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

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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, $[(CH_3)_2NC(S)S]_2$) 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 GHtz, intensity - 10^{-16}–10^{-10} W cm^{-2}, 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:

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Fig. 1. Physical configuration of EMF EHF device.

1. Peptone agar (g l^{-1}: peptone – 100, KH_2PO_4 – 5, glucose – 0.3, $MgSO_4$ – 0.1) – this medium is used to detect ammonifiers;

2. Aamylum-ammoniac agar (g l^{-1}: $(NH_4)_2SO_4$ – 2, K_2HPO_4 – 1, $MgSO_4$ $7H_2O$ – 1, NaCl – 1, $CaCO_3$ – 3, amylum – 10, agar – 20) – this medium is used to detect actinomycetes and bacteria using mineral nitrogen;

3. Ashbi medium (g l^{-1}: K_2HPO_4 – 0.2, $MgSO_4$ – 0.2, NaCl – 0.2, KH_2PO_4 – 0.1, $CaCO_3$ – 5, mannitol – 20, agar – 20) – this medium is used to detect nitrogen-fixing microorganisms;

4. Giltai medium, composed of two solutions: A: (g l^{-1}) KNO_3 – 2, asparagine – 1, H_2O – 250 ml; and B: sodium citrate – 2.5, $MgSO_4$ – 2, KH_2PO_4 – 2, $CaCl_2$ – 0.2, H_2O – 500 ml. Solution A and B should then be mixed and bromine thymol blue (2 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 denitrificators;

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 ± 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 denitrificators (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 microorganisms (oligonitrophyles and ammonifiers, bacteria using mineral nitrogen) acts in favour of nitrogen enrichment of soils available to plants.

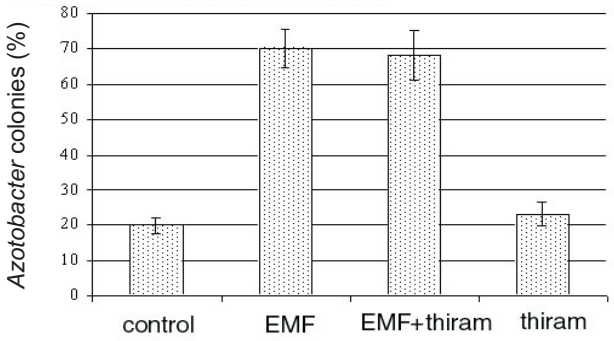

Fig. 2. Alteration in the amount of *Azotobacter* bacteria colonies due to various presowing treatments.

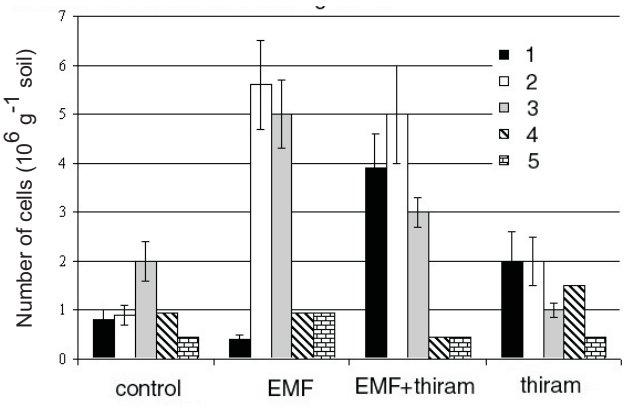

Fig. 3. Influence of various treatments on the number of different microorganisms in the rhizosphere of pine seeds; 1 – heterotrophs, 2 - oligonitrophyles, 3 – bacteria using mineral nitrogen, 4 – denitrificators, 5 – ammonifiers.

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 (λ = 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.

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