Influence of Some Beneficial Microorganism on the Development of Pepper Seedlings

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Abstract

In soil there are a large number of microorganisms which colonize the root system and stimulate the growth of plants. The main purpose of this article is to study the effect of some effective *microorganisms on the development of peeper seedlings. The experiments were carried out using the pepper varieties Kurtovska kapia 1619 and Bulgarski rotund. The plants were sown and grew on peat substrate, which was preliminarily inoculated with the bacteria Bacillus subtilis strain A1, Pantoeae agglomerans strain B_{43} and the fungus Trichoderma viride strain T_6 in the following doses: 50 ml, 100 ml and 150 ml. The second treatment was made ten days after the pricking. The morphological characteristics of the plants such as weight and volume of the root system, weight, height and thickness of the stem, weight and number of the leaves and number of the flower buds were investigated before planting. A well developed root system was observed when applying 150 ml of Bacillus subtilis A_1 and Pantoeae agglomerans B_{43}. The plants treated with Bacillus subtilis A_1 and Trichoderma viride T_6 had the highest stems. When applying 150 ml of Pantoeae agglomerans $B_{43,}$ there was an increase of the leaf weight and the number of the leaves as well, which also affected the generative characteristics. The total vegetative mass is the largest when using the highest doses of Bacillus subtilis and Pantoeae agglomerans B_{43}.*

Key words: morphology, varieties, doses, Trichoderma viride, Pantoeae agglomerans, Bacillus subtilis

Introduction

Soil microflora plays an important role when creating, maintaining and improving the fertility of the soil. The growth, development and yield of plants depend on the soil, the rhizosphere microflora and their components – root exudates and metabolic substances which are used to satisfy the needs of plants (Anderson et al., 1993). The theory regarding the influence of the useful microorganisms was established by Higa (1991) and it has been proven that the inoculation which is made using them improves the condition of the soil, the vegetative growth, the productivity rate and the quality of crops. Kloepper et al. (1980) and Asghar et al. (2004) reported that the microorganisms which colonize the root system have the capacity to enhance the growth of plants and induce resistance to some pathogens, due to which they are called beneficial microorganisms.

A number of authors use the soil living bacteria, either individually or in combination, and observe enhancement of flowering, fruiting and ripening in a number of agricultural crops - Garcia et al. (2004); Antoun, H., D. Provost (2006). According to Anandaraj and Sarma (1995) and Sawanth et al. (1995), the fungi *Trichoderma* sp. stimulates the growth and productivity of pepper. Similar results were obtained by Herman et al. (2008) when applying *Bacillus subtilis* and *Bacillus amyloliquefaciens*. The height of pepper plants, the volume of the root system, the number of branches and their productivity increases as a result of the treatment with *Trichoderma viride* in a quantity of 6 g/kg for the seeds and 10 g/m^2 for the soil. Kumar et al., (2006) and Domenech et al. (2006) established that the treatment using different kinds of the genus *Bacillus* has a better influence on peppers compared to tomatoes. It also has a positive effect on the development of pepper seedlings, Yobo et al. (2004).

The main purpose of this study was to establish the effect of the bacteria *Bacillus subtilis* strain A_1, *Pantoea agglomerans* strain B_{43} and the fungus *Trichoderma viride* strain T_6 on the growth and development of pepper seedlings.

Methodology applied

The experiments were carried out during the period from 2007 to 2009 in the Experimental fields of the Department of Horticulture as well as in the laboratory of the Department of Microbiology and Ecological Biotechnologies of the Agricultural University of Plovdiv, Bulgaria. Bulgarian varieties of pepper were used - Kurtovska kapia 1619 and Bulgarski rotund. The pricked seedlings were grown in a heated greenhouse in accordance with the adopted technology regarding the conditions in South Bulgaria. The plants were

sown in the middle of March in a peat substrate which was preliminarily inoculated with the following types effective microorganisms: *Bacillus subtilis* strain A_1 and *Pantoea agglomerans* strain B_{43} and the fungus *Trichoderma viride* strain T_6 in doses of 50, 100 and 150 ml/l (1.5 liter of a solution per 3,0 kg substrate).

Microbial strains *Trichoderma viride* T6, *Bacillys subtilis* A1, and *Pantoea agglomerans* B43, taken from the collection of Laboratory of microbial technology, Agricultural University – Plovdiv, were previously selected for their ability to stimulate plant growth. Bacteria were grown on tryptic soy agar. Bacterial suspensions used in the different experiments were prepared in 500 ml flask containing 200 ml of tryptic soy broth (TSB). The flasks were inoculated with 1ml of an overnight culture of the bacteria grown in TSB. The flasks were incubated on a rotary shaker (150 rev min^{-1}) at 28°C for 24 h. *T. viride* T6 tested was grown on potato dextrose agar (PDA). T6 was cultivated in liquid medium containing glucose and liquid corn extract for 48h in 500ml flask containing 200 ml of medium on a rotary shaker (150 rev min^{-1}) at 28°C. Plant growth substrates were inoculated with $3{,}5.10^9$ cfu/ml, for the bacterial and $1{,}2.10^7$ spores/ml.

During the two-leaf phenophase, the plants were pricked out using pots with a diameter of 8 cm. Ten days later the plants were treated again. Just before planting, 15 randomly chosen plants were examined and measurements were made of the weight and the volume of the root system, the height, the weight and the diameter of the stem, the number and the weight of the leaves, the total vegetative weight and the number of the flower buds as well.

The statistical analysis was performed using ANOVA. The data presented contains the averages obtained as a result of three-year experiments.

Results obtained

The beneficial microorganisms affect the development of pepper seedlings. There was a genotype responses resulting from their effect. All the results from the development of the root system have been presented in table 1. In variety Kurtovska kapia 1619, the biggest weight and volume of the roots were observed when inoculating the peat substrate with *Bacillus subtilis* A_1 -150 ml. The increase compared to the control plants was respectively 1.13 g and 1.39 cm^2. Similar results have been achieved by Martinez et al. (2008) and Harris (1999), who have observed a greater development of the root system of pepper after applying *Bacillus subtilis*. When using the same quantity of *Pantoea agglomerans* B_{43}, the highest values of the aforementioned parameters were observed for Bulgariski rotund. The weight of the roots was 4.08 g or 2.57 g more compared to the control plants and the volume was 4.54 cm^3 compared to 2.34 cm^3 for the non treated plants. In all cases when microorganisms were used, both varieties were influenced positively. However, the effect observed when using *Trichoderma viride* T_6 was smaller.

The applied microorganisms stimulate the development of the stem of the seedlings (table 2). In most of the variants the values registered were higher than those of the control plants. The stems of the plants of variety Kurtovska kapia 1619 were the highest when they were grown in a substrate with *Bacillus subtilis* A_1 -150 ml – 10.49 cm, followed by *Pantoea agglomerans* B_{43}-150 ml – 10,27 cm. In Bulgarski rotund, this effect was established under the influence of *Trichoderma viride* T_6 – 100 ml – 9.55 cm. In the aforementioned variants, the weight of the stem was also the biggest. Kumar (2006) established that the fungus *Trichoderma viride* significantly increase the height of the pepper plants. Only when applying *Pantoea agglomerans* B_{43} - 50 ml on Bulgarski rotund and *Trichoderma viride* T_6 – 150 ml on Kurtovska kapia 1619 there was a decrease in the height of the stem compared to the control plants. Pietr et al (2002) observed a different reaction when applying *Trihoderma sp*. There was an insignificant inhibition of these two indexes when using the highest concentration of *Trichoderma viride* T_6 on Kurtovska kapia and *Pantoea agglomerans* B_{43}-50 ml on Bulgarski rotund.

The diameter of the stem was slightly increased in all variants of the treatment.

The growth of leaves is one of the main morphological characteristics. The received data can use as a conclusion about the development of the plants under the influence of various agricultural and technological practices and their effect as well. The data are present in table 3. The number of leaves is increased when applying all three microorganisms. The best results were achieved when using *Pantoea agglomerans* B_{43} -150 ml and 100 ml - 11.20 for Kurtovska kapia 1619 and 10.18 for Bulgarski rotund compared to the control plants, 8.53 and 7.40 respectively. As the concentration was increased, the number of leaves in most variants was also increased. When using a concentration of 150 ml of *Trichoderma viride* T_6 for both varieties as well as 150 ml of *Bacillus subtilis* A_1 for Bulgarski rotund, the values obtained were lower.

Table 1 Morphological characteristics of root system of peppers seedlings

Variants	Kurtovska kapia 1619		Bulgarski rotund	
	weight (g)	volume (cm^3)	weight (g)	volume (cm^3)
Control	**2.23**	**2.79**	**1.51**	**2.10**
Bacillus subtilis A_1 -50 ml	**2.38**	**3.19**	**3.18**	**3.95**
Bacillus subtilis A_1 -100 ml	**3.00**	**3.50**	**2.98**	**3.41**
Bacillus subtilis A_1 -150 ml	**3.36**	**4.18**	**1.81**	**2.69**
Pantoea agglomerans B_{43}-50 ml	**2.26**	**3.28**	**1.91**	**2.43**
Pantoea agglomerans B_{43}-100 ml	**3.03**	**3.93**	**3.34**	**4.18**
Pantoea agglomerans B_{43}-150 ml	**3.25**	**4.10**	**4.08**	**4.54**
Bacillus subtilis A_1 -150 ml	**3.25**	**4.10**	**4.08**	**4.54**
Trichoderma viride T_6 – 50 ml	**3.36**	**3.65**	**3.56**	**4.00**
Trichoderma viride T_6 – 100 ml	**2.66**	**3.63**	**3.66**	**4.24**
Trichoderma viride T_6 – 150 ml	**2.81**	**3.38**	**2.40**	**2.91**
GD 5.0 %	1.22	1.16	1.2	1.36
GD 1.0 %	1.67	1.59	1.64	1.86
GD 0.1 %	2.28	2.16	2.24	2.54

Table 2 Development of the peppers seedlings stem

Variants	Kurtovska kapia 1619			Bulgarski rotund		
	height (cm)	diameter (mm)	weight (g)	height (cm)	diameter (mm)	weight (g)
Control	**8.98**	**0.33**	**0.55**	**6.47**	**0.30**	**0.43**
Bacillus subtilis A_1 -50 ml	**9.54**	**0.35**	**0.90**	**8.06**	**0.35**	**0.64**
Bacillus subtilis A_1 -100 ml	**10.05**	**0.38**	**0.88**	**8.58**	**0.40**	**0.88**
Bacillus subtilis A_1 -150 ml	**10.49**	**0.41**	**0.94**	**7.00**	**0.39**	**0.50**
Pantoea agglomerans B_{43}-50 ml	**9.22**	**0.37**	**0.80**	**6.23**	**0.32**	**0.33**
Pantoea agglomerans B_{43}-100 ml	**9.07**	**0.36**	**0.65**	**8.31**	**0.41**	**0.78**
Pantoea agglomerans B_{43}-150 ml	**10.27**	**0.40**	**0.88**	**8.96**	**0.42**	**0.75**
Trichoderma viride T_6 – 50 ml	**9.50**	**0.34**	**0.80**	**8.99**	**0.38**	**0.75**
Trichoderma viride T_6 – 100 ml	**9.18**	**0.35**	**0.63**	**9.55**	**0.38**	**0.93**
Trichoderma viride T_6 – 150 ml	**8.36**	**0.35**	**0.50**	**7.93**	**0.35**	**0.78**
GD 5.0 %	1.45	0.04	**0.27**	2.16	0.06	0.43
GD 1.0 %	1.99	0.05	0.37	2.96	0.09	0.60
GD 0.1 %	2.71	0.07	0.51	4.03	0.12	0.82

Table 3 Morphological behaviors of the peppers seedlings leaves

Variants	Kurtovska kapia 1619		Bulgarski rotund	
	number	weight (g)	number	weight (g)
Control	**8.53**	**1.09**	**7.40**	**0.87**
Bacillus subtilis A_1 -50 ml	**9.25**	**1.68**	**9.60**	**1.21**
Bacillus subtilis A_1 -100 ml	**9.63**	**1.81**	**9.40**	**1.46**
Bacillus subtilis A_1 -150 ml	**10.65**	**2.13**	**8.63**	**0.98**
Pantoea agglomerans B_{43}-50 ml	**10.40**	**1.40**	**8.15**	**0.70**
Pantoea agglomerans B_{43}-100 ml	**9.73**	**1.34**	**9.38**	**1.45**
Pantoea agglomerans B_{43}-150 ml	**11.20**	**1.78**	**10.18**	**1.58**
Trichoderma viride T_6 – 50 ml	**9.18**	**1.24**	**8.88**	**1.54**
Trichoderma viride T_6 – 100 ml	**9.38**	**1.31**	**9.40**	**1.34**
Trichoderma viride T_6 – 150 ml	**8.88**	**1.38**	**8.50**	**1.17**
GD 5.0 %	1.36	0.52	1.86	0.65
GD 1.0 %	1.87	0.71	2.55	0.90
GD 0.1 %	2.55	0.97	3.47	1.22

As a result of applying the investigated microorganisms, the weight of the leaves was increased, with the exception of *Pantoea agglomerans* B_{43}-50 ml where a decrease was registered for the variety Bulgarski rotund.

The beneficial microorganisms clearly demonstrated their stimulating effect on the growth of the total vegetative weight (table 4). Considering the data presented, it is obvious that for the Kurtovska kapia 1619 the highest vegetative weigh is obtained when applied *Bacillus subtilis* A_1 -150 ml. These results confirm the conclusions made by Domenech et al. (2006), Sid et al. (2004) and Harris (1999), who repoirted that the bacteria of the genus *Bacillus* enhance the vegetative growth of the pepper seedlings. Woo et al. (2006) established that the positive effect of *Bacillus subtilis* on the growth and the development of plants is probably due to the influence of the cellulose gene celH. The total vegetative weight of Bulgarski rotund was the biggest when using 150 ml of *Pantoea agglomerans* B_{43}. The root system plays an important role for forming this parameter, 52.25% and 63.65% respectively. This may be due to the metabolic products secreted as a result of the influence of those microorganisms.

The formation of flower buds on the plant seedlings indicates their better development, which ensures earlier and bigger yield. The stimulating effect on the number of flower buds was established for Bulgarski rotund, where their number is higher compared to the control plants. The highest values for all three microorganisms were observed when using a concentration of 100 ml - 3.85, 3.40 and 3.23 respectively for *Pantoea agglomerans* B_{43}, *Bacillus subtilis* A_1 and *Trichoderma viride* T_6. The stimulating effect for the other variety was achieved when using 50 ml of *Bacillus subtilis* A_1 and 150 ml of *Pantoea agglomerans* B_{43}.

The summarized results show that in variety Kurtovska kapia 1619 better growth and development was observed in all variants with applying *Bacillus subtilis*. Preeti V. et al. (2002) and Yobo et al. (2004) also emphasized that microbial formulations based on *Bacillus sp*. are more suitable when producing pepper seedlings.

Conclusions

As a result of the experiments with bacteria *Bacillus subtilis* strain A_1, *Pantoea agglomerans* strain B_{43} and the fungus *Trichoderma viride* strain T_6, the growth and development of pepper seedlings was improved.

The development of the root system was better when using the biggest doses (150 ml) of *Bacillus subtilis* strain A_1 for Kurtovska kapia 1619 and *Pantoea agglomerans* strain B_{43} for Bulgarski rotund.

The growth of the plants in a substrate inoculated with *Bacillus subtilis* strain A_1 -150 ml and *Trichoderma viride* strain T_6 -100 ml leaded to the formation of the highest stems of both varieties.

The highest number of the leaves has been obtained when using 100 ml and 150 ml of *Pantoea agglomerans* strain B_{43} for both varieties. The highest doses of *Bacillus subtilis* strain A_1 and *Pantoea agglomerans* strain B_{43} induced the formation of the highest total vegetative weight of the peppers seedlings. The studied microorganisms have a greater effect on the setting of flower buds in Bulgarski rotund.

As results of the carried out experiments could be recommended that *Bacillus subtilis* strain A_1 and Pantoea agglomerans strain B_{43} in dose 150 ml are suitable for grow the pepper seedlings with better morphological behaviors.

Table 4 Total weight of seedlings plant and number of flower buds

Variants	Kurtovska kapia 1619		Bulgarski rotund	
	weight (g)	number	weight (g)	number
Control	**3.87**	**0.73**	**2.80**	**1.25**
Bacillus subtilis A_1 -50 ml	**4.96**	**0.75**	**5.03**	**2.58**
Bacillus subtilis A_1 -100 ml	**5.69**	**0.25**	**5.32**	**3.40**
Bacillus subtilis A_1 -150 ml	**6.43**	**0.63**	**3.29**	**2.50**
Pantoea agglomerans B_{43}-50 ml	**4.45**	**0.38**	**2.94**	**1.45**
Pantoea agglomerans B_{43}-100 ml	**5.01**	**0.53**	**5.57**	**3.85**
Pantoea agglomerans B_{43}-150 ml	**5.91**	**1.48**	**6.41**	**3.17**
Trichoderma viride T_6 – 50 ml	**5.40**	**0.38**	**5.85**	**2.75**
Trichoderma viride T_6 – 100 ml	**4.60**	**0.00**	**5.93**	**3.23**
Trichoderma viride T_6 – 150 ml	**4.69**	**0.50**	**4.35**	**1.73**
GD 5.0 %	1.85	0.51	2.1	1.48
GD 1.0 %	2.54	0.7	2.88	2.03
GD 0.1 %	3.46	0.96	3.92	2.77

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