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Original Research

Using Biofertilizer to Improve Seed Germination and Early Development of Maize

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Abstract

In this study, the effect of a living bacteria (*Bacillus megaterium var. phosphaticum*, *Azotobacter chroococcum*) containing biofertilizer, made in Hungary, was investigated on the germination and dry matter production of maize seedlings in germination tests. The biofertilizer was applied in concentrations of 1 $ml \cdot L^{-1}$ and 3.5 $ml \cdot L^{-1}$. Seed and filter paper treatments were used in the experiments, completed with autoclaved biofertilizer treatment. Germination and weight of shoots and roots were evaluated. It was observed that the seed-and-filter paper treatments with biofertilizer significantly increased – by more than 20% – the numbers of the germinated seeds in comparison to the untreated control. The dry weight of the shoot and root was higher by more than 7% than the control in the case of treatments with biofertilizer. Based on this result, it was concluded that there is a positive effect of PGPB on germination, as well as it is supposed, that the applied biofertilizer treatments stimulated the germination and growth of maize by reason of excreting phytohormones and enhancing the nutrient mobilization from the seed.

Keywords: biofertilizer, maize, germination, seed-, filter paper treatment

Introduction

Today the advantage of several microorganisms and their association with crop plants are taken for the production of biofertilizers. The application of bacteria (PGPB – plant growth promoting bacteria) as biofertilizer is increasing due to the low level of animal husbandry and the utilization of organic fertilizers. As a consequence, soils become poor in useful bacteria. PGPB indicate a group of bacteria actively colonizing plant roots, thus increasing the growth and yield of the plant [1]. The *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, and *Serratia* genus belong to this group [2].

One of the ways to improve germination is to use seed priming. The major aim of seed priming is to partially hydrate the seed to a point where the germination processes starts but does not finish. Several ways of seed priming exist, such as hydropriming, halopriming, osmopriming, thermopriming, solid matrix priming, and biopriming [3]. These inspire the question: how to improve seed germination by using biological compounds to hydrate the seeds, and what are the advantages and disadvantages of this

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Table 1. Applied direct seed and filter paper treatments and their mark.

Treatments	
Direct seed treatments	Control (H_2O) – Control
	Biofertilizer in the concentration of 1 ml·L^{-1} – Biof. 1 ml·L^{-1}
	Biofertilizer in the concentration of 3.5 ml·L^{-1} – Biof. 3.5 ml·L^{-1}
	Autoclaved biofertilizer in the concentration of 1 ml·L^{-1} – A. biof. 1 ml·L^{-1}
	Autoclaved biofertilizer in the concentration of 3.5 ml·L^{-1} – A. biof. 3.5 ml·L^{-1}
Filter paper treatments	Control (H_2O) – Control
	Biofertilizer in the concentration of 1 ml·L^{-1} – Biof. 1 ml·L^{-1}
	Biofertilizer in the concentration of 3.5 ml·L^{-1} – Biof. 3.5 ml·L^{-1}
	Autoclaved biofertilizer in the concentration of 1 ml·L^{-1} – A. biof. 1 ml·L^{-1}
	Autoclaved biofertilizer in the concentration of 3.5 ml·L^{-1} – A. biof. 3.5 ml·L^{-1}

Biof. – biofertilizer, A. biof. – autoclaved biofertilizer

method? The priming techniques with biofertilizers have been investigated, but there is a lack of knowledge of the effects of PGPR on germination and early development.

It has been published [4, 5] that PGPR bacteria can be used as seed treatment to examine the effects of biofertilizers on germination and yield in a paper towel an experiment using maize as experimental plant. After sterilization the seeds were coated by the bacteria suspension with the help of gum arabic adhesive. The seed treatment with bacteria significantly increased the germination and vigor of maize up to 18.5% in comparison to the non-treated control. It was examined [6] that the seed inoculation by bacteria resulted in interaction between the bacteria and the germinated seed, as well as the plant-microbe association determining the germination and the extension of the root in the initial stage.

Azospirillum, *Pseudomonas*, and *Azotobacter* strains could affect germination and seedling growth [7]. Seeds were immersed into the bacteria solutions and after drying it resulted that the seeds inoculated by effective microorganisms (EM) and biofertilizer significantly increased the germination and vigour in carrot, cucumber, pea, beet, and tomato [8]. The effect of bacterization of seeds was investigated in the case of several plant species with *Azotobacter vinelandii* on germination using the soaking method and more dilution of biofertilizer, which stimulated the germination and seedling development to different degrees when the seed-bacteria treatment was applied [9]. It was demonstrated that the filter paper soaked into the prepared solutions of biofertilizers after seed sterilization in order that the germination rate of cucumber increased by 20-25% in all biofertilizer treatments compare to the control [10]. The wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation [11].

It was proposed to examine the effect of a biofertilizer on the germination of maize seeds and on the growth of the maize seedlings in early stages.

Experimental Procedures

In this study maize (*Zea mays* L. – Dekalb DKC 4490) was used as the experimental plant. Filter paper was applied as germination medium. It was applied using a commercially available ready-to-use biofertilizer manufactured in Hungary containing microbiological active compounds, the following two bacteria stain: *Bacillus megaterium var. phosphaticum* (1-2×10^8 pieces·cm^{-3}), and *Azotobacter chroococcum* (1-2×10^9 pieces·cm^{-3}), which was applied in the concentrations 1 ml·L^{-1} and 3.5 ml·L^{-1}. Distilled water was used in the case the of control treatments. The seeds were sterilized by 18% H_2O_2 for 25 min. and soaked in 5×10^{-3} M $CaSO_4$ for 4 hours.

Two different biofertilizer treating methods (direct seed treatments [6, 7, 9] and filter paper treatments [4, 5, 10]) were used in the experiments as it published previously in the literature. Direct seed treatment was applied to inoculate the seeds with the different biofertilizer concentrations using 35 µl bacteria-solutions per seed and cold room storage for 24 hours before the experiment started. Distilled water was used in the case of control seeds. In the case of the direct seed treatment the filter papers were treated with distilled water and the treated seeds were used. In the filter paper treatments the filter papers were treated with the bacteria solution in the amount of 100 ml solution per filter paper. Filter papers each with 24 seeds were placed in a closed plastic box and incubated in the dark for 7 days at 25ºC with 4 replicates per treatment (the number of investigated seeds was 96) in a randomized experimental design. The treatments can be seen in Table 1.

The numbers of the germinated seeds were counted daily at the same time. Seedlings were harvested on the 7th day. The shoot and root dry weight was determined using an Ohaus (Switzerland) type analytical balance. Microsoft® Excel 2007 and SigmaPlot 11.0 (One Way ANOVA) were used for statistical evaluation of the data.

Table 2. The effects of the direct seed and filter paper treatments on the numbers of germinated seeds (pieces·seed^{-1}, from 24 seeds, n=96±s.e.).

Treatments		Days					
		2	3	4	5	6	7
Direct seed treatments	Control	18.25±0.96 ab	21.25±1.71 ab	22.50±0.58	22.50±0.58	22.50±0.50	23.75±0.50
	Biof. 1 ml·L^{-1}	19.50±0.58 ab	22.50±0.58 ab	22.75±0.96	22.75±0.96	22.75±0.96	22.75±0.96
	Biof. 3.5 ml·L^{-1}	23.00±0.82 a	23.50±0.58 a	23.50±0.58	23.50±0.58	23.50±0.58	23.50±0.58
	A. biof. 1 ml·L^{-1}	19.75±0.96 ab	22.25±1.50 ab	22.75±1.26	22.75±1.26	24.00±0.00	24.00±0.00
	A. biof. 3.5 ml·L^{-1}	18.50±1.29 ab	21.00±1.15 ab	22.50±1.00	22.50±1.00	23.50±0.58	23.50±0.58
Filter paper treatments	Control	16.75±0.50 b	19.75±1.71 b	21.75±1.26	21.75±1.26	23.50±1.00	23.50±1.00
	Biof. 1 ml·L^{-1}	23.25±0.50 a	23.75±0.50 a	24.00±0.00	24.00±0.00	24.00±0.00	24.00±0.00
	Biof. 3.5 ml·L^{-1}	22.50±1.91 a	23.75±0.50 a	23.50±1.00	23.50±1.00	23.50±1.00	23.50±1.00
	A. biof. 1 ml·L^{-1}	19.75±2.87 ab	22.00±1.41 ab	22.00±0.82	22.00±0.82	23.00±0.00	24.00±0.00
	A. biof. 3.5 ml·L^{-1}	18.50±4.36 ab	22.00±1.41 ab	22.00±1.41	22.00±1.41	23.00±0.00	24.00±0.00

Biof. – biofertilizer, A. biof. – autoclaved biofertilizer

Results

The homogeneous seed emergence and the intensity of germination determine the homogeneity of crops and their ripening. Data of Table 2 shows the effect of the direct seed and filter paper treatments on the number of germinated seeds. It was observed that the germination of the seeds started on the second day in the case of all treatments. Significant differences were observed among the treatments on the 2nd and 3rd days, because the given biofertilizer treatments increased the number of the germinated seed in the case of the direct seed and filter paper treatment as well. There was not a significant difference on the other days. However, the biofertilizer treatments increased the germination on the other days as well. The explanation of these results can be that the environmental circumstances were controlled and in this case the optimal factors promoted germination. In the case of more complex field conditions the biofertilizer application may result in greater differences for the benefit of bacteria. The applied biofertilizer treatments made the germination more intensive in the first two days.

On the second measuring day the direct seed treatment with 3.5 ml·L^{-1} biofertilizer significantly – by 37% – increased the numbers of the germinated seeds in comparison to the untreated control. The direct seed treatment with 1 and 3.5 ml·L^{-1} biofertilizer enhanced the germination of the maize seeds by 7% and 37%, respectively, compare to the control seed treated with water.

The numbers of the germinated seed were similar in the case of control and the sterilized biofertilizer direct seed treatments.

Seeds directly treated with biofertilizer in concentrations of 1 and 3.5 ml·L^{-1} germinated 6% and 11% more than the control, respectively, on the 3rd day. Seeds treated with sterilized biofertilizer showed a similar result as the control on the 3rd day of germination as well. 93% and 98% of the seeds germinated under direct seed treatment with 1 and 3.5 ml·L^{-1} biofertilizer for the 3rd day.

The filter paper treatments with biofertilizer significantly increased – by more than 34% – the germination of the seeds in comparison to the control on day 2. The numbers of the germinated seeds were higher – by more than 10% – than the control in the case of the sterilized filter paper treatments, which indicate the basic nutrient content of the biofertilizer, which has some positive effect on germination.

The numbers of the germinated seeds increased by 20% in the case of filter paper treatments with biofertilizer compared to the control on the third measuring day. The germination of the seeds grown in filter paper treated with sterilized biofertilizer was higher by 11% than the untreated control in order to the nutrient content of the biofertilizer. 99% of the seeds were already germinated in the case of the filter paper treatments with biofertilizer, 17% more than in the control.

Higher differences were measured in the case of filter paper treatments with biofertilizer than in the case of the direct seed treatments with biofertilizer in comparison to controls in all cases up to the 4th measuring day. The numbers of the germinated seeds were higher by more than 1% and 10% than the control when the seeds and the filter papers were treated with biofertilizer on days 4 to 7.

The dry weight of the shoots was higher by 10% and 7% in comparison to the control in the case of the seed and filter paper treatments with biofertilizer, respectively (Table 3). There was no concentration effect of biofertilizer on the dry weight of shoots, because the same values (direct seed treatment: 0.032±0.003/0.002; filter paper treatment: 0.029±0.003/0.005) were measured in both concentrations of biofertilizer treatments.

The effect of the sterilized biofertilizer treatments on the shoot dry weight was similar to the control.

Table 3. The effects of direct seed and filter paper treatments on the dry weight of the shoots of 7-day-old maize seedlings and the ratio of shoot and root (g·seedling^{-1}, n=94-96±s.e.).

Treatments		Shoot	Shoot/root ratio
Direct seed treatments	Control	0.029±0.004	1.17 ab
	Biof. 1 ml·L^{-1}	0.032±0.003	1.18 ab
	Biof. 3.5 ml·L^{-1}	0.032±0.002	1.09 ab
	A. biof. 1 ml·L^{-1}	0.029±0.003	1.63 ab
	A. biof. 3.5 ml·L^{-1}	0.027±0.002	1.52 ab
Filter paper treatments	Control	0.027±0.004	1.69 a
	Biof. 1 ml·L^{-1}	0.029±0.003	0.99 b
	Biof. 3.5 ml·L^{-1}	0.029±0.005	1.04 ab
	A. biof. 1 ml·L^{-1}	0.028±0.003	1.59 a
	A. biof. 3.5 ml·L^{-1}	0.028±0.002	1.48 ab

Biof. – biofertilizer, A. biof. – autoclaved biofertilizer

The root dry weight increased by more than 7% and 43% in comparison to the control when biofertilizer was applied as direct seed and filter paper treatment, respectively (Fig. 1).

Significant differences were observed in the shoot-root ratio. The filter paper treatment with biofertilizer in 1 ml·L^{-1} caused the lowest value (0.99), which was significantly lower than the control and the autoclaved biofertilizer treatment in 1 ml·L^{-1}. Data can be seen in Table 3.

Discussion of Results

In this study plant growth promoting bacteria (PGPB) containing biofertilizer was used to examine its effect on the germination of maize seeds and on the growth of the maize seedlings in early development stage.

In accordance with the literature [4, 5, 9, 10], the applied biofertilizer stimulated the germination in comparison to the control and sterilized biofertilizer treatments on all measuring days, but significant differences were observed among the treatments only on the 2nd and 3rd days.

The direct seed treatment with 3.5 ml·L^{-1} biofertilizer significantly increased the numbers of the germinated seeds by 37% in comparison to the control, as well as enhanced the germination of the maize seeds by 26% compare to the control seed treated with water on the 2nd day.

The filter-paper treatments with biofertilizer significantly – by more than 34% – increased the numbers of the germinated seeds in comparison to the control on the second measuring day. The numbers of the germinated seeds were higher than the control by more than 20% on the third measuring day.

The favorable effect of tested bacteria presumably comes from the feature of bacterial metabolism as reported previously [1]. The possible mechanism of PGPB on the germination process is that these useful bacteria can excrete phytohormones such as auxines and gibberellines, etc., thereby improving seed germination and early development. Besides, during metabolism the bacteria excrete organic acids (citric-, malic acid etc.) as well, thus helping nutrient uptake at a later stage of growth.

However, the biofertilizer treatments increased the germination on the other days as well.

The differences in the numbers of the germinated seeds between the control and the sterilized biofertilizer treatments were negligible. However, the sterilized biofertilizer treatments increased germination by 10% compared to the untreated control caused by the basic nutrient content of the biofertilizer, although this is unimportant in comparison to the biofertilizer treatments.

Higher differences were measured in the case of filter paper treatments with biofertilizer than in the case of the direct seed treatments with biofertilizer in comparison to the controls in all cases up to the 4th measuring day. The explanations can be that filter paper was more optimal for bacteria to live than the surface of the seeds or the filter paper could carry more bacteria, which was more effective.

According to the results of [8] and [5], authors positive effect of biofertilizer treatments was observed on germination. A favorable effect of direct seed and filter paper treatment on germination, which concurs with these author's results.

As was published by Kloepper et al. [11], the *Azotobacter* and *Bacillus* inoculation increased wheat yield up to 43% and 30%, respectively. Based on our results the biofertilizer treatments – containing *Azotobacter* and *Bacillus* strains – had a favorable effect on shoot and root growth appearing in the dry weight, which agrees with the results of the mentioned authors. The dry weight of the shoot was higher by 10% and 7% in comparison to the control in the case of the direct seed and filter paper treatments with biofertilizer, respectively. There was no concentration effect of biofertilizer on the dry weight of shoots.

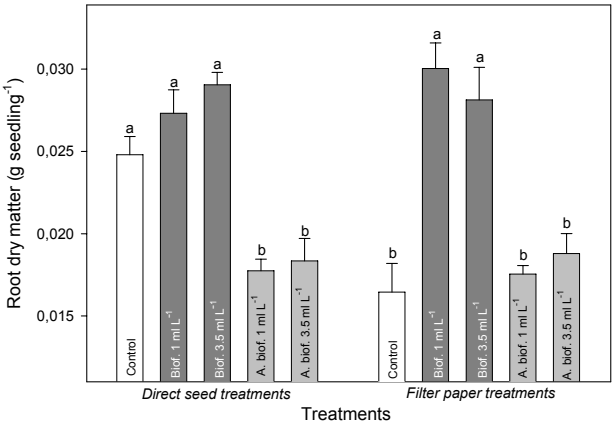

Fig. 1. The effect of direct seed and filter paper treatments on the dry weight of the roots of 7-day-old maize seedlings (g·seedling^{-1}, n=94-96±s.e.).
Biof. – biofertilizer, A. biof. – autoclaved biofertilizer.
A significant difference at level p>0.05 between the columns marked a and b.

When biofertilizer was applied as direct seed and filter paper treatment the root dry weight was increased by more than 7% and 43% in comparison the control, respectively. The effect of the sterilized biofertilizer treatments on the shoot dry weight was similar to the control. The filter paper treatment with biofertilizer in 1 $ml \cdot L^{-1}$ caused the lowest value (0.99) in the shoot-root ratio by reason of the relative high (0.030±0.003) root weight.

Conclusions

Based on the results of these experiments the following can be concluded: the biofertilizer made the germination more effective, thus significantly increasing the number of the germinated seed in comparison to the control in the case of the direct seed and filter paper treatment as well. The biofertilizer treatment influenced root growth positively, which has an important role in nutrient uptake.

There is a purpose for further examination on interaction between the bacteria treatment and germination, as well as development in the early stage to determine more useful knowledge for seed priming.

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