



## Evaluation of innovative and environmentally safe growing practice suitable for sustainable management of plum orchards

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### ABSTRACT

Intensive agricultural development based on the long-term and excessive use of chemical nitrogen fertilizers significantly contributes to a series of undesirable effects and results in environmental pollution. In line with the above, there is a pressing need for major changes in agricultural production management. Bearing in mind that fertilization strategy, among other practices, plays an important role in improving the growing technology of different fruit crops, we considered that the above mentioned problems could be overcome by introducing an environmentally safe and innovative practice in plum growing technology as well. Accordingly, a comparative study was conducted to evaluate the effects of biofertilizer (a combination of nitrogen-fixing and phosphorus-mineralizing bacteria including *Azotobacter chroococcum*, *Bacillus megatherium* and *Bacillus subtilis*) and chemical fertilizer (a water-soluble chemical fertilizer supplemented with the microelements B, Cu, Fe, Mn and Zn) on 'Čačanska Lepotica' and 'Stanley' plum cultivars. Morphometric characteristics (fruit weight, length and width), internal quality traits (soluble solids content and firmness) and chemical properties (total phenolic content and total antioxidant activity) of the fruit of the tested plum cultivars were assessed. The obtained results indicate that the substitution of chemical fertilization with biofertilization in 'Čačanska Lepotica' and 'Stanley' is a justified practice. Furthermore, this approach seems to have potential as an appropriate technique in commercial plum production, which may improve yield-attributing characteristics and the phytochemical content of plum fruits.

*Keywords: microbial fertilizer, chemical fertilizer, plum, productivity, quality.*

### ИЗВОД

Интензивни развој пољопривредне производње заснован на дугорочној и прекомерној употреби минералних азотних ђубрива значајно доприноси низу нежељених ефеката и резултира загађењем животне средине. У складу са тим, неопходне су крупне и брзе промене у пољопривредној производњи. Имајући у виду да исхрана игра важну улогу у технологији гајења различитих врста воћака, сматрали смо да би се наведени проблеми могли превазићи увођењем иновативних и по животну средину безбедних производа и техника у постојећу технологију гајења различитих врста воћака, укључујући и шљиву.

Сходно томе, у засаду шљиве сорти 'Čačanska Lepotica' и 'Stanley' спроведена су упоредна проучавања утицаја биофертилизације (комбинација азото-фиксирајућих и фосфо-минерализирајућих бактерија: *Azotobacter chroococcum*, *Bacillus megatherium* и *Bacillus subtilis*) и употребе минералног хранива (водотопиво минерално храниво са додатком микроелемената: B, Cu, Fe, Mn и Zn). Испитивања су обухватала оцену масе, дужине, ширине и чврстине плода, као и садржаја растворљиве суве материје, укупних фенола и укупне антиоксидативне активности наведених сорти шљиве. Добијени резултати указују на чињеницу да је замена минералних хранива биофертилизаторима код сорти шљиве 'Čačanska Lepotica' и 'Stanley' оправдана мера. Поред тога, може се рећи да наведена технологија представља потенцијал и за комерцијалну производњу шљиве и може допринети побољшању приноса и фитохемијском саставу плодова шљиве.

*Кључне речи: микробиолошко храниво, минерално храниво, шљива, продуктивност, квалитет.*

### 1. Introduction

The plum (*Prunus domestica* L.) is the most widespread fruit crop in the Republic of Serbia of high economic and social importance. In the cultivar structure, the leading place is occupied by cultivars developed at the Fruit Research Institute Čačak (Republic of Serbia) such as 'Čačanska Lepotica' and 'Čačanska Rodna'. In addition, an important position is held by the 'Stanley' cultivar developed in the USA.

The Republic of Serbia is the third largest producer of plums in the world, just behind China and Romania, accounting for 4.1% of global production (Vlahović and Zdravković 2016). However, in Serbia, the plum is mostly used for processing into a traditional plum alcoholic drink called 'Šljivovica' (Milošević et al. 2010), while its use for fresh consumption is quite small. Since consumers' requests for fresh plum fruits are constantly increasing due to their health-promoting effects, recent trends have encouraged the development of new growing technologies associated

with an increased synthesis of phenolic compounds, as well as with obtaining fruits rich in phytochemicals.

Fertilization is one of basic cultural practices in orchards, which contributes most to the efficiency and intensity of production. However, improper fertilization (excessive and indiscriminate use of chemical fertilizers) causes not only economic inefficiency but also damage to the environment and, in certain situations, harms the plants themselves and human beings who consume them (Singh et al. 2012). As determined by Bockman et al. (1990), more than 50% of the applied chemical fertilizers are not adopted by plants, but are lost in different ways instead. Pešaković et al. (2007; 2012) pointed up that a high application rate of chemical fertilizers (above 600 kg ha<sup>-1</sup> of NPK) in plum growing technology results in a decrease of the number and diversity of microorganisms in plum rhizosphere and reduces the production capacity. Therefore, we thought it was important to find a new fertilizer strategy in order to achieve and maintain an optimum balance between growth and productivity and obtain high nutritional quality of the fruit, which will further promote the existing plum growing technology, and at the same time preserve the environment.

Current trends, among other things, point to biofertilization, primarily the application of living cells of microorganisms. Living cells of microorganisms accelerate the mineralization of organic residues in soil, thereby making nutrients more available. At the same time, due to the effect of living microbial cells from biofertilizer, the uptake of heavy metals decreases (Lévai et al. 2008). Mosa et al. (2014; 2015) considered biofertilization in fruit growing a healthy alternative and/or supplement to chemical fertilizers. Von-Bennwitz and Hlusek (2006) also found biofertilization to be a beneficial technique in stimulating growth and fruiting of pomes and stone fruits. The promoting effect of yeast on growth characteristics, nutritional status as well as physical and chemical characteristics of fruits of 'Kelsey' plum trees were also observed by Mansour et al. (2011). Pešaković et al. (2016; 2017) emphasized that biofertilization is an important factor that significantly affects productivity as well as antioxidant features in cv. 'Čačanska Lepotica'.

In line with the aforementioned, the present study aimed to improve the existing plum growing technology and encourage sustainable plum production through a comparative examination of the effects of bacterial fertilizer and chemical fertilizer on quality traits of plum cvs. 'Čačanska Lepotica' and 'Stanley'.

## 2. Material and methods

### 2.1. Plant material and experimental design

The study was conducted on plum cvs. 'Čačanska Lepotica' and 'Stanley' during two consecutive seasons (2016–2017 and 2017–2018). The plum orchard was established in autumn 2003 in the village of Gornja Gorevnica near Čačak, Western Serbia (20°57'48" N; 20°19'31" E; 396 m a. s. l.). The experiment was replicated thrice in a randomized block design. Myrobalan seedling (*Prunus cerasifera* Ehrh.) was used as a rootstock. Planting space was 4 × 2 m (1,250 trees ha<sup>-1</sup>). The trees were trained to the spindle bush

system. Standard cultural practices typical of a high intensity growing system, except irrigation, were used.

### 2.2. Treatments

Treatments included two fertilizer types i.e. biofertilizer and chemical fertilizer. For the purpose of biofertilization, a liquid fertilizer consisting of nitrogen-fixing and phosphorus-mineralizing bacteria was used (*Azotobacter chroococcum*, *Bacillus megatherium*, *Bacillus subtilis*). Bacterial titer in the inoculum ranged from 20–40 × 10<sup>6</sup> CFU cm<sup>-3</sup>. For chemical fertilization, a water-soluble fertilizer commercially named Murtonik, K+N (EFTHYMIADIS S.A., Greece) supplemented with microelements (B, Cu, Fe, Mn, Zn) was used. Both types of fertilizers were applied by watering at the beginning of the growing season and by spraying during the growing period from May until mid-July every 20 days. Untreated trees served as control.

### 2.3. Parameters tested

To determine fruit morphometric traits, twenty five fruits were sampled at harvest maturity in the experimental field. Samples were taken from the south-facing side of trees 1–1.5 m above the ground. In representative fruit samples, average fruit weight was determined using an Adventurer Pro AV812M technical scale (Ohaus Corporation, Switzerland) and fruit dimensions (length, width) were taken by a digital caliper (0–150 mm, Kronen GmbH, Kehl am Rhein, Federal Republic of Germany). In the same samples, internal fruit quality traits were evaluated by determining fruit impact firmness using a hand-held shore-type penetrometer (FT 327, Effegi, Italy) and soluble solids content was measured by a binocular refractometer (Carl Zeiss, Germany). The data were expressed in g, mm, N and °Brix, respectively. Total phenolic content (TPC) was assessed by a modified Folin-Ciocalteu colorimetric method and total antioxidant capacity (TAC) by the DPPH method reported by Brand-Williams et al. (1995) with modifications (Sanchez-Moreno et al. 1999). The data were expressed as milligrams of gallic acid equivalents (GAE/100 g fresh weight) and Trolox equivalent antioxidant capacity (μmol TE/100 g FW), respectively.

### 2.4. Statistical analysis

Statistical analysis was performed using the statistical software package Statgraphics18 (Manugistics, Inc., Rockville, MD, USA). The data were subjected to one way analyses of variance (ANOVA, F test), followed by a comparison of means according to the Duncan test. Treatments were declared different at  $p = 0.05$  level of significance. The analyses were performed in three replications and the obtained values were expressed as the means ± standard error.

### 3. Results

#### 3.1 Morphometric traits of plum fruits

The present study revealed that fertilizer type and cultivar are important factors that significantly affect the morphometric traits of plum fruits, with the

exception of the influence of cultivar on fruit width (Table 1). The largest fruits were recorded in biofertilizer treatment (weight – 37.96 g; length – 47.99 mm; width 37.28 mm). With regard to the influence of cultivar, higher values for fruit weight (37.14 g) and length (51.02 mm) were observed in 'Stanley'.

**Table 1**

The influence of fertilizer type on fruit morphometric traits of plum cultivars

Factor		Weight (g)	Length (mm)	Width (mm)
<b>Fertilizer type (A)</b>	Biofertilizer	37.96±0.87 a	47.99±2.10 a	37.28±0.28 a
	Chemical fertilizer	35.66±1.56 ab	47.28±2.23 a	36.68±0.47 ab
	Control	32.43±1.38 b	45.01±1.064 b	35.70±0.37 b
<b>Cultivar (B)</b>	'Čačanska Lepotica'	33.57±1.09 b	42.50±0.48 b	36.56±0.31 a
	'Stanley'	37.14±1.19 a	51.02±0.82 a	36.54±0.44 a
<b>ANOVA</b>				
<b>A</b>		*	*	*
<b>B</b>		*	*	ns
<b>A × B</b>		ns	ns	ns

Data represent the means of three replicates ± standard error. Values within each column followed by the same small letter are not significantly different at  $p \leq 0.05$  by Duncan's test.

#### 3.2 Internal fruit quality

The analysis of variance showed a significant effect of fertilizer type and cultivar on fruit internal quality with the exception of the influence of fertilizer type on the soluble solids content (Table 2). The highest fruit

firmness (44.21 N) was recorded in biofertilizer treatment. With regard to the influence of cultivar on the internal quality traits of the fruit, a higher value of soluble solids content (19.98 °Brix) was recorded in plum cv. 'Stanley', while fruit firmness was higher in 'Čačanska Lepotica' (46.98 N).

**Table 2**

The influence of fertilizer type on internal fruit quality traits of plum cultivars

Factor		Soluble solids (°Brix)	Firmness (N)
<b>Fertilizer type (A)</b>	Biofertilizer	17.90±1.16 a	44.21±3.59 a
	Chemical fertilizer	17.74±1.19 a	37.52±4.61 ab
	Control	17.23±1.17 a	35.12±4.06 b
<b>Cultivar (B)</b>	'Čačanska Lepotica'	15.26±0.09 b	46.98±1.49 a
	'Stanley'	19.98±0.58 a	30.92±2.54 b
<b>ANOVA</b>			
<b>A</b>		ns	*
<b>B</b>		*	*
<b>A × B</b>		ns	ns

Data represent the means of three replicates ± standard error. Values within each column followed by the same small letter are not significantly different at  $p \leq 0.05$  by Duncan's test

### 3.3 Total Phenolic Content (TPC) and Total Antioxidant Capacity (TAC)

The results of our study related to the effects of fertilizer type on TPC and TAC in fruits of investigated plum cultivars are shown in Table 3. Fertilizer type showed a significant effect on TPC, as well as on TAC. Greater values of TPC and TAC were determined under

biofertilizer treatment (275.51 mg/100 g FW and 3.10 Trolox, mmol/100 g FW, respectively). The other studied factor (cultivar) also showed a significant effect on TPC and TAC. The value of TPC (273.35 mg/100 g FW) was higher in 'Stanley', while higher TAC (3.03 Trolox, mmol/100 g FW) was found in 'Čačanska Lepotica'.

**Table 3**

The influence of fertilizer type on TPC and TAC in fruits of plum cultivars

Factor		Total phenolics (mg/100 g FW)	Total antioxidant capacity (Trolox, mmol/100 g FW)
Fertilizer type (A)	Biofertilizer	275.51±32.27 a	3.10±0.45 a
	Chemical fertilizer	220.75±32.66 b	2.60±0.35 b
	Control	134.81±21.34 c	1.44±0.13 c
Cultivar (B)	'Čačanska Lepotica'	147.36±17.65 b	3.03±0.35 a
	'Stanley'	273.35±24.55 a	1.72±0.17 b
<b>ANOVA</b>			
A		*	*
B		*	*
A × B		ns	ns

Data represent the means of three replicates ± standard error. Values within each column followed by the same small letter are not significantly different at  $p < 0.05$  by Duncan's test.

## 4. Discussion

### 4.1 Morphometric traits of the fruit

Fruit size has been discussed as one of the main components of yield. Due to their attractiveness, fruits of large size are preferred for the fresh market. Moreover, large sized fruits improve hand-harvest efficiency. The largest fruits recorded under biofertilizer treatment in both studied cultivars might be related to the stimulatory activity of microorganisms introduced into the soil by the biofertilizer used, thus resulting in increased nutrient ability and better solute uptake by the plants. Additionally, it may be due to the translocation of photosynthates to the fruit. In this regard, Kamatyanatti et al. (2019) reported that applications of *Azotobacter* and phosphate solubilizing bacteria in the rhizosphere of subtropical plum cultivar 'Kala Amritsari' enhanced the availability of N and P to the plant roots while also increasing their rate of translocation from roots to flowers by intensively developing an extensive extraradical mycelium which helps the plants exploit mineral nutrients and water from the soil. As determined in their study, the free living nitrogen fixer (*Azotobacter*) can affect plant growth not only by fixing nitrogen but also by altering microbial balance, solubilizing fixed soil phosphorus, suppressing pathogenic microorganisms and by producing metabolites that stimulate plant development. Singh et al. (2017) reported that the applications of *Azotobacter* may promote nitrogen fixation and biosynthesis of plant growth regulators (viz. IAA, GA3) and hence positively affect the growth of fruit trees. Some authors found that the positive impact

of biofertilization can be further enhanced if these fertilizers are applied together with chemical fertilizers, FYM, green manures and/or vermicompost. In this regard, Thakur and Thakur (2014) recorded the highest plum yield in the combined application of biofertilizers, chemical fertilizers, FYM, vermicompost and green manures. Positive effects of the combined use of bio- and chemical fertilizers, and vermicompost on the growth of plum trees were also reported by Chauhan (2008). Von-Bennowitz and Hlusek (2006) also revealed positive effects of the combined application of chemical fertilizers (100% NPK, 75% NPK and 50% NPK) and bio-fertilizers (*Azotobacter*, *Azospirillum* and vesicular arbuscular mycorrhiza) on the vegetative growth, productivity and quality traits of mango fruits.

### 4.2 Fruit internal quality

Bearing in mind that the tested cultivars are primarily intended for fresh consumption and the fact that soluble solids and fruit firmness are the two indicators of plum internal quality that most affect consumer acceptance (Paz et al. 2008), we thought that it was important to investigate the significance of the effect of fertilizer type on these parameters. Soluble solids content is an important factor which determines the eating quality of fruit. Vangdal et al. (2007) reported that plums with less than 12.5% of soluble solids were not acceptable for most consumers. On the other hand, fruit firmness is a good way to monitor fruit softening and to predict bruising damage during harvest and postharvest handling. Numerous authors recorded moderate to significant effects of orchard management practices on the soluble solids content

and firmness of plum fruits. While studying the fruit quality parameters of several Japanese plum cultivars in organic and conventionally managed orchards, Daza et al. (2012) reported similar values of soluble solids concentration in both orchards. In their study, plum fruits under organic management showed equal or slightly greater firmness than under conventional treatment. Kamatyanatti et al. (2019) reported maximum firmness in treatment with biofertilizer along with 75% of N + 12.5% N through vermicompost + 12.5% N through FYM in their study of the effect of integrated nutrient management on growth, flowering, firmness and yield of subtropical plum cultivar 'Kala Amritsari'.

### 4.3 Total Phenolic Content (TPC) and Total Antioxidant Capacity (TAC)

Due to their health benefits, the demand for fruits with high levels of phytochemicals, in particular phenolic compounds, has received increasing attention from breeders, pharmaceutical companies and consumers. Plums contain a wide range of phenolics (Tomić et al. 2019; Sahamishirazi et al. 2017; Jaiswal et al. 2013; Miletić et al. 2012; Cevallos-Casals 2006; Kim et al. 2003; Stacewicz-Sapuntzakis 2001) which have been implicated in improving human health. The greatest benefit for human health is attributed to their antioxidant, anti-carcinogenic, antimutagenic, antimicrobial, anti-inflammatory and neuroprotective characteristics (Nile and Park 2014). However, the quantity and quality of bioactive compounds in fruits are strongly related to genotype (Tomić et al. 2019; Vizzotto et al. 2007; Scalzo et al. 2005), pre- and post-harvest factors (Melgarejo et al. 2012; Díaz-Mula 2009) and also to the applied growing technologies (Peck et al. 2006; Veberic et al. 2005; Carbonaro et al. 2002). All these studies confirmed that fruits produced without the use of synthetic products (fertilizers and plant protection products) have a higher amount of micronutrients and health-related secondary metabolites, such as phenolic compounds. When studying the effect of organic and conventional management on the bio-functional quality of thirteen plum cultivars (*Prunus salicina* Lindl.), Cuevas et al. (2015) recorded 5–10 % higher TPC and TAC of plum fruits under organic production management. In their study, an increased synthesis of phenolic compounds might be attributed to the production without pesticides and chemical fertilizers. The increase of the TPC and TAC of plum fruits could be explained by similar reasons. Our study revealed that 'Stanley' was the richest in TPC, whereas higher TAC was found in 'Čačanska Lepotica'. The pronounced influence of genotype was confirmed by Tomić et al. (2019) in their study on the phytochemical assessment of 18 plum cultivars, with 'Stanley' showing the highest content of total phenols, thus being categorized into a group of cultivars with a high content of total phenols.

### 5. Conclusion

The results presented in this study reveal that fertilizer type is an important factor that significantly affects plum production. Applications of biofertilizers containing beneficial microorganisms (*Azotobacter chroococcum*, *Bacillus megatherium* and *Bacillus subtilis*) in plum growing technology had a positive

effect on fruit morphometric traits, soluble solids content and firmness, as well as on TPC and TAC in both studied cultivars. Since this technique has been shown to be sustainable, it can be recommended for use in commercial plum production. However, further field trials should be conducted to establish a proper rate and frequency of applications in order to obtain the most beneficial influence on yield and fruit quality.

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