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# ASSESSMENT OF SOME ABIOTIC FACTORS ON MICROBIAL BIOPRODUCTS USEFUL IN BIOCONTROL OF PHYTOPATHOGENS

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## ABSTRACT

The environmental factors may influence the growth of microorganisms, by favoring their growth or slowing their multiplication rate and the synthesis of different metabolites. Parameters such as temperature, aeration, nutrients, pH or tolerance to NaCl can become limiting factors for microorganisms survival. *Bacillus subtilis* and related species can grow in variable pH conditions, maintaining the cytoplasmically pH in a relatively close range, stable to the synthesis of proteins and nucleic acids.

The aim of this work was to assess the influence of some abiotic factors on the biocontrol activity of microbial bioproducts, based on beneficial strains from *Bacillus* sp..

The bioproducts were tested *in vitro* against soil borne fungi at different temperatures and pH conditions. The results showed that the antagonistic activity of the biopreparates, tested at 27°C and 25°C, against phytopathogenic fungi released antifungal metabolites which inhibited the fungal growth. Also, when different pH values were analyzed, the results reflected that at pH 5.5 and pH 8.5 the bioproducts maintained the same antagonistic effect as in the control variant (pH 7.0).

## Indexing terms/Keywords

Microbial biocontrol products; *Bacillus* sp.

## Academic Discipline And Sub-Disciplines

Agriculture, Plant protection;

## SUBJECT CLASSIFICATION

SB 974-978 (Library of Congress)

## TYPE (METHOD/APPROACH)

Original article

## INTRODUCTION

The environmental conditions may influence the growth of microorganisms, by favoring their growth or slowing their multiplication rate and the synthesis of different metabolites [1]. Parameters such as temperature, aeration, nutrients or pH can become limiting factors for microorganisms survival. *Bacillus subtilis* and related species can grow in variable pH conditions, maintaining the cytoplasmic pH in a relatively close range, stable to the synthesis of proteins and nucleic acids [1, 2]. Biocontrol activity of antagonists may be influenced by the specific pathogen, host commodity and particularly by environmental conditions [3, 4]. An important matter in formulating bioproducts it the fate of the beneficial microorganism in diverse environmental conditions. Therefore, it is essential to study the impact of climatique changes and varied environmental factors on the efficacy of biocontrol attivity of formulated microorganisms. Overall, it is important to learn as much as possible regarding the ecology of these biocontrol organisms and their interactions with phytopathogens, host plant and environmental factors [5, 6, 7]. The fatty acids are essential for bacteria adaptation to different temperatures. The increase/decrease of fatty acids concentration and their composition, such as degree of saturation, ramification and length of the chains, influence bacterial ability to tolerate a lower or higher temperature [8, 9]. In bacteria, the motility and biofilm forming ability is dependent on temperature and temperature changed, such as freeze-thaw conditions frequently encounted during winter in the upper layer of soils [10]. Another aspect, is the bacterial endospores germination process. This is particularly important, considering that *Bacillus* strains are preponderant formulated as endospore state. Some germination studies performed on five different species of endospore forming *Baciilus*, showed an increased germination rate at 30-33°C than 3-8°C [11]. Due to the obvious influence of abiotic factors towards microorganism and their survival and metabolic activity, we focused our study on the influence of temperature, and pH on the biocontrol activity of two *Bacillus* sp.beneficial strains formulated as bioproducts.

## MATERIAL AND METHOD

**Biologic material.** Two bacterial bioproducts, formulated as concentrated suspensions, were used in there study. These bioproducts are based on *Bacillus subtilis* Bce2 and *Bacillus* sp. 83.2s beneficial strains with biocontrol ability. The bacterial inoculum was prepared in laboratory contitions. The antagonistic bacteria were cultivated on Luria Bertani broth (LB), at 28°C and 150 rpm, in Erlenmeyer flasks of 100ml. After 48 h growth, the bacterial biomass was harvested by centrifugation at 10°C and 3750 rpm for 15 minutes. Each bacterial strain was than formulated as concentrated suspensions of 1.3 x $10^8$cfu/mL.

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The antagonistic activity of the beneficial bacteria was studied atainst three soil borne fungal pathogens of forest interest: *Fusarium solani* (indigenous strain isolated from spruce seedling of *Picea abies* L., H. Karst.), *Fusarium oxysporum* f.sp. *radicis lycopersici* ZUM 2407 strain (from IBL Holland Collection) and *Rhizoctonia solani* DSM63002 strain (from Germany DSMZ Collection).

***In vitro* study of antifungal antivity in different temperatures conditions**

The antifungal efficacy of bacterial bioproducts was tested on PDA medium, in Petri dishes (ø = 9cm) using the double cultures technique. Mycelia disks, calibrated at 5 cm in diameter, were takened from active cultures, and placed on one side of a Petri dish. Subsequently, a bacterial spot of 10µl was placed on the other side of the Petri dish, equidistant from the centre (figure 1).

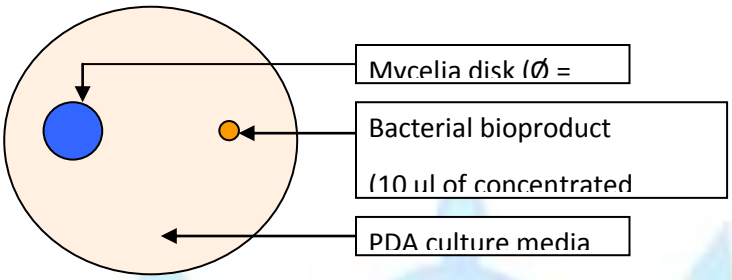


**Fig 1: The set up of the experiment**

The inoculated plates were incubated at 25°C and noted for antagonistic activity against the phytopathogenic fungi, after 2, 4, 7 and 10 days. Simultaneously, control plates were incubated at optimum growth temperature (27°C). The experiment was carried out in three replicates. After 2 to 10 days of incubation, the inhibition zones were recorded. The inhibitory activity of bacterial bioproducts was evaluated by calculating the percentage inhibition of radial growth (PIRG) [3]:

$$PIRG(\%) = \frac{R_1 - R_2}{R_1} \times 100$$

where: R1 is the radius of the fungal colony in control plates and R2 is the radius of the fungal colony in the presence of antagonistic bacteria, expressed in centimeters.

***In vitro* study of bacterial bioproducts efficacy at variable pH**

The antifungal activity of the bacterial bioproducts was tested at pH values of 5.5 for *Bacillus* sp. 83.2s and 8.5, respectively, for *Bacillus subtilis* Bce2 strains, by adjusting pH of the basal medium with 0,1N NaOH. The co-inoculated plates were incubated at 27°C. Control plates with the pH of 7.0 were also prepared. The observations were performed at intervals of 2 to 10 days after incubation, by noting the clear inhibition area of the phytopathogen due to the antifungal activity expressed by the bacterial bioproduct.

## RESULTS AND DISCUSSIONS

**The bacterial antifungal activity expressed at different temperatures conditions.** The *in vitro* test results reflected the inhibitory activity expressed by the bacterial bioproducts against tested phytopathogenic fungi (figure 2 a, b; figure 3 a). However, the variation of temperatures did not affect the antifungal potential of the formulated bacteria.

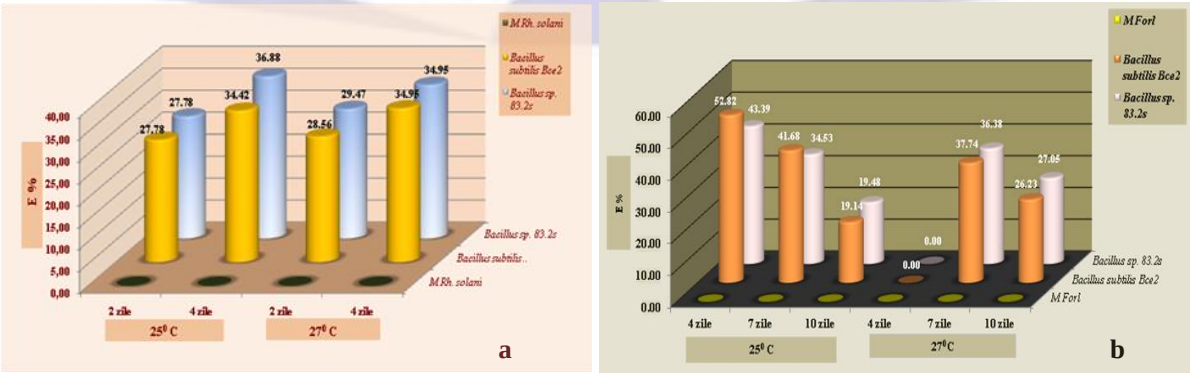


**Figure 2.** *In vitro* efficacy of the bioproducts against phytopathogenic fungi: *Rhizoctonia solani* (a), and *Fusarium oxysporum* f.sp. *lycopersici* (b), at different incubating temperatures (25°C and 27°C).

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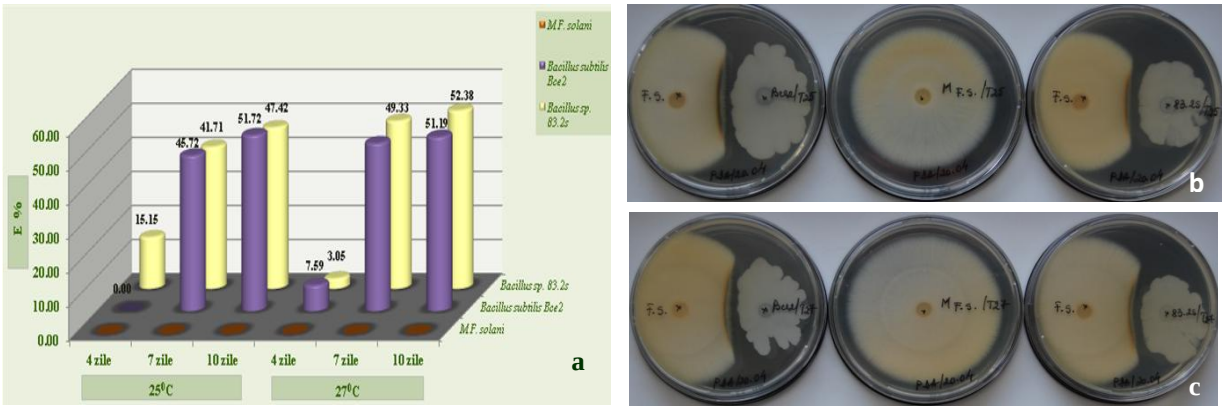


**Figure 3.** *In vitro* efficacy of bacterial bioproducts against fungal phytopathogen *Fusarium solani* (a), and their antagonistic activity at 25°C (b) and 27°C (c).

The microorganisms sensitivity to temperature is mainly related to the activation and / or inactivation of enzymatic systems. Most antagonists can grow at temperatures between 27 to 35°C. The two tested bioproducts produced antifungal metabolits at both tested temperatures, 27°C (optimal for bacteria growth) and 25°C (figure 3 b,c). However, it was shown that a temperature decrease with only two Celsius degrees slightly reduces the antifungal activity of *Bacillus* sp. 83.2s against *Fusarium solani* compeering to optimal thermic conditions.

***In vitro* antifungal activity of bacterial bioproducts in variable pH conditions**

The bacterial based bioproduct revealed to maintain an antifungal effect against the forest specific pathogens (figure 4 and 5). Although the bacterial strains *Bacillus* sp. 83.2s and *B. subtilis* Bce2 can growth better at the pH of 5.5 and 8.5 respectively (data not shown), when analyzing the antifungal effect at these pH values it has been observed that the biological activity is decreased.

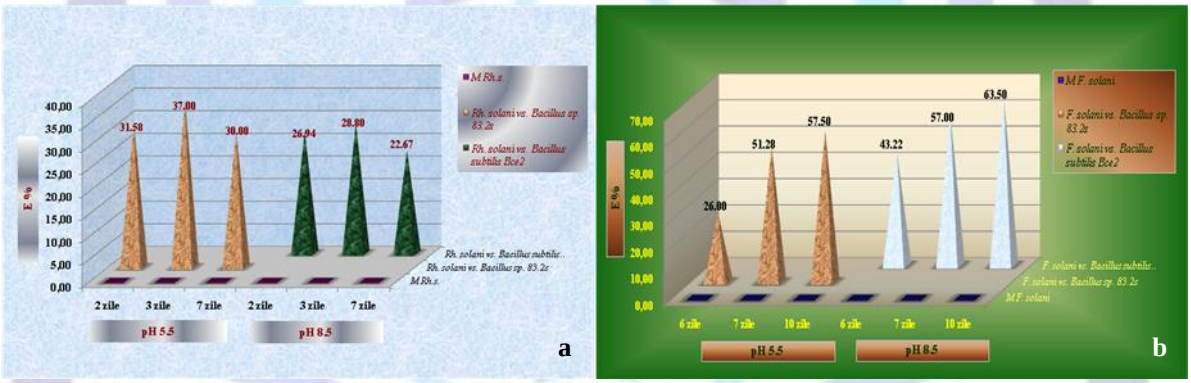


**Figure 4.** *In vitro* efficacy of the bioproducts against phytopathogenic fungi: *Rhizoctonia solani* (a) and *Fusarium oxysporum* f.sp. *lycopersici* (b), at the pH of 5.5 and 8.5, respectively.

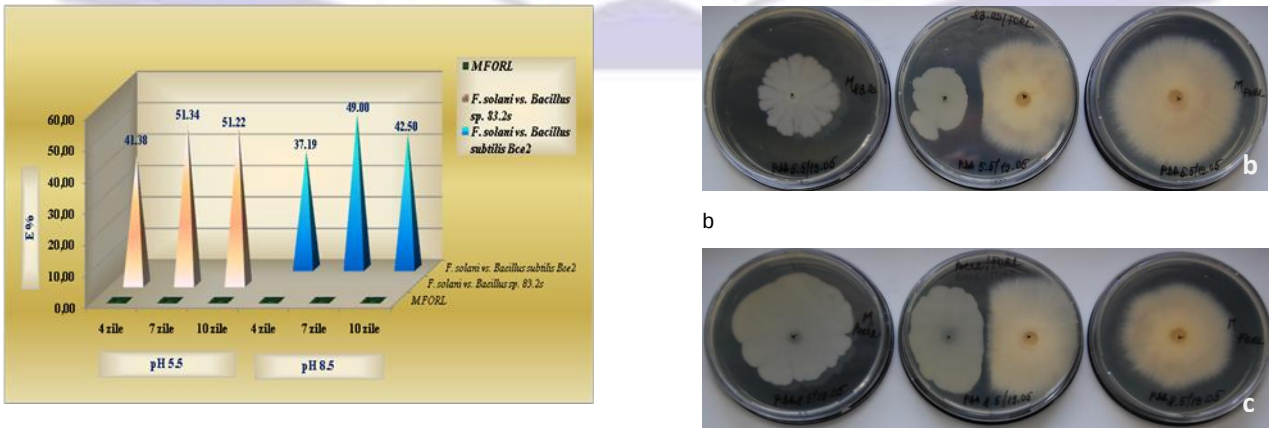


**Figure 5.** *In vitro* efficacy of the bioproducts against fungal phytopathogen *Fusarium solani*. a - The efficacy of mycelial growth inhibition; b – pH 5.5; c – pH 8.5

The pH is an important parameter which influence the ability of the microorganisms to grow in diverse environmental conditions. Overall, bacterial growth is decreased at acidic pH values [12]. However, bacterial bioproducts maintained their capacity to produce antifungal compounds at both pH values, as shown by the presence of inhibition zones against phytopathogenic fungi (figure 5 b, c), even if the antifungal efficacy was slightly reduced.

## CONCLUSIONS

- *In vitro* testing of bacterial bioproducts for their antagonistic capacity against phytopathogenic soil fungi, at temperature of 25 and 27$^0$C, highlighted antifungal metabolites production by the appearance of inhibition zone.
- At pH values of 5.5 and 8.5 the production of antifungal compounds by bacterial bioproducts was not affected, the antagonistic effect being maintained at similar level to the optimal values.
- The results demonstrate the adaptability of the tested bioproducts and the possibility to use them for crop protection against soil born fungi.

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## Author' biography with Photo

**Sorina Dinu** is scientific researcher worked on research topics regarding biological control and plant nutrition, collaborator in the frame of COST - 830 position (Development of bacterial inoculants with beneficial effects on crop legumes, cereals and technical plant), NUCLEU (Plant protection microbiological means, sustainable alternative to chemical products) programs.

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