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VEGETATIVE BEHAVIORS AND PRODUCTIVITY OF PEPPER AFTER APPLICATION OF PLANT GROWTH PROMOTING MICROORGANISMS

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Abstract

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The main purpose of the carried out pots experiments was to establish the vegetative development and productivity of pepper plants Kurtovska kapia 1619 and Bulgarian rotund varieties when they are cultivated in an substrate, inoculated with PGPM (Plant growth promoting microorganisms). The bacteria strains *Bacillus subtilis* A_1 and *Pantoea agglomerans* B_{43}, and the fungi strain *Trichoderma viride* $T_{6,}$ in concentrations of 50, 100 and 150 ml were used. The microorganisms were applied six days after the seeds planting. The general vegetative weight, the weight and volume of the root system, height, weight and diameter of the stem, number and weight of leaves, number of flowers, and number of fruits were monitored. The total yield per one plant was recorded. The highest growth of the root system and stem were observed under the effect of *Trichoderma viride* T_6 – 50 ml, and for the leaves and flowers, after the inoculation of *Bacillus subtilis* A_1. The number of formed fruits also increased. The productivity of Kurtovska kapia 1619 is highest after the application of 150 ml of *Bacillus subtilis* A_1, and of Bulgarian rotund – of 100 ml *Trichoderma viride* T_6.

Key words: pepper, PGPM microorganisms, vegetative development, *Bacillus*, *Trichoderma*, *Pantoea*

Introduction

The microorganisms in the rhizosphere of the plants function as regulators of the soil eco-systems and play significant role for their nutrition and soil fertility. There is a large variety of bacteria, that colonize the root system of the plants – *Pseudomonas, Panthoea, Klebsiella*, *Azotobacter, Agrobacterium, Enterobacter,* etc. (Kennedy, 2005). Indisputable proves of the role and significance of rhizobacteria for the improvement the development of the plants (PGPR bacteria) and development of ecological agriculture that can be expressed by the reduction of pesticides and chemical fertilizers (Rodriguez and Fraga, 1999; Vessey, 2003).

Along with the use of several organic products for the growth stimulation and increase the productivity of vegetable crops (Cholakov et al., 2006; Petkova and Todorova, 2007; Haytova, 2009; Boteva and Cholakov, 2010; Boteva et al., 2010; Vlahova et al., 2010), the application of different bacteria and fungi in the plants rhizosphere, that affects their development and growth also plays a significant role (Gholami et al., 2009; Klopper, 1992). Herman et al. (2008) established that the bacterias *Bacillus subtillis* and *Bacillus amyloliquefaciens* improve the growth and accelerate the development and productivity of pepper. In experiments with four types of bacterias *Pseudomonas putida* NA_{40}, *Pseudomonas putida* NA_{41}, *Bacillus subtillis* A_{13} and *Bacillus amyloliquefaciens* A_{31}, that were carried out, Harris (1999) observed enhanced growth of the root system of that culture. Kleifeld and Chet (1992) and Paulitz et al. (1986) reported about accelerated growth, induced by the fungi *Trichderma* sp. with different plant types. With some vegetable cultures, the accelerated growth consists in shorter period of germination or in the increase of height, dry weight and leaves area of the plant. There are scientific reports about accelerated growth of different crops under the influence of some fungi species *Trichoderma* gender as a *Tr. harzianum* (Chang et al., 1986; Inbar et al., 1994) and *Tr. viride* (Ousley et al., 1993).

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The researches on the effect of the PGPM on the agricultural crops, and especially, on pepper are limited, and in Bulgaria are almost missing. This is the reason why a targeted scientific research was required in order to clarify the effect of the microorganisms on this crop.

The purpose of the current study was to establish the effect of two bacterial strains (*Bacillus subtilis* A_1 and *Pantoea agglomerans* B_{43}) and one fungi strain (*Trichoderma viride*, T_6) on the vegetative development and productivity of pepper.

Materials and Methods

The experiments were carried out in 2007–2009 in the scientific field of Horticultural department of the Agricultural University – Plovdiv with two Bulgarian pepper varieties – Kurtovska kapia 1619 and Bulgarian rotund. The effect of the following plant growth promoting microorganisms (PGPM) in three concentrations was tested:

1. Control – untreated
2. *Bacillus subtilis* A_1 – 50 ml
3. *Bacillus subtilis* A_1 – 100 ml
4. *Bacillus subtilis* A_1 – 150 ml
5. *Pantoea agglomerans* B_{43} – 50 ml
6. *Pantoea agglomerans* B_{43} – 100 ml
7. *Pantoea agglomerans* B_{43} – 150 ml
8. *Trichoderma viride* T_6 – 50 ml
9. *Trichoderma viride* T_6 – 100 ml
10. *Trichoderma viride* T_6 – 150 ml

The sowing was carried out in peat-perlite substrate, enriched with 1.5 l. 3 kg^{-1} substrate of the above mentioned microorganisms and concentrations and the control sample was watered with the same quantity of water. On the fifth day after the pricking in the pots 60.0 ml solution of the tested strains was applied and the control plants were watered with the same quantity of water. In polyethylene containers, with volume of 40 kg, containing 35 kg alluvial-meadow soil, enriched with 3.2 g triple-super phosphate, 1.6 g potassium sulphate and 2.24 g ammonium nitrate per container and with cultural liquid of the tested microorganisms in three concentrations of 1.0 l per container were planted three plants per container in the second decade of May. In the containers with the control plants was added 1.0 l of water. The experiments were set in four replications, and each replication consisted of six containers. Six days after the planting process, each container was inoculated with 4.0 l of cultural solution, containing the studied PGPM in three concentrations, and the control plants were watered with the same quantity of water. During the vegetation period, the required cultural practices, ensuring the normal development of the plants were implemented. In the phases of mass flowering and the beginning of fruit formation, the plants were fertilized with ammonium nitrate with quantity of 1.1 g/container. The mineral fertilizers doses were set according the quantities required per one decare for medium-early field production of pepper, recalculated according to the volume of soil in each container.

The microbe strains were received by the laboratory of "Ecological bio-technologies" of Agricultural University – Plovdiv. The bacteria were sustained in nutrition medium – Triptic soybean agar (TSA), containing Triptic soybean brought – 20.0 g.l^{-1} and agar – 20.0 g.l^{-1}. From the storage test-tubes they were cultivated on fresh nutrition TSA medium and were incubated at temperature of 28°C for a period of 24-hours. The inoculation material was prepared in 500 ml flasks with 200 ml nutrition medium (TSB-20.0 g. l^{-1}), that were sterilized in autoclave at temperature of 121°C for 20 min. When the nutrition medium cooled down to room temperature, we inoculated the flasks with 3.0 ml bacterial suspension that was accumulated by adding 5.0 ml sterile water to the test-tubes with cultivated bacteria and stirred them at vortex apparatus. Using the same method, we prepared the cultural liquid of the fungi *Trichoderma viride* (T_6) that was maintained in test-tubes on potato-dextrose agar. The fungi isolate were re-sowed on a fresh nutrition medium KDA and cultivated in thermostat at temperature of 28°C for 48-hours. We placed the inoculated flasks on a shaking machine (190 rpm) at temperature 28°C for a period of 24-hours for the bacteria and 72-hours for the fungi. The resulting cultural liquids that we used for inoculation had the following titre: for bacteria 10^9–10^{10} cfu ml^{-1}, and for fungi, it was 10^8 spores ml^{-1}.

During the mass flowering period morphometric analysis – weight and volume of the root system at depth and diameter of 30 cm, height, thickness and weight of the stem, number and weight of leaves, total vegetative weight of one plant were investigated. The number of flowers per one plant in mass flowering stage and number of fruits/plan from all harvests were determinated. The productivity of the plants was established. In 2007 and 2009 we did 4 harvests and in 2008 – three.

The statistical analyses were made by ANOVA. Data of the study were subjected to analysis of variance, and least significant differences between means were calculated by the Fisher test at $p = 0.05$.

Due to the similarity in the development and in productivity of the plants, the presented results are averaged values of the three years experiments.

Results

The vegetative behaviors are one of the first indicators that show the occurring changes as a result of stimulating influence. Table 1 shows the results of the development of the root system in the pepper plants. In Kurtovska kapia

Table 1

Weight (g) and volume (cm³) of root system of pepper plants

Variants	Kurtovska kapia 1619		Bulgarian rotund	
	weight	volume	weight	volume
Control	9.63	9.75	7.00	7.00
Bacillus subtilis A_1 – 50 ml	13.19	13.13	7.63	8.77
Bacillus subtilis A_1 – 100 ml	13.73	13.54	8.25	8.38
Bacillus subtilis A_1 – 150 ml	11.33	10.77	8.13	8.00
Pantoea agglomerans B_{43} – 50 ml	9.72	9.95	8.00	8.38
Pantoea agglomerans B_{43} – 100 ml	13.13	13.25	8.75	9.19
Pantoea agglomerans B_{43} – 150 ml	12.88	12.56	9.13	9.25
Trichoderma viride T_6 – 50 ml	14.81	14.25	9.38	9.25
Trichoderma viride T_6 – 100 ml	14.56	14.31	8.38	8.63
Trichoderma viride T_6 – 150 ml	12.81	13.25	9.94	10.94
LSD p 0.05%	3.19	5.13	2.79	2.78

1916, the plants treated with *Trichoderma viride* T_6 – 50 ml were with the biggest root weight, and the volume is highest with the next concentration of 100 ml. The stimulating effect with Bulgarian rotund was most strongly visible after the inoculation with *Trichoderma viride* T_6 also, but in the highest studied dosage of 150 ml. The most significant effect of the bacteria applying was seen in dose of 100 ml, with the exception of Bulgarian rotund for *Pantoea agglomerans* B_{43}, where the effect was visible in dose of 150 ml.

Stem with highest weight (Table 2) was developed by the plants of both pepper varieties after the inoculation with *Bacillus subtilis* A_1 – 50 ml, where the growth for Kurtovska kapia 1619 reached to 33.06 g, and for Bulgarian rotund – 28.67 – with 22.11 g and 22.75 g for both control plants, respectively. The effect of *Trichoderma viride* T_6 in dose of 100 ml for the first variety and 150 ml for the second variety can be put on second place. Differences in regards of the concentrations were reported in the application of *Pantoea agglomerans* B_{43}, where the stimulating effect for Kurtovska kapia 1619 was highest in dose of 150 ml, and for the Bulgarian rotund in dosage of 50 ml. The differences between the variants are with statistical significance.

With highest stem growth are characterized the plants from the variants with *Trichoderma viride* T_6, where for the variety of *var. kapia* it was with dosage of 100 ml – 45,13 cm, and for the *var. rotundum* in dosage of 150 ml – 35.36 cm, and the increase compared to the control was with 17.98% and with 11.86% higher. More significant increase was observed also and under the effect of 150 ml *Pantoea agglomerans* B_{43}. The impact of the PGPM was less on the stem diameter. Despite of the above fact, the effect on this index was much stronger after the implementation of *Trichoderma viride* T_6 for Kurtovska kapia 1619 in 100 ml, and for Bulgarian ratund in 150 ml.

Table 2

Vegetative behaviors of stem of pepper plants in stage of mass flowering

Variants	Kurtovska kapia 1619			Bulgarian rotund		
	weight (g)	height (cm)	diameter (cm)	weight (g)	height (cm)	diameter (cm)
Control	22.13	38.25	0.95	22.75	31.61	0.94
Bacillus subtilis A_1 – 50 ml	33.06	41.13	1.15	28.67	31.24	0.96
Bacillus subtilis A_1 – 100 ml	28.92	41.33	1.03	24.06	30.13	0.97
Bacillus subtilis A_1 – 150 ml	30.10	41.58	1.02	26.63	33.00	0.95
Pantoea agglomerans B_{43} – 50 ml	27.81	41.29	1.06	26.81	31.60	1.06
Pantoea agglomerans B_{43} – 100 ml	26.60	39.88	1.01	24.13	32.00	1.04
Pantoea agglomerans B_{43} – 150 ml	29.94	42.00	1.07	24.50	33.11	1.00
Trichoderma viride T_6 – 50 ml	32.00	41.63	1.14	24.63	32.03	1.04
Trichoderma viride T_6 – 100 ml	32.81	45.13	1.16	22.85	32.10	0.96
Trichoderma viride T_6 – 150 ml	26.83	42.38	1.10	28.56	35.36	1.06
LSD p 0.05%	3.12	2.61	0.25	2.35	2.32	0.22

Under the effect of 50 ml *Bacillus subtilis* A_1, the Kurtovska kapia 1619 plants developed the highest number of leaves (Table 3) 158.75 and the leaves were with highest weight of 50.75 g with 91.25 pcs. and 32.25 g for the control plant. When the concentration was increased, the values of these two characteristics decreased. Similar effect was observed with the other bacteria – *Pantoea agglomerans* B_{43}, while about *Trichoderma viride* T_6 the increase of the number and weight of leaves was observed to concentration up to 100 ml. In the Bulgarian rotund plants, the highest values for number of leaves was established for 50 ml Bacillus subtilis A_1 – 122.96, and for their weight in 150 ml *Trichoderma viride* T_6 – 37.63, with 27.09% and with 36.83% above the control plants. As with the other pepper variety, with the increase of the concentration of *Bacillus subtilis* A_1 and *Pantoea agglomerans* B_{43}, the number and weight of the set leaves decreased, and when the fungi was applied, the highest values were registered with the highest tested dose.

All variants are characterized with development of higher total vegetative weight, compared to the control plants, where the increase for Kurtovska kapia 1618 was highest after the inoculation with 50 ml of *Bacillus subtilis* A_1 – 27.09% above the control plants, and for Bulgarian rotund in *Trichoderma viride* T_6 – 150 ml – the augmentation was 32.97%. The differences are statistically proven.

Apart from the vegetative growth, the PGPM also improve the generative development of the pepper plants (Table 4). The highest number of flowers for both pepper plant varieties during the mass flowering stage was observed after the using of *Bacillus subtilis* A_1 in dosage of 50 ml. But the increasing the quantity of the inoculums in Bulgarian rotund suppressed the setting of flowers, but their number, com-

Table 3

Numbers and weight (g) of leaves and total vegetative weight (g) of pepper plants

Variants	Kurtovska kapia 1619			Bulgarian rotund		
	Nr. of leaves	weight of leaves	total weight	Nr. of leaves	weight of leaves	total weight
Control	91.25	32.25	64.01	96.75	27.50	57.25
Bacillus subtilis A_1 – 50 ml	158.75	50.75	97.00	122.96	32.50	68.80
Bacillus subtilis A_1 – 100 ml	124.42	46.81	89.46	116.25	31.29	63.60
Bacillus subtilis A_1 – 150 ml	103.08	40.96	62.39	113.13	29.06	63.82
Pantoea agglomerans B_{43} – 50 ml	103.79	41.02	78.56	100.25	29.94	64.75
Pantoea agglomerans B_{43} – 100 ml	101.25	41.06	80.79	98.5	29.13	62.01
Pantoea agglomerans B_{43} – 150 ml	100.63	42.88	85.70	97.38	28.00	61.63
Trichoderma viride T_6 – 50 ml	94.75	41.33	88.14	97.88	29.06	63.07
Trichoderma viride T_6 – 100 ml	107.88	46.19	93.56	103.38	33.81	65.04
Trichoderma viride T_6 – 150 ml	103.46	42.19	81.83	110.00	37.63	76.13
LSD p 0.05 %	8.25	6.12	9.22	4.16	4.44	4.08

Table 4

Generative behaviors and productivity of pepper

Variants	Kurtovska kapia 1619			Bulgarian rotund		
	Nr. of flowers	Nr. of fruits	yield (g per plant)	Nr. of flowers	Nr. of fruits	yield (g per plant)
Control	4.88	5.63	347.25	3.00	6.50	431.00
Bacillus subtilis A_1 – 50 ml	8.75	7.50	355.63	6.75	8.96	518.00
Bacillus subtilis A_1 – 100 ml	6.70	9.38	374.38	4.00	9.12	558.75
Bacillus subtilis A_1 – 150 ml	6.67	12.25	436.88	3.88	8.13	538.75
Pantoea agglomerans B_{43} – 50 ml	5,00	7.38	361.13	3.88	8.00	541.25
Pantoea agglomerans B_{43} – 100 ml	5.67	9.38	365.63	3.25	8.00	511.50
Pantoea agglomerans B_{43} – 150 ml	2.63	7.00	394.38	3.88	10.38	591.75
Trichoderma viride T_6 – 50 ml	6.08	8.00	324.63	3.38	10.88	531..75
Trichoderma viride T_6 – 100 ml	5.63	8.00	372.88	4.75	13.38	605.25
Trichoderma viride T_6 – 150 ml	5.63	9.58	399.63	5.88	9.63	407.50
LSD p 0.05 %	2.11	2.28	22.62	1.28	2.09	23.12

pared to non-treated plants was higher. The using of *Pantoea agglomerans* B_{43} – 150 ml had suppressing effect over Kurtovska kapia 1619.

With all tested PGPM, the number of set fruits was significantly higher than those of the controlled plants. Kurtovska kapia 1619 plants formed and developed the highest number of fruits under the effect of *Bacillus subtilis* A_1 – 150 ml – 12.25, with 5.63 for the control plants, and for Bulgarian rotund after the application of *Trichoderma viride* T_6 – 100 ml – 13.38 against 6.50 of the control plants. Trichoderma *viride* T_6 – 150 ml showed insignificant reduction, but without the presence of inhibition in the last mentioned variety.

The most important indicator of the effect of one stimulating activity is the productivity of the plants. *Bacillus subtilis* A_1 in concentration of 150 ml and *Trichoderma viride* T_6 in concentration of 100 ml mostly increased the production of one plant, respectively with 25.81% for Kurtovska kapia 1619 and with 40.42% for Bulgarian rotund. With the kapia variety plants, the second place was for *Trichoderma viride* T_6 and *Pantoea agglomerans* B_{43} in highest dosage. The improvement of Bulgarian rotund after the treatment with *Pantoea agglomerans* B_{43} – 150 ml was significant. Reduction of the yield, compared to the control plants was observed with treatment with 150 ml of *Trichoderma viride* T_6 for Bulgarian rotund. The statistical significant of the data was established.

Discussion

The microorganisms in rhizosphere have effect over the accessibility and mobility of nutrition elements, which, according to Antoun and Provost (2006) play a role for the improvement of the growth and development of the plants. The tested strains improved the development and productivity of pepper plants. Individual indicators though, are influenced in various extends, depending on the microorganism species used for inoculation as well as the concentration applied. The fungi strain with both pepper variety contributed to development of the most powerful root system and pepper plants with stem that was highest stem and biggest in diameter. Differences in the both genotypes were observed in relation to the implemented dosage – in Kurtovska kapia 1619, the impact was most powerful under the effect of lower concentrations – 50 and 100 ml and for Bulgarian rotund, the effect was stronger in dosage of 150 ml. Similar differences, when this microorganism was used, were observed by Pietr at al. (2002), and according to the authors, the stimulating effect is related to the increase of the activity of the fenilalaninamonilase. *Bacillus subtilis* A_1 caused better development of the stem weight, especially on the number of leaves in lowest concentration. Kloper et al. (2002) and Preeti et al. (2002) pointed out that the pepper plants develop extremely well after the inoculation with *Bacillus subtilis* or agents in which it is contained.

The plants that are cultivated in soil, inoculated with PGPM, developed bigger total vegetative weight, which is an indicator for their generally more stable development. It also depends on the species of the relevant microorganism as well on the concentration applied. As it was mentioned, the stronger development of the plants of the Bulgarian rotund is induced by the implementation of higher concentrations. The weight of the leaves plays bigger role in the formation of that indicator and the tendency was not changed under the impact of the tested bacteria and fungi, which is a proof that their effect does not affect the normal and harmonious development of the plants.

Together with that a positive effect was observed in the generative events, resulting in the increase of number of flowers and fruits and the effect is dependent on the implemented concentrations and is more significant for Kurtovska kapia 1619 type. In relation to these two characteristics, the productivity of pepper is significantly influence and with all tested variants stimulation was observed. The yield increased with increasing of concentration. A well-defined genotype response was observed over that indicator. The fruit production of Kurtovska kapia 1619 was stimulated better by the bacteria *Bacillus subtilis* A_1, and for Bulgarian rotund by the fungi *Trichoderma viride* T_6. A lot of researchers (Anandaraj and Sarma, 1995; Herman et al., 2008; Sawanth et al., 1995) underline, that the significant increase in the pepper yield is achieved by the incorporation of these two microorganisms, which are widely used with cultivated plants.

The prominent positive effect of the impact of PGPM in the rhizosphere of the pepper is possible to be related to their direct impact mechanisms. According to Egamberdiyeva (2007), Martinez-Viveros et al. (2010); Whitelaw (2000) and Shaharoona (2006) they present themselves in reinforced synthesis of growth regulators, reduction of ethylene levels in the plants, increase of nitrogen fixation and increase of solubility of mineral phosphates. The researchers emphasize that all of the above reflects over the improvement of vegetative growth and mostly over the productivity of cultivated plants.

Conclusions

The implementation of the tested PGPM in the rhizosphere of pepper plants caused more accelerated vegetative development of the plants. The highest growth of the root system and stem were observed under the effect of *Tricho-*

derma viride T_6, and the leaves and set flowers, after the application of *Bacillus subtilis* A_1. The number of formed fruits also increased.

Significant stimulation over the productivity was observed and the effect was higher with the increase of concentration and for Kurtovska kapia 1619 it was highest after the implementation of *Bacillus subtilis* A_1 150 ml, and for Bulgarian rotund after the inoculation of *Trichoderma viride* T_6 100 ml.

The effect over the different behaviors of the pepper plants in result of inoculation of PGPM depends on their species as well as on the dosage used, and is characterized by well – defined genotype response.

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