, 2, 68-72 .
- Betskii O.V. and Kislov V.V., 1988.** Waves and Cells (in Russian). In: Fizika, Znanie Press, Moscow, Russia.
- Brown F.A. and Chow C.S., 1973.** Interorganismic and environmental influences through extremely weak electromagnetic fields. Biol. Bull., 144, 437-461.
- Gilligan C.A., 2007.** Sustainable agriculture and plant diseases: an epidemiological perspective. Philosophical Trans. Royal Society of London, Series B – Biological Sci., September 7 [Epub ahead of print] DOI 10.1098/rstb.2007.2181].
- Hazell P. and Wood S., 2007.** Drivers of change in global agriculture. Philosophical Trans. Royal Society of London, Series B – Biological Sci., July 26 [Epub ahead of print] DOI:10.1098/rstb.2007.2166].
- Morozov G.A., Stakhova N.E., Vedernikov N.M., Ratushnyak A.A., Andreeva M.G., and Nazirov A.A., 2007.** A method to increase of nitrogen-fixing bacteria of *Azotobacter* genus in rhizosphere of pine and fir seedlings. Proposal No. 2006140773 (044528) dated 17.11.06). Decision on patent from Federal Service for Intellectual Property, Patents and Trademarks. October 8, Moscow, Russia.
- Pandita V.K., Anand A., and Nagarajan S., 2007.** Enhancement of seed germination in hot pepper following presowing treatments. Seed Sci. Technol., 35, 282-290.
- Parlevliet J.E., 2002.** Durability of resistance against fungal, bacterial and viral pathogens, present situation. Euphytica, 124, 147-156, (DOI:10.1023/A:1015601731446).
- Pietruszewski S., 1999.** Influence of pre-sowing magnetic biostimulation on germination and yield of wheat. Int. Agrophysics, 13, 241-244.
- Pietruszewski S., Muszyński S., and Dziwulska A., 2007.** Electromagnetic fields and electromagnetic radiation as non-invasive external stimulants for seeds (selected methods and responses). Int. Agrophysics, 21, 95-100.
- Presman A.S., 1970.** Electromagnetic Fields and Life. Plenum Press, New York, USA.
- Pretty J., 2007.** Agricultural sustainability: concepts, principles and evidence. Philosophical Trans. Royal Society of London, Series B – Biological Sci., July 25 [Epub ahead of print] DOI: 10.1098/rstb.2007.2163].
- Reddy M.V.B., Raghavan G.S.V., Kushalappa A.C., and Paultiz T.C., 1998.** Effect of microwave treatment on quality of wheat seeds infected with *Fusarium graminearum*. J. Agric. Eng. Res., 71, 2-11.

- Soriano-Martín M.L., Porrás-Piedra A., Porrás-Soriano A., Marcilla-Goldaracena I., and Porrás-Soriano M.L., 2005.** Microwave oven as a clean technology for the eradication of fusariosis in melon. *Agron. Sust. Develop.*, 25, 417-423.
- Tambiev A.Kh., Kirikova N.N., and Luk'yanov A.A., 2002.** Application of active frequencies of electromagnetic radiation of the millimeter-wave and centimeter-wave bands in microbiology (in Russian). *Naukoyomkie Tekhnologii*, 1, 34-53.
- von Hoersten D. and Luecke W., 2001.** Thermal treatment of seeds – comparison of different methods for eradicating seedborne fungi. *Proc. ASAE Annual Meeting*, Paper No. 016165.
- Wójcik S., 1995.** Effect of the pre-sowing magnetic biostimulation of the buckwheat seeds on the yield and chemical composition of buckwheat grain. *Current Advances in Buckwheat Res.*, 12, 667-674.