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Influences of *Bacilus suptilis* and *Trichoderma harzianum* to productivity and fruits quality of strawberry cultivar 'Clery'

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Abstract

In the study were examined an influence of different microorganisms to improve yield and quality of fruits strawberry cultivar ‘Clery’. The microbiological preparations were applied by drip irrigation system in doses of 5 l/ha, two and four weeks after planting, and also, next spring four and two weeks from the expected beginning of blooming. The following properties of productivity were analyzed: the number of crowns per plant, diameter of crowns, the number of inflorescences per crowns, the number of fruits per inflorescence, the total number of fruits per plants and the yield per plants (total and separately per harvesting in kg). According UPOV Code for strawberry the following phenological properties were studied. Plants treated with *T. harzianum* had significantly higher and numbers of inflorescences per crowns. A percentage of harvested fruits in first harvest, in regard to total fruits per plants, were between 19.5% (control plants) to 25.3% (*B. suptilis*). In the second harvest, to all treatments were recorded an increased yield of harvested fruits compared to first harvest. A total yield of marketable fruits per plants ranged between 0.38 kg (control) to 0.56 kg (*T. harzianum*). The highest content of TSS had fruits from plant treated with *T. harzianum* (10.7%), while the lowest had fruits treated with *B. suptilis* (10.2%). In the first harvest values of ascorbic acids in fruits ranged between 32.6 mg to 35.2 mg. Fruits from control plants had significantly higher values of AA. Generally, fruits from the second harvest had a decreased of contents of chemical properties.

Key words: strawberry, microorganisms, fruits quality, yield

Introduction

The cultivated strawberry (Fragaria × ananassa Duch.) is the most important soft fruit worldwide (Natsheh et al., 2015). The strawberries are economically important owing to their unique flavour of fruits, health benefits, and nutritional aspects (Shen et al., 2019). Also, strawberry is an excellent source of natural antioxidants, such as carotenoids, phenolics, vitamins, anthocyanins and flavonoids, with a remarkably high capacity for scavenging free radicals (Giampieri et al., 2012). Annual production of strawberry is more than 8.1 million tons (FAO, 2022) but, loses during postharvest strawberry storage are estimated to be as high as 40% (Duran et al., 2016).

At last two decades the new agro-techniques have been developed with special emphasis on the use of plant growth-promoting microorganisms (PGPMs). Biofertilizers or biostimulants, containing beneficial microorganisms, possess the ability to aggressively colonize the rhizosphere, plant roots or both when applied to seed or crops. Those microorganisms have shown potential to promote plant growth by the release of metabolites into the rhizosphere which stimulating growth. Their activity is reflected in plant growth through the secretion of plant growth hormones such as auxin, which enhances root hair growth and lateral root development in plant. The improved of root volume increases the rate of nutrient uptake from the soil, which leads to enhanced plant growth and fruit yield and support plant development under natural or stressed conditions, as well as increasing yield and quality of strawberry (Cho and Lee, 2013; Vejan et al., 2016). Moreover, they can improve plant nutrition, increased fruit quality, in terms of taste and nutritional value (Kundan et al., 2015; Morais et al., 2019).

However, biopesticides and biostimulants also have certain disadvantages, including: more difficult implementation, narrower spectrum of action, slower action compared to synthetic pesticides, exclusively preventive action, shorter shelf life, incompatibility with other compounds, and need for frequent application (Khirallah et al, 2016). However, the effect of plant growth-promoting microorganisms on strawberry yield and quality has been poorly studied due to the difficulties associated with field experiments (Chebotar et al., 2022). Nevertheless, we found only a few publications connected to this topic. A combined application of PGPMs strains *Rhizobium* sp., *Bacillus* sp. and *Herbaspirillum* sp. significantly increased the productivity of strawberries and reduced the use of chemicals. Similar results were obtained when using PGPR strains *Pseudomonas* sp (Anuradha et al., 2020; Bayramoglu et al. 2020). The goals of this study were examined an influence of different microorganisms to improve yield and quality of fruits strawberry cultivar ‘Clery’.

Materials and Methods

Plant, research design and environmental measurements

The research was performed in the strawberry open field plantation, which was established in 2018 year on an area of 3 ha, in the village of Selevac, Central Serbia. Plantation was built in the first half of August, by planting cold stored (frigo) plants. A single-row system of cultivation on banks has been applied. The distance of planting hedges in the banks is 16.5 cm, that is, 6 plants were planted per meter of the bank. The orchard was managed according to the integrated cultivation system, on sandy loam soil type, with an average pH of 6.3. The experiment was set up according to a complete block design with four repetitions (30 plants per repetition). During period close to a blooming (first half of September) all of flowers were hand removed. The researche were examined an influence of following microbiological preparations: *Bacillus subtilis* 10^9 **CFU/ml (**colony-forming unit per millilitre) **and** *Trichoderma harzianum* (10^{10} **CFU/ml)**, on productively properties of strawberry plants and physical and chemical quality of fruits. The microbiological preparations were applied by drip irrigation system in doses of 5 l/ha, two and four weeks after planting, and also, next spring four and two weeks from the expected beginning of blooming. Fruit were harvested at commercial maturity stage and transported to our laboratory within 2 h. The fruits were selected based on uniformity of size, shape, and color, and the absence of external physical damage and fungal infection.

Determination of plant phenological properties, productivity and physical properties of fruit

According UPOV Code for strawberry the following phenological properties were studied: beginning of blooming - when 10% of flowers are open, full blooming - when 90% of flowers are open, and beginning of harvest – when 90% of the surface showing red color. The following properties of productivity were analyzed: the number of crowns per plant, diameter of crowns, the number of inflorescences per crowns, the number of fruits per inflorescence, the total number of fruits per plants and the yield per plants (total and separately per harvesting in kg). The examined of physical traits of fruits after harvesting included: mass of fruit (g), a length and width of fruit (mm) and firmness (kg/cm^{-2}).

The firmness of fruits was determined with table digital firmness analyser (AMSTAT with diameter of 4.0 mm). The parameters for firmness analysis were as follows: trigger force = 10 g and penetration depth = 5 mm. Physical properties of fruits were determined with four repetitions, and each repetition included 15 fruits. Analysis of variance has been done with STATISTICA 9 software package. The significant differences between means determined at

P<0.05, measured with Duncan's test.

Determination of total soluble solids (TSS), titratable acidity (TA) and ascorbic acid (AA)
After harvesting, the fruits were stored at 3° C and analysed within 24 h. In total, 500 g of undamaged berries were selected and manually crushed to obtain the juice. For analyses, juices were centrifuged at 14,000× *g* for 20 min. The chemical properties of fruits were determined with four repetitions Total soluble solids (TSS) content of berries was determined using by refractometer (Atago, pocket PAL-1. Kyoto, Japan). Titratable acidity (TA) was determined by titrating the berry juice with 0.1 N NaOH up to pH 7.0. Acidity was expressed as percent of malic acid. Ascorbic acid was quantified using a reflectometer set from Merck Co. (Merck RQflex, Darmstadt, Germany). Fruit samples (5 g) and 20 mL oxalic acid (1%) were mixed, homogenized for 1 min and filtered. Polyvinyl-polypyrrolidone (PVPP) (500 g) was added to 10 mL of the filtered sample to remove phenols, and 6–7 drops of H_2SO_4 (25%) were added, to reduce the pH level below 1.14. Results were expressed as mg ascorbic acid 100 g^{-1} FW.

Results and Discussion

The plant growth-promoting microorganisms had influence to phenological properties of strawberry plant (Figure 2.). The control plants had early beginning of blooming compared to others treatments. Nevertheless, all plants supplied with PGPMs had early harvesting stages compared to control plants.

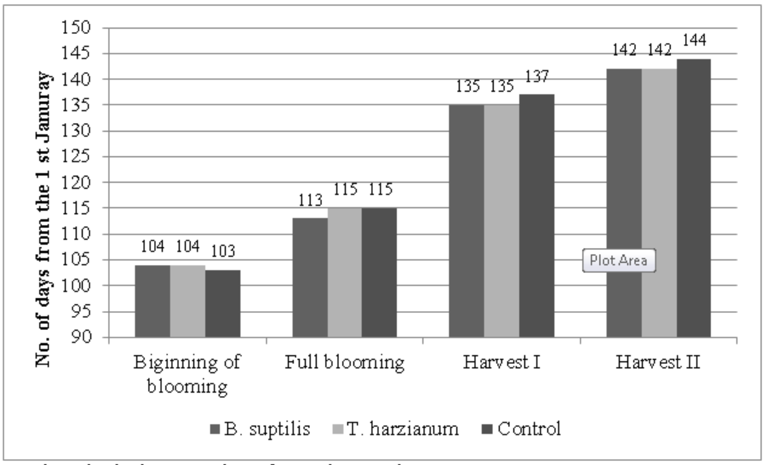

Figure 2. Phenological properties of strawberry plants

The plant growth-promoting microorganisms had significantly influence to strawberry plant productivity (Tab. 1). The number of crowns per plant was between 2.6 and 2.9. Plants supplied with *T. harzianum* and *B. suptilis* had significantly higher diameter of crowns and numbers of inflorescences per crowns compared to control plants. The numbers of flowers per inflorescences was between 7.0 (control plants) to 9.8 (plants treated with *B. suptilis*). The applied a PGPMs improved and enhanced the root system development of strawberry plants which contributed of high production of crowns per plants. Auxins are responsible for the division, extension, and differentiation of plant cells and tissues and are known to increase the rate of xylem and root formation (Chebotar et al., 2022).

Table 1. Productivity properties of strawberry plants treated with PGPMs

Treatment	Number of crowns per plant	Diametre of crowns (mm)	No of inflor. per plant	No of fruits per inflor.	Number of leaves per plants	Yield of 1st harvest (kg)	Harvested fruits I harvest (%)	Yield of 2nd harvest (kg)	Harvested fruits II harvest (%)	Total yield (kg)
B. suptilis	2.8 a	15.5 a	2.9 a	9.8 a	23.3 b	0.18 a	22.2 ab	0.24 b	38.5 b	0.42 b
T. harzianum	2.9 a	17.2 a	3.1 a	9.7 a	29.5 a	0.22 a	25.3 a	0.34 a	46.2 a	0.56 a
Control	2.6 a	13.4 b	2.1 b	7.0 b	24.8 b	0.12 b	19.5 b	0.26 b	40.8 b	0.38 b
lsd	0.5	2.0	0.6	1.6	3.6	0.04	5.6	0.04	3.2	0.08

Data are means of four replications; Different letters in columns indicate significant difference between cultivars according to Duncan's test at 5% level ($p < 0.05$)

The plants treated with *T. harzianum* had the highest yield of fruits in first harvest (0.28 kg), and followed plats treated with *B. suptilis* (0.18 kg). All applied PGPMs contributed to higher yield harvested fruits in first harvest compared to control plants. Further, a percentage of harvested fruits in first harvest in regard to total fruits per plants were between 19.5% (control plants) to 25.3% (*T. harzianum*). In the second harvest, to all treatments were recorded an increased yield of harvested fruits compared to first harvest. Plants treated with T. *harzianum* had the highest yield in second harvest (0.34 kg), while plant treated with *B. suptilis* had the lowest (0.24 kg). Also, percentage of harvested fruits in second harvest was higher compared to first harvest. The highest percentage harvested fruits had plants treated with *T. harzianum*, while the lowest plants treated with *B. suptilis*. A total yield of marketable fruits per plants ranged between 0.38 kg (control) to 0.56 kg (*T. harzianum*). Plants treated with T. *harzianum* had significant higher values of this property compared to other treatments. Also, results of other authors show that plants grown promoting bacteria such as *Bacillus simplex*, *Paenibacillus polymyxa* and *Bacillus* spp**.,** have the potential to increase the yield and growth of strawberries (Erturk et al., 2012). PGPMs specifically activate growth regulators such as auxin, gibberellins, cytokinin, inorganic phosphorus solubilization and nutrient mineralization along with symbiotic N-fixation (Zahir et al., 2004).

In the first harvest the highest mass of fruits had plants treated with *T. harzianum* (34.2 g), while the lowest had control plants (Tab. 2). According to Mikiciuk et al. (2019) applied species of rhizosphere bacteria and mycorrhizal fungi increase the yield and mean weight of strawberry fruit, due to improved activity of roots which contributed uptake of nutrient from soil. Also, enhances root hair growth and lateral root development in strawberry plants had influence increased of yield (Meng et al., 2016). The firmness of fruits harvested from plants treated with T. *harzianum* was significantly higher compared to fruits from control and plants treated with *B. suptilis*. The firmness of fruits in first harvest ranged between 3.4 kg/cm^2 to 4.7 kg/cm^2. In the second harvest the highest mass of fruits had plants treated with *T. harzianum* (22.5 g), while the lowest had control plants. To all treatments in second harvest were recorded higher values of fruits firmness compared with fruits picked in the first harvest. Similar like in the first harvest, a firmness of fruits harvested from plants treated with *T. harzianum* (4.9 kg/cm^2) was significantly higher compared to fruits from control and plants treated with B. suptilis (4.1 kg/cm^2 and 4.3 kg/cm^2, respectively).

Table 2. Physical properties of fruits harvested in the first and second harvest

Treatment	Fruits mass (g)	Fruits lenght (mm)	Fruits width (mm)	Firmness (kg/cm2)	Fruits mass (g)	Fruits lenght (mm)	Fruits width (mm)	Firmness (kg/cm2)
	I harvest				II harvest			
B. suptilis	33.2 ab	52.4 a	41.6 b	3.4 b	21.3 ab	32.5 a	31.2 ab	4.3 b
T. harziaunum	34.2 a	48.5 b	43.2 a	4.7 a	22.5 a	31.2 a	32.5 a	4.9 a
Control	31.1 b	48.2 b	41.2 b	3.4 b	19.2 b	28.4 b	29.6 b	4.1 b
lsd	2.7	3.1	1.4	0.8	3.2	2.7	2.4	0.4

Data are means of four replications; Different letters in columns indicate significant difference between cultivars according to Duncan's test at 5% level ($p < 0.05$)

The plant growth-promoting microorganisms had significantly influence to fruits chemicals quality in the first harvest (Tab. 3). The highest content of TSS had fruits from plants treated with T. *harzianum* (10.7%), while the lowest had fruits treated with *B. suptilis* (10.2%). In the first harvest values of ascorbic acids in fruits ranged between 32.6 mg to 35.2 mg. Erturk et al. (2012) were noticed that bacteria also increased soluble solid content (SSC) and vitamin C in strawberry. Based on the assessment of the condition of the photosynthetic apparatus and the analysis of chlorophyll "a" fluorescence indices, including hierarchical cluster analysis, the rhizosphere bacteria were found to have favorable effects on strawberry plants which can likened with improved chemical properties of strawberry fruits (Paliwoda et al., 2022). Fruits from control plants had significantly higher values of AA. Generally, fruits from the second harvest had a decreased of contents of chemical properties compared to fruits from the first harvest. The highest decreased of chemicals traits in fruits of second harvest were recorded to values of total acids and ascorbic acids.

Table 3. Chemical properties of fruits harvested in the first and second harvest

Treatment	TSS (%)	TA (%)	AA (mg/100 g f.w.)	TSS (%)	TA (%)	AA (mg/100 g f.w.)
	I harvest			II harvest		
B. suptilis	10.2 b	0.84 a	35.6 a	10.8 a	0.81 a	31.6 b
T. harzianum	10.7 a	0.76 a	33.4 b	10.7 a	0.47 b	30.5 b
Control	10.5 ab	0.86 a	34.2 b	10.4 ab	0.72 ab	33.6 a
lsd	0.4	0.18	1.1	0.3	0.32	1.5

Data are means of three replications; Different letters in columns indicate significant difference between cultivars according to Duncan's test at 5% level ($p < 0.05$)

Conclusion

According to ours results used plant growth-promoting microorganisms had significantly influence to plants productivity and fruits quality of strawberry cultivar 'Clery'. A plants supplied with *T. harzianum* had significantly higher values of examined productivity properties, especially when it comes to yield in second harvest and total yield of marketable fruits. Further, plants supplied with *B. suptilis* had significantly higher values of chemicals properties of fruits. Also, applied PGPMs had influences to improve physical properties of fruits. Due to improved of productivity properties and increased of fruits quality apply of plant grown promoting microorganisms it should be part of practice in strawberry growing.

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