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# Grain yield and rhizosphere microflora of alternative types of wheat in organic production

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## Abstract
*This study examines the impact of organic growing technologies on the rhizosphere microflora value and grain yield alternative types of wheat. Research is based on three varieties of different alternative types of wheat (Triticum spelta, Triticum compactum and Triticum durum) and one conventional variety of ordinary soft wheat (Triticum vulgare). The experiment was set up on less carbonate-chernozem, according to the random block design with four replicates for each treatment ($F_1$-microbiological fertilizer, $F_2$-the combined use of microbiological fertilizer and organic fertilizer, $F_0$-control (no fertilizer application)). Research results showed that fertilization significantly influenced the increase in the total number of microorganisms to the level of 3.7% and 28%, and the number of Azotobacter to the level of 2.8% and 19.1%, with the proven higher efficiency of mixed application of fertilizers. Analysis of variance of the total number of microorganisms and Azotobacter showed a statistically significant difference between the tested varieties. Grain yield of spelt (4,540 kg $ha^{-1}$) was significantly higher compared to other varieties. The greatest variation in yield in the three-year period was noted for conventional variety ordinary soft wheat. The examined variants of fertilization had a significant effect on increasing grain yield, from the level of 13.9% to 36.4%.*

**Keywords:** organic production, alternative types of wheat, microorganisms, yield

## 1. Introduction
In addition to wheat, barley, rye and oats, as some of the most important agricultural plants for human consumption, there are other forms of small grains for special purposes, grown on smaller areas and often called alternative crops. Into this group, of the genus *Triticum*, the following sub-types of wheat belongs: durum wheat (*Triticum durum*), spelt wheat (*Triticum spelta*), the compactum wheat (*Triticum compactum*) and Khorasan wheat (*Triticum turanicum*). These types are old, well tolerating the lack of nutrients in the soil (KONVALINA & al. [11]), demonstrating the ability to be competitive towards weeds (BOND & GRUNDY [2], MURPHY & al. [15]) and are commonly associated with alternative agriculture systems such as the organic production.
The availability of the nutrients amounts required is a decisive factor for plant growth and yields achieving in organic production. Optimal levels of nutrients are provided mostly from alternative sources which, inter alia, include the use of organic and microbial fertilizers. Application of organic fertilizer influence improving the physical and chemical characteristics of rhizosphere soil (SIX & al. [19]), the increase in biomass and microbial activity (BULLUCK & al. [3], PIMENTEL & al. [16]; ŠIMON & CZAKÓ, [20]), and, thus, the content and availability of nutrients in the soil. In organic agriculture the presence of beneficial microorganisms in the soil is very important for the mineralization of organic substances and fixation of atmospheric nitrogen. It is estimated that the association between

wheat and diazotrophs can provide 10-30 kg $ha^{-1}$, or 10% of the total wheat requirements for nitrogen (KENNEDY & al. [10]) JARAK & HAJNAL [9] found that the use of micro-organisms in crop production can increase yields, reduce the consumption of mineral fertilizers and increase the soil microbial activity.

The main objective of this paper is to examine the dynamics of the number of rhizosphere microflora, as well as the productivity of alternative types of winter wheat in terms of organic production.

## 2. Materials and methods

The method used in this research is randomized block system with four replications, and the surface of the elemental plot was $6m^2$. An experiment was carried out in the "Radmilovac" ($44^o$45'N, $20^o$35'E Serbia) experimental school estate of the Faculty of Agriculture in Zemun.

*Meteorological and soil conditions of plant growth*. The research was conducted during three seasons in the period 2009/2010-2011/2012 (factor A). In the first year of investigation (2009/2010) precipitation and average air temperature was higher compared to the second and third year (table 1). A large amount of rainfall in June (180 mm) and high temperature in the second decade of the month shortened and hindered the process of winter wheat grain filling. In the second year (2010/2011) somewhat lower air temperature in October (10,5°C), December (2,5°C) were recorded compared with the same months in the first and third year of the research, while in May (63 mm) a smaller deficit rainfall was recorded. Autumn period in the third year (2011/2012) was characterized by colder weather with little precipitation. In late January and early February a heavy snowfall occurred, so the winter cereals had protection from extremely low ground-frosts. Very hot weather (24.6°C) with considerably less rainfall (14 mm) in June affected the faster flow of the initial stages of ripening winter wheat.

Table 1. The average monthly temperature and precipitations during the vegetation period (2009/2010-2011/2012)

| Year | Temperature (ºC) | | | | | | | | | | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | X | XI | XII | I | II | III | IV | V | VI | VII | |
| 2009/2010 | 14.0 | 10.4 | 4.9 | 1.0 | 3.9 | 8.7 | 13.9 | 18.3 | 21.4 | 24.3 | 12.1 |
| 2010/2011 | 10.5 | 12.2 | 2.5 | 2.0 | 1.4 | 8.2 | 14.6 | 17.3 | 22.4 | 24.1 | 11.5 |
| 2011/2012 | 12.9 | 5.0 | 5.8 | 2.7 | -2.5 | 10.1 | 14.4 | 17.9 | 24.6 | 27.1 | 11.8 |
| | Precipitations (mm) | | | | | | | | | | |
| 2009/2010 | 101 | 62 | 122 | 89 | 111 | 46 | 41 | 85 | 180 | 41 | 878 |
| 2010/2011 | 49 | 45 | 61 | 40 | 53 | 26 | 11 | 63 | 40 | 107 | 495 |
| 2011/2012 | 35 | 6 | 49 | 82 | 62 | 3 | 67 | 128 | 14 | 39 | 485 |

The experiment was carried out in field conditions, on leached chernozem soil type. Soil chemical properties were as follows: pH ($H_2O$) of 8.04, the total nitrogen content is 0.13% $P_2O_5$ 22.18 mg, 19.10 mg $K_2O$, content of humus in the topsoil layer ranges from 2.09% to 2.82%. Organic technology of wheat growing included conventional tillage, fertilization with organic and microbiological fertilizers without chemical crop protection and by use of four varieties of different types of winter wheat. Crops replacement in four - crop rotation was as follows: maize - winter wheat - spring barley + red clover - red clover.

Objects of the investigation were three varieties of different alternative types of wheat (Bambi, Nirvana and Dolap) and one common variety of soft wheat (NS 40S), has been selected primarily for intensive, conventional production (factor B).

Nirvana (*Triticum spelta*) late wheat variety, very resistant to winter with hulled grain. This type of wheat is used for special bread preparation that is significantly faster digested compared to regular wheat bread.

Dolap (*Triticum durum*) - winter, medium early durum wheat, good winterhardiness. Dolap is intended exclusively for the production of pasta, spaghetti, macaroni and the like.

Bambi (*Triticum compactum*) - late varieties of wheat resistant to winter with hulled grain. Bambi is a variety of wheat intended solely for preparing tea and hard biscuits.

NS 40S (*Triticum vulgare*) - medium-early variety of soft common wheat with good resistance to winter, drought-tolerant, high yield potential, excellent resistance to lodging.

Sowing was done by hand at the end of the second decade of October. The original seed of the Institute for Small Grains in Novi Sad was used.

To maintain and increase biological soil fertility two variants of fertilization were examined (factor C):

$C_1$ - fertilization using only microbiological fertilizer for nourishment (5.0 l $ha^{-1}$);

$C_2$ - biohumus fertilization (3.0 t $ha^{-1}$) and the microbiological fertilizer for nourishment (5.0 l $ha^{-1}$);

$C_0$ - control - without the use of fertilizers.

The organic fertilizer which is used in the study is characterized by a pH of 8.63 and the minimum content: 2.2% N; 4.8% $P_2O_5$ and 2.8% $K_2O$. The organic ferilizers is tilled in an amount of 3.0 t $ha^{-1}$ with a basic treatment. During the spring nutrition in the tiller phenophases of development microbiological fertilizer at a dose of 5 l $ha^{-1}$ was used, which contains min *Bacillus megaterium* $10^{-6}$ $cm^3$, *Bacillus licheniformis* $10^{-6}$ $cm^3$, *Bacillus suptilis* $10^{-6}$ $cm^3$, *Azotobacter chtoococcum* $10^{-6}$ $cm^3$, *Azotobacter vinelandi* $10^{-6}$ $cm^3$, *Derxia* sp. $10^{-6}$ $cm^3$.

Soil sampling for microbiological analysis was done in the spring, after the nutrition of the crops, at the end of the tiller phenophases. Analyses conducted related to the biological value of the land in the microbiology laboratory of the Institute of Field and Vegetable Crops in Novi Sad. The number of microorganisms was determined using the method of agar plates on a suitable nutrient medium, while soil suspension was prepared using a dilution series. The total number of soil microorganisms - was determined on an agarised soil extract (POCHON & TARDIEUX, [17]), from the $10^{-7}$ dilution. Number of *Azotobacter spp*. - was determined by the method "fertile drops" (ANDERSON [1]), from the $10^{-1}$ dilution. The sown bases were incubated at a temperature of 28°C. The incubation period for the total number of microorganisms was seven days and 24 hours for *Azotobacter*. After incubation, the colonies were counted and the number was converted per gram of dry soil (WOLLUM [22]).

Harvesting is done by special wheat combine harvester, customized for harvesting small experimental plots. Grain yield is measured from the entire elementary plot, converted to 14% moisture and expressed in kg $ha^{-1}$.

The data were processed by a mathematical statistical procedure using the statistical package Statistica 6.0. ANOVA statistical method was used to determine lowest significant differences for average values and confidence intervals of 95% (LSD 0.05% and 0.01%).

## 3. Results and discussion

Analysis of variance of the total number of microorganisms and the mixture on the basis of LSD test showed that the main sources of variation (age, variety and fertilization) and their interactions have very important ($P<0.01$) impact on the biological value of rhizosphere soil layer in the conditions of organic production.

Many soil properties, especially its biological phase, are subject to change due to meteorological factors. The favorable ratio of average air temperature and rainfall in the second year had a positive impact on the microbial community, and the total number of microorganisms in this year was the highest ($336.50 x 10^{-7}$ g soil) (table 2). The high impact on the total number of microorganisms' variability in the soil had observed cultivars, and the resulting differences were statistically highly significant. Overall, the cultivars Bambi and NS 40S a greater total number of microorganisms compared to varieties Dolap and Nirvana was noted (table 2).

Table 2. The total number of microorganisms ($10^{-7}$ g soil) in the rhizosphere tested species/varieties of wheat during the period 2009/2010 - 2011/2012.

| Year A | Types/varietes of small grains B | Fertilization C | | | Average | |
|---|---|---|---|---|---|---|
| | | $C_0$ | $C_1$ | $C_2$ | AB | A |
| 2009/2010 | *Triticum vulgare* – NS 40S | 296.40 | 208.65 | 319.20 | 274.75 | 289.70 |
| | *Triticum durum* - Dolap | 206.60 | 298.60 | 297.80 | 267.67 | |
| | *Triticum compactum* - Bambi | 305.35 | 355.45 | 369.75 | 343.52 | |
| | *Triticum spelta* - Nirvana | 246.90 | 228.30 | 343.45 | 272.88 | |
| | AC | 263.81 | 272.75 | 332.55 | | |
| 2010/2011 | *Triticum vulgare* – NS 40S | 453.70 | 477.20 | 449.70 | 460.20 | 336.50 |
| | *Triticum durum* - Dolap | 191.40 | 220.40 | 225.20 | 212.33 | |
| | *Triticum compactum* - Bambi | 411.50 | 378.30 | 428.20 | 406.00 | |
| | *Triticum spelta* - Nirvana | 253.20 | 268.20 | 281.00 | 267.47 | |
| | AC | 327.45 | 336.03 | 346.03 | | |
| 2011/2012 | *Triticum vulgare* – NS 40S | 239.10 | 340.10 | 388.70 | 322.63 | 281.24 |
| | *Triticum durum* - Dolap | 191.80 | 171.80 | 275.40 | 213.00 | |
| | *Triticum compactum* - Bambi | 349.20 | 332.60 | 411.30 | 364.37 | |
| | *Triticum spelta* - Nirvana | 140.60 | 128.40 | 405.90 | 224.97 | |
| | AC | 230.18 | 243.23 | 370.33 | | |
| BC | *Triticum vulgare* – NS 40S | 329.73 | 341.98 | 385.87 | 352.53 | B |
| | *Triticum durum* - Dolap | 196.60 | 230.27 | 266.13 | 231.00 | |
| | *Triticum compactum* - Bambi | 355.35 | 355.45 | 403.08 | 371.29 | |
| | *Triticum spelta* - Nirvana | 213.57 | 208.30 | 343.45 | 255.11 | |
| | C | 273.81 | 284.00 | 349.63 | | |

| | | A | B | C | AB | AC | BC | ABC |
|---|---|---|---|---|---|---|---|---|
| F-test | Level | ** | ** | ** | ** | ** | ** | ** |
| LSD | 5% | 2.641 | 3.096 | 2.641 | 5.912 | 4.932 | 5.912 | 14.405 |
| | 1% | 3.534 | 4.181 | 3.534 | 8.494 | 6.915 | 8.494 | 26.442 |

While researching the impact of seed inoculation on the yield of three different conventional varieties of common wheat, MILOŠEVIĆ & GOVEDARICA [13] also noted a different total number of microorganisms in the rhizosphere tested varieties.

The efficiency of applied fertilizers differed significantly between the examined variants of fertilization. The organic fertilizer in combination with microbiological fertilizer ($C_2$) had a better effect on the dynamics of rhizosphere microflora, so the number of microorganisms on this variant was higher by 28% compared to control. On variants with a separate application of microbial fertilizers ($C_1$) the difference compared with the control was significantly lower (3.7%) but still statistically significant. Investigations of other authors also point to a significant influence of organic fertilizers application on the number and diversity of microorganisms in the soil (CHAUDHRY & al. [4], HARTMAN & al. [8]).

Application of fertilizers in organic production, depending on the variant and varieties, influenced the increase in the total number of microorganisms in the level of 13.4% (Bambi) to 60.8% (Nirvana). Overall, the variant $C_2$ in all sorts achieved better effects compared to $C_1$ when it comes to increasing the total number of microorganisms. CVIJANOVIĆ & al. [5] also found that the use of different types and levels of various nutrients affect the dynamics and activity of microflora in the root` s rhizosphere of wheat.

Members of the genus *Azotobacter* are real aerobic microorganisms outnumbered in aerated soils. In addition to oxygen and moisture levels there may be the limiting factor for the growth and activity of these microorganisms. The conditions for mineralization of organic matter in the third year were very favorable, so the number of *Azotobacter* was significantly higher (154.15x$10^{-1}$ g soil) compared to the previous two years (table 3).

Table 3. The number of *Azotobacter* ($10^{-1}$ g soil) in the rhizosphere of tested species/varieties of wheat during the period 2009/2010 - 2011/2012.

| Year A | Types/varietes of small grains B | Fertilization C | | | Average | |
|---|---|---|---|---|---|---|
| | | $C_0$ | $C_1$ | $C_2$ | AB | A |
| 2009/2010 | *Triticum vulgare* – NS 40S | 127.15 | 130.40 | 145.45 | 134.33 | 138.34 |
| | *Triticum durum* - Dolap | 108.35 | 128.70 | 118.90 | 118.65 | |
| | *Triticum compactum* - Bambi | 145.20 | 136.95 | 180.50 | 154.22 | |
| | *Triticum spelta* - Nirvana | 131.40 | 149.90 | 157.15 | 146.15 | |
| | AC | 128.03 | 136.49 | 150.50 | | |
| 2010/2011 | *Triticum vulgare* – NS 40S | 180.60 | 160.90 | 182.80 | 174.77 | 145.97 |
| | *Triticum durum* - Dolap | 111.50 | 136.70 | 122.40 | 123.53 | |
| | *Triticum compactum* - Bambi | 115.70 | 115.30 | 147.70 | 126.23 | |
| | *Triticum spelta* - Nirvana | 122.20 | 128.30 | 227.50 | 159.33 | |
| | AC | 132.50 | 135.30 | 170.10 | | |
| 2011/2012 | *Triticum vulgare* – NS 40S | 191.70 | 179.90 | 198.10 | 189.90 | 154.15 |
| | *Triticum durum* - Dolap | 135.20 | 130.70 | 125.40 | 130.43 | |
| | *Triticum compactum* - Bambi | 154.70 | 161.93 | 213.30 | 176.64 | |
| | *Triticum spelta* - Nirvana | 110.60 | 121.50 | 126.80 | 119.63 | |
| | AC | 148.05 | 148.51 | 165.90 | | |
| BC | *Triticum vulgare* – NS 40S | 166.48 | 157.07 | 175.45 | 166.33 | B |
| | *Triticum durum* - Dolap | 118.35 | 132.03 | 122.23 | 124.21 | |
| | *Triticum compactum* - Bambi | 138.53 | 138.06 | 180.50 | 152.36 | |
| | *Triticum spelta* - Nirvana | 121.40 | 133.23 | 170.48 | 141.70 | |
| | C | 136.19 | 140.02 | 162.17 | | |

| | Level | A | B | C | AB | AC | BC | ABC |
|---|---|---|---|---|---|---|---|---|
| F-test | | ** | ** | ** | ** | ** | ** | ** |
| LSD | 5% | 2.113 | 2.478 | 2.113 | 4.731 | 3.947 | 4.731 | 11.527 |
| | 1% | 2.828 | 3.346 | 2.828 | 6.797 | 5.534 | 6.797 | 21.160 |

Analysis of variance in the number of *Azotobacter* in rhizosphere zone area showed a statistically significant difference between the tested varieties. Variety of ordinary soft wheat NS 40S (166.33x$10^{-1}$ g soil), which is designed in conventional production conditions, had a significantly higher average number of *Azotobacter* in the rhizosphere compared to alternative types of wheat varieties. The variability of genotypes according to the representation of this group of microorganisms indicates an increase in the ability to make associations by the selection of wheat.

Researched variations of fertilizers applied had a significant effect on number of *Azotobacter* increasing, whereby the efficiency of $C_2$ variants was higher (19.1%) compared to a variant of $C_1$ (2.8%). In the total amount of variation greatest impact on *Azotobacter* in the rhizosphere soil layer had an interaction year x genotype. In years with less rainfall and favorable air regime of soil (2010/11 and 2011/12), the number of *Azotobacter* for conventional varieties NS 40S was significantly higher compared with the varieties of alternative crops (table 3).

Applied fertilizers, depending on the variant and varieties are differently affected by the number of *Azotobacter*. On average, the variety of alternative types of wheat are better responding to applied fertilizers in comparison with the conventional variety of ordinary soft wheat. In addition, with the combined application of fertilizers better results have been achieved, compared to the standalone application of microbiological fertilizer related to increasing number of *Azotobacter*. The negative and statistically significant effects of independent application of microbial fertilizers to the number of *Azotobacter* were found in the variety NS 40S.

In the total amount of variation the greatest impact on grain yield had age and genotype. By studying the influence of rainfall at harvest time on the yield of winter wheat, MLADENOV & al. [14] found that each l $m^{-2}$ affect the yield reduction of 10 kg $ha^{-1}$, or 0.12%. In this study a greater influx of rainfall in the first year also had negative effects on grain yield (2,479 kg $ha^{-1}$) compared to the second (3,897 kg $ha^{-1}$) and third year of tests (4,417 kg $ha^{-1}$) (table 4).

The highest average yield in the applied organic cultivation technology had a variety of spelt, Nirvana (4,450 kg $ha^{-1}$), which was significantly higher compared to other varieties (table 4). Furthermore, in conventional varieties NS 40S was found with higher variation of grain yield in the three-year period compared with the varieties of alternative crops. Due to the interaction of genotype x environment in terms of organic production conventional varieties are not always the most effective. MURPHY & al. [15] also point to lower yields than conventional varieties of wheat in terms of organic cultivation.

The lowest yield in these studies was obtained at variety of durum wheat Dolap (2,473 kg $ha^{-1}$), in which he recorded the smallest total number of microorganisms and *Azotobacter*. Research of SINGH & al. [18] have shown that the efficiency of utilization of nutrients and water at durum wheat can be increased by choosing varieties that are compatible with beneficial microorganisms.

On average, in the three-year period, the application of fertilizers yield increased significantly, by 13.9% in the variant $C_1$ and 36.4% in the variant $C_2$ as compared to controls. The results obtained indicate a significant effect of organic fertilizers on wheat yield which is consistent with studies of other authors (ČERNÝ & al. [6], VERMA & al. [21]). Similar results on the impact of microbial and organic fertilizers on the yields of alternative cereals were announced by other authors (DOLIJANOVIĆ & al. [7], KOVAČEVIĆ & al. [12]).

Table 4. Average yield (kg $ha^{-1}$) of tested species/varieties of wheat during the period 2009/2010 - 2011/2012.

| Year A | Types/varietes of small grains B | Fertilization C | | | Average | |
|---|---|---|---|---|---|---|
| | | $C_0$ | $C_1$ | $C_2$ | AB | A |
| 2009/2010 | *Triticum vulgare* – NS 40S | 2,021 | 2,333 | 2,372 | 2,242 | 2,479 |
| | *Triticum durum* - Dolap | 1,563 | 1,835 | 2,161 | 1,853 | |
| | *Triticum compactum* - Bambi | 1,649 | 1,659 | 3,007 | 2,105 | |
| | *Triticum spelta* - Nirvana | 2,988 | 3,490 | 4,664 | 3,714 | |
| | AC | 2,055 | 2,329 | 3,051 | 2,479 | |
| 2010/2011 | *Triticum vulgare* – NS 40S | 4,264 | 5,575 | 5,671 | 5,170 | 3,897 |
| | *Triticum durum* - Dolap | 2,213 | 2,882 | 4,321 | 3,138 | |
| | *Triticum compactum* - Bambi | 3,454 | 3,994 | 4,113 | 3,854 | |
| | *Triticum spelta* - Nirvana | 4,002 | 4,899 | 5,636 | 4,846 | |
| | AC | 3,483 | 4,338 | 4,935 | 4,252 | |
| 2011/2012 | *Triticum vulgare* – NS 40S | 4,951 | 5,342 | 6,542 | 5,611 | 4,417 |
| | *Triticum durum* - Dolap | 2,145 | 2,275 | 2,867 | 2,429 | |
| | *Triticum compactum* - Bambi | 4,375 | 4,558 | 5,405 | 4,780 | |
| | *Triticum spelta* - Nirvana | 4,752 | 4,867 | 5,567 | 5,062 | |
| | AC | 4,056 | 4,261 | 5,095 | 4,471 | |
| BC | *Triticum vulgare* – NS 40S | 3,745 | 4,417 | 4,862 | 4,341 | B |
| | *Triticum durum* - Dolap | 1,974 | 2,331 | 3,116 | 2,473 | |
| | *Triticum compactum* - Bambi | 3,159 | 3,404 | 4,175 | 3,579 | |
| | *Triticum spelta* - Nirvana | 3,914 | 4,419 | 5,289 | 4,540 | |
| | C | 3,198 | 3,642 | 4,361 | | |

| | Level | A | B | C | AB | AC | BC | ABC |
|---|---|---|---|---|---|---|---|---|
| F-test | | ** | ** | ** | ** | * | ns | ** |
| LSD | 5% | 132.990 | 192.101 | 132.990 | 240.893 | 366.781 | - | 893.665 |
| | 1% | 177.276 | 259.412 | 177.276 | 329.982 | 526.984 | - | 1,640.445 |

The efficacy of fertilizers applied and their effect on the yield of tested species and varieties of wheat depends on the variant and agro-ecological conditions of the season. It is interesting that the differences between the fertilizing variants are more common in the second year (2010/2011), with favorable weather conditions. In a year with less rainfall (2011/2012) due to lack of moisture the full effect of the microbiological fertilizer activity is missing, so the resulting differences were smaller.
To the yield of tested wheat varieties significant influence of the interaction of all three sources of variation (AxBxC) is manifested. This points to the complexity of the interactions studied factors and that optimal organic growing technology, in terms of variety of choice and proper application of nutrients can only be created by taking into account the weather conditions during the year.

## 4. Conclusion

Based on these results it can be concluded that in the organic production system impact of agro-meteorological factors and genotype on rhizosphere microflora is significant. In addition, the results of these tests have clearly shown the importance of the application of organic fertilizers in organic production. The best effects on the number examined groups of the microorganisms and grain yield of different species/varieties of wheat have been achieved by using a combination of organic and microbial fertilizers. In terms of low investment the biggest yield was given by the variety of spelt. The research has indicated the higher yield variation of common wheat conventional varieties, which favors the cultivation of alternative wheat types in terms of organic production.

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