Soil fertility management in apple orchard with microbial biofertilizers

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Abstract. Intensification of horticulture in Russia involves planting of new high-density orchards with drip irrigation and fertigation as well as intensification of the exploitation of traditional orchards. This approach involves an increase in mineral fertilizer application imposing the risk of soil fertility loss. For several reasons, the use of traditional organic fertilizers like manure in orchards is currently marginal. Although bacteria-based biofertilizers cannot substitute mineral fertilizers completely, they can significantly reduce the need for mineral fertilizer application. The effect of microbial biofertilizers of the brands "Azotovit" (*Azotobacter chroococcum*), "Phosphatovit" (*Bacillus mucilaginosus*), as well as a mixture of bacteria and the fungus, "Organic" (*Azotobacter chroococcum*, *Bacillus subtilis*, *Bacillus megaterium*, *Trichoderma harzian*) was studied in two field experiments. In the experiment #1, the preparations "Azotovit" and "Phosphatovit" were delivered through a drip irrigation system in various combinations with mineral fertilizers. In experiment #2, the preparation "Organic" was also applied to the soil with irrigation water, also in combination with the mineral fertilizer. When solely applied, none of the studied preparations changed significantly the soil nutrient content and yield as compared with the variant fertilized by the mineral fertilizer at the maximum studied application rate. The combination of the microbial biofertilizer and mineral fertilizers applied at a low rate ensured the yield commensurate to that obtained under high-rate application of the mineral fertilizer.

1 Introduction

The number of apple orchards, including high-density intensive orchards, is rapidly increased in Russia. In intensively managed orchard, optimum supply of nutrients is a prerequisite for ample high-quality yields. In turn, this requires increased fertilizer application rates adjusted for the actual soil nutrient content, crop load, etc. Overapplication of fertilizers leads to environmental pollution, washout of the nutrients to groundwater [1, 2, 3], and to soil fertility degradation via acidification [4, 5] or salinization (mainly due to fertigation) [6].

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An excellent opportunity to decrease the chemical fertilizer application rate is their augmentation by microbiological biofertilizers based on bacterial and fungal cultures. Bacterial fertilizers are around for a long time [7, 8] but still are not widespread in commercial orchards. Still, certain bacterial species could steadily supply available nutrients to plants [9]. Plant growth-promoting bacteria (PGPB) dwell in the rhizosphere, where their abundance is increased due to the presence of the root exudates [10]. The effect of PGPB on plants stems from the transformation of nitrogen, phosphorus, and potassium compounds into the forms available to plants. They also produce plant hormone-like compounds, enzymes, and other substances with biopesticide and biostimulant activity [11].

Azotobacter synthesizes auxin-, cytokinin-, and gibberellic acid-like substances that directly affect plant growth and development [12]. The key function of *Azotobacter* as the fertilizer is fixing atmospheric nitrogen and converting it into ammonium available for absorption by plant roots [13]. *Bacillus mucilaginosus* is a bacteria that mobilizes soil potassium [14] and phosphorus [15] converting them into bioavailable forms. The plant phosphorus supply is also improved by *Bacillus subtilis* [17] and *Bacillus megaterium* [18] inoculation. *Trichoderma harzianum* is a soil fungus that promotes plant growth and development (PGPF) upon soil inoculation [18]. Different species of *Trichoderma* establish symbiotic relationships with higher plants increasing stress tolerance of their plant symbionts [19, 20].

Inoculation with *Azotobacter, Pseudomonas*, and *Bacillus* cultures increased yield of apple cvs. 'Granny Smith' and 'Čadel' as compared to artificial fertilization [21]. Biofertilizer application (based on *Bacillus, Pseudomonas, Lysobacter* and *Ohtaekwangia* species) increased the yield as well as microbiological and biochemical activity of the soil in the long-term study in China [21]. Monoculture inoculation of chernozem soil with *A. chroococcum* and *B. mucilaginosus* enhanced the soil microbial activity and optimized its available nutrient content, but the yield was significantly lower than in the orchard fertilized by chemical fertilizers at a maximal studied rate (broad applied $N_{120}P_{60}K_{120}$ and fertigated with $N_{35}P_{25}K_{35}$) [23]. To broaden the application of microbiological biofertilizers in modern orchard management practice, one must find economically viable approaches commensurate in terms of yield to the conventional fertilization in the intensive fruit production. Monoculture-based microbiological biofertilizer application could not accomplish the goal, so this study aimed at studying the combined application of microbiological and chemical fertilizers in intensive orchard on chernozem soil and its effect on yield and soil primary nutrient content.

2 Materials and Methods

The study was carried out in the experimental apple orchard of I.V. Michurin Federal Scientific Centre (Michurinsk, Tambov Region, Russia). Experiment #1 (2015-2017): cv. 'Zhigulevskoye' grafted on B396 rootstock, 2222 tr. ha^{-1} (4,5 x 1 m), planted in 2007, drip irrigated. Experiment #1 design: Control 1 (without fertilizing); Control 2 ($N_{25}P_{20}K_{3}5$); PGPB1 (*A. chroococcum*); PGPB2 (*B. mucilaginosus*); PGPB1 + PGPB2; PGPB1 + PGPB2 + $N_{10}P_5K_{15}$. Experiment #2 (2018-2019): cv. Berkutovskoye grafted on B118, 800 tr. ha^{-1}, planted in 2014. Experiment #2 design: Control 3 (without fertilizing); Control 4 ($N_{90}P_{30}K_{120}$, broad application); PGPBF (*A. chroococcum, B. subtilis, B. megaterium, T. harzianum*); PGPBF + $N_{30}P_{10}K_{40}$; PGPBF + $N_{45}P_{15}K_{60}$; PGPBF + $N_{90}P_{30}K_{120}$. Soil samples were taken in the middle of August. The assays included hydrolyzable nitrogen (Kjeldahl method, AKV-20), plant-available phosphorus (molybdenum blue method, KFK-3.01), exchangeable potassium (flame photometer, FPA-2.01), exchangeable calcium – complexometric with Trilon B [24]. Flower clusters were counted as well as small (20-25 mm) fruits (immediately after June drop), and fruits before harvest. The data were statistically treated according to Fisher's method [25], the lowest significant difference (LSD) was calculated at the level $P < 0.05$: the differences above the calculated LSD we considered as significant.

3 Results and Discussion

Monoculture inoculation of biofertilizers and chemical fertilizer application stimulated a significant increase of cluster number in both cultivars (Table 1).

Table 1. Fertilizer effects on productivity components under the influence of fertilizers

	Number of clusters per tree	Number of fruitlets per tree	Number of fruits per tree	Fruitset, %	Average fruit weight, g	Yield, kg tr.$^{-1}$
Experiment #1						
Control 1	65^{b}	73^{b}	57^{b}	$17{,}5^{b}$	$185{,}2^{b}$	$11{,}7^{b}$
Control 2	77^{a}	98^{a}	84^{a}	$21{,}8^{a}$	$210{,}2^{a}$	$17{,}8^{a}$
PGPB 1	80^{a}	89^{ab}	75^{ab}	$18{,}7^{b}$	$183{,}6^{b}$	$13{,}8^{ab}$
PGPB 2	74^{a}	77^{b}	68^{ab}	$18{,}3^{b}$	$180{,}7^{b}$	$12{,}3^{b}$
PGPB 1 + PGPB 2	71	97^{a}	78^{a}	$22{,}0^{a}$	$187{,}2^{b}$	$14{,}7^{ab}$
PGPB 1 + PGPB 2 + $N_{10}P_{5}K_{15}$	71	92^{a}	83^{a}	$23{,}4^{a}$	205,3	$16{,}8^{a}$
LSD_{05}	7	8	6	2,8	22,2	1,6
Experiment #2						
Control 3	57^{b}	57^{b}	20^{b}	$5{,}2^{b}$	212,8	$4{,}3^{b}$
Control 4	75^{a}	130^{a}	32^{a}	$8{,}5^{a}$	225,3	$7{,}2^{a}$
PGPBF	69^{a}	109^{a}	27^{ab}	$7{,}8^{a}$	225,9	$6{,}0^{ab}$
PGPBF + $N_{30}P_{10}K_{40}$	56	75^{ab}	22^{b}	$7{,}9^{a}$	$241{,}5^{a}$	$5{,}3^{b}$
PGPBF + $N_{45}P_{15}K_{60}$	59	106^{a}	36^{a}	$12{,}2^{ac}$	$199{,}3^{b}$	$7{,}2^{a}$
PGPBF + $N_{90}P_{30}K_{120}$	46^{b}	87^{ab}	22^{b}	$9{,}6^{a}$	225,1	$5{,}0^{b}$
LSD_{05}	7	17	4	1,7	19,0	1,1

^{a} – significantly more than in the Controls 1 and 3; ^{b} – significantly less than in the Controls 2 и 4; ^{c} – significantly more than in the Controls 2 и 4

The increase of bloom intensity as an effect of PGPB was earlier noted in the study with strawberry [26]. Still, we recorded such an increase only in the treatments PGPB 1 and PGPB 2 but not in the other treatments of both experiments. The number of fruitlets in the cv. 'Zhigulevskoye' was significantly higher in all treatments than in Control 1 except PGPB 2. The application of the fertilizers and their combinations in the cv. 'Berkutovskoye' significantly increased the number of fruitlets remaining after the June drop. In the treatments with the PGPB monocultures, the fruitlet number of cv. 'Zhigulevskoye' was relatively low. Combining the biofertilizers, also with chemical fertilizers, increased the number of fruitlets to the level recorded in Control 2. The inoculation of cv. 'Berkutovskoye' with PGPBF brought about relatively high a number of fruitlets (at the Control 4 level). Almost the same result was obtained when PGPBF was combined with the mineral fertilizer ($N_{45}P_{15}K_{60}$). The monoculture inoculation (*A. chroococcum* and *B. mucilaginosus)* did not affect significantly the number of fruitlets, but combining the strains increased it considerably. The efficiency of the mineral fertilizer addition depended on application rate: over- or underapplication lead to a decline in the fruitlet number in comparison with the Control 4. Consequently, the combined application of bio- and artificial fertilizers is complicated by the problem of determination of the correct application rate for the chemical fertilizer.

Table 2. Effect of fertilizers on primary soil nutrient contents pH (0-40 cm)

	Hydrolyzable N, mg kg^{-1} soil	P_2O_5, mg kg^{-1}soil	K_2O, mg kg^{-1}soil	CaO mmol/100g^{-1} soil	pH
Experiment #1					
Control 1	118,8	134,5	141,3	21,2	5,4
Control 2	169,5	183,2	192,3	23,1	5,6
PGPB 1	143,7	122,3	137,3	22,8	5,5
PGPB 2	104,5	152,1	155,7	25,6	5,5
PGPB 1 + PGPB 2	146,8	157,8	182,4	21,7	5,6
PGPB 1 + PGPB 2 + $N_{10}P_5K_{15}$	154,6	162,5	198,5	22,7	5,4
Experiment #2					
Control 3	68,6	154,7	172,1	18,3	5,2
Control 4	148,4	184,9	290,6	18,1	5,1
PGPBF	95,2	178,2	205,1	17,2	5,1
PGPBF + $N_{30}P_{10}K_{40}$	106,4	145,6	195,5	18,0	5,4
PGPBF + $N_{45}P_{15}K_{60}$	97,1	123,3	155,4	19,1	5,0
PGPBF + $N_{90}P_{30}K_{120}$	120,4	164,8	167,9	18,8	4,9
Optimal values for chernozem soil [30]	151-200	151-200	121-180	-	5,5-7,5

[a] – significantly more than in the Controls 1 and 3; [b] – significantly less than in the Controls 2 и 4;
[c] – significantly more than in the Controls 2 и 4

The fruit set from free pollination (ratio of fruit number to the number of flowers) increased in the treatments with mineral fertilizer application and combination of different microbial preparations and chemical fertilizers on cv. ‘Zhigulevskoye’. This parameter was higher in the cv. ‘Berkutovskoye’ in all fertilizer treatments as compared to the Control 3. As compared to the high-rate mineral fertilizer application, the fruit set did not increase in the cv. ‘Zhigulevskoye’ regardless of the treatment. In the cv. ‘Berkutovskoye’, a significant increase of fruit set was documented in PGPBF + $N_{45}P_{15}K_{60}$. The mineral fertilizer (also combined with biofertilizer) application increased the average fruit weight in ‘Zhigulevskoye’. Inoculation with the bacterial monocultures and their combinations had no discernible effect.

The critical manifestation of the efficiency of the studied treatments is yield. Thus, PGPB had a positive impact on the yield of strawberry and other *Rosaceae* fruit crops [27, 28, 29]. Regional climatic conditions modulate the efficiency of biofertilizers. Application of biofertilizers increased the yield in the cv. ‘Zhigulevskoye’ as compared to Control 1. The combined application of PGPB with low-rate mineral fertilizing increased the yield to the level of high-rate chemical fertilizing (Control 2). The efficiency of combination of the chemical fertilizers with the PGPBF inoculation on younger trees of cv. ‘Berkutovskoye’ had no discernable effect at the low and the high rates compared with the Control 3. The inoculation with PGPBF only increased significantly the yield as compared to Control 3, but it was lower than that in the treatment with the high-rate mineral fertilizing (Control 4). Only in the treatment PGPBF + $N_{45}P_{15}K_{60}$ the yield was the same as in Control 4.

The fertilizer application optimized the soil primary nutrient content (Table 2.). Thus, *A. chroococcum* adjusted hydrolyzable nitrogen concentration to the optimum level. The amounts of available phosphorus and exchangeable potassium in the soil increased in the treatment with *B. mucilaginosus*. The mixed culture PGPBF preparation also stimulated the soil phosphorus and potassium; a similar but lesser effect was recorded for soil nitrogen.

We demonstrated that a decrease of the mineral fertilizer application rate by combining them with biofertilizers is entirely possible and favorable for the apple trees. Monoculture microbiological fertilizers did not exert the expected effect, so the using of the combination fertilizing is a prerequisite. The most serious problem was associated with determination of the correct rate of the mineral fertilizer application in the combined fertilization since the traditional diagnostic methods are not efficient enough for this.

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