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Magnetic Fields Improve *Trichoderma Harzianum*'s Capability for Plant Growth Stimulation

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ABSTRACT

Trichoderma species are known by having the ability to stimulate the growth of crops. The production of a growth promoter from *Trichoderma harzianum* is a viable alternative for replacing chemicals that represent a high cost in the production of vegetables.

In this study it is determined that the application of the electromagnetic field for 30 minutes at 110 mT, produce an increase of 39 % in spore production compared to untreated fungus. It is also proved the possibility of using *Trichoderma harzianum* as a growth promoter.

With the application of the inoculum magnetically treated for 30 minutes at 110 mT to bean seeds, it was obtained an increase of the main root length by 55% and the number of secondary roots by 86% compared to untreated inoculum.

Keywords: *Trichoderma harzianum*, magnetic field, growth stimulation, roots length, bean seeds, Cuba.

1. INTRODUCTION

The use of fungal antagonists is being actively investigated due to the ability of these fungi to stimulate plant growth and to activate local and systemic defense mechanisms. Among the fungi species, the genus *Trichoderma* present genetic and phenotypic stability acceptable for scaling production processes and is reported to be safe to man and other species and do not harm the ecosystem (Hermosa *et al.*, 2012). The soil fungus *Trichoderma harzianum* is a well known biocontrol agent of fungi that cause serious diseases on tomato, lettuce, pepper, beans and others plant species (Cruz, 2007).

Besides the effect of pathogen biocontrol, it has been found that inoculation of *T. harzianum* promotes the growth and development of crops producing metabolites which stimulate plant growth processes, and have the ability to multiply in the soil, colonizes plant roots releasing growth factors (auxins, cytokinins and gibberellins) that stimulate germination and growth of plants (Cubillos *et al.*, 2009).

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For this reason this fungi have been used for over 70 years, but only until recently these strains have begun to acquire significant commercial value, due to effective results obtained during application and the emergence of new technologies for mass production and the development of products based on this fungus (Elósegui, 2006).

Growth regulators are endogenous bioactive substances in plants, which control various processes of plant development. The practical applications of these compounds are very diverse and their current use in agriculture is common and constantly increasing (Montaño *et al.*, 2009). The agrochemical industry provides a wide range of plant growth regulators that, in general, are obtained by chemical synthesis. Moreover, industrial agriculture is facing a major problem due to increased sensitivity of society to environmental problems and a greater appreciation of organic products by consumers in developed countries. There is therefore an increasing interest in developing sustainable agriculture to replace the massive use of pesticides, fertilizers and synthetic growth regulators (phytohormones analogues) by natural products (Castillo, 2005).

As an alternative for higher efficiency on biotechnology systems, electromagnetic fields (EMF) have been used on cellular cultures. Intermediate intensity magnetic fields (up to 0.1 T) have been reported to increase spores production on a 10-70 % (MÁS *et al.*, 2003). However, although the application of magnetic fields is a technique that has been used successfully in many areas such as chemical engineering, biomedical engineering and agriculture, few studies have found application to microorganisms (Oliveira *et al.*, 2010).

The scope of this work is to compare results of *Trichoderma harzianum* cultured under static electromagnetic field on its ability to stimulate germination of red beans roots (*Phaseolus vulgaris*).

2. MATERIALS AND METHODS

The experimental work was developed in the laboratory of Microbiology at the National Center of Applied Electromagnetism (CNEA), Universidad de Oriente, Cuba. The experiments were carried out as shown in Figure 1 (Más, 2006).

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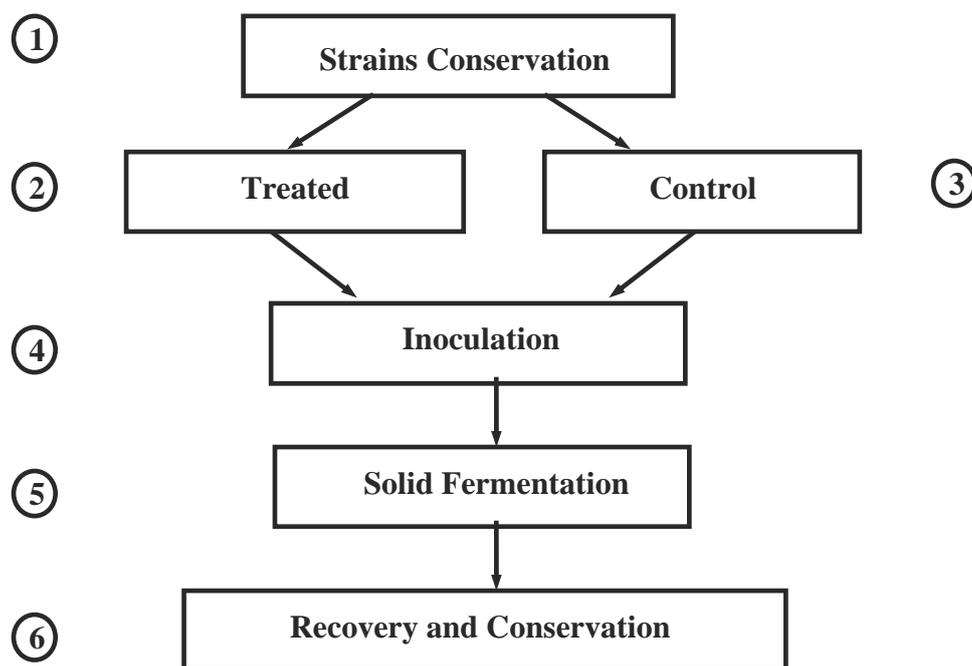


Fig.1 Stages in the production and formulation of *Trichoderma harzianum*

Strains of *Trichoderma harzianum* (IMI 314381), conserved at the Federal University of Santa Catarina (UFSC), Brazil, at Agar Czapeck medium (Herrera, 1985), were cultured 72 h on an enriched substrate: sugar cane bagasse, 1.5 - 3.0 mm particle size, (80 % w/w) and powdered rice (20 % w/w). Culture media were sterilized at 1 atm, 120 °C, during 20 minutes. Quality control was done determining its pathogenicity and strain purity (Kuhls et al., 1997).

Magnetic treatment was applied with a ferritic permanent magnet device. A steady, non-homogeneous field was achieved by a permanent magnet magnetizer (IP 100012, Cuba), designed and patented at the National Centre of Applied Electromagnetism (CNEA). It was characterized using a Gaussmeter (410-HCAT, Lakeshore) which has been calibrated against patterns of Lakeshore Cryotronics Inc.

A 2² factorial design was carried out, using as independent variables:

X₁: Magnetic field induction (92 mT to 110 mT)

X₂: Exposure time (15 min and 30 min)

Seed germination: The extract obtained was used in the red beans (*Phaseolus vulgaris*) seed germination. Quality control was performed in the Mycology Laboratory of the Provincial Plant Laboratory. Seed germination was performed taking into account the operating procedure of the Mycology Laboratory at Provincial Plant Laboratory for the

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growth of seeds in a moist chamber. Length of principal roots and quantity of secondary roots were measured.

Statistical processing was done using Statistica 7.1 on Windows 7 Ultimate. In all cases the method was the comparison of means, after testing for normality and homogeneity of variances.

3. RESULTS AND DISCUSSION

Means values of spore concentration were determined for the different treatments are presented on Table 1. Means followed by the same letter do not differ significantly from one another ($p \leq 0.05$). It is observed that, for all treatments, the spore concentration was in the order of 10^9 spores/g, achieving its maximum value for 110 mT and 30 min. exposure time with 5.85×10^9 spores/g. The minimum value was found in the treatment of 92 mT and 15 min. exposure time, producing 3.29×10^9 spores/g.

Table 1. Spore count achieved by *Trichoderma harzianum* under a magnetic field

| Treatments | Spore concentration (spores.g ⁻¹) |
|-------------------|---|
| Control | $3.59 \cdot 10^9 \pm 5.83 \cdot 10^8$ a |
| 92 mT and 15 min | $3.29 \cdot 10^9 \pm 8.23 \cdot 10^8$ a |
| 92 mT and 30 min | $4.82 \cdot 10^9 \pm 4.76 \cdot 10^8$ b |
| 110 mT and 15 min | $5.60 \cdot 10^9 \pm 7.56 \cdot 10^8$ b, c |
| 110 mT and 30 min | $5.85 \cdot 10^9 \pm 8.95 \cdot 10^8$ c |

Best results were achieved in increasing spore production with the application of a magnetic field during 30 minutes at 110 mT, where an increment of a 39 % is achieved with respect to the non treated fungi. These results are similar to those reported by Nagy (1993).

In all cases, magnetically treated fungus achieved higher spores production values than the non-treated experiments. These results were similar to those reported previously by Más (2001) for the growth of *Trichoderma viride* under a magnetic field of induction ranging from 10 to 60 mT and exposure times of 15 and 30 min., in which the highest concentration of spores obtained was for treatment of 60 mT and 15 min. exposure, showing that better results are obtained for higher values of magnetic induction, which led to the experimental range used in this work.

Table 2 shows the average values of measurements made on the main root of bean seeds to which was added the product of *Trichoderma harzianum* magnetically treated and the control experiment. Reported results include not only the effect of the metabolites, but the effect caused by the fungi as well.

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Table 2 Length of main root of bean seeds treated with *T. harziamun* extract.

| Treatments | Main root length (mm) |
|-------------------|-----------------------|
| Control | 53.78 ± 12.04 |
| 92 mT and 15 min | 46.35 ± 13.45 |
| 92 mT and 30 min | 53.13 ± 10.99 |
| 110 mT and 15 min | 111.68 ± 9.12 |
| 110 mT and 30 min | 120.63 ± 2.72 |

From Table 2 it can be observed a root length greater for the seeds treated with induction of 110 mT with respect to control, this being a statistically significant result. The increase in root length obtained for the induction treatment of 110 mT and 30 min. of exposure time may be due to a better establishment of magnetically treated microorganism on the bean root, enhancing its development. This is an important fact for the adaptation of plants to the nursery, as greater root development will have a greater influence on their capacity for absorption of salts and nutrients. Figure 2 shows the values of main root length of seeds treated with the *T. harziamun* extract.

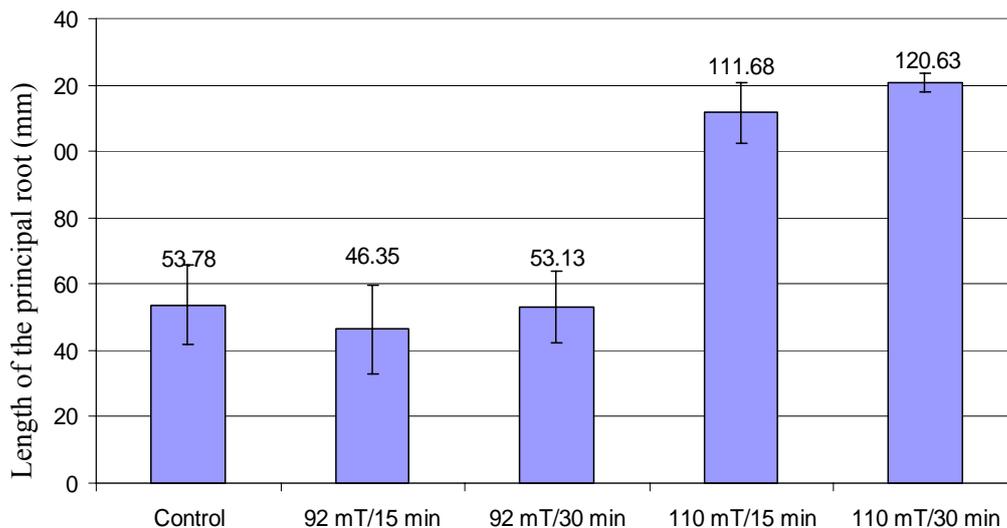


Fig.2 Root length measured at the seventh day of germination.

The treatment of the inoculum during 30 minutes at 110 mT and its application to red bean seeds increases the length of principal root upon a 55 % over the non treated inoculum and the quantity of secondary roots increased in an 86 %.

The linear equation which describes the behavior is:

$$\text{Root length (mm)} = -178.1528 + 2.2010 X_1 + 0.3822 X_2 + 0.0112 X_1 X_2 \quad (\text{Ec.1})$$

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Where:

X_1 : Magnetic field induction (mT)

X_2 : Exposure time (min)

It follows that to achieve an increment in root length the more influencing variable is the induction of the magnetic field. This is due to the stimulation of the growth of *T. harzianum* by the magnetic field that, in turn increase the *Trichoderma* ability of colonizing roots allowing greater absorption of nutrients by the plant thereby increasing their growth.

These results are consistent with studies done by Cubillos (2009), although they did not treat the inoculum with a magnetic field. They explained the obtained increase in the germination of maracuyá seeds with the production of growth factors (auxins, cytokinins and gibberellins), which *T. harzianum* released into the medium and stimulate the germination and development of plants.

Figure 3 shows the average quantity secondary roots. The results obtained for 110 mT was significantly different to the control treatment, being better for 110 mT and 30 min. of field exposure.

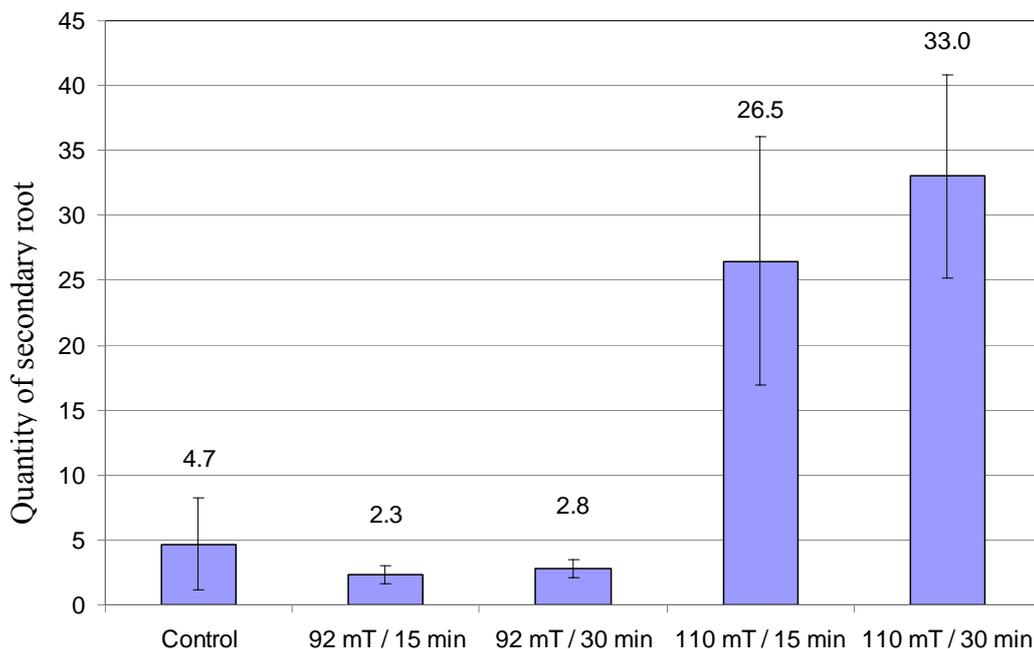


Fig.3 Quantity of secondary root measured at the seventh day of germination.

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A mathematical equation describing the system was obtained:

$$\text{Number of roots} = -97.6389 + 0.1069 X_1 - 1.8074 X_2 + 0.0020 X_1 X_2 \quad (\text{Ec.2})$$

Where:

X_1 : Magnetic induction (mT)

X_2 : Exposure time (min.)

According to the model obtained the statistically significant variable is the magnetic induction.

Under the action of magnetic fields changes are produced in ion channels in cell membranes, causing a change in the transport of ions within the cell which may result in biological changes in organisms (Gilart, 2008). The stimulation of cell metabolism has a direct involvement in the stimulatory effects observed.

Whereas biological samples have a large variability in response to stimuli, this result has a predictive value for the utility of the use of the magnetic field as a promoter of production growth stimulators from *T. harzianum*.

4. CONCLUSIONS

Magnetic fields applied to *Trichoderma harzianum* inoculum at 110 mT and 30 minutes improve its capability for plant growth stimulation, achieving longer principal roots and more secondary roots.

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